

Site-Specific Profiles of Estrogenic Activity in Agricultural Areas of California's Inland Waters

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Received August 25, 2009. Revised manuscript received October 30, 2009. Accepted November 4, 2009.

To evaluate the occurrence and sources of compounds capable of feminizing fish in agriculturally impacted waterways of the Central Valley of California, water samples were extracted and subjected to chemical analyses as well as in vitro and in vivo measurements of vitellogenin in juvenile rainbow trout (*Oncorhynchus mykiss*). Among the 16 sites sampled, 6 locations frequently exhibited elevated concentrations of estrogenic substances with 17 β -estradiol equivalents up to 242 ng/L in vitro and 12 μ g/kg in vivo. The patterns of activity varied among sites, with two sites showing elevated activity only in vitro, two showing elevated activity only in vivo, and two showing elevated activity in both assays. Sequential elution of solid-phase extraction (SPE) disks followed by bioassay-guided fractionation was used to characterize water samples from the two locations where activity was observed in both bioassays. The highest estrogenic activity was observed in the most nonpolar fractions (80–100% methanol eluent) from the Napa River, while most of the activity in the Sacramento River Delta eluted in the 60% methanol eluent. Quantitative analyses of SPE extracts and additional HPLC fractionation of the SPE extracts by GC–MS/MS and LC–MS/MS indicated concentrations of steroid hormones, alkylphenol polyethoxylates, and herbicides that were at least 1–3 orders of magnitude below bioassay 17 β -estradiol equivalent calculations. Given the different patterns of activity and chemical properties of the estrogenic compounds, it appears that estrogenic activity in

these agriculturally impacted surface waters is attributable to multiple compounds. Further investigation is needed to identify the compounds causing the estrogenic activity and to determine the potential impacts of these compounds on feral fish.

Introduction

Starting in the 1990s, scientists began reporting the presence of male fish with an ovotestis and elevated concentrations of the blood serum protein vitellogenin in surface waters in which a significant fraction of the overall flow consisted of wastewater effluent (1–3). Subsequent surveys of feral fish in effluent-impacted waters in Europe, North America, and Japan confirmed the presence of intersex or feminized fish (4–6).

Coincident with studies of effluent-impacted waters, scientists began to document the occurrence and effects of estrogenic contaminants in agricultural watersheds. Initial studies focusing on hydrophobic compounds, such as DDT and its metabolites, indicated that sediment-associated pesticides could cause endocrine disruption in alligators (7). More recent studies have documented the presence of steroid hormones at concentrations high enough to feminize sensitive species of fish in runoff from confined animal feeding operations (8–11) and grazing rangelands (12). Furthermore, runoff from cultivated fields may contain naturally occurring estrogenic compounds, such as mycotoxins (13), while some commonly used pesticides (14) and nonionic detergents (used as wetting agents in pesticide formulations), can be converted to estrogenic compounds either in the environment or in the liver (15).

In vivo bioassays employing caged fish (16), flow-through aquaria (17, 18), static renewal (18), and intraperitoneal injection (18, 19) have been used to assess the estrogenicity of specific contaminants, to compare the estrogenicity of municipal wastewater effluent (20), and to evaluate temporal and spatial variations of estrogenic contaminants in surface waters (21, 22). However, the large volume of water required and limited throughput of these assays has precluded their widespread use for bioassay-directed fractionation. To identify the compounds responsible for fish feminization, inexpensive in vitro bioassays that require relatively small volumes of water or water extracts, such as the yeast estrogen screen (23) and the trout liver hepatocyte assay (24), have been developed. Studies that have used these bioassays indicate that steroid hormones (i.e., ethinylestradiol, 17 β -estradiol, and estrone) account for most of the in vitro estrogenic activity in wastewater effluent and in effluent-impacted waters (25, 26). In some cases, detergent metabolites (e.g., nonylphenol and nonylphenol ethoxylates) also contribute to the estrogenic activity (27).

Several recent studies have considered the possibility that estrogenic compounds in agricultural runoff could feminize fish in agricultural watersheds, but thus far results have been ambiguous. For example, Hinck et al. (28) observed intersex fish at several sites impacted by agricultural runoff along the Colorado River basin, but simultaneous measurements of pesticides did not indicate the presence of elevated concentrations of estrogenic compounds at locations where feminized fish were observed. The use of in vitro bioassays has indicated estrogenic activity in waters impacted by agriculture, but most of the activity was attributed to endogenous steroids excreted by the animals (9–11) despite the many other potential sources of estrogenic compounds in the agricultural watersheds. Preliminary data from caged

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TABLE 1. EEQs Determined in the Selected Sampling Sites by an in Vitro Bioassay^a

code	site	July 2006	September 2006	November 2006	January 2007	March 2007	April 2007
1	Upper Sacramento River	1.8 ± 0.6	3.7 ± 2.9	1.1 ± 0.5	1.7 ± 1.7	bdl	bdl
2	Battle Creek	bdl	0.2 ± 0.1	bdl	bdl	bdl	0.2 ± 0.1
3	Upper Feather River	1.2 ± 1.1	0.9 ± 0.5	4.8 ± 2.8	0.6 ± 0.4	0.5 ± 0.1	0.6 ± 0.3
4	Yuba River	bdl	12.5 ± 11.2	10.4 ± 11.9	0.4 ± 0.3	1.8 ± 0.9	0.9 ± 0.1
5	Lower Feather River	0.3 ± 0.1	15.3 ± 7.0	na	na	bdl	na
6	Lower Sacramento River	bdl	bdl	1.2 ± 0.2	0.9 ± 0.5	bdl	bdl
7	Lower American River	bdl	bdl	bdl	bdl	na	bdl
8	Sacramento River Delta	164.0 ± 117.7	8.6 ± 6.1	51.2 ± 31.9	107.5 ± 35.6	40.1 ± 11.9	71.3 ± 5.8
9	Mokelumne River	bdl	bdl	bdl	bdl	bdl	bdl
10	Stanislaus River	bdl	bdl	bdl	0.5 ± 0.3	bdl	bdl
11	San Joaquin River	bdl	bdl	bdl	bdl	bdl	bdl
12	Tuolumne River	91.6 ± 50.1	241.8 ± 46.3	24.6 ± 4.1	129.5 ± 47.1	68.5 ± 6.3	43.9 ± 14.1
13	Merced River	6.4 ± 3.4	56.1 ± 27.8	0.9 ± 0.4	10.9 ± 7.3	bdl	0.4 ± 0.2
14	Napa River	0.2 ± 0.1	68.3 ± 22.6	13.6 ± 14.1	2.3 ± 0.9	6.8 ± 3.1	10.1 ± 5.4
15	Clifton Court Forebay	bdl	bdl	bdl	bdl	bdl	bdl
16	Butte Creek	na	na	2.8 ± 0.6	6.5 ± 2.0	1.4 ± 0.3	1.6 ± 0.7

^a Data are presented in units of nanograms per liter and as the mean ± SD (*n* = 4). Abbreviations: bdl, below the detection limit (<0.15 ng/L); na, not analyzed.

TABLE 2. EEQs Determined in the Selected Sampling Sites by an in Vivo Bioassay^a

code	site	July 2006	September 2006	November 2006	January 2007	March 2007	April 2007
1	Upper Sacramento River	4.8 ± 2.8	0.3 ± 0.1	2.5 ± 1.2	1.2 ± 0.3	3.2 ± 0.03	1.1 ± 0.3
2	Battle Creek	bdl	bdl	bdl	bdl	bdl	bdl
3	Upper Feather River	bdl	0.2 ± 0.02	bdl	bdl	bdl	bdl
4	Yuba River	0.2 ± 0.01	0.3 ± 0.01	bdl	bdl	bdl	bdl
5	Lower Feather River	7.7 ± 0.2	3.4 ± 2.4	na	na	bdl	na
6	Lower Sacramento River	bdl	0.3 ± 0.03	bdl	bdl	bdl	bdl
7	Lower American River	bdl	bdl	bdl	bdl	na	bdl
8	Sacramento River Delta	4.6 ± 5.2	bdl	2.4 ± 0.5	3.1 ± 0.2	5.1 ± 0.7	4.1 ± 1.3
9	Mokelumne River	0.2 ± 0.01	0.2 ± 0.02	bdl	bdl	bdl	bdl
10	Stanislaus River	0.3 ± 0.01	0.2 ± 0.01	bdl	bdl	bdl	bdl
11	San Joaquin River	0.2 ± 0.01	0.2 ± 0.1	bdl	bdl	bdl	bdl
12	Tuolumne River	bdl	bdl	bdl	0.8 ± 0.1	0.3 ± 0.1	bdl
13	Merced River	0.2 ± 0.01	0.7 ± 0.03	0.4 ± 0.01	0.7 ± 0.4	bdl	bdl
14	Napa River	0.2 ± 0.01	12.4 ± 0.8	5.2 ± 0.6	0.2 ± 0.01	0.4 ± 0.02	3.1 ± 0.04
15	Clifton Court Forebay	0.3 ± 0.02	0.2 ± 0.02	bdl	bdl	bdl	bdl
16	Butte Creek	na	na	bdl	bdl	bdl	bdl

^a Data are presented in units of micrograms per kilogram (ww) and as the mean ± SD (*n* = 3–5). Abbreviations: bdl, below the detection limit (<0.15 µg/kg ww); na, not analyzed.

mental Section in the Supporting Information). Biologically active as well as inactive SPE fractions were evaluated for 51 current use pesticides and alkylphenol surfactants (mixture centered around nonylphenol C1–10 ethoxylates) by the California Fish and Game Laboratory in Rancho Cordova, CA, using established methods (see Table S4a for results and Table S4b for the full list of analytes, Supporting Information). Biologically active SPE fractions were subjected to HPLC fractionation as described above, and all HPLC fractions from the positive control, Napa River, and Sacramento River Delta were evaluated for the compounds listed in Table S5 (Supporting Information) using previously published methods (33–35).

Results

A total of 101 surface water samples were analyzed from the 16 sites between July 2006 and April 2007. The water quality parameters and chemical analyses indicated good water quality with relatively low concentrations of suspended solids (median 5 mg/L), low concentrations of dissolved organic carbon (median 2 mg/L), and the infrequent presence of low concentrations of herbicides and other trace organic compounds (Tables S1 and S3, Supporting Information). The concentrations of compounds most frequently associated with feminization of fish (i.e., selected steroid hormones,

alkylphenol polyethoxylates, and alkylphenols) were well below the threshold values for steroids and for alkylphenols (36) for feminization of sensitive species, such as rainbow trout.

Estrogenic activity was detected consistently at 6 of the 16 sites in the two bioassays (Tables 1 and 2). The highest estradiol equivalents (EEQs) measured with the in vitro bioassay were observed at the Sacramento River Delta (site 8; 8.6–164 ng/L), Napa River (site 14; 0.2–68.3 ng/L), Tuolumne River (site 12; 24.6–242 ng/L), and Merced River (site 13; <0.15–56.1 ng/L) sites (Table 1). Elevated EEQs were observed throughout the year in these locations. The highest in vitro estrogenicity was observed in the Tuolumne River in September 2006, and the highest activity in the Sacramento River Delta was observed in July 2006.

The in vivo bioassays indicated the highest EEQs in the Sacramento River Delta (<0.15–5.1 µg/kg ww), Lower Feather River (site 5; <0.15–7.7 µg/kg ww), Upper Sacramento River (site 1; 0.3–4.8 µg/kg ww), and Napa River (site 14; 0.2–12.4 µg/kg ww) (Table 2). Estrogenic activity was consistent throughout the entire year, but was more variable relative to the in vitro bioassay.

The Tuolumne River (site 12) and the Merced River (site 13) sites exhibited measurable EEQs in the in vitro assay but had estrogenicity at or below the limits of detection in the

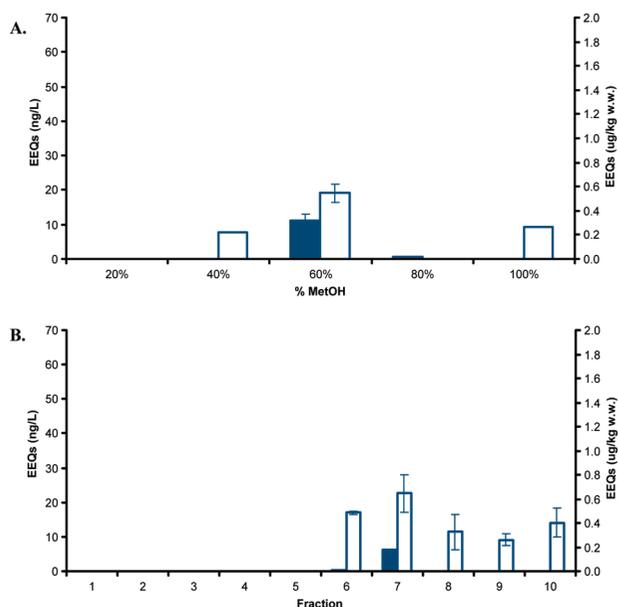


FIGURE 2. In vitro and in vivo estrogenic activities of fractions resulting from methanol elution of water samples from the Sacramento River Delta following solid-phase extraction (A) and subsequent HPLC fractionation of the 60% methanol eluent (B). Data are expressed in EEQs for in vitro (dark bars; ng/L) and in vivo (clear bars; µg/kg ww). Each value represents the mean average of 3–4 replicate measurements ± SD.

in vivo bioassay. Two different sites, the Upper Sacramento River (site 1) and the Lower Feather River (site 5), had elevated in vivo activity but low in vitro activity.

The Sacramento River Delta and the Napa River samples had the highest EEQs in both in vitro and in vivo bioassays. Consequently, they were chosen for additional characterization with HPLC fractionation coupled with bioassay analyses. Solid-phase extraction with sequential methanol/water elution was carried out on two sets of samples taken at different times (July 2007 and August 2008). The two sets of samples yielded similar results (see Figures S2 and S3, Supporting Information). The majority of the estrogenic activity measured by in vitro and in vivo bioassays using the Sacramento River Delta sample extracts eluted in the 60% methanol fraction (Figure 2A). In samples from the Napa River, most of the estrogenic activity was observed in the 80% and 100% methanol fractions. The highest in vitro estrogenicity was observed in the 80% methanol fraction, while the highest in vivo activity was observed in the 100% methanol fraction (Figure 3A). In the first positive control sample, extracts of river water amended with 100 ng/L E2 yielded an in vitro EEQ of 32 ng/L and an in vivo EEQ of 0.4 µg/kg in the 60% methanol fraction. In a second positive control (i.e., dechlorinated tap water with 30 ng/L E2), the in vitro (54 ± 8 ng/L EEQ) and in vivo estrogenicities were largely restricted to the 60% methanol fraction, with some carryover into the 80% fraction (Figure 4A). Chemical analysis of the second set of positive controls indicated 16 ± 1 ng/L E2 in the 60% methanol fraction after fractionation and 29 ± 3 ng/L when the cartridge was extracted with 100% methanol in one step. Evaluation of SPE extracts of dechlorinated tap water without E2 spiking or distilled water without E2 yielded no measurable estrogenic activity in either bioassay (data not shown).

In an attempt to identify other potential agents responsible for the estrogenic activity, additional chemical analyses of 51 pesticides and nonylphenol C1–10 ethoxylates (NPEOs) were conducted in the bioactive fractions from the Sacramento River Delta, the Napa River, and the 30 ng/L E2 positive control (Table S4, Supporting Information). Both surface water samples contained low concentrations of herbicides:

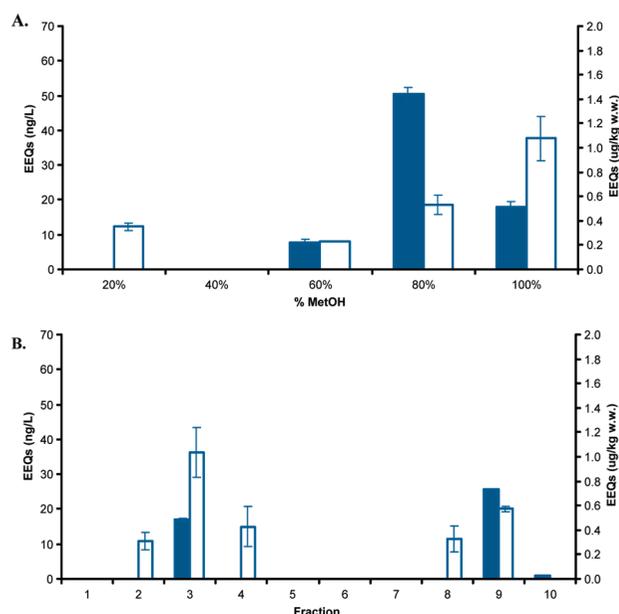


FIGURE 3. In vitro and in vivo estrogenic activities (EEQs) of fractions resulting from methanol elution of water samples from the Napa River following solid-phase extraction (A) and subsequent HPLC fractionation of the 80% methanol eluent (B). Data are expressed in EEQs for in vitro (dark bars; ng/L) and in vivo (clear bars; µg/kg ww). Each value represents the mean average of 3–4 replicate measurements ± SD.

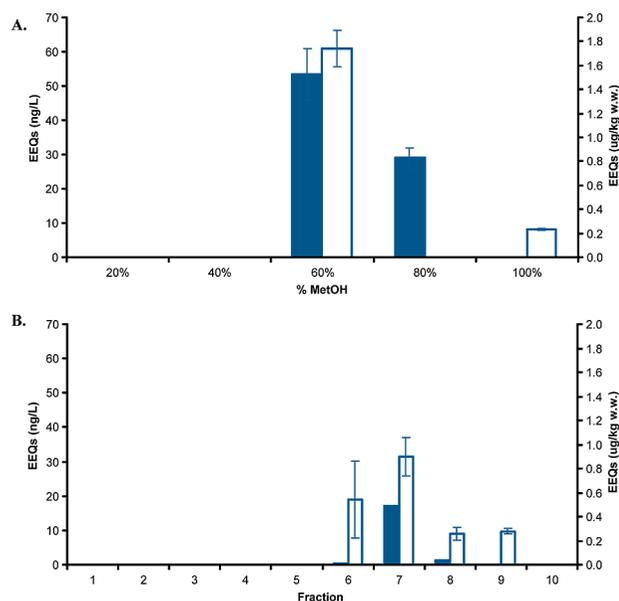


FIGURE 4. In vitro and in vivo estrogenic activities (EEQs) of fractions resulting from methanol elution of water samples from dechlorinated tap water amended with 30 ng/L 17β-estradiol following solid-phase extraction (A) and subsequent HPLC fractionation of the 60% methanol eluent (B). Data are expressed in EEQs for in vitro (dark bars; ng/L) and in vivo (clear bars; µg/kg ww). Each value represents the mean average of 3–4 replicate measurements ± SD.

the 80% methanol fraction from Napa River contained 6.2 ng/L diuron, 4.1 ng/L simazine, and 2.8 ng/L 2-hydroxyatrazine, while the 60% methanol fraction from the Sacramento River Delta contained 2.5 ng/L diuron and 0.2 ng/L 2-hydroxyatrazine. A mixture of the NPEOs (i.e., 421 ng/L) was only detected in the 80% methanol fraction from the Sacramento Delta extract.

To further characterize the active fractions, the 60% methanol fraction from the Sacramento River Delta, the 80%

methanol fraction from the Napa River, and the 60% methanol fraction from the positive control were subjected to HPLC fractionation (Figures 2B, 3B, and 4B). Fraction 7, which corresponded to a retention time of 19–21 min from the Sacramento River Delta possessed the highest *in vitro* and *in vivo* estrogenic activities (Figure 2B). Similar levels of *in vivo* activity were observed in fractions 6, 8, 9, and 10. After HPLC fractionation, recovery of *in vitro* activity (i.e., the sum of the activity from the fractions) was approximately 80% of that measured in the extract that was not subjected to sequential elution, but recovery of *in vivo* activity was >300%.

In the Napa River samples, *in vitro* activity was observed in fractions 3, 9, and 10 (Figure 3B). *In vivo* activity was observed in fractions 2, 3, 4, 8, and 9. Recovery of *in vitro* activity was approximately 80% and *in vivo* recovery was >500% after HPLC fractionation.

In the positive control, fraction 7 (corresponding to 18–21 min) also showed the highest EEQs *in vitro* and *in vivo* with some *in vivo* activity in fractions 6, 8, and 9 (Figure 4B). The retention time for a 17 β -estradiol standard in this HPLC method was 20.4 min, corresponding to fraction 7. Overall recovery of E2, as measured by GC–MS/MS was 110% (33 ng/L) from the 60% methanol fraction (30 ng/L) of the SPE extraction. The recovery of estradiol, as measured by the *in vitro* bioassay (i.e., 22 ng/L EEQ), was 73%.

All HPLC fractions were analyzed for 30 compounds commonly observed in domestic wastewater by LC–MS/MS and GC–MS/MS (Table S5, Supporting Information). Six human pharmaceuticals with no known estrogenic activity were detected in fractions 1–5 at low concentrations (Table S5a). No compounds were detected in the bioactive fractions from the Sacramento Delta.

For the Napa River samples, estrone (<1 ng/L total) was observed in fractions 8, 9, and 10 and carbamazepine (0.6 ng/L) was observed in fraction 8 (Table S5b, Supporting Information).

Discussion

Occurrence of Estrogenic Substances. Estrogenic activity was repeatedly observed at 6 of 16 locations in the inland waters that drain into San Francisco Bay. At the remaining sites, estrogenic activity was near or below the detection limit of the assay. Measured concentrations of selected steroid hormones and APEs could not explain the biological observations, and screening for modern use pesticides and wastewater-derived contaminants did not indicate contamination (concentrations were <10 ng/L). In most previous studies in which *in vitro* bioassay-guided fractionation data were coupled with chemical analyses, steroid estrogens were the class of compounds responsible for most of the activity (26, 36, 37). For example, studies of wastewater effluent in Switzerland indicated that the calculated estrogenicity from chemical analyses was of the same order of magnitude as that calculated from YES activity and other *in vitro* assays (38). However, the previous studies were almost exclusively focused on municipal wastewater or effluent-impacted surface waters. In contrast, the sites targeted in the current study were primarily within agriculturally impacted areas.

In contrast to the calculated EEQs from chemical analyses (typically less than 1 ng/L), bioassay-derived EEQs for *in vitro* activity averaged 52 ng/L. When samples from locations where municipal wastewater is not the source of estrogenic activity are considered, the calculated chemical EEQs rarely correspond to the EEQs measured with bioassays. For example, Pawlowski et al. (40) observed higher YES activity than that predicted from chemical analyses in surface waters from the Rhine River in Germany. Other authors also have reported discrepancies between measurements from *in vitro* bioassays and EEQs based on chemical analyses of selected steroid hormones or other known estrogenic chemicals (41).

In waters impacted by agricultural activities, estrogenic activity may result from the presence of pesticide mixtures and/or their degradates as well as phytoestrogens, adjuvants, and other compounds with multiple endocrine targets and modes of action (37, 39, 42).

While YES and *in vitro* estrogen receptor (ER)-based assays frequently used in studies of this nature are rapid and cost-effective, the ability of these ER-based assays to detect mechanisms of feminization other than direct binding to the receptor is limited. For example, compounds that require biotransformation to a metabolite that activates the receptor, such as the organochlorine insecticide methoxychlor, require demethylation to phenolic metabolites prior to interaction with the estrogen receptor (43). The inability of *in vitro* assays or cell lines to detect these compounds was illustrated by comparisons of feminization caused by methoxychlor and nonylphenol in fish relative to MCF-7 cell lines where the estrogenic signal in fish was 1000 times more sensitive (44). In this regard, the use of isolated hepatocytes from fish circumvents this issue because the full contingent of biotransformation enzymes are present to potentially activate or deactivate putative estrogens as would occur *in vivo*. When hepatocyte-based *in vitro* assays have been used in bioassay-guided fractionation studies to identify estrogenic compounds in surface and wastewater effluents at other locations, estrogenic activity has been observed in fractions that do not have steroid estrogens (45, 46).

The occurrence of estrogenic activity in surface waters of central California was initially reported by Johnson et al. (47), who observed estrogen receptor activation from water extracts in agricultural regions. De Vlaming et al. (48) found limited *in vivo* estrogenic activity in a study that included a larger number of rural and urban sites throughout central and northern California, with activity being detected in only 6 of 113 samples. The low frequency of detection in the de Vlaming study may have been due to shorter exposure durations, which raised the detection limits for the assay to 5 ng/L EE2. EE2 is up to 10 times more potent than estradiol in rainbow trout estrogenic responses (49). If this value for the estrogenicity of EE2 is used, the LOEC of the study would be approximately 50 ng/L for E2, which significantly exceeds biological thresholds for E2 in fish (0.35 ng/L) (50).

Discrepancies between *in vitro* and *in vivo* responses clearly show that the causative agent(s) responsible for feminization differ in mode of action, as well as identity. Estrogenicity observed with *in vivo* bioassays but not *in vitro* bioassays suggests that the causative agent(s) affects circulating estrogen biosynthesis or disposition. For example, an *in vivo* response that would not be observed in the hepatocyte bioassay could be caused by one or more compounds that increase the release of gonadotropins or inhibit elimination of estrogens within the organism (51). Compounds that are active *in vitro* but not *in vivo* may undergo detoxification and elimination through extrahepatic biotransformation or may be rapidly cleared prior to distribution to tissues where estrogen receptors are located. For example, the androgen testosterone has been shown to induce vitellogenin in hepatocytes when cells are exposed to high concentrations (2×10^{-5} M) due to transformation to E2, but the transformation does not occur when animals are treated *in vivo* (52) or if the cells are exposed to lower concentrations (10^{-13} – 10^{-7} M) (53). Additional characterization is needed to resolve this complex issue.

Characterization of Estrogenic Substances. A fractionation procedure guided by the two bioassays used for Sacramento River Delta and Napa River samples that had both elevated and consistent estrogenic activity in both bioassays provided insight into the chemical properties of the estrogenic compounds. Bioactive fractions from sequential elution from SPE cartridges followed by HPLC separation

differed significantly between the two sites. SPE separation indicated most of the activity from the Napa River (site 14) was associated with the two most hydrophobic fractions, whereas the Sacramento River Delta sample indicated activity in the less hydrophobic 60% methanol fractions, where the steroid estrogens would be eluted. Chemical analyses did not indicate the presence of compounds in either sample at sufficient concentrations to explain the observed high levels of estrogenic activity.

Fractionation of the sample extracts enhanced *in vivo* biological activity 2–5-fold in each of the three samples. SPE fractionation enhanced *in vitro* activity of the E2-spiked tap water more than 2-fold, and HPLC fractionation of the Sacramento Delta and Napa River SPE fractions enhanced *in vivo* activities 3–5-fold. Since extraction of dechlorinated tap water or distilled water failed to elicit responses, these data suggest that fractionation may separate estrogenic compounds from antagonistic compounds that dampen their effects in the whole extracts. Similar results have been reported previously in TIE experiments carried out in wastewater effluents (25, 26) and indicate that bioassay-guided fractionation may not allow mass balance comparisons even though methods are useful for qualitative end points. The identities of these antagonistic materials are unknown, but the interaction of antagonistic compounds with the ER and estrogenic response is well established (54). Alternatively, variability associated with quantification of bioassay signals at the limits of detection (0.15 µg/kg), especially in the *in vivo* assays, also may have contributed to our inability to obtain a mass balance.

As a result of the difficulties associated with identification of the compound(s) responsible for the observed estrogenic activity, future efforts to identify the sources of the unknown compounds may need to focus on the behavior of the compounds in the TIE experiments. The differences in activity patterns (i.e., Figures 2 and 3) between the two sites, which were identical in two separate years, suggest that different compounds may be responsible for the estrogenic response (i.e., more hydrophobic compounds seem to be responsible for estrogenic activity at the Napa River site). Potential candidates include unknown degradation products of pesticides and phytoestrogens. While preliminary efforts to identify the compounds by GC- and LC-MS/MS have proven unsuccessful, use of high-resolution mass spectrometry and different ionization techniques may help identify the causative agent(s).

Acknowledgments

This work was supported by the CALFED Bay Delta Program (Grant No. U055C031) and the UCR Agricultural Experimental Station (D.S.). We thank Brett Vanderford, Rebecca Trenholm, Doug Mawhinney, and Janie Zeigler-Holady of the Applied Research and Development Center of SNWA for their aid in processing the samples.

Supporting Information Available

Additional technical details (standards and reagents, sample preparation for bioassays and chemical analysis, analysis of steroid estrogens, alkylphenol ethoxylates, current use pesticides, water quality analyses, estrogenicity bioassays, fractionation methods, and statistical procedures), additional tables (description of sampling sites, water quality parameters, steroid hormones and APEs, current use pesticides in SPE fractions, and phytoestrogens, pharmaceuticals, pesticides, and potential endocrine disruptors in HPLC fractions), and additional figures (standard curves for *in vitro* and *in vivo* estrogenicity bioassays and *in vitro* and *in vivo* estrogenic activities of samples from the Sacramento River Delta, the Napa River, and E2-spiked water). This information is available free of charge via the Internet at <http://pubs.acs.org>.

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ES902583Q