Bull. Environ. Contam. Toxicol. (1993) 51:361–366

Environmental Contamination and Toxicology

Estrogen as an Environmental Pollutant

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Steroidal estrogens and testosterone from human and animal sources are constantly excreted into the environment. For example, a pregnant woman may excrete 10 μmol/day of the steroidal estrogens, estradiol and estrone (Fostis 1987) and chicken manure can contain over 1μ mol/g of either testosterone or estrogen (Shore et al. 1988; Shore and Shemesh 1992). Other animals such as cows, swine, horses and goats also excrete large amounts of estrogen into the environment (Knights 1980). However, there is no information on the environmental fate of these hormonal steroids. We recently reported (Shore et al 1992) that estrogen appears in readily measurable concentrations in sewage water used for irrigation even following treatment in sedimentation ponds. Furthermore, we found that estrogen concentrations of 0.02 - 2 nmol/L significantly increased the growth of alfalfa plants while 200-2000 nmol/L of estrogen significantly decreased growth compared to controls (Shore et al. 1992). In the present work, we attempted (1) to determine the stability of estrogen in the environment by determining the effect of treatment in an advanced sewage disposal unit and (2) to determine estrogen concentrations in lake and drinking water in Israel.

MATERIALS AND METHODS

The water samples were obtained from the following sources: (1) From a sewage disposal plant (Central plains, Tel Aviv area) (a) before treatment, (b) within the digestive tank (both aerobic and anaerobic digestion), (c) after treatment and (d) following percolation through sand for 3 mon. The samples a,b,c were all from the same batch of sewage water and the treatment lasted about 20 hr. Samples were taken each week for 5 wks in the late summer or early fall of 1991 when there was severe restrictions on water usage due lack of precipitation. Additional samples were taken in 1992 after a very wet winter and easing of water restrictions. (There is no precipitation in Israel during the summer months.) (2) From Lake Kinneret in Northern Israel. Samples were taken from (a) the shore line and (b) from the

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drinking water. The source of the drinking water (obtain from taps) was about 500 m offshore. Samples were taken in 1990, 1991 and 1992. 1991 was the third year of extreme drought in Israel and the level of the lake was at a record low. The samples from 1992 were taken after an extremely wet winter which caused the lake to overflow. At its lowest level the lake was nearly -213 m below sea level as opposed to -209 m when the lake was full. It has been calculated that the difference represent a reduction of 680 million m^3 from the total volume of 4 billion cubic m^3. (3) Eleven samples of water drawn from wells in Northern Israel. (4) Irrigation water - effluent from domestic (farm or cities) sources which had to been treated in oxygenation and sedimentation ponds.

Samples of water (50-100 mL) were centrifuged at 800 g. The supernatants were then mixed with an equal volume of 0.1 M sodium acetate buffer, pH 5.0, prior to column extraction. The columns used for steroid purification were solid phase extraction column containing 500 mg of bonded C-18 (Alltech Associates, Inc., Deerfield, Ilinois, USA). The columns were first washed with 10 mL of methanol followed by 10 mL of the sodium acetate buffer (pH 5.0) by use of a suction vacuum. The sample was loaded on the column, allowed to dry for 30 sec, and the column was washed with 10 mL of 10% aqueous methanol. This last step was necessary to remove impurities which could interfere with the assay. The column was then dried for two min and eluted twice with 5 mL 80% aqueous methanol. The eluate was dried under a vacuum and the residue redissolved in 2 mL of the buffer used for steroid analysis. One hundred µL aliquots were then taken for radioimmunoassay. Since the sensitivity of the assay was 10 pg/tube and a 1/20 aliquot was assayed, 200 pg/100mL (7 pmol/L) represented the limit of detectablility. The radioimmunoassay for testosterone and estrogen was performed as previously described (Shore and Shemesh 1981). The recovery of a known fortified amount of steroid (1 to 10 ng) to the sample prior to extraction was 101±7.6%SD (n=10) for testosterone and 97±3.1% for estradiol 17-β or estrone. The inter- and intra-assay coefficients of variation were 8.9 and 6.7% respectively. Since the antibody for estradiol-17β cross-reacted 25% with estrone, these results are expressed as nmol or pmol estrogen/L.

RESULTS AND DISCUSSION.

The concentration of estrogen in raw sewage water originating from the Tel Aviv area in 1991 (3rd year of drought) ranged from 0.2 to 0.5 nmol/L (48-141 ng/L) and testosterone ranged from 0.8 to 1.1 nmol/L (208-320 ng/L) (Table 1) but these concentrations were considerably lower in 1992 after the drought was over. Even higher levels of estrogen were found in sewage water from agricultural settlements (1.3 nmol/L; Table 2). Following anaerobic (supernatant of activated sludge) and aerobic digestion (reaction mixture) in sewage disposal plant, the level of estrogen was 0.1-0.2 nmol/L while testosterone was 0.2-0.5 nmol/L (Table 1). These data indicated that although the amount of steroidal

hormone reaching the effluent was related to the concentration of steroid hormone in the raw sewage water entering the tank, the relative percentages of testosterone (60-77%) and estrogen (20-88%) removed or their relative concentrations in the digestion tank fluids suggest they are processed by different mechanisms. However, the concentrations of both steroidal hormones found ever after percolation for 3 months through sand, was extensively reduced to nearly undetectable levels of estrogen (9±3 pmol/L) and testosterone (5±2 pmol/L) and was not different from that found in well water (10±4 and 3±1 pmol/L respectively). Estrogen was present in lake water at 0.08-0.09 nmol/L and in the drinking water (same as used for irrigation) obtained from the lake at 0.05-0.08 nmol/L (Fig. 1) during a period of extreme drought. The testosterone concentration in the lake at this time ranged from 0.031 to 0.069 nmol/L.

Table 1. Effect of treatment in a modern sewage disposal plant on estrogen concentrations in the water.

Treatment	Estrogen pmol/L	Testosterone pmol/L
Raw Sewage		
July 1991	176±11	760±17
Sept. 1991	518±48	1111±52
Sept. 1992	200±15	66±10
Digestion Tank		
1. reaction fluid		
July 1991	143±11	409±34
Sept. 1992	26± 6	33± 8
2. activated sludge		
July 1991	234±14	600±41
Sept. 1992	69±10	35± 8
Effluent		
July 1991	140±11	173±10
Sept. 1991	184±11	451±38
Sept. 1992	24± 6	25± 8

Raw sewage was tested at the beginning and end of the summer of 1991 during which there was no precipitation and there were severe restrictions on water usage. Samples were tested before, during and after aerobic and anaerobic digestion. Data are presented as means±SEM, n=5.

Estrogen therefore appears to be a persistent compound in the environment. It is present both in lake water and sewage water used for irrigation in concentrations we have shown to affect the growth of plants (0.02-2 nmol/L). This is in agreement with earlier reports that the common bacteria present in soil or feces cannot destroy estrogen (Zondek and Sulman 1943; Gregers-Hansen 1964). Indeed, we have shown that in chicken manure even after 6 mon of silaging, there is no decrease either in the concentration of testosterone or estrogen (Shore and Shemesh 1992). There is no evidence in the literature that steroidal estrogens present in water can affect animals. However, since

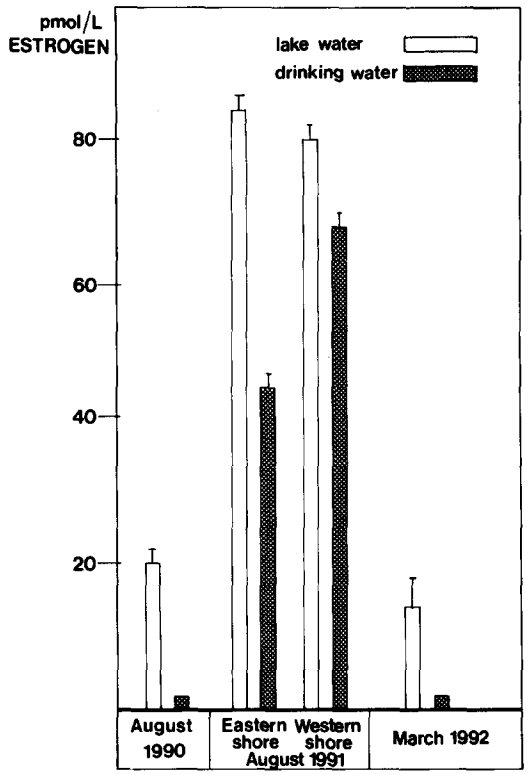

Figure 1. Measurement of estrogen levels in Lake Kinneret. Samples were taken over the years 1990-1992. 1991 was the third year of a severe drought and the Kinneret was at its lowest historical level. Following record breaking rains in the winter of 1991-1992, the lake was full in March 1992. The bars represent the mean±SEM, n=5. Columns without bars were below the limit of detectability.

Table 2. Estrogen concentrations in treated sewage water used for irrigation.

Source	Time of year	Estrogen pmol/L
Farm	summer	1260±38
Farm	winter	562±22
Municipal effluent	summer	427±17
Municipal effluent	winter	145±14

Samples were taken following sedimentation from small sewage treatment units on farms and municipal effluents during various times of the year (1990). (Precipitation in Israel occurs only from September to April). Data are expressed as mean±SEM of 5 different sampling sites; each determination was done in triplicate.

milk from pregnant cows, which contains about 4 nmol/L estrogen (Erb et al 1977; Koldovsky and Thornburg 1987) is ingested by large populations of humans without noted effect it is unlikely that 0.08 nmol/L present in drinking water would have a readily detectable effect on humans.

Testosterone was also present in the samples of sewage water as well as in lake water. The significance of this observation is not known as there is no literature on such low concentrations of testosterone affecting either plants or animals. It is not known how testosterone leaves the environment; however, it does not appear to be similar to that of estrogen. Specifically, preliminary experiments in vitro with labelled steroids added to 1 gm of heavy soil from a field irrigated several months with sewage water indicated that testosterone is readily washed out by aqueous solutions (98±1.4%; n=5) while 56±2% of the estradiol and 59±2% of estrone was strongly bound by the soil and only extractable by organic solvents. This suggests that the phenolic group of estrogen may be involved since phenolic groups would increase the binding to organic matter in the soil.

The source of the estrogen and testosterone found in lake water is not known. However, considering the comparatively high concentrations found in sewage water, pollution by sewage water would appear to be the most logical source. However, the contribution of fish can not be ignored. Preliminary experiments with an aquarium containing 15 tropical fish (water sampled every wk) indicated that after 4 wks of exposure the level of testosterone reached 10 pmol/L and remained constant at this level for six mon.

As stated in the introduction, the purpose of the paper was to determine if estrogen in concentrations we have observed to affect alfalfa growth is widespread in the environment. Since such concentrations were found in a wide variety of water sources used for irrigation, it can be concluded that this is the case. However this type of analysis of hormones in water sources may also be useful to (1) determine the source and level of pollution in drinking water and (2) give some idea of the hormone status of fish in ponds.

Acknowledgments. This work was funded by grant no. 3772 from the Israeli Ministry of Science and Technology.

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Received July 1, 1992; accepted March 1, 1993.