Chemical and Bioassay Analysis of Water and Soil Extracts from South Texas

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Abstract

There has been concern regarding a cluster of birth defects in Southwest Texas and the possible role of dietary and/or environmental factors. An extensive water and soil sampling program has been initiated in this area and extracts are being investigated by chemical analysis and bioassays. Initial studies showed that water extracts contained minimal levels of various chemicals as determined in both assays. In contrast, soil extract R188 significantly induced proliferation of MCF-7 human breast cancer cells at a 1/5 dilution of the original extract and this same sample also induced ethoxyresorufin *O*-deethylase (EROD) activity in rat hepatoma H4IIE cells. The induction response observed for this sample (30.4 ± 5.3 pmol/min/mg) was less than that observed for 2 x 10^{-9} M 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; maximal-inducing concentration). The chemical composition of this soil extract was complex; however, the results obtained in the bioassays are consistent with identification of several polynuclear aromatic hydrocarbons (AhR agonists) as well as isomeric nonylphenols (1.2 to 1.9 ppm) which have been previously identified as xenoestrogens.

Introduction

Neural tube defects (NTDs), specifically anencephaly and spina bifida, are common congenital malformations that contribute substantially to childhood morbidity and infant and fetal mortality (Waitzman et al., 1994). The birth prevalence of NTDs varies from approximately 0.7 per 1,000 births in most areas of the United States to 3.5 per 1,000 in Mexico (Elwood et al., 1992; Shaw et al., 1994). In the spring of 1991, the staff at the Valley Regional Medical Center, in Brownsville, Texas, was alarmed by the appearance of 6 anencephalic births in a 6 week period. Based upon the Centers for Disease Control (CDC) Atlanta Study (CDC, 1988), fewer than 2 anencephalic births out of the 6,000 recorded annually were expected in one year for all of Cameron County. Brownsville, the largest city in Cameron County, is located at the extreme southern tip of Texas. The geography of the area is that of a flat coastal plain, with little significant variation in elevation. Like much of the Lower Rio Grande Valley, its economy depends primarily on agriculture and there is extensive use of a wide variety of agricultural chemicals. Opposite Brownsville on the Mexican side of the border is the city of Matamoros. Matamoros is a highly industrialized city with numerous Maquiladoras (foreign-owned companies) located in and around the city. Maquiladoras are common along the entire border of Texas and Mexico. The Maquiladoras produce a wide variety of materials ranging from electronics to chemicals and pesticides (BEDC, 1990). Many of the Maquiladoras along the Rio Grande River discharge untreated industrial waste directly into the river.

Research in our laboratories has been investigating the possible role environmental and/or dietary factors may have had in this observed cluster. A case-control study of NTDs in three South Texas counties (Cameron, Hidalgo and Nueces) with predominantly Hispanic populations (74%) has been initiated and a detailed environmental sampling of drinking water, surface water, and soil is now in progress. To assess folate in diet, the study utilizes a nutritional questionnaire (in Spanish and English), specifically tailored to the Mexican-American diet found in South Texas. Additionally, sampling of sediments from surface water was included at the time of water sampling.

Materials and Methods

Environmental Exposure Assessment

As part of this ongoing study, a detailed evaluation was conducted of water, soil and sediments in the three study counties. Utilizing 7.5 minute United States Geological Survey Quadrangle maps, counties were divided into approximately 100 km^2 quadrants. Each quadrant had at least one sampling location and only quadrants which had residential areas were included in the sampling. Samples have been taken from all major surface water sites in the three counties. Multiple locations were sampled along the Rio Grande to allow for consideration of differences due to input of material into the river. Two annual samples were taken from all locations and quarterly samples from a subset.

These samples were collected in glass containers that had been washed and rinsed a minimum of three times in deionized water to minimize contamination of the sample with organic compounds. Samples were stored in a -70°C freezer (this is necessary to minimize degradation of the sample). Field blanks, spiked samples, and a minimum of two analytic runs per sample were performed. Samples were prepared by liquid and solid phase extraction for GC or GCMS analysis using procedures based on published EPA guidelines and detailed analytical procedures will be published elsewhere.

Cell Culture and EROD Assay

Rat hepatoma H4IIE cells were grown as a continuous cell line in a minimum essential media (Sigma, St. Louis, MO) supplemented with 2.2 g/L sodium bicarbonate, 10% fetal bovine serum, and 10 ml/L antibiotic/antimycotic solution. Stock culture cells were grown in 150 cm^2 plates at 37°C in a humidified air/carbon dioxide (95/5%) atmosphere. Cells were seeded into 48 well plates at a density of 80,000 cells per well in 0.5 ml media. After 24 hr, plates were treated with 1 µl per well of the environmental extracts in DMSO (1 µl) or DMSO alone. Treatments were run in triplicate and at least three replicates per treatment group were analyzed. EROD activity and protein concentrations were determined on a CytoFluor 2350 plate reader. Plates were read at 530 nm/590 nm for resorufin production and 400 nm/460 nm for fluorescamine protein determination.

Cell Culture

MCF-7 cells were obtained from the American Type Culture Collection (ATCC) and grown in MEM supplemented with 10% fetal bovine serum plus $NaHCO_3$ (2.2 g/L), gentamycin

(2.5 mg/L), penicillin/streptomycin (10,000 units/L and 10 mg/L), amphotericin B (1.25 mg). Cells were maintained in 150 cm^2 tissue culture flasks/plates and incubated at 37°C in a humidified mixture of 5% CO_2 and 95% air under atmospheric pressure. Cells were seeded at 7.5 x 10^4 cells/well in 6-well plates in media containing 2 ml DME/F12 without phenol red, supplemented with 5% FBS treated with dextran-coated charcoal (FBS-DCC). After 24 hr, the media was changed to serum free and cells were treated with E2, or the environmental extracts in DMSO or DMSO alone (control). The medium was changed and cells were redosed every 48 hr. The cells were harvested and counted using a Coulter Z1 cell counter. All determinations were carried out in triplicate, and results are expressed as means ± SD.

Results and Discussion

Five field samples from the Rio Grande Valley were analyzed by GC-MS. The three water samples contained relative low ppb levels of several compounds; however, only phthalates were consistently detected in these extracts (4 to 23 ppb). The samples were also analyzed as inducers of EROD activity in rat hepatoma H4IIE cells [sensitive for aryl hydrocarbon receptor (AhR) agonists] and as mitogens in the MCF-7 human breast cancer cell proliferation assay for estrogenic compounds. No significant activity was observed for any of the water extracts. In contrast, soil extract R188 significantly induced proliferation of MCF-7 cells at a 1/5 dilution of the original extract (Fig. 1) and this same sample also induced EROD activity in H4IIE cells. The induction response observed for this sample (30.4 ± pmol/min/mg) was less than that observed for 2 x 10^{-9} TCDD (maximal-inducing concentration). The chemical composition of this soil extract was complex; however, the results obtained in the bioassays are consistent with identification of several polynuclear aromatic hydrocarbons (AhR agonists) as well as isomeric nonylphenols (1.2 to 1.9 ppm) which have been previously identified as xenoestrogens.

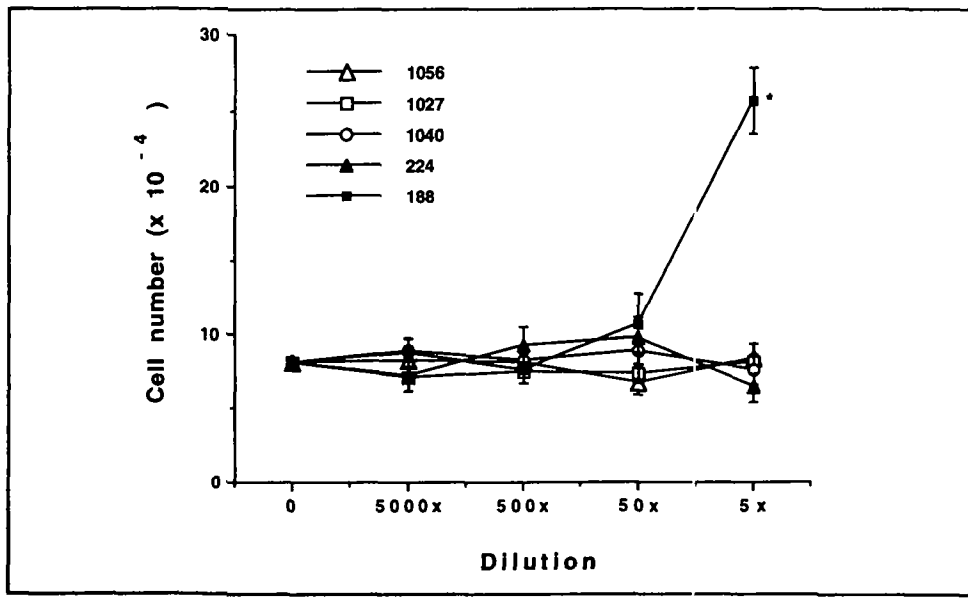

Figure 1. Induction of MCF-7 cell proliferation by water and soil extracts from Southwest Texas (*significant induction, $p < 0.05$).

Current ongoing studies are focused on determining the distribution, concentration and biological activities of soil and water extracts from diverse locations in South Texas.

Acknowledgements

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References

Brownsville Economic Development Council. *Brownsville Texas Data Book,* October, 1990.

Center for Disease Control. *Congenital Malformations Surveillance.* Report Jan. 1982 - Dec. 1985. 1988.

Elwood, J.M., Little, J. and Elwood, J.H. Epidemiology and control of neural tube defects. Oxford University Press, Oxford, 1992.

Shaw, G.M., Jensvold, N.G., Wasserman, C.R. and Lammer, E.J. Epidemiologic characteristics of phenotypically distinct neural tube defects among 0.7 million California births, 1983-87. *Teratology* 49:143-149, 1994.

Waitzman, N.J., Romano, P.S. and Scheffler, R.M. Estimates of the economic costs of birth defects. *Inquiry* 31:188-205, 1994.