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Kathryn Barr April 10, 2023

The Effect of Farming Techniques on Degradation of DDT in Historical Cotton Farms

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a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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p,p'-Dichlorodiphenyltrichloroethane (DDT) was a popular pesticide in the mid 20th century for commercial crop and livestock production. DDT remains in the environment for decades as either the original compound or its dechlorinated environmental metabolites p,p'-dichlorodiphenyldichloroethylene (DDE) and p,p'-dichlorodiphenyldichloroethane (DDD) which can bioaccumulate and translocate. Many studies have correlated these compounds (collectively referred to as DDX) with human health and environmental effects. DDX are xenobiotics that can disturb endocrine function resulting in infertility, premature births, delayed sexual development, and other hormone-mediated effects in wildlife. Several studies have reported that the rate of degradation of DDT into its metabolites is affected by various farming techniques like tillage, irrigation, and fertilizers. Georgia consistently has been one of the major farming states, specifically a large producer of cotton since the 19th century, thus is likely to have high levels of DDX in the soil of historical farms. Therefore, in this study, we aimed to determine if different farming techniques affect the decomposition of DDT in Walton County, Georgia, where farms historically grew cotton. In this study, several Walton County farms were sampled for soil, and churches were sampled as control sites. The extensive land history of the farms was recorded, and the soil levels of p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, and o,p'-DDE were measured using gas chromatography-tandem mass spectrometry. The farm sites had detectable levels of p,p'-DDT, p,p'-DDE, and p,p'-DDD, while few sites had detectable levels of o,p'-DDT and o,p'-DDE. Control church sites had detectable levels of p,p'-DDT, p,p'-DDE, and p,p'-DDD, but lower levels of each analyte were detected compared to the farm sites. Tillage was found to speed up p,p'-DDE degradation, but there was no effect on p,p'-DDT degradation. Plowing caused an increased fraction of decomposition, but no metabolite was significantly increased. The largest difference in the degradation of DDT was based on the fertilizer type. Natural fertilizer sped up degradation of p,p'-DDT and p,p'-DDE; synthetic fertilizer increased p,p'-DDE degradation, but not p,p'-DDT degradation. Farmers could potentially use some of these farming practices to encourage further degradation of DDX to reduce the contamination in their soil.

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Introduction

History of DDT

1,1,1- trichloro-2,2-bis(p-chlorophenyl)ethane (DDT), the first modern pesticide to be widely distributed, was first synthesized by an Austrian student, Othmar Zeidler in 1874.[1] In 1939, Swiss chemist Paul Müller discovered the insecticidal properties of DDT, and in 1948, he won a Nobel Prize for this invention.[1] DDT is a powerful pesticide that was historically used to combat malaria by killing mosquitos and helped eliminate malaria in Europe, the United States, and Australia.[1] Quickly, DDT became commonplace in agriculture and households to control boll weevils in cotton crops, houseflies, and other pests in the home.[1] However, there were multiple environmental concerns about DDT. In 1962 Rachel Carson wrote The Silent Spring where she detailed the negative impacts of the powerful insecticide, such as the thinning of bird eggshells and its hypothesized role as a carcinogen. Carson's novel revolutionized environmental policy, and it led to the ban of DDT in the United States in 1972 and worldwide in 2004 at the Stockholm Convention, with exceptions for areas with high malaria rates.[1] While DDT has been banned for over 50 years, the half-life of DDT in soil can be as long as 15 years. Therefore, areas with heavy historical use of DDT can have residual levels that may result in current negative health and environmental impacts.

The use of synthetic pesticides has increased yearly since gaining popularity in both agricultural and public health applications after World War II. DDT was one of the first post-war era insecticides, becoming widely used by the end of the 1940s.[2] After its creation, DDT was used to control vector-borne diseases like malaria, typhus, and dengue.[2] Furthermore, it was a

popular pesticide for commercial crop and livestock production and for personal use in homes and gardens.[2]

Mode of Action

Like most insecticides, DDT is neurotoxic by design, acting to modulate sodium channel voltages at the neuronal axon, resulting in acute toxicity in insects and other non-target species.[3] DDT causes insects to exhibit hyperactivity and convulsions because of overstimulation of neurons and ultimately causes death.[3] DDT is easily absorbed through the insect's exoskeleton, and it is lodged in the pores of the nerve membranes.[4] The altered membranes allow sodium ions to leak so the cell cannot control impulse transmissions.[4] However, in mammals, DDT is far less toxic because it does not absorb into the skin.[5] Instead, the main pathway exposure and absorption of DDT into mammals is by ingesting plants with DDT residue or eating animals (or their dairy products) with DDT in the fatty tissue.[5] DDT is fat soluble, so when it is ingested, it is readily stored in adipose tissue and remains in equilibrium with all biological lipid-rich matrices (e.g., serum, brain tissue, adipose).[5] Furthermore, the body has no mechanism to remove DDT in the body (except breastfeeding in lactating females), so as an animal continues to consume DDT, it accumulates higher levels of the pesticide.[6] This phenomenon leads to bioaccumulation, where species higher in the food chain have increased levels of DDT because they absorb the biomagnified DDT in their prey.[6] Therefore, many species experience chronic toxicity of DDT through the accumulation of it in their body.

Human health and environmental impacts

In 1972, the newly formed US Environmental Protection Agency (EPA) banned the use of DDT due to its potential environmental and human health risks resulting from its biological and environmental persistence.[2] Many studies have correlated DDT with human health and environmental effects. DDT is a xenobiotic that can disturb endocrine function.[7] In a previous study, various known xenobiotic pesticides were introduced to rabbit uteri to determine if the pesticides inhibit binding of steroids to the estrogen and androgen receptors.[7] DDT decreased the binding of the steroid to the estrogen receptors by 60-75%.[7] Furthermore, for the androgen receptors, DDT and DDE were the strongest inhibitors, and they prevented the binding of the steroid by 80-100%.[7] Therefore, this study concluded that DDT and DDE were the most potent endocrine disrupting xenobiotic, out of the various pesticides tested.[7]

Since DDT and DDE are strong endocrine disrupting compounds (EDCs), human exposure to them have been associated with lowered sperm counts,[7,8] premature births,[9,10] and spontaneous abortions.[11,12] In 1949, DDT crop dusters were found to have reduced sperm counts, and those who worked at a pesticide manufacturing company also had low sperm counts and became impotent.[13] In addition, in a study of 44,000 pregnant women from 11 different university hospitals between 1959-1966, serum DDT and DDE levels were associated with a significant increase in preterm births and small-for-gestational-age infants.[10] Moreover, a few studies have linked higher levels of DDT in serum to an increased probability of spontaneous abortion.[11,12]

In addition, DDT exposure has been linked to cancer of the prostate, uterus, and breasts.[14] The most researched correlation between cancer and DDT exposure is for breast

cancer. A meta-analysis of four studies determined that increased exposure to DDT is correlated with a higher probability of breast cancer.[15] One of the studies analyzed found that there was a 50% increase in DDT levels in the 20 patients that were diagnosed with breast cancer in comparison to the 20 control patients[16], and similar results were found in another study.[17] Moreover, many studies have concluded that DDT exposure can cause prostate cancer. Specifically, in the large Agricultural Health Study that included 52,000 male pesticide applicators in Iowa and North Carolina found that more frequent use of chlorinated pesticides over a lifetime was correlated with a higher probability of prostate cancer.[18]

Other cancers have also been correlated with DDT exposure. A cohort study of 5,000 workers at DDT manufacturing plants determined that increased DDT exposure led to a risk of pancreatic cancer that was 7.4 times higher than normal.[19] In addition, another study found that those who sprayed DDT or worked in its manufacture had an increased risk of lung cancer.[20]

Many studies have demonstrated that estrogen and other sex hormones are vital in the immune response. A few studies have found that DDT is associated with a decreased white blood cell count[21] and a hypersensitive type 2 helper cell (Th2) cytokine response.[22]

One study determined the correlation between a mother's DDT exposure and the white blood cell levels in their child.[21] A Japanese study quantified the DDT and DDE levels in the breast milk of 108 mothers, and they measured the T cell levels in the blood of their infants.[21] They concluded that the higher levels of DDT in breast milk led to a lower number of T cells in the infants.[21] Furthermore, a South African study found that serum DDT levels were associated

with higher levels of inflammation biomarkers, resulting in a hypersensitive Th2 cytokine response in those individuals.[21]

However, DDT does not only affect human health; it also has negative effects on wildlife. Often DDT has a larger health effect on animals due to their smaller weights and shorter developmental times.[11] DDT can cause thinning of eggshells,[11,23] reduce animal fertilization, and delay sexual development of wildlife which reduces their populations.[11] In 1992, a study of the fish eagle in Zimbabwe collected 20 egg clusters from nests around Lake Kariba and found measurable concentrations of DDT in all the eggs.[24] Furthermore, higher levels of DDT in the shell corresponded to a significant decrease in the shell thickness.[24] The thin shells can be easily broken during the incubation time, which leads to the death of the underdeveloped bird.[24]

Fate of DDT

DDT can be deposited in the environment in two different ways. First, it can be directly applied over a specified area or it can volatilize into the atmosphere and deposit onto an area that was never directly sprayed with DDT.[25] While there are no conclusions on the temperature that volatilization occurs, DDT can be volatilized as low as 15°C.[26] An estimated 54-72% of DDT evaporates from the initial application and travels throughout the air to colder climates due to the change in density, a phenomenon known as the "grasshopper effect."[25] Ambient temperatures in warmer climates allow DDT to evaporate from the soil or crop it was sprayed on then rises with the warm air and is carried by the wind patterns.[27] The DDT is, then, deposited as the air cools and sinks or as rain carries it back to the surface.[27] Since DDT does

not degrade quickly, it remains as the original compound as it travels and is redeposited in the environment. DDT can undergo multiple cycles of translocation, so it can be found far away from the original source and is often found at higher concentrations near the Arctic basin.[27] Since persistent organic pollutants (POPs) such as DDT predominantly sequester in organic parts of soil once it is redeposited, areas that never received a direct application of DDT will likely still have detectable concentrations of the pesticide because of drifting and translocation.[28,29]

Also, DDT remains in the environment for decades as either the original compound or its dechlorinated environmental metabolites 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethene (DDE) and 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (DDD). DDT, DDE, and DDD are highly ecotoxic because of the chlorine atoms disruption of biological pathways, the low solubility of the molecules, and their lipophilic nature.[30] Various types of bacteria can convert DDT into its metabolites, and many of them thrive in anaerobic conditions.[31] The efficiency and speed of decomposition of DDX are dependent on the type and abundance of bacteria.[32] Many of the bacteria that degrade DDT flourish in alkaline environments, so soils in the neutral to the slightly alkaline range have increased decomposition rates of DDT because there are higher populations of bacteria that can quickly degrade it.[32]

The mechanism of degradation of DDT into DDE and DDD is not fully known. Various types of bacteria, fungi, and animals process DDT in different ways, so there is no singular pathway for degradation. In one proposed pathway for Gloeophyllum trabeum, a brown-rot fungi, DDT dechlorinates to create DDE.[33] Then, DDE undergoes hydrogenation to become DDD.[33] The reaction continues as oxidative dechlorination of DDD yields 4,4-dichlorobenzhydrol

(DBH).[33] However, in Daedalea dickinsii, another brown-rot fungus, the DDD becomes 2,4-dichloro-1-[2-chloro-1-(4-chlorophenyl)ethenyl]benzene (DDMU) from another mechanism of dechlorination.[33] In Fomitopsis pinicola, the fungus is unable to further transform, so the final decomposition product is DDD (Figure 1).[33]

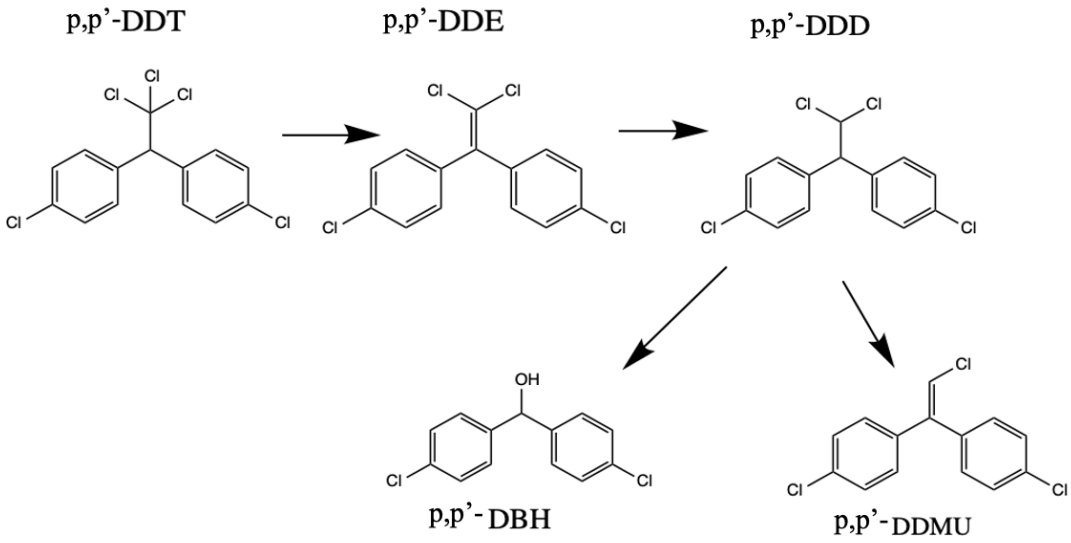

Figure 2: Proposed Pathways of Decomposition of p,p'-DDT

DDT has two possible structural isomers, p,p'-DDT and o,p'-DDT. Each isomer has isomer-specific degradates because the isomers cannot interconvert. Therefore, p,p'-DDT degrades into p,p'-DDE and p,p'-DDD, while o,p'-DDT degrades into o,p'-DDE and o,p'-DDD. The World Health Organization (WHO) specified that technical grade DDT must contain 70% p,p'-DDT.[34] The remaining 30% contains p,p'-DDE, p,p'-DDD, o,p'-DDT, and o,p'-DDE.[34] Therefore, the major pathway of DDT degradation is from p,p'-DDT.

Factors of DDT Degradation

In addition to bacteria, earthworms can affect the degradation of DDT. A study conducted at South China Agricultural University in Guangzhou, China, measured levels of DDX in previously contaminated soil with and without earthworms.[35] The drilosphere matrices, the gut and burrow lining of the earthworms, specifically sped up the decomposition of DDT.[35] The plots with earthworms had a DDT half-life of 8 weeks, while the half-life in the other plots was over 14 weeks.[35] The uptake of the DDT by the earthworms was not a major cause of the decrease, as the earthworms' tissues only contained about 0.03% of the DDT that was in the soil.[35] In the gut by reductive chlorination, the major DDX compound found was DDE, while in the burrow linings, the major compound was DDD and DDMU by oxidative degradation.[35]

The rate of decomposition of DDT into DDE and DDD can be affected by the environment. For instance, the climate can speed up or slow down the degradation of DDT. Warmer temperatures[36] and a wetter environment[37] will speed up the degradation of DDT. In a study, contaminated pans of soil with identical concentrations of DDT were analyzed for their degradation. The soil was placed in areas with varying temperatures and water levels.[36] After 140 days, the DDE degradate found in the soil that was in a 30˚C area was only 0.4% of the original DDT concentration.[36] However, the DDE in the soil that was in a 60˚C area comprised 17% of the original DDT, as detected by thin layer chromatography.[36] Higher temperatures increase the rate of decomposition of DDT into its metabolites, presumably because the increase in energy allows for the chemical reactions to surpass the activation energy barrier[36]

Furthermore, the study concluded that a wetter environment increases the speed of the decomposition of DDT.[37] While the dry soil at 30˚C degraded 0.4% of the original DDT, the

flooded soil at the same temperature degraded 5.7% of the DDT.[37] Also, the flooded soil at 60˚C degraded 31.6% of the DDT, while the dry soil at that temperature had only 17% of the DDT degraded.[37] Two hypothesized reasons may explain why a wetter climate decreases levels of DDT in soil. First, the input of precipitation can allow for the leaching of DDT into the lower levels of the soil, and it can allow for more run-off of DDT into other areas, like lakes and streams.[37] In addition, the wetter climate can create a waterlogged environment, which is anaerobic and allows for certain bacteria to thrive and decompose the DDT.[37]

In addition to the climate and environment, a few studies have studied the effect of various farming techniques on the rate of decomposition of DDT in soil. A New Zealand study used a highly DDT-contaminated field to determine the effects of various agricultural practices on DDT levels.[38] They separated the field into three sites, where the soil was subjected to various agricultural techniques.[38] Continuous irrigation resulted in 37% more degradation than the control area.[38] Another study in California analyzed the levels of DDX in a contaminated field and recorded the agricultural techniques the soil underwent over 40 years.[39] Flooding of the land increased the rate of degradation and volatilization of DDT.[39] The authors hypothesized that this increase was due to the anaerobic environment that allows for certain bacteria to thrive.[39] It is unlikely that the increase in irrigation causes the leaching of DDT into lower sections of the soil because DDT is not water soluble, so it would not readily travel with water.[38] Flooding increases the decomposition of DDT more than other types of irrigation because of the large anaerobic environment it creates.[39] Furthermore, the uptake from plants is not a major pathway to decrease levels of DDT in the irrigated soil because plants do not absorb a significant amount of DDT from the soil.[38]

Likewise, fertilizers and tillage could decrease levels of DDT in agricultural soils. Synthetic phosphate fertilizers[38] and natural manure fertilizers[40] can increase the population of bacteria in the soil compared to no fertilizer use, which increases the degradation of DDT. The addition of synthetic[38] and natural fertilizers[41] is an established bioremediation practice for other pesticides, and it is a plausible pathway of DDT degradation. Additionally, tillage decreases the levels of DDX in the soil.[38] Tillage increases volatilization and photodegradation of DDT because it brings the DDX in the lower levels of the soil in contact with air and light.[38] However, in the New Zealand study, there was no significant loss observed from tillage.[38] In a Californian study, the plowed plots had a larger ratio of DDE:DDT than the unplowed plots.[39] In the plowed areas, 85% of the DDT degraded to DDE, but for the unplowed areas, 78% of the DDT had become DDE.[39] While the study was not statistically significant, the trend suggests that plowing can increase degradation of DDT.

Georgia History

In Georgia, agriculture has been the most prominent industry for centuries. Georgia concentrated on agriculture from its original colonization up to World War II.[42] In the latter half of the 20th century, many parts of the state that were historically farmland rapidly developed into metropolitan areas.[42] According to the 1959 census of agriculture, when DDT was still in use, there were 106,350 farms in Georgia resulting in 19,657,615 acres of farmland.[43] In Walton County specifically, there were 1,137 farms, which had 139,695 acres of farmland combined.[43]

Moreover, in 1959, during the peak DDT application period, there were 1,834,255 acres of cotton in Georgia, which made up 32% of all crop-based farming in Georgia.[43] In Walton

County specifically, there were 13,459 acres of cotton, which was 40% of all the cropland in the county.[43] According to a study of organic contaminants in cotton fields in Georgia and South Carolina, concentrations of DDT, DDE, and DDD were found in almost all the soil samples at varied concentration.[44] They found that the DDX concentrations averaged 11 ng/g dry weight, but the concentrations reached as high as 45 ng/g, with the highest concentrations found in Statesboro, Georgia in Bulloch county.[44]

Historically and currently, cotton farms are one of the crops that use the highest levels of pesticides worldwide. An study in Indian found that 45% of its pesticide use was for cotton farms, even though cotton farms made up only 5% of the total farmland in India.[45] In 2014, 38 million pounds of pesticides were used for cotton farms alone, worldwide.[45] Cotton farms used 16.1% of all the pesticides sold globally.[46]

While much is known about DDT use in rural Georgia in particular, studies evaluating farming practices and their relation to DDT degradation are lacking. This study aimed to quantify levels of DDT, DDE, and DDD in historical cotton farms in Walton County, Georgia and determine the effect of agricultural techniques on the rate of degradation of DDT in soil. Walton County, Georgia was chosen because of its historic high use of DDT and one county was selected to reduce the factor of soil composition and weather patterns on the decomposition rate of DDT. Furthermore, Walton County contained 40% of all croplands in Georgia, and it remains a largely agricultural county, today. In addition, churches were used as control sites as they were known areas without direct DDT application due to the long history of the churches selected. The soil levels of DDT, DDE, and DDD found in church soil were considered as baseline levels.

This project includes several important innovations for understanding the variation of levels of DDT in Georgia. This is the first study that looks at the effects of agricultural practices in Georgia soils. Previous studies that have evaluated DDX levels of southern US soils did not assess the factors that catalyzed the degradation of the pesticide. In addition, previous studies that researched the decomposition of DDT from agricultural practices often used a controlled environment, where the factors were manipulated, but this study utilizes current farms to better illustrate the real-world effects of agriculture. Furthermore, most research on this topic was completed in the mid to late 20th century, with outdated techniques. This study uses modern, optimized, and efficient techniques to quantify DDT in the soil. Overall, this study aims to close multiple literature gaps in our understanding of degradation of DDT in farm soils.

Materials and Methods

Chemicals and Reagents

Both the o,p' and p,p' isomers of DDX were obtained in their unlabeled and $^{13}C_{12}$-ring labelled forms (Figure S1) from Cambridge Isotope Laboratories (Andover, MA). Analytical grade dichloromethane (DCM), n-hexane, and nonane were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Ethyl acetate and methanol were purchased from Fisher Scientific (Waltham, MA). Sodium chloride crystal was purchased from Macron Fine Chemicals (Radnor, PA). Trisodium citrate dihydrate was purchased from Beantown Chemical (Hudson, NH). Disodium hydrogen citrate sesquilhydrate was purchased from Thermo Fisher Scientific (Ward Hill, MA). Magnesium sulfate anhydrous was purchased from Sigma Aldrich (Burlington, MA). Isolute primary secondary amine was purchased from Biotage (Uppsala, Sweden). Water was generated using a Milli-Q Ultrapure water purification system (Millipore, Billerica, MA). The

standard reference material (SRM 1944, organic contaminants in house dust) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). Helium and nitrogen gases were of 99.999 percent ultra-high purity and obtained from nexAir, Inc. (Suwanee, GA).

Study Design and Population

Soil samples (n=33) were taken in Walton County, Georgia, from five sites that were reported as historical cotton farms by the owners of the farms between 1945-1972. A map of the sites is included below in Figure 2. Extensive land use history was recorded from a questionnaire, which asked the crops farmed, livestock presence, irrigation type and frequency, fertilizer type and frequency, pest control type and frequency, tillage frequency, and plowing frequency. The land history was used to determine decision units (DUs) (n=11), areas with different agricultural techniques in each site. Questionnaire data collected information on recent and current farming practices, current and historic crops grown, crop rotation, fertilizer use, pesticide use, tillage frequency, and plowing frequency.

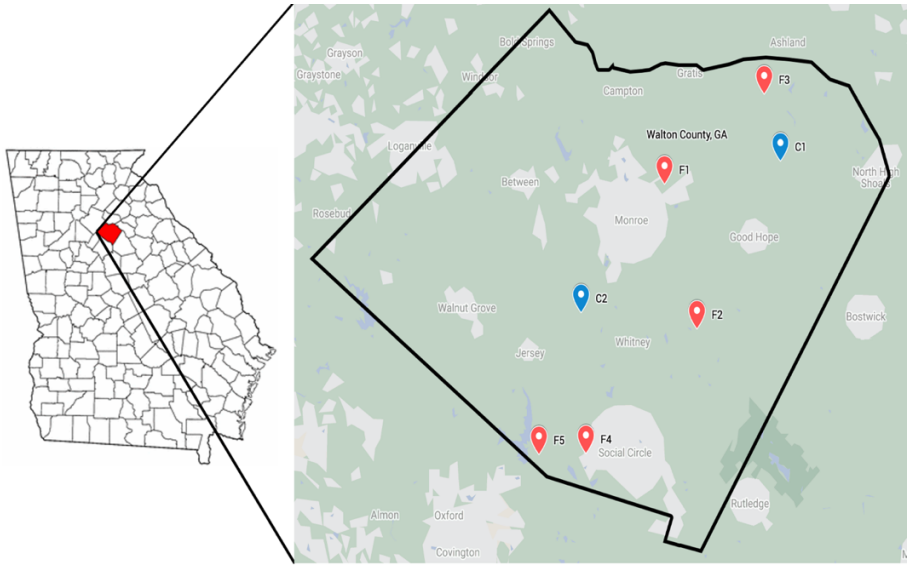

Figure 3: Map of Georgia county borders with Walton County in red (Left) A zoomed in map of Walton County Georgia with the soil sample sites (Right). Black border (Walton County boundaries), Red (Farm sites), and Blue (Control sites).

For control soil samples (n=6), churches (n=2) in Walton County, Georgia were sampled. All samples were taken in Walton County, Georgia, to avoid introducing additional variables, such as soil composition and weather into the analysis. All control sites were verified by owners to have not been directly sprayed with DDT. The control soil samples were used to determine background levels of DDX and never had any DDT application.

Soil Collection

Incremental soil sampling methodology was used.[47] As seen in Figure 3, each site was divided into different DUs based on suspected variation in the DDT levels from differing agricultural techniques. Each DU was divided into 30 equal zones. Instead of a 2-inch-deep sample as detailed in the method, a 4-inch-deep soil sample was taken from each zone with a hand shovel because tillage and plowing can bury the topsoil with the largest DDX concentrations, and irrigation and rainfall can cause leaching of DDX. The samples taken from

each zone were compiled into one composite sample. Two more samples were taken from each square and were put in two more composite samples. Sampling squares were decided by dividing each zone into a grid and using a random number generator to decide where on the grid to sample. At the end of the sampling, there were three composite samples per DU, which corresponded to a certain area of the grid of all zones. The soil pans were dried at ambient temperature and sieved first to 2 mm and then to 150 µm using trace-cleaned brass frame sieves with stainless-steel mesh (Number 10 and Number 100 respectively; Gilson, Lewis Center, OH). The samples were then weighed and put into amber jars to store for analysis and delivered to the Laboratory for Exposure Assessment and Development in Environmental Research (LEADER) at Emory's Rollins School of Public Health for further processing.

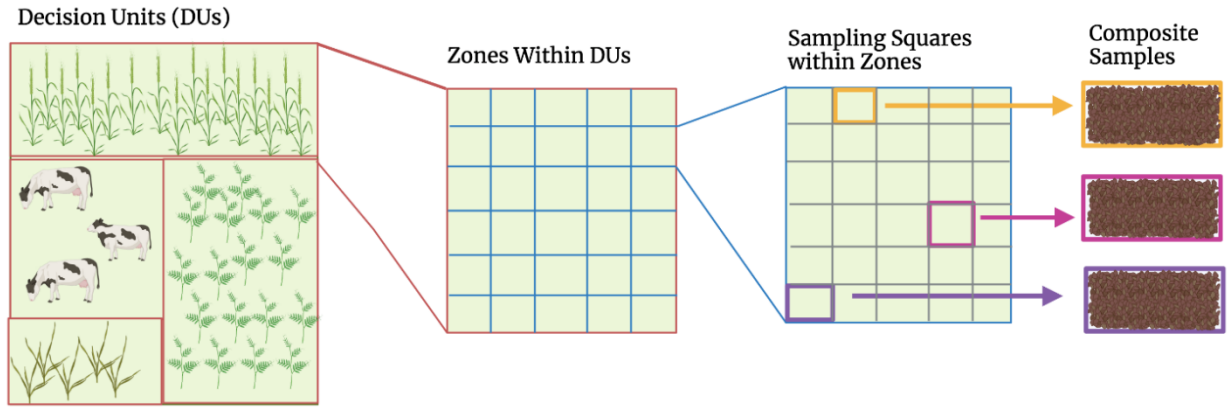

Figure 3: Illustration of the Incremental Soil Sampling Methodology

Farming History Questionnaire

Each farmer completed a farming history questionnaire for each DU (Table S1). The questionnaire prompted the farmer to input the current and previous crops grown in the area; if livestock were present; types and frequency of irrigation used currently and historically; type,

composition, and frequency for fertilizer used; type and frequency of pest control use; and frequency crop rotation, tillage, plowing, and mulching.

Preparation of Standard Calibrants, Labeled Standard Spiking Solutions

Calibration standards were created to quantify the target analytes. Stock solutions of unlabeled p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, and o,p'-DDE with concentrations of 100 ppm were diluted to 400 ng/mL in nonane. Then, a set of serial dilutions created calibration standards with a range of 0.01 ng/mL to 400 ng/mL in nonane.

In addition, a labeled standard spiking solution for the calibration curve was prepared in nonane. Stock solutions of labeled p,p'-DDT, p,p'-DDE, and p,p'-DDD with concentrations of 100 ppm were diluted to 100 ng/mL.

Furthermore, a labeled standard spiking solution for the soil samples was prepared in methanol. A stock solution of labeled p,p'-DDT, p,p'-DDE, and p,p'-DDD with concentrations of 100 ppm were diluted to 100 ng/mL. All standard solutions and spiking solutions were aliquoted into amber vials and stored at 4°C until used.

Quality Control and Assurance

To determine potential contamination, a reagent blank sample was analyzed with the unknown samples.

Also, a soil pool was made by mixing 1 gram of each composite soil sample together in an amber jar with a screw cap. Four pooled samples were prepared and analyzed alongside the unknown samples to determine method precision. Three pooled samples were spiked with one

of three quality control (QC) spiking solutions (QC High, QC Medium, and QC Low) with known concentrations of unlabeled p,p'-DDT, p,p'-DDE, and p,p'-DDD, o,p'-DDT, and o,p'-DDE and one pooled sample was not spiked with the unlabeled DDX derivatives. The QC spiking solutions were prepared in methanol. Unlabeled p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, and o,p'-DDE with original concentrations of 100 ppm were diluted to 200 ng/mL and serial diluted to 40 ng/mL and 20 ng/mL. The QC spiking solutions were prepared into amber vials and stored at 4°C until used.

In addition, two samples of the NIST (SRM) 1944 which contains standard concentrations of p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, and o,p'-DDE were also prepared and analyzed to ensure the accuracy of the data.

Sample Preparation and Analysis

A modification of the Quick Easy Cheap Effective Robust and Safe (QuEChERS) method was used. For analysis, 2.5 grams of the sieved soil sample was weighed into a 50 mL polypropylene tube with a screw cap. The samples were spiked with 50 µL of the isotopically labeled analogues. 10 mL of Milli-Q water was added, and the samples were vortex mixed for 10 seconds and sat for 10 minutes. Then, 10 mL of acetonitrile was added, and the samples were vortex mixed for 10 seconds and sat for 10 minutes. A pre-weighed container of salts (4 grams of anhydrous magnesium sulfate, 1 gram of sodium chloride, 1 gram of trisodium citrate, and 0.5 grams of disodium citrate) was added to the samples and shaken until the salts dissolved (the tube became very warm to the touch). Next, the samples were shaken for 30 minutes and centrifuged for 10 minutes at 2500 rpm. The supernatants were extracted and

transferred into 15 mL glass tubes. The sample looked yellow as some soil was still in the sample. 50 mg of primary secondary amine sorbent and 150 mg of magnesium sulfate anhydrous were added to the supernatants. The samples were shaken for 30 minutes and centrifuged for 10 minutes at 2500 rpm. The supernatants were now a pale clear yellow. The supernatants were taken and transferred into 15 mL glass tubes. The samples were evaporated to dryness under nitrogen at 50°C and 15 psi. The samples were reconstituted in 100 µL of nonane and sat for 3 minutes to equilibrate before being vortex mixed for 30 seconds and saved for gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis. Some samples had remaining salts in the bottom, and those samples were reconstituted, centrifuged, and the supernatant was taken for analysis. A flowchart of the method is outlined in Figure S2.

The GC system was fitted with a Zebron DB5 (5%-Phenyl)-methylpolysiloxane analytical column (30 m x 25um x 0.025 um film thickness, Phenomenex, Torrance, CA) for optimum separation. A 2 µL injection was used with an injection port temperature set to 280 °C with pulsed splitless at 3 minutes. The helium carrier gas flow rate was 1 mL/minute through the end of the run; the collision gas was He as well. The oven temperature program was 90 °C (0.5 minute), ramped to 245 °C (25° C/minute), ramped to 270 °C (5 °C/minute), ramped to 325°C (40°C/minute), and held for 4 minutes. The total run time was 17.08 minutes. Transfer line, source, and quadrupole temperatures were set to 280 °C, 230 °C, and 150 °C, respectively. Two transitions were monitored for each native analyte for quantification and confirmation. Only one transition was selected for each labeled analyte. All transitions were monitored in a multi-segment analysis using multiple reaction monitoring (MRM) mode, with a unit resolution for

MS1 and wide resolution for MS2. These MRM transitions and associated parameters are summarized in Table S2.

Calibration plots derived from linear regression analysis of the standard concentrations plotted again the area of the native standard per area of the labeled standard were used to quantify unknown concentrations. Samples were determined valid if the native analyte were at the same retention time as the labeled analogue and agreed with concentrations calculated from quantification and confirmation ions. In addition, the quantified NIST (SRM) 1944 concentration had to be within 20% of the known concentration.

The modified QuEChERS method was evaluated using the pooled soil samples and NIST standards to determine effectiveness before the farm samples were quantified.

The analytes measured are seen below (Figure 4).

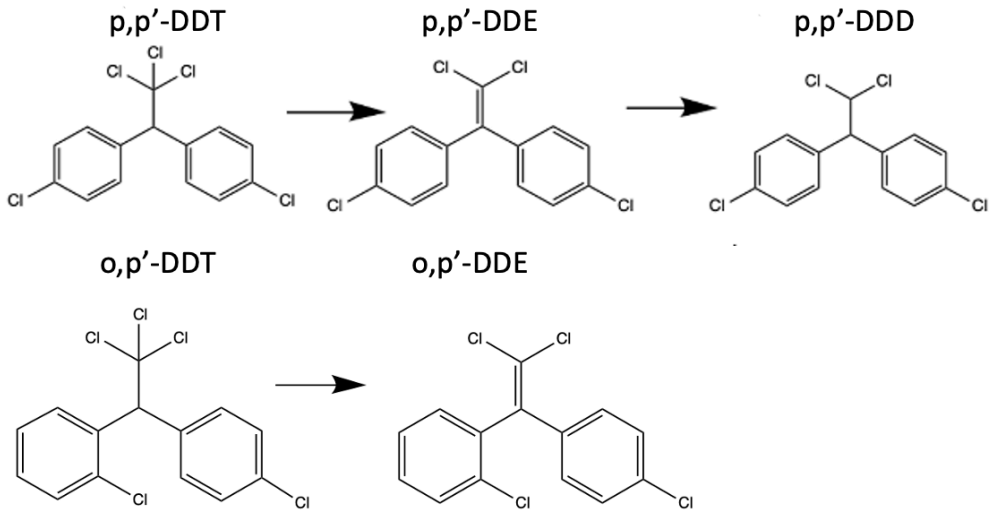

Figure 4: Analytes measured and their degradation pathway.

Validation

The limit of quantification (LOQ, formally defined as $10*s_0$ where s_0 is the standard deviation at 0 concentration) was decided from the lowest calibrant in the calibration curve that met the following requirements: had an accuracy +/- 20%, a precision of 20%, and a concentration that was 5 times greater than the blank samples. The accuracy was determined from the agreement of the quantified NIST concentration with the certified concentrations. The precision was calculated as the relative standard deviation (RSD) from the repeated pooled soil samples spiked with each QC concentration.

Statistical Analysis

The data were processed and analyzed in R Studio Integrated Development Environment Version 2022.07.1 (Posit Software, PBC, Boston, MA) and R Programming Language Version 4.2.1 (The R Foundation, Vienna, Austria). Prior to data analysis, samples with concentrations below the LOQ were assigned a value equal to $\mathrm{LOQ}/\sqrt{2}$. The LOQ was 0.02 ng/g, so the value inputted for those under the LOQ was 0.01414 ng/g.

Ratios of p,p'-DDT:p,p'-DDE, p,p'-DDD:p,p'-DDE, p,p'-DDD:p,p'-DDT, and fraction of decomposition (FD) were calculated for each sample to measure to amount of degradation of DDT and DDE. FD was calculated by $\frac{\mathrm{p,p'DDE+p,p'DDD+o,p'DDE}}{\mathrm{p,p'DDT+p,p'DDE+p,p'DDD+o,p'DDT+o,p'DDE}}$. The geometric means (GMs) and standard deviations (STDs) were calculated for each DU. Welch two-sample t-tests were executed between the experimental and control sites, and an Analysis of Variance (ANOVA) was calculated for p,p'-DDT/p,p'-DDE and p,p'-DDD/p,p'-DDE ratios and fertilizer type, tillage usage, plowing usage, and pest control usage. Furthermore, a Pearson Correlation Test

was evaluated between p,p'-DDT, p,p'-DDE, and p,p'-DDD concentrations. The fertilizer types and p,p'-DDT/p,p'-DDE and p,p'-DDD/p,p'-DDE ratios were evaluated with Welch two-sample t-tests between synthetic fertilizer, natural fertilizer, and no fertilizer.

Results

Validation

The LOQ was determined to be 0.02 ng/g for each analyte, as it was the lowest concentration within the calibration curve that met the predetermined criteria. This is sufficient for a LOQ for the quantification of the analytes because the calibrant sample was +/- 20% of the known concentration and the RSD was below 20%. The percent accuracy of the extraction method was calculated as the ratio of the calculated concentration to the known concentration of each analyte in the NIST (SRM) 1944 soil samples then multiplied by 100. o,p'-DDT could not be calculated for accuracy, as the NIST (SRM) 1944 soil samples did not contain the analyte. The precision was calculated as the relative standard deviation of the two QC materials. The accuracy of the analytes ranged from 90.3-102.7%, and the average RSD for each analyte was below 10% (Table S3).

Extraction recoveries were calculated by spiking a group of soil samples before extraction with isotopically labeled standards and spiking another group of soil samples after extraction with isotopically labeled standards of each analyte. The percent recovery was then calculated by dividing the post-extraction spiked group by the pre-extraction spiked group. The extraction recoveries were found to range from 66-84%, which was sufficient to provide low LOQs for soil pesticide detection within a soil sample. Further, because isotope dilution

calibration was used, the samples were automatically corrected for extraction recoveries. Table S4 displays the GM of the percent extraction recoveries at each concentration of spiked isotopic label.

Farm Characteristics

The farms were reported as cotton farms within 1945-1972. However, over the past 50 years, each farm was utilized for farming various crops (wheat, cotton, corn, and millet). The farms had varying agricultural techniques, but all of the farms had not changed the agricultural techniques significantly in the past 30 years, so only current historical techniques were analyzed. 45% of the farms used natural fertilizer (sample IDs: F1_1, F1_3, F3_2, F3_3, F5_1), 27% of the farms used synthetic fertilizer (sample IDs: F2_1, F2_2, F3_1), and 27% of the farms used no fertilizers (F1_2, F4_1, F5_2). 73% of the farms used tillage (sample IDs: F1_1, F1_3, F2_1, F2_2, F3_1, F3_2, F3_3, F4_1) and 55% utilized plowing (sample IDs: F1_1, F1_3, F3_1, F3_2, F3_3, F4_1). Irrigation was only used by 9% of the farms (sample IDs: F2_2), so the effect of irrigation could not be analyzed in this study. Table S5 reports the farming practices and the response percentage.

DDX Concentrations

The distribution of DDX concentrations detected in the soil samples in farm sites is provided in Table 1. The levels detected of p,p'-DDT, p,p'-DDE, p,p'-DDD in the control sites were found to be 0.19-0.27 ng/g, 0.20-0.62 ng/g, and 0.05-0.09 ng/g, respectively. The control sites did not have detectable levels of o,p'-DDT and o,p'-DDE. The frequency of detection

ranged from 38.5-100%, with o,p'-DDT and o,p'-DDE having the lowest detection rates. p,p'-DDT, p,p'-DDE, and p,p'-DDD were detected in almost every sample, although each had a large variation between the farm sites. p,p'-DDT, p,p'-DDE, and p,p'-DDD concentrations in the soils ranged from 0.04-34.85 ng/g, 0.09-53.11 ng/g, and 0.02-18.34 ng/g, respectively. The large variation suggests the rate of degradation varied between each farm site.

Table 1: Distribution of DDX in farm sites, Georgia 2021-2022

Analyte	FOD %	GM (ng/g)	Median (ng/g)	95^{th} (ng/g)	Maximum (ng/g)	Minimum (ng/g)
p,p'-DDT	97.4%	6.89	0.69	27.56	34.85	0.04
p,p'-DDE	100%	11.31	0.92	42.69	53.11	0.09
p,p'-DDD	100%	3.42	0.26	14.30	18.34	0.02
o,p'-DDT	38.5%	2.75	1.66	5.48	0.41	0.29
o,p'-DDE	38.5%	0.16	0.13	0.37	6.02	0.03

FOD=frequency of detection; GM=geometric mean; 95th=95th percentile

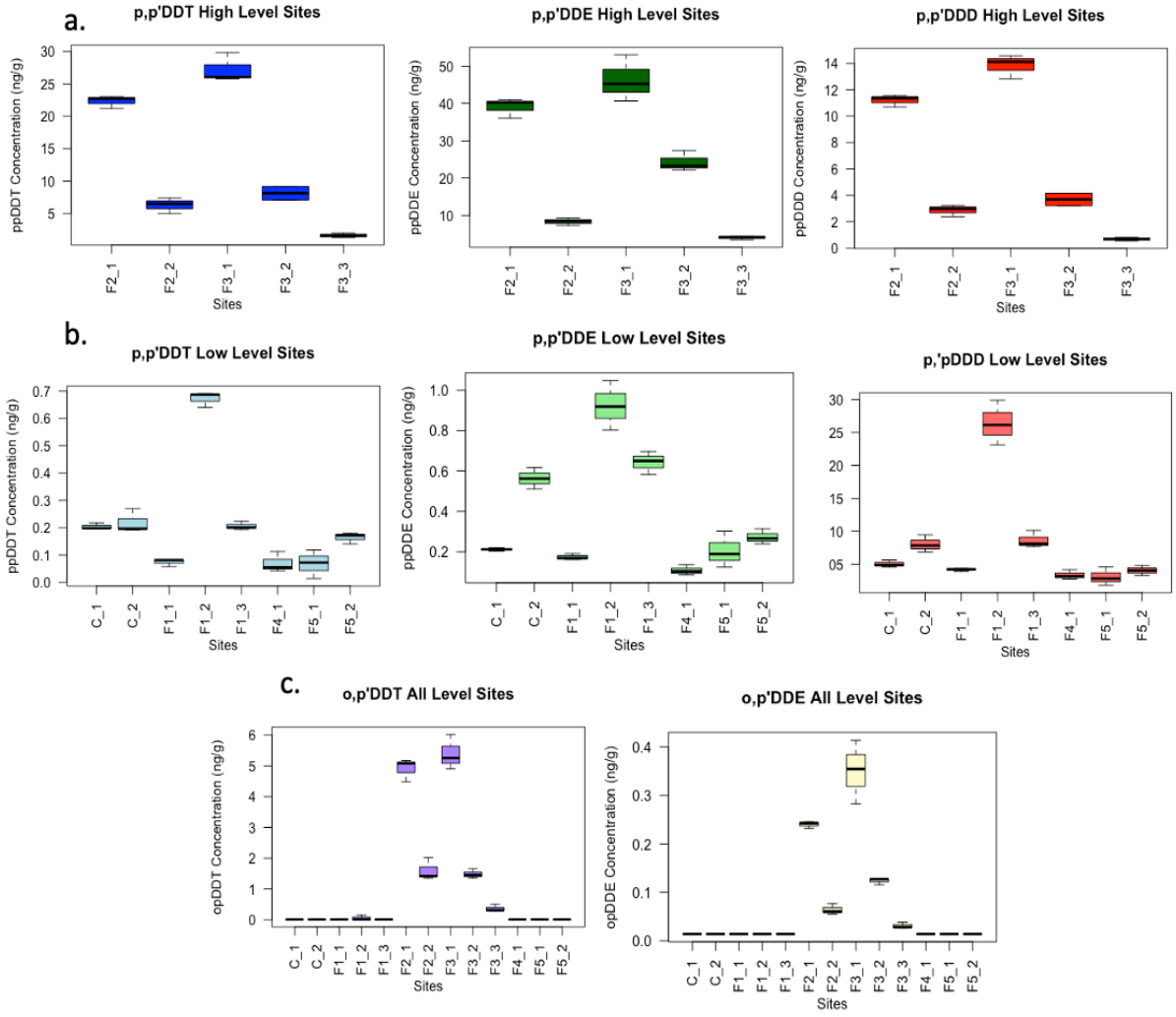

Figure 5: a. Soil concentration(ng/g) of p,p'-DDT (blue), p,p'-DDE (green), p,p'-DDD (red) in sites with high levels (2-30 ng/g). b. Soil concentration (ng/g) of p,p'-DDT (blue), p,p'-DDE (green), p,p'-DDD (red) in sites with low levels (0-2 ng/g). c. Soil concentration (ng/g) of o,p'-DDT (purple) and o,p'-DDE (yellow) in all sites. [1]

1. For the sample identification, the letter is C (control) or F (farm). The first number indicates a specific site, and the second number indicates the specific DU within the site. However, since the control site does not have multiple DUs within each site, the control only includes the site number. Each sample ID follows the patterns: Control_ Number or Farm Site Number _ DU Number.

All analytes significantly differed in the control sites vs farm sites at 95% confidence level, with the farm sites having increased levels of DDX (Table 2). The largest difference was for

p,p'-DDE with the control having an average of 0.39 ng/g and the agricultural sites having an average of 11.31 ng/g.

Table 2: Analyte Concentration between Control and Farm sites, Georgia 2021-2022

Analyte	Control Site GM (ng/g)	Farm Site GM (ng/g)	p-value
p,p'-DDT	0.21	6.89	0.001043
p,p'-DDE	0.39	11.31	0.000704
p,p'-DDD	0.07	3.42	0.001288
o,p'-DDT	0.01	0.16	0.0009006
o,p'-DDE	0.01	2.75	0.001526

A Pearson correlation determined that the concentration of p,p'-DDT, p,p'-DDE, and p,p'-DDD were highly correlated (Figure S4). The correlation coefficients between the three analytes ranged from 0.9834-0.9985 ($p<0.0001$). The highest correlation was between p,p'-DDT and p,p'-DDD with a correlation coefficient of 0.9985 ($p<0.0001$).

DDX Degradation

According to Welch's Two Sample T Test with unequal variance in R programming language, the p,p'-DDE/p,p'-DDT ratio, p,p'-DDD/p,p'-DDE ratio, and the FD did not significantly vary at the 95% confidence level between the control sites and the farm sites ($p>0.05$). However, the p,p'-DDD/p,p'-DDT ratio did significantly differ at a 95% confidence interval ($p=0.0086$). In addition, p,p'-DDD/p,p'-DDE ratio also significantly varied at a 90% confidence interval ($p=0.0739$). Table 7 includes the GM for p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-

DDD/p,p'-DDT, and the FD for the control (church) soil samples and the experimental (farm) soil samples.

Table 3: GM of p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT and FD for the control and experimental groups.

	Control GM	Experimental GM	p-value
p,p'-DDE/p,p'-DDT	1.82± 0.08	2.0 ±0.7	0.6285
p,p'-DDD/p,p'-DDE	0.19±0.02	0.3±0.1	0.07389
p,p'-DDD/p,p'-DDT	0.31±0.04	0.5±0.2	0.008195
FD	0.65±0.02	0.68±0.06	0.4436

Within the farm sites, the ANOVA for p,p'-DDE/p,p'-DDT displayed significant variation between the three fertilizer types (no fertilizer, synthetic fertilizer, and natural fertilizer) at 95% confidence ($p=0.0010$). The ANOVA did not show significant variance for tillage use, plowing use, or pest control use. For the p,p'-DDD/p,p'-DDE ratio, there was significant variation between the three fertilizer types ($p<0.0001$) and between tillage use and no tillage use at the 95% confidence level ($p=0.0461$). Plowing usage and pest control usage did not have a significant variance for the p,p'-DDD/p,p'-DDE ratio. Furthermore, for p,p'-DDD/p,p'-DDT ratio, fertilizer type ($p=0.00000361$) and tillage ($p=0.0305$) had significant variance at the 95% confidence interval, while plowing and pest control usage did not. And, for fraction of degradation, there was a significant difference in fertilizer types ($p<0.0001$) and plowing ($p=0.0127$), while tillage and pest control usage did not.

The GMs of p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT, and FD for tillage use, no tillage use, plowing use, and no plowing use are shown in Table 7. Sites with no tillage use had a lower average of p,p'-DDD/p,p'-DDE ratios, but a higher average of p,p'-DDD/p,p'-DDT ratio. Plowing use had a higher fraction of degradation compared to no plowing use.

Table 4: GM of of p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT, and FD for tillage use, no tillage use, plowing use, and no plowing use.

	Tillage Use GM	No Tillage Use GM	Plowing Use GM	No Plowing Use GM
p,p'-DDE/p,p'-DDT	2.2±0.6	3±2	2.4±0.6	2±2
p,p'-DDD/p,p'-DDE	0.3±0.1	0.19±0.06	0.3±0.2	0.23±0.09
p,p'-DDD/p,p'-DDT	0.49±0.07	0.4±0.3	0.49±0.09	0.5±0.3
FD	0.70±0.08	0.68±0.07	0.72±0.07	0.66±0.08

Further analysis of the fertilizer type on p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT, and the FD was conducted. p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, and the FD were found to have a statistical difference with 95% confidence between natural fertilizer usage and synthetic fertilizer usage, but the p,p'-DDD/p,p'-DDT did not have any statistical variation. The natural fertilizer usage having a higher p,p'-DDE/p,p'-DDT ratio ($p=0.0040$) and FD ($p<0.0001$) and a lower p,p'-DDD/p,p'-DDE ratio ($p=0.0367$).

In addition, the natural fertilizer usage and no fertilizer usage sites were statistically different at a 95% confidence interval for p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDT, and fraction

of degradation. The natural fertilizer usage had the higher p,p'-DDE/p,p'-DDT ratio ($p=0.004704$), p,p'-DDD/p,p'-DDT ($p=0.0394$), and FD ($p<0.0001$). However, the p,p'-DDD/p,p'-DDE ratio had no statistical difference ($p>0.05$).

Synthetic fertilizer usage and no fertilizer usage sites had a similar p,p'-DDE/p,p'-DDT ratio and FD ($p>0.05$), but the p,p'-DDD/p,p'-DDE ratio($p=0.0006866$) and p,p'-DDD/p,p'-DDT ratio ($p=0.001792$) were statistically different. Synthetic fertilizer use had a higher p,p'-DDD/p,p'-DDE ratio and p,p'-DDD/p,p'-DDT ratio than no fertilizer use. The GM of p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT, and FD for each fertilizer type is found in Table S6.

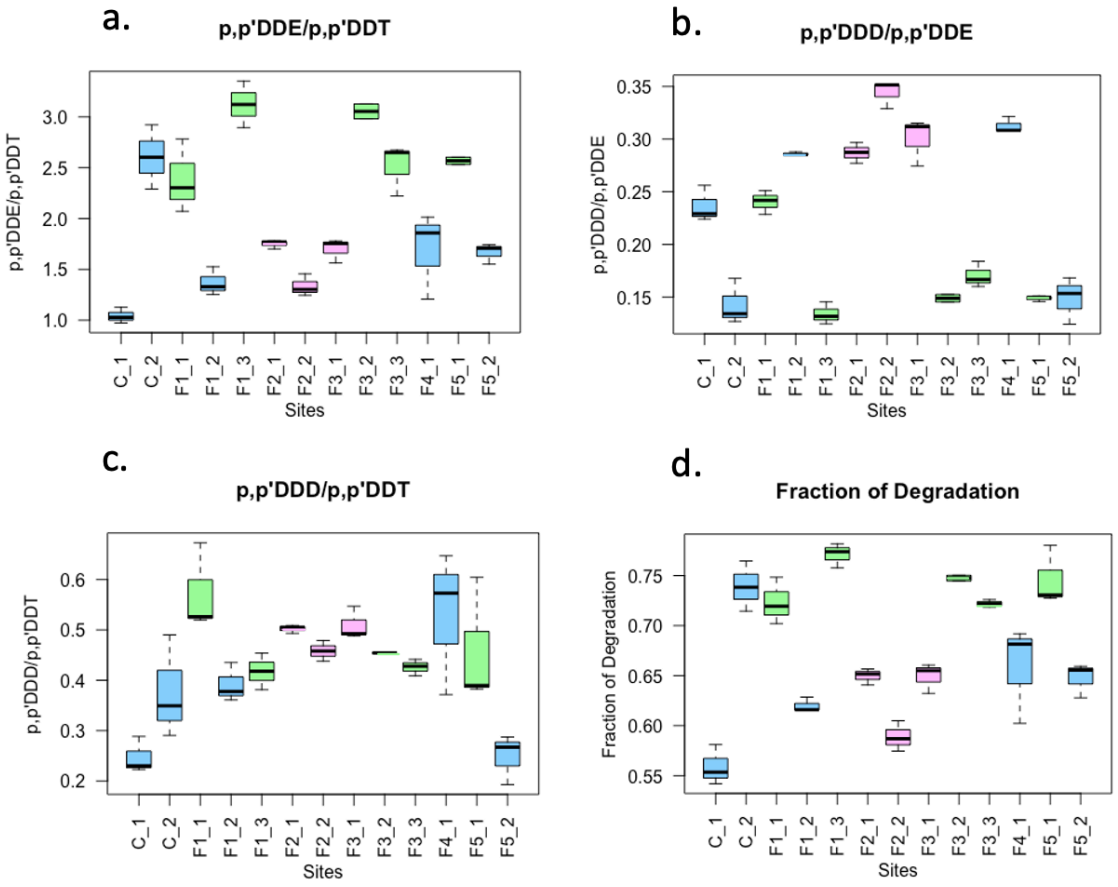

Figure 6: a. p,p'-DDE//p,p'-DDT ratio in all sites based on fertilizer type (blue: no fertilizer usage, green: natural fertilizer usage, pink: synthetic fertilizer usage). b. p,p'-DDD/p,p'-DDE ratio in all sites based on fertilizer type (blue: no fertilizer usage, green: natural fertilizer usage, pink: synthetic fertilizer usage). c. p,p'-DDD//p,p'-DDT ratio in all sites based on fertilizer type (blue: no fertilizer usage, green: natural fertilizer usage, pink: synthetic fertilizer usage). d. fraction of degradation in all sites based on fertilizer type (blue: no fertilizer usage, green: natural fertilizer usage, pink: synthetic fertilizer usage).

Discussion

Validation

All validation metrics of this method were consistent with those required in EPA's Method 1699: Pesticides in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, December 2007.[48] According to this method, the LOQs must be <10 pg/g, and the RSDs must be <30% for each analyte. The percent extraction and the percent accuracy are within the

recommended ranges. Therefore, the QuEChERS method was an accurate method for extraction of DDX in soil.

DDX Concentration

Almost every soil sample had detectable levels of p,p'-DDT, p,p'-DDE, and p,p'-DDD because each site had historical DDT pesticide use. However, o,p'-DDT and o,p'-DDE were only detected at 38.5% of the sites. This can be due to the lower concentration of o,p'-DDT in the original DDT technical pesticide mixture, as p,p'-DDT and o,p'-DDT cannot switch between the structural isomers. Since o,p'-DDT and o,p'-DDE are in much lower concentrations and cannot be formed from the high amount of p,p'-DDT, the sites were expected to have much lower levels. Also, the sites that had detectable levels of o,p'-DDT were the same sites on which o,p'-DDE was detected, which is logical because o,p'-DDE can only form as a degradate or metabolite from o,p'-DDT.

Within the experimental farm sites, the levels of each analyte varied greatly. This suggests that each farm sprayed differing amounts of DDT, even though each farm was growing cotton at the time of DDT use. Since each farm most likely had differing initial levels of DDT, the concentration of each analyte is not useful in determining the amount of degradation within each farm. Therefore, for the aim of determining if farming techniques affect decomposition of DDT, the ratios between the analytes (p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT, and FD) were found to be more useful.

The church control sites had detectable levels of p,p'-DDT, p,p'-DDE, p,p'-DDD, but did not have detectable levels of o,p'-DDT and o,p'-DDE. The detectable levels of some of the

analytes could be due to direct application of DDT to the area, but the low levels of each analyte could make this unlikely. Another reason for the detection of some analytes in the control sites could be from atmospheric redeposition of volatilized DDX from high concentration soils. The control sites had much less variation of each analyte between the sites than the farm sites. This could be due to a much smaller sample size (2 sites), or the control sites could have had similar amount of application from wind. The p,p'-DDE concentration varied more significantly between the two controls, which could be due to difference in wind application. Control site 2 had higher levels of DDE than control site 1. As seen in Figure 2, control site 2 is west of the farms while control site 1 is east of the farm sites. This result was opposite from what was expected because the direction of the wind in the area is from west to east, and it was expected that the wind might carry the p,p'-DDE to control site 1 rather more than control site 2 after volatilization, which would explain the variance in the levels of p,p'-DDE in both sites. However, there are many farms west of Walton County in the other nearby counties that could deposit p,p'-DDE to control site 2.

The correlation of p,p'-DDT, p,p'-DDE, and p,p'-DDD were all highly positively correlated. This is most likely due to the initial levels of DDT. From this study's correlation test, if there was a high starting amount of DDT sprayed in the area, there would be higher amounts of p,p'-DDT, p,p'-DDE, and p,p'-DDD currently. The correlation does not represent the degradation of DDT, which would have a negative correlation because higher amounts of a metabolite would cause lower amounts of the original compound. The degradation is most likely not represented in the correlation due to the high variation of each analyte in the soil. A site with more degradation

could have lower levels of a metabolite than a site with lower degradation due to lower initial amounts of the starting compound.

DDX Degradation

The p,p'-DDE/p,p'-DDT ratio was greater than 1 for every sample, which indicated historical use of DDT. The half-life of p,p'-DDT in soil is 2-15 years,[49] so the ratio of p,p'-DDE/p,p'-DDT was expected be greater than 1. The p,p'-DDD/p,p'-DDE ratio was less than 1 for each sample. The half-life of p,p'-DDE in soil is 5.7 years,[50] so the p,p'-DDD/p,p'-DDE ratio was expected to be higher. However, a previous study found that for repeated historical use of DDT, DDE concentrations may remain constant for more than 20 years,[51] so the p,p'-DDD/p,p'-DDE ratio could be expected to be less than 1. In addition, p,p'-DDD has a half-life of 160 days, where the most common fate of the p,p'-DDD is volatilization,[26] and some bacteria are able to further convert DDD into DBH and DDMU. Therefore, the low levels of p,p'-DDD, which results in a p,p'-DDD/p,p'-DDE ratio less than 1, could have been due to volatilization or further degradation of the compound.

The control sites had similar values to the farm sites for p,p'-DDE/p,p'-DDT and FD, which could indicate that the degradation of DDT is not affected by agricultural techniques. However, the control sites had lower values for p,p'-DDD/p,p'-DDT and p,p'-DDD/p,p'-DDE, which can indicate that agricultural techniques can affect the production of DDD in soil. Since the decomposition ratios do not have the same conclusion, there could be an outside factor that is affecting some of the ratios. Outside factors, like soil composition and weather, were limited by selecting control and experimental sites within the same county, but deposition of

DDT, DDE, and DDD from the air could not be limited. Uneven deposition of these compounds could cause false similarities or false differences between the decomposition ratios. Therefore, further analysis between agricultural techniques were conducted.

Tillage usage appeared to increase the degradation of DDT, as shown in the higher p,p'-DDD/p,p'-DDE and p,p'-DDD/p,p'-DDT ratios, but it had no effect on p,p'-DDE/p,p'-DDT and FD. However, the higher ratios of p,p'-DDD/p,p'-DDE and p,p'-DDD/p,p'-DDT could have been due to the increased volatilization of DDE from the tillage, which has been reported in previous studies.[38] Therefore, the use of tillage may not have increased the degradation of DDT, but increased loss of DDE, which can cause a higher ratio of p,p'-DDD/p,p'-DDE and p,p'-DDD/p,p'-DDT. The p,p'-DDE/p,p'-DDT for the sites with tillage usage, while not statistically different than from the sites with no tillage usage, was lower than the ratio of p,p'-DDE/p,p'-DDT of the sites with no tillage usage, which further pointed to volatilization of DDE from the usage of tillage. In addition, the FD was similar for both of the sites with and without tillage, so it was unlikely that the tillage is increasing the degradation, but instead affected the levels of DDE specifically. Since all four ratios did not show a difference with tillage usage, there could be no definitive conclusion on the effect of tillage on rate of degradation of DDT.

Plowing usage appeared to speed up the degradation, since the FD was higher for the sites with plowing usage. However, p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT ratios were not altered by plowing usage. Possibly, the o,p'-DDT and o,p'-DDE levels, which were used to calculate the FD, could have caused the differences between plowing use and no plowing use groups. The few sites that had detectable levels of o,p'-DDE underwent tillage

usage, since not all samples detected o,p'-DDE. Any detection of the analyte can skew the data set of the FD.

The type of fertilizer used had a large impact on DDT degradation. There was a difference between the types of fertilizer used for each degradation ratio. When compared to no fertilizer use, natural fertilizer had higher values for p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDT, and FD, which indicated that both p,p'-DDT and p,p'-DDE degradation were enhanced by natural fertilizer use. Since natural fertilizer is often manure, the addition of diverse microbes could have increased the overall degradation of DDT in the soil because bacteria are known to decompose DDT. Results of increased microbial activity due to fertilizer has been concluded previously, but they have not distinguished the type of fertilizer.[38,40,41]

Synthetic fertilizer use increased the degradation of p,p'-DDE. Compared to no fertilizer use, synthetic fertilizer had higher values for p,p'-DDD/p,p'-DDE and p,p'-DDD/p,p'-DDT, but no difference for p,p'-DDE/p,p'-DDT ratio and FD. Since the two decomposition ratios that were affected incorporate p,p'-DDD, it can be concluded that synthetic fertilizer increased p,p'-DDD levels by increasing the degradation of p,p'-DDE, but p,p'-DDT degradation was not affected. Synthetic fertilizer's effect on p,p'-DDE degradation could have been an increase in microbial activity. However, unlike natural fertilizer, which had an addition of microbes, synthetic fertilizer increases the nutrients available for the bacteria, so the already existing bacteria can boom in population.[38]

When comparing natural and synthetic fertilizer use, natural fertilizer increased only the degradation of p,p'-DDT, as can be seein in the higher ratio of p,p'-DDE/p,p'-DDT and FD. However, synthetic fertilizer had a higher p,p'-DDD/p,p'-DDE value, and p,p'-DDD/p,p'-DDT

were similar for both fertilizers. Therefore, p,p'-DDT degradation is considered to be enhanced for natural fertilizer, while there is increased p,p'-DDE degradation in synthetic fertilizer. However, to conclude the reasoning behind the differences in each fertilizer's effect on degradation, further studies must be conducted. A hypothesis would be that the fertilizer type can affect the microbial composition, which could favor the degradation of p,p'-DDT or p,p'-DDE.

Conclusions

DDX Concentration

The farm sites had detectable levels of p,p'-DDT, p,p'-DDE, and p,p'-DDD, while few sites had detectable levels of o,p'-DDT and o,p'-DDE. The levels of each analyte largely varied, so pesticide application was not consistent from farm to farm. Control church sites had detectable levels of p,p'-DDT, p,p'-DDE, and p,p'-DDD, which could have been deposited through direct application or deposition from the greenhouse effect. Overall, there was a high positive correlation between p,p'-DDT, p,p'-DDE, and p,p'-DDD, so the increased levels of each degradate most likely are due to increased levels of initial DDT application.

DDX Degradation

The degradation of DDT suggests that each farm has historical use of DDT because each p,p'-DDE/p,p'-DDT value was more than 1. p,p'-DDD/p,p'-DDE degradation ratio was less than 1 for all sites, which is similar to the past studies, and it might be due to a large amount of volatilization of p,p'-DDD in the soil. Furthermore, the control sites had similar degradation

ratios to the farm sites. The degradation ratios have large variation within the experimental groups, so agricultural techniques may still have an effect on the degradation of DDT.

Tillage was found to speed up p,p'-DDE degradation, most likely due to photodegradation, but there was no effect on p,p'-DDT degradation. Plowing caused higher FD, but the other degradation ratios did not have a difference, so there can be no conclusion on the effect of plowing on DDT degradation. The largest difference in the degradation of DDT was based on the fertilizer type. Natural fertilizer sped up degradation p,p'-DDT and p,p'-DDE, which could have been due to the addition of microbes that participate in the decomposition of DDT. Synthetic fertilizer increased p,p'-DDE degradation, but not p,p'-DDT degradation. Synthetic fertilizer may have affected the microbial activity by increasing the nutrients for the microbes. Natural fertilizer increased p,p'-DDT degradation more effectively, while synthetic fertilizer increased p,p'-DDE degradation more effectively. Therefore, the type of fertilizer may have affected the microbes in the soil, which would affect the type of degradation that was preferred. However, since the sample size of farms was limited, no definite conclusions can be made for the effect agricultural techniques on DDT degradation.

Future studies

Future studies can further elucidate the effect of agricultural techniques on DDT degradation. A larger sample size of farms and control sites would allow for more subtle effects due to agricultural techniques to be differentiated. In addition, experimental fields with known spiked concentrations of DDT could be used with a singular agriculture technique to isolate the effects of one agricultural practice. Furthermore, future studies could expand to urban,

suburban, and non-agricultural rural areas to make a more robust model about soil DDX concentrations throughout Georgia. Moreover, DDX in other crops could be quantified to allow for better estimation of DDT application in multiple crops. Current-use pesticides, such as chlorpyrifos and other organophosphate pesticides, could also be analyzed in soils to determine the decomposition within varying soil compositions and weather patterns. Lasty, DDX concentrations can be quantified in the same site areas after phytoremediation or bioremediation to study the efficiency and quality of remediation options. In general, this study is a launching point for multiple projects studying the residue levels of pesticides throughout Georgia.

Supplemental Information

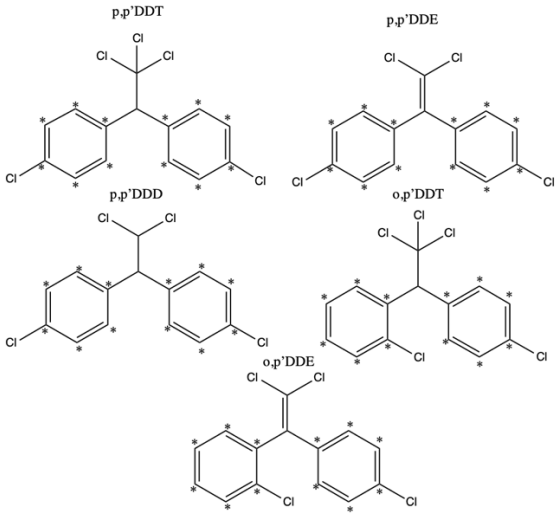

Figure S1: Structures of isotopically labeled analytes with ^{13}C on each ring.

Table S1: Land Use History Questionnaire

What crop or crops are farmed in this area? If applicable: please describe which are farmed together and how often the crops are rotated.
Are there livestock present? If so, what kind and how many?

What type of irrigation was historically used in the area? What type of irrigation is currently used in the area?
Is fertilizer used? If so, what type? If synthetic fertilizer is used, what major chemicals are found in the fertilizer? How often is fertilizer used?
What kind of pest control is used?
Select all of the practices that were historically used in the area? (Crop rotation. Intercropping, Tillage, Conventional Plowing, Contour Plowing, Mulching) Please indicate how often and long the practices selected are used?
Select all of the practices that are currently used in the area? (Crop rotation. Intercropping, Tillage, Conventional Plowing, Contour Plowing, Mulching) Please indicate how often the practices selected are used?

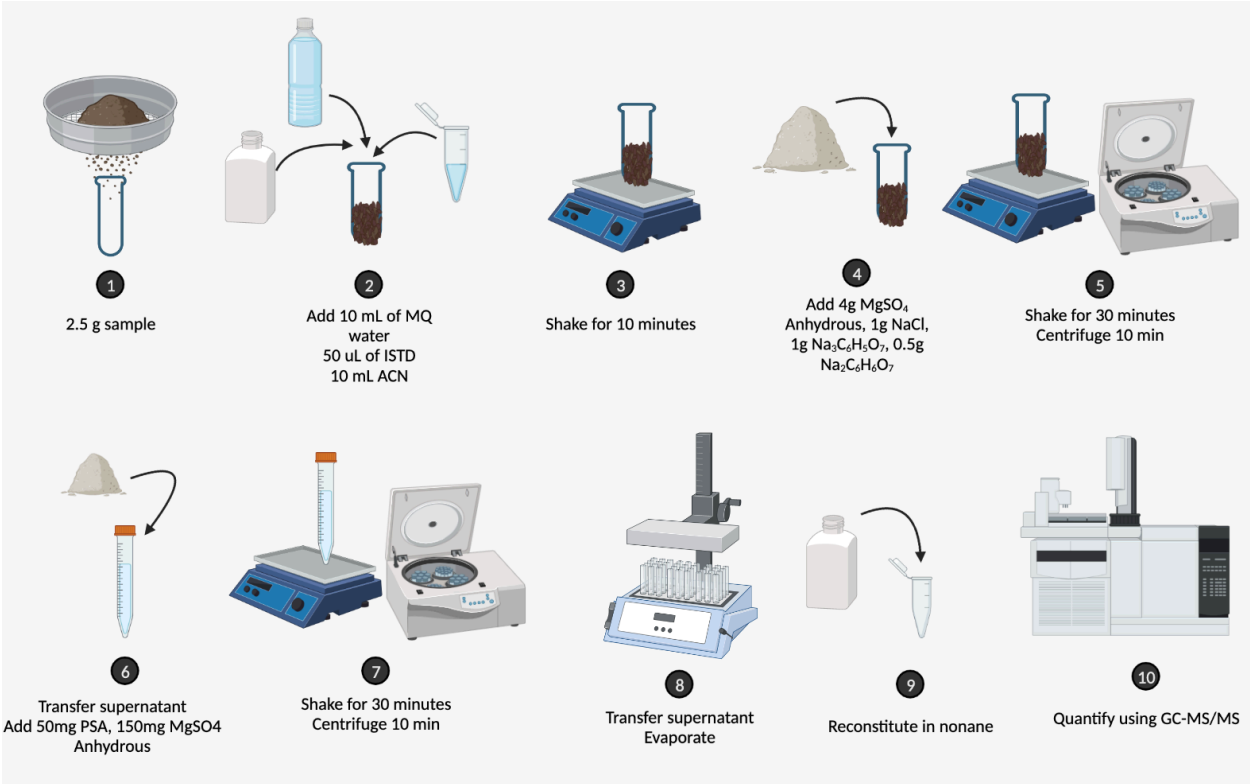

Figure S2: QuEChERS Extraction Method

Table S2: MRM Transitions and Parameters

Analyte	Ion Type	Precursor Ion	Product Ion	CE (V)	Retention Window	RT (min)
p,p'-DDE	L	257.8	188.2	35	1	9.05
p,p'-DDE	Q	245.9	176.0	35	1	9.05
p,p'-DDE	C	247.9	176.0	35	1	9.05

p,p'-DDT	L	247.0	177.2	25	2	10.2
p,p'-DDT	Q	234.9	165.0	25	2	10.2
p,p'-DDT	C	236.9	165.0	25	2	10.2
p,p'-DDD	L	247.0	177.0	25	2	9.6
p,p'-DDD	Q	234.9	165.0	25	2	9.6
p,p'-DDD	C	234.9	165.0	25	2	9.6

L=labeled ion, Q=quantitative ion, C=confirmation ion, CE=collision energy in volts, RT=retention time

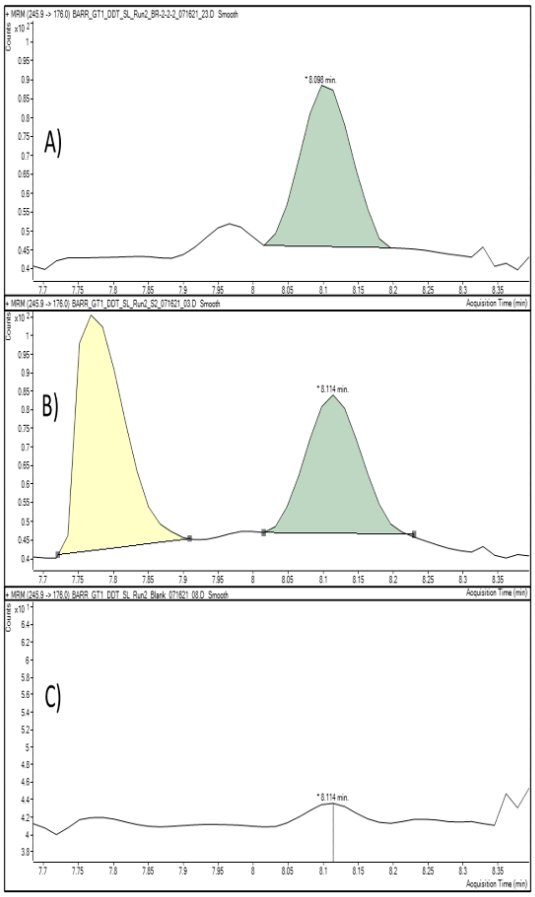

Figure S3: Mass chromatograms of DDE in a soil sample (A), calibrant (B), and blank (C).

Table S3: LOQ, Percent Accuracy, and Average Percent RSD of QCs for Each Analyte

Analyte	LOQ (ng/g)	% Accuracy	Average % RSD of QCs
p,p'-DDT	0.02	100.0	7 ± 4
p,p'-DDE	0.02	95.24	2 ± 2

p,p'-DDD	0.02	102.7	5 ± 4
o,p'-DDT	0.02	N/A	10 ± 3
o,p'-DDE	0.02	90.3	2 ± 2

LOQ=limit of quantification; STD=standard deviation; RSD=relative standard deviation; QC=quality control

Table S4: GM of repeated percent extraction recoveries at 1, 5, and 10 ng/mL of spiked isotopic labels of p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, o,p'-DDE

Concentration (ng/mL) of spiked isotopic label	%Recovery of p,p'-DDT	% Recovery of p,p'-DDE	% Recovery of p,p'-DDD	% Recovery of o,p'-DDT	% Recovery of o,p'-DDE
1	66 ± 7	80 ± 5	70 ± 10	70 ± 10	71 ± 7
5	73 ± 4	80 ± 6	70 ± 10	70 ± 10	77 ± 2
10	79 ± 6	84 ± 4	79 ± 6	80 ± 10	80 ± 6

Table S5: Agriculture techniques and response percentage for the farm sites

Agriculture Technique	Response
Irrigation	9.0%
No Fertilizer	27.2%
Natural Fertilizer	45.5%
Synthetic Fertilizer	27.3%
Tillage	72.7%
Plowing	54.5%

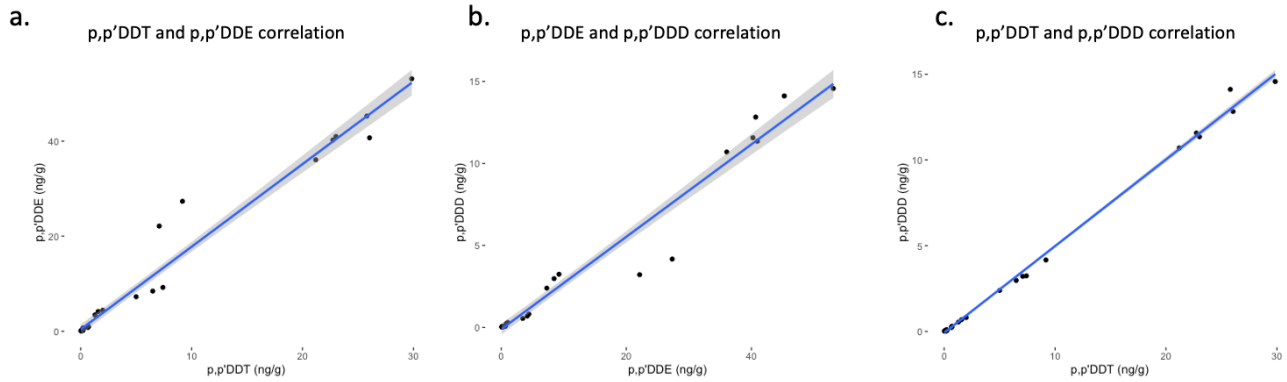

Figure S4: a. scatter plot of p,p'-DDT (x) and p,p'-DDE (y) with line of best fit. b. scatter plot of p,p'-DDE (x) and p,p'-DDD (y) with line of best fit. c. scatter plot of p,p'-DDT (x) and p,p'-DDD (y) with line of best fit.

Table S6: GM of p,p'-DDE/p,p'-DDT, p,p'-DDD/p,p'-DDE, p,p'-DDD/p,p'-DDT, and FD for each fertilizer type

	No Fertilizer Use	Natural Fertilizer Use	Synthetic Fertilizer Use
p,p'-DDE/p,p'-DDT	1.5 ± 0.3	3 ± 2	1.59 ± 0.2
p,p'-DDD/p,p'-DDE	0.25 ± 0.08	0.2 ± 0.2	0.31 ± 0.03
p,p'-DDD/p,p'-DDT	0.4 ± 0.1	0.5 ± 0.2	0.49 ± 0.03
FD	0.64 ± 0.03	0.76 ± 0.06	0.03 ± 0.03

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