

Environmental fate of chlorothalonil in a Costa Rican banana plantation

Alicia Chaves, Damian Shea, W. Gregory Cope *

Department of Environmental and Molecular Toxicology, North Carolina State University, Campus Box 7633, Raleigh, NC 27695, USA

Received 24 October 2006; received in revised form 22 March 2007; accepted 27 March 2007

Available online 10 May 2007

Abstract

The environmental fate of chlorothalonil (CHT) and its metabolites were studied under field-variable conditions in a commercial banana plantation in Costa Rica. Weather conditions were representative of a tropical environment and the fungicide applications were typical of those in banana production. The test plots were treated with Bravo 720[®] at 1.2 l ha⁻¹ of formulated product. Field persistence of CHT in soil and on banana leaves was measured during five consecutive months and after three aerial applications of the fungicide. Residues were analyzed in soil, sediment, water, banana leaves and drift cards by gas and liquid chromatography coupled to mass spectrometry. In soil and on the surface of banana leaves, CHT dissipated rapidly with half-lives of 2.2 and 3.9 d, respectively. Soil residues persisted and were detected 85 d after application. The main metabolite found in soil, 4-hydroxy-chlorothalonil, accounted for approximately 65% of residues detected and was measured up to 6 d after application.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Fungicide; Dissipation; Half-life; Soil

1. Introduction

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CHT) is a broad spectrum, non-systemic foliar fungicide used extensively to control fungal and bacterial infestations in many crops, including food, feed and non-food crops; outdoor residential applications and even in adhesives, coatings and paints. In the United States, it is mainly used on peanuts (about 34% of its usage), potatoes, tomatoes, paint and golf courses (US Environmental Protection Agency, 1999). In recent years, there have been reports of potential adverse ecological effects in the southeastern United States from CHT runoff after application to peanuts (US Environmental Protection Agency, 1999). The toxicity of CHT has been previously studied (Sherrard et al., 2003). For example, it is known that once in the environment, CHT is highly toxic to fish, birds and aquatic invertebrates (Caux et al., 1996). CHT is acutely toxic to freshwater fish

at concentrations as low as 10.5 µg l⁻¹, whereas chronic toxicity may occur at concentrations of 2 µg l⁻¹ (Caux et al., 1996). For rainbow trout, a 21-d LOEC and NOEC of 4.9 and 2.3 µg l⁻¹, respectively have been reported (Caux et al., 1996). In humans, CHT can cause dermatitis, severe eye and skin irritation and gastrointestinal problems (Draper et al., 2003). More recently, a toxicity assessment of pesticides used in Costa Rica concluded that chlorothalonil and terbufos are responsible for more than 90% of human toxicity (Humbert et al., 2007).

Several studies have been published that dealt with understanding the fate and behavior of CHT in the environment, as well as the possible adverse effects associated with its usage. Most of the main findings were compiled by the US Environmental Protection Agency on its Reregistration Eligibility Decision (RED) document (US Environmental Protection Agency, 1999). However, these studies were all conducted in temperate environments and under conditions that do not apply to other climates that are wetter and warmer, like those in portions of Central and South America. In the tropics, CHT is applied

* Corresponding author. Tel.: +1 919 515 5296; fax: +1 919 515 7169.
E-mail address: greg_cope@ncsu.edu (W.G. Cope).

extensively for the prevention and treatment of many crop diseases. Of special concern is its usage on banana plantations where we observed applications as frequently as 45 times per year at rates of up to 1.41 ha^{-1} of formulated product and others have reported application frequencies up to 60 times per year (Lustig, 2004).

A review of the fate of CHT in tropical environments revealed that little research has been conducted directed at understanding the environmental behavior of this compound (Van Eeden et al., 2000; Regitano et al., 2001). Much uncertainty exists about the extent of environmental pollution from CHT and whether toxic effects to biota are occurring. Moreover, no studies have been conducted on the fate and behavior of CHT and its metabolites in banana growing areas, although a report has identified the presence of CHT in water bodies draining banana fields (Castillo et al., 2000).

CHT is considered to be moderately persistent in soil, although data in the literature exhibit high variability. For instance, soil half-lives in field situations ranged from 4 d to 6 month (US Environmental Protection Agency, 1996). In water, half-lives ranged from 0.18 to 8.8 d. This variation in reported data is likely a reflection of the effects of different environmental and experimental conditions on degradation data. This also indicates that to investigate the fate and behavior of CHT in banana plantations, site-specific studies need to be conducted and that extrapolation of data from temperate environments may result in erroneous assessments.

Another limiting factor in understanding pesticide fate in the environment is the lack of studies that involve not only the parent compound but also its degradation products. In the case of CHT, several degradation products have been identified (Ballee et al., 1976; Sato and Tanaka, 1987; Rouchaud et al., 1988) and several degradation pathways have been reported (Sato and Tanaka, 1987; Roberts and Hutson, 1999; Putnam et al., 2003). In soil, CHT is mainly metabolized to the more toxic and persistent 4-hydroxy-2,5,6-trichloroisophthalonitrile (metabolite II) (Davies, 1988). The presence of this metabolite may cause inhibition of CHT degradation, due to its toxicity to microorganisms (Motonaga et al., 1996). Other major metabolites include dechlorinated as well as substituted forms of CHT, like 1, 3-dicarbamoyl-2,4,5,6-tetrachlorobenzene (metabolite III), 2,5,6-trichloro-4-methoxyisophthalonitrile (metabolite IV), 1-carbamoyl-3-cyano-4-hydroxy-2,5,6-trichlorobenzene (metabolite V); 2,4,5-trichloroisophthalonitrile (metabolite VI), 2,5,6-trichloro-4-methylthioisophthalonitrile (metabolite VII) and 1,3-dicyanobenzene (metabolite VIII); metabolite I is not described (Putnam et al., 2003). More recently, Regitano et al. (2001) and Carlo-Rojas et al. (2004) studied the degradation of CHT in tropical soils in laboratory studies. The study of Carlo-Rojas et al. (2004) reported the occurrence of anaerobic degradation of CHT in microcosms with sandy, acidic soil from a banana plantation. However, the degradation products were not identified in this study. This paper reports the findings of

a field dissipation study of CHT, and for the first time, of its metabolites in banana plantations.

2. Materials and methods

2.1. Study area

This study was conducted in a 33 ha section of a commercial banana farm located in Costa Rica's Atlantic Region and was part of a major research project aimed at evaluating the use of multimedia fate models to estimate CHT transport and fate in banana plantations.

The experimental site was part of a banana plantation that had been used for commercial cultivation for about four decades. A wide range of pesticides and fertilizers had been applied to the land during that time, including CHT. Previous to this study, CHT was applied an average of 45 times a year at a rate of 1.21 ha^{-1} until 1998. CHT application ceased in September 1998 and did not resume until the beginning of this study in February 1999. Water input into this area was exclusively due to rainfall and excess water was drained out of the cultivation area via a series of natural and manmade channels. Some channels were covered by vegetation as a means to reduce runoff and aerial drift during pesticide application. Major channels were drained by smaller channels at several points along the study area. Live fish and crustaceans were observed in several of the channels. Meteorological data were collected daily from a weather station located inside the plantation.

2.2. Fungicide application and sample collection

The field research was conducted from February to June 1999, corresponding to the dry season in Costa Rica. During this time, fungicides were applied by airplane on a weekly basis following grower recommended practices. Three applications of CHT (Bravo 720[®]) at a rate of 1.21 ha^{-1} (formulated product) were made at two week intervals starting on February 9 and ending on March 11. Before 1999, Bravo 720[®] had been intensively applied, with as many as 45 applications per year. However, it was not applied during the four months preceding this study. During that period, which corresponded to the heart of the rainy season, this area experienced heavy rainfall due to the effects of Hurricane Mitch. Fertilization and application of other pesticides on the plantation was carried out following established practices for this crop.

Dissipation of CHT under field conditions was studied in the soil and on the surface of banana leaves, which were collected following the protocol described herein. To address the question of how much fungicide reaches the soil surface, drift cards were collected from the ground, the top of the banana plants and from the water channels, including those that were buffered by vegetation and those that were not.

Five experimental plots were randomly selected inside the study area following a grid sampling design (Crepin

and Johnson, 1993). Within each plot, eight sub-sampling stations were randomly selected. One sample was collected at each location and all eight samples in each plot were composited to obtain a single sample per plot. The day of the application, sampling started immediately after the 2-h safety re-entry period. A set of soil and banana leaf samples was collected in the study area approximately one week before the first application with CHT. These samples were used to measure background concentrations. Reference control samples for soil were collected from a nearby forestry reserve and foliage control samples were taken from nearby wild banana plants, both with no history of pesticide applications. Sampling in the five plots was conducted for 7 d after the first two consecutive applications. For the third application, soil and banana leaves were sampled at days 4, 16, 30 and 44 after application. Soil samples were taken additionally at days 57, 72 and 85.

Banana leaf samples. Following the sampling protocol previously described, eight banana plants of similar height and age were chosen in each plot. Five fully opened leaves were chosen from each plant and one disc was taken from each leaf using a 2.5-cm metallic hole punch, for a total of 40 discs per plot. Punches were taken from the center of the leaf and near the base. The five leaves selected had an angle of approximately 45° with respect to the stem. All of the discs taken from each plot were composited, wrapped in aluminum foil, placed in sealable plastic bags and held on ice in the field and stored frozen (−20 °C) until analysis.

Soil samples. Soil samples were taken from the uppermost 5 cm of the soil with a stainless steel coring tube. This soil zone is where strongly sorbing pesticides like CHT are likely to accumulate. Using the marked banana plants as a reference, soil sampling sites were located approximately 1.5 m away from each plant and from an area of maximum exposure to aerial spray. Composite samples were prepared by carefully mixing the eight soil cores from each plot. In two of the plots, soil profiles from depths of 5–10, 10–20 and 20–30 cm were collected once, 3 d after the second application with Bravo® 720. All the samples were collected in solvent cleaned glass containers, labeled and stored frozen until analysis.

Water samples. Water samples were collected at five different locations along main channels inside the plantation. Samples were taken from slow as well as from faster flowing channels. The day of the application, water samples were taken approximately 3 h after application. Afterwards, they were collected every time there was a rain event. Sampling was done manually by immersing 50-ml dark glass bottles directly into the stream. Samples were preserved immediately by adding 0.5% of a solution of 5 M H₂SO₄ (v/v).

Aquatic sediments. Sediment samples were collected manually from the uppermost 2 cm of the sediments from the channels. Sampling was conducted twice on February 13 and 25 in two of the plots (3 and 7).

Spray deposition. During the first and second applications of CHT, spray deposition at the top of the plant

canopy, as well as on the soil surface was determined. Drift samples were collected with water sensitive paper (Novartis Crop Protection AG, Basel, Switzerland). Following the sampling protocol described previously, two drift cards were placed at each sub-sampling site. One card was placed on the ground at the same location as for the soil samples. The other card was placed on top of the plants, in the same location as for the banana leaf samples. About 2 h after application, the drift cards were collected and each card was wrapped in aluminum foil, placed in a sealable plastic bag, held on ice in the field and stored frozen until analysis. Spray deposition was also evaluated along the water channels. Drift cards were placed at the water level in open areas of the channels, as well as in areas protected by vegetation (buffer zones). Samples were also taken from outside the plantation to obtain an estimate of drift out of the field.

All of the samples were stored frozen at −20 °C on site and were shipped by overnight courier to the Analytical Toxicology Laboratory, Department of Environmental and Molecular Toxicology at North Carolina State University, USA for analysis. All samples arrived frozen and in good condition.

Meteorological data combined with field measurements were used to calculate the rate of fungicide loss under tropical conditions. They were also used to quantify the amount of fungicide that was deposited to the soil, water and to the canopy compartments during application, allowing for an estimation of off target contamination. Water measurements were also used to evaluate possible runoff of fungicide from the field into the water channels. The decline of fungicide residues was calculated as an exponential decay function of the form:

$$\text{residue}_t = \text{residue}_{t_0} \times \exp^{-kt},$$

where k is the decay rate and t is days after application. The decay rate k and the correlation coefficient (r^2) were calculated with regression analysis of the concentration against the days after application for each curve, using the slope parameter as the least squares estimate of k . The half-life in days was calculated as $[\ln(0.5)]/k^{-1}$. The slopes of each curve, as well as the calculated half-lives, were compared with analysis of variance using SAS statistical software (SAS Corporation, Cary, NC, USA).

2.3. Test chemicals and analytical procedures

For pesticide applications, the water-based liquid formulation of CHT (2,4,5,6-tetrachloroisophthalonitrile), Bravo 720® (720 g a.i. l⁻¹) was used. Analytical grade CHT, hexachlorobenzene (HCB), pentachloronitrobenzene (PCNB), 2,4,5,6-tetrachloro-*m*-xylene (TCMX), 4,4'-dibromooctafluorobiphenyl (DBOBF, 2000 µg ml⁻¹ in *t*-butyl methyl ether), diuron, linuron, caffeine and 1,3-dicyanobenzene (metabolite VIII) had purities of over 97% and were obtained from ChemService Inc. (West Chester, PA, USA). 4-Hydroxy-2,5,6-trichloroisophthal-

nitrile (metabolite II, 97%) and 5-hydroxy-dicamba (98%) were obtained from the US Environmental Protection Agency (US EPA) National Pesticide Standard Repository (Fort Meade, MD, USA). Milligram amounts of 1,3-dicarbamoyl-2,4,5,6-tetrachlorobenzene (metabolite III, 97%) and small amounts of stock standard solutions in toluene of 2,5,6-trichloro-4-methoxy-isophthalonitrile (metabolite IV); 1-carbamoyl-3-cyano-4-hydroxy-2,5,6-trichlorobenzene (metabolite V); 2,4,5-trichloroisophthalonitrile (metabolite VI) and 2,5,6-trichloro-4-methylthioisophthalonitrile (metabolite VII) were donated by Dr. Raymond Putnam (University of Massachusetts, Amherst, MA, USA). Milligram amounts of 1,3-dicarbamoyl-2,4,5,6-tetrachlorobenzene were also synthesized (Rouchaud et al., 1989). Solvents (HPLC-grade) for gas and liquid chromatographic analysis were purchased from J.T. Baker (Phillipsburg, NJ, USA). Distilled methyl *tert*-butyl ether (MtBE) was purchased from EM Science (Gibbstown, NJ, USA). Water (HPLC-grade) was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Sodium chloride (NaCl), sodium sulfate (Na₂SO₄), ammonium formate, acetic, formic and sulfuric acids, and ammonium hydroxide (NH₄OH) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Disposable 1 ml OASIS[®] HLB SPE cartridges were obtained from Waters Inc. (Milford, MA, USA).

The analytical methods used in this study included a combination of gas and liquid chromatographic techniques, designed to reduce solvent consumption and to avoid the characteristic limitations of each technique without sacrificing selectivity and sensitivity. New methods for the analysis of CHT and metabolites in water and soil were developed using liquid chromatography coupled to mass spectrometry (LC–MS) atmospheric pressure chemical ionization (APCI) (Chaves, 2001). Other methods included modifications to existing methods (Ballee et al., 1976; Rouchaud et al., 1988; Putnam et al., 2003).

Banana leaves were analyzed for foliar dislodgeable residues of CHT, HCB, PCNB and 1,3-dicyanobenzene (metabolite VIII). Soil and sediment samples were analyzed for CHT, HCB, PCNB, and metabolites IV, VI, VII and VIII with gas chromatography coupled to mass spectrometry (GC–MS) and for metabolites II and III with LC–MS. Water samples were analyzed for CHT with an enzyme linked immunosorbent assay (ELISA) (RaPID Assays, Strategic Diagnostics, Inc., Newark, DE, USA). Positive ELISA samples were confirmed by solid phase extraction on OASIS[®] HLB cartridges, concentration under nitrogen and analysis by GC–MS. Drift cards were analyzed for CHT and HCB. Detailed descriptions of the analytical procedures, quality assurance protocol and instrumentation used in this study are found in Chaves (2001).

3. Results and discussion

Soil characteristics differed in the plots in the study area in particle size composition, textural class and total organic

Table 1

Soil characteristics of the experimental plots from the banana plantation in Costa Rica used in fate studies with chlorothalonil

Plot	Sand ^a (%)	Silt ^a (%)	Clay ^a (%)	Textural class	TOC ^b (%)	pH ^c
Control	13	24	64	Clay	NA	NA
3	32	54	14	Silt loam	4.4	6.1
4	42	38	20	Loam	4.1	5.5
6	40	47	13	Loam	4.4	6.2
7 (0–5 cm)	39	38	24	Loam	5.0	5.7
7 (5–10 cm)	28	34	38	Clay loam	3.2	NA
7 (20–30 cm)	27	29	45	Clay	1.4	NA
11	32	34	35	Clay loam	2.9	5.8

^a Analysis by hydrometer.

^b TOC = total organic carbon, analysis by flame ionization.

^c Analysis as described by Carter (1993); NA = not analyzed.

carbon (Table 1). For example, total organic carbon content in sediment samples ranged from 1.4% to 5.0%. In addition, average air temperatures in the area ranged from 22 to 26 °C. Maximum air temperatures were as high as 32 °C with minimum temperatures of 17 °C. Humidity ranged from 80% to 100% and soil temperature remained constant at 26 °C during the study. Rainfall amounts (mm) were recorded daily at midnight. The overall rainfall during this study was 442 mm. However, approximately 33% of the rainfall occurred during the first week of the study (142.9 mm), as compared to only 6% (28 mm) during the first week after the second application.

Analysis of background samples collected one week before the first application showed detectable levels of CHT in the soil, with maximum concentrations of 15 ng g⁻¹ (dry weight). CHT and metabolites were below detection limits in the leaf samples as well as in the reference control samples. Even though CHT had been intensively applied during previous years, with the last application only four months before the beginning of this study, the low background concentrations found in the soil may be due to the formation of soil-bound residues that prevent it from being biodegraded, as has been previously reported for this compound (Regitano et al., 2001).

3.1. Dissipation of CHT and metabolites in soil

CHT dissipated rapidly from the soil under tropical conditions with a half-life of 2.2 d (Fig. 1a). Dissipation was especially rapid during the first 24 h after application of the fungicide, with losses of up to 44% of the initial concentration. CHT continued rapid dissipation during the first week after application, following first-order exponential decay ($r^2 = 0.9682$, $p < 0.01$). After this period, CHT degraded slowly, with residues of 8 ng g⁻¹ ± 2.6 found 85 d after the third application (Fig. 1b). These results were in agreement with the mean background concentration of CHT of 12.8 ng g⁻¹ ± 2.3 (dry weight) found approximately four months after the last application in 1998. The half-life observed in this study was much faster than the 4–90 d commonly reported in the literature for

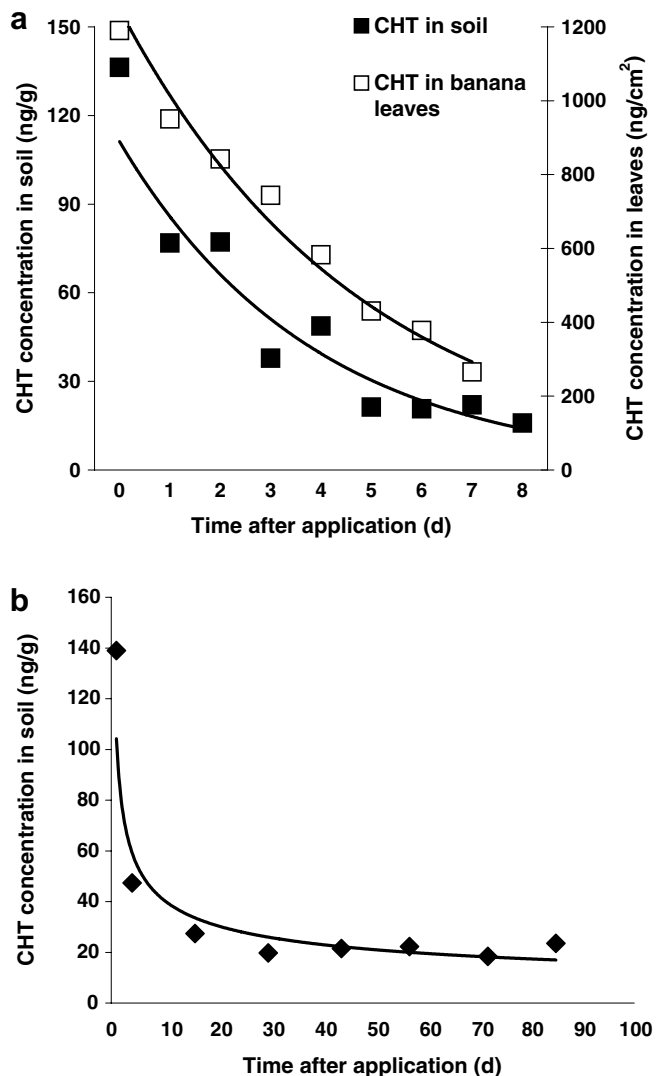


Fig. 1. (a) Dissipation of chlorothalonil (CHT) in soil and banana leaves over 7 d after the first application at a banana plantation in Costa Rica. (b) Dissipation of CHT in soil over 85 d after the third application.

temperate environments (US Environmental Protection Agency, 1999). Our results were more in agreement with a recent study by Potter et al. (2001) on the dissipation of CHT after foliar application to peanuts in Georgia, USA, in which they established a soil half-life of <1–3.5 d in laboratory incubation studies at 30 °C.

Sampling was not conducted during the first 4 d after the third application due to technical constraints. However, if an initial concentration equal to the average of the first two applications is assumed, a degradation curve that shows the long-term behavior of CHT can be constructed (Fig. 1b). In this case, CHT exhibited a very rapid exponential decay at the beginning, followed by a slow linear decay starting approximately two weeks after application.

A laboratory study (Regitano et al., 2001) reported that the dissipation of CHT in three different tropical Brazilian soils was very fast, with half-lives ranging from 0.4 to 13 d and varied by soil type. Dissipation was more rapid in soils

with high organic carbon content, due to faster formation of soil-bound residues and to increased microbial degradation. They also found that a significant fraction of the loss of CHT was due to the formation of soil-bound residues and not to biodegradation. However, they considered soil-bound residues as those that were not extracted by a very mild procedure that consisted of two extractions with 40 ml of acidic acetone for 15 min on a rotary shaker. This relatively weak extraction may have led to incomplete recovery of CHT and therefore, led to an overestimation of what was defined as “soil bound residues”. In this study, residues were extracted with 40 ml of a mixture of hexane–dichloromethane by shaking for 2 h on a rotary shaker. Several additional samples were extracted for 6 h without improving recovery, showing that extraction was complete. The formation of soil bound residues is of environmental concern, as tightly bound residues are less prone to undergo degradation or to be transported. Therefore, bound residues should not be considered the result of dissipation of the compound, as may have been reported by Regitano et al. (2001). Additional research is needed to evaluate the long-term effects of frequent applications of CHT on soil quality.

Analysis of CHT, HCB and metabolites in the different soil profiles are presented in Fig. 2. Soil profiles were collected 2 d after the second application of CHT and after an accumulated rainfall of 190 mm. We found that all CHT compounds were capable of leaching through the soil. The top 5 cm of the soil contained approximately 60% of the CHT residues, but some (12%) were detected as deep as 20–30 cm (Fig. 2). In contrast to CHT, concentrations of metabolite II increased with increasing soil depths, reaching the highest concentrations at 20–30 cm (85%). These results support batch equilibrium and aged

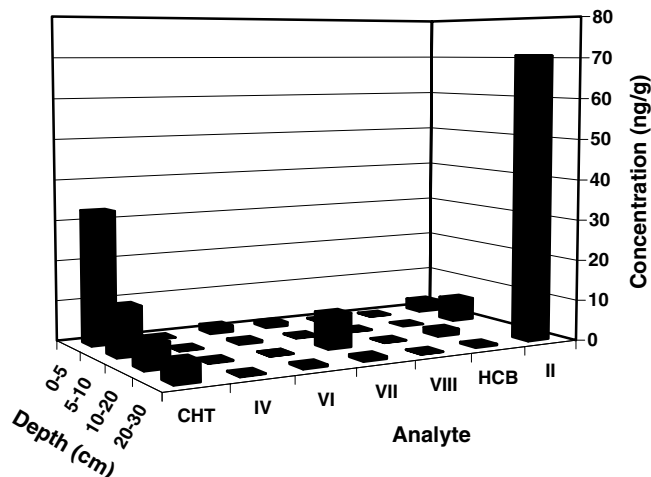


Fig. 2. Concentration of chlorothalonil (CHT) and metabolites in soil sampled 3 d after the second CHT application at selected depths; II, 4-hydroxy-2,5,6-trichloroisophthalonitrile; IV, 4-methoxy-2,5,6-trichloro-1,3-dicyanobenzene; VI, 2,4,5-trichloro-1,3-dicyanobenzene; VII, 4-methylthio-2,5,6-trichloro-1,3-dicyanobenzene; VIII, 1,3-dicyanobenzene; HCB, hexachlorobenzene.

column studies reported in the literature, in which metabolite II leached in several soil types, while CHT was only slightly mobile in silt clay loam, silt and sandy loam soils (US Environmental Protection Agency, 1999).

Metabolite II. Metabolite II was the dominant metabolite (Fig. 3b), reaching peak concentrations 6 d after the second application of CHT, accounting for as much as 65% of the total residues and reaching concentrations as high as 34.7 ng g^{-1} (dry weight). Residues of this metabolite were detected even 85 d after the third application. These results suggest that metabolite II is more persistent than the parent compound. Mobility in the soil, might also explain why the highest concentrations were achieved by the end of the first week after the second application; a period when rainfall amounts were very low.

Metabolite VI. Metabolite VI was the second most abundant metabolite detected in the soil (Fig. 3b), with concentrations as high as 8.5 ng g^{-1} (dry weight) 4 d after application. Residues persisted and were detected 85 d after application at an average concentration of 0.3 ng g^{-1} . Concentrations of this metabolite seemed to have increased with increasing amounts of rainfall. Moisture may lead to

increased hydrolysis of CHT in the soil, but also may cause transformation products to be formed on the surface of banana leaves that can be washed off and reach the soil surface. For example, previous reports have indicated that temperature and moisture are important parameters affecting the degradation of CHT and the formation of metabolites (US Environmental Protection Agency, 1999). However, there are no comparable studies on the degradation and formation of metabolite VI in soil. Metabolite VI has been previously identified in cranberry fruit at 28 and 45 d after two applications, and at concentrations of less than 25 ng g^{-1} (Putnam et al., 2003). No residues in fruit were detected 76 d after application in their study, but soil residues persisted and were detected in small amounts 76 d after application.

Metabolites III, IV, VII and VIII. Residues of metabolite III were below detection limits in all samples. Metabolite III has been reported as one of the main metabolites in soil from cranberry bogs, accounting for 41% of residues 76 d after application (Putnam et al., 2003). Rouchaud et al. (1988) also reported that metabolite III was one of the main metabolites in soil and cabbage crops. More recently, metabolite III has been identified as an intermediate form leading to the formation of 3-carbaryl-2,4,5-trichlorobenzoic acid (Roberts and Hutson, 1999). Regitano et al. (2001) reported 3-carbaryl-2,4,5-trichlorobenzoic acid as the predominant breakdown product of CHT in Brazilian soils, accounting for as much as 25% of the residues found 56 d after application. In our study, this breakdown product was not analyzed. However, because metabolite III is an intermediary in the formation of the acid, it is likely that if it was not detected in our samples, then the acid was not likely present. Moreover, the analysis of full scans in LC-MS and GC-MS showed no evidence of other significant metabolites.

Metabolites IV, VII and VIII were also detected in small concentrations in the soil (Fig. 3b) during the three applications. Small amounts of metabolites VII and VIII have been reported (Putnam et al., 2003) in soil 76 d after application. No reports on residues of metabolite IV were found in the literature.

The average concentrations of CHT, metabolite II and the summation of all the other metabolites during the three applications varied as a function of time (Fig. 3a and b). Overall, metabolite levels seemed to remain constant during the study period, suggesting that they may be more persistent than the parent compound. Metabolite II levels, on the other hand, seem to be more variable. Summation of all the residues did not account for the mass of CHT lost with time. Several factors may have contributed to the unaccounted loss of CHT in this study. First, the formation of soil-bound residues may not have been efficiently extracted by the analytical method used in this study. Potter et al. (2001) reported that less than 15% of the total CHT residues were recovered after three 30-min acetone extractions in laboratory incubations. They also reported an unpublished registrant study in which the soil-bound

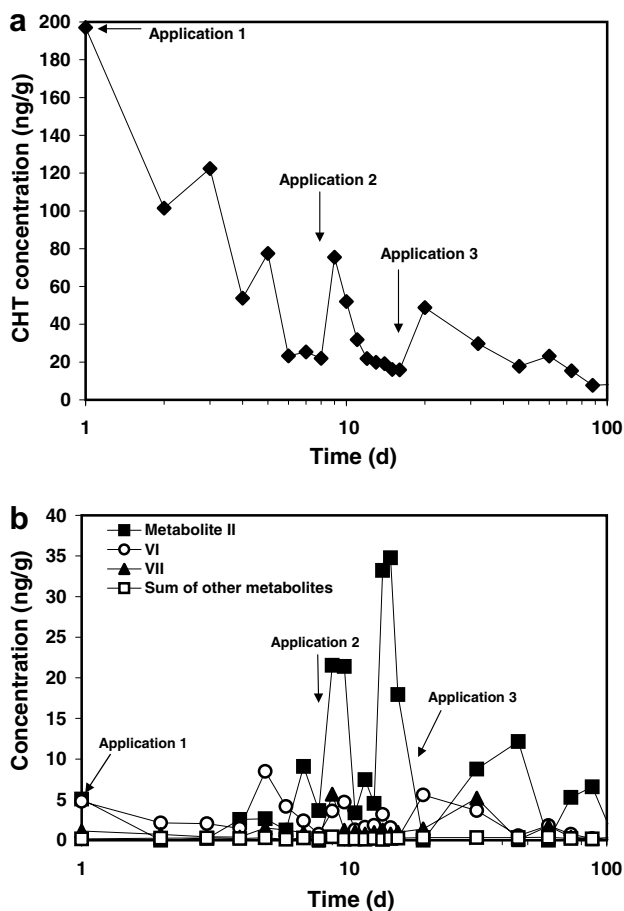


Fig. 3. (a) Concentrations of chlorothalonil (CHT) and (b) metabolites II, VI, VII and sum of all other metabolites in soil after three consecutive applications of Bravo[®] 720. Arrows indicate the point of application dates; II, 4-hydroxy-2,5,6-trichloroisophthalonitrile; VI, 2,4,5-trichloro-1,3-dicyanobenzene; VII, 4-methylthio-2,5,6-trichloro-1,3-dicyanobenzene.

fraction of CHT accounted for 40–75% of the initial dose after 90 d. Secondly, the formation of other major degradates, such as the 3-carbaryl-2,4,5-trichlorobenzoic acid (Regitano et al., 2001) were not analyzed in this study. However, this metabolite has been reported to be the result of further degradation of metabolite III and would have likely been detected in our study had the benzoic acid form been a major metabolite. Finally, several major factors that might have contributed to the loss of these metabolites from the soil are leaching and surface runoff, especially because of the identified mobility of these metabolites and the high amounts of rainfall in the area.

3.2. Field dissipation of CHT on the surface of banana leaves

CHT dissipated very rapidly on the surface of banana leaves (Fig. 1a), following an exponential decline, according to first-order kinetics ($r^2 = 0.9944$, $p < 0.01$) and with a half-life of 3.9 d. This half-life was unexpectedly short because CHT has been reported to degrade in cranberry foliage at a half-life of 12.7 d (Putnam et al., 2003).

The exponential decay parameters for CHT dissipation in soil and on banana foliage showed half-lives of 2.2 and 3.9 d, respectively (Table 2). Analysis of variance (ANOVA) showed no significant differences in the decay rates (k) and the half-lives ($t_{1/2}$) of CHT ($p < 0.01$) in the different plots within the same application as well as between applications 1 and 2. Therefore, the overall degradation rate, as well as the half-life of CHT in soil and on banana leaves, was calculated using all the individual measurements from the plots during applications 1 and 2 ($n = 70$). These parameters were not calculated after the third application, as sampling started 4 d after application, with no data on the previous days.

Table 2
Exponential decay parameters of chlorothalonil in soil and on banana leaves after the second application of Bravo 720® in a Costa Rican banana plantation

Compartment	Initial concentration	SD of initial concentration	k	r^2	Half-life (d)
Soil	136.3 (ng g ⁻¹)	95	0.31	0.9682	2.2
Banana leaves	1190.1 (ng cm ⁻²)	469	0.18	0.9944	3.9

k = decay rate constant; r^2 = correlation coefficient; SD = standard deviation.

Table 3
Deposition of applied chlorothalonil on the top of banana plants and to the soil surface

Application date	TA (μg cm ⁻²)	Top of the plants			Soil surface			Total (%)
		MA (μg cm ⁻²)	SD	Recovery (%)	MA (μg cm ⁻²)	SD	Recovery (%)	
February 9	8.71	3.08	1.16	35.5	2.24	1.31	25.7	60
February 23	8.71	2.72	0.62	31.2	1.51	0.59	17.3	48

TA = theoretical application; MA = measured application; SD = standard deviation.

3.3. Analysis of CHT in surface water and aquatic sediments

Concentrations of CHT were significantly greater ($p < 0.01$) in channels with slow-flowing water as compared to those with faster flows. In laboratory experiments, CHT has been found to degrade very fast under aerobic aquatic conditions. Depending on different experimental and environmental conditions, half-lives have been reported to range from 2 to 200 h (US Environmental Protection Agency, 1999). This might account for the relatively rapid loss of CHT in slow-flowing channel water. However, dissipation was even faster in the fast flowing channels, and this difference might be explained by the reduced residence time expected in channels with faster flows.

Sediments in the water channels were sampled twice during each of the first two applications at days 4 and 2, respectively. During the first sampling, CHT and metabolite II residues averaged 12 ng g⁻¹ (dry weight) whereas after the second one, CHT was detected at concentrations as high as 234 ng g⁻¹. Metabolite II levels remained constant at 9.6 ng g⁻¹ over this period.

3.4. Deposition of CHT to plantation soil

The recovery of applied CHT was calculated as a percentage of the application rate using the mean measured concentrations (Table 3). About 36% (±13%) and 26% (±15%) of the applied CHT was recovered from the top of the canopy and from the soil surface during the first application, respectively. Recoveries were less during the second application (Table 3). Deposition on the soil surface was less than that on top of the canopy; however, they did not differ significantly. These differences may have been due to the placement of drift cards on areas of the ground where maximum exposure to drift may have occurred. These results are less than deposition rates reported in other studies (Putnam et al., 2003); however, none of the previous studies were conducted in banana plantations. Ernst et al. (1991), who studied the deposition of CHT on a pond after aerial application, found that only 67–88% of the application rate was recovered with 20.3 × 25.4 cm glass fiber filters. Their results were slightly greater than the reported maximum of 70% (Maybank et al., 1976) under similar application conditions. Drift deposits on the plantation soil were expected to be low, as the canopy in a banana plantation is very dense.

However, the low recoveries from on top of the banana plants may have been due to the location of the drift cards. The drift cards located on the canopy were not in areas of maximum exposure to the applied CHT as banana plants are very tall and it was very difficult to reach the highest leaves. This may have accounted for lower values as the upper leaves might have interfered with the deposition of the drops.

Among some of the newer strategies to reduce contamination of surface water during aerial application of pesticides is the establishment of field borders, which includes the growing of bushes and other vegetation along the borders of the channels. These bushes grow at similar heights as the banana plants and have a very dense canopy. Concentrations of CHT on drift cards located at water level and below the bushes were significantly less, as compared to those obtained at water level in open channels. Deposits on the open channels were approximately 82 times greater than those in channels covered by bushes.

4. Conclusions

Concentrations of CHT, HCB and metabolites were measured in the soil for 7 d after the first two applications, starting approximately 2 h after spraying of the fungicide. For the third application, sampling did not start until 4 d after spraying. Interestingly, HCB and metabolites were detected in these post-application samples.

These results suggested that the source of these compounds might have been the formulation itself. Several degradation products of CHT, as well as HCB, have been identified in the fungicide formulation. The presence of metabolites II, V, VI and VIII in the formulation Bravo 720[®] (54% CHT), at 2.07%, 0.016%, 0.008% and 0.095%, respectively has been reported (Putnam et al., 2003). HCB is a known formulation by-product found at concentrations ranging from 0.02% to 0.05% (US Environmental Protection Agency, 1999). Measured concentrations of metabolite II, VI, VIII and HCB were 5.07, 4.78, 0.09 and 0.57 ng g⁻¹ (dry weight), respectively. Estimated concentrations calculated using the reported percentages of analytes in the formulation were 4.1, 0.02, 0.19 and 0.10 ng g⁻¹, respectively. Measured metabolite II levels correlated very well with the expected amounts (1.2 times greater). Metabolite VIII concentrations were two times less than the expected values. Measured concentrations of metabolite VI and HCB were 300 and six times greater, respectively, than the estimated concentrations. These differences might have been the result of differences in the handling of the mixtures of pesticides, as well as to the higher temperature and humidity characteristic of tropical environments that may enhance the formation of degradation products. The reported presence of HCB and CHT metabolites in the formulation, as well as the actual detection of residues found in this study, should be considered in the evaluation of pesticide residues in the environment. Additional studies should be conducted to carefully

examine the amount of these metabolites that may be directly broadcasted during the application of the parent compound.

Finally, CHT degraded very rapidly under tropical conditions. In soil, 24 h after application, approximately 45% of the initial concentration of CHT had already dissipated. Degradation was very rapid during the first week after application; however, CHT persisted and residues were detected 85 d after application. Half-lives in the soil and on the surface of the banana leaves were 2.2 and 3.9 d, respectively, assuming first-order degradation. The analysis of metabolites in soil showed that metabolite II was the main degradation product. Similar results have been reported for CHT elsewhere in the literature. Other metabolites were identified, however only in smaller quantities.

Acknowledgements

We thank Mr. Rafael Chaves for arranging shipment of samples from Costa Rica to North Carolina State University and for coordination with the many local Costa Rican agencies in obtaining all necessary permits. We thank Dr. Robert Bringolf for assistance with preparation of the figures.

References

- Ballee, D.L., Duane, W.C., Stallord, D.E., Wolfe, A.L., 1976. Chlorothalonil. In: Zweig, G., Sherma, J. (Eds.), *Analytical Methods for Pesticides and Plant Growth Regulators*. Academic Press, New York, NY, pp. 263–274.
- Carlo-Rojas, Z., Bello-Mendoza, R., Figueroa, M.S., Sokolov, M.Y., 2004. Chlorothalonil degradation under anaerobic conditions in an agricultural tropical soil. *Water Air Soil Pollut.* 151, 397–409.
- Carter, M.R. (Ed.), 1993. *Soil Sampling and Methods of Analysis*. Lewis Publishers, Boca Raton, FL.
- Castillo, L.E., Ruepert, C., Solis, E., 2000. Pesticide residues in the aquatic environment of banana plantation areas in the North Atlantic zone of Costa Rica. *Environ. Toxicol. Chem.* 19, 1942–1950.
- Caux, P.Y., Kent, R.A., Fan, G.T., Stephenson, G.L., 1996. Environmental fate and effects of chlorothalonil: a canadian perspective. *Crit. Rev. Environ. Sci. Technol.* 26, 45–93.
- Chaves, A., 2001. *Agrochemical environmental fate and behavior in banana plantations: a modeling approach*, Ph.D. Dissertation, North Carolina State University, Raleigh.
- Crepin, J., Johnson, R., 1993. *Soil Sampling for Environmental Assessment*. Lewis Publishers, Boca Raton, FL.
- Davies, P.E., 1988. Disappearance rates of chlorothalonil (TCIN) in the aquatic environment. *Bull. Environ. Contam. Toxicol.* 40, 405–409.
- Draper, A., Cullinan, P., Campbell, C., Jones, M., Taylor, A.N., 2003. Occupational asthma from fungicides fluazinam and chlorothalonil. *Occup. Environ. Med.* 60, 76–77.
- Ernst, W., Jonah, P., Young, J., Julien, G., Hennigar, P., 1991. The toxicity of chlorothalonil to aquatic fauna and the impact of its operational use on a pond ecosystem. *Arch. Environ. Contam. Toxicol.* 21, 1–9.
- Humbert, S., Margni, M., Charles, R., Torres Salazar, O.M., Quirós, A.L., Jolliet, O., 2007. Toxicity assessment of the main pesticides used in Costa Rica. *Agric. Ecosyst. Environ.* 118, 183–190.
- Lustig, T., 2004. *The search for the perfect banana*. Swedish Society for Nature Conservation, Stockholm, Sweden.

- Maybank, J., Yoshida, K., Shewchuck, S., Grover, R., 1976. Comparison of swath deposit and characteristics of ground-rig and aircraft herbicide spray systems. Report of the 1975 Field Trials, Saskatchewan Research Council, Saskatchewan, Canada.
- Motonaga, K., Takagi, K., Matumoto, S., 1996. Biodegradation of chlorothalonil in soil after suppression of degradation. *Biol. Fert. Soils* 23, 340–345.
- Potter, T.L., Wauchope, R.D., Culbreath, A.K., 2001. Accumulation and decay of chlorothalonil and selected metabolites in surface soil following foliar application to peanuts. *Environ. Sci. Technol.* 35, 2634–2639.
- Putnam, R.A., Nelson, J.O., Clark, J.M., 2003. The persistence and degradation of chlorothalonil and chlorpyrifos in a cranberry bog. *J. Agric. Food Chem.* 51, 170–176.
- Regitano, J.B., Tornisielo, V.L., Lavorenti, A., Pacovsky, R.S., 2001. Transformation pathways of C-14-chlorothalonil in tropical soils. *Arch. Environ. Contam. Toxicol.* 40, 295–302.
- Roberts, T., Hutson, D., 1999. Metabolic pathways of agrochemicals. Part 2: insecticides and fungicides. The Royal Society of Chemistry, Cambridge, United Kingdom.
- Rouchaud, J., Roucourt, P., Vanachter, A., Benoit, F., Ceustermans, N., 1988. Hydrolytic biodegradation of chlorothalonil in the soil and in cabbage crops. *Toxicol. Environ. Chem.* 17, 59–68.
- Rouchaud, J., Roucourt, P., Metsue, M., Herin, M., Moulart, C., 1989. Nucleophilic reactions of OH⁻ and CH₃O⁻ with tetrachloroisophthalonitrile. *Bull. Soc. Chim. Belg.* 98, 211–214.
- Sato, K., Tanaka, H., 1987. Degradation and metabolism of a fungicide, 2,4,5,6-tetra-chloroisophthalonitrile (TPN) in soil. *Biol. Fert. Soils* 3, 205–209.
- Sherrard, R., Murray-Gulde, C., Rodgers, J.J., Shah, Y., 2003. Comparative toxicity of chlorothalonil: *Ceriodaphnia dubia* and *Pimephales promelas*. *Ecotox. Environ. Safe.* 56, 327–333.
- US Environmental Protection Agency, 1996. Chlorinated herbicides by GC using methylation or pentafluorobenzoylation derivatization, Washington, DC.
- US Environmental Protection Agency, 1999. Chlorothalonil: reregistration eligibility decision (RED), EPA738-R-99-004, Washington, DC.
- Van Eeden, M., Potgieter, H.C., Van der Walt, A.M., 2000. Microbial degradation of chlorothalonil in agricultural soil: a laboratory investigation. *Environ. Toxicol.* 15, 533–539.