Updated recommendations for monitoring current-use pesticide toxicity in water and sediment in the Surface Water Ambient Monitoring Program

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SWAMP Technical Memorandum
SWAMP-TM-2015-0001
September 2015 **(updated July 2018)**

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BACKGROUND – Changing Pesticides

A decade of evidence from the Surface Water Ambient Monitoring Program has indicated that toxicity to invertebrates is most often caused by pesticides (Anderson et al., 2011). As patterns of urban and agricultural pesticide use change in California, the species used to monitor water and sediment toxicity in SWAMP programs should be selected to properly evaluate these variations. While past data showed that much of the surface water toxicity was due to organophosphate pesticides such as diazinon and chlorpyrifos, these have largely been replaced by pyrethroids in most watersheds. In addition, recent data suggest new classes of pesticides are increasing in use, including phenylpyrazoles such as fipronil, and neonicotinoids such as imidacloprid. Decisions regarding toxicity monitoring for these pesticides should be based on their use patterns, and their relative toxicity to different test species and protocols. In addition, the decision to monitor in water and/or sediment depends on the solubility and stability of these pesticides, which dictates their environmental fate. The following discussion provides guidance for application of appropriate test species and protocols to

SWAMP monitoring coordinators interested in incorporating toxicity testing into their monitoring designs. Emphasis is placed on monitoring in freshwater habitats but two protocols are also recommended for marine receiving systems.

RELATIVE SPECIES SENSITIVITY

Four classes of pesticides that continue to be detected at toxicologically relevant concentrations in California streams are organophosphates (e.g., diazinon, chlorpyrifos, malathion), pyrethroids (e.g., bifenthrin, permethrin, cypermethrin), phenylpyrazoles (e.g., fipronil and its degradates), and neonicotinoids (e.g., imidacloprid, clothianidin, thiamethoxam). The relative acute toxicity of selected pesticides from these classes to standard test species is presented as 96-hour median lethal concentrations (LC50s) in Table 1. These data show that at 96 hours, the amphipod *Hyalella azteca* is the most sensitive to pyrethroids such as bifenthrin, the midge *Chironomus dilutus* is most sensitive to fipronil and its degradates, and the cladoceran *Ceriodaphnia dubia* is most sensitive to organophosphates such as chlorpyrifos. Both *C. dubia* (48-hour LC50) and *C. dilutus* have comparable acute sensitivities to imidacloprid, but evidence suggest that *C. dilutus* is more sensitive in chronic exposures. *Hyalella azteca* is also relatively sensitive to the organophosphate pesticide chlorpyrifos. Table 1 also lists a column of fathead minnow (*Pimephales promelas*) LC50 values to demonstrate the lower sensitivities of this vertebrate to current use pesticides. The other component of U.S. EPA three-species testing, the algae *Selenastrum*, does not respond to these pesticides, but could be used for monitoring involving potential toxicity caused by herbicides.

Because pesticides are usually detected in mixtures (U.S.G.S., 2006), the use of more than one toxicity test organism is recommended if multiple pesticides are present or suspected, and if the monitoring budget allows for it. Pesticide mixtures can be additive, synergistic, or antagonistic. Lydy et al. (2004) provides a review of challenges in regulating pesticide mixtures with differing modes of action and relative toxicities to aquatic organisms. Surface waters containing current use pesticides may include mixtures containing the parent compound and its toxic degradates. Phillips et al. (2014) demonstrated that monitoring the single active ingredient of the organophosphate mosquito control pesticide naled did not capture all of the potential impacts to receiving systems because the primary degradate, dichlorvos, was more toxic than the parent compound. This characteristic also applies to fipronil, where the degradates fipronil sulfone and fipronil sulfide are more toxic to *Chironomus dilutus* (Weston and Lydy, 2014). Toxicity testing integrates the effects of mixture toxicity from different pesticides, as well as active ingredient and degradates.

Acute tests measure lethality, whereas chronic tests measure sub-lethal effects such as reduced reproduction, growth, or development. The differences between acute and chronic exposures in water column tests are typically defined by the protocol endpoint and test duration. Some pesticides demonstrate greater chronic toxicity to certain species so selection of chronic vs. acute toxicity test protocols should consider this characteristic. For example, there is little difference in 10 day and 28 day sediment exposures of *H. azteca* to the pyrethroid pesticide bifenthrin (Table 2; (Anderson et al., 2015)), but the difference in sensitivity between a 96 hour and 10 day water exposures of *H. azteca* to the neonicotinoid imidacloprid is much greater. The sensitivity of *C. dilutus* to imidacloprid in chronic water exposures is greater than that of *H. azteca*, and even *C. dubia*. Monitoring programs for pyrethroids will be adequately protective using the 96 hour water or 10 day sediment test protocols (note: water vs. sediment monitoring is discussed in the following section). Neonicotinoids, such as imidacloprid, demonstrate greater toxicity in longer term chronic toxicity tests (Table 2; see review (Morrissey et al., 2015)). Therefore, monitoring with longer-term tests using *C. dilutus* is recommended for receiving systems where imidacloprid is of concern (e.g., 10 day and 28 day water test protocols). Recent data by the California Department of Pesticide Regulation suggest that the highest concentrations of imidacloprid have been measured in agricultural watersheds (Starner and Goh, 2012), so chronic testing in agriculture-dominated watersheds is a current priority. Although the imidacloprid 28 day LC50 for *C. dilutus* is 0.91 µg/L, Morrissey et al., (2015) suggest 0.1 µg/L for chronic sublethal effects. These authors also suggest a long-term chronic protective value based on a probabilistic risk assessment of 0.035 µg/L.

A source of acute and chronic benchmarks for standard test species used for the evaluation of pesticide registration is the U.S. EPA Office of Pesticide Programs (OPP) Aquatic Life Benchmarks Database. The database is maintained by OPP and provides acute and chronic endpoints for over 300 parent pesticide compounds and degradates in surface waters. These benchmarks are developed using data from ecological risk assessments for pesticide registration decisions. The results of toxicity tests using standard species are reported and these species typically include one or more species of fish, invertebrates, and both vascular and non-vascular plants.

Table 1. Acute water toxicity of representative pesticides to standard test species in water.

	96 hour water LC50 (µg/L)			
Pesticide	*Ceriodaphnia dubia*	*Hyalella azteca*	*Chironomus dilutus*	*Pimephales promelas*
Bifenthrin	0.142 [a]	0.0093 [e]	0.069 [i]	1.90 [k]
Fipronil	17.7 [b]	0.728 [f]	0.033 [f]	398 [k]
Imidacloprid	2.07 [c]	65.4 [g]	2.65 [j]	>1,000 [l]
Chlorpyrifos	0.053 [d]	0.086 [h]	0.29 [i]	203 [m]

[a] (Wheelock et al., 2004), [b] (Konwick et al., 2005), [c] 48-hour LC50 (Chen et al., 2010), [d] (Bailey et al., 1997), [e] (Anderson et al., 2006), [f] EC50 (Weston and Lydy, 2014), [g] (Stoughton et al., 2008), [h] (Phipps et al., 1995), [i] (Ding et al., 2012), [j] (LeBlanc et al., 2012), [k] (Beggel et al., 2010)(24-hour LC50), [l] (Lanteigne et al., 2015), [m] (Holcombe et al., 1982)

Table 2. Acute versus Chronic LC50s for bifenthrin and imidacloprid toxicity to *H. azteca* and *C. dilutus*. ND indicates not determined.

	Hyalella azteca			*Chironomus dilutus*	
Pesticide and Matrix	96 hour	10 day	28 day	96 hour	28 day
Bifenthrin in Sediment (ng/g)	ND	9.1 [a]	9.6 [a]	60.2 [c]	Uknown
Imidacloprid in Water (µg/L)	65.4 [b]	7.01 [b]	7.08 [b]	2.65 [d]	0.91 [b]

[a] (Anderson et al., 2015), [b] (Stoughton et al., 2008), [c] (Maul et al., 2008), [d] (LeBlanc et al., 2012)

*Morrissey et al., 2015 suggest 0.1 ug/L for chronic sublethal effects; these authors suggest a long term chronic protective value based on a probabilistic risk assessment of 0.035 ug/L.

WATER and SEDIMENT MATRICES and RECOMMENDATIONS

The environmental fate of current use pesticides largely depends on their relative stability and solubility in water. The octanol water partitioning coefficient (K_{ow}) is a laboratory derived parameter used as a surrogate measure for the potential of organic chemicals to accumulate in tissues; it is also used as an indicator of relative solubility. Pesticides with high log K_{ow} values are hydrophobic and pesticides with lower log K_{ow} values are more soluble. Pyrethroid pesticides like bifenthrin are highly hydrophobic and therefore readily partition to particles in water and accumulate in sediments. Urban stormwater and agriculture monitoring programs also routinely detect pyrethroids in water. Based on this, and the relative sensitivity of test species, the primary environmental compartment and matrix recommended for monitoring pyrethroids would be sediments using the 10-day *H. azteca* protocol (Table 3). Depending on resources, water toxicity testing for pyrethroids also provides useful information and the 96-hour water test with *H. azteca* is appropriate for this application. Fipronil and its degradates have moderate log K_{ow} values and therefore can be expected to accumulate in sediments and be detected in water. As with pyrethroids, they can be monitored in both matrices depending on resources. Toxicity testing should be conducted with the midge *C. dilutus* based on its greater sensitivity to this pesticide. For sediment, the 10-day test is applicable. For water, the 96 hour and 10 day tests are applicable, but the 10 day test is likely more sensitive (Table 3). Since fipronil is not registered for use in agriculture, monitoring for this pesticide should be restricted to urban watersheds. Neonicotinoids are highly soluble and are therefore not expected to accumulate in sediments. Because they are sufficiently stable to persist in receiving waters and exhibit greater potential for chronic toxicity to chironomids (testing at longer durations), water testing for this pesticide should use the 10-day test with *C. dilutus*.

Table 3. Log K_{ow} partitioning coefficients for selected current use pesticides, likely environmental compartments and recommended monitoring matrices.

Pesticide Class	Representative Compounds	Usage	Solubility (Log Kow)	Primary Recommended Test Species and Test	LC50 for species and exposure
Pyrethroids	Bifenthrin	Urban/Ag	6.4	*H. azteca* - 10-day Sediment	12.9 ng/g
	Cyhalothrin	Urban/Ag	7.1	*H. azteca* - 10-day Sediment	5.6 ng/g
	Cypermethrin	Urban/Ag	6.8	*H. azteca* - 10-day Sediment	14.9 ng/g
	Permethrin	Urban/Ag	6.3	*H. azteca* - 10-day Sediment	201 ng/g
Phenylpyrazoles	Fipronil	Urban	4.1	*C. dilutus* - 10-day Sediment	0.90 ng/g
	Fipronil Sulfide	Urban		*C. dilutus* - 10-day Sediment	1.11 ng/g
	Fipronil Sulfone	Urban		*C. dilutus* - 10-day Sediment	0.83 ng/g
Neonicotinoids	Imidacloprid	Ag/Urban	0.57	*C. dilutus* - 10-day Water	0.91-2.65 ug/L
Organophosphates	Chlorpyrifos	Ag	4.7	*C. dubia* - 96-hour Water	53 ng/L
	Diazinon	Ag	3.8	*C. dubia* - 96-hour Water	320 ng/L
	Malathion	Ag	2.4	*C. dubia* - 96-hour Water	2,120 ng/L

MARINE and ESTUARINE TESTING

The amphipod *H. azteca* is tolerant of a relatively wide range of salinities and can therefore be tested in estuarine systems up to 15‰. Standard U.S. EPA protocols using euryhaline species with high sensitivity to pesticides include the 10-day sediment test with the amphipod *Eohaustorius estuarius,* and the 96-hour acute and 7-day chronic water tests with the mysid *Americamysis bahia*.

STATUS of U.S. EPA PROTOCOLS

The U.S. EPA describes acute toxicity test methods for *C. dubia* in its freshwater acute toxicity test manual (U.S. EPA, 2002). This method allows a range of test durations from 24 to 96 hours. In addition, the manual includes a supplemental list of test species, including the amphipod *H. azteca* and the midge *C. dilutus*.

The U.S. EPA and United State Geological Survey describe 10-day, and 42-day sediment toxicity test protocols for *H. azteca* and *C. dilutus* (U.S. EPA, 2000). The 10-day sediment exposure procedure can be adapted for use as a 10-day water-only static renewal exposure with both *H. azteca* and *C. dilutus* (this is the procedure currently used at the UCD Granite Canyon Lab for water testing with these species).

Long term tests can also be adapted for shorter durations, such as the 28-day exposures with *H. azteca* (measuring growth and survival), and *C. dilutus* (measuring growth, survival and, potentially, emergence). U.S. EPA and USGS are currently in the process of updating the U.S. EPA 2000 sediment toxicity manual, which will include methods for testing both species in water and sediment using different exposure durations that range from 10 to 42 days for *H. azteca*, and 10 to ~50 days for *C. dilutus*. This revision is currently undergoing internal review within these agencies (personal communication, C. Ingersoll, USGS, Columbia, Missouri).

SUGGESTED CITATION

Anderson BS, Phillips BM, Denton D, Stillway M, Deanovic L, Lyons M, Hamilton M. 2015. Updated recommendations for monitoring current-use pesticide toxicity in water and sediment in the Surface Water Ambient Monitoring Program. SWAMP Technical Memorandum. SWAMP-TM-2015-0001.

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