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Pesticides and PCB Contaminants in Fish and Tadpoles from the Kaweah River Basin, California

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The potentially harmful impacts of increasing air pollution to wildlife and plants in the Sierra Nevada mountain range of California and Nevada represent a growing concern, Pollution resulting from urban development, agricultural operations and industrial activity in the Central Valley and coastal metropolitan centers of California may be transported eastward to the mountain range. There is accumulating evidence of transport and deposition of airborne pollutants including ozone (Cahill et al. 1996) particulate matter (Ewell et al. 1989) nitrogen oxides (Coats and Goldman 1993) nitrogen and phosphorus (Jassby et al. 1994) and pesticides (Zabik and Seiber 1993; Aston and Seiber 1997; Datta 1997a) to the Sierra Nevadas.

While the potential significance of airborne pollutants in contributing to the stress load of the Sierra Nevada ecosystem has been recognized (Cahill et al. 1996; Jennings 1996; Drost and Fellers 1994) there are few data on organic contaminants in air, water, precipitation and biota in this mountain range. Knowing the types and concentrations of contaminants in various Sierra Nevada ecosystem compartments is important to understanding the impact these pollutants might have on the Sierran plants and wildlife. We recently found that polychlorinated biphenyls (PCBs) are present in air, surface water, snow and fish (lake trout [*Salvelinus namaycush*] and rainbow trout [*Oncorhynchus mykiss ssp.*]) in the Lake Tahoe basin in the central Sierras (Datta et al. 1997b). The objective of this study was to determine concentrations of PCBs and selected pesticides in brook trout (*Salvelinus fontinalis*) and tree frogs (*Hyla regilla*) in the Kaweah River basin, and to compare the concentrations of these pollutants with those from other locations in California.

MATERIALS AND METHODS

The Kaweah River receives its water from a group of glacier lakes near the crest of the Sierra Nevada Mountains and is the primary source of water to the Kaweah Reservoir (Fig. 1). Sycamore Creek, a shallow tributary to the Kaweah River, is located in Sequoia National Park at 610 m elevation, Brook trout (*Salvelinus fontinalis*) from the Kaweah River were caught by hook and line from Buckeye

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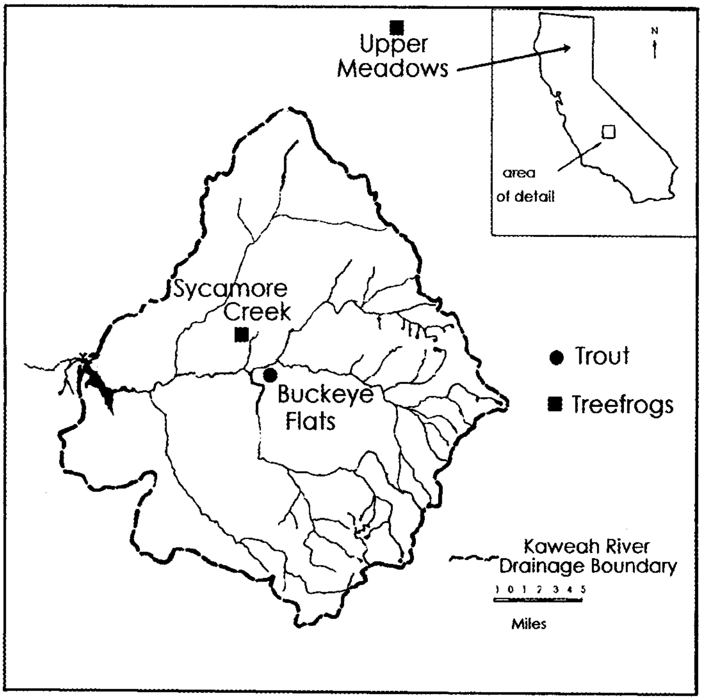

Figure 1. Map of Kaweah River basin showing sampling locations.

Flats on July 10, 1996. Freshly caught fish were wrapped in aluminum foil and stored on ice until transported to the laboratory where they were stored frozen at - 14°C. Pacific tree frog (*Hyla regilla*) tadpoles were collected from field enclosures on Sycamore Creek on June 22, 1996, and July 9, 1996, where they had been held *in vivo* following deposition as egg masses, and then stored in the laboratory at -20°C. *Hyla regilla* egg masses from Lassen Volcanic National Park were collected in Upper Meadow at 2,243 m on July 2, 1996, stored in glass jars with Teflon caps, and placed on wet ice until later storage at -20°C. Pacific tree frog tadpoles from the University of California, Davis (UC Davis), CA were collected on June 11, 1996 with dip nets from the Institute of Ecology adjacent to experimental agricultural fields and the university airport, 2 km west of Highway 113 and 3 km north of Highway 80. This site was chosen to compare non-remote and remote frog habitats for residues.

The method of Ribick et al. (1982) was employed for extraction and clean-up of trout and tree frog samples. For tadpole and egg mass samples, the gel permeation step was omitted. All PCB congeners were analyzed with a Hewlett Packard 5890A gas chromatograph (GC) equipped with an electron capture detector. A 60 m DB-5 capillary column of 0.25 mm i.d. and 0.25 µm film thickness (J&W Scientific) was used. PCB congener identification and quantitation procedures were based on the method of Mullin (1984). Using the same type of DB-5 column of 30 m, mass spectrometry work for the pesticides was performed using a Hewlett Packard 5890 GC coupled to a Hewlett Packard 5890A mass spectrometer in the negative chemical ionization, selected-ion-monitoring mode

Instrumental detection limit for PCB congeners was approximately 1 pg. Analytical detection limits were based on the method of Leister and Baker (1994) in which sample data is reported only if it is more than three times the mass of the analyte determined in the blanks. Mass of t-PCBs in blanks varied between 2.5 to 21.5 ng of t-PCBs (average 14.8 +/-10.6 ng). For both trout and tree frogs, the sample masses were over three times the average blank mass of 14.8 ng. Trout samples ranged from 48.4 to 81.0 ng (average 64.5 +/-16.3) t-PCBs and tree frog samples ranged from 47.2 to 322.7 ng (average 253.9 +/-368.4 ng) t-PCBs. Method recovery based on recovery of three surrogate PCB congeners was: #14 (108+/-22%); #65 (114+/- 17%) and #166 (125+/-22%). Limit of detection for pesticides calculated using the average blank plus three times the standard deviation of the blank, ranged from 0.13 to 2.3 ng.

RESULTS AND DISCUSSION

Concentrations of t-PCBs in brook trout from the Kaweah River at Buckeye Flats ranged from 4.8 to 8.1 ppb, wet weight (Table 1) and were similar to those found in lake trout from Lake Tahoe and rainbow trout from Marlette Lake (Datta et al. 1997b). Congener profiles of Kaweah River brook trout also closely resemble those found in trout from Lake Tahoe and Marlette Lake. Figure 2 shows the distribution of PCB congeners in brook trout from Kaweah River with 66+95, 56+60, 105 +132 +153 and 187+182 as dominant congener groups. Sycamore Creek tadpoles had wet weight concentrations of t-PCBs of 12.0 ppb (June 22 collection) and 23.8 ppb (July 9 collection) (Table 1). Unlike the brook trout, tree frog tadpoles and egg masses showed enriched levels of tri- and tetrachlorinated biphenyl congeners (Figure 2). Differences in the PCB congener profiles may reflect variations in the metabolism, or the lack of metabolism of PCBs by tree frog egg masses, tree frog tadpoles and brook trout. No comparative fish or tadpole data were available with the Lassen Meadow egg masses.

Further sampling is needed to determine if concentrations between brook trout and tree frogs are statistically different. But based on these tentative results we can speculate that the variability may be due to different levels of PCBs in surface waters, or it may indicate differences in metabolism, diet and habitat. Because the concentration of PCBs may be higher in surface and shallow waters than in deeper waters, egg masses and tadpoles may be receiving greater exposure than fish due to their respective habitats. *Hyla regilla* eggs are laid on emergent vegetation in shallow water and as tadpoles they bask in shallow waters. Additionally, due to their heavily vascularized and highly porous skin (Duellman and Trueb 1994) frogs may have a high propensity for dermally absorbing lipophilic compounds. Tissue concentrations of both PCBs and pesticides in tadpoles from the UC Davis

Table 1. Concentration of t-PCBs and chlorinated pesticides (wet wt) in brook trout and tree frogs tadpoles from the Kaweah River Basin, and in tree frog egg masses from Upper Meadow in Lassen National Park.

	Collection Date	Sample Composite	Length (cm)	Mass (g)	t-PCBs ppb	p,p'-DDE ppb	chlorothalonil ppb	chlorpyrifos ppb
Pacific Treefrog								
Sycamore Creek	6/22/96	31 tadpoles	-	4.5	12.0	9.6	47.7	10.4
Sycamore Creek	7/9/96	44 tadpoles	-	4.6	23.8	9.3	33.3	17.4
UC Davis	7/2/96	32 tadpoles	-	3.5	229.3	258.0	12.7	9.2
Upper Meadow	7/11/96	8 egg masses	-	5.2	9.1	nd	4.5	<lod
Kaweah Fish								
brook trout	7/10/96	1 fish	19.6	103.5	6.4	40.1	na	na
brook trout	7/10/96	1 fish	23.4	159.3	8.1	64.2	na	na
brook trout	7/10/96	1 fish	22.0	146.9	4.9	65.7	na	na

na = not analyzed
nd = not detected
<lod = below limit of detection

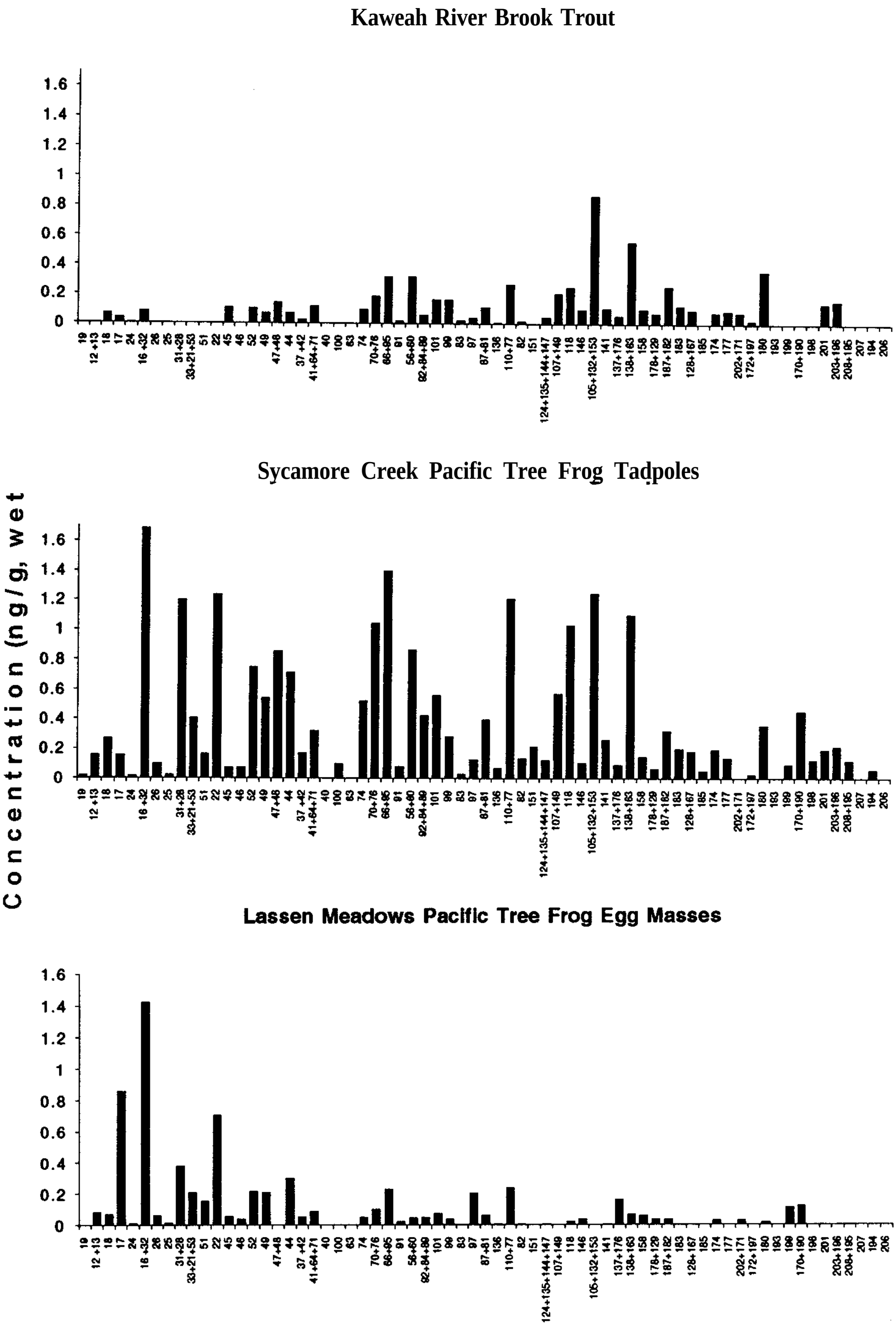

Figure 2. Distribution and concentration of PCB congeners in Kaweah River brook trout, Sycamore Creek tree frog tadpoles (collected June 22, 1996), and Lassen Meadows egg masses.

Institute of Ecology were notably higher (approximately 10 fold) than those measured in tadpoles from Sycamore Creek (Table 1). High levels of PCBs and p,p'-DDE at this wetland site may be due to deposition of airborne pollutants arising from the nearby airport and general local urbanization.

Levels of p,p'-DDE in Kaweah River trout ranged from 40.1 to 65.7 ppb and were one order of magnitude greater than t-PCBs. These higher concentrations of p,p'-DDE relative to PCBs may in part be explained by the past heavy use of DDT for cotton production in the counties of Fresno, Madera, Tulare and Kings that are immediately west (upwind) of Sequoia National Park. Concentrations of t-PCBs and p,p'-DDE measured in tadpoles at Sycamore Creek are similar to levels found in a recent Canadian study of PCBs and DDE in green frogs (*Rana clamitans*) at their two sampling sites, Long Point (11.8 ppb estimated t-PCBs and 3.54 ppb p,p'-DDE) and Hillman Marsh (33.7 ppb estimated t-PCBs and 5.84 ppb p,p'-DDE) in southern Ontario (Russell et al. 1997).

Analyses for currently used pesticides in tadpoles revealed the presence of chlorpyrifos, an organophosphate pesticide, and chlorothalonil, a chloronitrile fungicide. In all of the samples analyzed for pesticides, chlorothalonil was present at slightly higher levels than chlorpyrifos (Table 1). Total combined application of chlorpyrifos (504,853 kg, representing 59% of total California usage in 1995) and chlorothalonil (304,705 kg, representing 32% of total California usage in 1995) in counties of Fresno, Madera, Tulare and Kings which are in the vicinity of the Kaweah River basin is high relative to other counties (California Environmental Protection Agency, 1995). The presence of chlorpyrifos in tadpoles may be especially significant for several reasons: (a) Intense large scale production of citrus fruits in the foothills of the southern Sierras to which chlorpyrifos is heavily applied; (b) chlorpyrifos has been reported to enter fresh water ecosystems in the form of spray drift, and with eroded soil particles (U.S. Environmental Protection Agency, 1986); and (c) Sequoia National Park and Kings Canyon National Park are home to many amphibian species, including a large portion of the range of *Rana muscosa* (Bradford et al. 1993). The closest location of intense pesticide usage occurs just below the Kaweah Reservoir dam, approximately 15 km from Sycamore Creek where the tadpoles were collected. Pesticides may originate from further away in the valley.

Although Kaweah River brook trout were not tested for pesticides, tadpoles from farther up the watershed were found to contain the same pesticides as trout from the Kaweah River and Lake Tahoe basin (Datta 1997a). All of the contaminants measured in brook trout and tree frog tissues including PCBs, p,p'-DDE, chlorpyrifos and chlorothalonil are aromatic halogenated compounds. PCBs and p,p'-DDE are known estrogen mimics (Bitman and Cecil 1970; Bergeron et al. 1994) and this may have implications for the decline in populations of some amphibian species in the Sierra Nevada. Aquatic organisms that inhabit the Sierra Nevada Mountains, such as fish and frogs, appear to be recipients of airborne pollutants, which originate in the intensively farmed valleys of California and the coastal urbanized areas. This study is the only recent report of PCB, p,p'-DDE and some current-use pesticides in frogs from the Sierra Nevada, expanding on the original work of Cory et al. (1970) and is suggestive of a connection between the usage of chemicals and their transport and deposition to locations far from the actual sites of application. Clearly much more sampling and analysis must be carried out in conjunction with population studies and dose-response testing to reach definitive conclusions.

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