

ACCUMULATION OF PESTICIDES IN PACIFIC CHORUS FROGS (*PSEUDACRIS REGILLA*) FROM CALIFORNIA'S SIERRA NEVADA MOUNTAINS, USA

KELLY L. SMALLING,\*† GARY M. FELLERS,‡ PATRICK M. KLEEMAN,‡ and KATHRYN M. KUIVILA†

†California Water Science Center, US Geological Survey, Sacramento, California

‡Western Ecological Research Center, US Geological Survey, Point Reyes National Seashore, Point Reyes, California

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**Abstract:** Pesticides are receiving increasing attention as potential causes of amphibian declines, acting singly or in combination with other stressors, but limited information is available on the accumulation of current-use pesticides in tissue. The authors examined potential exposure and accumulation of currently used pesticides in pond-breeding frogs (*Pseudacris regilla*) collected from 7 high elevations sites in northern California. All sites sampled are located downwind of California's highly agricultural Central Valley and receive inputs of pesticides through precipitation and/or dry deposition. Whole frog tissue, water, and sediment were analyzed for more than 90 current-use pesticides and pesticide degradates using gas chromatography–mass spectrometry. Two fungicides, pyraclostrobin and tebuconazole, and one herbicide, simazine, were the most frequently detected pesticides in tissue samples. Median pesticide concentration ranged from 13  $\mu\text{g}/\text{kg}$  to 235  $\mu\text{g}/\text{kg}$  wet weight. Tebuconazole and pyraclostrobin were the only 2 compounds observed frequently in frog tissue and sediment. Significant spatial differences in tissue concentration were observed, which corresponded to pesticide use in the upwind counties. Data generated indicated that amphibians residing in remote locations are exposed to and capable of accumulating current-use pesticides. A comparison of *P. regilla* tissue concentrations with water and sediment data indicated that the frogs are accumulating pesticides and are potentially a more reliable indicator of exposure to this group of pesticides than either water or sediment. *Environ Toxicol Chem* 2013;32:2026–2034. © 2013 SETAC

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## INTRODUCTION

A variety of factors including habitat loss, predation, disease, and pollution are thought to be involved in amphibian population declines [1–4]. Agricultural chemicals, whether acting singly or in combination with other stressors, are receiving attention as one of several potential causes of amphibian population declines [5]. Surveys of natural populations have shown correlations between population declines and proximity to agricultural lands [2]. Moreover, anthropogenic stressors, such as the use of agrochemicals, are considered important cofactors that have the potential to decrease an amphibian's immune system and thereby increase disease prevalence in local populations [6–7].

Although the occurrence of a few pesticides (atrazine, endosulfan, and organophosphate insecticides) has been studied extensively in amphibian habitats [2], little is known about the exposure and potential effects on amphibian health of many other current-use pesticides. There is, however, increasing evidence that pesticide exposure may impact amphibians directly. For example, glyphosate formulations are considered highly toxic to amphibians [8–9], while effects on larval development have been observed after exposure to the pyrethroid, cypermethrin [10–11]. Another recent study described the effects of several previously untested fungicide formulations on tadpoles and juvenile frogs [12]. The cumulative relationship between current-use pesticides and other variables on amphibian health is complex.

Synergism can occur with pesticide mixtures being more toxic than the sum of individual compounds [13], and other stressors (such as disease) can enhance pesticide effects [6–7]. Although a clear link between pesticide exposure and population declines has not been established [14], we cannot rule out the role of pesticides in amphibian survival, especially as the use of pesticides continues to increase.

Amphibian population declines have been well documented in the Sierra Nevada Mountains over the last few decades [3,15–17], and several studies have observed a potential link between declines and agricultural land use [4,18]. In California, studies have focused primarily on the effects of organophosphate insecticides (chlorpyrifos, diazinon, and malathion), endosulfan, and their metabolites on the foothill yellow-legged frogs and the Pacific chorus frogs [14,19–20]. The Sierra Nevada mountains lie downwind of the Central Valley, which is one of the most intensively cultivated areas in the United States. Atmospheric transport and deposition of pesticides from the Central Valley has also been documented in these remote high elevation locations considered to be suitable and sustainable amphibian habitats despite recent population declines [3,14–15,21–25]. Amphibians residing in remote locations are exposed to a mixture of pesticides throughout the year [24], and the occurrence/effects of many of the newly registered compounds, including several fungicides, has not been well documented.

The overall objective of the present study was to determine if current-use pesticides accumulate in the tissues of frogs living in remote locations in the Sierra Nevada Mountains. The data generated from the present study adds to existing data on the occurrence of pesticides in the Sierra Nevada that suggests that atmospheric transport is the source of pesticides at these locations. The data also suggest that for many current-use

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\* Address correspondence to ksmall@usgs.gov.

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pesticides, tissue concentrations are a more reliable indicator of organismal exposure than rigorously sampled matrices such as water and sediment.

## MATERIALS AND METHODS

### Study area and sample collection

The sampling sites for pesticide analysis followed a north/south transect ranging from Lassen Volcanic National Park in the north to Giant Sequoia National Monument (adjacent to Sequoia National Park) in the south. A total of 7 sites were sampled throughout 5 areas, including Lassen Volcanic National Park, Lake Tahoe, Stanislaus National Forest, Yosemite National Park, and Giant Sequoia National Monument (Figure 1). All locations selected are located at varying distances from California's Central Valley and contain breeding populations of *Pseudacris regilla* (Pacific chorus frog; Figure 1). This species was selected for sampling because it is widespread

and abundant throughout the study area [3,26]. Sampling was conducted during the breeding season (May–July) in 2009 and 2010. The 2 sites in Yosemite National Park (Summit Meadow and Tioga Pass) were sampled only in 2010. Samples were collected from seasonal or perennial ponds (Table 1) ranging in size from less than 10 m to approximately 30 m in diameter and with relatively small contributing watershed areas (6.5–515 ha).

At each site, approximately 15 male frogs were collected, euthanized using a 0.2% benzocaine solution [26], weighed, measured, wrapped in aluminum foil, and frozen on dry ice. Surface water and bed sediment samples were also collected at each site during each visit. Water was collected again 10 to 12 wk following the first event, when pesticide application was at its peak. Approximately 4 L of water was collected from the edge of each pond, filtered through a glass fiber filter (Whatman), extracted, and analyzed for a suite of 94 pesticides and degradates. Bed sediment samples were collected from areas of active deposition located near the pond edge and analyzed for 93 pesticides. A detailed discussion of the methods used and the

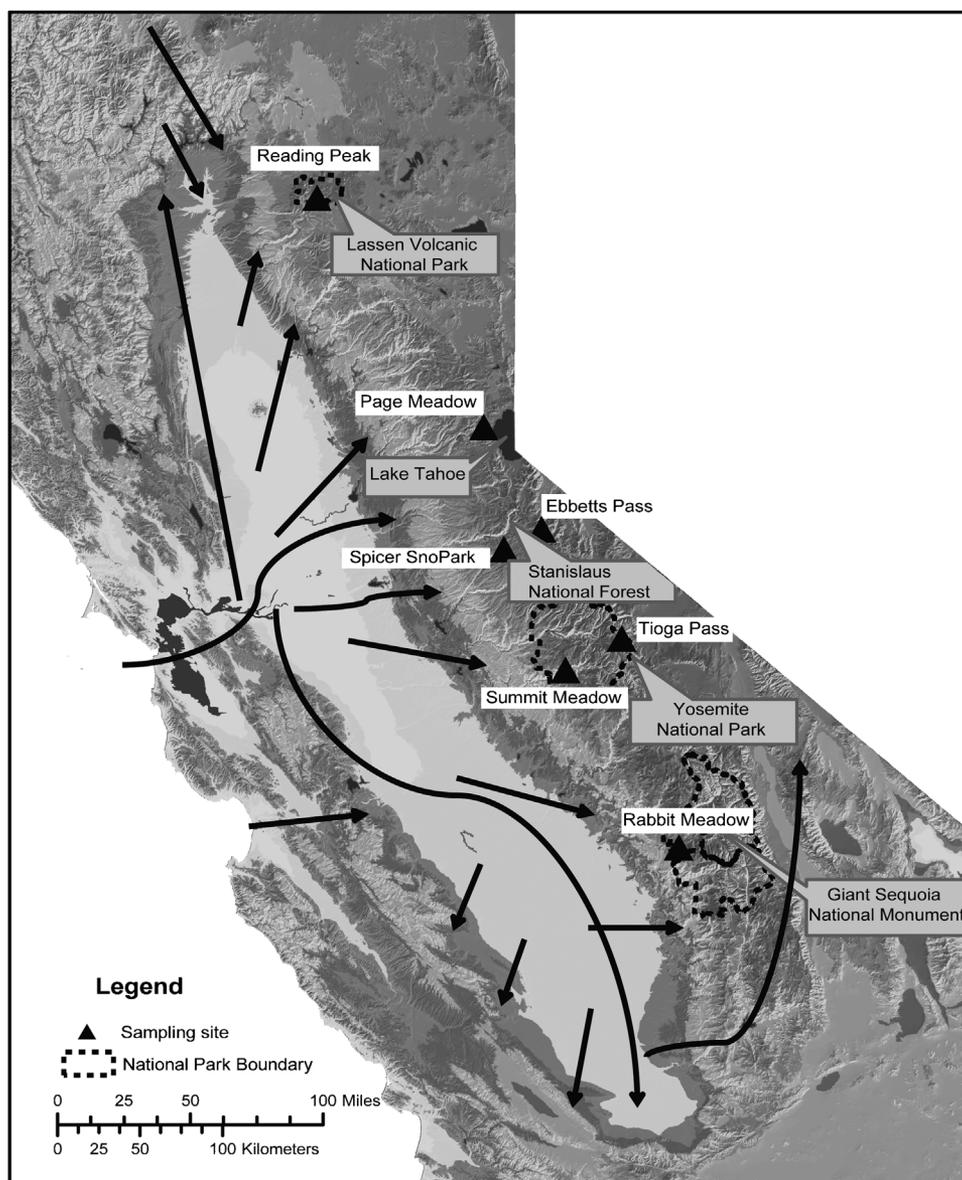


Figure 1. Location of water, sediment, and *Pseudacris regilla* tissue sampling sites. Seven sites were sampled in 5 different areas (denoted in grey boxes). Also shown are the key predominant wind directions based on California Air Resources Board streamline wind maps [17]. Sites in Yosemite National Park were not sampled in 2009.

Table 1. Location (including area sampled) of water, sediment, and *Pseudacris regilla* tissue sites sampled in 2009 and 2010

US Geological Survey station name	Site name	Areas sampled	Elevation (m)	Watershed size (ha)
Pond SE of Reading Peak at Lassen National Park	Reading Peak	Lassen Volcanic National Park	2230	9.0
Pond at Page Meadows near Tahoe City	Page Meadow	Lake Tahoe	2100	35.5
Pond at Spicer Sno-Park near Tamarack	Spicer Sno-Park	Stanislaus National Forest	2060	6.4
Pond at Ebbetts Pass near Markleville	Ebbetts Pass	Stanislaus National Forest	2660	5.0
North Summit Meadow at Yosemite National Park <sup>a</sup>	Summit Meadow	Yosemite National Park	2220	160
Pond at Tioga Pass at Yosemite National Park <sup>a</sup>	Tioga Pass	Yosemite National Park	3020	20.6
Rabbit Meadow at Sequoia National Park	Rabbit Meadow	Giant Sequoia National Monument	2360	17.0

<sup>a</sup>Sampled in 2010 only.

results from the water and sediment analyses can be found in Smalling et al. [27].

#### Tissue extraction and analysis for pesticides

Frog tissue samples were extracted and analyzed for 98 pesticides and pesticide degradates (Supplemental Data, Table S1) based on minor modifications to methods described in Smalling et al. [28]. Individual whole frogs (3–5 g) were thawed and homogenized with sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) using a clean, solvent-rinsed mortar and pestle. Samples were spiked with trifluralin-d<sub>10</sub>, ring-<sup>13</sup>C-*p,p'*-DDE and phenoxy-<sup>13</sup>C-*cis*-permethrin as recovery surrogates and extracted 3 times with dichloromethane using a Dionex 200 accelerated solvent extractor. Following extraction, sample extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and reduced to 1 mL. Ten percent by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.001 g using a microbalance. A majority of the lipid was removed using gel permeation chromatography followed by 6% deactivated Florisil previously activated at 550 °C for 16 h. Prior to analysis, samples were reduced to 200 µL, and a deuterated internal standard containing [<sup>2</sup>H<sub>10</sub>] acenaphthene was added to each extract.

Sample extracts were analyzed on an Agilent 7890 gas chromatograph coupled to an Agilent 5975 mass spectrometer (GC–MS) operating in electron ionization mode. Data for all pesticides were collected in selective ion monitoring mode with each compound having one quantifier ion and 1 to 2 qualifier ions (Supplemental Data, Table S1). For more detailed information on the extraction and analysis methods used, please see the Supplemental Data.

#### Quality control

All sample glassware was hand-washed and rinsed with tap water followed by acetone and hexane prior to use. All solvents and other reagents were ACS grade or better (Thermo Fisher Scientific). Pesticide standard materials were purchased from Chem Service, Riedel-de Haën, Supelco, and Ultra Scientific or were donated by the US Environmental Protection Agency (USEPA) National Pesticide Repository. Purities ranged from 95% to 99%. Internal standards ([<sup>2</sup>H<sub>10</sub>] acenaphthene) and surrogates (trifluralin-d<sub>10</sub>, ring-<sup>13</sup>C-*p,p'*-DDE and phenoxy-<sup>13</sup>C-*cis*-permethrin) were purchased from Cambridge Isotope Labs. Neat pesticides were dissolved in acetone or methanol for an initial concentration of 1 mg/mL.

Performance-based quality assurance and quality control included the parallel analysis of procedural blanks, matrix spikes, and replicates. The GC–MS was calibrated using standards that spanned the linear range of instrument response (0.025–5.0 ng/µL). Instrument response was monitored every 6 to 8 samples with midlevel check standards. Procedural blanks

consisting of 10 g to 20 g of baked Na<sub>2</sub>SO<sub>4</sub> run with every batch of 17 samples did not contain detectable levels of pesticides. Mean (± standard deviation) recoveries of trifluralin-d<sub>10</sub>, ring-<sup>13</sup>C-*p,p'*-DDE and phenoxy-<sup>13</sup>C-*cis*-permethrin added prior to sample extraction as recovery surrogates were 88.2 ± 11.5%, 89.8 ± 10.5%, and 89.1 ± 11.8%, respectively. Fifteen matrix spikes were analyzed and the recovery ranged from 70% to 132% with a median of 92%. Water samples were held for no longer than 48 h at 4 °C prior to extraction. Sediment and tissue were stored frozen at –20 °C and held for no longer than 2 yr prior to extraction.

Method detection limits for all compounds in tissue ranged from 0.5 µg/kg to 4.2 µg/kg wet weight (Supplemental Data, Table S1). Analytes can be identified at concentrations less than the method detection limits with lower confidence in the actual value and are reported as estimates. Limits of detection for all pesticides measured were also calculated and can be defined as the amount of analyte in the spiked sample that produced a signal greater than 3 times the background signal (Supplemental Data, Table S1).

#### Statistical analysis

An alpha level of 0.05 and a 95% confidence interval was used for all statistical analyses. A Kruskal–Wallis one-way analysis of variance by ranks was used to determine if there was a significant difference in pesticide concentrations between areas and years. A Tukey's rank sum multiple comparison was then performed to determine which area pairs were significantly different from each other. Censored values (i.e., non-detections expressed as lower than the limit of detection) were ranked as ties below the lowest detected concentration. Letters a, b (above the *x* axis, in Figure 4) indicate statistical significance, and areas with no letters in common are significantly different from one another (Tukey's test, *p* < 0.05). Statistical analyses were performed using S-Plus software (SPlus Technologies).

## RESULTS AND DISCUSSION

#### Occurrence of pesticides

Nine pesticides and 3 pesticide degradates were detected in male *P. regilla* samples collected in 2009 and 2010 (Supplemental Data, Tables S2–S3). Pyraclostrobin and tebuconazole (fungicides) and simazine (herbicide) were detected frequently (Table 2) with median detectable concentrations ranging from 13 µg/kg to 74 µg/kg wet weight (Figure 2) and maximum concentrations ranging from 64 µg/kg to 363 µg/kg wet weight. To our knowledge, this is the first field study that has reported the occurrence of pyraclostrobin, simazine, and tebuconazole in frog tissue. The highly persistent and bioaccumulative metabolite of *p,p'*-DDT, *p,p'*-DDE, was the most frequently detected pesticide

Table 2. Frequency of detection<sup>a</sup> in frog tissue for all pesticides detected at each site during the study<sup>b</sup>

Compound	Type	log $K_{OW}$ <sup>c</sup>	Lassen Volcanic	Lake Tahoe	Stanislaus National Forest		Yosemite National Park		Giant Sequoia
			National Park		Spicer Sno-Park	Ebbetts Pass	Summit Meadow <sup>e</sup>	Tioga Pass <sup>e</sup>	National Monument
			Reading Peak	Page Meadow					Rabbit Meadow
			30 <sup>d</sup>	24 <sup>d</sup>	30 <sup>d</sup>	27 <sup>d</sup>	15 <sup>d</sup>	15 <sup>d</sup>	30 <sup>d</sup>
3,4-DCA	D	NA	10	4	nd	4	7	nd	nd
3,5-DCA	D	NA	nd	4	nd	7	nd	nd	nd
Bifenthrin	I	7.3	nd	nd	13	nd	nd	7	nd
Carbofuran	I	1.8	nd	nd	nd	22	nd	nd	nd
Diazinon	I	3.7	nd	nd	nd	33	nd	nd	nd
Iprodione	F	3.1	nd	nd	nd	11	nd	nd	nd
Myclobutanil	F	2.9	23	nd	20	nd	nd	nd	nd
<i>p,p'</i> -DDE	D	6.5	57	25	43	22	60	nd	93
Propyzamide	H	3.3	nd	nd	23	15	nd	nd	17
Pyraclostrobin	F	4.0	7	13	27	48	nd	20	63
Simazine	H	2.3	nd	4	nd	59	nd	nd	37
Tebuconazole	F	3.7	60	54	30	52	nd	33	57

<sup>a</sup>Detection frequency is represented as a percent.

<sup>b</sup>Data for 2009 and 2010 was combined. The log  $K_{OW}$  for each detected compound was also included.

<sup>c</sup>Pesticide Properties Database, 2013; <http://sitem.herts.ac.uk/aeru/footprint/index2.htm>.

<sup>d</sup>Number of samples.

<sup>e</sup>Sampled in 2010 only.

DCA = dichloroaniline; D = degradate; F = fungicide; H = herbicide; I = insecticide; NA = not applicable; nd = not detected.

in frogs from all sites (Table 2). Although banned in the United States in 1972, DDT and its degradates persist in the environment and are biologically available for uptake by aquatic organisms. Median *p,p'*-DDE concentrations in frogs from Giant Sequoia National Monument (Rabbit Meadow) were similar to those observed in frogs collected in the late 1990s [3]. The absence of the parent compound suggests that *P. regilla* has the potential to metabolize the parent to *p,p'*-DDE or that all the environmental DDT has been weathered and converted almost

entirely to *p,p'*-DDE. Currently, no data are available on the toxicity of DDE to *P. regilla*; however, our results and those of other studies [3,29] suggest that DDE is persistent in amphibians collected from sites previously contaminated by DDT.

With the exception of *p,p'*-DDE and diazinon, none of the other pesticide detected during the present study have been reported in frog tissue. This is also the first study to measure tebuconazole and pyraclostrobin in samples collected from remote locations in the United States. Based on pesticide use

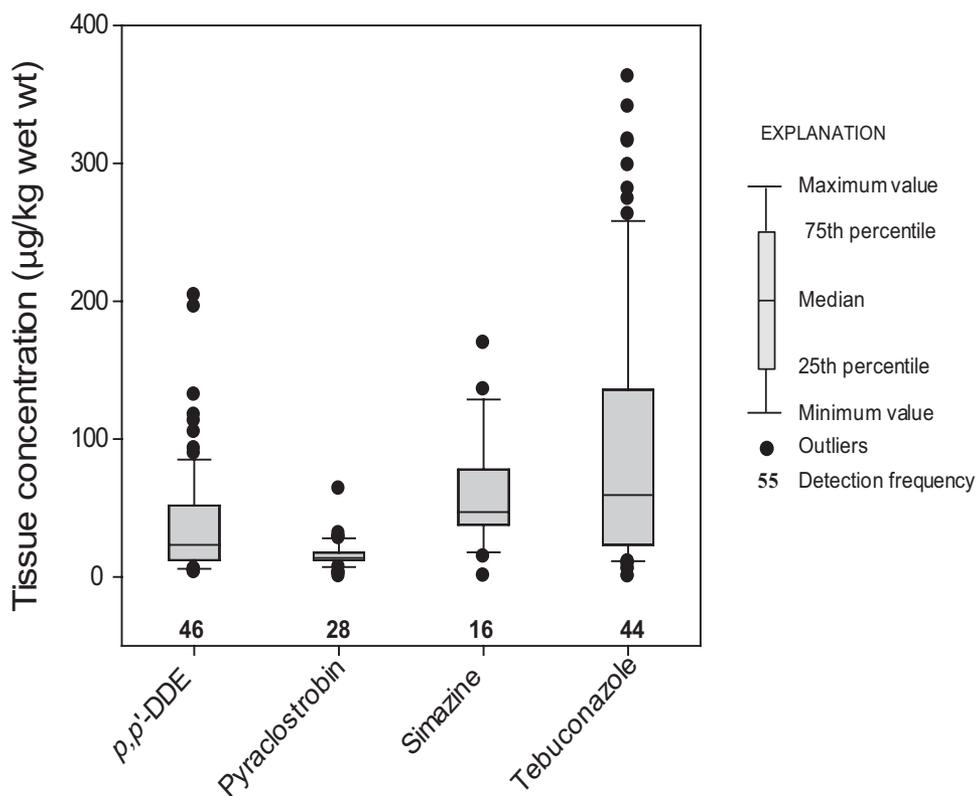


Figure 2. Individual *Pseudacris regilla* tissue concentrations ( $\mu\text{g}/\text{kg}$  wet wt) of the 4 most frequently detected pesticides. Only detected concentrations are included in the box plots.

records from 2008 to 2010, none of the pesticides detected during the study were applied directly to the area where they were observed [30]. Based on the nearest upwind county application data, many of the current-use pesticides detected during the present study were applied in the Central Valley (Table 3). The herbicide simazine was detected frequently in frog tissue from Rabbit Meadow (Giant Sequoia National Monument) and Ebbetts Pass (Stanislaus National Forest) in both 2009 and 2010 and in samples from Page Meadow (Lake Tahoe) in 2009 (Table 2), with a median detected concentration of 48  $\mu\text{g}/\text{kg}$  wet weight (Figure 2). Of the compounds detected, simazine has one of the highest uses in the Central Valley (Table 3), has been reported frequently in precipitation in agricultural areas throughout the United States, and was one of the most frequently detected pesticides in winter and early spring rain from California [31]. Simazine was also detected frequently in alpine lakes sampled in the Sierra Nevada, particularly when snow melt was dominant [32]. Temporal patterns of simazine and other herbicides in alpine lakes may reflect atmospheric inputs from areas sprayed months previously [22,32]. In contrast, little is known about the occurrence, fate, and transport of fungicides, and very few studies have reported the environmental occurrence of these compounds in the United States. In agricultural areas in Europe, tebuconazole was one of the most frequently detected pesticides in air samples [33]. Schummer et al. [33] reported the detection of tebuconazole predominantly in the gas phase, indicating its limited capacity for long-range transport, suggesting that the source of this compound to remote sierran sites is more localized and could be the Central Valley. Unlike tebuconazole, many pesticides such as atrazine and metolachlor are mostly present in the particle phase, will generally have higher atmospheric lifetimes [34], and therefore may be more susceptible to long-range transport. Both tebuconazole and pyraclostrobin are used in the Central Valley (Table 3), have the potential for atmospheric transport, and were some of the most frequently detected compounds in frog tissue.

Six pesticides were detected in water samples collected during 2009 and 2010 with concentrations ranging from 0.2 ng/L to 67 ng/L (Supplemental Data, Table S4; [27]). Other studies have detected low concentrations of organophosphate insecticides (chlorpyrifos and diazinon) and endosulfan in water at low parts per trillion levels in Sequoia National Park [3]. Similar compounds have also been detected in larval amphibians in the Klamath region near Lassen Volcanic National Park, including chlorpyrifos, endosulfan, and dacthal [35]. In the present study, water was not a good indicator of exposure because none of the pesticides detected in the frog tissue were observed in the water samples (with the exception of one very low DDE detection). This could be because of sampling frequency, extremely low (or undetectable) pesticide concentrations in the water, or because organisms metabolize the compounds observed in the water quickly or they do not accumulate in tissue.

Seven pesticides were detected in bed sediment samples from all sites with concentrations ranging from 2.5  $\mu\text{g}/\text{kg}$  to 430  $\mu\text{g}/\text{kg}$  dry weight (Supplemental Data, Table S5; [27]). Tebuconazole was one of the only compounds consistently detected in both frog tissue and sediment collected from the same site (Figure 3). However, it was only detected in both compartments (sediment and tissue) half the time, and a greater number of detections was observed in frog tissue compared with sediment (Figure 3). The concentration observed in the sediment from a specific site was also not directly related to the concentrations and number of tissue samples in which it was detected (Figure 3). The remaining compounds detected (Table 2) were only observed in tissue samples from each site. Water and sediment samples are expected to provide information on the source of exposure to organisms such as frogs; however, in the present study, the types of pesticides observed in the environment were not indicative of the body burden in chorus frogs. In remote locations, the sources of pesticides are difficult to pinpoint. Many studies in the Sierra Nevada range suggest, however, that aquatic organisms are being exposed to pesticides via wet and dry

Table 3. Pesticide usage data for upwind counties, 2008–2010 (kg active ingredient)<sup>a</sup>

	Total use	Bifenthrin	Diazinon	Chlorpyrifos	Myclobutanil	Propyzamide	Pyraclostrobin	Simazine	Tebuconazole
<b>2008</b>									
Giant Sequoia National Monument <sup>b</sup>	21 815 048	11 737	13 857	247 133	7675	5252	9990	84 908	5220
Yosemite National Park <sup>c</sup>	5 650 495	3702	3729	52 132	1931	110	5446	19 319	1109
Stanislaus National Forest <sup>d</sup>	5 654 454	3718	3729	52 185	1937	110	5459	18 928	1109
Lake Tahoe <sup>e</sup>	3 274 303	6039	7291	23 930	660	1	3429	3 258	169
Lassen Volcanic National Park <sup>f</sup>	603 273	318	514	7 165	8	28	155	1 374	0
<b>2009</b>									
Giant Sequoia National Monument	22 068 847	19 865	5824	168 444	8295	2323	8374	79 771	5516
Yosemite National Park	6 403 528	5191	3566	65 544	2680	137	6632	25 662	1781
Stanislaus National Forest	6 399 503	4805	3566	65 690	2685	137	6634	25 321	1785
Lake Tahoe	3 490 946	6521	7974	21 252	611	–	4378	5103	271
Lassen Volcanic National Park	569 431	387	397	7241	10	22	164	1791	0
<b>2010</b>									
Giant Sequoia National Monument	22 759 339	43 017	3727	241 274	8365	106	10 857	69 502	7109
Yosemite National Park	6 994 714	10 581	8882	56 609	2554	2	7385	20 941	738
Stanislaus National Forest	6 995 134	10 452	8882	56 769	2560	2	7401	20 712	737
Lake Tahoe	3 588 581	14 566	7713	19 922	724	–	3887	3037	643
Lassen Volcanic National Park	569 026	431	85	6390	9	20	438	1868	<1

<sup>a</sup>Includes all counties that are potential sources of pesticides to each of the sampling areas [30].

<sup>b</sup>Kings, Fresno, Tulare counties.

<sup>c</sup>San Joaquin, Stanislaus, Mariposa, Tuolumne counties.

<sup>d</sup>San Joaquin, Stanislaus, Calaveras, Alpine counties.

<sup>e</sup>Yolo, Colusa, Sutter, Placer counties.

<sup>f</sup>Tehama, Shasta, Lassen counties.

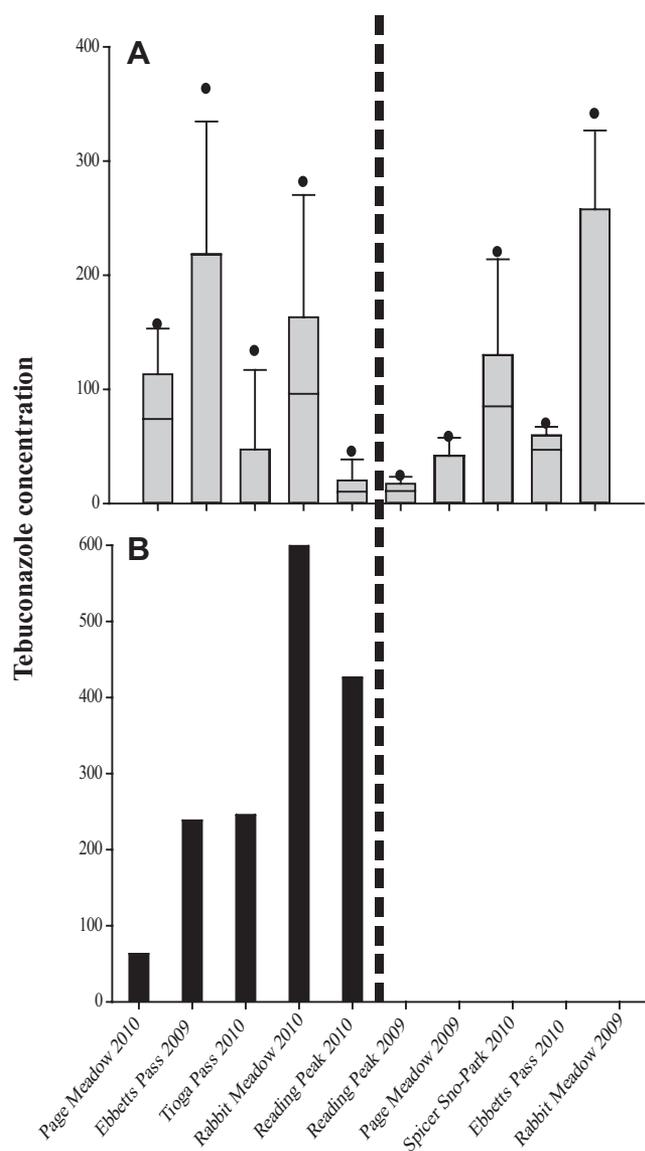


Figure 3. Tebuconazole concentrations in (A) *Pseudacris regilla* tissue ( $\mu\text{g}/\text{kg}$  wet wt) and (B) sediment ( $\mu\text{g}/\text{kg}$  dry wt) collected from all sites in 2009 and 2010. The dashed line separates sites where tebuconazole was detected in both tissue and sediment and sites where it was only detected in tissue. Tissue box plot includes detects and nondetects assigned a value of one-half the limit of detection. Sediment was only sampled once per site. Yosemite National Park (Tioga Pass) was only sampled in 2010.

deposition from heavy use in upwind counties [14,21–25]. If the interest is the organism, measuring accumulation into tissue may be a more robust metric for understanding contaminant exposure, especially in remote areas.

#### Temporal and spatial patterns

There were no significant temporal differences in pesticide concentrations of the 4 most frequently detected compounds in frogs. Average annual rainfall at each site was similar in 2008 and 2009, and pesticide use in the Central Valley did not change dramatically during the 2-yr period (Table 3). Pesticide concentrations in frog tissue did vary spatially by area, and concentrations in frogs from Giant Sequoia National Monument were significantly higher ( $p < 0.05$ ) than all of the other areas sampled (Figure 4). This is not surprising because pesticide concentrations have been detected frequently in wet

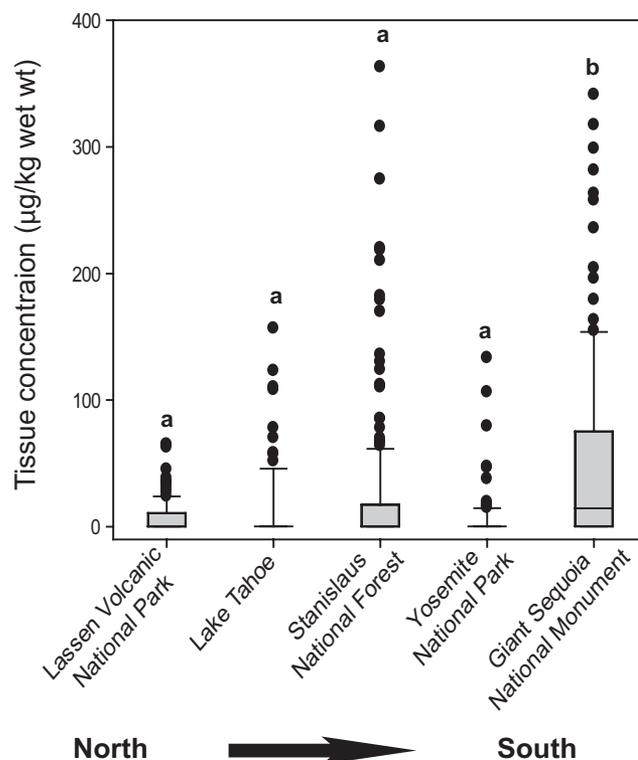


Figure 4. North–south spatial comparison of individual *Pseudacris regilla* pesticide concentrations ( $\mu\text{g}/\text{kg}$  wet wt) observed in the 5 major areas sampled in 2009 and 2010. Yosemite National Park was only sampled in 2010 and includes the Tioga Pass and Summit Meadow sites. Stanislaus National Forest includes both the Ebbetts Pass and Spicer Sno-Park sites. Nondetects were assigned a value of one-half the limit of detection for statistical analysis. Letters above the box plot represent statistical significance, and areas with no letters in common are significantly different from one another (Tukey's test,  $p < 0.05$ ).

deposition [22], dry deposition, and surface water [23] in Sequoia and Kings Canyon National Parks near the southern end of the Central Valley. The US National Park Service has also noted that these parks periodically experience some of the worst air quality in the national park system. The greatest numbers of pesticides were detected in frogs from Stanislaus National Forest followed by Lake Tahoe and Giant Sequoia National Monument. The number of pesticides detected and the median detectable concentrations in frogs were the lowest at Yosemite and Lassen Volcanic National Parks. A similar north–south spatial pattern was observed by Sparling et al. [20], where the lowest concentrations and detections were observed at Lassen Volcanic National Park, and some of the highest concentrations were detected in Giant Sequoia National Monument. Pyraclostrobin and tebuconazole were detected in samples collected from all areas (except Summit Meadow in Yosemite National Park); whereas simazine was only detected in samples from Lake Tahoe, Stanislaus National Forest, and Giant Sequoia National Monument. Significant spatial differences in tebuconazole concentrations were also observed, and the highest concentrations were measured in areas where use in the upwind counties was the highest (Supplemental Data, Figure S1). Tebuconazole was detected frequently at Lassen Volcanic National Park (Table 2) but at much lower concentrations compared with Giant Sequoia National Monument and Stanislaus National Forest. Although its use in the upper Central Valley is limited, the source of tebuconazole may be coming from agricultural regions upwind, possibly including areas in southern Oregon, where

detailed county level use data was not available. Pesticide detection and distribution could be a function of elevation, distance from source (upwind counties), timing of pesticide application, as well as complex pathways for airborne transport from the source to remote montane areas. Biological factors such as species life history traits (i.e., overwintering patterns) or compound specific metabolism could also influence differences in pesticide occurrence and distribution between sites and between animals at the same site.

Previous studies have reported the occurrence, persistence, and accumulation of pesticides in many of the locations sampled during the present study [3,11,25,32,35]. In California, Sequoia and Kings Canyon National Parks have received the most attention in the past 2 decades, and many studies have observed current-use and legacy pesticides in wet-dry deposition, vegetation, fish, and frogs [3–4,22,24,32]. Of the current-use pesticides, endosulfan has been one of the most frequently detected compounds at low part per billion (sediment, tissue) and low part per trillion levels (water, wet deposition). In previous studies, endosulfan was detected in sierran frogs collected in August [3,20,35], as well as in alpine lakes where peak concentrations were observed in the late summer, a time that corresponded to increased use in the upwind counties. Over the past 10 yr, endosulfan use has decreased in the Central Valley (46 000 kg to ~15 000 kg in Fresno, Kings, and Tulare counties) [30]. Furthermore, in 2010, the USEPA began to phase out the use of endosulfan in the United States because of its toxicity to a wide range of aquatic organisms. Peak endosulfan application in 2009 occurred in mid- to late August, with the majority of use occurring in the southern Central Valley (Fresno, Kings, and Tulare counties; [30]) upwind of Giant Sequoia National Monument. Chorus frogs were collected from Giant Sequoia National Monument in early June prior to peak endosulfan applications. Endosulfan was not analyzed in the present study because of several factors, including: 1) decreased use in the Central Valley, 2) the breadth of knowledge available in the literature, 3) cost limitations, and 4) timing of collection (prior to pesticide application).

#### *Effects of pesticides on amphibians*

Evaluating the biological effects of contaminants in tissues of field-caught organisms is complicated by many factors, including stressors that may go unmeasured. Typical laboratory-based studies are designed to measure the exposure of an organism to known chemical concentrations in water rather than assessing risk directly using tissue concentration. In the past, many toxicological tests included only standard test species and did not focus on native amphibians [36–37]. It is important to note that sensitivity to contaminants are often species-specific; therefore, laboratory studies conducted on other species may not apply directly to the field-collected species such as *P. regilla* collected in the present study. Furthermore, Fellers et al. [3] concluded that the effect of pesticides appeared to be more pronounced with longer exposure durations. This is important when considering the life histories of the species of interest. In the field, adult *P. regilla* are associated with water throughout their lives and remain proximal to ponds and lakes throughout much of the year, thus increasing their potential for exposure. Recently, more realistic exposure regimes have used native amphibian species [5,10,38] to compare field-collected data with laboratory exposure results.

Previous laboratory studies have focused on the effects of the herbicide glyphosate [9]; the insecticides carbaryl, chlorpyrifos,

diazinon, and endosulfan on many different species of anurans [5,19,39–40]; and the fungicide formulations containing pyraclostrobin [12,41]. Fungicides were detected frequently in frog tissue collected from many of the sites, and more information is needed to understand the effects of these compounds. Although certain pesticides have been shown to have an effect on many different species of anurans, the concentrations are orders of magnitude higher than what has been reported in the environment, especially in remote locations. It is also important to note that only frogs with sublethal concentrations of contaminants are available to sample because lethal concentrations would, by definition, remove the frogs from the pool of available samples. Therefore, the effects of the pesticides detected in the present study on populations of wild amphibians in the Sierra Nevada can only be inferred from limited information. Additional field and laboratory studies may be needed to confirm the hypothesized effects.

To understand the effects of pesticides on anurans and other species better, it is important to consider the compounds detected in tissue and their concentrations. In remote areas, water and sediment do not appear to be good predictors of anuran exposure because of the physical–chemical properties of the compounds ( $\log K_{OW}$ , environmental half-life), metabolism within the organism, as well as limitations in sampling and method performance. A direct measure of the accumulation of pesticides in tissue may give us a better understanding of what organisms are being exposed to in their environment and what they are capable of accumulating. Moreover, understanding tissue concentrations limits the amount of biological information necessary, such as pesticide bioaccumulation potential and metabolism within the organism, to model direct uptake and exposure.

#### CONCLUSION

Results from the present study show that current-use pesticides, particularly fungicides, are accumulating in *P. regilla* tissues. Many of the compounds detected in the present study have not been analyzed previously in the Sierra Nevada; in particular, pyraclostrobin and tebuconazole have not been measured in amphibian tissues at any location to our knowledge. This is one of the first studies to report fungicides in field-collected amphibians; however, the biological effects of these compounds on *P. regilla* and other amphibians are unknown. Current-use pesticides have the potential to be transported to remote locations via precipitation or dry deposition. The pesticides detected in tissue varied spatially and concentrations were highest in samples collected from Giant Sequoia National Monument, whereas the lowest concentrations were observed in Lassen Volcanic and Yosemite National Parks. Data generated from the present study expands our knowledge of current-use pesticides in amphibians and their habitats by increasing the number and types of compounds analyzed and comparing these results with previous studies.

It is difficult to evaluate the biological effects of contaminants measured in frog tissue because laboratory studies are typically based on exposure to known chemical concentrations in water and do not report tissue concentrations resulting from these exposures. Thus, the effects of environmentally relevant concentrations of pesticides on amphibians and their role in population decline are still unknown. The hypothesis that pesticides are one of many stressors responsible for amphibian population declines continues to present a challenge because of the large number of pesticides in use, the continual changes in

pesticides used, and the difficulty in determining routes of exposures in the wild. In addition, the biological effects of chemical mixtures are difficult to replicate in the laboratory. Their close association with wetlands makes amphibians potentially more sensitive to pesticides because they are exposed to seasonal changes in pesticide use. Even if concentrations are not high enough to be lethal, sublethal effects such as decreased resistance to disease may affect amphibian populations. To understand the effects of pesticides on amphibians, more coordinated field and laboratory studies are needed, the analytical methods and sampling techniques must be sensitive enough to measure low concentrations of pesticides, and the types of pesticides examined must evolve as pesticide use continues to change.

#### SUPPLEMENTAL DATA

##### Tables S1–S5.

##### Figure S1. (2.3 MB DOC).

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