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Organophosphate Flame Retardants in Needles of *Pinus ponderosa* in the Sierra Nevada Foothills

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Haloalkyl phoshates act as flame retardants when added to polymers. They are used extensively in important commercial products such as textiles, building materials, and packaging materials. Tris(2-chloroethyl)phosphate (TCEP) is used as a flame retardant additive in such products as polyurethane and polyisocyanurate foams, c t backing, flame retardant aints, lacquers, coatings, resins and adhesives (Hutzinger et al., 1976; USEPA, 1988; Green, 1993). Tris(1,3-dichloroisopropyl)phosphate (TDCPP) and tris(2-chloropropyl)phosphate (TCPP) are also used as flame retardant additives in flexible plastic products such as polyurethane foam (Figure 1.).

Because production volumes of these haloalkyl phosphates are considered confidential business information, exact amounts produced annually in the U. S. are not available. The annual consumption of flame retardants in the U. S. was predicted to be 20,000 to 40,000 tons in 1980 (Hutzinger et al., 1976). Regardless of how much is produced and where these chemicals are used, they are found to be widespread throughout the environment. Contamination by TCEP has been reported at levels ranging from 17 to 350 ppt in river water in Japan (Ishikawa et al., 1985) at 8.7 ppt in river water in Canada and 1.7 ppt in the Great Lakes (Williams and LeBel, 1981; Williams et al., 1982), at 0.7 ppb in well water in Massachusetts (Bedient et al., 1983), and at detectable levels in both the Maas and Rhine rivers in Europe (Meijers and van der Leer, 1976). Low levels of TCEP have been detected in fruit and fish (USEPA, 1988). TDCPP has also been found in the environment at even lower concentrations than those of TCEP. The primary medium in which it has been identified is water, but it has also been found in shellfish, house dust (Sellstrom and Jansson, 1987) and human adipose tissues (LeBel et al., 1989). There is no information on the occurrence of TCPP in the environment, nor is there evidence that it has been sought. It is unlikely that these phosphate esters are produced naturally, thus these environmental residues must have origins stemming from the manufacturer’s use or disposal of the compounds themselves or the products into which they are incorporated. Little information exists regarding the physical properties of these compounds. TCEP is quite soluble in water (7943 mg/L) (Yoshioka et al., 1986) while TDCPP is reported to be soluble at only 7 mg/L at 24°C (Hollifield, 1979) and 1000 mg/L at 30°C(Stauffer, 1979). The log K_{ow}’s of these two compounds are 1.7 and 3.8, respectively (Yoshioka et al., 1986; Sasaki et al., 1981). Vapor pressure and K_{oc} data are not available. No physical constants are available for TCPP. These OP esters are generally assumed to degrade slowly in the environment because they are quite resistant to hydrolysis and oxidation (USEPA, 1988). Bioconcentration factors for TCEP and TDCPP have been estimated to be about 1 and 47, respectively, in killifish, although both compounds can be eliminated within hours from the

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$O{=}P{-}(OCH_2CH_2Cl)_3$

TCEP

$O{=}P{-}(OCH_2CHClCH_3)_3$

TCPP

$O{=}P{-}(OCH(CH_2Cl)_2)_3$

TCDPP

Figure 1. Structures of organophosphate flame retardants

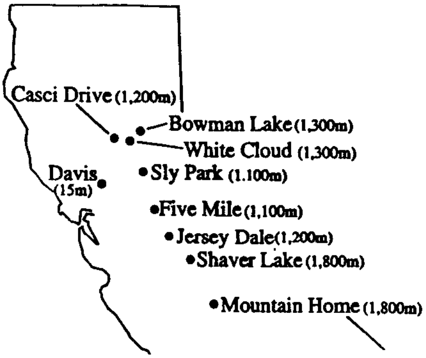

Figure 2. Location and elevation of Sierra Nevada foothill sampling sites.

organism (Sasaki et al., 1982). Toxicity tests have shown these compounds to be toxic to aquatic organisms (Sasaki et al., 1981). The EPA has listed these compounds on the Priority Testing List as provided by the Toxic Substance Control Act (TSCA) because of their structural similarity to aryl phosphates that are suspected carcinogens (USEPA, 1988). Moreover, the three compounds were listed for priority testing under TSCA because of the lack of information about their physical characteristics and their environmental behavior (USEPA, 1988). Of special interests to the EPA are the effects of chronic exposure of organisms in aquatic and terrestrial ecosystems.

Our recent investigation focused on detecting organophosphates insecticides in needles of the ponderosa pine (*Pinus ponderosa*) collected in California's Sierra Nevada foothills. Here, we report the finding of the flame retardants (TCEP, TDCPP, and TCPP) in this matrix. This was unexpected because they are not approved for use as forest fire retardants (USDA, 1994). After initially identifying these compounds in samples taken in the summer of 1993, we returned the next summer to the site where they were found in the highest concentrations to confirm their continued presence and to obtain more samples from the region. The analysis scheme utilized was designed to analyze the needles by compartments (Aston and Seiber, Submitted). The compartments consisted of a polar surface compartment (distilled water wash (DI)), a nonpolar surface compartment (surfactant wash (SW)), the cuticular wax (CW) and the remainder needle (RN). Results of recovery studies for these three compounds in the four compartments of the pine needles are also presented.

MATERIALS AND METHODS

Reagents used in this experiments were all RESI-ANALYZED grade (J. T. Baker Inc., Phillipsburg, NJ.) Organophosphate flame retardants (97% purity for TCEP, 99% purity for TCPP, and 93-97% purity for TDCPP) were obtained from Pfaltz & Bauer Inc., (Waterbury, CT) and were diluted with ethyl acetate. Ponderosa pine needle samples were collected in 1993 from foothill sites used as ozone monitoring stations by the California Air Resources Board (ARB). These sites were: White Cloud, Sly Park, Five Mile, Jersey Dale, Shaver Lake, and Mountain Home . In 1994 samples were collected from the vicinity of the White Cloud Forest station and at new sites at Casci Drive, approximately 5 miles west of White Cloud, and Bowman Lake, 40 miles east of White Cloud. The elevations of the sites range from 1,100 m at Five Mile to 1,800 m at Shaver Lake and Mountain Home (Figure

2). Exposure of the sites to air masses moving up slope from the Central Valley differs as does the number and proximity of the local population.

Sampling was conducted by removing 50 to 100 grams of one to four year old needles by gloved hand from various parts of four trees. The needles were first mixed together to ensure homogeneity then separated into four 30 g subsamples. Distilled water washes were performed at the site by spraying each subsample with approximately 330 ml double distilled water from a spray bottle. The rinse water was collected in an amber glass bottle fitted with a funnel. Needles were placed in 1 liter mason jars. Both the distilled water samples and needles were placed on dry ice and transported to the lab where they were stored at -20°C until they were extracted.

Single subsamples from each site were extracted into four compartmental fractions by the following methods. The distilled water samples were extracted using 6 ml, 0.5g C-18 "Bond Elute" (Varian, Harbor City, CA) solid phase extraction (SPE) cartridges. The cartridges were conditioned with 6 ml each of ethyl acetate, methanol, and double distilled water. After extracting the samples, the cartridges were eluted with 10 ml of ethyl acetate. Sample volumes were adjusted by nitrogen evaporation. For the nonpolar surface compartment the needles were brought to room temperature and placed in a flask with 200 ml of a solution of 1:25,000 dilution of sodium dioctylsulfosuccinate (ICN Biomedicals Inc. Costa Mesa, CA) in water. The needles were shaken for one hour on a rotary shaker, then the surfactant decanted. The surfactant was extracted using the same method as for the distilled water fraction.

For the cuticular wax fraction, the needles were washed two times in 100 ml of chloroform by shaking for 45 seconds. The wash solutions were filtered over anhydrous sodium sulfate and combined. After reducing the sample volume to 1 ml by rotary and nitrogen evaporation, the samples were transferred to 40 ml graduated centrifuge tubes. Cold acetonitrile (10 ml) was pipetted into the chloroform/wax mixture, and the tube was placed on an ice bath for five minutes. The precipitated wax was then filtered through Whatman No. 1 filter paper, and the filtrate was blown just to dryness and brought up to the desired volume in ethyl acetate.

The remaining needles were cut into 1-2 cm pieces, ground for one minute in 250 ml benzene using a high speed tissue grinder (Ultra Turrex, IKA-Work, Cincinnati, OH), and the benzene extracts were filtered over anhydrous sodium sulfate. The sample volume was reduced to 1 ml and applied to a 9 cm column of Florisil (Fisher Scientific, Pittsburgh, PA) conditioned at 130°C for 24 hours. The sample was eluted with 40 ml of 15% diethyl ether:benzene followed by 40 ml of 20% acetone:benzene. The collected eluate was rotary evaporated to near dryness, and brought up to the desired volume in ethyl acetate. Further details of the extraction methods are in Aston and Seiber (1996).

Recovery experiments were performed in triplicate at two spiking levels (75 ng/g and 150 ng/g) on 10 gram needle samples collected from Ponderosa pine trees from the Davis Arboretum located on the campus of the University of California, Davis. These needles were determined to be adequate blanks by extracting them by the methods described above, and analyzing them by GC-MSD. For each fraction, the addition of the spiking solution was done at a point when the needle matrix had been removed from the extract. The spiking solution consisted of a quantitative mixture of the three analytical standards in a 50:50 solution of acetone and ethyl acetate.

Extract of each of the four compartmental fractions from each sample was analyzed in triplicate by a Hewlett Packard 5890 gas chromatography equipped with a flame photometric detector with a phosphorus filter. All the samples were injected with a Hewlett Packard 7673A Automatic Liquid Sampler. External standards injected throughout the run were used for quantification. The column was a 30 m DB-17 megabore column (0.53 mm i.d., 1.0 µm film thickness) (J&W Scientific, Folsom, CA.) Injector and detector temperatures were both 250°C. The oven temperature program started at 180°C ramped to 200°C at 5°C/min., held for 5 min., ramped to 260°C at 20°C/min., and held for 6.5 min. The total temperature program ran for 16.5 min. Confirmation tests were performed by a Hewlett Packard 5890 gas chromatograph with a 5970 Mass Selective Detector Fitted with a 30 m DB-120 capillary column (0.25 mm i.d., and 0.25 µm film thickness) (J&W Scientific, Folsom, CA.). Injector and detector temperatures were set at 250°C and the transfer line temperature was set at 260°C. The oven temperature program started at 180°C for 2 min., ramped to 190°C at 5°C/min., ramped to 250°C at 30°C/min., held for 3 min., ramped to 255°C at 5°C/min. and held for 2 min. The total temperature program ran for 12.2 min. Detection was performed by Selective Ion Monitoring (SIM). Ions monitored were m/z 249 and 251 for TCEP, m/z 201, 237, 277, and 279 for TCPP, and m/z 303, 381, and 383 for TDCPP.

RESULTS AND DISCUSSION

Recoveries for the three flame retardants spiked separately in the four needle compartments are shown in Table 1. Recoveries were adequate (between 70% and 120%) from at least three of the four compartments for each compound. TCEP and TDCPP were recovered from all compartments except the remainder needle compartment within the range of 86.0% to 110%. At the 150 ng/g spiking level, TCPP was recovered adequately from the distilled water, surfactant wash and cuticular wax compartments. For the 75 ng/g spiking level, however, recoveries from those three compartments were inconsistent. Matrix and equipment blanks extracted simultaneousl to the spiked samples, showed no residues of TCEP and TDCPP in any of the four compartments. TCPP did appear at low levels in the matrix and equipment blanks of the cuticular wax fraction. The amount found however, was very low and recoveries from the spiked samples were not corrected for these residues. Recoveries of the three flame retardants from the remainder needle compartment ranged from 41.9% to 73.6% for both spiking levels. Spike and recovery studies were conducted only to show the performance of the methods used; residue values found in field samples are not corrected for recoveries. Limits of quantification (Table 1) were calculated by determining the quantity of residue represented by the smallest standard injected multiplied by the final volume of the sample extract and divided by the original needle mass (wet weight).

Table 1. Percent Recoveries of OP flame retardant from four compartments of ponderosa pine needles.

Compound		Spiking Level (ng/g)	% Recovery			
			Distilled Water Rinse	Surfactant Rinse	Cuticular wax	Remainder Needle
TCEP	n=3	75†	102 (9)*	107 (4)	86 (18)	74 (16)
	n=3	150	102 (3)	110 (7)	90.1 (9)	49 (150)
TCPP	n=3	75	248 (75)	242 (55)	138 (13)	72 (30)
	n=3	150	112 (3)	127 (6)	117 (7)	42 (130)
TDCPP	n=3	75	100 (13)	98 (10)	94 (10)	71 (16)
	n=3	150	107 (9)	110 (10)	97 (14)	56 (170)

† Limit of quantification (L.O.Q.) for each compartment is 7.5 ng/g except for the Remainder Needle Compartment for which L.O.Q. = 30 ng/g.

* Numbers in parentheses are percent relative standard deviations (n=3).

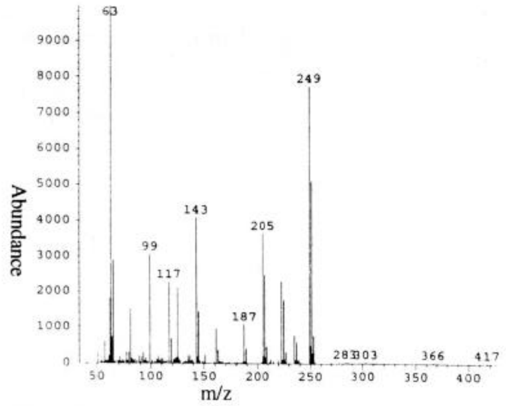

Figure 3. GC-MSD spectra of TCEP was obtained from distilled water wash compartment of White Cloud 1993.

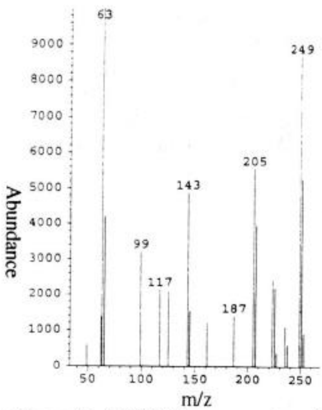

Figure 6. NIST library spectra of TCEP.

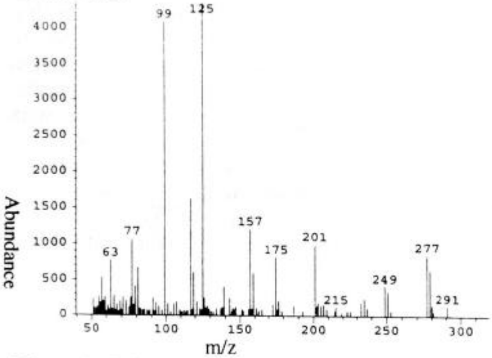

Figure 4. GC-MSD spectra of TCPP from distilled water compartment of White Cloud 1993.

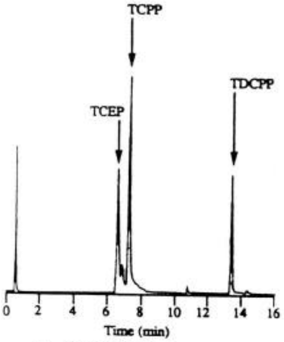

Figure 7. GC-FPD chromatogram of analytical standard of TCEP, TCPP and TDCPP.

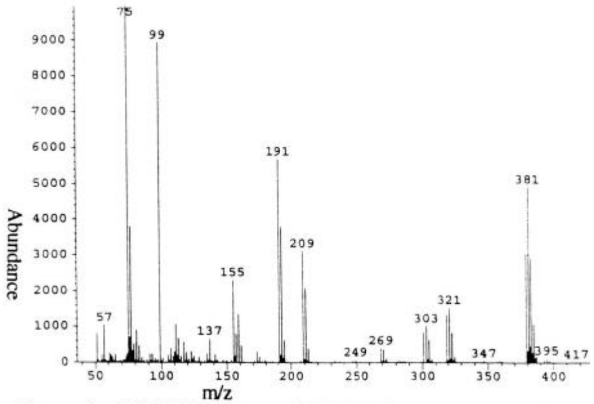

Figure 5. GC-MSD spectra of TDCPP from distilled water compartment of White Cloud 1993.

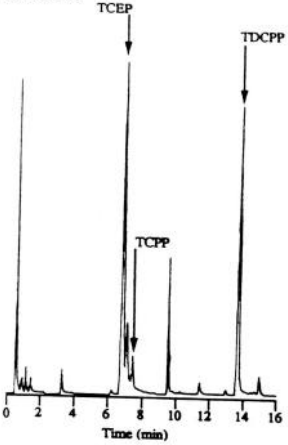

Figure 8. GC-FPD chromatogram ot TCEP, TCPP and TDCPP from surfactant wash compartment of White Cloud 1993.

Figures 3-5 show the mass spectra of TCEP, TCPP and TDCPP in 1993 samples from White Cloud. These compounds were initially identified in these samples by matching their spectra to those in the National Institute of Standards and Technology's mass spectral library. An example of a match is shown in Figure 6. Chromatograms of analytical standards and a surfactant wash compartment of the 1993 White Cloud sample are shown in Figure 7-8. Analytical results of the samples collected from the Sierra foothill sites are presented in Table 2. Highest total residue concentrations were found in the 1993 White Cloud samples: 1320 ppb (wet weight) TDCPP and 763 ppb TCPP. The 1993 Sly Park sample contained the highest residue concentrations of TCEP at 1950 ppb. TCEP in the Sly Park sample was found primarily in the remainder needle compartment whereas the TCPP and TDCPP in the White Cloud samples were found in the distilled water wash (surface) compartment. The samples from Jersey Dale and Shaver Lake showed relatively high concentrations of TCEP, 281 and 248 ppb, respectively. Again these residues were found in the remainder needle compartment of these samples.

TCEP, with the higher water solubility and thus presumably the greater polarity, would be expected to be found either in the aqueous surface compartment, or the polar remainder needle compartment, while the other compounds with higher log K_{ow} values would be expected to be found in the lipophilic wax compartment (Green, 1993). That TCPP and TDCPP are found in the distilled water rinse must indicate either that they are recently deposited vapors on the surface of the needle, residues bound to dust particles which are unable to migrate to the cuticular wax compartment, or are not polar enough to significantly partition into the wax compartment. 1994 samples from White Cloud showed only 43.2 ppb TDCPP and levels of TCEP and TCPP were below the limit of quantification. Casci Drive and Bowman Lake samples taken in 1994 from the vicinity of White Cloud, showed levels of flame retardant residues well below the levels found at White Cloud. All samples with residues quantitated by the primary method at levels of 20 ppb or greater were confirmed by GC-MSD.

Comparing White Cloud residues in 1993 to those in 1994 samples, the results show that the compounds are persistent, but do not remain at high concentrations in or on the needles. This may be due to their relatively high water solubility. The surfaces of the needles are subjected to the natural forces of wind, rain, snow and dew year round. Compounds deposited in 1993 may have washed off the needles after dissolving in such water. If the residues in the surface compartment are mainly bound to dust particles, wind may erode the residues. We did not detect any phosphorus containing breakdown products or metabolites in the samples.

These three compounds were found on pine needles at several sites in the Sierra Nevada foothills at orders of magnitude 1 to 3 times larger than those reported for aqueous matrices in other studies (Ishikawa et al, 1985; Williams and LeBel, 1981; Williams et al., 1982). Possible explanations for their presence include that, like pesticidal organophosphates, these compounds may have volatilized from another location and been transported in the air to the needles of these trees. This type of movement has been demonstrated for organophosphate pesticides used in the Central Valley moving into the Sierra Nevada mountains (Zabik and Seiber, 1993). Yet, while the organophosphate pesticides are extensively used on California's orchards and fields, the source for haloalkyl organophosphate vapors is unknown. One uninvestigated source of these chemicals may be then release into the air when materials such as polyurethane foam are burned, or when the materials degrade in land fill sites. Given their high water solubility, it may be possible that these compounds partition from the atmosphere into rain water and snow, which then deposit on the needles, leaving the residues after the water evaporates. Soil at these sites have not been analyzed for the presence of the flame retardants. Compounds which are nonpolar have been shown not to move appreciably through plants

Table 2. Concentrations (ng/g) (wet weight) of OP flame retardant in pine needles.

Location (Date)	Compartment	**TCEP**	**TCPP**	**TDCPP**
White Cloud (6/29/93)	DI Water Rinse	187	759	1260
	Surfactant Rinse	27.0	<L.O.Q.*	25.8
	Cuticular Wax	3.12	4.44	33.1
	Remainder Needle	193	<L.O.Q.	<L.O.Q.
	Total Amount	410	763	1320
Sly Park (7/28/93)	DI Water Rinse	8.13	73.2	510
	Surfactant Rinse	<L.O.Q	<L.O.Q.	17.4
	Cuticular Wax	<L.O.Q.	<L.O.Q.	17.5
	Remainder Needle	1940	16.4	17.4
	Total Amount	1950	89.6	562
Jersey Dale (7/28/93)	DI Water Rinse	<L.O.Q.	<L.O.Q.	3.73
	Surfactant Rinse	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Cuticular Wax	2.65	<L.O.Q.	<L.O.Q.
	Remainder Needle	278	<L.O.Q.	<L.O.Q.
	Total Amount	281	<L.O.Q.	3.73
Shaver Lake (7/28/93)	DI Water Rinse	2.89	3.14	41,8
	Surfactant Rinse	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Cuticular Wax	N.D.†	N.D.	2.51
	Remainder Needle	245	N.D.	<L.O.Q.
	Total Amount	248	3.14	44.3
Mountain Home (7/29/93)	DI Water Rinse	2.73	<L.O.Q.	8.20
	Surfactant Rinse	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Cuticular Wax	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Remainder Needle	11.9	<L.O.Q.	11.6
	Total Amount	14.6	<L.O.Q.	19.8
Five Mile (10/25/93)	DI Water Rinse	5.27	8.33	9.86
	Surfactant Rinse	4.76	<L.O.Q.	<L.O.Q.
	Cuticular Wax	<L.O.Q.	<L.O.Q.	2.74
	Remainder Needle	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Total Amount	10.0	8.33	12.6
White Cloud (5/24/94)	DI Water Rinse	<L.O.Q.	<L.O.Q.	29.0
	Surfactant Rinse	<L.O.Q.	<L.O.Q.	8.55
	Cuticular Wax	<L.O.Q.	<L.O.Q.	5.63
	Remainder Needle	N.D.	N.D.	<L.O.Q.
	Total Amount	<L.O.Q.	<L.O.Q.	43.2
Bowman Lake (5/24/94)	DI Water Rinse	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Surfactant Rinse	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Cuticular Wax	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Remainder Needle	N.D.	N.D.	<L.O.Q.
	Total Amount	<L.O.Q.	<L.O.Q.	<L.O.Q.
Casci Drive (5/24/94)	DI Water Rinse	<L.O.Q.	<L.O.Q.	2.94
	Surfactant Rinse	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Cuticular Wax	<L.O.Q.	<L.O.Q.	<L.O.Q.
	Remainder Needle	N.D.	N.D.	N.D.
	Total Amount	<L.O.Q.	<L.O.Q.	2.94

* Limit of quantification (L.O.Q.) for all compartments is 2.5 ng/g (wet weight) except for the remainder needle compartment for which L.O.Q. = 10 ng/g.

† N.D. = Not Detected. The peak of interest was not detected in the sample.

systemically. If such compounds have high enough vapor pressures, movement from soil into plants may occur by the uptake of vapors which volatilize from the soil into the plant's foliage (Bromilow and Chamberlain, 1995). However, no uses of these compounds have been found that would indicate that they may have been applied to the soil.

The results of this study indicate that residues of three haloalkyl phosphate flame retardants were present in Ponderosa pine needles in the Sierra Nevada foothills at levels higher than those reported in other matrices. The source of the contamination is unknown, but is suspected to involve aerial transport and deposition perhaps from nearby point sources where the chemicals were released during incineration of waste plastic articles. Further studies of these compounds in soil, air, streams and watersheds, and of potential sources, are necessary in order to understand the significance of these compounds and their means of transport in the environment. Such information, along with results of acute and chronic toxicity tests, would tell us if these compounds present any on-going danger to sensitive organisms in the Sierra Nevada's terrestrial and aquatic ecosystems.

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