

" FRIENDS of the RUSSIAN RIVE

2002 303(d) List Update
Reference #23

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Friends of the Russian River preserve, restore and enhance the natural systems of the Russian River, its tributaries, riparian corridors and uplands through citizen action, public education, scientific research and expert advocacy

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5550 Skylane Blvd. Suite A
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RWQCB
REGION 1

MAY 15 2001

RE: CWA 303(d) Water Quality Information

<input type="checkbox"/> LAM	<input type="checkbox"/> CRJ	<input type="checkbox"/>
<input type="checkbox"/> RLT	<input type="checkbox"/> SAW	<input type="checkbox"/> KAD
<input type="checkbox"/> FCR	<input type="checkbox"/> RSG	<input type="checkbox"/>

Impairment:

Diazinon

Water Body:

Santa Rosa Creek, Laguna de Santa Rosa, Lower Russian River

Sources:

Urban stormwater run-off

Narrative:

Diazinon is found in every single urban tributary to the San Francisco Bay that has been tested. Residential and urban use contribute large amounts due to impervious surfaces. In addition it has been recently demonstrated that Diazinon is present in urban sewage influent and passes through tertiary treatment with little removal. In the lone Department of Pesticide Regulation study of the Russian River, Diazinon was detected in a sample from Midway Beach. Diazinon use in Sonoma County during the DPR study period was 2,200 lbs. of commercial application. According to DPR figures 1999 Diazinon use in Sonoma was 5,618lbs more than double over the study period. Neither figure includes residential use of diazinon that is used for ants, lawns and airborne insects. Unfortunately, the DPR study discarded all positive tests below MDLs. In 2000, a study was released which showed sub-lethal concentrations of Diazinon disable Salmonids sense of smell negatively affecting their migration and anti-predatory instincts.

Reference:

SWDB study 14. Ganapathy, C., C.Nordmark, K.Bennett, A.Bradley, H.Feng, J.Hernandez, and J. White. 1997. **Temporal distribution of insecticide residues in four California Rivers.**

Environmental Hazards Assessment Program, Environmental Monitoring and Pest Management Branch, California Department of Pesticide Regulation, Sacramento, CA. Report EH97-06.

(Russian River Data enclosed)

Singhasemanon, N., C. Nordmark, and T. Barry. 1998. **Diazinon and chlorpyrifos in the Central Contra Costa Sanitary District Sewer System, Summer 1996.**

(exec summary enclosed)

Nathaniel L. Scholz, Nathan K. Truelove, Barbara L. French, Barry A. Berejikian,

Thomas P. Quinn, Edmundo Casiss, and Tracy K. Collier. 2000 **Diazinon Disrupts Antipredator and homing behaviors in chinook Salmon**, Canadian Journal of Fisheries and Aquatic Sciences (enclosed)

SWDB study 47. Kratzer, C.R. 1998. Pesticides in storm runoff from agricultural and urban areas in the Tuolumne River basin in the vicinity of Modesto, California. USGS National Water-Quality Assessment Program, water-resources investigations report 98-4017.

Department of Pesticide Regulation, 1999 Annual Pesticide Use Report Preliminary Data, Sonoma County, Ca

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Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*)

Nathaniel L. Scholz, Nathan K. Truelove, Barbara L. French, Barry A. Berejikian, Thomas P. Quinn, Edmundo Casillas, and Tracy K. Collier

Abstract: Neurotoxic pesticides are known to contaminate surface waters that provide habitat for salmonids, including some listed for protection under the U.S. Endangered Species Act. Despite their widespread use, the impacts of these pesticides on the neurological health of wild salmon are not well understood. Of particular concern are the organophosphate and carbamate insecticides that block synaptic transmission by inhibiting neuronal acetylcholinesterase. Here we assess the effects of diazinon, an organophosphate insecticide, on alarm pheromone induced antipredator responses and homing behavior in chinook salmon (*Oncorhynchus tshawytscha*). Nominal exposure concentrations (0.1, 1.0, and 10.0 $\mu\text{g}\cdot\text{L}^{-1}$) were chosen to emulate diazinon pulses in the natural environment. In the antipredator study, diazinon had no effect on swimming behavior or visually guided food capture. However, the pesticide significantly inhibited olfactory-mediated alarm responses at concentrations as low as 1.0 $\mu\text{g}\cdot\text{L}^{-1}$. Similarly, homing behavior was impaired at 10.0 $\mu\text{g}\cdot\text{L}^{-1}$. Our results suggest that olfactory-mediated behaviors are sensitive to anticholinesterase neurotoxicity in salmonids and that short-term, sublethal exposures to these insecticides may cause significant behavioral deficits. Such deficits may have negative consequences for survival and reproductive success in these fish.

Résumé : On sait que les pesticides neurotoxiques contaminent les eaux de surface qui constituent l'habitat des salmonidés, notamment de certains poissons désignés comme protégés par la loi américaine sur les espèces en danger («Endangered Species Act»). Malgré leur usage répandu, on ne connaît pas bien les impacts de ces pesticides sur la santé neurologique des saumons sauvages. On s'inquiète particulièrement des effets des insecticides à base d'organophosphate et de carbamate, qui bloquent le transfert synaptique en inhibant l'acétylcholinesterase (AChE) dans les neurones. Nous évaluons ici les effets du diazinon, un insecticide organophosphaté, sur les réactions antiprédateur induites par les phéromones d'alarme et sur le comportement de retour chez le saumon quinnat (*Oncorhynchus tshawytscha*). Nous avons choisi des concentrations nominales d'exposition (0,1, 1,0 et 10,0 $\mu\text{g}\cdot\text{L}^{-1}$) pour simuler les administrations de diazinon dans le milieu naturel. Dans l'étude sur le comportement antiprédateur, le diazinon n'avait aucun effet sur le comportement de nage ni sur la capture de nourriture à vue. Toutefois, le pesticide inhibait de façon significative les réponses d'alarme médiées par l'olfaction à des concentrations de 1,0 $\mu\text{g}\cdot\text{L}^{-1}$. De même, le comportement de retour était troublé à 10,0 $\mu\text{g}\cdot\text{L}^{-1}$. Nos résultats permettent de penser que les comportements médiés par l'olfaction sont sensibles à la neurotoxicité anticholinesterase chez les salmonidés, et que des expositions sublétales de courte durée à ces insecticides peuvent causer d'importants déficits comportementaux, déficits qui peuvent avoir des conséquences négatives pour la survie et le succès de reproduction chez ces poissons.

[Traduit par la Rédaction]

Introduction

Wild Pacific salmon (*Oncorhynchus* sp.) populations are declining throughout the western United States (e.g., Myers et al. 1998). For example, several salmon stocks (defined as evolutionarily significant units) in the states of California,

Oregon, Washington, and Idaho have recently been listed for protection under the U.S. Endangered Species Act (ESA). A significant factor in these declines is the deterioration or loss of freshwater habitat (National Research Council 1996). Land-use activities (e.g., forestry, agriculture, grazing, mining, and urban and industrial development) have complex

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and mostly negative consequences for salmon productivity. The effects of physical disturbances such as diking, erosion, channelization, sedimentation, loss of riparian vegetation, and loss of large woody debris on salmon productivity are reasonably well known (National Research Council 1996). However, many western river systems also receive substantial inputs of toxic chemicals, and the impacts of these compounds on salmon survival and reproductive health are poorly understood.

Rivers and streams in the western United States are routinely contaminated by a diverse mixture of insecticides, herbicides, and other biocidal compounds. Recent monitoring studies by the U.S. Geological Survey found 49 different pesticides in the surface waters of California's Central Valley (Dubrovsky et al. 1998), 50 in Oregon's Willamette basin (Wentz et al. 1998), and 23 in urban streams in the Puget Sound basin (U.S. Geological Survey 1999). Although these compounds are prevalent in streams and rivers that provide habitat for ESA-listed salmonids, the effects of individual pesticides and pesticide mixtures on salmon health are largely unknown. Consequently, there is considerable scientific uncertainty with respect to what role, if any, pesticides play in the decline of wild populations.

In general, the organophosphate and carbamate classes of insecticides are more acutely lethal to fish than other pesticides (EXTOXNET; pesticide information available at <http://ace.orst.edu/info/extoxnet/>). These compounds are neurotoxins that inhibit the activity of acetylcholinesterase (AChE), an enzyme that terminates acetylcholine-mediated, or cholinergic, neurotransmission. The enzyme is widely expressed throughout the fish nervous system (Weiss 1958; Ferenczy et al. 1997; Sturm et al. 1999). Correspondingly, AChE inhibition can lead to a distributed loss of nervous system function. At high concentrations, exposures to anti-cholinesterase neurotoxins produce spasms, paralysis, and eventual death (Post and Schroeder 1971). Organophosphates and carbamates frequently contaminate western rivers; for example, they account for 12 of 19 insecticides detected in the surface waters of California's Central Valley (Dubrovsky et al. 1998).

Diazinon, an organophosphate insecticide, is commonly detected in the aquatic environment (e.g., Dubrovsky et al. 1998). Diazinon is transported into rivers largely via storm-water runoff (Domagalski 1996), with rain events producing pesticide pulses in rivers and streams. These pulses are typically narrow and well defined, lasting from a few days to weeks (Kuivila and Foe 1995). While in-stream concentrations of diazinon have been measured as high as $36.8 \mu\text{g L}^{-1}$ (Menconi and Cox 1994), they are more commonly less than $10.0 \mu\text{g L}^{-1}$. A typical pulse of diazinon lasting for only a few days at concentrations of less than $10.0 \mu\text{g L}^{-1}$ is unlikely to kill fish outright. The acute toxicity (96-h LC_{50}) of diazinon has not been determined for chinook salmon (*Oncorhynchus tshawytscha*), but the value is probably similar to the known LC_{50} values for congeneric rainbow trout (*Oncorhynchus mykiss*) ($839 \mu\text{g L}^{-1}$) or cutthroat trout (*Oncorhynchus clarki*) ($2620 \mu\text{g L}^{-1}$) (Novartis Crop Protection, Inc. 1997). If so, there is a substantial (approximately 2–3 orders of magnitude) difference between concentrations of diazinon commonly found in the environment and those

required to produce acute lethality in the laboratory. Chronic toxicity (e.g., reduced egg production) has been reported in sheepshead minnow (*Cyprinodon variegatus*) at diazinon concentrations as low as $0.47 \mu\text{g L}^{-1}$ (Goodman et al. 1979). However, chronic exposures can last for 1 or more months, which rarely, if ever, occurs under natural conditions. Thus, based on the results of conventional acute and chronic toxicity assays, it has been concluded that Pacific salmon are not at risk from the direct effects of diazinon in freshwater habitat (Novartis Crop Protection, Inc. 1997).

However, the conventional toxicity studies on which that conclusion was based were not explicitly designed to measure nervous system function. For example, conventional assays do not consider potential injury to the fish olfactory system despite the fact that sensory neurons are directly exposed to dissolved toxicants in the water. In fish, the detection of chemical cues (signal transduction) occurs within the olfactory rosettes, which are the principal structures in the fish olfactory organ. Olfactory receptor neurons, which bind odorant molecules, are embedded within a sensory epithelium that lines the folded lamellae of the rosettes (Zeiske et al. 1992). Chemosensory perception can be altered when dissolved neurotoxins either (i) compete with natural odorants for binding sites on membrane-associated receptor proteins, (ii) modulate the activation properties of these receptor proteins, or (iii) are translocated to the cytosol of the sensory neuron where they modify intracellular signalling events (Klaprat et al. 1992). The olfactory nervous system is sensitive to a wide range of dissolved contaminants (Sutterlin 1974; Brown et al. 1982; Klaprat et al. 1992), and a loss of olfactory capacity could interfere with many important behaviors in fish, including feeding, defense, schooling, migration, and reproduction (Kleerekoper 1969).

Recently, Moore and Waring (1996) found that environmentally relevant exposures to diazinon can desynchronize the reproductive physiology of prespawning Atlantic salmon (*Salmo salar*) by inhibiting the male's perception of a priming pheromone. Exposures to carbofuran, another anti-cholinesterase pesticide, resulted in a similar loss of function (Waring and Moore 1997). Sublethal exposures to carbofuran have also been shown to inhibit AChE enzymatic activity in the olfactory rosettes and the olfactory bulb of the common carp (*Cyprinus carpio*) (Haubruge and Toutant 1997). Collectively, these studies suggest that AChE plays an essential role in signal transduction within the olfactory epithelium. In addition, AChE inhibition may interfere with the normal integration of chemical cues within the olfactory forebrain.

Because chinook salmon rely on a wide range of olfactory-mediated behaviors for survival and reproductive success, a loss of olfactory capacity, while subtle, could have important negative consequences for the health of individual fish. In addition, olfactory cues provide the basis for imprinting and homing, which are critical for maintaining the integrity of wild populations (Dittman and Quinn 1996). Here, we use specific behavioral assays to determine whether diazinon disrupts olfactory nervous system function in chinook salmon. We show that sublethal, environmentally relevant exposures are sufficient to impair both alarm pheromone induced antipredator behavior and homing behavior in juvenile

and adult animals, respectively. These findings are discussed in the context of freshwater habitat quality and the management of ESA-listed species in western river systems.

Materials and methods

Antipredator study

Chemical alarm signal induced antipredator behaviors were measured using an experimental design modified from Berejikian et al. (1999). Eyed chinook salmon eggs were obtained from the Minter Creek Hatchery, Pierce County, Wash. The eggs were incubated and hatched in a model stream at the National Marine Fisheries Service Manchester Experimental Station (Manchester, Wash.). The fish were reared in a circular tank (1.2 m in diameter) on a 16 h light : 8 h dark cycle and fed a standard commercial salmon diet several times daily, 5 days per week. A stock conspecific skin extract (alarm stimulus) was prepared by homogenizing approximately 100 cm² of skin from 23 fish in 1 L of deionized water. The extract was filtered twice through a polyester filter floss, aliquoted, and stored at -20°C.

Behavioral trials were carried out indoors in ten 170-L aquaria positioned within two nearly identical flumes (9.0 m long by 1.5 m wide; see Berejikian et al. (1999) for a complete description of tank design). In brief, each aquarium was constructed of opaque glass on three sides. The tanks were lined with gravel, and uniform overhead lighting was provided by a solid bank of wide-spectrum fluorescent lights. A 15-cm² tile was situated approximately 5 cm above the gravel to provide overhead cover, and a 2.5-cm-high plastic ring was placed around the tile to add lateral cover. The water used during the maintenance, exposure, and testing of fish was gravity-fed from an on-site well. Water temperatures ranged between 10 and 14°C, and chemical properties were pH 8.0, total hardness (as CaCO₃) 65 mg·L⁻¹, conductivity 150 µmho·cm⁻¹, sodium 5.2 mg·L⁻¹, magnesium 7.3 mg·L⁻¹, and calcium 14 mg·L⁻¹. Water was introduced at a rate of 6 L·min⁻¹ via a polyvinyl tube that terminated middepth on one side of the aquarium, and depth was maintained at 25 cm by a double siphon on the opposite side of the tank.

It has been previously shown in Atlantic salmon parr that a brief exposure to diazinon (30 min at 1 µg·L⁻¹) is sufficient to reduce the responsiveness of the olfactory epithelium to an odor stimulus. In addition, the olfactory epithelium does not recover within the first few hours postexposure (Moore and Waring 1996). Based on these findings, we chose to expose chinook salmon parr to diazinon for 2 h and allow them to recover for 1 h before initiating the antipredator behavioral trials. Diazinon exposures were conducted in 20-L opaque glass aquaria. Exposure concentrations (0.1, 1.0, and 10.0 µg·L⁻¹) were prepared fresh each day by dissolving an analytical-grade stock of diazinon (Chem Services) in a small volume of acetone (0.01% final concentration). The diazinon-acetone mixtures were then diluted into 10 L of well water. Acetone alone (0.01%) was used as a vehicle control.

Diazinon exposures and behavioral testing were conducted in the following sequence. On the day prior to testing, a single juvenile chinook salmon was placed in each of the 170-L aquaria. The fish were allowed to acclimate for at least 24 h, during which they were fed a single meal (1.5% of body weight). On the day of the trial, individual fish were removed from their tanks and transferred to separate 20-L exposure aquaria that had been previously treated with diazinon or acetone as a vehicle control. After a 2-h exposure, the fish was returned to the 170-L test aquarium and allowed to reacclimate for 1 h. Subsequently, approximately 3 mL of live *Daphnia* (collected fresh from a local pond) was introduced into the tank via the inflow tube. After a 2-min interval, prestimulus feeding behavior was recorded for 8 min. The conspecific skin ex-

tract (100 µL) was then added through the water inflow tube. After a 10-s delay, antipredator behaviors were recorded during an 8-min poststimulus interval.

Prestimulus feeding behaviors and the poststimulus antipredator response were recorded using behavioral data acquisition software (Observer 2.0). Specifically, we measured the frequency of food strikes, the amount of time spent motionless, and the amount of time spent in the lower, middle, and upper third of the water column. Fish were defined as motionless if they remained stationary (moved less than 1 cm) for 3 s. During the alarm response, many animals maintained a stationary position in the water column by rapidly fanning their pectoral fins. The frequency of darting behavior (burst swimming) and amount of time spent under cover were also measured; however, as reported previously (Berejikian et al. 1999), these behaviors were infrequent and we did not consider them significant antipredator behaviors.

All trials were conducted blind, i.e., the observer had no knowledge of the treatment conditions. Overall, 17–20 individual juvenile chinook salmon were tested at each exposure concentration for a total of 75 fish (fork lengths between 4.7 and 6.3 cm, mean = 5.5 cm). Each fish was used only once, and the relationship between diazinon treatment and test aquarium was randomized between trials. In preliminary experiments, three control fish were exposed to a higher concentration of skin extract. The poststimulus behaviors of these animals were not included in the analysis.

Homing study

Homing behavior has previously been used to measure olfactory capacity in chinook salmon (Quinn et al. 1988). In the present study, a total of 160 maturing chinook salmon were collected from the University of Washington hatchery between October 9 and November 10, 1998. The pond was seined twice weekly, and returning males (age 1) were captured and transferred to a concrete raceway. The fish (fork lengths between 14.8 and 30.1 cm, mean = 23.7 cm) were in good condition, without lacerations or obvious signs of disease.

After each day of seining, the fish captured were immediately divided into four treatment groups and fin-clipped for identification. The following day, static 24-h exposures to diazinon (0.1, 1.0, and 10.0 µg·L⁻¹ nominal concentrations) or a vehicle control (acetone, final concentration <0.01%) were conducted in four 800-L tanks filled with lake water. Diazinon dilutions were prepared fresh each day from an analytical-grade stock solution (Chem Services). Each tank was fitted with a Living Stream[®] chiller to maintain aeration and a constant water temperature (13–14°C).

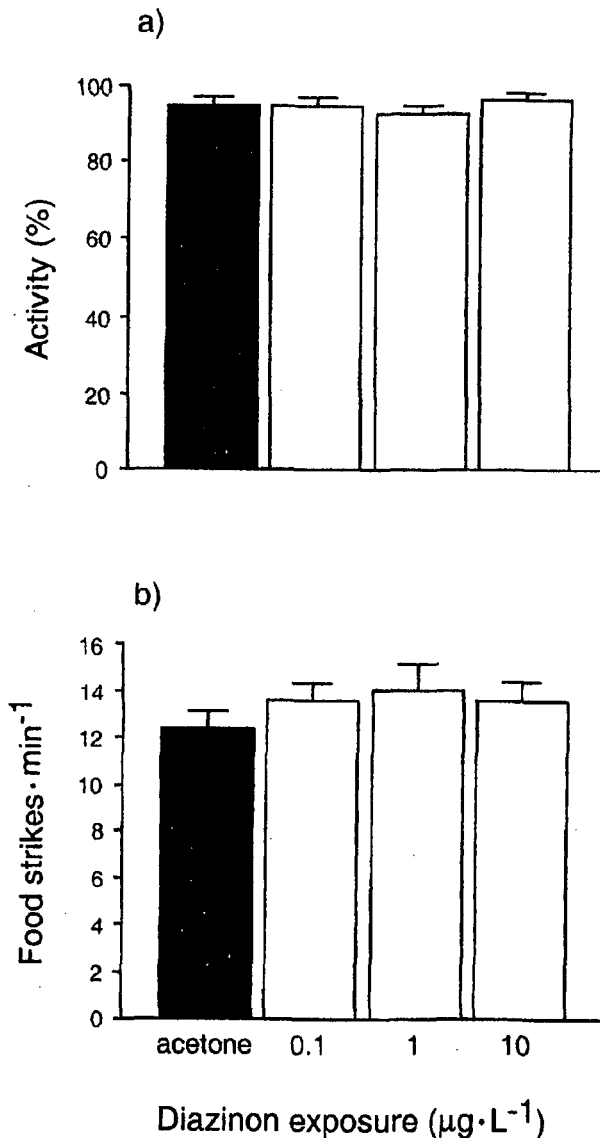
Following exposures, fish were transferred to a 775-L stainless steel tank and transported to a freshwater release site approximately 2 km downstream from the hatchery. A total of 160 fish (40 per treatment group) were treated and released during the course of the experiment. Homing fish (i.e., those returning to the hatchery) were identified during subsequent seining operations at the hatchery that lasted until November 23. In addition, a total of 20 fish (five per treatment group) were placed together in a concrete raceway at the hatchery. These animals were in the same size range as the fish that were treated and released. They were monitored throughout the study to ensure that the diazinon exposures did not grossly reduce the longevity of animals used in the homing experiment.

Results

Antipredator study

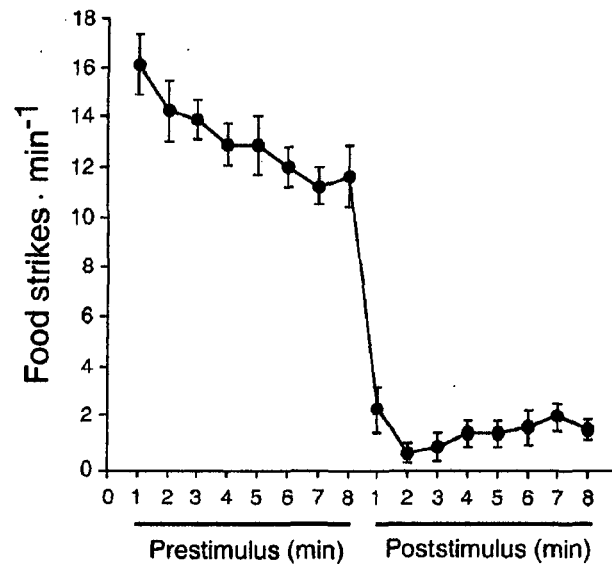
A 2-h diazinon exposure at nominal concentrations of 0.1, 1.0, and 10.0 µg·L⁻¹ had no significant effect on either basal foraging behavior or swimming activity (Fig. 1). In general, individual fish fed actively throughout the 8-min prestimulus

Fig. 1. Short-term diazinon exposures have no effect on (a) swimming activity or (b) feeding behavior in chinook salmon parr. Individual fish were removed from their aquaria and exposed to diazinon (0.1, 1.0, and 10.0 $\mu\text{g}\cdot\text{L}^{-1}$ nominal concentrations) or a vehicle control (acetone) for 2 h. The fish were subsequently returned to their aquaria and allowed to reacclimate for 1 h. Food (live *Daphnia*) was added to the tank and the behavior of each fish was recorded (see Materials and methods) for 8 min. Values represent the mean \pm SEM for 20 animals. Each animal was tested only once. There were no significant differences among treatment groups ($p > 0.05$, Fisher's test).



interval (Fig. 2). As previously reported (Berejikian et al. 1999), juvenile chinook salmon show stereotypic anti-predator behaviors in response to a chemical alarm signal. Qualitatively, animals moved towards the bottom of the tank and became motionless when a conspecific skin extract was added to the tank. In addition, control fish abruptly stopped feeding in response to the skin extract (Fig. 2). The alarm

Fig. 2. Conspecific skin extract elicits an abrupt reduction in feeding behavior in control chinook salmon. Feeding activity was recorded for 8 min before (prestimulus) and after (poststimulus) the addition of the skin extract to the aquaria. Values are data from 1-min intervals and represent the mean \pm SEM for 17 animals.



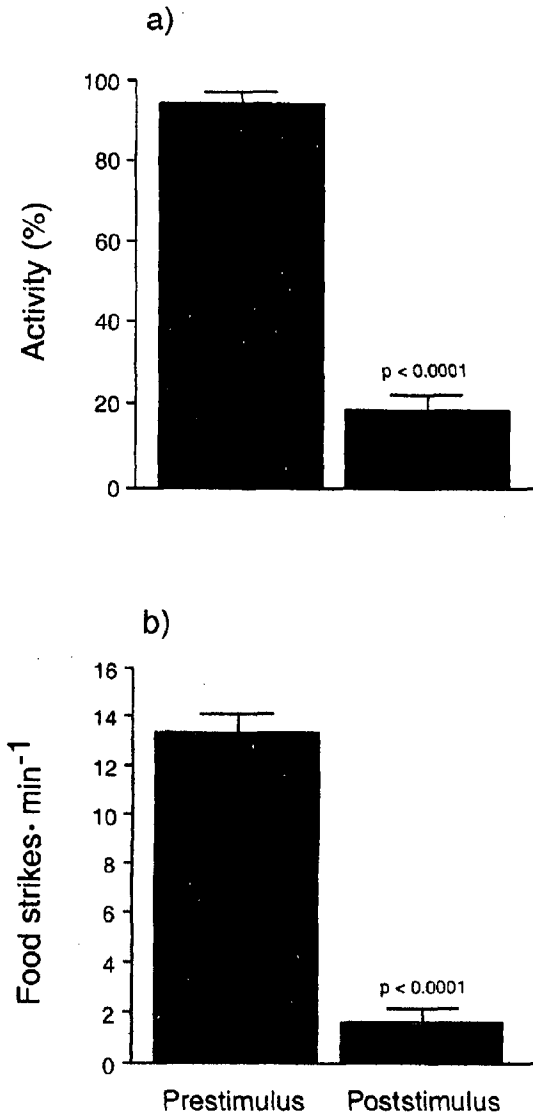
signal elicited a significant decrease in both swimming activity ($p < 0.001$, Fisher's test; Fig. 3a) and feeding behavior ($p < 0.001$, Fisher's test; Fig. 3b) in controls. The fish also tended to move to the lower third of the water column. However, several fish were already foraging in the lower part of the water column when the extract was introduced, so the pre- and post-stimulus differences in this measure were less pronounced.

Compared with control animals, diazinon-treated fish remained more active and fed more frequently when exposed to the conspecific skin extract (Fig. 4). Although some treated fish exhibited antipredator behaviors, many continued to forage in the presence of the alarm signal. The effect of diazinon on swimming and feeding behavior was significant at concentrations of 1 and 10.0 $\mu\text{g}\cdot\text{L}^{-1}$ ($p = 0.05$, Fisher's test), with the exception of the 10.0 $\mu\text{g}\cdot\text{L}^{-1}$ dose, which had a marginal effect ($p = 0.06$) on poststimulus swimming activity.

Homing study

Fewer diazinon-treated chinook salmon returned to the hatchery compared with control fish (Fig. 5). Overall returns were 40% (16 of 40) for control fish, 30% (12 of 40) for the 0.1 and 1.0 $\mu\text{g}\cdot\text{L}^{-1}$ exposure groups, and 15% (six of 40) for the 10.0 $\mu\text{g}\cdot\text{L}^{-1}$ exposure group. The effect of diazinon on homing success was significant at the 10.0 $\mu\text{g}\cdot\text{L}^{-1}$ exposure ($p < 0.01$, chi-square contingency analysis). Diazinon did not appear to have any gross effects on chinook salmon longevity. Compared with controls, there were no significant differences in the survival of diazinon-treated fish ($n = 5$ for each treatment group) that were held in raceways at the hatchery. Survival durations (mean \pm SEM) for the different treatment groups were 26 \pm 9 days for controls, 31 \pm 11 days at 0.1 $\mu\text{g}\cdot\text{L}^{-1}$, 24 \pm 13 days at 1.0 $\mu\text{g}\cdot\text{L}^{-1}$, and 25 \pm 12 days at 10.0 $\mu\text{g}\cdot\text{L}^{-1}$.

Fig. 3. Control chinook salmon significantly decrease both (a) swimming activity and (b) food capture behavior in response to the alarm stimulus ($p < 0.001$, Fisher's test). Values represent the mean \pm SEM for 17 animals, and the data from an entire interval (prestimulus or poststimulus) are combined.

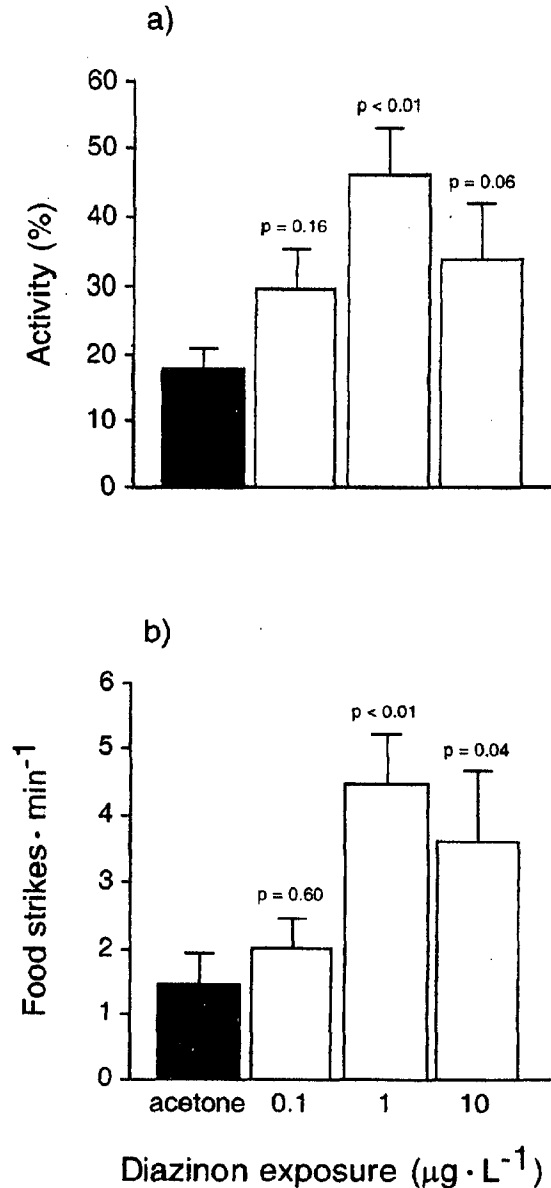


We were unable to test the hypothesis that diazinon-treated fish take longer to return to the University of Washington hatchery than controls. Returning fish were collected two or three times each week as part of normal hatchery operations. Consequently, we did not obtain precise temporal data on when fish from different treatment groups reentered the pond. Moreover, the small males used in this study occasionally avoided the net during seining. However, we found no significant differences in time to recapture (mean \pm SEM) between control fish (11.8 ± 2.2 days) and animals exposed to diazinon at $0.1 \mu\text{g L}^{-1}$ (11.0 ± 2.8 days), $1.0 \mu\text{g L}^{-1}$ (11 ± 2.1 days), and $10.0 \mu\text{g L}^{-1}$ (7.5 ± 3.0 days).

Discussion

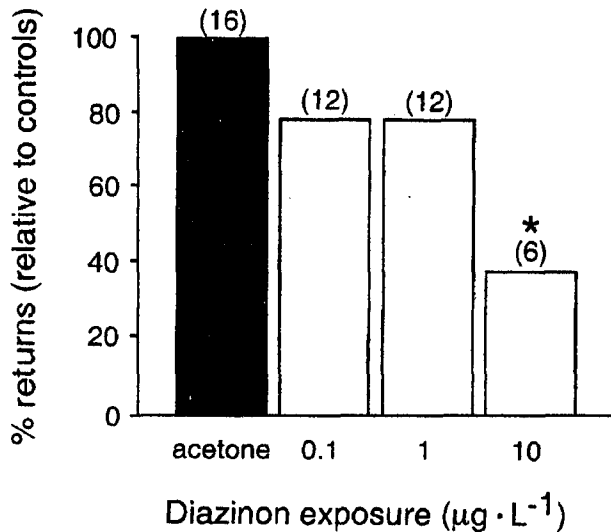
Olfaction plays an important role in the complex life his-

Fig. 4. Antipredator behaviors are reduced in diazinon-exposed chinook salmon. Control fish responded to the conspecific skin extract by reducing their foraging activity and freezing, i.e., poststimulus fish were (a) largely inactive and (b) fed only infrequently (solid bar in Figs. 4a and 4b, respectively). The magnitude of the antipredator response was reduced in diazinon-exposed fish (2 h at 0.1 , 1.0 , and $10.0 \mu\text{g L}^{-1}$), and they were (a) more active and (b) fed more often than controls. The effect of diazinon was significant at the 1.0 and $10.0 \mu\text{g L}^{-1}$ exposures ($p = 0.05$, Fisher's test) with the exception of the marginal ($p = 0.06$) effect of $10.0 \mu\text{g L}^{-1}$ on poststimulus swimming activity.



ories of Pacific salmon. We found that environmentally realistic exposures to the insecticide diazinon significantly disrupt olfactory-mediated behavior in chinook salmon. Our findings, together with recent studies of Atlantic salmon (Moore and Waring 1996, 1998; Waring and Moore 1997), demonstrate that the salmon olfactory nervous system is par-

Fig. 5. A 24-h exposure to diazinon disrupts homing in chinook salmon males. Compared with controls, fewer diazinon-exposed fish returned to the University of Washington hatchery from a release site approximately 2 km downstream. A total of 40 fish were released in each treatment group. Numbers in parentheses indicate the total number of fish returning. The effect of diazinon was significant at the 10.0 $\mu\text{g}\cdot\text{L}^{-1}$ exposure ($p < 0.01$, chi-square contingency analysis).



ticularly sensitive to dissolved neurotoxins. Diazinon has now been shown, in salmonids, to impair reproductive priming (Moore and Waring 1996) as well as predator avoidance and homing behaviors (present study). The survival and reproductive success of wild Pacific salmon may, therefore, be compromised in river systems that are periodically contaminated with diazinon and other neurotoxic pesticides.

Antipredator behavior

Overall, the results of the antipredator experiment are consistent with the hypothesis that diazinon disrupts olfactory nervous system function in juvenile fish. Our findings confirm earlier studies (Brown and Smith 1997, 1998; Berejikian et al. 1999) demonstrating a chemical alarm system in salmonids. Moreover, when compared with controls, diazinon-exposed chinook salmon parr showed a reduced antipredator response to the conspecific skin extract (they were more active and fed more often in the presence of the alarm stimulus). Alarm behaviors reduce the likelihood that an animal will be preyed upon during an encounter with a predator (Smith 1992). For example, Mathis and Smith (1993) found that fathead minnows (*Pimephales promelas*) exposed to a conspecific alarm pheromone survived predation by a northern pike (*Esox lucius*) for approximately 40% longer than fathead minnows that were not exposed to the pheromone. Although not tested directly, the disruption of the alarm response by diazinon would likely increase the vulnerability of chinook salmon parr to predation.

In general, environmental toxins can reduce the ecological performance of a prey animal by adversely affecting its behavior (Mesa et al. 1994). In chinook salmon parr, we have shown that diazinon interferes with the early olfactory detection of a nearby predator, which is critical for escape and

survival (Mesa et al. 1994). As a consequence, diazinon-exposed parr would be in a substandard condition at the time of a predatory interaction. For example, the alarm substance elicited subtle shifts in behavior during the poststimulus observation period. Individual fish would sometimes continue to search for food, but in a more restricted area, or they would continue to strike at food, but only if a *Daphnia* drifted near their stationary position. Presumably, the decision to forage in the presence of an alarm signal reflects a balance between each animal's appetitive or motivational state and its individual perception of predation risk. Decision-making, whereby fish balance the trade-off between food acquisition and the risk of mortality, is an important factor in predator-prey interactions (Mesa et al. 1994). For example, juvenile coho salmon (*Oncorhynchus kisutch*) restrict their foraging behavior following the visual detection of a predator (Dill and Fraser 1984; Martel and Dill 1993). In the present study, control fish sharply reduced their feeding activity in response to the alarm signal. By contrast, some diazinon-treated fish continued to forage at levels that were inappropriate for the situation, perhaps because they were unable to accurately gauge the potential of a predatory encounter.

The results from the antipredator study suggest that the olfactory nervous system is more sensitive to anticholinesterase neurotoxins when compared with the motor nervous system (e.g., the networks that underlie swimming behavior). Diazinon had no observed effect on swimming activity or visually guided food capture behavior at concentrations that significantly disrupted alarm signal evoked antipredator behaviors. Swimming performance is a common behavioral measure of sublethal anticholinesterase toxicity in salmonids and other fishes (e.g., Cripe et al. 1984; Little et al. 1990; Van Dolah et al. 1997). Although previous studies have reported anticholinesterase-induced swimming impairment, the thresholds for behavioral dysfunction were generally at concentrations or durations that exceed conditions in the natural environment. By these measures, it could be interpreted that anticholinesterases pose little risk for wild salmon. However, our results suggest that motor networks may be relatively insensitive to anticholinesterases. Consequently, toxicity thresholds derived from swimming assays may underestimate the susceptibility of the nervous system as a whole.

Homing

In the homing study, fewer diazinon-treated fish returned to the University of Washington hatchery compared with controls. Chinook salmon rely on olfactory cues to guide their upstream movements during the freshwater phase of their homeward migration (Hasler and Scholz 1983), and the weight of evidence suggests that diazinon-exposed fish were less able to smell and thus were less able to navigate back to their natal stream (Moore and Waring 1996). However, the results of the homing study should be viewed as preliminary for several reasons. First, the overall return of maturing males to the hatchery in the fall of 1998 was low, which limited the number of fish in each treatment group. Second, posttreatment returns were small in all four exposure groups. Only 40% of control fish returned to the hatchery, compared with approximately 80% in previous studies of homing in older salmon (e.g., Quinn et al. 1988). The reason for this

discrepancy is not clear. It may reflect differences in the ages of the fish used or environmental conditions or both. Third, the hatchery pond was seined biweekly, and without precise temporal return data, we were unable to determine whether diazinon-treated fish took longer to home relative to controls. Finally, although our longevity experiment indicates that it is unlikely that diazinon exposures caused a delayed gross mortality, we cannot rule out the possibility that exposures may have subtly diminished postrelease survival. This could also account for the observed differences in returns. Nevertheless, significantly fewer fish returned to their natal stream to spawn after a short-term exposure to diazinon.

Dissolved neurotoxins may increase the incidence of straying among wild and hatchery-reared salmon that must traverse pesticide-contaminated rivers during their homeward migration. Straying, in which fish return to nonnatal streams to spawn, is an adaptive mechanism for colonizing new habitat and avoiding unfavorable local conditions (reviewed by Quinn 1993). However, straying of hatchery fish could compromise the genetic integrity of wild populations (Quinn 1993). Reciprocally, straying of wild salmon can diminish the number of animals remaining to spawn in a given stream. A loss of olfactory nervous system function would presumably lead to an increase in straying, since anosmic (nares-occluded) fish are unable to home (Wisby and Hasler 1954). Since sublethal exposures to pesticides and other contaminants can render fish functionally anosmic (reviewed by Klapprat et al. 1992), the presence of these toxicants in the freshwater environment could increase straying, thereby undermining the genetic integrity of ESA-listed species. This represents an important area for future research, especially since the factors that determine normal patterns of straying in wild and hatchery salmon are still poorly understood (Quinn 1993).

Management implications

Conventional chronic and acute toxicity studies, and the LC_{50} experimental paradigm in particular, may underestimate thresholds for neurobehavioral toxicity in salmonids. Consequently, the use of mortality data may not be appropriate for risk assessment in the context of the ESA. For example, our data and the study by Moore and Waring (1996) indicate that environmentally relevant exposures to diazinon can disrupt olfactory capacity in the context of survival and reproductive success, both of which are key management considerations under the ESA. These findings contrast with an ecological risk assessment of diazinon in the Sacramento and San Joaquin River basins (Novartis Crop Protection, Inc. 1997), which concluded that salmon are unlikely to be at risk from direct acute effects of diazinon residues in the water. Importantly, the latter study relied extensively on mortality (LC_{50}) measurements, an approach that has several drawbacks in the context of the ESA. First, to our knowledge, there have been no reports of diazinon-induced fish kills in western streams. Given this, acute mortality has little or no relevance to conditions in the natural environment. Second, LC_{50} studies are not explicitly designed to measure nervous system function. Consequently, it is impossible to accurately predict from LC_{50} values the exposures at which diazinon could cause sublethal behavioral disorders in wild salmon. Third, the LC_{50} values for diazinon in representative salmonids are approximately 2–3 orders of magnitude higher

than the highest concentrations of pesticide that are commonly detected in the aquatic environment. This represents a perceived margin of safety that, when taken alone, may significantly underestimate the actual risk that ambient levels of diazinon and related pesticides pose for salmon.

Diazinon pulses are common in western rivers and streams. This insecticide was detected, at concentrations greater than $0.01 \mu\text{g L}^{-1}$, in 71% of the surface water samples from California's San Joaquin and Tulare basins (Dubrovsky et al. 1998) and in 35% of the samples taken from Oregon's Willamette basin (Wentz et al. 1998). Similarly, diazinon was recently found at 100% of the sites sampled in an analysis of urban streams in Puget Sound, Washington State (U.S. Geological Survey 1999). Although in-stream pulses of diazinon alone may reach levels high enough to cause behavioral dysfunction in wild salmon, diazinon is only one of many current-use pesticides that co-occur in the aquatic environment. Organophosphates and carbamates both inhibit AChE (Millard and Broomfield 1995), and they are likely to have an additive impact on AChE-mediated functions in the fish nervous system. In the surface waters of California's Central Valley, the organophosphates azinphos-methyl, chlorpyrifos, diazinon, disulfoton, fonofos, malathion, methyl parathion, and terbufos and the carbamates aldicarb, carbaryl, carbofuran, and methomyl all co-occur (Dubrovsky et al. 1998). The additive effects of these pesticides on the salmon nervous system have not been studied, and it remains an important area for future research.

In conclusion, a properly functioning nervous system is critical for the expression of animal behavior. We have shown that environmentally relevant exposures to diazinon are sufficient to disrupt olfactory-mediated behaviors in chinook salmon. These behavioral deficits may, in turn, have negative consequences for the survival and reproductive success of animals under natural conditions. Since diazinon and other neurotoxic pesticides with a similar mode of action are known to contaminate river systems in the western United States that provide freshwater habitat for several ESA-listed species, they represent an unknown but potentially significant obstacle to salmon recovery efforts.

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EXECUTIVE SUMMARY
of Report EH 98-05 Entitled
"Diazinon and Chlorpyrifos in the Central Contra Costa Sanitary
District Sewer System, Summer 1996"

Environmental Monitoring and Pest Management Branch
Department of Pesticide Regulation

BACKGROUND

Historically, agricultural activities have been the focus of investigations into pesticide impacts on water bodies. In recent years, however, pesticide use in urban areas is increasingly being examined as a potential source of aquatic pollutants. Although applications of pesticides in urban areas are typically on a small scale, the wide variety of chemicals used and the frequency of applications can result in a substantial amount of pesticides used. Urban-use pesticides can move off application sites and enter storm drains which route surface runoffs into urban creeks. These pesticides can also end up in urban sewage which then travels to wastewater treatment plants.

Although conventional wastewater treatment techniques employed by Publicly Owned Treatment Works (POTWs) may remove certain pesticides with high efficiency, others may not be sufficiently removed. Thus, these pesticides can be present in the treated effluent and eventually be released into a receiving water body. Under the California Porter Cologne Act, the Regional Water Quality Control Boards (RWQCBs) regulate the quality of treated effluent by issuing wastewater discharge permits to POTWs. These permits prohibit toxic substances in the treated effluent at concentrations that may cause harm to aquatic species.

In 1986, the San Francisco Bay RWQCB asked the Central Contra Costa Sanitary District (CCCSD) in Martinez, California, to initiate an Effluent Toxicity Characterization Program. The aim of this program was to help characterize the toxicity of CCCSD's treated effluent on selected aquatic test species. Twelve of the 18 tests performed under this program revealed that the treated effluent was acutely toxic to the water flea, *Ceriodaphnia dubia*. The RWQCB then requested that CCCSD perform toxicity identification evaluation (TIE) studies to determine the cause of the toxicity. The TIE studies suggested that two organophosphate insecticides, diazinon and chlorpyrifos, were responsible for the toxicity in CCCSD's effluent. Diazinon and chlorpyrifos are commonly found in consumer and commercial products marketed for urban uses. These uses are generally related to lawn/garden

care, indoor pest control, and pet care products.

PURPOSE

This study was jointly conducted by CCCSD and the Department of Pesticide Regulation to: 1) characterize the mean daily diazinon and chlorpyrifos concentrations and flow in the sewage of residential areas, selected commercial sites, and CCCSD treatment plant influent; 2) determine the relative importance of residential and commercial sources to the total influent load; and 3) compare the mean daily concentration and mass of CCCSD's influent to those of two other POTWs.

STUDY METHODS

Source sampling efforts were focused on the residential sector (single-family and multi-family residences) which contributes about 82% to CCCSD's influent load, and the commercial sector which contributes about 6%. Five residential areas were sampled daily in conjunction with daily sampling of CCCSD's influent for a one-week period. The areas contained as few as 829 and as many as 2,079 residences. Residential sampling occurred July 9-15, 1996.

Twelve commercial sites in the CCCSD service area (consisting of pet groomers, kennels, and pest control businesses) were also sampled. These three business types were selected because reconnaissance sampling by CCCSD had shown notable amounts of diazinon and/or chlorpyrifos in their effluent. Although sewage from other business types such as nurseries, restaurants, waste management facilities, and industrial facilities may also contain varying amounts of the two active ingredients, limited resources excluded their investigation. Unannounced sampling of selected commercial sources was done from July 18 through September 8, 1996.

The CCCSD influent samples were taken on a semi-weekly basis from June 22 through September 10; daily sampling occurred during July 9-16, August 4-11, and August 31 through September 7, 1996. For one week of the study, influent samples were collected from two other treatment plants in an effort to compare diazinon and chlorpyrifos concentrations among the POTWs: Union Sanitary District (USD) in Alameda County, and the Palo Alto Regional Water Quality Control Plant (RWQCP) in Santa Clara County. Simultaneous daily sampling of the USD and RWQCP occurred from August 5-11, in conjunction with the daily sampling of CCCSD.

The general sampling period was chosen so that warmer months

would be included. Insect problems and subsequent urban organophosphate use were expected to be greater during this period. Sewage samples were collected using programmed automatic samplers. For residential and commercial sampling, automatic samplers were suspended underneath manhole covers during operating periods and filled with blue-ice packs. Influent samplers were refrigerated units that were housed at the point of sewage entry into the treatment plants. All of the samples analyzed were flow-proportionally composited samples.

Representative flow data for residential, commercial, and influent sampling sites were also collected to allow the estimation of mass loads. Residential and CCCSD influent flow data were generated by CCCSD's flow modeling program known as the Sewer Network Analysis Program (SNAP). Commercial, USD influent, and RWQCP influent flow data were collected using inline flowmeters.

RESULTS

Influent Sampling:

Diazinon and chlorpyrifos were detected (reporting limit = 50 parts per trillion or ng/L) in all 37 of CCCSD's wastewater influent samples during the sampling period. The mean concentrations (calculated as the Uniformly Minimum Variance Unbiased estimator) of influent diazinon and chlorpyrifos were 310 ppt (parts per trillion) and 190 ppt, respectively. Influent diazinon concentrations ranged from 103 to 940 ppt. There are no diazinon and chlorpyrifos compliance criteria for treatment plant influent and other raw sewage concentrations. The treatment plant effluent; however, has to meet the "no toxicity" criteria enforced by the RWQCB.

Residential Sampling:

Residential sampling for diazinon and chlorpyrifos from five neighborhoods over a seven-day period (July 9-15, 1996) yielded 35 samples. The mean daily diazinon concentrations for each neighborhood were 740, 420, 120, 110, and 340 ppt. For chlorpyrifos, the mean daily concentrations for the same neighborhoods were 550, 110, 80, 110, and 180 ppt. Residential area diazinon concentrations ranged from none-detected to 4,300 ppt. Residential area chlorpyrifos concentrations ranged from none-detected to 1,200 ppt. The CCCSD service area's total daily residential mass loads for diazinon and chlorpyrifos were projected from sampling results to be approximately 42 g and 24 g, respectively.

Commercial Sampling:

Sampling of selected commercial businesses within the CCCSD occurred from July 18 through September 8, 1996. Of the 12 sites monitored, both diazinon and chlorpyrifos were found at seven sites. At two sites, only diazinon was detected. At two other sites, only chlorpyrifos was detected. Neither active ingredients were detected at the remaining commercial site. Daily concentrations of diazinon and chlorpyrifos were also variable. The highest level of diazinon (20,000 ppt) was found in the sewage from a kennel. The highest chlorpyrifos level (38,000 ppt) was found in the sewage from a pet groomer. Diazinon was detected on 17 out of the 32 days sampled. Chlorpyrifos was detected on 23 out of the 32 days sampled. CCCSD service area's total pet groomers, kennels, and pest control operators daily mass loads for diazinon and chlorpyrifos were projected from sampling results to be approximately 2.2 g and 2.3 g, respectively.

Concurrent POTW Sampling:

Concurrent sampling of the CCCSD, USD, and the RWQCP showed that the mean daily influent diazinon concentrations for the three POTWs were 300, 230, and 150 ppt, respectively. For chlorpyrifos, the mean daily influent concentrations were 190, 230, and 110 ppt, in the same order. Influent diazinon concentrations for CCCSD, USD, and RWQCP ranged from 130 to 750 ppt, 91 to 530 ppt, and 66 to 240 ppt, respectively. Influent chlorpyrifos concentrations ranged from 140 to 230 ppt, 130 to 330 ppt, and none-detected to 150 ppt, in the same order. The only statistically significant finding among the POTWs was that the median influent chlorpyrifos concentration at RWQCP was less than those of CCCSD and USD.

CONCLUSIONS

Mass balance estimates revealed that residential sewage contributed the majority of the diazinon and chlorpyrifos to CCCSD's influent. Although much higher concentrations were occasionally seen in the sewage of selected commercial sources, the larger residential area flows translated to a greater residential contribution. Therefore, a source reduction strategy that focuses on reducing diazinon and chlorpyrifos loads from residential sources would be the most effective strategy. If such a source reduction program can successfully increase the pollution prevention awareness of service area residents, input from commercial and unknown sources may also decrease. The reduction of diazinon and chlorpyrifos influent loads will likely result in increased compliance with waste discharge permits.
