SCREENING OF ENVIRONMENTAL SAMPLES FOR AN ESTROGENIC POLLUTANT: DDT

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ABSTRACT

Soil samples collected from three farm communities in southeast Missouri were analyzed for the presence of dichlorodiphenyltrichloroethane (DDT) and its metabolites, using commercially available DDT in a soil test kit. The soil test kit is based on the use of polyclonal antibodies that bind either DDT or a DDT-enzyme conjugate. The same numbers of antibodies are immobilized to the walls of the test tubes. When DDT is present in samples, it competes with the DDT-enzyme conjugate for a limited number of antibody-binding sites. The presence of DDT is determined by a colorimetric reaction in the test tubes that results in the formation of a blue solution. Based on the binding of the DDT molecules, a low concentration of DDT produces a dark blue solution, and conversely, a high concentration of DDT allows fewer DDT-enzyme conjugate molecules to be bound to the antibodies, resulting in a lighter blue solution. Methanol extracts of 11 soil samples were tested. Nine of the samples showed a level of 0.2 ppm or greater of p'-DDT. Only two samples had levels below 0.2 ppm.

Key words: *dichlorodiphenyltrichloroethane, estrogens, polyclonal antibodies, biomagnification*

INTRODUCTION AND BACKGROUND

The world around us has become contaminated with many synthetic chemicals that are believed to affect the activity of sex hormones such as estrogen. At least 45 environmental contaminants, including DDT (Figure 1), that have chemical structures similar to estrogen, have been reported to cause changes in the reproductive systems of animals. It is postulated that estrogenic pollutants can replace natural estrogen (Figure 2) on cell receptors, which may result in a variety of abnormal responses such as the feminizing of male alligators and deformities in frogs.

DDT was one of the most extensively used pesticides in the United States prior to the 1970's. It was widely used on crops and in communities around the world for controlling disease-carrying insects such as mosquitos. When populations of ospreys, cormorants, and bald eagles declined, research revealed that degradation products of DDT accumulating in the bodies of the affected birds were the cause. Subsequently, DDT usage in the United States was banned in 1972 (Enger et al., 1998). The complex structure of DDT makes it very persistent, and small quantities can remain in the environment for a long time. The chemical can enter the food chain through crops and fish and may be ultimately transferred to humans. Studies of selected populations exposed to DDT revealed the presence of DDT metabolites in body fat, urine, semen, breast milk, and blood (Lu,1985). DDT has a half-life in the body between 2-15 years. Additionally, research data has shown a relationship between DDT and breast cancer. DDT is listed in the National Toxicology Program's Fifth Annual Report on Carcinogens as a "*substance which may reasonably be anticipated to be carcinogenic"*

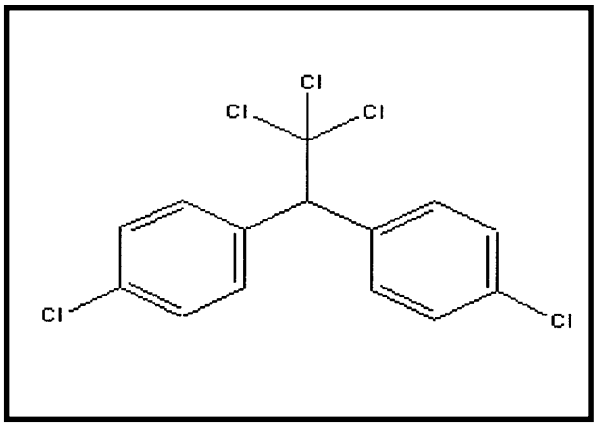

Figure 1. Chemical structure of DDT.

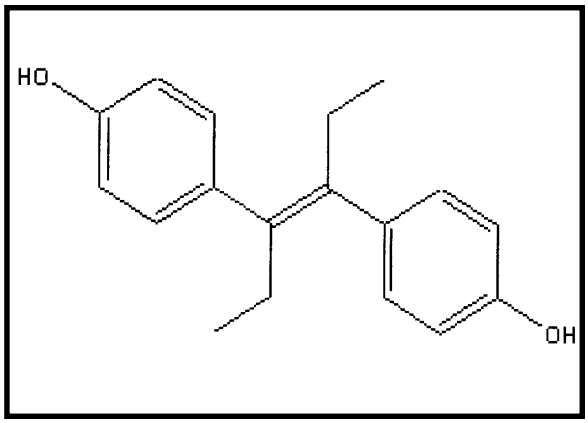

Figure 2. Chemical structure of estrogen.

and the Environmental Protection Agency categorizes DDT and its metabolites as probable human carcinogens (Environmental Health Center, 2000).

It has been recognized by some nations that DDT contamination is a global problem that requires a global solution. Global attention is needed because usage of DDT in some developing nations continues. Studies that reveal the continued presence of various quantities of DDT in the environment are relevant and significant in light of known adverse environmental and health impacts.

For this study, methanol extracts of soil were analyzed according to procedures of the Enviro-Gard ™ DDT in Soil Test Kit. This semi-quantitative enzyme immunoassay allowed rapid and reliable screening for DDT at concentrations as low as 0.2 parts per million. The procedures in the kit are based on EPA method 4042. With this kit, samples can be screened with a 95% confidence of no false negatives at specified action levels.

MATERIALS AND METHODS

Soil collection

Soil samples were collected from 11 homes sites located in New Madrid County, Missouri. A 12-inch stainless steel corer was used to collect each sample, which was taken from the A-horizon of the soil profile. The 12-inch core of soil was sliced into three, 4- inch sections. Each section was placed in a labeled plastic bag and stored on ice until transported back to the laboratory. The weight of each collected sample was recorded before analysis. The samples that were analyzed for DDT were taken from the top portion of the core to a depth of four inches.

Chemicals

The extracting solvent was laboratory-grade methanol purchased from the Sigma Corporation, St. Louis, Missouri. The **EnviroGard™** DDT in Soil Test Kit was purchased from Strategic Diagnostics.

Extraction of DDT

The extraction procedure was a modification of procedures recommended in the Strategic Diagnostics Inc., EnviroGard™ DDT in Soil

Test Kit. A 1:4 ratio (5 g of soil with 20 ml of methanol) was mixed and placed in screw-capped 50-mL Erlenmeyer flasks. The flasks containing the soil-solvent mixture were allowed to shake overnight (12 hours) on an Eberbach shaker. After overnight shaking, the soil slurry was centrifuged at 8,000 rpm for 15 minutes at 4°C. The solvent supernatant was carefully decanted into sterile, labeled test tubes under an exhaust hood.

The EnviroGard™ DDT in soil test kit (EPA Method 44042)

Before use, all components were stored at 4°C. On the day of analysis, the test kit components were allowed to come to an ambient temperature of 26°C before proceeding with the test. The test components consist of the following: 20 12 x 75mm antibody-coated test tubes; one vial of assay diluent; one vial as a negative control (methanol); one calibrator vial containing 0.2 ppm DDT in methanol; one 1.0 ppm DDT in methanol calibrator vial; one of 10.0 ppm DDT in methanol calibrator vial; one DDT-enzyme conjugate vial; one vial of substrate; and a 20-place test tube rack. The solvent extracts were analyzed according to test kit procedures.

RESULTS AND DISCUSSION

DDT determination

The uniqueness of the EnviroGard™ DDT in Soil Test Kit is that it is based on the use of polyclonal antibodies that bind to either DDT or *DDT-enzyme conjugate.* All coated test tubes have the same number of antibody-binding sites and receive a matching number of *DDT-enzyme conjugate* molecules. When the *DDT-enzyme conjugate* is added to the antibody-coated test tubes, it competes with DDT in the sample for antibody-binding sites. The color reaction that occurs when the substrate molecules are added to the test tubes allows for visual comparisons. Samples that contain very low concentrations of DDT bind to very few antibodies in the test tube, so large numbers of the prepared *DDT-enzyme conjugate* molecules bind with the antibodies and cause the development of a dark blue solution. Samples with high concentrations of DDT bind with more antibodies so fewer molecules of the *DDT-enzyme conjugate* bind to the antibodies and the solution has a lighter blue color. After the *stop solution* is added, photometric readings can be made using a spectrophotometer. The calibration curve, plotted from the prepared DDT calibrators, is used to extrapolate the concentrations of the samples.

Occurrence of residues

The amounts of DDT residues in the form of DDE for each sample are recorded in Table 1. Data indicates the presence of DDT in all soil samples collected from the three southeast Missouri communities. Three out of the eleven samples collected contained concentrations of DDT greater than 10 ppm. Two out of eleven samples contained concentrations of DDT lower than 1ppm. The site with the highest concentration was located in North Lilbourn, Missouri, with a concentration of 16.8 ppm.

Possible health impact

The discovery of any quantity of DDT in the environment is of concern. DDT can enter the body mainly through consumption of con-

taminated foods, and by inhalation of contaminated air. Since the body's natural hormonal balance is necessary to maintain normal body activities, estrogenic contaminants that enter the body may affect that balance. Estrogen and other hormones cause their effects by binding to receptor molecules in tissues of the breast, uterus, brain, and testis. The action of DDT is known to mimic the female sex hormone by triggering or blocking a response to the body's natural hormones. The mimicking effects of DDT disrupt normal estrogen metabolism in the body, which can probably cause premature breast development in young girls; infertility in men and women; and other diseases of the nervous system, liver, and blood.

Table 1. Concentrations of DDT and/or its metabolites in methanol extracts of soil samples collected from three southeast Missouri farm communities.

Sample	DDT and/or Metabolites - DDE and DDD *(parts per million/5 grams of soil)*
S1	0.4
S2	8.0
S3	7.2
S4	6.0
S5	16.8
S6	6.4
S7	6.8
S8	9.0
S9	12.8
S10	2.0
S11	0.4

Ecological effects

The problem with DDT is a that it is a chemical that can be biomagnified. Synthetic estrogens are insoluble in water, are soluble in fat, and are slowly biodegraded by natural processes. This means that they become more concentrated in the fatty tissue of an organism at higher trophic levels in the food chain. As noted in the introduction, research has shown that populations of predatory birds such as falcons, hawks, and eagles have declined because DDT accumulated in their bodies and altered normal reproductive processes.

CONCLUSION

Since the 1940's, four billion pounds of DDT have been used worldwide and 80% of that has been used in agriculture (Fransis and Magnus, 1994). Our data indicates that DDT was broadly used in the three southeast Missouri farm communities we studied. Because DDT is very persistent in the environment, having a half-life between 2-15 years, we were able to detect DDT in the collected soil samples. We can also reasonably conclude that DDT may be present in water and wildlife in the area. Future studies will be made to confirm this speculation. The data from this study will be shared with the communities in hopes that it will help them develop appropriate strategies to protect human health and improve the quality of their environment.

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