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Environmental Estrogens in Agricultural Drain Water from the Central Valley of California

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The estrogenic activity of some chemicals (e.g., Diethylstilbesterol) have been known for a long time (McLachlan 1985). Concerns about these compounds stem from their known or presumed effects on humans and wildlife. In humans, links between cancers and estrogen are established (McLachlan 1985), and the effects of estrogens on reproduction of wildlife are also well documented in the laboratory (Fry 1995). However, there are only a few investigations of adverse effects due to exposure to large doses of estrogenic compounds in nature including the feminization of alligators in Lake Apopka (Gillette et al. 1994), the feminization of gulls due to exposure to DDT (Fry and Toone 1981), and deformities in birds in the Great Lakes (Giesy et al. 1994).

The exposures of the alligators and birds arose from unusually high levels of contaminants being discharged in specific areas (Kendall and Dickerson 1996). A major question that remains is if estrogenic chemicals are found over wide geographic areas at concentrations that could cause adverse effects in wildlife (Kendall and Dickerson 1996). The potential for the exposure to environmental estrogens exists since many chemicals, such as pesticides, are known to be estrogenic and are applied over wide areas. While many of these chemicals possess a relatively short half-life (e.g., dicofol = 60 d), many of the breakdown products may also possess estrogenic activity (e.g., α- and β-endosulfan and endosulfan sulfate). Consequently, it remains to be determined if wildlife near agricultural areas are exposed to substantial quantities of estrogenic compounds. A potential route of exposure to terrestrial and aquatic wildlife at the interface of natural ecosystems and agricultural systems is by contact with contaminated surface waters. Pesticides can enter surface water by a variety of mechanisms including direct spray drift (Longley et al 1997, Longley and Sotherton 1997), overland flow after runoff events (Wauchope 1978), or infiltration of pesticides into shallow ground water where they are transported via agricultural drains tostreams, rivers, or storage ponds. Wan (1989) found that some pesticides applied to fields in British Columbia were found in farm ditches that eventually emptied into local rivers. Because sampling occurred primarily during the rainy season, the presumed mode of transport was via overland flow after rainfall events.

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In the Central Valley, agricultural drain canals may receive estrogenic chemicals due to the widespread application of a variety of pesticides. These drains are used by numerous species of wildlife, because during the summer and fall, these small canals represent a major source of free water. Consequently, there exists the potential for the exposure of wildlife to chemicals with estrogenic activity. We undertook a brief survey of agricultural drains in the Central Valley of California to determine if water and sediment from these drains contained chemicals with the ability to directly interact with an estrogen receptor.

MATERIALS AND METHODS

Information was obtained from the California Integrated Pest Management Program (IPM) on the total pounds of chemicals with known or suspected estrogenic activity, applied to all counties in the San Joaquin Valley in 1991, the most recent data available for this study. We identified those counties with high application rates of dicofol, endosulfan, nonylphenolpolyoxyethylene-ethanol, nonyl-phenoxypolyethyleneoxyethanol, and octylphenoxy-polyethoxyethanol. The highest use of these chemicals were on cotton, grapes, and nut trees. San Joaquin county was selected for study. In 1995, the major crops in the county included grapes (65,600 acres), melons and other vegetables (48,850 acres), almonds (40,206 acres), walnuts (36,761 acres), and tomatoes (32,570 acres) (California Farmer, 1996). In 1991, 7615 kg of dicofol, 1998 kg of endosulfan, and 2391 kg of octylphenoxypolyethoxyethanol were applied to the crops in the county, primarily from June to August.

Using United States Geological Survey 1:24,000 topographic maps, agricultural drains canals were located and sample sites established prior to going to the field. Sampling occurred during the months of August and September 1994. Prior to the main sampling effort, one initial sampling occurred at Sites 1-3 to determine if estrogen receptor binding chemicals existed in sediment. Subsequent sampling of water and sediment at all sites were collected in liter glass containers and plastic centrifuge tubes respectively. No attempt was made to filter the water prior to analysis. Samples were transported to the laboratory and processed within 48 hours. All samples were refrigerated between collection and analysis.

Sediment and water samples were extracted with chloroform using 30ml/g of sediment and 100 ml/00 ml of water. For analysis, the solvent was extracted under nitrogen and dried samples resuspended with 250 µ1 of dioxane and diluted 2 ml total volume with dimethylsulfoxide. Additionally, a pure 12.5 % dioxane-Dimethylsulfoxide mixture was prepared as a negative control.
Calf uteri were obtained from a local slaughterhouse and cytosol was prepared in HEDGM buffer (25 mM Hepes, pH 7.4, 1 mM Ethyelenediaminetetraacetic acid, 1 mM Dithiothreitol, 20 mM sodium molybdate and 10% glycerol (v/v)) as previously described (Denison et al. 1986) and stored at -80°C until use. Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin as the standard.

The presence of estrogen receptor binding chemicals in the water and sediment was determined by measuring the ability of the solvent extracts of these samples to competitively inhibit the specific, high affinity binding of [^{3}H]estradiol to calf uterine cytosolic estrogen receptors. Competitive binding to the estrogen receptor was measured using the Dextran-coated charcoal assay. Cytosol(3 mg/ml) was incubated with 2 nM [^{3}H]17β-estradiol(47.2 Ci/mmole, New England Nuclear) in the presence or absence of 200 nM diethylstilbesterol (DES) or an aliquot (15 µ1) of the sample extract for 2 h at -4°C. The binding reaction was subsequently added to a Dextran-coated charcoal pellet (0.25 mg Norit A charcoal/0.025 mg dextran), incubated for an additional 15 min and the Dextran-coated charcoal pelleted by centrifugation. The amount of radioactivity present in an aliquot (0.3 ml) of the supernatant was quantitated by liquid scintillation counting. Total specific binding of the [^{3}H]estradiol to the estrogen receptor was computed by subtracting the amount of [^{3}H]estradiol bound in the presence of Diethylstilbesterol from the amount of [^{3}H]estradiol bound in the absence of a competitor. Decreased specific binding of the [^{3}H]estradiol in the presence of a sample extract would suggest that the sample contains chemical(s) which can competitively bind to the estrogen receptor ligand binding site. Ligand binding activity was calculated as the number of fmoles of [^{3}H] estradiol specifically bound per mg of protein. The relative estrogen receptor binding activity of a sample extract was expressed as a percent of the total estrogen receptor binding (determined using Diethylstilbesterol as the competitor) averaged over the three replicates.

RESULTS AND DISCUSSION

Identification of estrogenic chemicals can be accomplished at several levels including measurement of ligand binding (competitive), estrogen receptor transformation and DNA binding, or estrogen receptor-dependent activation of gene expression. Although each of these assays will allow detection of estrogenic chemicals, the latter two assays do not easily allow detection of chemicals with antiestrogenic activity. Although the ligand binding assay has the advantage of allowing detection of both estrogenic and antiestrogenic estrogen receptor ligands, it does not differentiate between these two classes of chemicals. However, since both of these classes of chemicals can have significant adverse effects on endocrine function, detection of both in a simple screening assay is preferred. In addition, the ease, rapidity, sensitivity, and low cost of the estrogen receptor competitive dextran-coated charcoal binding assay are advantages over the other assay systems. Final confirmation of the specific estrogenic and/or antiestrogenic activity of a chemical or sample extract will require subsequent analysis.

Using the estrogen receptor assay, competitive ligand binding activity was detected in both sediment and water samples (Table 1). Estrogen receptor binding for the three sites (1, 2, 3) sampled initially to determine if estrogen receptor binding chemicals were present, was 25.4% for Site 1,28.6% for Site 2, and 14.3% for Site 3 (unreplicated samples). In general, binding of the remaining samples ranged from 30% to 80% of the controls indicating the presence of moderately weak tomoderately strong estrogen receptor binding chemicals. Water

Table 1. Competitive estrogen receptor ligand binding of chemicals extracted from sediment and water samples from agricultural drains sites in San Joaquin county.

Site	Collection Date	Sediment Extract	Water Extract
		Competitive Binding	Competitive Binding
1	09/19/94	11.1 ± 1[a]	NC[b]
2	"	11.1 ± 1	NC
3	"	13.9 ± 3	NC
4	08/31/94	47.9 ± 2	Contaminated[c]
5	"	43.8 ± 6	Contaminated
6	09/02/94	38.2 ± 2	Contaminated
7	"	48.9 ± 2	72.2 ± 4
8	"	48.9 ± 4	42.6 ± 0
9	08/31/94	45.8 ± 2	78.6 ± 1
10	09/02/94	47.5 ± 18	41.1 ± 4
11	"	42.2 ± 4	52.5 ± 2
12	08/31/94	52.1 ± 6	82.1 ± 0
13	09/02/94	35.6 ± 7	46.4 ± 2
14	"	39.3 ± 2	67.9 ± 4

a. Values are expressed relative to the total amount of specific binding estimated using 1 μM DES as the estrogen receptor ligand binding competitor and are expressed as the mean ± SD.
b. Samples not collected
c. Samples became contaminated and were discarded.

samples contained slightly higher levels of estrogen receptor binding chemicals than sediment, most probably as a result of the high load of organic matter in the water and/or the presence of water-soluble estrogen receptor ligands. In addition, the composition of the sediment varied substantially depending on the collection site. Some drains were lined with cement which not only reduced the sediment accumulation, but the sediment collected at several of these sites was very sandy, providing less of an opportunity for the sorption of organics.

Sites 1-3, which were sampled initially for estrogen receptor binding chemicals, were sampled a second time approximately one month later. Specific binding for Sites 1 and 2 decreased substantially (Table 1) indicating either or both of two situations. First, water containing higher levels of estrogens from the first sample period had already moved into larger drain canals and eventually into drainwater holding ponds. With the reduced application rate of pesticides later in the growing season, a lower amount of estrogen receptor binding chemicals would be reaching the drains immediately adjacent to the fields. Second, there was some degradation of the estrogen receptor binding compounds over time. In the former case, the bulk of exposure to wildlife would occur at the larger drainwater holding ponds over a relatively long period of time, and in the latter case, exposure would be limited to a shorter window of time. Both waterfowl and shorebirds use the drains during their fall migration through the Central Valley. Determining which of the explanations is correct (or if a combination of the two is correct) is important because the level of exposure of migrating waterfowl and shorebirds would vary depending on the amount of estrogen receptor binding compounds present through the period of migration.

The movement of the estrogen receptor binding chemicals from the fields to the agricultural drain canals during the summer is most likely through infiltration into the soil and movement through the shallow ground water or drain tile systems. Rain is rare in the Central Valley of California during June through August, and all water found in the drains is the result of irrigation. Pesticide applications to the major crops are through spraying, and some of the pesticides fall onto the ground immediately. Irrigation of crops can be by a variety of methods including furrow, drip, flood, and sprinkler. Regardless of the irrigation method, there is little overland flow of water and pesticides to drain canals during the summer months. Water applied during sprinkler irrigation may wash pesticide residues from the plant surfaces. All irrigation practices move pesticides from the upper layers of the soil through the vadose zone and eventually into the drain tile systems.

The fact that chemicals can survive the transport process while maintaining their activity is not unexpected (Antonious and Byers 1997). For example, endosulfan is relatively resistant to degradation by soil microorganisms (Goebel et al. 1982) and does not readily photodegrade in water. Once the chemicals reach the drain canals, they can persist for several weeks to several months. In British Columbia, Wan (1989) found pesticide residues (dinoseb, endosulfan) in small farm ditches up to seven months after the last spraying event. Also, the compounds detected in our sample are likely to be bioavailable. Our analytical technique primarily detects the bioavailable fraction of environmental estrogens in the water and sediment by extracting almost all of the hydrophobic chemicals present in the sample. Since water-soluble estrogen receptor ligands or ligands that are tightly bound to particulate matter in the water might not be extracted by this procedure, our results may represent an underestimate of the total amount of estrogen receptor ligands present in these samples. Overall, our results demonstrate the presence of chemicals in water and sediment samples from a variety of agricultural sites in San Joaquin County that competitively bind to the estrogen

receptors. The identification of these chemicals with their estrogenic and/or antiestrogenic activity and their toxicological and biological potency remain to be determined.

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