A Refined Ecological Risk Assessment for California Red-legged Frog, Delta Smelt, and California Tiger Salamander Exposed to Malathion

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ABSTRACT

The California red-legged frog (CRLF), Delta smelt (DS), and California tiger salamander (CTS) are 3 species listed under the United States Federal Endangered Species Act (ESA), all of which inhabit aquatic ecosystems in California. The US Environmental Protection Agency (USEPA) has conducted deterministic screening-level risk assessments for these species potentially exposed to malathion, an organophosphorus insecticide and acaricide. Results from our screening-level analyses identified potential risk of direct effects to DS as well as indirect effects to all 3 species via reduction in prey. Accordingly, for those species and scenarios in which risk was identified at the screening level, we conducted a refined probabilistic risk assessment for CRLF, DS, and CTS. The refined ecological risk assessment (ERA) was conducted using best available data and approaches, as recommended by the 2013 National Research Council (NRC) report “Assessing Risks to Endangered and Threatened Species from Pesticides.” Refined aquatic exposure models including the Pesticide Root Zone Model (PRZM), the Vegetative Filter Strip Modeling System (VFSMDO), the Variable Volume Water Model (VWWM), the Exposure Analysis Model (EXAMS), and the Soil and Water Assessment Tool (SWAT) were used to generate estimated exposure concentrations (EECs) for malathion based on worst-case scenarios in California. Refined effects analyses involved developing concentration–response curves for fish and species sensitivity distributions (SSDs) for fish and aquatic invertebrates. Quantitative risk curves, field and mesocosm studies, surface-water monitoring data, and incident reports were considered in a weight-of-evidence approach. Currently, labeled uses of malathion are not expected to result in direct effects to CRLF, DS or CTS, or indirect effects due to effects on fish and invertebrate prey.

Keywords: Probabilistic risk assessment Malathion Endangered species SWAT model

INTRODUCTION

Malathion is a general-use, organophosphorus insecticide and acaricide that is used to control a variety of aphids, beetles, grasshoppers, and worms. First registered in the United States in 1956, malathion is widely used in agriculture, ornamental nurseries, pastures and rangelands, forestry, residential areas, public-health mosquito control, and rights-of-way. Additional details on the use of malathion in California are described in Supplemental Data Section S1.

Pursuant to the 2006 October 20 Settlement Agreement between the Center for Biological Diversity vs. USEPA (Case No. 07-cv-2794-JCS), the US Environmental Protection Agency (USEPA) conducted effects determinations for 3 species that are potentially exposed to malathion and listed by the US Fish and Wildlife Service (USFWS) under the US Endangered Species Act: California red-legged frog (CRLF; Rana draytonii), Delta smelt (DS; Hypomesus transpacificus), and California tiger salamander (CTS; Ambystoma californiense) (USEPA 2007, 2010a).
Using deterministic approaches in all 3 assessments, the USEPA concluded that malathion was “likely to adversely affect” the CRLF, DS, and CTS (USEPA 2007, 2010a). Based on their assessment, a request for consultation with the services (USFWS) was initiated under the ESA, which to date has not yet been acted upon or further reviewed.

Since the publication of USEPA’s assessments for CRLF, DS and CTS, the National Research Council (NRC) has conducted a review of the US government’s approaches for assessing risks posed by potential exposure to pesticides to listed species (NRC 2013). In addition to identifying the need to better characterize “best available data,” the NRC indicated that deterministic screening-level risk assessments should be followed by refined probabilistic assessments to appropriately characterize risk. Following the release of the NRC (2013) recommendations, the USEPA, USFWS, National Marine Fisheries Service (NMFS), and US Department of Agriculture (USDA) published the “Interim Approaches for National-Level Pesticide Endangered Species Assessment” (Agencies 2013). In a letter to Congress, the Agencies (2014) made it clear that they intended to use the NRC (2013) recommendations to conduct endangered species assessments, specifically their most recent biological evaluation (BE) for malathion (USEPA 2017). In the BE, USEPA determined the CRLF, DS, and CTS to be “likely adversely affected” by uses of malathion. Despite USEPA’s claims to follow the newly recommended approach to endangered species risk assessment, the risk designation conclusions in the BE retained the use of risk quotients. Risk quotients were described by NRC (2013) as being “not scientifically defensible for assessing the risks to listed species posed by pesticides…” As such, based on the NRC (2013) recommendations, the changes to the ESA assessment process, and the availability of many new studies for malathion, we have performed a screening-level ecological risk assessment (ERA) (Supplemental Data Section S2) and a subsequent refined probabilistic risk assessment for these 3 species, considering updated use patterns of malathion and best available data (Breton et al. 2013, 2014, 2015).

METHODS

Contaminants of concern and environmental fate

The focus of the present assessment was on the technical-grade, parent compound malathion. Based on available studies, most potential degradation products of malathion are much less toxic to nontarget organisms than is malathion itself (USEPA 2007). However, 1 minor degrade, malafoxon, is more toxic than malathion for some aquatic species (Supplemental Data Table S24). Despite its toxicity, malafoxon degrades quickly, has been infrequently detected, and when detected is found only at small percentages (0.2% to 10%) of malathion applied in both aquatic and terrestrial environments (Supplemental Data Section S3). In addition, a risk assessment of malathion and malafoxon found that risk conclusions for aquatic species did not differ substantially when malathion was assessed alone or in combination with malafoxon (Supplemental Data Section S3).

Malathion is degraded quickly in aquatic and terrestrial environments. Half-lives for aerobic metabolism of malathion in water are pH dependent and range from 0.3 to 3.3 d (Walker 1976; Knoch 2001a; Hiler and Mannella 2012), whereas half-lives in soil range from 0.5 to 14.3 h (Blumhorst 1990; Nixon 1995; Knoch 2001b). Similarly, 90th percentile foliar dissipation half-lives ranged from 4.10 to 5.73 d for non-ULV and ultra-low volume (ULV) applications of malathion, respectively (Moore, Clemow et al. 2014). Malathion’s estimated log $K_{ow}$ of 2.75 and solubility of 142 mg/L in water suggest that it is soluble and has a low potential for bioaccumulation in wildlife. Furthermore, malathion is rapidly metabolized via carboxylesterases, and overall removal half-lives of 17, 3.44, and 3.32 h have been estimated for fish, birds, and mammals, respectively (Reddy et al. 1989; Cannon et al. 1992, 1993; Kammerer and Robinson 1994).

Identification of ecological receptors

Three listed species that inhabit aquatic ecosystems in California were considered in the present risk assessment: CRLF, DS, and CTS. The designated critical habitat for each of these species is presented in Figure 1, and complete species profiles are presented Supplemental Data Section S2.

California red-legged frog. The CRLF is the largest native frog in the western United States (Wright and Wright 1949) and is endemic to the State of California and Baja California in Mexico (USFWS 2002). The aquatic-phase CRLF occurs in lentic or low-flowing water bodies with surrounding dense riparian or emergent vegetation (Hayes and Jennings 1988). The diet of larval aquatic-phase CRLF has not been well studied, but they are primarily algal grazers and they may also consume organic debris, plant tissue, and minute organisms (USFWS 2002; NatureServe 2016).}

Less is known about the habitat of terrestrial-phase CRLF. They likely disperse from breeding habitats to forage and seek shelter in riparian and upland habitat such as the burrows of small mammals, moist leaf litter, undersides of boulders, rocks, various agricultural features, and cracks at the bottom of dried ponds (USFWS 2002, 2010a). Adult CRLF consume a variety of invertebrate and vertebrate species found along the shoreline and on the water surface and are thought to forage indiscriminately. Gut-content analysis of CRLF reported prey from 42 taxa, with the most common being water striders, beetles, spiders, and larval alderflies (Hayes and Tennant 1985). Although analyses of gut contents suggested that underwater feeding is limited, similar studies for ranid frogs have reported the consumption of fish. Thus, this forage item should not be disregarded (Hayes and Tennant 1985). The largest prey items observed include Pacific tree frogs (Pseudacris regilla) and California mice (Peromyscus californicus).

Approximately 182 201 hectares have been designated as critical habitat for the CRLF (USFWS 2010a; Figure 1). Thirty-four core areas have been identified for CRLF, which
represent areas with long-term viability for existing populations as well as for potential re-establishment of historical populations (USFWS 2010a).

**Delta smelt.** The DS is an annual fish endemic to the San Francisco Bay and Sacramento–San Joaquin Delta Estuary ("Delta") in California (USFWS 2010b). Critical habitat for the DS has been designated in Suisun Bay; in Goodyear, Suisun, Cutoff, First Mallard, and Montezuma sloughs; and in the existing contiguous waters of the Delta (USEPA 2010a; Figure 1). Delta smelt are semi-anadromous (Bennett 2005) and spend a large part of their life in the freshwater edge of the "mixing zone," where the salinity is approximately 2 g/L (USFWS 1994, 1995). The diet of the DS is composed almost entirely of copepods (Bennett 2005), but they also consume small portions of rotifers, cladocerans, amphipods, insect larvae, and *Neomysis mercedes* (Nobriga 2002; Bennett 2005).

**California tiger salamander.** The CTS is endemic to the Central Valley and adjacent foothills of California where there are cool, wet winters and hot, dry summers (Loredo et al. 1996; USEPA 2010a; Orloff 2011). There are 3 distinct population segments (DPSs) of the CTS: Sonoma County, Santa Barbara County, and Central California. Currently, the CTS population in Sonoma County is found only in and around Santa Rosa (USFWS 2011). All CTS within Santa Barbara County occur in the Santa Maria Basin Geomorphic Province (USFWS 2009). The Central California populations are located in the East Bay Area, Central Valley, Central Coast, and Southern San Joaquin Valley (USFWS 2005).
The aquatic-phase of the CTS occurs in seasonal and perennial ponds, vernal pools, and stock ponds (USFWS 2004). During the wet winter season, CTS migrate to seasonal breeding pools approximately 0.15 to 1 m in depth (Cook et al. 2005, 2006). Terrestrial-phase CTS primarily inhabit burrows of the California ground squirrel (Spermophilus beecheyi) and Botta’s pocket gophers (Thomomys bottae) in surrounding upland areas, most commonly in open grasslands or under isolated oaks and less commonly in oak woodlands (Shaffer et al. 1993; Barry and Shaffer 1994; Loredo et al. 1996; Trenham 2001; USFWS 2009).

Aquatic-phase CTS are opportunistic feeders and consume zooplankton, arthropods, amphipods, mollusks, insect larvae, and small tadpoles of Pacific tree frogs (Pseudacris regilla), California red-legged frogs (R. draytonii), and toads (Dodson and Dodson 1971; USEPA 2010a, 2010b).

Risk hypotheses

Based on the screening-level risk assessments for CRLF, DS, and CTS conducted prior to the present refined ERA, malathion is not expected to cause acute or chronic direct adverse effects to aquatic-phase CRLF or CTS, aquatic-phase amphibian prey of CTS (Supplemental Data Section S2), terrestrial-phase CRLF or CTS (Breton et al. 2013, 2014), or the aquatic and terrestrial habitat of CRLF, DS, and CTS (i.e., aquatic and terrestrial plants; USEPA 2007, 2010a). The screening-level risk assessment results suggest no risk of chronic direct effects to the DS. However, acute direct effects could not be excluded at the screening level. Therefore, only potential direct acute effects to DS and indirect acute and chronic effects to CRLF, DS, and CTS due to reduction in fish (CRLF only) and/or aquatic invertebrate prey (CRLF, DS, and CTS) are assessed herein.

Analysis plan measures of exposure

Five refined exposure models were used to estimate exposure of aquatic species to malathion in worst-case scenarios for each species within California: the Pesticide Root Zone Model (PRZM) (Young and Fry 2014), Vegetative Filter Strip Modeling System (VFSMOD) (Muñoz-Carpena and Parsons 2004), Exposure Analysis Modeling System (EXAMS) (Burns 2000), Soil and Water Assessment Tool (SWAT) (Neitsch et al. 2011), and Variable Volume Water Model (VWWM) (Young 2014). Descriptions of these models and full details on the specific modifications made to the models, geographical locations considered, and exposure estimates that were derived for each species are described in the Supplemental Data Sections S5, S6, and S7. All models were run probabilistically (through Monte Carlo simulation) to account for uncertainty in the spatial and temporal distribution of malathion applications, hydrological characteristics, and spray drift inputs. The daily time series generated from each Monte Carlo simulation were combined to determine annual maximum estimated exposure concentrations (EECs) for various exposure durations (e.g., 48 h, 96 h, 21 d) at each site.

Exposure to CRLF. Using data from the California Pesticide Use Reporting Database (CA PUR) (CDPR 2012), California Natural Diversity Database (CNDD) (CNDD 2009), 2010 Cropland Data Layer (CDL) (Boryan et al. 2011), and recent (1993–2011) aerial photography and geographic information system (GIS) data from the area, the refined risk assessment incorporated spatially explicit use of malathion in California and with specific aquatic areas within the CRLF habitat areas (Figure 1). Based on the present analysis, approximately 95% of malathion used in CRLF critical habitat occurs in 2 core areas: the Watsonville Slough (WVS)/Elkhorn Slough (core area 19) and the Santa Maria/Santa Ynez rivers (core area 24) (Hanzas et al. 2013). Three conservative exposure scenarios were then modeled for specific aquatic features in these 2 areas:

1) Three ponds in the WVS/Elkhorn Slough core area
2) Streams and rivers in the WVS watershed
3) A tributary drainage ditch to the WVS.

For Scenario-1, PRZM/EXAMS was used in combination with VFSMOD to estimate EECs in 3 connected natural ponds of the McClusky Slough within the WVS core area. These ponds are surrounded by 18 agricultural fields with high historical use of malathion. The PRZM/EXAMS model was parameterized to account for area-specific soil and topography, local wind and weather patterns, locations and sizes of surrounding fields, pond volumes, and pesticide degradation. The VFSMOD accounted for the reduction in loads of malathion reaching the ponds due to trapping within the buffer between the treated fields and the water.

Scenario-2 EECs were generated using the SWAT model, which predicts flow, sediment, and pesticide outputs at multiple scales and locations throughout a watershed. Concentrations of malathion were estimated for 10 different subbasins, including 2 ponds and outlets of 8 stream segments located near Salinas, California. Each subbasin was parameterized using site-specific soil characteristics, flow, slope, hydrology, and pesticide use data. The SWAT model was also calibrated to account for the environmental fate of malathion during transport between subbasins. The resulting model produced 30-y time series (1981–2010) of daily concentrations of malathion at each of the 10 subbasins.

The SWAT model was also used to generate EECs for Scenario-3, which represents a worst-case ditch that contributes to the WVS watershed near Salinas, California. This ditch is within 6 m of agricultural land and is prone to runoff due to high clay content soils (50%); CRLF have been observed at this location. The SWAT model was parameterized to represent the size, hydrology, land use, soil, weather, agronomic practices, malathion use, and spray drift contributions for the ditch.

Because it was not practical to evaluate all labeled agricultural uses of malathion, 2 use patterns of malathion were investigated in the refined exposure analyses: strawberries and lettuce (Supplemental Data Tables S1 to S3). These 2 crops were selected because together they account for the majority (83.2%) of the cropland in the WVS (Hanzas et al. 2013). Further details of the refined modeling exercises,
input parameters, and results are provided in Supplemental Data Sections S4 and S5 and Tables S27 to S29.

Exposure to DS. A SWAT modeling approach was used to predict aquatic EECs in the flowing water bodies within the DS critical habitat. Only drainage areas below reservoirs draining the Sierra Nevada and coastal mountain ranges were included in the modeled watershed area (Figure 1). This area was associated with 97.9% of malathion use within the Delta watershed. The Delta watershed was divided up into 344 subbasins, each of which had an associated stream or river reach (n = 59) where daily predictions of concentrations of malathion were made (i.e., 59 subbasins had areas within or partially within DS habitat). The subbasin boundaries were designed to align with standard 12-digit Hydrologic Unit Code (HUC12) boundaries, and capture the main stem rivers entering the Delta (i.e., the Sacramento and the San Joaquin rivers), major tributaries, as well as smaller backwater sloughs within the DS critical habitat. Additional refinements were made to the SWAT model for reach-159 and reach-197 to more accurately account for the water volumes in those reaches (Supplemental Data Section S6). The structure of the model allows for the prediction of flow, sediment, and pesticide outputs at multiple scales and locations throughout a watershed. The resulting model produced 29-y time series of daily concentrations of malathion within multiple flowing-water bodies throughout the DS critical habitat.

Exposure to CTS. Given the unique aspects of CTS aquatic habitat (i.e., vernal pools, 0.15 to 1 m in depth), a custom approach to modeling concentrations of malathion in surface waters inhabited by CTS was developed for the present analysis. Using the USEPA standard landscape (PRZM version 5; Young and Fry 2014) and water body (VVWM; Young 2014) models provided as part of the Surface Water Concentration Calculator (Fry et al. 2014), input parameters were customized to reflect soils, slopes, weather, crop, and pond geometries observed in CTS habitat range. Pesticide burial and overflow were omitted to generate more conservative predictions of concentrations. Variable volume of water was not simulated so that volume of the vernal pool could be simulated at various estimates of constant volume based on observed surface areas and assumed depths. Modeling was conducted in 3 stages in which model input parameter assumptions were varied at each stage:

- Stage I: Best conservative exposure estimates for overall CTS range. One simulation ensemble (a collection of simulations with input parameters randomly sampled based on observed probability distributions) was conducted to determine the probability of malathion exposure to the overall CTS range including habitat in all population segments given best conservative assumptions about habitat and environmental conditions. Conservative assumptions included the use a crop footprint based on all agricultural use sites, and the assumption that malathion was applied aerially at the maximum labeled rate and minimum retreatment interval for strawberry (i.e., use site co-occurring with CTS habitat with the highest labeled rate). This modeling stage characterized exposure to the overall species range and included the potential for some pools to have no exposure based on their geographic proximity to use sites of malathion.

- Stage II: Conservative exposure estimates for varying drift and pool-depth assumptions for all potentially exposed CTS aquatic habitat. This modeling was conducted to evaluate the sensitivity of concentration predictions to 2 factors: spray drift and pool depth. Exposure was characterized only for the fraction of the aquatic habitat with some potential for exposure via spray drift and/or runoff (i.e., percent cropped area [PCA] greater than 0). Six unique simulation ensembles were computed: 3 with conservative estimates of spray drift deposition, assuming an aerial application at the maximum rate, and 3 with no spray drift. Each of the 3 ensembles with and without spray drift assumed 3 different pool depths at the minimum (0.15 m), median (0.575 m), and maximum (1 m) of the estimated range relevant for CTS.

- Stage III: Exposure estimates for distinct crops and CTS population segments. This modeling was conducted to evaluate exposure in more geographically specific population segments and for individual crops labeled for use of malathion. This is in contrast to Stage I modeling that characterized all population segments and all agricultural crops. Ensembles were simulated for the uses of malathion with the greatest extent of overlap with CTS vernal pool watersheds for each DPS. As in Stage II modeling, pools without any potential for exposure were not included in the ensembles.

Additional details on the input parameters and probability distributions used in PRZM5/VVWM modeling, as well as characteristics of the modeled water bodies are provided in Supplemental Data Section S6.

Effects assessment

As per recommendations of NRC (2013), acute and chronic toxicity data from open-literature and registrant-sponsored studies were reviewed, and endpoints that met quality criteria such as the high purity of the compound tested (as per current technical specifications), use of acceptable study protocols, application of appropriate statistical methods, and ecological relevance were selected for use in the refined assessments. A summary of all data considered and their subsequent ratings can be found in Breton et al. (2014, 2015).

Indirect effects to CRLF, DS, and CTS. Because CRLF, DS, and CTS are generalist feeders, the present assessment was not restricted to the most sensitive prey species. As long as overall productivity of prey species is protected, indirect effects to CRLF, DS, and CTS are not expected. To determine overall effects on prey productivity, the preferred measure of effect is at the community level of organization (e.g.,
community structure) (Aldenberg et al. 2002; Posthuma et al. 2002; NRC 2013). The data used to fit the acute species sensitivity distributions (SSDs) for fish and aquatic invertebrates and their corresponding probit slopes are presented in Supplemental Data Tables S10 and S11, respectively, and displayed in Figure 2A. Acute SSDs were generated using SSD Master v3.0 (Rodney et al. 2013), an Excel-based tool that fits 5 different cumulative distribution functions (normal, logistic, extreme value, Weibull, and Gumbel) in both log and arithmetic space. The Gumbel model using log LC50 and/or EC50 values (Supplemental Data Equation S2) was the best-fitting model for both fish and aquatic invertebrate data according to the Anderson-Darling (AD) goodness-of-fit test statistic and various graphical plots of model residuals (e.g., p-p and q-q plots).

Model parameters for the fish and aquatic invertebrate SSDs are presented in Supplemental Data Tables S10 and S11, respectively. The fish and aquatic invertebrate SSDs were used as the acute effects metrics for community structure (Figure 2A). Acute-to-chronic ratios (ACRs) were generated to assess chronic effects to aquatic invertebrates. Both acute and chronic toxicity data were available for 2 aquatic invertebrate species: the water flea (Daphnia magna) and saltwater mysid (Americamysis bahia). Based on these data, an ACR for the water flea of 7.95 was calculated by dividing the acute 48-h EC50 of 0.72 µg a.i./L (Gries and Purghart 2001) by the chronic EC10 of 0.0881 µg a.i./L (Blakemore and Burgess 1990), and an ACR for the saltwater mysid (A. bahia) of 29.3 was calculated by dividing the acute 48-h LC50 of 7.8 µg a.i./L (Brougher et al. 2014) by the 39-d EC10 of 0.266 µg a.i./L (Claude et al. 2012). The methods used to estimate EC10 values from Blakemore and Burgess (1990) and Claude et al. (2012) are described in Supplemental Data Section S8. Using USEPA’s guidelines for deriving water quality criteria (Stephan et al. 1985), a geometric mean of the 2 ACRs (15.3) was calculated for the refined assessment and used to derive the chronic SSD.

Direct acute effects to DS. Due to uncertainty regarding the sensitivity of DS to malathion, a concentration–response

Figure 2. Acute SSDs for aquatic invertebrate and fish species exposed to malathion (A). Acute concentration–response curve used to assess direct effects to DS (B). DS = Delta smelt; SSD = species sensitivity distribution.
The curve representative of the 5th percentile of the fish SSD was derived as a conservative effect metric. Probit slopes from all fish studies were estimated using the PROC PROBIT procedure in SAS software (Version 9.1, SAS/STAT 2004). The most sensitive probit slope was used to parameterize the probit concentration–response curve to estimate effects to DS. The concentration–response curve was derived using the fish SSD hazardous concentration for 5 percent of species (HC5) of 12.3 μg a.i./L as the LC50 and the most conservative slope of 2.81 for the sheepshead minnow (Supplemental Data Table S10). The fitted model is presented in Figure 2B, and the model parameters are μ = 1.09 and σ = 0.356.

Risk characterization

Risk curves were derived by integrating exposure distributions with an effects function to indicate the probability of exceeding effects of differing magnitudes (ECOFRAM 1999; USEPA 2004; Giddings et al. 2005). Acute risk curves for fish were derived by integrating the acute SSD with 96-h EEC distributions, and for aquatic invertebrates the acute and chronic SSDs were integrated with 48-h and 21-d EECs, respectively. The areas under the risk curve (AUC) were then used to categorize risk as de minimis, low, intermediate, or high based on the criteria described in Moore et al. (2010); Moore, Teed et al. (2014); and Whitfield Aslund et al. (2016), in which risk categories are defined as follows:

- If the area under the risk curve is less than the AUC associated with the curve produced by risk products (risk product = exceedance probability × magnitude of effect) of 0.25% (e.g., 5% exceedance probability of 5% or greater effect = 0.25%), then the risk was categorized as de minimis. The AUC for risk products of 0.25% is 1.75%;
- if the AUC was equal to or greater than 1.75%, but less than 9.82% (i.e., the AUC for risk products of 2%), then the risk was categorized as low;
- if the AUC was equal to or greater than 9.82%, but less than 33% (i.e., the AUC for risk products of 10%), then the risk was categorized as intermediate; and
- if the AUC was equal to or greater than 33%, then the risk was categorized as high.

RESULTS AND DISCUSSION

California red-legged frog

The 90th percentiles of annual maximum EECs were calculated for 48-h, 96-h, and 21-d exposure periods for each of the exposure scenarios modeled (Table 1). Of the 3 ponds modeled in the WVS core area, pond-1 had the greatest 48-h, 96-h, and 21-d 90th percentile EECs of 0.102, 0.0863, and 0.0689 μg a.i./L, respectively. This pond had the greatest total area of water (106 108 m²) and was the shallowest of the 3 ponds considered (0.78 m compared to 0.85 and 1.18 m for pond-2 and pond-3, respectively). It was also the pond that has the greatest distance perpendicular to the field (184 m) and greatest distance parallel to adjacent fields (577 m compared to 565 and 542 m for pond-2 and pond-3, respectively). Therefore, it is expected to receive most of the malathion via drift and runoff after application. For the 10 modeled subbasins in the WVS watershed, 48-h and 96-h 90th percentile concentrations ranged from 0.0240 to 1.81 μg a.i./L and from 0.0145 to 0.989 μg a.i./L, respectively, whereas 21-d 90th percentile EECs ranged from 0.00299 to 0.246 μg a.i./L (Table 1). For the tributary drainage ditch of the WVS, the estimated 48-h, 96-h, and 21-d 90th percentile EECs were 1.71, 1.17, and 0.278 μg a.i./L, respectively (Table 1).

Risk curves were derived for each of the exposure scenarios modeled and were used to categorize risk to aquatic invertebrates and fish as de minimis, low, intermediate, or high. Acute risk to fish was determined to be de minimis for all exposure scenarios modeled including the 3 ponds, the 10 WVS streams and sloughs, and the drainage ditch (Table 1). For aquatic invertebrates, results indicate de minimis risk of acute effects in all 3 ponds and in 5 of the 10 subbasins in the WVS watershed (Figures 3A and 3B). Acute risk was low for the other 5 subbasins (AUCs ranging from 1.77% to 4.64%; Figure 3B) and for the drainage ditch modeled (AUC of 5.733) (Table 1 and Figure 3A). Chronic risk for the 3 ponds was categorized as de minimis (AUC of 0.692%), low (AUC of 5.72%), and intermediate (AUC of 10.7%) for pond-3, pond-2, and pond-1, respectively (Table 1 and Figure 3A). For the streams and sloughs of the WVS, risk was calculated as de minimis for 3 subbasins and low for the other 7 (AUCs ranging from 2.88% to 7.42%; Figure 3B). Intermediate risk of chronic effects to aquatic invertebrates was identified in the drainage ditch (AUC of 10.3%) (Table 1 and Figure 3A).

The tributary drainage ditch is considered to be the most vulnerable water body in the entire WVS watershed due to its collection of runoff and irrigation tail waters from agricultural land. Additionally, it consists of soils having high clay content that facilitate runoff from crops within 6 m of adjacent fields (Hanzas et al. 2013). The 3 ponds from McClusky Slough represent a natural pond system, in which CRLF have been observed, surrounded by land receiving high agricultural use and subject to some of the greatest historical uses of malathion in California (Hanzas et al. 2013). Of the 3 ponds located in McClusky Slough, 1 of the ponds (pond-1) was found to pose potential intermediate risk to aquatic invertebrates (a dietary item of adult CRLF). Results from the estimated risk curves can also be used to describe the probability of exceeding various percentages of effects. The estimated risk curve for chronic effects to invertebrates in pond-1 shows that 88% of the aquatic invertebrate community would not be chronically affected (i.e., zero probability of exceeding the EC10). For the ditch scenario in which intermediate risk was described, there is a 15% probability of chronic effects to 25% of aquatic invertebrate populations, and a negligible probability that 75% of the community will experience chronic effects.

Delta smelt

The 90th percentiles of 48-h, 96-h, and 21-d annual maximum EECs for the 10 worst-case reaches are presented...
Critical habitat reaches had 48-h 90th percentile annual maximum EECs ranging from 0.000013 to 1.65 \( \mu \text{g a.i./L} \) and 21-d 90th percentile annual maximum EECs ranging from 0.0000014 to 0.491 \( \mu \text{g a.i./L} \) (Supplemental Data Table S34). After refinements to the SWAT model were made to better account for water volumes in reach-159 and reach-197, the 90th percentile 48-h average and 21-d average annual maximum concentrations were reduced to 0.0111 and 0.00469 \( \mu \text{g a.i./L} \), respectively, for reach-159, and 0.0122 and 0.00511 \( \mu \text{g a.i./L} \), respectively, for reach-197. Concentrations in the higher end of the range generally occurred in reaches corresponding to the subbasins with the greatest use of malathion and in headwater reaches with the smallest drainage areas and thus the lowest flow volumes, making them particularly susceptible to spray drift deposition.

Risk curves were derived for the 10 worst-case reaches of the DS SWAT model (Table 2), which were identified by ranking the 50th and 90th percentiles of annual maximum EECs for the 59 modeled reaches. There was de minimis risk of acute effects to DS residing in the 10 worst-case reaches of the Delta. The AUCs ranged from 0.00000005% for reach-159 and reach-197 to 0.218% for reach-155 (Table 2). The probability that SWAT EECs exceeded concentrations associated with 1% mortality to freshwater and marine fish was less than 4% for all reaches, and there was less than a 1% probability of observing a 5% effect on mortality.

### Table 1. Risk categories for fish and aquatic invertebrate prey of CRLF exposed to the 3 refined worst-case exposure scenarios in CRLF habitat using annual maximum EECs

| Scenario modeled | Subbasin | Acute effects to fish | | Acute effects to aquatic invertebrates | | Chronic effects to aquatic invertebrates |
|------------------|----------|-----------------------|-------------------------------|-------------------------------------|-------------------------------------|
|                  |          | 90th percentile 96-h EEC (\( \mu \text{g/L} \)) | AUC (%) | Risk category | 90th percentile 48-h EEC (\( \mu \text{g/L} \)) | AUC (%) | Risk category | 90th percentile 21-d EEC (\( \mu \text{g/L} \)) | AUC (%) | Risk category |
| 1) Three ponds in the WVS core area | Pond 1 | 0.0863 | 5.00 \( \times \) 10\(^{-8} \) | De minimis | 0.102 | 0.361 | De minimis | 0.0689 | 10.7 | Intermediate |
| | Pond 2 | 0.0572 | 5.00 \( \times \) 10\(^{-8} \) | De minimis | 0.0648 | 0.0822 | De minimis | 0.0437 | 5.72 | Low |
| | Pond 3 | 0.0171 | 5.00 \( \times \) 10\(^{-8} \) | De minimis | 0.0199 | 0.00261 | De minimis | 0.0107 | 0.692 | De minimis |
| 2) Eight streams and 2 sloughs in the WVS watershed | Reach 1 | 0.138 | 1.31 \( \times \) 10\(^{-5} \) | De minimis | 0.262 | 0.638 | De minimis | 0.0308 | 1.23 | De minimis |
| | Reach 2 | 0.58 | 0.00417 | De minimis | 1.04 | 2.47 | Low | 0.144 | 4.32 | Low |
| | Reach 3 | 0.989 | 0.0160 | De minimis | 1.81 | 4.64 | Low | 0.246 | 7.42 | Low |
| | Reach 4 | 0.343 | 0.00112 | De minimis | 0.596 | 1.91 | Low | 0.0898 | 3.85 | Low |
| | Reach 5 | 0.0208 | 0.00181 | De minimis | 0.0317 | 0.765 | De minimis | 0.00427 | 1.40 | De minimis |
| | Reach 6 | 0.0145 | 7.69 \( \times \) 10\(^{-6} \) | De minimis | 0.024 | 0.289 | De minimis | 0.003 | 0.651 | De minimis |
| | Reach 8 | 0.29 | 0.0567 | De minimis | 0.533 | 2.28 | Low | 0.0625 | 3.82 | Low |
| | Reach 11 | 0.28 | 0.0130 | De minimis | 0.409 | 1.77 | Low | 0.0853 | 4.93 | Low |
| | Slough 1 | 0.174 | 1.32 \( \times \) 10\(^{-6} \) | De minimis | 0.217 | 0.481 | De minimis | 0.0593 | 2.88 | Low |
| | Slough 2 | 0.398 | 1.04 \( \times \) 10\(^{-6} \) | De minimis | 0.524 | 1.35 | De minimis | 0.117 | 4.77 | Low |
| 3) Tributary drainage ditch to the WVS | Ditch | 1.17 | 0.0118 | De minimis | 1.71 | 5.73 | Low | 0.278 | 10.3 | Intermediate |

AUC = area under the risk curve; CRLF = California red-legged frog; EEC = estimated exposure concentration; WVS = Watsonville Slough, California, USA.

*Risk category boundaries: De minimis-low: 1.75% AUC; Low–intermediate: 9.82% AUC; Intermediate–high: 33.0% AUC.*
For 4 of the 10 worst-case reaches, there was de minimis risk of acute effects to aquatic invertebrates (AUCs of 0.000269% to 1.73%). Low risk of acute effects (AUCs of 1.79% to 4.37%) was observed for the remaining reaches (Table 2 and Figure 3C). Seven of the 10 worst-case reaches had low risk, and AUCs ranged from 3.98% (reach-153) to 8.43% (reach-210). Intermediate risk of chronic effects to aquatic invertebrates was observed for reach-209, where there is a 52% probability that EECs will exceed the chronic effects metrics for 5% of aquatic invertebrate species, a 15% probability of exceeding 25% of species’ effects metrics, and a 0.2% probability of exceeding 50% of species’ effects metrics (Figure 3C). However, the chronic AUC for reach-209 (10.7%) is only slightly higher than the low-intermediate risk boundary (9.82%). Furthermore, there is limited overlap between reach-209 and DS critical habitat as shown in Supplemental Data Figure S7.

California tiger salamander

For the conservative modeling assumptions, most representative of the entire range of CTS habitat conditions (Stage I modeling), the probability of CTS exposure to malathion was low. More than half (55%) of CTS aquatic habitats were not exposed to malathion (i.e., predicted concentrations were negligible). These habitats were outside of the range of malathion transport via spray drift (792-m buffer around pool) and runoff based on a conservative estimate of potential use sites from the all-agriculture crop footprint. The 90th percentile annual maximum 48-h and 21-d average concentrations were 0.83 and 0.423 μg a.i./L, respectively (Table 3).

The sensitivity of annual maximum concentrations to vernal pool depth, spray drift rate, and PCA was evaluated further in the next stages of modeling. For Stage II and Stage III modeling ensembles, the resulting exposure concentration distributions presented correspond to only 45% of habitat areas that have potential exposure. These habitats had potential exposure via deposition of spray drift from 0.001% up to 14.1% of the application rate. Stage II modeling was conducted with and without spray drift at 3 different pool depths individually. When spray drift was included, the simulation ensemble with the shallowest depth (0.15 m) had the highest concentrations whereas the simulation with the greatest depth (1 m) had the lowest concentrations. The 90th percentile annual maximum 48-h and 21-d average concentrations were 0.83 and 0.423 μg a.i./L, respectively (Table 3).
concentrations ranged from 2.31 μg a.i./L (1 m) to 11.4 μg a.i./L (0.15 m) and from 1.24 μg a.i./L (1 m) to 6.09 μg a.i./L (0.15 m), respectively (Table 3). These results show that vernal pool depth was significant in determining the volume of the vernal pools for dilution of the chemical. Modeling was repeated for these ensembles but with spray drift exposure assumed to be zero in all simulations. The 90th percentile concentrations were nearly the same for all depths (0.147 to 0.157 μg a.i./L and 0.036 to 0.045 μg a.i./L for 48-h and 21-d averages, respectively), indicating that pool depth was not an

Table 2. Risk categories for DS and aquatic invertebrates exposed to malathion in the 10 worst-case reaches within the DS critical habitat*  

| Reach | 90th percentile 96-h EEC (μg/L) | AUC (%) | Risk category | 90th percentile 48-h EEC (μg/L) | AUC (%) | Risk category | 90th percentile 21-d EEC (μg/L) | AUC (%) | Risk category |
|-------|---------------------------------|---------|---------------|---------------------------------|---------|---------------|---------------------------------|---------|---------------|
| Rch 144 | 0.467                          | 0.0118  | De minimis    | 0.744                           | 2.09    | Low           | 0.216                           | 8.01    | Low           |
| Rch 153 | 0.297                          | 0.0141  | De minimis    | 0.547                           | 1.79    | Low           | 0.0796                          | 3.98    | Low           |
| Rch 155 | 0.912                          | 0.218   | De minimis    | 1.65                            | 3.63    | Low           | 0.491                           | 8.10    | Low           |
| Rch 159 | 0.00815                        | 5.00 × 10⁻⁸ | De minimis | 0.0111                          | 2.69 × 10⁻⁴ | De minimis | 0.00511                        | 0.0825               | De minimis |
| Rch 197 | 0.00991                        | 5.00 × 10⁻⁸ | De minimis | 0.0122                          | 0.00263 | De minimis    | 0.00469                        | 0.0978               | De minimis |
| Rch 201 | 0.334                          | 0.00601 | De minimis    | 0.537                           | 1.73    | De minimis    | 0.128                           | 6.13    | Low           |
| Rch 203 | 0.766                          | 0.170   | De minimis    | 1.51                            | 4.37    | Low           | 0.160                           | 6.54    | Low           |
| Rch 207 | 0.235                          | 0.00554 | De minimis    | 0.399                           | 1.40    | De minimis    | 0.110                           | 6.06    | Low           |
| Rch 209 | 0.644                          | 0.0473  | De minimis    | 1.18                            | 3.95    | Low           | 0.283                           | 10.7    | Intermediate |
| Rch 210 | 0.375                          | 0.0349  | De minimis    | 0.638                           | 2.47    | Low           | 0.167                           | 8.43    | Low           |

AUC = area under the risk curve; DS = Delta smelt; EEC = estimated exposure concentration.

*Risk category boundaries: De minimis–low: 1.75% AUC; Low–intermediate: 9.82% AUC; Intermediate–high: 33.0% AUC.

Table 3. Refined risk characterization results for aquatic invertebrates exposed to malathion in vernal pools within CTS critical habitat  

| Modeling stage | Application rate | Drift | Depth (m) | 90th percentile 48-h EEC (μg/L) | AUC (%) | Risk category | 90th percentile 21-d EEC (μg/L) | AUC (%) | Risk category |
|---------------|------------------|-------|-----------|---------------------------------|---------|---------------|---------------------------------|---------|---------------|
| I             | Strawberry       | Aerial | Uniform distribution (0.15, 0.575, 1) | 0.83    | 3.23          | Low                      | 0.423   | 7.46          | Low |
| II            | Strawberry       | Aerial | 0.15      | 11.4                            | 11.1    | Intermediate  | 6.09                             | 21.5    | Intermediate  |
|               | Strawberry       | 0.575  |           | 3.66                            | 6.73    | Low           | 1.97                             | 16.3    | Intermediate  |
|               | Strawberry       | Aerial | 1         | 2.31                            | 5.23    | Low           | 1.24                             | 14.1    | Intermediate  |
|               | Strawberry       | None   | 0.15      | 0.147                           | 0.779   | De minimis    | 0.0363                           | 2.18    | Low           |
|               | Strawberry       | None   | 0.575     | 0.155                           | 0.818   | De minimis    | 0.0433                           | 2.46    | Low           |
|               | Strawberry       | None   | 1         | 0.157                           | 0.824   | De minimis    | 0.0446                           | 2.51    | Low           |
| III           | Strawberry       | Aerial | Santa Barbara | 6.08                            | 8.65    | Low           | 3.46                             | 19.8    | Intermediate  |
|               | Grape            | Aerial | Santa Barbara | 1.67                            | 4.47    | Low           | 0.675                           | 10.7    | Intermediate  |
|               | Grape            | Aerial | Sonoma    | 2.25                            | 5.08    | Low           | 0.919                           | 12.4    | Intermediate  |
|               | Vegetable        | Aerial | Santa Barbara | 1.50                            | 3.83    | Low           | 0.545                           | 9.29    | Low           |

AUC = area under the risk curve; CTS = California tiger salamander; EEC = estimated exposure concentration.
important factor when drift exposure potential was removed (Table 3).

In Stage III modeling, 1 ensemble of simulations was generated for each crop and DPS by sampling from the PCA distribution specific to that crop and DPS, instead of the PCA distribution for all agriculture and the entire habitat range that was sampled in Stage I. In addition, crop-specific use patterns were applied rather than using the worst-case strawberry use pattern, as was done in Stage I modeling (Supplemental Data Table S36). The crop–DPS combinations for strawberry–Santa Barbara, grape–Sonoma, grape–Santa Barbara, and vegetable–Santa Barbara resulted in the highest 90th percentile annual maximum concentrations, and all other crop–DPS combinations had concentrations that were 1 or more orders of magnitude lower. As such, our Stage III ERA focused on these top 4 crop–DPS combinations. Strawberry use sites in the Santa Barbara DPS had the highest PCAs and, therefore, the highest concentrations with 90th percentile annual maximum 48-h and 21-d average concentrations of 6.10 μg a.i./L and 3.46 μg a.i./L, respectively (Table 3). Grape use sites in Sonoma and Santa Barbara DPSs, followed by vegetable use sites in the Santa Barbara DPS, resulted in the next highest concentrations with 90th percentile annual maximum 48-h average concentrations of 2.25, 1.67, and 1.50 μg a.i./L, and 21-d average concentrations of 0.919, 0.675, and 0.545 μg a.i./L, respectively.

Risk curves were derived for all Stage I, II, and III modeling ensembles (Figures 3D and 3E, Table 3). The Stage I scenario categorized risk to aquatic invertebrate communities for the full range of CTS, including areas with zero PCA, and thus no potential for exposure. Acute and chronic risk to aquatic invertebrate communities potentially exposed across the overall CTS range was categorized as low. The AUCs were 3.23% and 7.46% for acute and chronic risk, respectively (Figure 3D). These values indicate 5.21% and 12.6% probabilities of affecting 25% of aquatic invertebrates within the community.

In Stage II modeling, acute risk to aquatic invertebrates for ensembles that included spray drift was categorized as low for ponds that were 0.575 and 1 m deep (AUCs were 6.73% and 5.23%, respectively). However, acute risk was categorized as intermediate for ponds when a depth of 0.15 m was assumed (AUC = 11.1%, corresponding to 30% probability of exceeding the LC50s of only 10% of aquatic invertebrates). The acute AUC of 11.1% for this scenario is only slightly greater than the low-intermediate risk boundary of 9.82% (Table 3 and Figure 3D). In our exposure modeling, ponds were conservatively assumed to always be downwind of the field even though this is an unrealistic assumption given that wind direction is variable. Also, malathion labels state for agricultural uses, “Do not apply when weather conditions favor drift from target area” (USEPA 2009). Furthermore, Cook et al. (2005) monitored CTS breeding activity in 98 pools between 2000 and 2005 and concluded that the optimum maximum pool depth for CTS breeding was between 0.4 to 0.8 m. Given that CTS are more likely to breed in pools with depths above 0.15 m and the conservative assumptions regarding wind direction in exposure modeling, the predicted acute effects to aquatic invertebrate communities in vernal pools of this depth are unlikely to significantly affect aquatic-phase CTS populations.

Risk of chronic effects to aquatic invertebrates was relatively insensitive to depth, and risk was categorized as intermediate at all 3 pond depths. The AUCs ranged from 14.1% for the 1-m pond depth to 21.5% for the 0.15-m pond depth, indicating a 36% and 42% probability of exceeding the chronic effects metrics for 10% of aquatic invertebrate species (Table 3 and Figure 3D). The Stage II modeling results and risk curves are based on only the 45% of aquatic habitat that has potential for exposure to malathion and does not account for the 55% of aquatic habitat that is too far removed from potential use sites of malathion to be affected.

When spray drift was removed as a potential route of exposure in modeling, acute risk to aquatic invertebrates was de minimis and chronic risk was low for all pond depths. The AUCs ranged from 0.779% to 0.824% and from 2.18% to 2.51% for acute and chronic risk curves, respectively (Table 3 and Figure 3D). The corresponding probabilities of effect range from 2% to 8% probability of effects for 10% of aquatic invertebrates. These results indicate that reducing or eliminating spray drift would be the single most influential factor for reducing the risk of exposure indiscriminately over all DPSs.

Stage III modeling estimated risk to aquatic invertebrate communities in geographically specific population segments and for individual crops labeled for use of malathion. Ensembles were simulated for the uses of malathion with the greatest extent of overlap with CTS pond watersheds for each DPS. Analyses showed that 4 crop–DPS combinations drive the overall PCA distribution in the CTS habitat area (Supplemental Data Tables S41 and S44). Strawberry in the Santa Barbara DPS, grapes in the Santa Barbara and Sonoma DPSs, and vegetables in the Santa Barbara DPS had the highest PCA distributions and the highest exposure concentrations. Acute risk to aquatic invertebrate communities was categorized as low for these 4 crop–DPS combinations. Chronic risk was categorized as intermediate for strawberry in the Santa Barbara DPS and grapes in the Santa Barbara and Sonoma DPSs, and low for vegetables in the Santa Barbara DPS (Table 3 and Figure 3E). Thus, the crop–DPS combinations where use of malathion would have the greatest effects on aquatic invertebrate prey of CTS are strawberry use in the Santa Barbara DPS and grape use in the Santa Barbara and Sonoma DPSs.

Other lines of evidence

In addition to the modeling exercises, several other supporting lines of evidence including the results of environmental fate, toxicity, field and mesocosm studies, surface-water monitoring data, and incident reports were considered in assessing potential risks to CRLF, DS, and CTS. The results of aquatic field and mesocosm studies concluded that significant adverse effects to fish were not observed at concentrations of malathion ranging from 0.6 to 8500 μg a.i./L.
(Giles 1970; Tagatz et al. 1974; Shrestha et al. 1987; Kuhajda et al. 1996; Jensen et al. 1999) and significant effects to aquatic invertebrate communities were observed only at concentrations greater than 3.1 μg a.i./L (Ebke 2002; Relyea 2005, 2009; Groner and Relyea 2011; Hua and Relyea 2012, 2014; Halstead et al. 2014; Brogan and Relyea 2015). The most sensitive aquatic invertebrate taxon affected (i.e., Cladocera) recovered from single exposures of 10, 30, and 40 μg a.i./L within 2, 4, and 7 weeks, respectively (Ebke 2002; Hua and Relyea 2014), and recovered from pulse exposures of 18 and 36 μg a.i./L within 3 weeks (Brogan and Relyea 2015). Furthermore, more resistant invertebrate taxa (i.e., copepods, amphipods, and insects) were only affected by concentrations of malathion greater than 40 μg a.i./L, and copepod populations exposed to 40, 500, and 750 μg a.i./L recovered within 2 weeks (Shrestha et al. 1987; Relyea 2005; Hua and Relyea 2014). With the exception of 2 scenarios in the CTS modeling effort (Stage II/1 and Stage III/7b), the 90th percentile 48-h, 96-h, and 21-d EECs predicted by CRLF, DS, and CTS modeling were all below the lowest concentration at which effects to cladoceran abundance was observed in mesocosm studies (3.1 μg a.i./L; Groner and Relyea 2011). Furthermore, the maximum 48-h EECs predicted by SWAT and PRZM/EXAMS(VVWM) modeling for all exposure scenarios for CRLF, DS, and CTS were below 40 μg a.i./L, the level at which even sensitive cladoceran populations were found to recover within weeks (Hua and Relyea 2014). As such, even though effects on aquatic invertebrate communities in field and mesocosm studies have been noted at concentrations within the modeled exposure estimates, they are often within the upper end of the modeled EECs (i.e., 90th percentiles; Tables 1, 2, and 3) and would only be experienced by aquatic invertebrates in worst-case scenarios. Moreover, effects measured in the field occur to the most sensitive of aquatic invertebrate species (e.g., cladocerans; Ebke 2002) and effects are often transient in nature. Finally, CRLF, DS, and CTS have relatively few taxa of aquatic invertebrate species. It is, therefore, unlikely that a temporary decrease in only 1 or a few taxa of the aquatic invertebrate community will affect the overall diet of CRLF, DS, and CTS (Moore 1998; Suter et al. 2000).

California monitoring data for 2001 to 2015 were collected from 6 programs that measured concentrations of malathion ranging from 0.0013 to 22.5 μg a.i./L in surface-water samples collected near agricultural areas. Malathion was detected in 0% to 14% of surface-water samples (Supplemental Data Section S9, Table S52). Concentrations of malathion rarely exceeded the most sensitive chronic LOEL of 0.10 μg a.i./L for D. magna; the percent of samples exceeding 0.10 μg a.i./L range from 0% in the USDA Pesticide Data Program (PDP) database to 2.66% in the California Department of Pesticide Regulation (CDPR) Surface Water Database (SWD) database (Supplemental Data Section S9, Table S55). Thus, it is unlikely that exposure to malathion would be associated with impacts on aquatic invertebrate communities, and in the rare instances that effects do occur these populations are likely to recover (Shrestha et al. 1987; Ebke 2002; Hua and Relyea 2014; Brogan and Relyea 2015).

Incident reports collected by the USEPA’s Ecological Incident Information System (EIIS) and the USDA’s Boll Weevil Eradication Program (BWEP) were obtained and are described in detail in Supplemental Data Section S10. All incidents reported in the EIIS resulted from either misuse or unknown use of malathion. Only 2 aquatic incidents have been reported in the EIIS since 2000, and both were associated with misuse of malathion. Of the incidents reported following applications to nearby cotton fields as part of the BWEP, an intensive program with potentially worst-case applications of malathion, there was only 1 incident in which malathion was detected at concentrations high enough to cause the observed fish mortalities, and 2 incidents in which exposure to malathion in combination with other factors (e.g., low O levels and exposure to other chemicals) could have resulted in the observed mortalities. Although incident reports represent a weak line of evidence due to potential underreporting (e.g., aquatic invertebrate incidents are unlikely to be reported), the lack of recent incidents reported in the EIIS and the few malathion-related incidents for the BWEP suggest that the current malathion labels are protective of aquatic biota.

Finally, with regard to potential chronic effects of malathion to aquatic species, this exposure scenario is highly unlikely because malathion degrades quickly in aquatic environments, and in particular marine environments representative of DS habitat. Further, malathion degrades quickly (<1 d) in soil, thus reducing the likelihood of runoff into aquatic environments unless there is a major rain event soon after application. Malathion labels recommend not applying pesticide when rainfall is forecasted. As such, increased runoff potential due to rain is reduced. Several chronic aquatic toxicity studies conducted according to Good Laboratory Practice (GLP) using flow-through systems reported that concentrations of malathion decreased steadily to as low as 70% of nominal over the course of the experiments (Rhodes and Leak 1997; Palmer et al. 2011a, 2011b; Claude et al. 2012). The extended exposure to malathion over several weeks to months in chronic toxicity tests is inconsistent with exposure in the environment. Exposures are expected to peak with applications, and quickly dissipate in the environment within a matter of days. In a worst-case scenario, receptors may be exposed to pulse exposures when multiple applications are allowed during the growing season. But even under these conditions, fish and aquatic invertebrates can recover from exposure to malathion (Shrestha et al. 1987; Forbis and Leak 1994a, 1994b; Kammerer and Robinson 1994; Ebke 2002; Hua and Relyea 2014; Brogan and Relyea 2015).

Sources of uncertainty

Although the best available data and modeling approaches are used in the present ERA, as with any risk assessment, there are inherent uncertainties (Whitfield et al. 2016). The refined exposure assessments assume that end-use products of malathion are used at the maximum application rates with minimum time intervals between applications and applied using methods that would
result in the highest EECs. In practice, however, lower application rates with longer time periods between applications are more commonly used (Pai and Winchell 2016). The SWAT model realistically simulates hydrology and pesticide transport processes in flowing water systems. However, some uncertainties are still associated with the assumptions related to the agronomic practices used, contribution of spray drift to residues of malathion, flow conditions for some water bodies, and major transfers, diversions, and flood control structures. The impacts of many of these uncertainties were captured through the probabilistic representation of several key inputs. Others, such as structural uncertainty of the model, are difficult to quantify in terms of their impacts on predictions. In the refined PRZM/VWWM modeling for CTS, there were uncertainties associated with the depths and volumes of the vernal pools, which could not be readily estimated from available spatial data sets, the PCA calculation in modeling Stages I and II, which was based on a conservative all-agricultural lands crop footprint, and the assumption that spray drift and runoff entered directly into vernal pools without attenuation through vegetative buffers. The assumptions relating to the selected crop footprint and exclusion of vegetative buffers led to predicted concentrations with a high bias.

Uncertainties associated with the measures of effects selected for the refined assessments include the limited number of acceptable and supplemental studies available to derive effects metrics, relevance of the studied species to the target organisms being assessed, and use of ACRs based on 2 species to extrapolate from acute to chronic effects for aquatic invertebrates. More information on the sources of uncertainty identified in the exposure and effects assessments and their impact on modeled predictions is provided in Supplemental Data Section S11.

CONCLUSIONS

Exposure analyses were conducted using refined models to predict concentrations of malathion in areas representative of each species’ habitat. These refined exposure analyses suggest that direct acute effects to DS are de minimis, indicating little risk to DS. Chronic risk to the aquatic invertebrate prey of CRLF, DS, and CTS was categorized as “intermediate” for some of the worst-case scenarios evaluated. However, the uncertainties in the derivation of the refined EECs favor conservatism. Moreover, 21-d average concentrations were simulated in the refined exposure modeling and used to predict chronic risk. Due to its quick aquatic degradation, maintaining elevated concentrations of malathion in these water bodies between multiple applications is unlikely. Thus, the refined EECs used in our risk characterization may not represent the daily fluctuations of malathion over the course of multiple applications in which degradation and dissipation would occur over the 21-d duration. Aquatic invertebrates have been shown to recover from such “pulse” aquatic exposures, suggesting that a permanent decrease in food supply due to exposure for these species is not expected (Kuhajda et al. 1996; Brogan and Relyea 2015). Monitoring data measurements rarely exceeded the most sensitive chronic LOEL for D. magna, indicating minimal risk to the most sensitive aquatic invertebrates. Considering all lines of evidence in the present refined ERA, currently labeled uses of malathion are not expected to result in direct effects to DS, CTS, or CRLF or their fish prey. In addition, aquatic invertebrate prey of these species is rarely expected to be chronically affected.

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Data Accessibility—To access data, please contact the corresponding author Roger Breton by email at rbreton@intrinsik.com.

SUPPLEMENTAL DATA

Section S1. Use of malathion in California
Section S2. Aquatic screening-level ecological risk assessment
Section S3. Aquatic screening-level ecological risk assessment of malaoxon
Section S4. Refined exposure assessment models
Section S5. CRLF refined aquatic modeling
Section S6. DS refined aquatic modeling
Section S7. CTS refined aquatic modeling
Section S8. Refined chronic effects assessment for aquatic invertebrates
Section S9. Monitoring data analysis
Section S10. Aquatic incident reports
Section S11. Sources of uncertainty
Section S12. References

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