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Toxicity of Stormwater Runoff After Dormant Spray Application of Diazinon and Esfenvalerate (Asana®) in a French Prune Orchard, Glenn County, California, USA

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Organophosphate pesticides (OPs), in particular diazinon and chlorpyrifos, have frequently been detected in toxic concentrations in waterways draining agricultural and urban areas in California's Sacramento and San Joaquin River watersheds (US Geological Survey 1997, Werner et al. 2000). Toxicity has in part been linked to stormwater runoff of OP pesticides applied during the dormant season on stonefruit and almond orchards (Foe and Sheipline 1993; Kuivila and Foe 1995). State Water Quality Plans have now been implemented by regulatory agencies to prevent movement of OPs into surface water, and growers have reduced the application of OPs. Simultaneously, the use of so-called reduced-risk alternatives, such as pyrethroid insecticides and *Bacillus thuringiensis* bloom sprays, has increased dramatically (Epstein et al. 2000).

Best management practices (BMPs) are aimed at reducing off-site movement of pesticides into surface waters. Pyrethroid pesticides, among them the widely used esfenvalerate (Asana®) are considerably more hydrophobic (solubility in water: 0.4 µg/L) than the relatively soluble OP pesticide diazinon (solubility in water: 40,000 µg/L; Extoxnet 2001). Although runoff of pyrethroids is believed to be minimal thus reducing pesticide impact on surface waters, esfenvalerate has been shown to be toxic to fish at extremely low concentrations (≤1 ug/L; Haya 1989; Clark et al. 1989; Lozano et al. 1992), and potentially poses a significantly higher risk to these organisms than OP pesticides. In addition, its potential to bioaccumulate and bioconcentrate is high (Smith and Stratton 1986). A second recommended method for reducing toxic runoff from orchards is the use of different orchard floor cover crops. Cover crops are believed to enhance water infiltration (Hargrove 1991).

This study was performed to measure the effectiveness of these two BMPs in reducing the toxicity of stormwater runoff. Experiments were carried out in a French prune orchard at the Talbot – Vereschagin Ranch, Glenn County, California.

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MATERIALS AND METHODS

Dormant sprays were applied to 42 rows of French prune orchard (Artois Orchard) as shown in Fig. 1. Rows 1-8, 21-25, and 38-42 were unsprayed. Rows 9-20 were sprayed with diazinon, and rows 26-37 were sprayed with esfenvalerate using 100 gallons of diazinon and esfenvalerate solutions per acre (= 0.1 L/m^2). Diazinon 4EC was applied at a concentration of 3 pints per 100 gallons (= 200.9 g/L active ingredient) and Asana XL was applied at 9.8 oz. per 100 gallons (6.2 g/L active ingredient). Four different covers were tested in three replicate rows each: (1) no cover (= bare), (2) perennial sod (= sod), (3) clover and (4) resident vegetation (= res. veg.).

Prior to sample collection, one half gallon jars were placed into the ground in each row. Jars remained capped until after the pesticides were sprayed. The orchard was sprayed on 18 February 2000. Caps were removed from the collection jars on 20 February 2000. Rainfall began late on 20 February, and water samples were collected on the following day. Samples from 3 replicate rows were combined, mixed and stored at 4°C. Aliquots for chemical analysis were frozen and stored at -20 °C.

Fathead minnows (*Pimephales promelas*) were obtained from Aquatox in Hot Springs, AK. Upon arrival, fish were acclimated to laboratory control water for six hours. Prior to the bioassays, water samples were mixed rigorously in the original container, filtered through a 60 µm screen, warmed to 25°C and aerated at a rate of 100 bubbles/minute until the dissolved oxygen concentration was approximately 8.5 mg/l (103% saturation). Ten 48-hr-old larvae were selected randomly and placed into each of three replicate 500 mL glass beakers containing 250 mL of water. The laboratory control water consisted of deionized water amended to U.S. EPA (1994) moderately hard standards. Minnows were fed *Artemia* nauplii three times daily. Two hundred mL of water were removed from each beaker and renewed with freshly aerated test or control water daily. Dead organisms (both fish and *Artemia*) were removed daily. At test termination (after 96 hours) mortality was recorded.

Sacramento splittail (*Pogonichthys macrolepidotus*) were obtained from the fish culture facility (S. Teh) at UC Davis. They were 6 days old when used for testing the water samples from the Talbot – Vereschagin Ranch, and 21 days old when LC50s were determined for diazinon and esfenvalerate. Upon arrival, fish were acclimated to laboratory control water for 6 hours. Bioassays were performed following the protocol described above for fathead minnows. Tests were conducted over a period of 96 hours.

Waterfiea *(Ceriodaphnia dubia)* were from an in-house culture maintained at the Aquatic Toxicology Laboratory, UC Davis, California. Prior to the bioassays, water samples were mixed rigorously in the original container, filtered through a 60 µm screen, warmed to 25°C and aerated at a rate of 100 bubbles/minute to

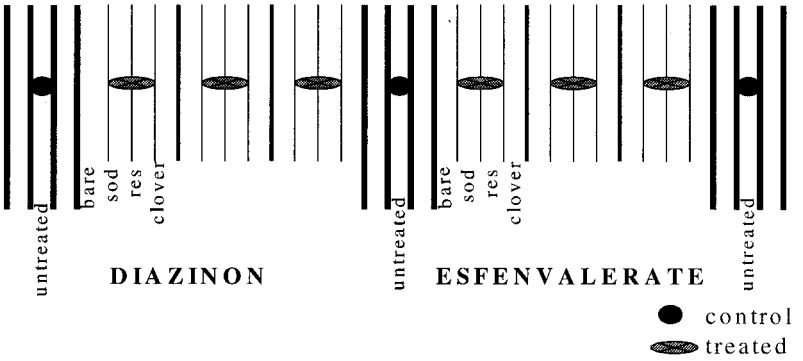

Figure 1. Experimental design at the Talbot – Vereschagin Ranch (Glenn County, CA). The sequence of four ground cover types was repeated three times for both pesticide treatments. Samples for toxicity testing were collected in three replicate rows per ground cover type and combined to yield one composite sample for toxicity testing.

reach a dissolved oxygen concentration of 8.5 mg/L (103% saturation). Toxicity tests were initiated within 24 h of sample collection. Organisms were maintained at 25±1°C under a 16:8 light to dark regime. Tests were set up according to US EPA (1994) protocol using 6-18 hr old *C. dubia* with the endpoint being mortality within 48 hours. One *C. dubia* was placed into each of 10 borosilicate vials containing 15 mL of sample. Sierra Springs water amended to US EPA (1994) moderately hard standards served as laboratory control water. Trout chow and algae (*Selenastrum capricornutum)* were added according to US EPA guidelines. Every 24 hours, each animal was transferred into a new vial containing 15 ml of sample. When 100% mortality occurred within 24 hours, dilutions of the respective water sample were tested to determine the lowest observed effect concentrations (LOEC) and the no effect concentrations (NOEC).

Toxicity was defined as a statistically significant difference ($p<0.05$) between water sample and laboratory control. Bartlett's test for homogeneity of variances was performed on all fish mortality data. When variance was homogeneous, data was compared to controls using analysis of variance and Dunnett's mean separation test. If variance was not homogeneous, data was transformed to relative ranks and analyzed using analysis of variance and Dunnett's mean separation tests. *C. dubia* mortality data was compared to controls using Fisher's exact test (Sokal and Rohlf 1981).

For chemical analyses by gas chromatography (GC), water samples were thawed at room temperature. An aliquot (100 mL) was passed through a sterile Millex^R-HV 0.45 µM filter to remove soil particles. Internal standards (ChemService, West Chester, PA, USA) were added, and the mixture was shaken for 1 minute, then allowed to sit for at least 3 minutes for phase separation. The organic phase was transferred and evaporated to ~ 5 mL under N_2 at 60 °C. The extract was

dried with anhydrous Na_2SO_4 and the volume adjusted to exactly 5 mL. Aliquots of 1 µL were injected into the GC. Diazinon was analyzed using a nitrogen phosphorus detector (NPD); esfenvalerate was analyzed using an electron capture detector (ECD). Percent recoveries were determined by analyzing laboratory water spiked with analytical standards.

Toxicity tests were performed according to the recommended guidelines and met the respective test acceptability criteria (US EPA 1994). Standardized procedures were followed in all aspects of research. For each set of bioassays, randomly chosen test samples were split and tested in duplicate for quality assurance. Monthly reference toxicant tests, consisting of five to six known concentrations of NaCl in laboratory control water, are routinely conducted for each standard bioassay species to monitor changes in animal sensitivity over time. Blind duplicated, blank, and spiked samples are tested on a regular basis for quality control. In addition, inter-laboratory precision is evaluated regularly by splitting samples with other toxicity testing laboratories. Chemical analysis of water samples by GC was performed in duplicate. GC response was linear over the range of the standards.

RESULTS AND DISCUSSION

Water quality parameters of orchard runoff samples and controls were within the normal physiological ranges of the test organisms (pH 7.13-8.10, conductivity 97-262 µmhos/cm, dissolved oxygen 8.1-9.6 mg/L). Chemical analyses of pesticides revealed high diazinon concentrations in runoff samples from diazinon treated orchard sections (Table 1). Detection limits were 0.5 µg/L for diazinon and 0.2 µg/L for esfenvalerate. Recoveries from the liquid-liquid extraction were 101.7 ± 4.6% and 88.4 ± 2.6% for esfenvalerate and diazinon, respectively. Runoff from rows without ground cover vegetation contained the highest concentrations of diazinon. Diazinon was also detected at low, however potentially toxic concentrations, in samples from unsprayed rows and esfenvalerate treated

Table 1. Results of chemical analyses of pesticides on water samples from Artois Orchard.

Treatment	Diazinon (µg/L)	Esfenvalerate (µg/L)
Laboratory Control	nd	nd
Unsprayed Res. Veg.	15.6	nd
Diazinon Bare	210.4	nd
Diazinon Sod	135.9	nd
Diazinon Res. Veg.	155.2	nd
Diazinon Clover	118.2	nd
Esfenvalerate Bare	3.6	nd
Esfenvalerate Sod	6.3	nd
Esfenvalerate Res. Veg.	3.9	nd
Esfenvalerate Clover	2.9	nd

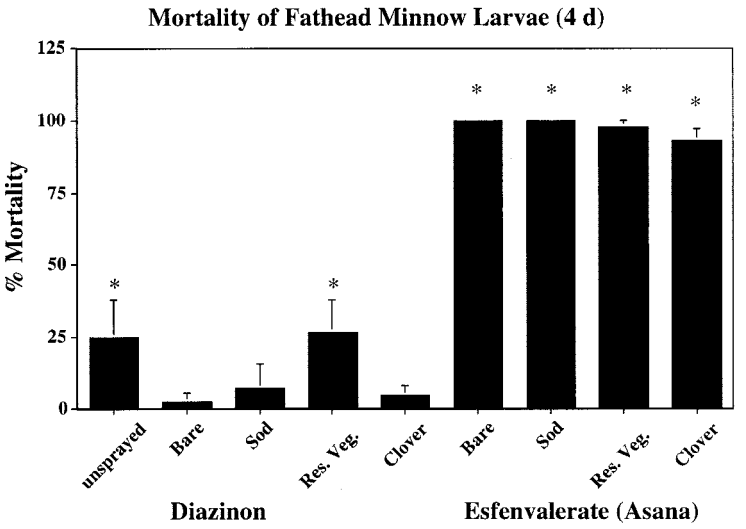

Figure 2. Percent mortality of fathead minnow larvae when exposed for 96 hours to orchard runoff. The laboratory control met the criteria for test acceptability. * = significant ($p<0.05$) increase in mortality compared to laboratory controls.

sections. We assumed that aerial drift was responsible for this widespread contamination. Esfenvalerate concentrations were below the detection limit of 0.2 µg/L in all water samples.

Orchard runoff samples from sections treated with esfenvalerate were highly toxic to fathead minnows (Fig. 2), although esfenvalerate concentrations were below the detection limit (Table 1) causing 93-100% mortality of test organisms within 96 hours of exposure. Significant mortality (25-26.8%) also occurred in water samples from diazinon treated rows with resident vegetation, and in samples from unsprayed rows with resident vegetation. The water sample from unsprayed rows contained diazinon at a concentration of 15.6 µg/L, which is 1/40 of the 96 hour-LC50 for this species. Control treatments did not cause mortality of fathead minnow larvae. Table 2 shows the results of our laboratory tests to determine LC50s for our test organisms when exposed to diazinon and esfenvalerate. Fathead minnows were more sensitive to both esfenvalerate and diazinon than Sacramento splittail.

Cross-contamination of orchard sections was apparent in runoff samples collected in orchard rows sprayed with esfenvalerate, where diazinon concentrations of 2.9-6.3 µg/L were detected. This was likely due to wind drift. Contamination with esfenvalerate may also have occurred at non-detectable levels, and could be responsible for the fish toxicity observed in water samples from diazinon sprayed and untreated rows. Diazinon concentrations measured in these samples were not high enough to account for the significant increase in fathead minnow mortality. Similarly, diazinon concentrations in the highly toxic water samples collected in

Table 2. LC50s of diazinon and esfenvalerate for waterflea (*C. dubia*), fathead minnow (*P. promelas*) and Sacramento splittail (*P. macrolepidotus*)

	96 hour-LC50	
	Diazinon (measured, µg/L)	Esfenvalerate (nominal, µg/L)
Waterflea	0.4	0.28
Fathead Minnow	6000	0.25*
Sacramento Splittail	7500	0.50*

* These LC50 values are for 21-day old fish.

esfenvalerate treated rows were an order of magnitude below the respective 96 hour-LC50 of fathead minnows, whereas the 96 hour-LC50 for esfenvalerate was close to the detection limit of 0.2 µg/L. It is possible that the combined effect of diazinon and esfenvalerate in these water samples caused the observed toxicity. In studies on the effects of chemical mixtures on fathead minnow larvae, toxic effects of diazinon and esfenvalerate have been shown to be synergistic (*Denton et al., submitted*).

Table 3. Percent mortality of Sacramento splittail (*P. macrolepidotus*) exposed for 96 hours to orchard runoff samples, and lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) of orchard runoff samples for waterflea (*C. dubia*, 48-hour test).

Treatment	Sacramento Splittail Mortality (%)		Waterflea Mortality	
	mean	se	NOEC (% FWS)	LOEC (% FWS)
Laboratory Control	2.5	3.0	NA	NA
Unspray Res Veg	3.3	3.0	1.25	2.5
Diazinon Bare	2.5	3.0	0.0625	0.125
Diazinon Sod	5.0	3.0	0.125	0.25
Diazinon Res. Veg.	2.5	3.0	0.125	0.25
Diazinon Clover	2.5	3.0	0.125	0.25
Esfenvalerate Bare	0.0	0.0	2.5	5
Esfenvalerate Sod	10.0	10.0	2.5	5
Esfenvalerate Res. Veg.	NA	NA	1.25	2.5
Esfenvalerate Clover	2.5	3.0	5	10

se = standard error, n = 4
FWS = field water sample

No significant mortality occurred in the 96 hour toxicity tests using Sacramento splittail (Table 3). As shown in Table 2, this fish species is less sensitive than

fathead minnow to both diazinon and esfenvalerate. However, significant chronic toxic effects such as histopathological lesions and increased levels of stress proteins (hsp70) were demonstrated in a subsequent study by Teh et al. (2001), where exposed fish from this experiment were raised in control water for an additional 3 months, then sacrificed and analyzed for sublethal effects.

Results of 48-hour tests with *C. dubia* are presented in Table 3. All water samples tested caused 100% mortality of the test organisms within 24 hours. Subsequently, water samples were tested in dilutions until the no effect concentration was determined. Shown above are the lowest observed effect concentrations (LOEC) and no observed effect concentrations (NOEC) using mortality as a test endpoint. The laboratory controls met the criteria for test acceptability. Runoff samples from rows treated with diazinon were 10-40 times more toxic than runoff from the esfenvalerate treated orchard sections. Runoff from esfenvalerate treated rows, which also contained diazinon (2.9-6.3 µg/L) was toxic after a 10- to 40-fold dilution with laboratory control water, which would result in diazinon concentrations (0.10-0.32 µg/L) that are below the 96-hour-LC50 (0.40 µg/L) for this species. Since LOECs were determined in 48-hour tests, it appears that the combined effects of esfenvalerate and diazinon were responsible for the observed toxicity.

An influence of ground covers on the toxicity of runoff samples was not evident. This may be due to the fact that the vegetation was not well developed in this first year of our field study. However, a study by Angermann (2001) on the hydrology of orchard runoff showed that infiltration of rainwater into soil planted with resident vegetation and ripped using 60 cm long shanks was approximately an order of magnitude greater than that for bare soil. When soil moisture was high (saturation > 0.80) the difference between runoff volumes was markedly reduced.

For the study presented here it is important to note that the sampling design was aimed at examining a 'worst case scenario'. Orchard runoff was collected directly in the orchard, and neither the influence of soil type on runoff nor the distance of the orchard from nearby surface waters was measured. The toxicity of orchard runoff was alarmingly high for fathead minnow larvae and waterflea. However, a quantification of hydrological parameters is essential for a more realistic assessment of what proportions of the runoff and pesticides may be discharged into a nearby lake or river.

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