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Use of nuclear and related techniques in studies of agroecological effects resulting from the use of persistent pesticides in Central America

Report of a final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Panama City, Panama, 20–24 April 1998

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FOREWORD

The use of pesticides for the control of pests of agriculture and vectors of human and animal diseases in the countries of Central America is the highest per capita and one of the most intense in the world. There are reports of acute toxicity and chronic effects among farm workers. There are also reports that pesticide residues in food frequently exceed the Codex Alimentarius Commission's maximum residue levels (MRLs) and shipments of foodstuffs have been rejected by importing countries due to the presence of excessive residues of pesticides. Pesticides are also implicated in the contamination of continental and coastal waters. The indiscriminate use of pesticides would be expected to also aggravate pest problems by adversely affecting populations of beneficial arthropods and causing the development of resistance in pest populations.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture initiated a co-ordinated research project in 1992 to generate information on residues of pesticides in the environment, their persistence under local conditions and effect on local species of beneficial arthropods in agricultural and adjacent areas in the countries of Central America. Such information could be used in the implementation of legislation to control the distribution and use of pesticides and the development and application of integrated pest management programmes. Scientists from Costa Rica, Guatemala, Honduras, Nicaragua, Panama and the United States of America participated in this project.

This TECDOC reports on the accomplishments of the project and includes the papers presented at the final Research Co-ordination Meeting held in Panama City, Panama, 20–24 April 1998.

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The IAEA officer responsible for this publication was M. Hussain of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY OF THE CO-ORDINATED RESEARCH PROJECT

INTRODUCTION

Central America is a region of seven countries with a combined population of 30 million people. Agriculture is the most important sector of national economies and the driving force behind regional economic and social development. Export of crops such as coffee, bananas, cotton and sugar as well as cattle continue to be the cornerstone of the region's economies

Pests of agricultural crops, which include insects, nematodes, disease organisms and weeds, are known to cause enormous losses of potential yield, if not controlled. Similarly, huge amounts of food are lost during storage as a result of attack by insects, rodents, birds and disease organisms. In addition, the insects, ticks and mites which infest animals and man carry and transmit diseases which cause immense suffering and financial losses.

Pesticides are used as an essential agricultural input to control pests. They are credited with alleviating some of the problems associated with food production and storage as well as protection of the health of humans and animals against vectors of diseases. For these reasons the use of pesticides has been increasing, especially in developing countries. In Central America the use of pesticides is one of the heaviest in the world. The export oriented agriculture of this region has become heavily dependent upon capital and technology intensive production strategies, which emphasise short term economic growth, paying little attention to longer term sustainability. The heavy use of pesticides has been a result of this production strategy [1]. The pesticide application rates in the region increased from an average of 8 per season in the 1950s to over 40 per season in the 1970s and 1980s as pest resistance and subsidised pesticide prices led to an extreme overuse of the technology.

PROBLEM

Pesticides have been deliberately developed to be toxic to some living organisms and because of this their use involves risks to human health and the environment. Excessive residues in foodstuffs could have deleterious acute or long term effects on the health of the consumers. Pesticides may also be hazardous to non-target species. Chemicals applied to crops can be washed away with rain and be transferred into groundwater or lakes and rivers and from there into estuaries and harbours, where they may adversely affect fish, shrimp and other aquatic life [2]. In Central America the inappropriate use of pesticides has led to economic losses including high production costs and, in some cases, to unprofitable cultivation; risk to humans, animals, beneficial insects and other non-target species; contamination of the environment, including water resources; burdening of the foreign exchange with high cost of imported pesticides; and adverse impact on trade due to the rejection of agricultural products by importing countries because of the presence of excessive pesticide residues.

There are reports of acute toxicity and chronic effects among the farm workers of Central America from the use of pesticides, including identification of over 27 000 cases of acute poisoning in the 1980s by the Pan American Health Organization (PAHO). The pesticide related illness among the rural populace soared to among the highest per capita rate during the 1980s. By 1971 women in Guatemala were found to have 12.2 mg/kg of organochlorine residues in their breast milk, 250 times higher than maximum allowable residue levels for human consumption in cow's milk [3]. There are also reports that pesticide residues in food

frequently exceed Codex Alimentarius maximum residue levels (MRLs) and shipments of foodstuffs have been rejected by importing countries due to the presence of excessive residues of pesticides. Pesticides are also implicated in the contamination of continental and coastal waters, especially from the effluent coming from plantations and causing ecotoxicological problems. The indiscriminate use of pesticides would be expected to also aggravate pest problems by adversely effecting populations of beneficial arthropods and causing the development of resistance in pest populations. However, not much documented information is available on these problems.

OBJECTIVES

The short term objectives of the co-ordinated research project (CRP) were to support co-ordinated studies on the environmental effects of pesticides, in particular to generate information on residues of pesticides in the environment, their persistence under local conditions and their effect on selected species of beneficial insects in agricultural and adjacent areas.

The longer term objectives were to generate information which could be used in (i) the implementation of legislation to control the distribution and use of pesticides, and (ii) the development and application of integrated pest management programmes.

Project activities were also expected to result in improved institutional capacities and increased awareness in the region about the potential of pesticides to cause harm to human health and the environment.

APPROACH

Project activities focused on the following three areas:

- Monitoring pesticide residues in water and soil/sediment.
- Study of the persistence/degradation of pesticides and their uptake by plants.
- Study of the effect of pesticides on non-target species, especially the beneficial insects.

In addition to directing research effort towards these areas, the CRP was expected to provide information on the following aspects:

Pesticide monitoring:

(a) Assessment of each participating laboratory's capability in analysing the pesticides under study by chromatographic methods, including confirmatory analysis capabilities and reproducibility of results.
(b) Inter-laboratory comparison of analytical results.
(c) Water quality in the region.
(d) Identification of the principal pesticides found in the surface and ground waters of different countries.
(e) The relationship between pesticides found in the water with those used in the area.

On the study on the environmental fate of pesticides, the project would provide information on their persistence and metabolic profile in different areas. This type of information could then be used by farmers to choose proper timing for planting follow-up crops, by health authorities to protect public health, and by regulatory authorities for registration of pesticides.

Study of the effects of pesticides on beneficial insects would provide information on the safety of the pesticides used or the potential to cause harm to beneficial insects. This information would also be useful for regulatory authorities in pesticide registration and by farmers and government authorities to assure that pesticides used are compatible with the IPM systems in practice.

THE PROJECT

The project involved twelve research contracts, one in Guatemala, two in Nicaragua and three each in Costa Rica, Honduras and Panama. Five of the research contracts holders monitored pesticide residues, two studied their environmental fate and metabolism, and four studied the effects of pesticides on beneficial insects. In addition to these studies, two research agreements holders in the United States of America provided assistance to the contract holders in the introduction in their laboratories and validation of procedures for the extraction of pesticides from water using solid phase extraction (SPE) devices.

RESULTS

1. Monitoring pesticide residues in water and soil/sediment

Water samples from predetermined sampling locations from drainage ditches, streams and creeks within banana plantations, lakes, lagoons, rivers, estuaries and coastal areas of these countries were collected at predetermined sampling times and analysed for targeted pesticides (insecticides, fungicides and herbicides) including those which are currently used in agriculture and some of the older organochlorine pesticides which were used in the 1980s but are presently banned. Some samples of soil, sediment and aquatic organisms were also analysed. The analyses did not include metabolites and degradation products, some of which are also known to have acute and/or chronic toxicity to aquatic and/or terrestrial organisms.

Because of the warm and humid climate in these countries, fungal diseases of plants are a major problem. Therefore a variety of fungicides are used. For example in Costa Rica fungicides are sprayed on banana plantations 50 times a year by aircraft. Similarly, pesticides which are effective against insects as well as nematodes, the insecticides/nematocides, are applied as ground sprays several times a year. The herbicides are also quite frequently used in agriculture as well as banana plantations. While despite more frequent application the fungicides used have low toxicity to non target species, the insecticides/nematocides are generally more toxic, especially to mammals as well as the aquatic organisms. Therefore, residues of these compounds are of greater ecological and human health concern .

The analysis of water indicated widespread contamination with pesticides in surface waters from drainage ditches and creeks within the banana plantations as well as rivers, lakes, lagoons and coastal waters. In general the fungicides were present at high levels, and insecticides/nematocides at low levels. However, in some cases concentrations of insecticides exceeding their acute toxicity or LC50 levels (concentration which kills 50% individuals in a population) were also found.

In Costa Rica, water samples from drainage ditches in banana plantations and from the Rio Suerte Basin, which receives effluent from banana plantations, contained residues of pesticides used on banana plantations. In general, they were below the LC50 levels for daphnia or rainbow trout, the aquatic animals commonly used as aquatic toxicity indicator species. However, maximum concentrations of cadusafos (0.4 µg/L) and terbufos (1–2 µg/L)

in the drainage canal and creek water samples were close to their acute toxicity levels for fish. A majority of the water samples from the Suerte river, which receives effluent from the banana plantations, contained low level residues of cadusafos, chlorpyrifos and several other pesticides. Since some of the pesticides were found at a high frequency, and even though their concentrations were low, they may cause chronic toxicity. Cadusafos was found in 60% of the water samples from the Suerte river at concentrations ranging between 0.02 and 0.40 µg/L. The chronic toxicity reference value of this insecticide for daphnia is 0.16 µg/L, indicating that this organism is at risk. Organophosphate (cadusafos, chlorpyrifos and terbufos) and carbamate (carbofuran) insecticides have similar physiological action, i.e. they inhibit the enzyme cholinesterase in animals, and the pesticides with a similar physiological action can have additive effects. This is consistent with the legal concept of risk assessment which requires that a cumulative risk from each pesticide and from others of similar action be lumped together for a total risk.

This information about the widespread contamination of water in Costa Rica is not new or surprising. It confirms similar earlier findings. In 1992 Costa Rican authorities charged the Standard Fruit Company of USA, the major banana grower in the Valle de la Esterella, with polluting rivers and underground water of the area, and presented the case to the International Water Tribunal in Amsterdam. The charge was based on detection of chlorothalonil, chlorpyrifos, terbufos and ethprop pesticides in 88% of the surface and ground water samples. Some of the samples contained up to 40 µg/L chlorothalonil fungicide, which is close to its LC50 value of 44 µg/L for channel catfish, and far above the 3–6.5 µg/L level which is known to produce adverse reproductive effects in fish. The Tribunal had upheld the complaint and ruled that the Standard Fruit Company should phase out the use of pesticides recognised as being extremely hazardous and minimize the use of others.

Data in the present study show that chlorothalonil was present in 10% of the water samples from the Suerte river, at a concentration of 0.04 µg/L. Similarly, 2% of the drainage and creek samples in banana plantations contained chlorothalonil at a concentration of 0.9 µg/L. This indicates that the use of this fungicide has been reduced. However, it appears that chlorpyrifos, terbufos and cadusafos, all very toxic insecticides, are still commonly used in banana plantations and their residues are found at a high frequency in water samples. There is a need to find ecologically friendlier alternatives to these chemicals.

In Guatemala, surface waters were sampled along the coast from the Mexican border to the border with El Salvador and along the length of the largest rivers that drain into the Atlantic Ocean, both regions being of great agricultural activity. In addition, water samples from lagoons, a lake and municipal water system were analysed for 38 targeted pesticides. The pesticides most frequently found included cypermethrin, aldrin, chlordane, endrin, endosulfan, chlorpyrifos, diazinon, azinphos methyl and malathion. DDT and its metabolites DDD and DDE were also found in several water samples. Cypermethrin was found in concentrations up to 5 mg/L, which is alarming in view of its LC50 value of 2.8 mg/L for rainbow trout. Endosulfan, used as a general purpose insecticide and in coffee plantations, was also found in very high concentrations of up to 3.8 mg/L. This pesticide is also classified as highly toxic to fish. This means that two of the pesticides detected in high concentrations in water put the aquatic life at a great risk.

Some of the most frequently detected pesticides in Guatemala were not the ones regulated by the Guatemalan legislation on potable water, which is in fact copied from the US EPA regulation of 1964. The Guatemalan water quality legislation is under review to upgrade it to

the standard of the 1996 version of the US EPA regulation. This CRP has contributed data directly to the new Guatemalan water quality legislation.

In Honduras the water from wells, lagoons and springs and water and sediment from estuaries, lakes, and basins of Choluteca and Nacaome rivers were found to contain low concentrations of organochlorine and organophosphorus pesticides. More than 20% of the river, lagoon and well water and 30% of the sediment samples analysed contained low levels of pesticides. The contamination of the sediment increased from the northern areas, where more traditional agriculture is practised, to southern areas of more intensive agriculture, by about ten fold for some pesticide residues. The pesticides encountered most frequently included DDT and dieldrin in the sediment and chlorpyrifos, endosulfan and heptachlor in the water. Water samples collected during the dry season (December–May) had higher levels of residues than other time of the year.

Some river water samples had very high levels of contamination, including carbofuran (9.23 mg/L), diallate (2.8 mg/L), propiconazole (1.79 mg/L) and parathion (0.8 mg/L). Such a high level of carbofuran is suspected to be due to point source contamination. Concentrations of carbaryl, chlordane, 2,4-D, heptachlor, parathion and quinomethionate exceeding LC50 levels for some of the aquatic organisms were also found in several water samples from Choluteca river and estuaries. Similarly, most of the sediment samples from Choluteca river, estuaries and Lake Yojoba had high levels of organochlorine pesticides including chlordane (0.074 mg/kg), DDE (2.44 mg/kg), DDT (9.0 mg/kg) and heptachlor (0.09 mg/kg). The contamination of sediment samples with high frequency (30%) with the now banned organochlorine pesticides may be from their use in the 1980s in cotton cultivated in the southern part of the Choluteca river basin. However, residues of heptachlor were also detected at a high frequency in water samples from the Choluteca river and estuaries and from more than 20% of the lagoon and well water samples, which may indicate illegal use of this insecticide.

Muscle tissue from the river fish was contaminated with DDE and lindane at concentrations of 1.05 and 0.08 mg/kg, respectively. Pesticide residues were also found in the tissue of farm fish and these included aldrin, chlorpyrifos, chlordane, DDT, dieldrin, endrin, endosulfan, heptachlor and lindane insecticides at concentrations ranging between 0.003 and 0.27 mg/kg. DDT and lindane, each at a concentrations of 0.003 mg/L, were detected in cows milk and DDT (0.177 mg/kg) and dieldrin (0.06 mg/kg) were found in the kidney adipose tissue of the cow. Analysis of hay showed residues of endosulfan (0.06 mg/kg), HCB (0.056 mg/kg), methyl parathion (0.06 mg/kg) and quinomethionate (0.02 mg/kg). However, no detectable residues were found in the field samples of tomato, onion, cabbage, corn and cucumbers. Laboratory bioassay of endosulfan and lindane insecticides and propiconazol and tridemorph fungicides on marine shrimp confirmed that the two insecticides were more toxic to the shrimp than the fungicides.

Pesticide contaminated water from the Cholutecan watershed, where intensive melon cultivation takes place, flows directly into the Gulf of Fonseca, where shrimp cultivation is practised. The widespread contamination of the water with the pesticides puts shrimp cultivation at great risk. No systematic studies have been undertaken on this subject but there have been cases of massive shrimp kill in which pesticide contamination is suspected.

In Nicaragua well waters were monitored at three sites with a history of cotton production but where now sugarcane and ground nuts are grown. A number of organochlorine pesticides were

detected, alpha-BHC, beta-BHC, lindane, heptachlor, aldrin, dieldrin, DDT and its metabolites DDD and DDE, and toxaphene. In addition, ethyl parathion was found in two wells and ethion in one. In some cases concentrations were above values permitted by water quality standards. This information was shared with the Ministry of Health.

In Panama low levels (less than 0.1 μg/L) of alachlor, bromacil, chlorothalonil, chlorpyrifos and endosulfan were found in surface water samples from Chiriqui Viejo river. Traces of chlorothalonil and chlorpyrifos were also found in water samples from wells in the vicinity of the river.

Tests with solid phase extraction (SPE) devices for pesticide extraction from water

Pesticide residue analysis involves complex procedures and the use of sophisticated analytical instruments. Sustainable pesticide residue analytical operation depends on the skill of the analyst and the cost and availability of the instruments, spare parts and materials. Traditional analytical methods involve considerable use of organic solvents, some of which are hazardous for human health and the environment. The cost of procurement, storage and disposal of the organic solvents can be limiting factor for the entire operation, especially in developing countries.

Recently, new procedures have been introduced to improve the efficiency of extraction of pesticides from water by using solid phase extraction (SPE) devices. Instead of extracting pesticide contaminated water with large volumes of organic solvents, the water is passed through a SPE cartridge or a disk which retains the organic compounds, which can then be eluted with a small amount of an organic solvent and analysed for pesticides. The participants validated a pesticide extraction method introduced by Professor Anson Moye of the University of Florida, USA, based on the use of C-18 Empore™ SPE disks. The participants compared this method with other methods of pesticide extraction including a variety of SPE cartridges and disks as well as solvent-solvent extraction. In general extraction with C-18 Empore™ disks was satisfactory for most pesticides but not for all. The participants felt that for reliable analysis it is necessary to gain considerable experience in the optimisation of the technique. The disks need to be conditioned in ethyl acetate prior to use and this may limit their use in the field operation. Nevertheless, for many collaborators it was the first exposure to this valuable method and as it is projected to be the technique of the future in the environmental monitoring of pesticides, the project greatly benefitted from this exercise. It is now used in all participating laboratories.

2. Studies on the environmental fate of pesticides

Pesticides in the environment are subject to dissipation, which may be due to evaporation, leaching in the soil/sediment, degradation and transformation to other products/metabolites. Most of the older organochlorine pesticides are quite stable in the environment but the newer generation compounds are in general less persistent. Most of the transformation products/metabolites are less toxic, but some are more toxic than the parent compound. The persistence of pesticides and their metabolites is greatly influenced by factors such as the temperature, moisture, duration and intensity of light, and the pH and microbial compositionof the soil. Obviously, these parameters change from one location to another. Therefore, in order to have a realistic picture of the environmental fate of a pesticide, it is necessary to study it under the conditions where it is used. This information can be used by health authorities to protect public health, by regulatory authorities for the registration of pesticides, and by

farmers to choose proper timing for planting of crops in lands which have been previously treated with herbicides

Endosulfan is an organochlorine insecticide commonly used in the countries of Central America for the control of insect pests of fruit, vegetables, coffee and cereals. Unlike many of the other organochlorine pesticides, its use is not banned because it is not as persistent as DDT or some of the other organochlorines the use of which is now restricted/banned. However, the environmental and soil conditions profoundly effect the persistence and fate of a pesticide. Therefore, the degradation, leaching and binding of ^{14}C endosulfan insecticide in different types of soil in model lysimeters and persistence on melon plants was studied in Panama and its persistence on tomato plants and in soil was studied in Costa Rica.

The tests showed that the dissipation of endosulfan was faster in sandy loam soil, with a half-life of 38 d, than in silty clay soil, with a half-life of 61 d. In sand it was most stable, with a half life of 91 d. However, leaching took place only in the sand and not in the two agricultural soils. Six months after the application to the soil only 8.5% of the pesticide was recovered from the silty clay soil and 27% from the sandy loam, confirming that soil characteristics can greatly influence the persistence of this pesticide Most of the pesticide that remained in the soil was tightly bound and only 2–3% was extractable with organic solvents. The immobilised residues should, therefore, have a low potential for contamination of the ground water or adverse effects on non-target species. On melon plants the dissipation was fast. Only 10% of the applied insecticide remained on the leaves after 3 weeks and no detectable residues were found after 4 weeks.

Tests in Costa Rica showed that the dissipation of endosufan was rapid on tomato plants and in the soil. On the tomato plant the residues declined from 3.7 to 1.2 mg/kg and in the soil from 0.25 to 0.17 mg/kg between the 7th and the 19th day after the application. The residues on the plants comprised the parent insecticide as well as its sulphate metabolite, whereas in the soil the lactone and ether metabolites were also present. But only the sulphate metabolite is of toxicological significance. Analysis of tomatoes from the local market showed no trace of the insecticide or metabolites. The analyses were performed by using a validated analytical method under GLP (Good Laboratory Practice) environment. Terbufos is a relatively less persistent organophosphorus insecticide and nematocide which is commonly used for the control of soil dwelling arthropods and pest insects feeding in and on the plants. Greenhouse tests with radiolabeled terbufos insecticides showed that planting of corn enhanced the dissipation of the insecticide in the soil. Two months after the application 46% of the insecticide remained in the soil in which no plants had been grown and 31% in the soil in which corn had been grown.

3. Study of the effect of pesticides on non-target species

Study of the effects of insecticides on insect pests and on the yield of crops showed that although the insecticide applications temporarily relieve the pest pressure on the crop, pest populations resurge because of the adverse effects of insecticides on their natural enemies.

In Panama melons are grown for export. The crop is attacked by aphids among other insect pests. Several insecticides including endosulfan and fenitrothion are used for the control of aphids. However, they are also toxic to the natural enemies of the aphid, especially two insect species: Chrysoperla carnea and Cycloneda sanguinea, and therefore, insecticides interfere with the natural control process. Field experiments were performed in Los Santos Province of Panama to evaluate the effect of insecticide applications on the beneficial insects and the yield

of melons. The results indicated that although a mixture of six insecticides used by commercial producers was more effective than endosulfan or fenitrothion in reducing the populations of aphids, it also adversely affected the natural enemies of the aphids, and the yield of melons was somewhat greater with endosulfan or fenitrothion than with the mixture of insecticides used. Endosulfan was not persistent and its residues declined rapidly in the soil as well as on the melon leaves within ten days after the application. Most of the residue was in the form of endosulfan sulphate metabolite.

In Nicaragua two toxic insecticides carbofuran and chlorpyrifos are used for the control of insect pests of maize, which include two major pests, *Spodoptera frugiperda*, the armyworm, and *Dalbulus maidis*. Field experiments with chlorpyrifos applications to maize showed an increase in the yield of maize, but it was not significant. The insecticide reduced the population of the armyworms, which resurged afterwards. This is because of the adverse effects of the insecticide on parasitoids of the pest insect, as indicated by the reduced numbers of the parasitised armyworms. Insecticide sprays did not control the population of *D. maidis*, which increased more rapidly in the insecticide treated than the untreated plots. Populations of the beneficial arthropods, including *Cycloneda* spp., spiders, earwigs and ants decreased after each insecticide spray, indicating an adverse effect on their populations. These tests clearly indicate interference through insecticide applications of the natural pest control process in the maize agroecosystem.

In Costa Rica the effect of terbufos was evaluated on the larvae of *Diatraea saccharalis*, a pest of sugarcane and maize and commonly known as the sugarcane borer, and its Braconid parasitoid, *Cotesia flavipes*. Feeding on diet contaminated with sub-lethal doses of the insecticide caused the borer larvae to lose weight, prolonged the duration of the larval instars, and altered the sex ratio of the emerging adults, with fewer than normal females emerging. The insecticide, however, also adversely affected the parasitoid developing inside the poisoned host larvae, and its survival inside the host as well as after emergence was reduced. The emerging adult parasite wasps had reduced weight, which may adversely effect their ability to find and parasitize the host.

In Honduras a selected number of insecticides used for insect pest control in cabbage, corn, tomato and melons, were screened for toxicity to two beneficial insects: *Telenomus remus*, an insect parasite of armyworm, and *Chrysoperla carnea*, a common predatory insect. In general, organophosphorus and carbamate insecticides were more toxic than the organopyrethroids, difenthrin and abamectin insecticides.

These limited studies clearly indicate that the use of insecticides in agriculture in Central America has the potential to interfere with the natural control process by adversely affecting the insect parasites and predators of pests. The generated information, indicating differences in the toxicity of different insecticides to beneficial insects, can be utilised to intelligently select insecticides, if they must be used, to make sure they are compatible with the other components of integrated pest management and do not disrupt it. It would be even better to screen insecticides for their comparative toxicity to the pest in addition to the beneficial insects. Clearly, additional research is needed in this area, preferably on a regional basis.

CONCLUSIONS

- The results of this CRP demonstrate that there is widespread pesticide contamination of surface and groundwaters in Central America. This contamination is associated with

agricultural activities and the pesticides have entered the food chains and potable water resources. Some water sample contained concentrations of pesticides exceeding levels which are considered toxic to some aquatic organisms.

- It is of concern to find residues in water of pesticides such as aldrin, dieldrin, DDT, HCH, heptachlor and parathion, which are on the list of Prior Informed Consent (PIC) provision of FAO's Code of Conduct on the Distribution and Use of Pesticides. Most likely, they are residues from earlier use on cotton which was grown in the area. However, there is need to strengthen national environmental legislation to reduce further the chances for the use of pesticides which have the potential for harm to human health and the environment. An EC regulation of July 1992 makes compliance with PIC provisions of the FAO Code mandatory in EC member countries. Adoption of such a measure and incorporation of the provisions of the FAO's Code into national legislations of countries in Central America would make it legally binding in these countries and, thereby, eliminate the use/misuse of the pesticides on the PIC list.

- The major impact of this CRP is that the information generated is now being used by government authorities. In Guatemala the establishment of the analytical laboratory was influenced by the CRP and the information generated was directly used by the government to upgrade the water quality legislation. Some large producers of export crops and the local tobacco company have attempted to establish their own facilities for residue analysis but have not succeeded because of lack of suitably trained personnel. Consequently, there are clear opportunities for this laboratory to earn a significant income for the University from contract work and play an important role both in training and in providing service analyses. In Nicaragua results of research on the residues of pesticides in water and their effect on water quality are now shared with the Ministry of Health on a regular basis and potable water is also being sampled in collaboration with the local water company. In Panama the CRP stimulated the establishment of analytical laboratory at the University of Chiriqui and there is a good deal of local interest in the development of this laboratory notably from the Ministry of Health and the Municipal Water Authority as there is no possibility of analyzing water for pesticides elsewhere. This may provide an opportunity for the laboratory to establish a lucrative programme of contract analyses. The cattle producers association are also exerting pressure for political support as they regard the existing analytical facilities in Panama City as being too remote for their needs. The laboratory has been asked by the government to provide service to local cattle owners and farmers by analyzing pesticide residues in meat and fruits and vegetables, respectively.

- The information generated could be used by pest control personnel in improving the implementation of integrated pest management (IPM) practices. For example, a system to breed *Spodoptera* larvae in the laboratory has been developed which enables the crop to be infested artificially to supplement the natural infestation, if it is low. However, considerably more information needs to be generated. For, example, nothing seems to be known of the population dynamics of parasitoids in the absence of pesticides, so no baseline data are available. The test plots of 40 × 40 m used may be too small in relation to parasitoid mobility.

- In view of the magnitude and extent of the pesticide contamination of agro- and aquatic ecosystems there is a need to continue research in this area, preferably on a regional basis, to identify ecologically more compatible agricultural practices which result in reduced use of chemical pesticides. The pesticides used should not only be effective against agricultural

pests of economic importance, but they should be less persistent in the environment, have reduced potential to cause harm to human health and non-target biota and be compatible with the IPM schemes practised.

- As a result of participation in the CRP, analytical skills within participating laboratories were greatly improved and significant progress was made in the introduction of GLP and quality assurance and quality control (QA/QC) procedures. Awareness of the need to introduce management systems to assure the quality of data was enhanced, and in Costa Rica and Guatemala laboratories have now reached a high standard, with both laboratories planning to obtain accreditation soon. It would be most desirable that all participating laboratories fully implement GLP/ ISO 25 procedures and become accredited for pesticide residue analytical work. One other unique achievement of the CRP was to establish a regional co-operative research environment between pesticide chemists and national IPM staff, and great appreciation was expressed by the participants for the opportunity made available by FAO/IAEA and SIDA to them to jointly work on a problem of regional dimension.

- The participants expressed great satisfaction with the results achieved in research through this CRP despite a number of problems. These were generally the result of unreliable services, notably electricity and communications, and difficulty in obtaining supplies in a timely manner. Theft of equipment, and in one case, maize ears from field plots were other causes of difficulty, while a general problem was frequent turn over and shortage of trained staff.

In summary therefore, the main outputs and outcomes of this project were that extensive information was obtained on the scale of contamination of environmental matrices with pesticides and that this is being used by regulatory authorities to improve national environmental legislation. In addition, information was obtained on the toxicity of some pesticides to beneficial insects in the region and this will be used to design improved IPM programmes. Also, participants learned procedures for using solid phase devices for the extraction of pesticides in water and these are now being used in national programmes on environmental monitoring. And finally, the project has done much to stimulate inter-regional co-operation in pesticide monitoring and in building awarness of the potential harm to human health and the environment from the unwise use of pesticides.

RECOMMENDATIONS

The following recommendations were made by the participants of the CRP:

- Monitoring pesticide residues in the aquatic environment on a regional basis should be strengthened with special focus on possible impact of pesticides used in agriculture on other activities of economic importance (such as shrimp farming), on preserving the quality of water used for human consumption, and ecotoxicological effects on aquatic organisms. The use of biological indicator species would provide useful information on the possible impact of pesticides on fish, shrimp and other aquatic organisms of economic importance.

- Monitoring the aquatic environment should include analysis of not only the waters, but also of sediment and aquatic organisms. The mobility and fate of pesticides in the environment should be studied using radiotracer and chromatographic techniques. These are important for learning how different pesticides interact at the sediment water interface, how and to

what extent they are taken up by aquatic organisms, and to learn about the characteristics of their metabolites and the behaviour of these under local conditions.

- For such studies sampling and analytical protocols and procedures should be harmonised among the collaborating institutions to ensure high quality results. Methods employed should be fully validated, and this should include interlaboratory proficiency testing. All procedures should follow ISO 25 and the OECD guidelines on good laboratory practice (GLP) including full traceability, documentation and archiving, and should be based on sound technical methodology for sample preparation, GC and HPLC analysis and appropriate confirmatory techniques. Implementation of the QA/QC procedures should provide reliable and thus comparable results among the laboratories involved. This is important, not only to generate scientifically sound results, but also to ensure that they are legally defendable.

- Analytical infrastructure should be strengthened with adequate and modern instrumentation, especially for confirmatory analyses, to ensure reliable data that can withstand stringent quality assurance and legal scrutiny.

- Regional collaborative research projects should provide opportunities for inter-laboratory exchanges, training and consultancies to strengthen institutional capabilities.

- A regional data base should be generated to assist policy makers in developing a harmonised approach to environmental issues including legislation, education and information. The data base should make use of modern approaches such as satellite photography and geographical information systems (GIS) in order to generate a graphical distribution of pesticide contamination and thus be able to correlate agricultural practices with pesticide usage and its impact on the environment and human health.

- In order to facilitate the accomplishment of these goals it is important that collaborating laboratories are linked via internet and e-mail systems to exchange information and data, and have access to global databases and information systems on sampling and analytical methods and on reference materials as well as guidelines and manuals on GLP appropriate for ISO accreditation. This would require the supply of appropriate software and high speed hardware to the regional laboratories. Virtual discussions, immediate information transfer and electronic access to such a "virtual advice centre" would reduce the need for frequent travel and costly subscription to scientific publications and assist these and other countries meet international standards with respect to food and environmental contaminants. This, together with the operation of external quality assurance programmes (EQAPs) and co-ordinating research to validate simple low cost analytical technologies for analysis of pesticides and other contaminants covered by the FAO/WHO Codex Alimentarius food standards, is in essence the aim of the Training and Reference Centre for Food and Pesticide Control being established by FAO and IAEA within the framework of the Joint Division's programme.

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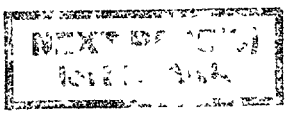
Part I

MONITORING PESTICIDE RESIDUES IN ENVIRONMENTAL SAMPLES



PESTICIDES IN SURFACE WATERS IN AREAS INFLUENCED BY BANANA PRODUCTION IN COSTA RICA

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Abstract

Banana production in Costa Rica is highly dependent on pesticide use. However, only a few studies have been undertaken regarding the presence and environmental impact of the agrochemical substances used in the banana culture on the aquatic ecosystem of the Atlantic Region of Costa Rica. This study was, therefore, undertaken in Rio Suerte Basin that drains into the 'Nature Conservation Area' of Tortuguero in the Atlantic lowlands of the country from June 1993 to December 1994. In order to investigate further the occurrence of pesticides in the water bodies located near the possible sources especially during worst-case situations, water samples were analysed following pesticide applications during 1995-1997. Pesticide residues were determined by GC equipped with an electron capture detector (ECD) and a nitrogen phosphorous detector (NPD). The study targeted 11 of the 21 pesticides used in banana production, the others were not analyzed.

The most frequently found compounds during the 1993-94 survey were the fungicide propiconazole and the nematocide cadusafos. Maximum concentrations measured after the pesticide applications were found in the main drainage canal and these were 2.1 ug/L carbofuran, 1.2 ug/L terbufos and 0.48 ug/L cadusafos. The peak concentration found shortly after the aerial application of the fungicide propiconazole was 13 ug/L in the creek leaving the banana plantation.

1. INTRODUCTION

Banana plantations cover 11.7% of the total cultivated land of the country, and it was 52, 165 ha in 1995 [1]. Although bananas have been one of the main foreign currency earners for the country, accounting for 20% of total export [2], they have been widely criticised because of the intensive use of pesticides. Banana production is highly dependent on pesticide use, it has been estimated than a third of the pesticide volume imported in Costa Rica is used on banana plantations with a range of 40 to 50 kg of active ingredient per hectare per year. The compounds applied include fungicides, nematicides, herbicides and insecticides. Fungicides are applied up to 50 times a year by aircraft, while highly toxic nematicides are applied two or three times a year directly to the ground. Herbicides are applied to the ground with backpack sprayers every 8 to 10 weeks. The highly toxic insecticide chlorpyrifos is impregnated in the plastic bags used to cover the banana bunches. Fungicides such as imazalil and thiabendazole are also used in the packing plants in order to protect the bananas for export. The intensive use of pesticides, together with the type of application, the toxicity of the compounds used, the drainage systems of the banana plantations and the high precipitation in the area are factors than make the water bodies of the area vulnerable, posing a threat to the organisms that inhabit these waters and to the human population living in the area. However, there are very few studies regarding the presence and environmental impact of the agrochemical substances used in the banana culture on the aquatic ecosystem of the Atlantic Region of Costa Rica.

2. METHODS

2.1. Study area

The first part of the study was conducted in the basin of La Suerte River, in the Atlantic lowlands of Limon, Costa Rica, an important banana producing area. Approximately ten thousand hectares

of bananas are grown in this basin. The annual precipitation in this area is more than 4 000 mm. The Tortuguero National Conservation Area, an area of great biological richness that protects a number of endangered species, is located downstream from the agricultural area. The sampling sites selected included four points in streams and main canals: one in the drainage canal of a packing plant, and three in the main river, one of which was located at the mouth of the La Suerte River into the lagoons of the Tortuguero Conservation Area.

The second part of the study (monitoring soon after the application of the pesticides) was conducted in a small banana plantation of about 40 ha. located in Siquirres, in the Atlantic Region of Costa Rica. Four sampling points were selected in the farm. The sampling points are coded and located as follows:

P-1 in a creek upstream of a secondary drainage ditch of the plantation;
P-2 in the drainage ditch within the plantation;
P-3 in the same ditch, about 5 m. before the junction with the creek;
P-4 in the creek downstream.

The drainage ditch receives water from a natural source that is born within the plantation.

2.2. Sampling

Samples were taken in the Rio Suerte Basin from June 1993 through December 1994. From June 1993 to September 1994 the sampling was conducted every 2 months, after September 1994 monthly sampling was carried out. Residues of four nematicides were monitored following the applications during June-July 1995, November-December 1995, November 1996 and February-March 1997. The pesticide application and sampling information is given in Table 1. The information on pesticide applications was obtained from the farm register book of the company.

The water samples were collected in pre-washed 1 liter glass bottles and preserved in the field by addition of dichloromethane. All samples were stored in ice until arrival at the laboratory in Heredia.

2.3. Analytical methods

Extraction of water samples was carried out within 24 hours of arrival at the laboratory. The nonpolar and semipolar pesticides were extracted from the water samples with dichloromethane. In the first extraction step sodium chloride was added. A further clean-up step was not usually necessary for the water extracts. Determination was carried out by capillary gas chromatography (GC) with electron capture (ECD) and nitrogen-phosphorus detection (NPD). Confirmation was obtained by injection on different columns and some of the extracts were sent for confirmation by GC-MS to the Organic Environmental Chemistry Section of the Swedish University of Agricultural Sciences in Sweden. Table 2 shows the main pesticides used in the banana culture and the analytical capability of the Laboratory for Pesticide Residue Analysis (LAREP) of IRET, where the analysis were carried out.

3. RESULTS ASND DISCUSSION

During the study in the Rio Suerte Basin 11 pesticides used in banana plantations were targeted for analysis, and 10 of them were found above the quantification limits. Results are shown in Tables 3 - 8.

The most frequently found compounds during the survey 93-94 were the fungicide propiconazole and the nematocide cadusafos. The frequency of occurrence and the concentration levels of the different pesticides were higher in the streams and main drainage canals. Thiabendazole was found

TABLE 1. LIST OF PESTICIDES USED AND SAMPLES COLLECTED

Period	Applications			Sample collection	
	date	active ingredient	rate (a.i.) kg /ha	date	number of samples collected
June-July 1995	20-6 21-6 22-6 27-6 15-7 18-7	Terbufos Terbufos Terbufos Terbufos Propiconazole** Terbufos	4.2	20-6 22-6 23-6 27-6 30-6 7-7 17-7	4 4 + 4 +4* 4 4 + 4* 4 3 4
November-December 1995	14-11 15-11 16-11 28-11	Cadusafos Cadusafos Propiconazole Cadusafos	3.7	15-11 16-11 17-11 20-11 22-11 29-11 13-12	3 + 3* 3 + 3* 3 3 3 3 3
November 1996	7-11 9-11 11-11 26-11	terbufos terbufos terbufos terbufos	5.6	9-11 13-11 27-11	6* 3 5
February-March 1997	22-02 27-02 28-02 6-3 8-3 10-3 14-3 15-3	carbofuran carbofuran carbofuran carbofuran carbofuran carbofuran carbofuran carbofuran	6.0	28-2 1-3 2-3 4-3 5-3 14-3 1-4	4 4 + 4 * 4 4 3 4 4

* Samples taken in the morning and in the afternoon
** No information was available

TABLE 2. LIST OF PESTICIDES MOST COMMONLY USED IN BANANA CULTIVATION IN COSTA RICA AND THEIR QUANTIFICATION LIMITS

Pesticides	Included in this study or not	Quantification limit (ug/L) for 1993-95 study	Quantification limit (ug/L) for 1995-97 study
FUNGICIDES			
Benomyl	no		
Bitertanol	no		
Propiconazole	yes	<0.1 - <0.5	<0.05 - <0.1
Tridemorph	no		
Chlorothalonil	yes	<0.04	<0.04
Mancozeb	no		
Imazalil	yes	<1.0	<1.0
Thiabendazole	yes	<1 - <5	<1 - <3
NEMATICIDES			
Cadusafos	yes	<0.02	<0.02 - <0.05
Carbofuran	yes	<0.5	<0.1 - <0.5
Ethoprophos	yes	<0.05 - <0.5	<0.04 - <0.1
Fenamiphos	no		
Oxamyl	no		
Terbufos	yes	<0.03	< 0.02 - <0.03
INSECTICIDES			
Bacillus thuringiensis	no		
Chlorpyrifos	yes	<0.03	<0.03
Diazinon	yes	<0.05 - <0.1	<0.04
HERBICIDES			
Ametryn	yes	<0.1	<0.1
Diuron	no		
Glyphosate	no		
Oxifluorfen	no		
Paraquat	no		

in concentrations up to 66 ug/L in the effluent of the packing plant in 5 of 6 samples and of 20 ug/L in streams and main drainage canals in 7 out of 47 (15 %) samples. Although the frequency and levels of pesticides found in the Mouth of the Suerte River were lower, 4 compounds were found in concentrations over the quantification limits, cadusafos was found in 3 out of 5 samples and propiconazole in 3 out of 10.

During the follow-up of applications the nematicides were found in maximum concentrations of 2.1 ug/L for carbofuran, 1.2 ug/L for terbufos and 0.48 ug/ for cadusafos in the main drainage canal. The peak concentration found shortly after the aerial application of the fungicide propiconazole was of 13 ug/L in the creek leaving the banana plantation.

The compounds that pose a higher risk for acute toxicity are the nematicides which have LC50 values of 0.3, 1.6 and 23.0 ug/L for terbufos, cadusafos and carbofuran respectively [3]. The residue levels of terbufos and cadusafos are close to the acute toxicity value, in one case the concentration of terbufos was above the LC50 reported for this compound. This indicates a potential impact to the water fauna. This is especially true if we take into account that we have used a very simple sampling strategy and we might have missed the peak concentrations. Also the farm we have studied is very small by banana production standards. An extensive application of terbufos in a bigger farm will probably have a greater risk for the aquatic fauna, especially if it happens to rain heavily, shortly after application. In recent years several fish kills have been reported after nematocide applications.

TABLE 3. CONCENTRATIONS OF PESTICIDES FOUND IN THE EFFLUENT FROM A PACKING PLANT

Active ingredient	Frequency positive samples %	Mean pos. samples ug/L	Range ug/L
Ametryn	17	0.2	0.2
Cadusafos			
Carbofuran			
Chlorothalonil			
Chlorpyrifos	33	0.05	0.03 - 0.07
Diazinon			
Ethoprophos			
Imazalil			
Propiconazole	33	0.20	0.1 - 0.3
Terbufos	17	0.09	0.09
Thiabendazole	83	24.4	6.7 – 66

TABLE 4. CONCENTRATIONS OF PESTICIDES FOUND IN DRAINAGE CANALS AND CREEKS

Active ingredient	Frequency (%) of positive samples	Mean concentration (ug/L)	Range of concentration (ug/L)
Ametryn	19	0.59	0.2 – 1.7
Cadusafos	87	0.45	0.05 - 2
Carbofuran	0	0	0
Chlorothalonil	2	0.9	0.9
Chlorpyrifos	11	0.04	0.03 – 0.05
Diazinon	9	0.17	0.05 – 0.3
Ethoprophos	4	0.20	0.05 – 0.34
Imazalil	21	6	1.1 – 17
Propiconazole	64	0.54	0.06 – 3.6
Terbufos	28	0.06	0.03 – 0.11
Thiabendazole	15	10.3	3.2 – 20

TABLE 5. CONCENTRATION OF PESTICIDES FOUND IN WATER SAMPLES FROM SUERTE RIVER

Active ingredient	Frequency (%) of positive samples	Mean concentration (ug/L)	Range of concentration (ug/L)
Ametryn			
Cadusafos	64	0.16	0.02 - 0.4
Carbofuran			
Chlorothalonil	5	0.3	0.3
Chlorpyrifos			
Diazinon	5	0.25	0.25
Ethoprophos	5	0.07	0.07
Imazalil			
Propiconazole	50	0.22	0.07 - 0.4
Terbufos			
Thiabendazole			

TABLE 6. PESTICIDES FOUND IN SAMPLES OF WATER TAKEN FROM THE MOUTH OF SUERTE RIVER

Active ingredient	Frequency (%) of positive samples	Mean concentration (ug/L)	Range of concentrations (ug/L)
Ametryn			
Cadusafos	60	0.09	0.06 - 0.15
Carbofuran			
Chlorothalonil	10	0.04	0.04
Chlorpyrifos	10	0.03	0.03
Diazinon			
Ethoprophos			
Imazalil			
Propiconazole	30	0.13	0.1 - 0.2
Terbufos			
Thiabendazole			

Although the toxicity of carbofuran is somewhat lower, because of the reasons stated above and the compounds solubility the risk of acute toxicity should not be disregarded. Furthermore because it is soluble in water and has a soil half-life between 3 and 60 days it is expected to have a high potential for groundwater contamination. No data is available about the half-life in the field of the granular formulation of carbofuran. Contamination to the surface water could be caused by direct run-off of the granulates or by infiltration to the groundwater and from there to the surface water. More investigation will be necessary to determine the main cause of the contamination.

TABLE 7 CONCENTRATION OF PESTICIDES DETECTED IN WATER SAMPLES TAKEN FOLLOWING THE APPLICATION OF NEMATICIDES

Period of nematicide applications	Pesticide found in the sample	Sampling point	Date	Concentration (ug/L)
June-July 1995	terbufos	P4	22-6	0 04/ 0 02/ 0 02*
		P3	22-6	1 2/ 0 15/ 0 13*
		P3	23-6	0 06
		P3	27-6	0 04/ 0 06*
		P3	30-6	0 1
		P2	22-6	0 02/ 0 03/ 0 02*
		P2	23-6	0 05
		P2	27-6	0 03
		P2	30-6	0 03
		P2	7-7	0 05
November-December 1995	cadusafos	P4	15-11	0 06/ 0 07*
		P4	16-11	0 27/0 3*
		P4	17-11	0 12
		P4	29-11	0 09
		P3	15-11	0 48/ 0 45*
		P3	16-11	0 17/ 0 46*
		P3	17-11	0 38
		P3	20-11	0 4
		P3	22-11	0 2
		P3	29-11	0 05
		P3	13-12	0 05
November 1996	terbufos	P3	9-11	0 07
February-March 1997	carbofuran	P4	28-2	0 15
		P3	28-2	2 1
		P3	1-3	0 91
		P3	2-3	0 15
		P3	4-3	0 14
		P3	1-4	0 14

TABLE 8 CONCENTRATION OF PROPICONAZOLE FOUND IN WATER SAMPLES TAKEN FOLLOWING THE PESTICIDE APPLICATION

Period of application	Pesticide found	Sampling point	Date	Concentration (ug/L)
November-December 1995	propiconazole	P4	16-11	13 0
		P4	17-11	1 1
		P4	20-11	0 3
		P4	29-11	0 1
		P3	17-11	1 5
		P3	20-11	0 3
		P3	22-11	0 15
		P3	29-11	0 07

Because of the frequency of occurrence cadusafos might have a higher risk for chronic toxicity. This compound was found in the Suerte River in around 60% of the samples in a range from 0.02 to 0.40 ug/L the chronic toxicity reference value for this compound is calculated in 0.16 ug/L [3] indicating a high risk.

The highest levels of pesticide residues found in water were detected after the application of propiconazole, 13 ug/L in the stream leaving the banana plantation. This concentration is about 3 orders of magnitude less than the LC50 values determined for fish and crustaceans. However this doesn't dismiss the possibility of chronic toxicity. In general propiconazole was found above detection limits in water samples up to 12 days after application. Propiconazole is used frequently over an extensive area and the data from the Suerte River Basin shows a constant presence of this compound. The chronic toxicity reference value for this compound is calculated in 20 ug/L [3].

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PRESENCE OF PESTICIDE RESIDUES IN WATER, SEDIMENT AND BIOLOGICAL SAMPLES TAKEN FROM AQUATIC ENVIRONMENTS IN HONDURAS

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Abstract

The objective of this study was to detect the presence of persistent pesticides in water, sediment and biological samples taken from aquatic environments in Honduras during the period 1995-98. Additionally, the LC_{50} for 2 fungicides and 2 insecticides on post-larval *Penaeus vannamei* was determined in static water bioassays. A total of 80 water samples, 16 sediment samples and 7 biological samples (fish muscle tissue) were analyzed for detection of organochlorine and organophosphate pesticide residues. The results of sample analyses indicate a widespread contamination of Honduran continental and coastal waters with organochlorine pesticides. Most detections were of low (< 0.050 ppm) or very low concentrations (< 0.010 ppm). The Nacaome and Choluteca Rivers, which include Tegucigalpa and other urban centers within their drainage basins, had the greatest number of pesticide detections and the highest concentrations among all the water samples tested. Carbofuran at 9.23 ppm and propiconizole at 1.79 ppm were detected in the sample taken from the Choluteca River in February of 1995. The April '95 sample from the Choluteca River contained 2.80 ppm of diallate and 0.26 ppm of heptachlor-epoxide. Samples from the Nacaome River contained high concentrations of diallate (1.400 ppm), carbaryl (0.560 ppm), 2,4-D (0.230 ppm) and quinomethinate (0.320 ppm). Intensive cultivation of melons and other export crops occurs throughout the drainage basin of this river in southern Honduras. All river and lake bottom sediments contained DDT or its metabolites. Four sediment samples had extremely high concentrations of these compounds in excess of 2.000 ppm. The insecticides endosulfan and lindane had much lower LC_{50} values and were therefore found to be much more toxic to the post-larval shrimp than the fungicides tridemorph and propiconazole.

1. INTRODUCTION

Pesticide use in Central America is intensive and greatly exceeds amounts applied in most developed countries of the world [1]. The 5 Central American Republics, Panamá and Belize have economies based primarily on agriculture production. During the past 15 years, an emphasis has been placed on developing non-traditional agricultural crops for export from Central America. Although laws and regulations for the protection of the environment exist [2], there are only scarce government resources for enforcement of these laws.

Marine shrimp and melon farming activities are located in areas surrounding the Gulf of Fonseca in southern Honduras [3]. Shrimp farms draw water from estuaries to fill ponds and to exchange the culture water during grow-out. Shrimp culture production activities are continuous throughout the entire calendar year. Farmers utilize river water to irrigate their melon plantations. Intensive melon farming activities begin each year in October and November and production continues until April or May. This period generally coincides with the Central American dry-season. Melons are exported fresh to the USA and other countries during the window of opportunity for this product during the northern hemisphere Winter. Cultured shrimp and melons are important export crops in the Honduran economy. During the 60's and 70's large tracts of land in southern Honduras were used for growing cotton. Cotton farming was generally abandoned in Honduras during the beginning the 80's [4]. Pesticide contamination of coastal waters has been implicated in the mass mortality of cultured shrimp and significant financial losses for farmers in Ecuador and other shrimp culture areas in Central America [3].

The Panamerican Agriculture School works with the local shrimp farmers association (ANDAH = Asociación Nacional de Acuicultores de Honduras) and with the Pond Dynamics/Aquaculture Collaborative Research Support Program (PD/A CRSP), an international research effort financed by USAID, to assure the sustainability of marine shrimp farming activities in this region. The PD/A CRSP focuses its research efforts on the principal water quality parameters that effect or limit aquaculture production.

The objective of this study was to detect the presence of persistent pesticides in water, sediment and biological samples taken from estuaries and other aquatic habitats in Honduras.

2. MATERIALS AND METHODS

2.1 Detection procedures

All pesticide residue determinations reported here were done at 2 Honduran laboratories. The "Centro para Estudios y Control de Contaminantes" is an analytical laboratory within the Secretary of Agriculture of the Honduran government. It performs routine analyses for the detection of chemical and biological contaminants in water, raw and processed foods, animal feeds, export products and other materials. The "Fondación Hondureña de Investigación Agrícola" (FHIA) performs routine analyses for the detection of chemical and other contaminants in water, soil, food and other types of samples. FHIA is a private non-profit organization.

Samples collected in 1995 and 1996 were analyzed for the presence of organochlorine and organophosphate pesticides. During 1997 and 1998, most samples were only analyzed for detection of the organochlorine group of compounds. All water samples were collected in amber glass bottles and kept refrigerated at 4° C until delivery to the analytical laboratory.

2.2 Water samples from aquatic habitats in southern Honduras

One-liter grab samples of surface water were taken from 3 estuaries and 2 rivers in southern Honduras from January '95 untill February of '97. Additional water and sediment samples were taken from Lake Yojoa, the Canique, Seguapa, Chamelecón and Ulua Rivers in central and northern Honduras during January of 1997. The Canique River was also sampled in February of '95.

2.3 Determination of the LC_{50} of 4 pesticides on marine shrimp

Laboratory studies were conducted to determine the LC_{50} of propiconazole, tridemorph, endosulfan and lindane to post-larval (P-Ls) shrimp (*Penaeus vannamei*). Under controlled conditions (salinity, temperature, light, NH_3, dissolved oxígeno), P-Ls were exposed to several concentrations of each pesticide in static water bioassays. The LC_{50} for each pesticide was calculated based on observed shrimp mortality and utilizing the Probit Analysis software.

3. RESULTS AND DISCUSSION

3.1. Pesticide residues detected in water samples from estuaries and rivers in southern Honduras

A total of 80 water samples, collected from 3 estuaries and 2 rivers in southern Honduras were analyzed as the first part of this study (January '95 thru February '97). Most samples were collected on a monthly basis. In general, the results of these analyses indicate a widespread

contamination of Honduran continental and coastal waters with organochlorine pesticides (Table I). Most pesticide detections were of low (< 0.050 ppm) or very low concentraciones (< 0.010 ppm).

The presence of pesticide residues was predominantly during the months corresponding to the Honduran dry-season (November-May) when 69% of the detections were registered (Table I). Reduced precipitation during the dry season would tend to concentrate any chemicals entering these water courses. Endosulfan, lindane, DDT and its metabolites, and endrin were detected in many samples but always at low concentrations. Aldicarb was detected only from samples taken from November '96 and February '97.

Pesticides detected at high concentration (> 0.050 ppm) included carbofuran, 2,4-D, heptachlor, diallate and parathion. These chemicals were detected only sporadically and were not commonly observed in the water samples analyzed. The presence of high concentrations of these chemicals in the coastal waters of Honduras is probably explained by point source contamination.

Of all aquatic habitats included in this study, samples taken from the Nacaome River Estuary and from the Choluteca River had the greatest number of pesticide detections. Most of these determinations were for organochlorine compounds at very low concentration (Table I).

However, on 2 occasions unusually high concentrations of pesticides were detected in water samples taken from the Choluteca River. In the February of '95 sample , carbofuran was detected at 9.23 ppm and porpiconizole at 1.79 ppm. In April of the same year, the sample contained 2.80 ppm of diallate and 0.26 ppm of heptachlor-epoxide.

The Nacaome River drainage basin includes important areas for cultivation of melons for export. Samples from the river and estuary had high concentrations of quinomethonate, 2,4-D, diallate and carbaryl during the months corresponding to intensive melon cultivation in southern Honduras.

3.2. Pesticides detected in river and lake bottom sediments

All sediment samples analyzed as part of this study contained detectable amounts of DDT and/or its metabolites (Table II). In several sediment samples the concentration of these compounds was very high, exceeding 2.000 ppm in 4 of the samples. These results demonstrate the long term persistence of the DDT family of compounds in tropical aquatic environments. Organochlorine pesticides are generally hydrophobic and they have low solubility in water. These compounds have a strong sorption tendency to river and lake bottom sediment particles [5]. They continue to be ecologically important because most of the compounds in this family are considered to be carcinogenic. During the 1980's use of these pesticides was banned in Central America.

3.3. Pesticide residues in biological samples

On two ocasions muscle tissue from fish were analyzed for pesticide residues (Table III). A snook (Family Centropomidae) captured (VII-96) in the El Pedregal Estuary in southern Honduras had lindane (0.080 ppm) and DDE-pp (1.050 ppm) in its mussle tissue. These concentrations exceed the recommended levels acceptable for human consumption. A concentration in excess of 1.000 ppm of DDT in water is considered detrimental to the health and general welfare of aquatic organisms [5].

TABLE I. RESIDUES OF PESTICIDES DETECTED IN WATER SAMPLES TAKEN FROM 5 AQUATIC HABITATS IN SOUTHERN HONDURAS DURING 1995-1997.

Description Habitat	Pesticide detected	Concentration (ppm)	Collection date
San Bernardo Estuary	Parathion	0.800	XI-94
	Endosulfan	0.010	I-95
	Lindane	0.012	I-95
	DDT-pp	0.012	I-95
	Endrin	0.011	IX-95
	Lindane	0.020	XI-95
	Endrin	0.006	XI-95
	Aldicarb	0.027	XI-96
	Aldicarb	0.027	II-97
El Pedregal Estuary	Endosulfan	0.010	I-95
	DDT-pp	0.014	I-95
	Carbofuran	0.090	II-95
	Propoxur	0.040	II-95
	2,4-D	0.200	IV-95
	Heptachlor-epoxide	0.010	V-95
	Lindane	0.020	XI-95
	Endrin	0.008	XI-95
	DDD-pp	0.001	VII-96
	Aldicarb	0.044	II-97
Nacaome River Estuary	α-Endosulfan	0.010	I-95
	β-Endosulfan	0.010	I-95
	DDT-pp	0.010	I-95
	Lindane	0.012	I-95
	Quinomethinate	0.320	IV-95
	2,4-D	0.230	IV-95
	Diallate	1.400	IV-95
	Lindane	0.010	V-95
	β-Endosulfan	0.030	VII-95
	Lindane	0.003	XI-95
	Carbaryl	0.560	II-97
Nacaome River	Lindane	0.018	I-95
	DDT-pp	0.029	I-95
	Heptachlor	0.010	V-95
	Aldrin	0.014	XI-95
	pp-DDD	0.026	XI-95
	Lindane	0.004	XI-95
	Carbaryl	0.150	II-97
Choluteca River	Carbofuran	9.230	II-95
	Propiconazole	1.790	II-95
	Diallate	2.800	IV-95
	Heptachlor-epoxide	0.260	IV-95
	β-Endosulfan	0.030	VIII-95
	Lindane	0.006	XI-95
	Endrin	0.003	XI-95

TABLE II. PESTICIDES DETECTED IN SEDIMENT SAMPLES COLLECTED DURING 1996-97 IN HONDURAS.

Description of the habitat	Pesticide detected	Concentration (ppm)	Collection date
San Bernardo Estuary	Heptachlor	0.040	VII-96
	Heptachlor	0.090	X-96
	Chlordane	0.074	X-96
	DDT-pp	9.000	X-96
	DDE-pp	0.207	II-97
	Aldrin	0.029	II-97
El Pedregal Estuary	DDT-op	0.120	VII-96
	Heptachlor	0.021	X-96
	DDT-pp	1.800	X-96
	Aldicarb	0.373	II-97
	DDE-pp	0.011	II-97
	Lindane	0.080	II-97
Nacaome River Estuary	Heptachlor	0.011	VII-96
	Aldrin	0.006	VII-96
	Heptachlor-epoxide	0.010	VII-96
	DDT-pp	4.181	X-96
	DDE-pp	0.031	II-97
Nacaome River	Heptachlor	0.009	VII-97
	DDT-pp	0.273	X-97
	DDE-pp	0.030	II-97
	Aldrin	0.010	II-97
Choluteca River	Heptachlor	0.013	VII-96
	Aldrin	0.006	VII-96
	DDT-pp	2.090	X-97
	DDE-pp	0.030	II-97
	Aldrin	0.010	II-97
Canique River	DDE-pp	0.106	I-97
Selguapa River	DDE-pp	0.034	I-97
	Endosulfan	0.012	I-97
Chamelecon River	DDE-pp	0.040	I.97
Ulua River	DDE-pp	0.020	I-97
Lake Yojoa	DDE-pp	2.440	I-97

In March of '98 fish fillets were purchased in supermarkets in Tegucigalpa. The muscle tissue analyzed contained only trace amounts (< 0.001 ppm) of several organochlorine compounds. These included heptachlor and endrin. The low concentrations of the compounds detected in the fish muscle tissue indicate that the fish are suitable for human consumption

3.4. Toxicity of propiconazole, tridemorph, endosulfan and lindane to post-larval marine shrimp

Fungicides were found less toxic than the insecticides in tests designed to determine the LC_{50} for post-larval marine shrimp (Table III). As arthropods, shrimp are presumably more

TABLE III. LC_{50} VALUES FOR POST-LARVAL MARINE SHRIMP EXPOSED TO 4 PESTICIDES [6] [7].

Pesticide	Estimated LC50	Mortality of control shrimp
Endosulfan (insecticide)	0.008 ppm at 8 hours	< 10%
Lindane (insecticide)	0.009 ppm at 12 hours	< 10%
Propiconazole (fungicide)	0.013 ppm at 72 hours	< 10%
Tridemorph (fungicide)	0.350 ppm at 72 hours	< 10%

susceptible to the action of insecticides, which are designed to kill insect pests, than to fungicides which are designed to interfere with the functioning of fungi.

The shrimp were somewhat tolerant to the effect of fungicides. The high concentrations of propiconazole and tridemorph required to cause the death of 50% of the shrimp population makes these products unlikely candidates for having caused the mass mortality of shrimp in farms in Ecuador and in other countries.

4. CONCLUSIONS

Aquatic habitats in Honduras have widespread contamination with organochlorine and other types of pesticides. Most of the compounds detected in this study were found at low concentration (< 0.050 ppm). On several occasions, high and extremely high concentrations of pesticides were detected in water, sediment and fish muscle tissue samples analyzed.

The LC50 for fungicides was considerably higher than the values for insecticides tested in this study. It is unlikely that tridemorph and propiconazole are involved with the mass mortalities of shrimp reported from Ecuador and other shrimp farming countries in Latin America.

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ORGANOCHLORINE PESTICIDES IN SEDIMENT AND BIOLOGICAL SAMPLES FROM THE COASTAL LAGOONS OF NICARAGUA

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Abstract

A study was carried out on the Pacific coast of Nicaragua to investigate the contamination of the coastal lagoons with residues of agricultural pesticides. Samples were taken during 1995 from the areas of Estero Real, Padre Ramos, Maderas Negras, Naranjo and Paso Caballos, and during 1996 from Aposentillo to Estero Barquito - Posoltega River. Analysis of the samples of sediment and aquatic life (fishes, oysters and bivalves) showed that they were contaminated with organochlorine pesticides. The pesticides found in the highest concentrations were toxaphene (1,734 $\mu g.kg^{-1}$) and p,p-DDE (275 $\mu g\ kg^{-1}$). These data indicate widespread contamination of the ecosystem with organochlorine pesticides in the main Pacific coastal lagoons of Nicaragua, resulting from intensive agricultural use of pesticides during the past decades. The contamination has been carried from the agricultural areas to the coastal lagoons by the rivers passing through the cultivated areas.

1. INTRODUCTION

The districts of Leon and Chinandega, located in the west coast of Nicaragua, have the highest agricultural activity in the country due to fertile soil of volcanic origin and favourable climatic conditions which help in growing a wide variety of crops. Also the main underground water supplies for the pacific coast are found in this region. Nevertheless, intensive farming for the last 40 years has caused damage to the soil, and the water has been contaminated due to generalization of single crop farming (cotton and sugar cane) . The soils superficial layer has been deprived of nutrients by single crop farming. The excessive use of pesticides has degraded and accelerated the contamination of soil and water.

Very little information is available regarding the contamination of coastal lagoons or estuaries with by pesticide residues in Nicaragua. Most of the estuaries, covered by vast mangrove forests, collect the drainage from the agricultural fields. The mangroves create an interface of sea and land area, and receive water from tides as well as the streams and surface run off from the land. Thus there is a continuous turn over of the soil and nutrients and organic matter, with an overall los from the land to the coastal water. In the the estuaries the rivers discharge silt, creating a platform of mud where the organic matter accumulates.

Atoya river is the main river that flows through Chinandega, and has a basin of 354 square kilometers. This river discharges within the system of coastal lagoons, which has an area of 155 square kilometers, and it is surrounded by a vast mangrove. Marine resources are exploited, and they represent a considerable fraction of the local populations diet. Therefore, contamination of these resources with pesticide residues would represent a potential health risk to the consumers sea food and fish from the lagoons. The aim of this study was to investigate the distribution, fate and effects of organochlorine pesticides in sediments, fish and bivalves tissues of the coastal lagoons system.

2. METHODS AND MATERIALS

Samples were obtained from the Pacific coast (Fig 1) during two field trips. In the December 1995 field trip sediment samples were collected from the following locations: Estero Real, Estero Padre Ramos, Estero Maderas Negras-Paso Caballos. In the September 1996 samples of sediments, fish, oysters and Anadara spp were collected from Estero Aserradores, Estero El Realejo, Estero Grande, Estero El Barquito, and the Atoya river.

2.1. Sampling of sediment

The superficial sediment samples were collected with the Eckman grabber, homogenized and transfered into previously washed glass jars labelled with precise information on the sampling sites. The collection of sediment at different depths was carried out with a soil core sampler.

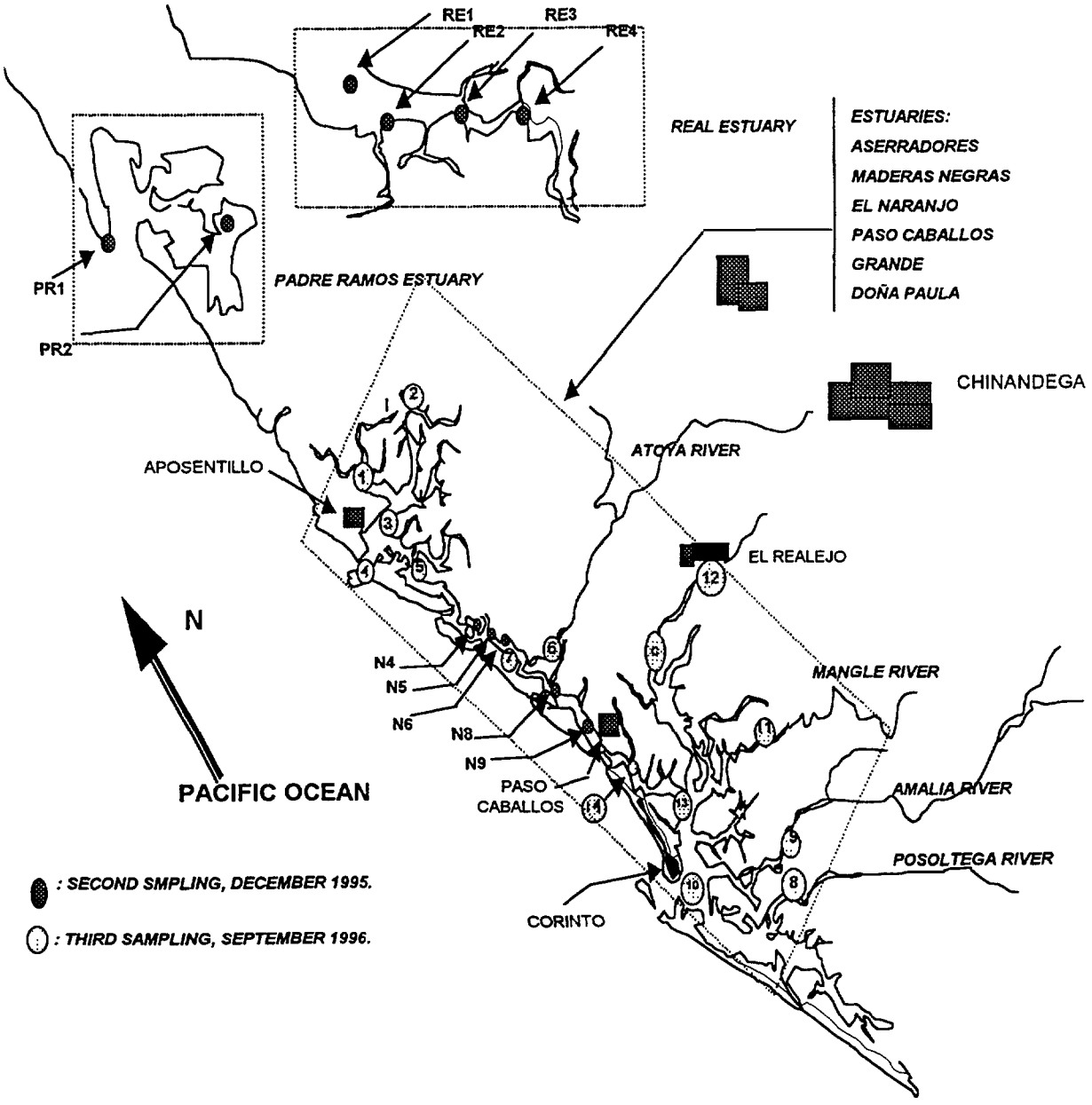

Fig. 1. Map of sampling points along the coast of Nicaragua

All sediment samples were kept cold during transport to the laboratory. The sediment cores were cut into 5 cm sections, weighed, and dried at room temperature. Sections of the same depth were pooled to obtain samples of the minimum weight requirement for the analysis of organochlorine pesticides [1].

2.2. Sampling of biological tissues

Fish and clam samples were collected with the aid of local fishermen in the following sites: Monte Redondo (Atoya River), Alemania Federal, El Realejo, La Lapa Estuary and Paso Caballo. The samples were transported to the Marine Environment Laboratory (MEL) in Monaco of International Atomic Energy Agency (IAEA), then dry-frozen to be sent to CIRA laboratory for the analysis of pesticides residues. Ten grams (dry weight)of tissue sample were used for extraction and residue analysis.

2.3. Analytical quality control

In order to ensure the precision of the analysis, known concentrations of pesticide standards were spiked in each group of sediment and biological tissue samples to be extracted. The percent of recovery of each spiked pesticide was calculated to test the performance of the analytical method used in this study.

Duplicate analysis for each group of sediment samples and biological tissues samples, were carried out to control the reproducibility of the analysis. A target, a fortified sample with known concentrations of organochlorine pesticides, and a fortified sample of toxaphene, were included in each group. An internal standard was added to all samples from the start of the analysis to assure that all organochlorine pesticides were detected. When the percentage of recovery for HCB was less than 70 %, the samples were analyzed again. The mean percentage of recovery for HCB in sediment samples was 74 %, and for biological tissues was 76 %. Another analytical quality control exercise in this work was the interlaboratory analysis of identical samples. This was carried out by the laboratories of CIRA/UNAN and the MEL, Monaco to compare the reproducibility of the analytical results.

2.4. Analytical procedures

The pesticides from the sediment or tissue samples were extracted with a mixture of hexane + methylene chloride, and the concentrated extract was analysed with a model 3400 Varian gas chromatograph(GC). The GC was equipped with an electron capture detector (ECD) and a DB5 capillary column with a length of 30 meters, and an internal diameter of 0.32 mm.

A micro liter of sample was injected, in the splitless mode. Hydrogen was used as a carrier gas and Nitrogen as the make up gas. The temperature program used for the analysis was the following : 80 ^{0}C (1 minute), 4 ^{0}C/min. to 200 ^{0}C, 3 ^{0}C/min. to 230 ^{0}C, 15 ^{0}C /min. to 250 ^{0}C (5 minutes) . The temperatures for the detector and the injector were 350 ^{0}C and 250 ^{0}C respectively. The peaks were identified comparing retention times with those for the best quality analytical standards, (Supelco Inc.) . The concentrations of the residues from pesticides were calculated on the basis of a calibration curve, using an internal standard.

3. RESULTS

3.1. Pesticide residues in sediment

In the samples taken during the second field trip during December 1995 fifteen organochlorine pesticides were targeted for analysis in the superficial and stratified sediment. The pesticides with

high concentrations were: toxaphene, p,p-DDE, p,p-DDD, lindane, dieldrin, and p,p-DDT. Endrin, α-endosulfan and heptachlor, were detected in lower concentrations. Aldrin, α-BHC, β-BHC, δ-BHC, β-ndosulfan and heptachlor epoxide were not detected in any of the samples analyzed.

Table 1 shows concentrations of organochlorine pesticides found in the superficial sediment samples taken during 1995. p,p-DDE was found in superficial sediment samples from all sampling sites, except sampling site PR1. The concentration of pp-DDE ranged from 0.87 to 130.34 $\mu g.kg^{-1}$. p,p-DDD was detected at sampling sites N4, N5 and N6, at a concentration ranging from 7.18 to 28.79 $\mu g.kg^{-1}$. Dieldrin was found at all sampling stations, except stations N5 and PR2, with concentrations ranging from 0.59 to 53.73 $\mu g.kg^{-1}$. Residues from p,p-DDT were detected at sampling stations N4, N5, N6 and N9, and the concentrations ranged from 1.63 to 32.22 $\mu g.kg^{-1}$. Endrin was found at stations N4 and N6 with concentrations of 1.97 and 2.90 $\mu g.kg^{-1}$ respectively. Heptachlor was detected at stations N4, N8, ER1, ER2 and ER3, with concentrations ranging from 0.56 to 0.76 $\mu g.kg^{-1}$ Toxaphene was detected only at station N4, with a concentration of 468.43 $\mu g.kg^{-1}$. This high concentration is probably due to the proximity to a town where cotton was cultivated. The detection of p,p-DDE at all sampling stations, except station PR1, is probably due to the wide spread use of the pesticide p,p-DDT in this area for a variety of crops, mainly cotton.

Sampling station N4 showed the highest degree of contamination with residues of organochlorine pesticides. Seven of the 15 pesticides targeted for analysis were found at this station, and this is most likely due to the proximity of the sampling site to the town of San Miguel where cotton was cultivated. All other sampling stations are located farther away from towns.

Table 2 shows concentrations of pesticides found in the stratified sediment core samples taken during 1995. The samples were taken at five increasing depths of 5 cm down to 25 cm and represent four sampling sites N4 (Naranjo 1), N5 (Naranjo 2), N8 (Santa Ana 1) and N9 (Paso Caballos). The results showed presence of p,p-DDE (18.53 to 274.64 $\mu g.kg^{-1}$), p,p-DDT (1.75 to 48.74 $\mu g.kg^{-1}$) and p,p-DDD was detected with a maximum concentration of 89.07 $\mu g.kg^{-1}$.

Endrin was detected at three sampling sites. The maximum concentration was 6.49 $mg.kg^{-1}$ at 5-10 cm at sampling site N4. This compound was not detected at the sampling site N8. Dieldrin was detected at all depths down to 25 cm at sampling sites N4 and N5, and the concentration ranged from 0.69 $\mu g.kg^{-1}$ to 17.29 $\mu g.kg^{-1}$. However, at sampling site N8 it was found only in the upper 5 cm layer, and at the sampling site N9 it was not detected at all. Heptachlor was present at sampling sites N4, N8 and N9. At N4 it was detected only in the upper 0-5 cm section of the stratified sediment. At N8 it was detected at all superficial and stratified sediments except at the 5-10 cm depth . Lindane was found in the sediments collected at a depth of 5-10 cm at N4. At N9 lindane was detected at the 10-15cm, 15-20 cm, and 20-25 cm in superficial and stratified sediments.

Residues of α-Endosulfan were present in a few samples of the sediment. These included the upper 10 cm sediment at sampling site N4, 15-20 cm section at N5, and 20-25 cm and sampling site N9. Toxaphene was found at two sampling sites: N4 and N5, but at all depth sampled at both sites.

Table 3 shows data on the residues of organochlorine pesticides found in surface sediment samples taken during 1996 from 14 sampling sites. Aldrin, heptachlor and lindane were detected at

TABLE 1. RESIDUES OF ORGANOCHLORINE PESTICIDES IN SURFACE SEDIMENT SAMPLES TAKEN AT SAMPLING SITES DURING 1995

Sampling sites	Organoclorine pesticides detected ($\mu g. kg^{-1}$). Heptachlor	Endrin	Dieldrin	p,p- DDT	p,p- DDE	p,p- DDD	Toxaphene
N4 (Naranjo 1)	0.68	1.97	2.33	32.22	130.34	28.79	468.43
N5 (Naranjo 2)	ND	ND	ND	10.46	38.64	13.81	ND
N6 (Naranjo 3)	ND	2.90	1.50	8.63	17.34	7.18	ND
N8 (Sta.Ana)	0.55	ND	1.29	ND	0.87	ND	ND
N9 (P.Caball.)	ND	ND	53.73	1.63	9.92	ND	ND
RE1	0.76	ND	30.33	ND	3.42	ND	ND
RE2	0.98	ND	1.38	ND	2.38	ND	ND
RE3	0.66	ND	4.23	ND	1.95	ND	ND
RE4	ND	ND	1.04	ND	5.77	ND	ND
PR1	ND	ND	0.59	ND	ND	ND	ND
PR2	ND	ND	ND	ND	2.19	ND	ND

ND: No detected

TABLE 2. RESIDUES OF ORGANOCHLORINE PESTICIDES IN STRATIFIED SEDIMENT SAMPLES TAKEN DURING 1995

Sampling sites		Organochlorine Pesticides detected µg. kg^{-1}								
		Heptach.	Lindane	a-Endosul.	Endrin	Dieldrin	p,p- DDT	p,p- DDE	p,p- DDD	Toxaphene
Naranjo 1" (N4)	0 -5	0.64	ND	1.7	2.32	7.99	38.07	107.59	38.72	553.61
	5-10	ND	1.24	2.81	6.49	8.65	45.80	127.84	48.93	740.66
	10-15	ND	ND	ND	4.94	5.86	40.59	192.36	45.06	1733.93
	15-20	ND	ND	ND	2.69	3.00	44.57	241.26	42.83	1216.15
	20-25	ND	ND	ND	2.04	1.98	19.92	127.73	19.91	457.89
Naranjo 2' (N5)	0-5	ND	ND	ND	3.02	9.22	36.49	257.73	52.28	1116.85
	5-10	ND	ND	ND	3.90	17.29	37.32	204.53	36.22	793.40
	10-15	ND	ND	ND	3.15	12.64	35.57	164.99	28.87	691.60
	15-20	ND	ND	2.30	1.73	6.06	45.37	128.33	29.62	646.70
	20-25	ND	ND	ND	2.30	11.51	48.74	274.64	89.07	487.63
Sta ANA 1' (N8)	0-5	0.43	ND	ND	ND	0.69	3.38	18.53	3.52	ND
	5-10	ND	ND	ND	ND	ND	1.75	26.25	4.45	ND
	10-15	0.32	ND	ND	ND	ND	2.48	30.97	4.76	ND
	15-20	0.20	ND	ND	ND	ND	2.54	61.09	11.55	ND
	20-25	0.09	ND	ND	ND	ND	2.79	39.89	4.79	ND
P.CABALLO 2 (N9)	0-5	0.07	ND	ND	ND	ND	4.32	74.63	9.62	ND
	5-10	ND	ND	ND	ND	ND	2.57	51.95	6.37	ND
	10-15	ND	23.86	ND	1.27	ND	2.34	49.80	10.23	ND
	15-20	0.15	2.15	ND	ND	ND	5.56	184.66	41.99	ND
	20-25	ND	71.29	1.89	3.75	ND	3.41	86.38	30.09	ND

TABLE 3. RESIDUES OF RGANOCHLORINE PESTICIDES DETECTED IN SURFACE SEDIMENT SAMPLES TAKEN DURING 1996

Sampling sites	Organochlorine pesticides detected (µg. kg $^{-1}$) Heptachlor	Lindane	Aldrin	Endrin	Dieldrin	pp'-DDT	pp'-DDE	pp'-DDD	Toxaphene
STATION 1	ND	ND	ND	ND	ND	2.05	31.17	3.21	ND
STATION 2	ND	ND	ND	ND	ND	ND	2.71	ND	ND
STATION 3	ND	ND	ND	ND	ND	1.26	18.02	2.18	ND
STATION 5	ND	ND	ND	ND	ND	0.95	16.47	2.24	ND
STATION 6	0.90	0.48	1.19	3.05	10.23	49.62	58.97	46.05	1,309.41
STATION 8	ND	ND	ND	1.01	8.30	35.77	58.73	26.23	589.44
STATION 9	ND	ND	ND	0.62	5.10	7.08	43.74	11.35	220.81
STATION 10	ND	ND	ND	ND	4.50	0.78	7.74	1.06	ND
STATION 11	ND	ND	ND	ND	4.19	8.45	59.21	9.73	184.45
STATION 12	ND	ND	ND	1.40	8.03	29.96	93.99	21.11	348.15
STATION 13	ND	ND	ND	ND	3.75	10.95	52.92	10.93	ND
STATION 14	ND	ND	ND	ND	8.39	3.06	20.57	4.73	ND

three sites only, and 6 of the other targeted pesticides were detected from more sites. The DDT group of compouds were detected with the greatest frequency. At sampling site 2 only DDE was detected. Absence of detectable quantities of the other pesticides from this site may be due to a low degree of farming in this area in the past. More pesticides have been detected at the other sampling sites, and this may be because the river water flowing through lands cultivated with cotton and sugarcane for decades and contaminated with pesticide residues would be expected to spread the contamination into these sampling sites.

Sampling sites 6, 8, 9, 11, 12 and 13 contained higher concentrations of p,p-DDE, p,p-DDD and p,p-DDT than the other sites, with maximum concentration of 93.99, 46.05 and 49.62 $\mu g.kg^{-1}$,respectively. The highest concentration of p,p-DDE was found at the sampling site 12, which is caracterized as a poor mangrove forest located near the town of Realejo and where sugar cane and cotton crops were cultivated. These compounds are resistant to breakdown and are readily adsorbed into sediments and soils, which can act both as sinks and as long-term sources of exposure for soil organisms [2]. The highest concentrations of p,p-DDD and p,p-DDT were found at site 6, and this may be due to the influence of the Atoya River and its tributaries (Sasama and Acome). Albone et al. [3] investigated the capacity of river sediments, from the Severn Estuary, United Kingdom, to degrade DDT. p,p'-DDT (^{14}C labelled) was applied to sediments either in situ on the mud flats or in the laboratory. Incubation in situ over 46 days led to very little metabolism of DDT in the sediments. Some p,p'-TDE was produced, but the ratio of DDT to TDE was 13:1 and 48:1 in two replicate experiments. Incubation of the same sediments in the laboratory, over 21 days, led to much greater metabolism (ratios of 1 : 1.1 and 1 : 3.3, DDT to TDE in replicate incubatios) and the production of some unidentified, further breakdown products.

Dieldrin was detected at sites 6-14. It was not detected at sites 1-5, probably due to the location of these sites where very little agricultural cultivation has been practiced in the past or possibly due to little use of dieldrin at these sites in past. The concentration of dieldrin at sites 6-14 ranged from 3.75 to 10.52 $\mu g.kg^{-1}$. Higher concentrations were found at the sampling sites 6, 8, 12 and 14, with the highest at site 6. This can be attributed to the intensive production of crops such as cotton, sugar cane in the past, and banana, sorghum, sesame seed, peanut and other crops at the present time.

Detectable residues of endrin were found only at sampling sites 6, 8, 9 and 12 in concentrations ranging between 0.62 and 30.55 $\mu g.kg^{-1}$. Lower residues indicate that this pesticide had been used in this area on a smaller scale.

Toxaphene was detected at the highest concentration of any pesticide during the 1996 sediment sampling campaign. It was detected at a concentration of 1309.41 $\mu g.kg^{-1}$ in sediment at site 6, which is located at the Atoya River. This indicates that toxaphene has been used widely in the past and may still be illegally in use, its use was banned during 1980. Another site with high concentration of toxaphene (589.44 $\mu g.kg^{-1}$) was site 8, which is situated at the Posoltega River. Intensive cotton cultivation was practiced in this area. Toxaphene was also found at sites 9, 11 and 12 but in low concentrations.

Table 4 shows concentrations of pesticides found in 5 cm sections of the stratified sediment core samples taken during 1996 at the sampling site Puerto Realejo. The detected pesticides included p,p-DDE, p,p-DDD, p,p-DDT, dieldrin, lindane, endrin, α-endosulfan were found in these samples. These results show a rather homogeneous deposition and provision of sediments in estuaries from this zone. Both p,p-DDE and dieldrin are found with higher concentrations in the

TABLE 4. RESIDUES OF ORGANOCHLORINE PESTICIDES IN STRATIFIED SEDIMENT SAMPLES TAKEN IN 1996

Section of sediment core (cm)	Organochlorine pesticides detected (µg. kg $^{-1}$).						
	Lindane	A-Endosulphane.	Endrin	Dieldrin	pp'-DDT	pp'-DDE	pp'-DDD
0-5	6.21	ND	1.04	4.92	5.48	64.69	6.91
5-10	5.85	0.66	ND	2.18	2.84	49.28	4.89
10-15	3.98	0.59	0.49	8.91	2.18	44.57	3.48
15-20	4.27	ND	0.74	19.14	1.71	44.55	2.79
20-26	5.57	0.29	0.89	22.36	2.65	82.24	5.55
26-32	0.83	0.77	ND	1.46	2.58	47.59	7.63

ND Not detected

layer from 20 to 26 cm. Above this level pesticide concentrations are homogenous probably due to the continuous deposition of the sediments. Geographical distribution of the pesticide contaminated sediment is shown in Fig 2.

Shin et al. [4] investigated the adsorption of DDT by soils of different types and by isolated soil fractions. A sandy loam, a clay soil and a high organic muck . Adsorption was least in the sandy loam and greatest in the muck (distribution coefficients [kd] were in the ratio 1:10:80 for sandy loam, clay soil and organic muck respectively).

3.2. Pesticide residues in biological tissues

The highest concentrations of p,p-DDE (118.64 $\mu g.kg^{-1}$) and α-endosulfan (4.13 $\mu g.kg^{-1}$) were found in tissues of lisa fish (*Rajidae spp)* from Monte Redondo. Also, high concentrations of p,p-DDE (77.62 $\mu g.kg^{-1}$) and p,p-DDD (3.63 $\mu g.kg^{-1}$) were found in pargo fish (*Sparidae spp)* from the same site. These results show that Atoya River and its tributaries have contaminated the Naranjo estuary and aquatic life in it. the data also suggest a direct relationship between the pesticide contamination in the aquatic life and the sediment at this site

Analysis of clams (*Anadara spp)* taken from Realejo showed residues of pp-DDE (44.25 $\mu g.kg^{-1}$, α-BHC (8.86 $\mu g.kg^{-1}$) and p,p-DDD (7.29 $\mu g.kg^{-}$). These residues may be a result of the feeding

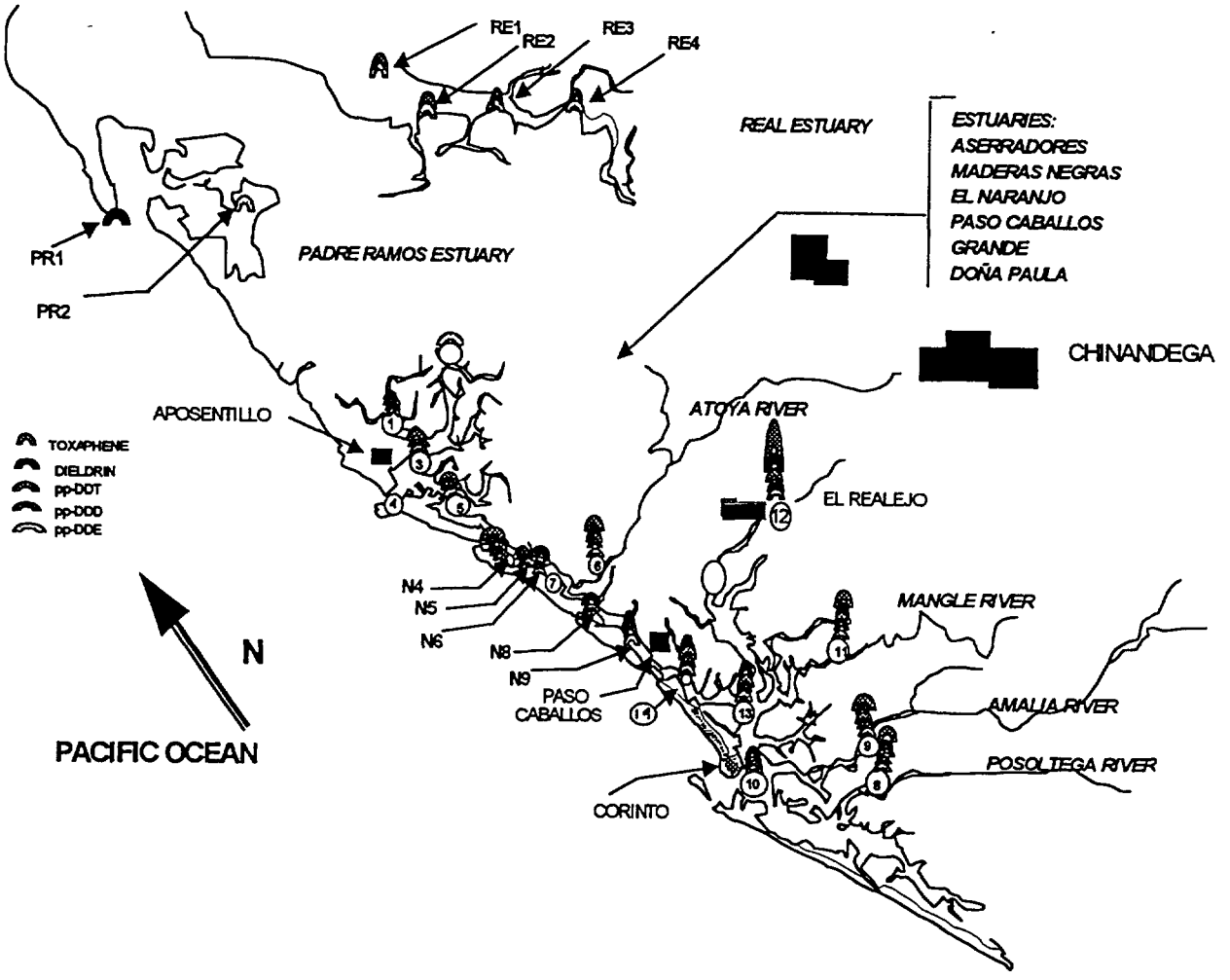

Fig. 2. Map showing distribution of organochlorine pesticides in surface sediment

behaviour of these organisms because they obtain their food through filtration, hence they are good indicators of contamination. Residues of p,p-DDE (38.78 $\mu g.kg^{-1}$), p,p-DDD (6.58 $\mu g.kg^{-1}$) and low levels of aldrin and heptachlor epoxide were also found in clams in La Lapa estuary.
The physical-chemical properties of DDT and its metabolites enable these compounds to be taken up readily by organisms. The rates of acumulation varies with the species, the duration and concentration of exposure, and with environmental conditions [2]. Different organisms metabolize DDT via different metabolic pathways. It may result in the formation of DDE or TDE. DDE is the more persistent metabolite, although not all organisms produce DDE from DDT. The alternative metabolism via TDE leads to more rapid elimination. Much of the retained DDT and its metabolites are known to store in lipid-rich tissues.

Other pesticides such as p,p-DDD, aldrin, α-endosulfan, heptachlor and α-BHC were also found in these tissues. The sampling site El Realejo shows the highest incidence of pesticides which correlate well with the sediment results. Organisms can acumulate these chemicals from the surrounding medium and from food. In aquatic organisms, uptake from the water is generally more important, whereas, in terrestrial fauna, food provides the major source. In general, organisms at higher trophic levels tend to contain more DDT-type of compounds than those at lower trophic levels.

4. CONCLUSIONS

The levels of contamination by organochorine pesticides found in different matrices in the second and third sampling journey show the advanced damage of a big area of the coastal lagoons in the western region of Nicaragua. This demonstrates an extensive use of these agrochemicals in past decades, which arrive with the runoff of cultivated soils to these coastal lagoons. On the other hand, residues of these agrochemicals have also been transported to these coastal lagoons through the most important rivers of the region, since, these rivers go through vast cultivated areas affected by erosion and human settlement. This contributes to the modification of the natural equilibrium of these aquatic ecosystems. The use of persistent organochlorine pesticides in cotton and sugar cane cultivation in the past has resulted in the contamination of aquatic life and sediment in the rivers and estuaries monitored. It can be concluded that the most contaminated sites in this area were Atoya River (site 6) and Posoltega River (site 8). The Atoya River is highly contaminated because it flows through the whole Chinandega Department, carrying along all kind of contaminants and especially residues of pesticides that were used in cotton cultivation. The confluence of Atoya River with Sasama, Acome and El Chiquito River; is another factor that has contributed to its contamination. Posoltega River has a large area of to runoff zones where the most important agricultural activity has been the cotton cultivation. It can be concluded from this study that all matrices of the coastal lagoon ecosystem are contaminated with the organochlorine pesticides resulting in an increase in damage to the coastal lagoon systems in the northwest region of Nicaragua.

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DETERMINATION OF PESTICIDES IN SURFACE AND GROUND WATER USED FOR HUMAN CONSUMPTION IN GUATEMALA

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Abstract

A 15 month sampling and analysis programme was carried out to monitor concentrations of 37 targeted organochlorine, organophosphorus and organopyrethroid pesticides in surface and ground water in Guatemala. The 80 sampling points included 4 points in a lake, one point in each of the four lagoons, 10 municipal water systems of major towns,. and 62 points along 52 rivers, most of which are located in the southern coast along borders with Mexico and El Salvador, and are one of the most productive areas.in the country. The sampling used provided only preliminary information on the pattern of pesticide contamination of surface and ground water. It showed contamination of surface water in Los Esclavos watershed, Motagua river watershed as well as Villalobos, lake Amatitlan and Michatoya river watershed. Cypermethrin was the ubiquitous pesticides in some areas present in concentrations exceeding toxic levels for fish and other aquatic organisms. Several of the other targeted organophosphorus and ECD detectable pesticides were also detected in surface water. Some municipal water samples also had low levels of pesticides.

1. INTRODUCTION

Guatemala is criss-crossed with many rivers which empty into the Pacific and Atlantic oceans. The watersheds receive water from some rivers passing through areas of high agricultural activity, where farmers frequently use a variety of pesticides. In many agricultural areas cotton was grown in the past and heavy use of organochlorine pesticides was practiced. These pesticides are known to persist in the environment and even though they have been banned since 1984 and 1988 there residues may still be found in the environment. Cotton has been replaced with exportable cash crops in many areas, and the persistent organochlorine pesticides are not used any more. However, some of the newer less persistent pesticides such as the organophosphorus compounds are highly toxic to aquatic organisms such as fish. Therefore, in order to protect the human health and the environment it is very important to monitor environmental matrices, especially water. In this project a 15 month sampling programme was undertaken to collect samples of river, lake, lagoon and municipal water samples to monitor residues of organophosphorus, pyrethroid and organochlorine pesticides. pesticides

2. METHODS AND MATERIALS

2.1. Sample handling and preparation

Water samples from the rivers were taken from the center of the rivers. Lake and lagoon water samples were taken along the shoreline. All water samples were taken at least 30 cm below the surface. Samples of municipal water systems were taken from public or domestic drinking water faucets. They were transported in amber glass bottles and kept cold with ice-water mixture. All samples were assigned code numbers for identification and traceability. Pesticides were extracted by adding 50g NaCl to 1 L water sample, stirring until dissolved and stirring with 100 mL dichloromethane for 1 hr. In each round of sample extraction a set of seven spiked laboratory water samples were run as control along with every 82 test samples.

The organic phase was separated, dried on 25g sodium sulfate and concentracted in a rotary evaporator at 40 ^{0}C and 30 mm Hg to 0.5 mL. The solvent was changed by adding 50 mL petroleum ether to the solution and concentrating to 0.5 mL. Samples were not allowed to concentrate to complete dryness during this process. The final volume was made to 1.0 mL with iso-octane. All organic solvents were pesticide grade quality and sodium sulfate was pesticide reagent grade, 12-60 mesh, heated at 130 °C and stored in a dessicator.

2.2. Preparation of solutions of analytical standards

Analytical standards of the targeted pesticides were purchased from Chem Service (USA) or Poly Science (USA). Solutions of standards were prepared according to their solubility in acetone or iso-octane. The stock solutions were prepared at a concentration of 1 mg/mL. Working solutions of appropriate concentration were prepared by further dilution of the stock solutions. .All solutions were stored at cold temperature and control sheets were maintained for all standards and solutions

2.3. Analysis

An in-house adapted and validated multi-residue analytical method was used for the analysis of water samples from rivers, lakes and municipal water supply systems. Samples were analysed by gas chromatography (GC) so only volatile compounds were detected and only those compounds were identified for which analytical standards were available. A number of compounds were detected but not identified due to the unavailability of analytical standards required to confirm their identity.

2.3.1. Gas chromatography

Samples were analysed by using a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with an electron capture detector (ECD), a flame photometric detector (FPD) and an HP-5 fused silica capillary column. The column was 60 m long, with 0.32 mm ID, and coated with a 0.25 μm thick film of stationary phase (5% phenyl, 95% methyl siloxane). Nitrogen,

TABLE 1. Gas chromatographic retention times (RT) for 37 targeted pesticides in the calibration solution

	μg/ml	t_{RET}		μg/ml	t_{RET}
DDVP	0 4	12 415	γ-CHLORDANE	0 1	43 399
FOSDRIN	0 4	18 862	o,p'-DDE	0 1	43 858
TRIFLURALINE	0 4	27 709	ENDOSULFAN I	0 1	44.535
PHORATE	0 4	28 352	α-CHLORDANE	0 01	44.795
a-HCH	0 03	28 665	DIELDRIN	0 1	[illegible]
HEXACHLOROBENZENE	0 02	29 162	p,p'-DDE	0 1	[illegible]
CYGON	0 4	29 535	o,p'-DDD	0 1	47 742
LINDANE	0 3	30 806	ENDRIN	0 1	49.256
TERBUFOS	0 4	31 102	ENDOSULFAN II	0 1	50 267
DIAZINON	0 4	31 857	p,p'-DDD	0 1	51 276
DISYSTON	4 0	32 149	o,p'-DDT	0 01	51 631
CHLOROTHALONIL	0 4	32 657	ETHION	0 8	51 886
PROPANIL	0 4	34 358	ENDOSULFAN SULFATE	0 3	55 176
METHYLPARATHION	0 2	34 985	p,p'-DDT	0 4	55 692
ALDRIN	0 1	35 518	PROPICONAZOLE	0 2	56 062
CHLORPYRIFOS	0 5	37 708	METHOXICHLOR	1 0	64 818
MALATHION	1 0	38 548	GUTHION	4 0	70 146
HEPTACHLOR EPOXIDE	0 1	41 315	CO-RAL	8 0	78 329
			CYPERMETHRIN	4 0	X=83 9

generated from a Claind ANG generator was used as the carrier and make up gas. The gas was passed through a series of traps set up in tandem and these included a Supelco high temperature trap, a charcoal trap, a moisture trap, an oxygen trap and finally an indicating moisture and oxygen trap. The flow rate for the gas was 1.3 mL/min. (measured at a temperature of 100 ^{0}C). The GC was operated in split/splitless mode, and the split vent was opened at 35 seconds after the injection. The split ration was adjusted at 1:5. Injections were made manually and by using Grob technique. The temperature programme was as following:

Oven initial temperature	115 °C
Initial hold time	5 min
Ramp 1	3.5 °C/min
Final temperature	210 °C
Final hold time	0.1 min
Ramp A	1.0 °C/min
Final temperature A	230 °C
Final hold time A	17 min
Ramp B	10 °C/min
Final temperature B	270 °C
Final hold time B	25 min

Due to long retention time for some of the pesticides each chromatographic run was for 90 minutes. Quantitation was based on a single point external standard calibration. Compound identification was based on absolute retention time, supported by the simultaneous detection by two detectors. This was accomplished by splitting the column effluent into two capillaries of equal length, each leading to a different detector. As both detectors responded at about the same time, it was possible to get some qualitative information about the compound detected. For example, a peak detected by ECD for an organophosphorus compound can be verified by the presence of a strong FPD signal at the same retention time.

2.3.2. Method performance tests and quality control procedures:

Quality control procedures included analysis of method performance control samples, recalibration of the GC after every 6 samples, use of control charts, and use of control forms for traceability. The calibration solution of 37 targeted pesticides was injected in the GC twice in the morning, the first for priming the instrument. During each round of analysis (80 samples) nine injections of the calibration solution were made and the results plotted on a control chart. This was done to verify that the analytical system was in control.

During the processing and analysis of field samples a fortified sample was prepared, extracted and analysed along with the field samples. The fortified sample was prepared by spiking laboratory water with acetone solution of the targeted pesticides. The recovery of extraction of the pesticides would give an estimate of the efficiency and reproducibility of the method.

3. RESULTS AND DISCUSSION

3.1. Quality control

The quality control chart for the results of the quantitative analysis of calibration solution of pesticides indicated that the analyses were repeatable as the results were within $\pm 2\ \sigma$ (standard deviation of the mean) for the pesticides in the calibration solutions used during the analysis of an entire sampling round which comprised of 80 samples. Quality control chart for GC retention time of pesticides in the calibration solution also verified repeatability of analyses.

The control charts verified that the analytical system was statistically under control quantitatively as well as qualitatively.

Figure 1 shows an example of a chromatogram with mirrored signals from the ECD and the FPD when a sample containing chlorpyrifos, diazinon and dimethoate was injected. Figure 2 shows chromatograms of calibration solutions of the 37 targeted pesticides detected by (a) ECD and (b) FPD. The retention times (RTs) for some pesticides were too close, for example α-endosulfan (44.535 min) and α-chlordane (44.795 min) as well as dieldrin (46.869 min) and p,p-DDE (47.033 min). The resolution of peaks for dieldrin and p,p-DDE was used as an indicator to test the performance of the column. The data on the recovery of the pesticides from the fortified water sample are shown in Table 2. It shows that the recovery of most pesticides was in the acceptable range (70-120%) with low CV (coefficient of variance) values. The exceptions were azinphos methyl and chlorothalonil. Azinphos methyl seemed to have an interfering compound co-eluting with it and it resulted in a false positive recovery value. Recovery of chlorothalonil, on the other hand, was very low, and the reason for this remained unknown. It may be the result of loss of the pesticide due to adsorption on septum in the sample vial cap.

3.2. Trends of pesticide contamination of rivers

The sampling frequency was too low to predict precise trends of pesticide residues in river, lake or municipal waters. Analysis of composite samples taken over a 24 h period with increased frequency would have given a more precise estimate of the levels and scale of pesticide contamination in Guatemalan waters. The sampling programme undertaken, therefore, gives at best only a rough idea about the extent or frequency of pesticide contamination. The results should, therefore, be taken as a general survey of the waters for pesticide contamination.

3.2.1. Pesticide residues in Los Esclavos watershed

Los Esclavos water shed receives water from three other rivers: Panal, Amapa and Utapa. The watershed lies in a region of heavy agricultural activity , with coffee as the main crop. Water samples were taken at sampling point Esclavos 1, located downstream from the merger of the three rivers with Eclovos, and sampling point Esclavos 2, located upstream. Water samples from sampling point Esclavos 1 contained cypermethrin (0.95 μg/L), γ-chlordane (0.17 μg/L), endosulfan (0.06 μg/L) and trace amount of coumaphos. Concentrations of pesticides varied from one sampling round to the next and the pattern of pesticide distribution was not consistent. The highest levels of pesticides were found in water samples taken during the second and third round of sampling. The concentration of cypermethrin in these water samples is quite high. This concentration is higher than that considered toxic to some of the fish and other aquatic organisms.

3.2.2. Pesticide residues in Motagua river

The Motagua river is one of the largest rivers in Guatemala, crossing from west to east and flowing into the Atlantic ocean. The Motagua river watershed receives water from 20 rivers of different sizes, some passing through agricultural areas. Water samples were taken at four sampling points. Residues of organophosphorus pesticides were detected more frequently and at higher concentrations than the other pesticides in these samples. This may be because of tobacco and melon cultivation in this area. Diazinon, with a mean concentration of 0.1 mg/L, was found in all water samples analysed. The other pesticides found included azinphos methyl (1.3 μg/L), disulfoton (1.0 μg/L at point 3 and 1.4 μg/L at point 4), malathion (between 0.25-

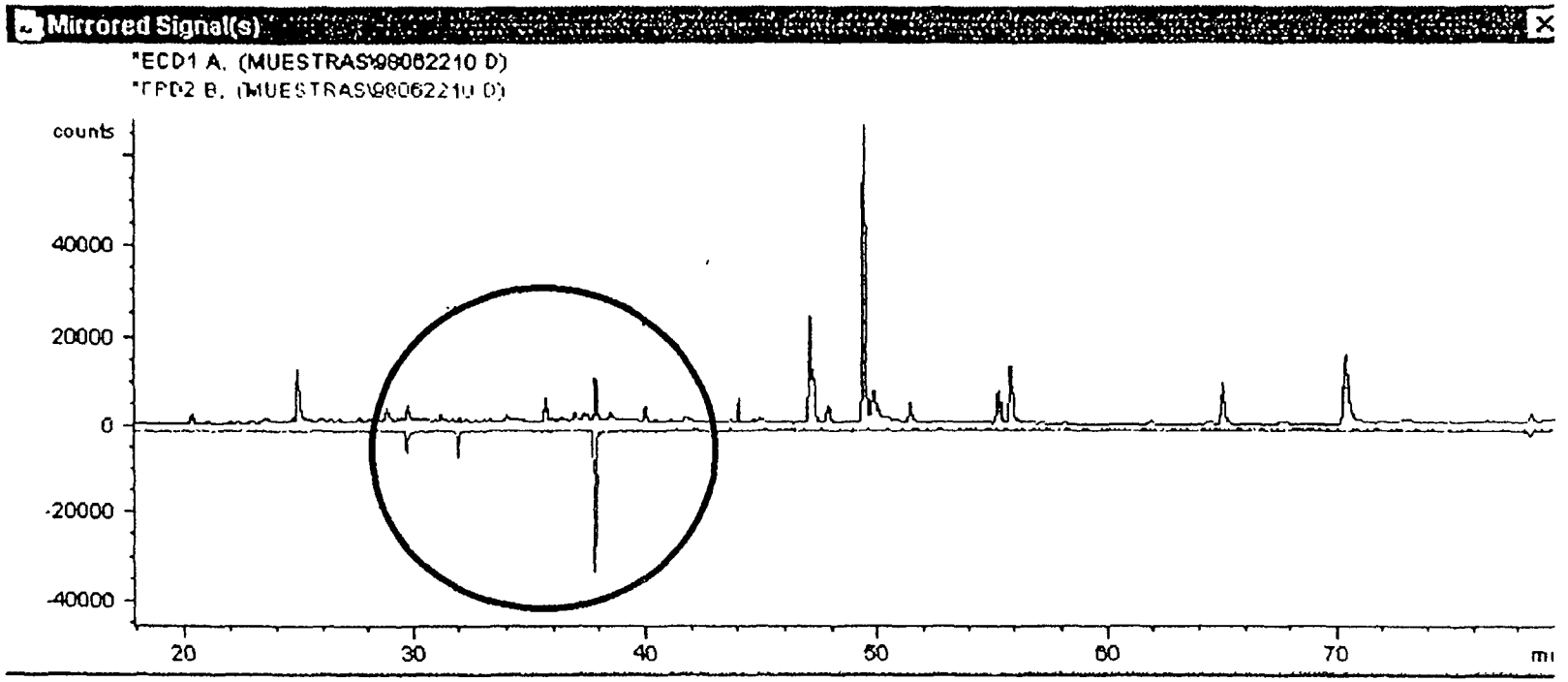
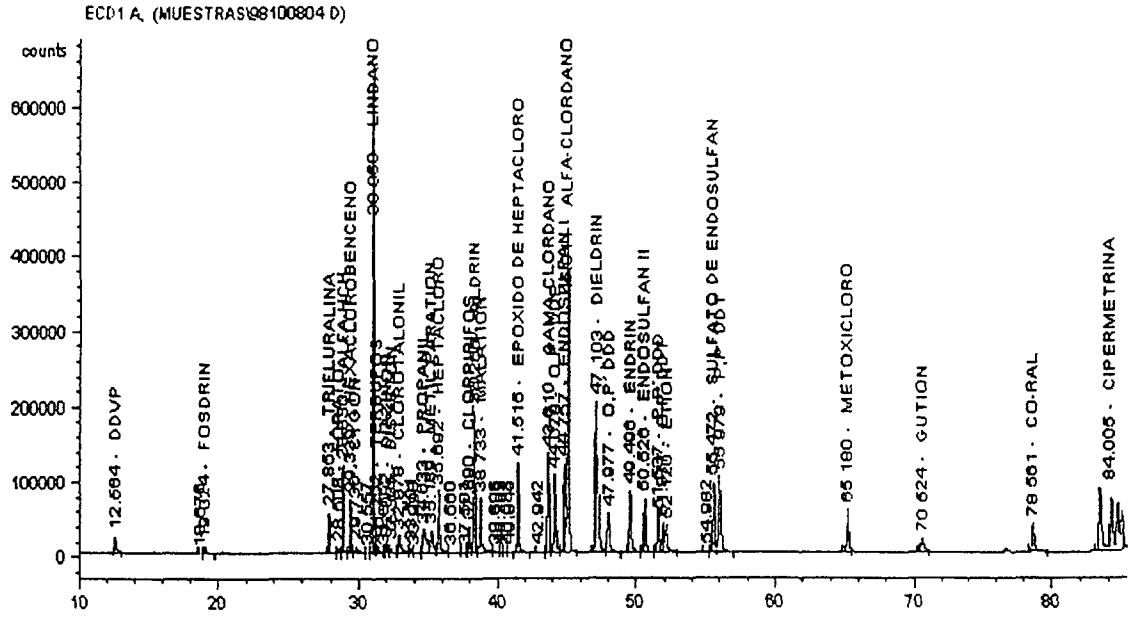


Fig. 1. GC chromatogram showing mirrored signals of three pesticides in a water sample simultaneously detected by ECD and FPD.

(a) ECD chromatogram of the calibration solution



(b) FPD chromatogram of the calibration solution

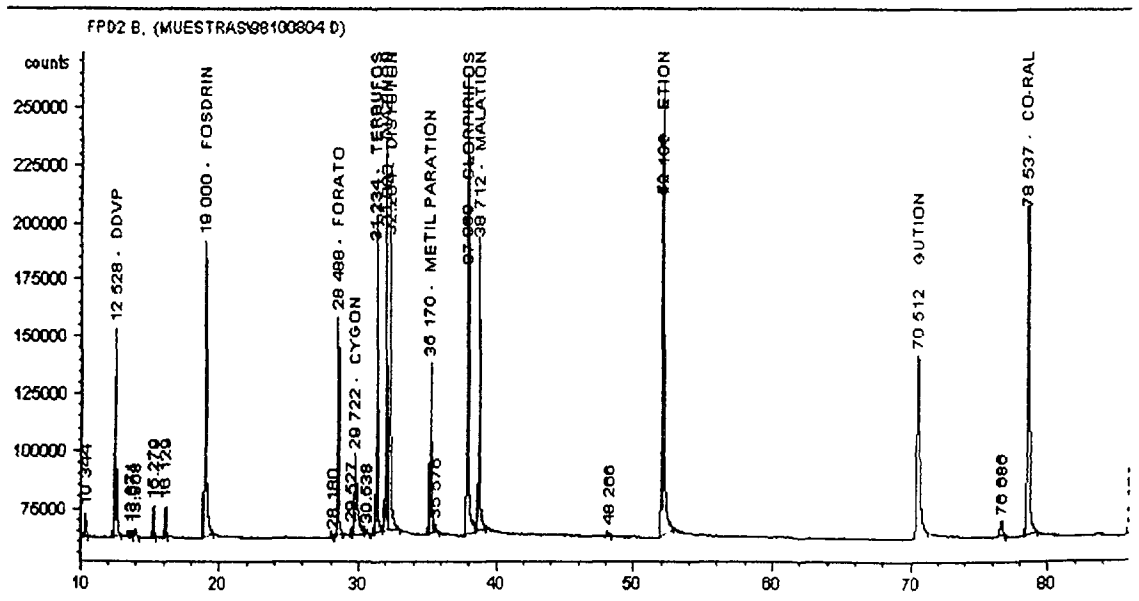


Fig 2. GC chromatograms showing signals for pesticides in the calibration solution detected by (a) ECD and (b) FPD.

0.35 μg/L at points 2 and 4), cypermethrin (0.1 μg/L) and trace amount of endosulfan and propanil.

TABLE 2. Concentration of 37 targeted pesticides determined in a water sample from the field and their recovery (%) from three fortified laboratory water samples

COMPOUND	98071711	fotn-1	fotn-2	fotn-3	% REC	% REC	% REC	MEAN	STD DEV	Coef. Var.
DDVP	24343	28480	29352	30853	116.99	120.58	126 74	121.44	4.93	4.06
FOSDRIN	21732	22234	23049	21220	102.31	106.06	97.64	102.00	4.22	4.13
TRIFLURALINE	44498	52587	57742	53638	118.18	129.76	120.54	122.83	6.12	4.98
PHORATE	8671	11262	12543	11157	129.88	144.65	128.67	134.40	8.90	6.62
a-HCH	29730	31903	33857	31701	107.31	113.88	106.63	109.27	4.01	3.67
HEXACHLOROBENZENE	25675	27827	27288	28727	108.38	106.28	111.89	108 85	2.83	2.60
CYGON	32965	28782	32303	26240	87.31	97.99	79 60	88.30	9.24	10.46
LINDANE	221939	237332	264262	237630	106.94	119.07	107.07	111.03	6.97	6.28
TERBUFOS	12693	11865	12595	11518	93.48	99.23	90.74	94.48	4.33	4.58
DIAZINON	16421	14522	15825	14994	88.44	96.37	91.31	92.04	4.02	4.36
DISYSTON	9842	9267	9770	8604	94.16	99.27	87.42	93.62	5.94	6.35
CHLOROTHALONIL	44405	2250	1209	2731	5.07	2.72	6.15	4.65	1.75	37.71
PROPANIL	128421	168234	177676	158992	131.00	138.35	123.81	131.05	7.27	5.55
M-PARATHION	76064	67331	73649	68537	88.52	96.83	90.10	91.82	4.41	4.80
HEPTACHLOR	48771	46096	47957	50775	94.52	98.33	104.11	98.99	4.83	4.88
CHLORPYRIFOS	59870	66715	69611	61890	111.43	116.27	103.37	110.36	6.51	5.90
ALDRIN	48664	44721	44329	45638	91.90	91.09	93.78	92.26	1.38	1.50
MALATHION	110259	109684	118597	107564	99.48	107.56	97.56	101.53	5.31	5.23
EPOX. HEPTACI	51831	46396	46189	48868	89.51	89.11	94.28	90.97	2.88	3.16
g-CHLORDANE	43450	36264	41278	42086	83.46	95.00	96.86	91.77	7.26	7.91
O,P'DDE	52963	40631	45052	53563	76.72	85.06	101.13	87.64	12.41	14.16
ENDOSULFAN I	52044	45984	51171	49379	88.36	98.32	94.88	93.85	5.06	5.39
a-CHLORDANE	147202	116444	128945	136118	79.10	87.60	92.47	86.39	6.76	7.83
DIELDRIN	139420	110682	125907	137022	79.39	90.31	98.28	89.33	9.48	10.62
P.P'DDE	49208	44773	47005	45461	90.99	95.52	92.39	92.97	2.32	2.50
O,P;DDD	58133	50199	53737	56884	86.35	92.44	97.85	92.21	5.75	6.24
ENDRIN	72288	69760	76930	73662	96.50	106.42	101.90	101.61	4.97	4.89
ENDOSULFAN II	42152	39224	44410	39915	93.05	105.36	94.69	97.70	6.68	6.84
P,P'DDD	87223	60734	66121	69133	69.63	75.81	79.26	74.90	4.88	6.51
O,P'DDT	21952	21563	24149	28494	98.23	110.01	129.80	112.68	15.96	14.16
EHTION	96222	83216	90726	90851	86.48	94.29	94.42	91.73	4.54	4.95
SULF. ENDO	80239	80717	87760	76705	100.60	109.37	95.60	101.85	6.97	6.85
P,P;DDT	63676	52668	57685	63484	82.71	90 59	99.70	91 00	8 50	9.34
METHOXICHLOR	131156	164059	177778	173100	125.09	135 55	131 98	130 87	5 32	4.06
GUTHION	147966	739610	713638	500405	499.85	482.30	338.19	440.11	88.70	20.15
CO-RAL	145182	135407	140630	129746	93.27	96.86	89 37	93 17	3 75	4 02
CYPERMETHRIN	497537	385100	427138	432557	77 40	85 85	86 94	83 40	5 22	6.26

3.2.3. Pesticide residues in the Villalobos river, lake Amatitlan and Michatoya river watershed

In this watershed the concentrations found were very consistent over the entire period of sampling. This fact distinguishes this watershed from those discussed in 3.2.1. and 3.2.2. It is noteworthy that Villalobos river receives effluent from the city of Guatemala and the associated industry as well as the surrounding agricultural areas. The river recieves upto 60% of the untreated effluent discharge from the city including domestic and industrial sewage This may be the reason for the presence of the pesticide residues in the river water throughout the year. Water samples from lake Amatitlan showed consistently low pesticide residues . This may be because the lake receives approximately 60% of its water from the underground

sources. Relatively higher concentration of pesticides were found in samples from Michatoya river, which may be due to intensive farming activity along this river.

In general, higher levels of pesticide residues were detected in the water from the southwestern region, where in some cases total pesticide residues reached a concentration of the ECD and FPD detectable pesticides of 2 to 3.5 μg/L, followed by northeastern region. It should be noted that the total pesticide concentrations referred to here do not include those pesticides which are not detectable by GC-ECD and FPD. Water in the southeastern region had on the average the lowest level of pesticide contamination.

3.2.4. Pesticide residues in water from lake Amatitlan and the lagoons

Analysis of water samples from lake Amatitlan indicated low level oc contamination with the ECD and FPD detectable pesticides. The total concentration of ECD and FPD detectable pesticides did not exceed 0.35 μg/L. This is important to know, because lake Amatitlan water will be used in the future as a reservoir to meet the needs of the growing population of Guatemala city. The residues in the lagoons were very low.

3.2.5. Pesticide residues in municipal water systems

The drinking water quality in 8 of the 10 municipalities, in terms of residues of organophosphates, complied even with the European legislation for potable water quality criteria which limits total pesticide residues to a maximum of 0.5 μg/L. However, in the municipal water from Amatitlin municipality the total pesticide concentration, including EC detectable and organophosphates, was 0.83 μg/L. Similarly, the municipal water samples from Villa Canales contained combined residues of EC detectable pesticides at 0.9 μg/L. High levels of cypermethrin were also detected in the municipal water distribution system of the cities of El Jicaro, Usumatlan and Estanzuela. These were above the 0.5 g/L limit for the sum of pesticides in potable water allowed under European legislation.

3.2.6. Relative frequency of ECD detectable pesticide residues in water

Results of the analysis of water samples taken during round 2 of sampling and analysed by GC-ECD showed a number of pesticides to be detected quite frequently. These included O,P-DDT in 12% of the samples, cypermethrin in 11%, propanil in 10%, endrin and heptachlor epoxide each in 9%, O,P-DDE in 8%, lindane in 7%, chlorothalonil, and α-HCH each in 6%, trifluralin in 5%, HCH in 4%, aldrin, α-chlordane and methoxychlor each in 2% and haptachlor, γ-chlordane, p,p-DDE, endosulfan sulfate and p,p-DDT each in 1% of the samples. Pesticides detected in samples with the frequency of less than 1% included α-and β-endosulfan, dieldrin, o,p-DDD and p,p-DDD

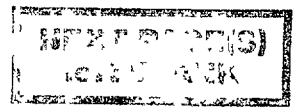
It is obvious that most of these compounds are old organochlorine pesticides which have been banned under Guatemalan law (DDT since 1984 and the rest since 1988). They have a very low water solubility, yet their presence in the environment is ubiquitous. This can be explained by assuming their presence in the soil and sediment, which is quite likely because, before the persistent organochlorine pesticides were banned, they were heavily used by cotton growers. It is likely that residues are now slowly partitioning from soil/sediment into water. Since many cotton growers are now cultivating exportable cash crops, they are showing increasing interest in having the soil tested for residues of the banned persistent pesticides prior to cultivation of new crops.

3.2.7. Relative frequency of residues of organophosphorus pesticides in water

Results of the analysis of water samples by GC-FPD showed the frequent detection of some of the organophosphorus pesticides. These included coumaphos in 34% of the samples, diazinon in 20%, chlorpyrifos in 12%, dimethoate in 8%, ethion, malathion, methyl parathion and mevinphos each in 5%, dichlorvos in 3%, disufoton in 2% and azinphos methyl in 1% of the water samples analysed.

4. CONCLUSIONS

Widespread contamination of surface water in rivers and lakes with ECD detectable and organophosphorus pesticides was found in a pesticide monitoring programme carried during 1997 and 1998. Cypermethrin was ubiquitous contaminant of river water and was also found in some of the municipal water supplies. It was detected in 11% of all the samples analysed and in some samples its concentration was as high as 0.90 - 0.95 μg/L which is greater than what is considered toxic to some fish and aquatic organisms. It is also in excess of the maximum limit (0.5 μg/L) for sum of pesticides set under European drinking water quality legislation. Several organochlorine pesticides were also detected in water samples, some as frequently as upto 12% of the samples analysed. The compounds most frequently detected included aldrin, α-chlordane, γ-chlordane, o,p-DDE, p,p-DDE, o,p-DDT, p,p-DDT, endosulfan sulfate, endrin, HCH, heptachlor epoxide and methoxychlor. Other pesticides most frequently detected in the water samples included chlorothalonil, triflralin, propanil, and organophosphorus insecticides azinphos methyl, chlorpyrifos, coumaphos, diazinon, dimethoate, disulfoton, malathion, methyl parathion and mevinphos.



THE USE OF SOLID PHASE EXTRACTION METHOD FOR ANALYSIS OF RESIDUES OF PESTICIDES USED IN BANANA PRODUCTION IN COSTA RICA

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Abstract

Different solid phase extraction devices were tested for the analysis of residues of eleven pesticides used in banana production in Costa Rica. The analysis was performed by using gas chromatograph equipped with NPD and ECD detectors. In general low recoveries and high variation coefficients were found for chlorothalonil, imazalil, terbufos and thiabendazole. For the other pesticides recoveries ranged between 60 and over 100%.

1. INTRODUCTION

Routine analyses of pesticides in water samples in our laboratory are done by liquid-liquid extraction (LLE) with dichloromethane followed by detection using GC. The use of solid phase extraction (SPE) techniques are becoming popular because they do not have several of the disadvantages of the LLE. Thinking of the ambient temperature we normally have in our laboratory between 25 and 30 °C, one of our main reasons for looking to alternatives to LLE is the use of dichloromethane which is known to be a hazardous solvent, and we want to reduce the risk of occupational exposure to it and other hazardous solvents. SPE-techniques could reduce the use of organic solvents to one third, thus reducing the associated risks and costs of use, storing and disposal. Other advantages could be more cost effective and faster extractions. It is also expected that SPE will be recommended in future official methods [1]. Different SPE-techniques are compared and described in several recent studies by Chiron [3], Schülein et. al. [4], Durand et. al. [5], De la Colina [6], and Di Gorcia [7]. In a recent book Barceló and Hennion covered an extensive review of SPE techniques used in the determination of pesticides in water [1].

The use of SPE-techniques during sampling of water samples could contribute to reduce losses of the contaminants by degradation (e.g. microbiological and UV light) and adsorption. Our field work often takes several days under tropical temperatures. Normally we preserve our water samples in 1 L bottles by adding in the field 25 mL dichloromethane and keep them cool in an icebox. Extracting the water samples in the field by a SPE-technique could offer several advantages, such as eliminating loss of pesticides due to elevated temperature and reduced use of hazardous solvents and shipping.

In this work a preliminary comparative study between several solid phase extraction techniques has been undertaken with pesticides normally screened in our banana-studies in order to determine recoveries and the possibilities of replacing the multi-residue screening LLE method.

In our ongoing-studies of impact of the banana production to the aquatic ecosystem we are analyzing routinely several pesticides used in the banana production. The pesticides included in this study are given in Table 1 with some physicochemical data and analytical remarks.

TABLE 1: PESTICIDES INCLUDED IN THE BANANA IMPACT STUDIES, WITH SOME PHYSICOCHEMICAL DATA AND ANALYTICAL REMARKS

Pesticides	Solubility (mg/L) [1,8]	Log Kow [8]	GC-detection	Remarks
FUNGICIDES				
Propiconazole	110	3.7	NPD, ECD	- frequently found in surface water samples
Chlorothalonil	0.6	2.5	NPD, ECD	
Imazalil	1400	3.8	NPD, ECD	- quantification limit 1 µg/l (NPD), with ECD lower - gives peak tailing with non-polar GC column - frequently found in effluent water from packing plant
Thiabendazole	50	1.9	NPD	- quantification limit 1 µg/l (NPD) - gives peak tailing with non-polar GC column - frequently found in effluent water from packing plant
NEMATICIDES				
Cadusafos	248	3.9	NPD	
Carbofuran	350	1.5	NPD	
Ethoprophos	700	3.6	NPD	- sometimes co-eluting with peak from blank
Terbufos	4.5	4.5	NPD	
INSECTICIDES				
Chlorpyrifos	0.4	4.7	NPD	
HERBICIDES				
Ametryn	185	2.8	NPD	

2. METHODS AND MATERIALS

2.1. Materials used

For the sample enrichment by solid phase extraction the following materials were used:

- ISOLUTE SPE ENV+ cartridges, IST (6 mL, 200 mg), polystyrene divinylbenzene (SDB) based polymer cartridges (extraction 2);
- ENVI-18 DSK, solid phase extraction disks Supelco (47 mm) (extraction 3);
- Empore C-18 extraction disks, J.T. Baker (47 mm, 500 mg) (extraction 4);
- Supelclean ENVI-18 SPE cartridges, Supelco (3 mL, 500 mg) (extraction 5);
  ISOLUTE sorbent C-18 bulk material, IST (40-70 µm) (extraction 6).

The disks and the bulk material were processed using a standard 47 mm all-glass filtration apparatus and vacuum. The within the IAEA project received single bell Speedman extraction

manifold for disks was only tested. The cartridges were connected with Teflon tubes and fittings to a vacuum flask.

The pesticide standards were obtained from Dr. Ehrenstorfer, Germany. Deionized water was used from a Milli-Q water purification system, Millipore. The solvents and the sodium sulfate (residue analysis grade) and other chemicals (analysis grade) for the elution, extraction, etc. were obtained from Merck, EM-Science or Riedel de Haen. Standard banana-mixtures (containing 11 or more pesticides) were prepared from individual pesticide stock solutions (50-100 mg in 100 mL acetone) and then diluted to 3 concentration levels with acetone/cyclohexane (1:9).

The standards and the extracts were analyzed with a Gas chromatograph, Shimadzu GC-9A modified for wide bore columns and equipped with a nitrogen-phosphorus detector NPD. Different 15 or 30 m x 0,53 mm GC-columns (containing SE54, BP-10 or HP-17 stationary phases) were used. The temperature program was 90 °C for 1 min, increased at 20 °C/min to 170 °C and then increased at 5 °C to 250 °C for 5 min. 1 µL of each standard or sample was injected by injection.

For the quantification external standard calibration graphs were constructed by injecting three different concentration levels of the standard pesticide mixture.

2.2. Extraction

Most of the extractions were done in du- or triplicate with Milli-Q or Milli-RO water samples spiked with one of the banana-mixture standards giving final concentrations of 0.05 - 5.2 µg/L in the water samples and one blank water sample. No pH adjustment was carried out.
Extraction 5 (see below) was done with spiked river water on three concentration levels. This experiment was executed at higher concentration levels than the other experiments.

2.2.1. Liquid-liquid extraction.

For the routine multiresidue extraction 1 L Milli-Q water samples were, after adding 100 g sodium chloride three times extracted with 50 mL dichloromethane. After concentration to 5 mL on a rotary evaporator, the extracts were concentrated to 0.1 mL in a gently stream of nitrogen. The extracts were transferred to acetone/cyclohexane (1:9) to a final volume of 1 mL.

2.2.2. Solid phase extraction on Isolute SPE ENV+ cartridges.

Two different experiments were executed with the cartridges: (a) The cartridges were washed with 10 mL ethyl acetate, conditioned with 5 mL methanol and were washed twice with 10 mL Milli-Q water. The 1 L of the spiked water sample was passed through the cartridge with a rate of 30 to 60 mL/min. The elution was carried out 3 times with 5 mL ethyl acetate. After drying the extract with sodium sulfate and concentration with nitrogen it was transferred to 1 mL acetone/cyclohexane (1:9). (b) The same procedure was followed as in (a) with one difference; the elution was carried out 3 times with 5 mL acetone/ethyl acetate.

2.2.3. Solid phase extraction on ENVI-18 disks.

Four different experiments were executed with these disks: (a) The disks were washed with 10 mL ethyl acetate, conditioned with 5 mL methanol and were washed twice with 10 mL Milli-Q water. The disks were maintained wet during conditioning. The spiked water sample (1 L) was passed through the disk with a rate of 30 to 60 mL/min. The elution was carried out 3 times with 5 mL acetone/ethyl acetate (1:1). After drying the extract with sodium sulfate and

concentration with nitrogen it was transferred to 1 mL acetone/cyclohexane (1:9). (b) The same procedure was followed as in (a) with one difference that 5 mL methanol was added to the spike water sample before passing through the disk. (c) The disks were washed with 5 mL methanol and 3 times with 10 mL acetonitrile, conditioned with 15 mL methanol and washed with 30 mL Milli-Q water (in two portions). The spiked water sample was passed through the disk with a rate of 30 to 60 mL/min. The elution was carried out 3 times with 10 mL acetone/ethyl acetate (1:1). The extract was dried with sodium sulfate, separated, concentrated first on a rotary evaporator and followed with nitrogen and then transferred to 1 mL acetone/cyclohexane (1:9). (d) The same procedure was followed as in (c) with one difference; 100 g sodium chloride was added to the spike water sample before passing through the disk.

2.2.4. Solid phase extraction on Empore C-18 disks.
Two different experiments were executed with these disks: (a) The cartridges were washed with 10 mL ethyl acetate, conditioned with 10 mL methanol. The 1 L of the spiked water sample was passed through the cartridge with a rate of 30 to 60 mL/min. (b) The elution was carried out 3 times with 10 mL ethyl acetate. After drying the extract with sodium sulfate and concentration with nitrogen it was transferred to 1 mL acetone/cyclohexane (1:9).

2.2.5. Solid phase extraction on ENVI-18 SPE cartridges [9].
The cartridges were washed with 3 mL ethyl acetate, conditioned with 3 mL methanol and were washed with 6 mL Milli-Q water. The 500 mL of the spiked river water samples (at three concentration levels) were passed through the cartridge with a rate of 15 to 20 mL/min.
The elution was carried with 5 mL ethyl acetate. After concentration with nitrogen the extract was made up to 1 mL ethyl acetate.

2.2.6. Solid phase extraction on C18-bulk
1 g of the C18-bulk material was placed on a PTFE membrane filter (1.0 μm) in the 47 mm filtration apparatus. The C18 bulk was washed with 10 mL ethyl acetate and conditioned with 10 mL methanol. The C18 was maintained wet during conditioning. To the spiked water sample (500 mL) was 2 mL methanol added and was passed through the filter with a rate of 50 mL/min.

The elution was carried out 3 times with 10 mL ethyl acetate. After drying the extract with sodium sulfate it was transferred after concentration with nitrogen to 1 mL acetone/cyclohexane (1:9).

3. RESULTS AND DISCUSSION

The recoveries obtained with the different methods are given in Table 2. The LLE showed good recoveries with most of the pesticides only within this case with rather high relative standard deviations (around 20-30%). Normally we get recoveries for most of the pesticides higher than 80% and with relative standard deviations below 10%.

The ISOLUTE ENV+ SPE cartridges (extraction 2) showed rather good recoveries for the more polar substances like imazalil and thiabendazole. For chlorothalonil the recovery was very low, it is not clear if this is caused by higher breakthrough volumes or by adsorption on the SDB material. For several compounds (especially for carbofuran and terbufos) high relative standard deviations were found, which could not be explained.

In the first assays (extraction 3a and 3b) with the SPE on the ENVI-C18 disks several problems occurred, among others: it was difficult to maintain the vacuum which caused fluctuating flow-rates and leakage occurred around the filter-assembly which causes losses during conditioning and elution. These problems could explain the low recoveries and the relative high deviations. The best results were obtained with the addition of salt to the water sample (extraction 3d). But still for chlorothalonil and terbufos the average recoveries were low.

TABLE 2: RECOVERIES AND VARIATION COEFFICIENT FOUND USING DIFFERENT TYPE OF EXTRACTION METHODS

Extraction		1. Liquid-		2a.SPE		2b.SPE ENV+c	
		n=3		n=3		n=3	
Pesticide	level	REC%	CV	REC%	CV	REC%	CV
Ametryn	0.57	102	19	101	16	57	19
Cadusafos	0.05	71	24	50	16	60	3
Carbofuran	0.54	80	17	67	55	75	7
Chlorothal	1.00	78	22	8	37	28	20
Chlorpyrifo	0.14	89	23	84	15	69	5
Diazinon	0.06	59	26	69	15	65	5
Ethoprofos	0.07	nd		54	18	61	5
Imazalil	2.51	111	32	92	20	32	6
Propiconaz	0.87	105	18	74	47	80	8
Terbufos	0.11	85	22	62	50	4	26
Thiabendaz	2.64	109	28	93	18	46	11

Extraction		3a. ENVI-		3b. ENVI-18 disk		3c. ENVI-18 disk		3d. ENVI-18 disk	
		n=3		n=2		n=2		n=2	
Pesticide	level	REC%	CV	REC%	CV	REC%	CV	REC%	CV
Ametryn	0.57	61	13	91	19	30	21	101	2
Cadusafos	0.05	40	44	51	28	37	20	62	8
Carbofuran	0.54	53	20	70	4	55	3	93	2
Chlorothal	1.00	39	10	49	18	9	141	24	52
Chlorpyrifo	0.14	46	34	59	19	55	5	89	8
Diazinon	0.06	33	44	54	7	36	5	78	8
Ethoprofos	0.07	nd		52	23	38	81	59	8
Imazalil	2.51	0	0	0	0	0	0	129	7
Propiconaz	0.87	52	23	79	17	76	7	111	3
Terbufos	0.11	31	150	3	141	3	133	19	8
Thiabendaz	2.64	0	0	0	0	69	9	104	1

Extraction		4. Empore C18	
		n=3	
Pesticide	level	REC%	CV
Ametryn	0.57	73	7
Cadusafos	0.05	65	13
Carbofuran	0.54	80	6
Chlorothal	1.00	69	9
Chlorpyrifo	0.14	72	11
Diazinon	0.06	69	9
Ethoprofos	0.07	80	22
Imazalil	5.03	31	1
Propiconaz	0.87	79	6
Terbufos	0.11	27	21
Terbufos-o-analogue sulfone	0.10	80	7
Thiabendaz	5.28	73	10

Table 2: (con't.)

Extraction		5. ENVI-18 SPE n=6	
Pesticide	level µg/L	REC%	CV
Ametryn	0.46-2.11	77	13
Carbofuran	0.40-2.09	74	14
Chlorothalon	0.44-2.06	69	12
Chlorpyrifos	0.09-0.41	82	15
Diazinon	0.10-0.48	81	12
Ethoprofos	0.09-0.46	79	14
Fenamifos	0.46-2.05	87	16
Propiconazol	1.07-4.69	90	17
Terbufos	0.08-0.41	60	20
Thiabendazol	4.92-20.1	78	15

Extraction		6. C18 bulk n=3	
Pesticide	level µg/L	REC%	CV
Carbofuran	2.1	59	5
Chlorpyrifos	0.41	63	8
Diazinon	0.48	47	9
Fenamifos	2.03	32	16
Propiconazol	4.63	73	7
Terbufos	0.41	23	26
Thiabendazol	19.76	65	5

Remarks:

extraction	refers to different techniques used, see text
level	spike level in water
REC%	average recovery in %
CV	variation coefficient

The EMPORE-C18 disks showed good average recoveries and relative standard deviations for most of the compounds low recoveries were found for imazalil and terbufos.

Also the ENVI-18 SPE cartridges (extraction 5) showed, at higher spike levels, good recoveries with spiked river water even for terbufos [9]. Imazalil was not included in the spike mixture.

The extraction 6 with the C18-bulk material showed low recoveries for terbufos and fenamifos. Only propiconazole gave a recovery above the 70 %.

In general we found lower recoveries than described in elsewhere in the literature, although for pesticides like chlorothalonil, terbufos, thiabendazole and imazalil no recovery data with SPE were found in the literature.

It is important to mention that the extractions were performed by different persons with different experience, which could contribute to several losses during the critical steps in the extractions like preconditioning, elution, concentration, etc.

4. CONCLUSIONS

The experiments done with the SPE-techniques showed that it is possible to obtain good recoveries for most of the pesticides analyzed in the banana impact studies. For some

substances the recoveries are low and in several cases high standard deviations were found. However, there is need for more experience in handling the equipment to avoid losses in the several critical steps like conditioning, elution and concentration.

Application of SPE needs method optimization, appropriate choice of solid phase materials and eluent are very important for pesticides of interest. Until now most of the experiments were done with Milli-Q water, more research will be necessary, also with real field samples, to see if the SPE-technique can replace the LLE. Also other sorbents such as Carbopack need to be tested.

Use of the Speedman Solid-Phase Extraction Manifold could eliminate the problems in handling the vacuum and avoid losses around the filter.

Besides the disadvantages of the LLE, the technique still has several advantages:
- because of relative high recoveries for a broad group of pesticides it is more versatile than SPE for screening purposes;
- sampling in the field (e.g. in a boat) is fast and relative simple.

Disadvantages of the SPE technique are:
-the optimization of the technique costs more time;
-the conditioning of the disks and cartridges under field conditions does not seems to be easy.

For the future additional experiments both with the C-18 disks and the cartridges will be executed.

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VALUATION OF SOLID PHASE EXTRACTION DISKS IN THE DETERMINATION OF PESTICIDE RESIDUES IN SURFACE AND GROUNDWATER IN PANAMA

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Abstract

In Panama large quantities of pesticides are used in agriculture and livestock farming and there is increasing concern about their impact on public health and the environment. Chiriqui is the Province that registers the largest number of producers whose activities have impact on the environment, especially on surface and groundwater. Systematic monitoring programmes are non-existent due, in part, to the high cost of laboratory determination of environmental residues of pesticides. Within the framework of the FAO/IAEA/SIDA Co-ordinated Research Programme, efforts were focused on evaluating and optimising the use of solid phase extraction C_{18} membrane disks in the analysis of surface and groundwater samples to determine pesticide residues. Factors studied were the effect of pre-washing and conditioning of the disks, flow rates, concentration level and matrix effects of field samples. Four pesticides, carbofuran, chlorothalonil, ametryn and chlorpyrifos were selected for these tests because preliminary analysis showed their presence in surface and groundwater. The technique significantly reduces the amount of organic solvents used as compared with the liquid-liquid extraction method. Quantifiable detection limits (Q_L) for the method were found to be 0.003 μg/L carbofuran, 0.016 μg/L chlorothalonil, 0.007 μg/L ametryn and 0.003 μg/L chlorpyrifos, when using standard spiked solutions. Recovery (%) was high when standard mixtures were used for the test runs but low when real surface water samples were tested, especially for chlorothalonil which was not recovered at all.

1. INTRODUCTION

Over 267 pesticides are distributed in Panama, of which 33.3% are insecticides, 27.9% are herbicides and 26.6% are fungicides. Imports are calculated to be in the order of 35 million US dollars [1]. Although an increasing number of farmers are using Integrated Pest Management techniques and more producers are resorting to organic farming, it is unlikely that the amount of agrochemicals will decrease significantly in the near future.

The Province of Chiriquí, located at the western region of the country, has the highest agricultural output and an important cattle-breeding tradition. A great percentage of the imported pesticides are used in this region, especially as crop protectors in banana plantations. Small and medium scale farming and cattle breeding account for many of the indiscriminate and improper use of pesticides. Due to heavy rainfalls and the relative fragility of tropical soils, many of these pesticides end up in surface and groundwater systems. An organochlorine pesticide residues study performed in 1989, on the waters of the Chiriquí Viejo River, which crosses highly fertile land, showed the presence of heptachlor epoxide, dieldrin, endrin and DDT with several samples showing residues above the maximum permissible limit [2]. These pesticides had already been banned from agricultural use.

In 1996 several water samples were taken from the Chiriquí Viejo River and the groundwater wells in the vicinity, and were extracted using solid phase membrane disks. The disks were analysed by the Food and Environmental Toxicology Laboratory of the University of Florida. Surface water samples had traces of chlorothalonil, alachlor, bromacil, chlorpyrifos and endosulfan, while groundwater samples had traces of chlorothalonil and chlorpyrifos. In both types of samples the levels were below 0.1 μg/L [3].

Even though pollution by pesticide residues does not seem to be a problem in Panama at the present time there is a need for monitoring food and the environment for residues of pesticides and other chemical pollutants by using cost effective and affordable analytical methods. In most of the pesticide analytical methods pesticides are extracted by using large quantities of expensive organic solvents. For example in Method 3510A of the U.S. Environmental Protection Agency [4], which is based on liquid-liquid extraction (LLE), pesticides are extracted from water by using large volumes of methylene chloride followed by subsequent Kuderna-Danish concentration to a reduced volume and final solvent exchange to hexane. At low analyte concentrations, the LLE procedures can result in analyte losses due to incomplete extraction, adsorption on the glass surface, and losses during concentrations and solvent exchange. . There is, therefore, a need for alternative methods of extraction based on the use of reduced quantities of solvents.

Many solid-phase extraction (SPE) methods and specially disk solid-phase extraction (DSPE) of pesticides from water provides a useful alternative to the traditional liquid-liquid extraction-base methods, since it reduces the amount of solvent required and shortens the time needed for extraction [5,6]. In the present research Empore C_{18} solid phase extraction disks was evaluated for extraction of four pesticides (carbofuran, chlorothalonil, ametryn and chlorpyrifos) from water prior to analysis by gas chromatography.

Because preliminary trials with ametryn, carbofuran, chlorothalonil, and chlorpyrifos had given poor recovery and low precision, modifications were made, which included disk-conditioning, change of extraction flow rate and solvent volume. The effect of spiking load and matrix effects was assessed.

2. METHODS AND MATERIALS

2.1. Solvents and reagents

All solvents used (acetone, toluene, methanol, iso-octane, ethyl acetate) and reagents (sodium sulphate anhydrous) were pesticide grade, and were purchased from J. T. Baker or Fisher.

2.2. Pesticides standards and solutions

Standards of ametryn, carbofuran, chlorpyrifos, and chlorothalonil were all 99% pure, and were obtained from Chem Service. Four standard mix solutions, containing 0.1, 0.2, 0.3 and 0.4 µg/mL of each of the four pesticides (ametryn, carbofuran, chlorothalonil and chlorpyrifos) in iso-octane was prepared for calibrations, and two standard mix solutions, containing 0.04 and 0.4 µg/mL of each of the four pesticides, in methanol was prepared for spiking. The solutions were kept at −10°C in amber screw cap vials with PTFE-silicone septa and open top phenolic closures.

2.3. Solid phase extraction disks

Empore™, 47-mm extraction disks with Bakerbond® octadecyl (C18) (J. T. Baker) were used in this study. A study protocol provided by Dr. Hugh Anson Moye [7] for the use of Empore™ SPE disks was used. The initial protocol established that the disks should be washed with 10 mL of ethyl acetate and conditioned with 15 mL of methanol, where the latter is allowed to saturate the membrane for 1 minute. Several researchers have used 10 mL methanol and a saturation time of 3 minutes to condition the membrane disks [8, 9, and 10]. It was, therefore, decided to do some tests using 10 and 15 mL methanol. The results showed that there was no

significant difference between the two volumes. Therefore, the disks were conditioned by using 10 mL of methanol and 3 minutes saturation time.

After several trials, pre-washing of the membranes with ethyl acetate was performed by placing the disks onto a petri dish, adding enough solvent to soak the membranes and leaving them covered for 6 hours. The disks were then placed on the filtration apparatus and dried by applying vacuum for 1 minute. Once dry, they were kept in aluminium foil until use.

2.4. Sample preparation

2.4.1. Extraction of pesticides from water

Glass distilled water, filtered through the Empore™ disks was used for sample preparation. One litre samples of water were spiked with 1 mL of standard mixture and filtered through the Empore™ disk, which had been previously conditioned with 10 mL of methanol. A Jr. Speedman system was used for extraction, which was kept at a flow rate of 30 mL/min. Once the sample was extracted, the disk was dried by applying vacuum for 20 minutes.

2.4.2. Elution of pesticides from the disks

Pesticides were eluted from the disks with ethyl acetate. Once the extraction of the disks was completed, they were placed on a 1 litre, glass filtering system (Wheaton TS 40/35), where a 200mm x 30mm glass tube, containing 1g of anhydrous sodium sulphate, was placed for collection of the extracts. The extracts were concentrated to approximately 4 mL and then transferred to a 10 mL Kuderna-Danish concentration tube where, with the aid of an adjustable Mini- Vap (Supelco 2-2970) concentrator/evaporator, the sample was evaporated to dryness, dissolved in iso-octane and then adjusted to a final volume of 0.2 mL for analysis.

2.5. Sample analysis

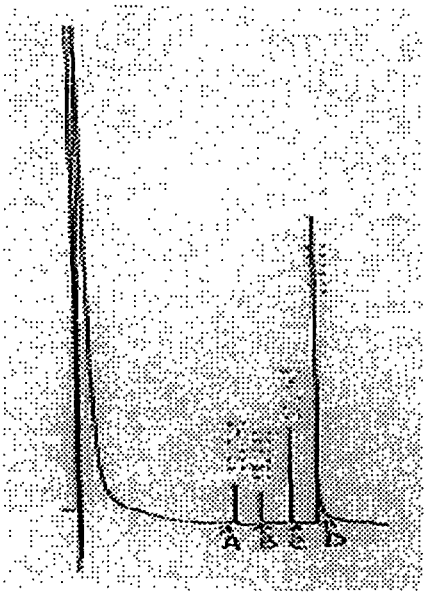
A Shimadzu model 14-B gas chromatograph equipped with direct injection system and a nitrogen-phosphorous thermionic detector (NPD) was used for the analysis. A fused silica, PTE-5®, wide bore capillary column, 30m x 0.53mm, with 0.53μm film thickness, (Supelco), was used for the separation of the pesticides. Direct manual injection, of 1μL, of both standards and samples were made with a 10μL (Hamilton #701) syringe. The operational conditions of the gas chromatograph were as follows:

Injector temperature (°C)	225
Column initial temperature (°C)	150
Column initial hold up time (min.)	3
Column temperature program (°C/min.)	4
Column final temperature (°C)	214
Column final hold up time (min.)	15
Detector temperature (°C)	300
Detector range	1
Detector current (nA)	6
Carrier gas flow (mL/min)	3 (Helium)
Backup gas flow (mL/min)	37 (Helium)
Hydrogen flow (mL/min)	3
Air flow (mL/min)	150
Attenuation	0

3. RESULTS AND DISCUSSIONS

3.1. Gas chromatography

The chromatogram of the analysis of the four pesticides is shown in Fig 1. The retention times for the pesticides were 13.7 min for carbofuran, 15.7 for chlorothalonil, 17.9 for ametryn and 19.9 for chlorpyrifos. The time span between injections was 45 minutes. Each sample was injected twice, and the standard pesticide mixture was run after every 6 injections to check the variability in the NPD response.



Std. mix: 0.2μg/mL (A: Carbofuran; B: Chlorothalonil; C: Ametryn; D: Chlorpyrifos)

FIG.1 Chromatogram of standard mixture of pesticides

The GC responce (peak height) was plotted against concentration for each pesticide, and the best-fitted linear regression lines were estimated. Detector response of the calibration solutions was linear over a range of concentrations of 0.1 to 0.4 μg/mL. The lowest response was given by carbofuran and the highest by chlorpyrifos (Fig. 2).

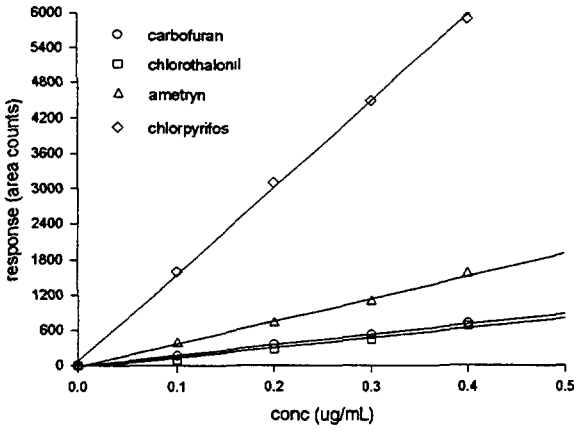


FIG. 2. Calibration curve of standard mixtures

The r^2 values for the linear regression model with non-zero intercept, of each of the four pesticides, ranged between 0.978 and 0.999. The limit of detection of the method calculated from the calibration curves, ($Q_L = 3s_{y/x}$ */slope*) was 0.003 μg/L for carbofuran, 0.016μg/L for chlorothalonil, 0.007 μg/L for ametryn and 0.003 μg/L for chlorpyrifos.

3.2. The effect of extraction speed on recovery of pesticides

Extraction speed is one of the main advantages of solid phase extraction, since it is reduced significantly when a comparison is made with other extraction techniques. Flow rates reported by H.A. Moye for water extraction averaged 234 mL/min, giving an extraction time of 4 to 5 min/L. Tests with this high flow rates gave poor results and a literature search revealed that several authors recommend a flow rate of 30 mL/min when using Empore™ C_{18} SPE disks [11]. This lower speed increased the extraction time to 33 minutes per liter.

Flow rate effects were tested by spiking six samples of distilled water with 1-mL standard mixture containing 0.04 μg/mL each of ametryn, carbofuran, chlorothalonil and chlorpyrifos. The results (Table I and Fig. 3) show an increase in recovery rates and reduced variation (<6%) when the extraction of the water sample is carried out at 30 mL/min.

TABLE I. RECOVERY (%) OF PESTICIDES FROM DISTILLED WATER BY USING TWO DIFFERENT FLOW RATES FOR EXTRACTION

Pesticide	200 mL/min					30 mL/min				
	1	2	3	mean	cv	1	2	3	mean	cv
Carbofuran	89.3	97.4	123.6	103.4	17.3	109.8	104.5	99.8	104.7	4.9
Chlorothalonil	56.1	26.7	50.5	44.4	35.1	58.3	64.5	64.0	62.3	5.6
Ametryn	21.6	24.0	14.8	20.1	23.7	67.0	63.8	65.3	65.3	2.5
Chlorpyrifos	44.1	40.6	55.6	46.8	16.8	95.3	85.0	87.8	89.3	5.9

3.3. The effect of fortification level on the recovery of pesticides

The rate of degradation of pesticides in water is affected by their concentration because of kinetic effects over certain reactions due to hydrolysis and photolysis [12]. This effect was, therefore, studied in the laboratory by spiking distilled water with standard solutions of different at two fortification levels of 0.04 μg/L and 0.4 μg/L of each pesticide. These levels were chosen so that they would fall below and above the maximum permissible limit in water of 0.1 μg/L.

For each trial run 1L distilled water was spiked with either a 0.04μg/L standard pesticide mixture or with a 0.4 μg/L spiking solution. Every run was done in triplicate and all fortified samples were extracted with an Empore C18 membrane at a 30 mL/min flow rate. The results (Fig. 4) show a significant reduction in the recovery percentages of the different pesticides when the fortification level is increased from 0.04 μg/L to 0.4 μg/L. Ametryn and Chlorpyrifos are the pesticides most affected, from 71.5% to 16.1% in the first case and from 95.8% to 48.9% in the latter. It was also observed that the coefficient of variation were lower than 10% for both concentration levels except for ametryn at the higher fortification level; recoveries were quantitative, with the notable exception of ametryn.

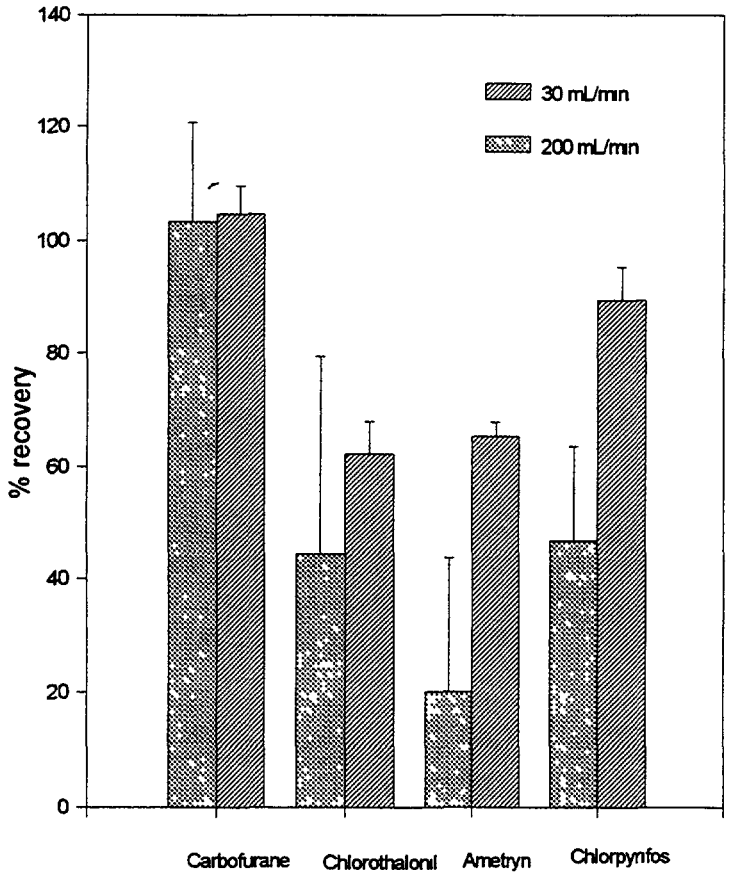


FIG. 3. Percent recovery of pesticides at two extraction flow rates (30 mL/min. and 200 mL/min.)

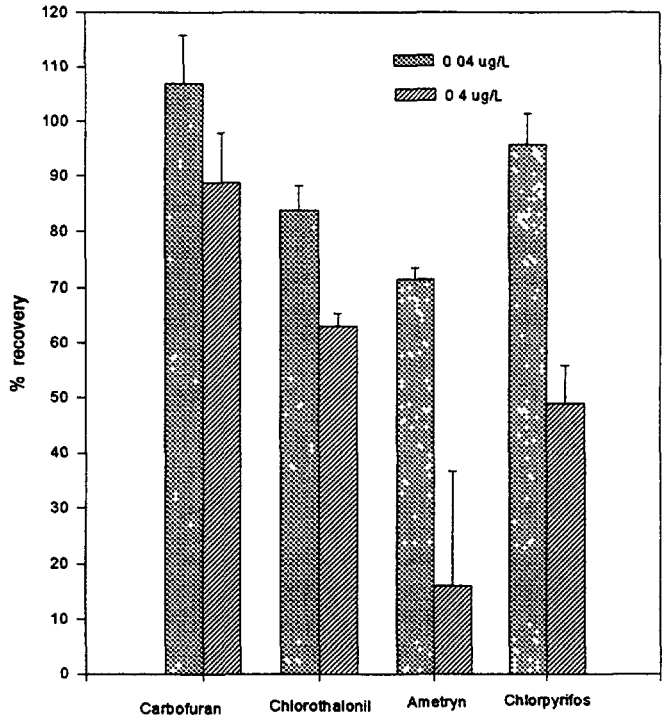


FIG. 4. Percent recovery of pesticides from distilled water at Two concentration levels (0.04μg/L and 0.4μg/L)

3.4. The effect of matrix on the recovery of pesticides

The effect of suspended particulate matter in the water on the extraction of the pesticides was studies by spiking 1L water samples from Platanares river with the four pesticides. The samples were spiked with a 1-mL aliquot of the standard pesticide mixture of 0.04 μg/L. A glass fibre pre-filter (Whatman GF/D) was placed above the Empore™ C_{18} membrane prior to filtration. Some difficulty was encountered during filtration due to the amount of suspended particles but a flow rate of 30 mL/min was achieved

Results in Fig. 5 show an increase in the recovery of carbofuran from 106.9% in distilled water to 126.5% in river water, Similarly, the recovery of ametryn increased from 71.5% in distilled water to 90.7% in river water. Chlorpyrifos, on the other hand, shows a reduction from 95.8% in distilled water to 77.3% in river water. No recovery, at all, was observed for chlorothalonil when river water was used. This could be due to sorption of chlorothalonil on organic matter suspended in the river water, a characteristic that has been reported for non-polar compounds (13,14).

An increase was also observed in the coefficient of variation (cv) when river water was used. However, they are lower than 20%, which means that the recoveries are quantitative, with the exception of chlorothalonil.

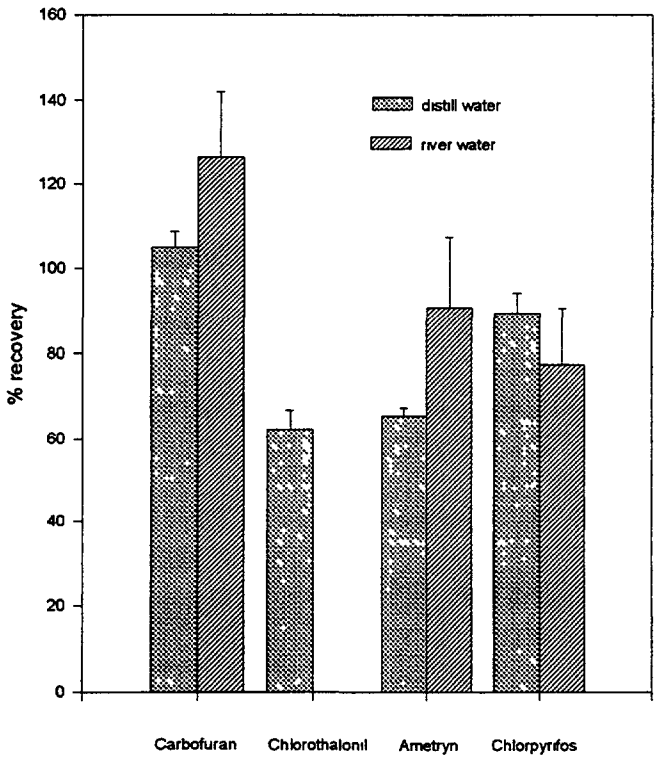


FIG. 5 Percent recovery of pesticides from surface water

4. CONCLUSIONS

The analytical method described in this work provided a rapid, quantitative, and convenient procedure for determining 4 pesticides at the low part per billion level. The extraction with the Empore™ C_{18} filter disks, permitted an average pesticide recovery, from distilled water, of

71.5% (ametryn), 83.7% (chlorothalonil), 95.8% (chlorpyrifos) and 106% (carbofuran), at a concentration level of 0.04 μg/L. But for real samples, recoveries vary significantly, especially in the case of chlorothalonil, whose recovery depends strongly on the amount of suspended organic matter. Finally, the disks proved to withstand several extractions, which makes it attractive for laboratories of low budget.

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PESTICIDE RESIDUE ASSESSMENT IN THREE SELECTED AGRICULTURAL PRODUCTION SYSTEMS IN THE CHOLUTECA RIVER BASIN OF HONDURAS

(Abstract)

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There is a basic lack of information about the presence of pesticide residues in the environment in Central America. Over the period of February 1995 to June 1997, river, well, lagoon and spring water samples, as well as soil, fish tissue, lagoon bed sediments and some foodstuffs were taken from the greater Cholutecan River Basin of Honduras and analyzed for pesticide residues. These were collected at three separate sites (La Lima, Zamorano and Choluteca), each characterized by differing agricultural production systems.

The main pesticide residues found in soil samples were dieldrin and p,p'-DDT, while river water samples were found to have detectable levels of heptachlor, endosulfan and chlorpyrifos, with lagoon and well water also being shown to contain heptachlor. These pesticides detected were in more than 20% of the samples assessed. In river water samples more pesticide residues at higher concentrations were found to be associated with areas of more intensive agricultural production. The fewest pesticides with lowest concentrations were found in the small sub-watershed associated with traditional agricultural production. Although the pesticides found in the soils at the three sites were generally similar they tended to be higher in the southern part of the Cholutecan watershed, followed by the central zone, with the lowest concentrations being found in the more traditional production zone. In lagoon and well water samples more pesticides, but mostly in lower concentrations were detected at the traditional production site than at the others.

Ten pesticide compounds were detected in fish tissue, mainly organochlorines, some of which were also found in lagoon sediments. In terms of food products, almost no pesticides were detected in vegetables, but the kidney adipose tissue taken from slaughtered cows was shown to have a tendency to contain some organochlorines. Spring water in the traditional agricultural production zone contained three organochlorine compounds as well as chlorpyrifos, but these were detected only in the rainy season.

This study indicates that pesticide residues are present and strongly associated with areas of intensive agriculture, although even in the areas practicing more traditional agriculture, pesticide residues were not absent. Further pesticide monitoring is necessary to obtain a more complete picture of the situation and based on this data an effective policy framework to assist in minimizing pesticide residue accumulation may be developed to reduce the adverse impact these compounds are known to have on the environment and human health. This snapshot of the pesticide distribution in three agriculturally distinct regions within the Choluteca watershed indicates that the situation is stable, but should be monitored to determine if pesticides residues will pose a health hazard in the future.

Part II

STUDIES ON DISSIPATION AND DEGRADATION OF PESTICIDES IN PLANTS AND SOIL

DISSIPATION, DEGRADATION AND UPTAKE OF ^{14}C-CARBOFURAN IN A PANAMANIAN ALFISOL SOIL

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Abstract

The dissipation, degradation and leaching of carbon-14 labelled carbofuran was studied in a micro-lysimeter system with disturbed and undisturbed soil cores of an Alfisol from El Ejido, Panama. The micro-lysimeters were conditioned under the environment prior to the application of the insecticide. Each lysimeter was treated with 14C-labelled carbofuran at a concentration of 1.7 μg carbofuran/g soil and maize seed were sown in the treated soil. Samples of soil were taken at 0,8,15,30,60,90 and 180 days after treatment. The plant material was separated and the soil was analyzed by radiometric techniques for total, extractable and non-extractable residues. The total 14C-radioactivity decreased with time to 30 % of the originally applied activity. Extractable residues decreased with time to 2.5 % whereas, the un-extractable residues increased to 35.5% of the original. Residues in the plant foliage were in the range of 0.5 to 0.9 μg/g and showed highest concentration during the first 30 days after germination. Extractable residues included carbofuran, 3-hydroxy-carbofuran and 3-keto-carbofuran. The amount of radioactivity leached was in the range of 19.2 to 22.8 % of original. It is concluded that carbofuran residues move easily in soil-maize system. Maize plants rapidly absorb the insecticide and C14-activity predominates 15 to 30 days post-treatment. .Dissipation of carbofuran occurs soon, with a halflife of 30 days.

1. INTRODUCTION

Carbofuran(*2,3-dihydro-2,2-dimethyl-benzofuran-7-yl-methylcarbamate)* is a systemic insecticide and nematicide with predominantly contact and stomach action. It is effective against soil inhabiting and foliar feeding arthropod pests that attack important crops [1] and was developed in USA by FMC [2]. Registration for uses on many crops have been accepted in the countries of Central America. Carbofuran as Curater was first registered in Panamá for use in agriculture in 1978. Today its use is recommended for crops such as fruits, vegetables, banana, sugar cane, rice, maize and potatoes [3].

Most of research on the fate of pesticides in soil has been carried out under temperate conditions [4]. However, agriculture in Central America makes substantial use of pesticides and there is great concern regarding the fate and effects of pesticides in Central American environment.

Loss of carbofuran from soil is possible from microbial and chemical degradation and dissipation by adsorption on fine soil particles. Therefore, special attention is required to understand the fate of this widely used insecticide in soil so that no contamination is experienced. A half-live of 30-60 days in soil have been reported [5]. No studies on the residues and dissipation of carbofuran in soil-maize ecosystem have been carried out in Panama. Therefore, we conducted experiments using ^{14}C-carbofuran in lysimeters under tropical field conditions with soil samples collected from the main maize commercial area of the country. The main purpose of these experiments was to gain information on the dissipation and degradation of carbofuran in model lysimeter systems following experimental protocols developed by FAO/IAEA [6] to evaluate the significance of pesticide residues which may be present in the soil following an insecticide application under tropical outdoor conditions typical of farming in Panama and Central America.

2. MATERIALS AND METHODS

2.1. Chemicals.

Carbofuran (*2,3-dihydro-2,2-dimethyl-benzofuran-7-yl-methylcarbamate)* uniformly ring-labelled with Carbon-14 was obtained from IAEA. Radiopurity was 94%-98% as checked by TLC. Analytical grade standards of carbofuran and degradation products were available commercially. All other chemicals used were of analytical grade.

2.2. Soil characteristics.

The soil used in this study was haplustalf alfisol characterized as clayish, fine isohiperthermic soil of moderate permeability, with a pH of 5.7. It contained 16.9% sand, 28.2% silt and 54.9% clay. The organic carbon content was 1.7% and maximal water holding capacity (1/3 bar) of 31.4%.

2.3Undisturbed soil columns.

Eighteen undisturbed soil cores were obtained in PVC cylinders of 30 cm length and 10 cm diameter from 0-25cm depth at the IDIAP Experimental Farm in El Ejido, in Los Santos Province. The upper 15 cm soil had been previously ploughed, but the 15-25 cm depth had not been ploughed. The total dry weight of the soil in each column was 1.5 kg. These microlysimeters (cylinders) were placed outdoors next to the laboratory and allowed to acclimatize before treatment with the insecticide.

2.4. Disturbed soil colums.

Soil was obtained from El-Ejido at a depth of 0-10 cm and passed through a sieve No. 10 which allowed passage of particles of less than 2mm size). After thorough mixing the soil was air dried and packed in each of the 18 PVC cylinders of 15x20cm dimension. Each microlysimeter (cylinder) had 3 kg air-dried soil. The microlysimeters were placed outdoors next to the laboratory and allowed to acclimatize for a period of two weeks before treatment with the insecticide.

2.5. Preparation of ^{14}C-labelled carbofuran formulation and treatment solution

A commercial granular formulation of carbofuran containing 10% a.i. was labelled with ^{14}C by mixing 20g formulation with 10 ml acetone and adding a solution of ^{14}C-carbofuran (833.3 KBq) in 10ml acetone. The mixture was swirled for 30 minutes and the solvent evaporated on a rotavapor. Radiochemical purity was 94% as determined on TLC. The radioactivity in the dried granules, as determined by combustion of 3 replicated samples of granules in a Harvey Biological Oxidizer OX-600, was 41.7 KBq/g. This preparation was used for the treatment of the microlysimeters containing undisturbed soil cores. Another treatment solution was prepared by dissolving 13.1 mg ^{14}C-carbofuran (specific activity 18.7 mCi/mmol) in 100ml acetone. The radioactivity in this treatment solution was 113.3 KBq/ml. This solution was used for the treatment of the lysimeter containing disturbed soil columns.

2.6. Treatment of lysimeters

Each microlysimeter with undisturbed soil core was treated with 1 gram ^{14}C-carbofuran granules. The radioactivity in the applied material was 41.67 KBq per lysimeter equivalent to a concentration of 1.7 mg carbofuran per gram soil. Three seeds of maize were planted at a depth of 2.5cm in each lysimeter.

The Lysimeters with disturbed soil were treated by addition of 3.0g of commercial carbofuran 10G granules in to a small hole in each microlysimeter, followed by 5ml of the treatment solution of ^{14}C-carbofuran containing 41.67 KBq. Three seeds of maize were added and the hole was covered with soil.

2.7. Sampling.

Soil samples from three microlysimeters were analysed at each sampling interval. The sampling intervals were 0, 8, 15, 30, 60, 90 and 180 days after treatment. The soil core was pushed out of each lysimeter, divided in to 3 sections 0-5cm, 5-15cm and 15-25cm and the fresh weight recorded. Soil from each section was then homogenized for 10 minutes, transferred onto aluminum foil and packed in a polyethylene bag which had been pre-labelled with a code number. The samples were stored at -20°C until the extraction and analysis were carried out. The moisture contents of the soil were determined by heating in an oven at 110 °C for 24h.

2.8. Determination of total C-14 residues.

Radioactivity in soil samples was determined by combustion of sub-samples in a Model OX-600 Harvey Biological Oxidiser. to carbon dioxide. The gas was absorbed in 10 ml carbon dioxide absorbant cocktail. The radioactivity in the sample was determined in a Packard Tricarb Model 1000 liquid scintillation counter. For determination of radioactivity in a solution, 1 mL solution was mixed with 10 ml Instagel liquid scintillation cocktail in a scintillation vial and the radioactivity estimated in the liquid scintillation counter. The external standard calibration technique was used to correct for quenching. The residues in soil were calculated on dry weight basis.

2.9. Determination of extractable residues.

Soil subsamples (50g) were extracted for 6 hour in a Soxhlet extraction apparatus with 200 ml methanol at a flow rate of 5-7 cycles per hour. The extract was concentrated to about 5 ml on a Buchi rotary evaporator. Radioactivity in the extract was estimated by mixing 1 mL of the extracted solution with 10 mL Instagel liquid scintillation cocktail and counting in a Packard Tricarb Model 1000 liquid scintillation counter. The extract was also analysed by TLC.

2.10. Determination of unextractable residues.

After the extraction of the radioactivity from the soil samples, the samples were air dried and 1.0g subsamples combusted in the oxidiser to determine the radioactivity which remained bound to the soil.

2.11. Thin layer chromatography(TLC).

TLC analysis was performed on precoated silica-Gel $60F_{254}$ chromatoplates (20x20cm; 0.25mm thickness, glass, E-Merck). The concetrated solution was directly applied at the botton of the central linear region of TLC plate while vertical channels on the two sides were used for reference authentic compounds. The plates were developed in a mixture of dichloromethane + chloroform + ether (1+1+1, v/v/v). The plate was autoradiographed on x-ray film to detect radioactive regions. The radioactive spots were scraped off the plate and radioassayed in the LSC after mixing with 10ml Instagel.

2.12. Leaching.

The leachate from the lysimeters was collected after rainfall. The volume from each lysimeter was measured and radioactivity determined by analysing 1 ml samples mixed

with 10 ml instagel liquid scintillation cocktail and counting in the liquid scintillation counter.. Radioactivity from the leachate was extracted by passing 500ml through a LC-18 soild phase extraction (SEP) cartridge. After elution with methanol the extract was concentrated to 1ml and analysed by HPLC and TLC.

3. RESULTS AND DISCUSSION

Levels of carbofuran residues in undisturbed soil cores are shown in Table I. The residues decreased steadly with time. Initially, the total C-14 carbofuran residues were in the upper soil layer and ranged from 1.3 to 1.7mg/g. But 60 days after the treatment the residues in soil decreased to 1/3 of the originally added radioactivity. After this period the radioactivity remained relatively constant. The slightly increased value at 90 days in the upper layer could be explained by the effect of movement by evapotranspiration related to rain during this period. Residue levels in the middle and lower soil layers increased slightly showing some movement downwards of the radioactivity. This was confirmed by the presence of ^{14}C-activity in leachates. After rainfall the radioactivity in the leachate increased with time. The leachate contained approximately 2% of the initial radioactivity.

Radioactivity in maize leaves is shown in Table II. Eight days after germination of the seed total residues in leaves were at a concentration of 0.5 to 0.9 mg/g. Thereafter the residues steadily decreased with time. It is during the first few weeks after the seed germination that carbofuran residue in the plat protect it aganist the attack of pest insects.

Carbofuran residues dissipate in disturbed soil more or less similar to that in undisturbed soil cores. However, dissipation is more rapidly during the first 15 days of treatment. After 30 days 45.9% of total added radioactivity was found in the disturbed soil samples. At this stage the amount of residues detected in undisturbed soil cores was 42.9%. Thereafter, the radioactivity decreased slowly to a value of 38.6% after 60 days (Table III).

Extractable residues decreased with time to 2.5% of initial amount after 60 days. Non extractable residues increased with time up to 35.5% (Table-IV) at 15 days and then decreased to 24.4% after 60 days.

Radioactivity in the leachate from the cores of disturbed soil is shown in Table-V. It did not make a significant difference whether or not the maize plants covered the soil; the amount of residues leached are in the same order of 19.2%-22.8% eight days after treatment and 3.2 and 3.3% after 30 days of the carbofuran application.

The extractable residues after 30 days treatment were composed of carbofuran, 3-hydroxy-carbofuran and 3-keto-7-phenol metabolite (Table-VI).

TABLE 1: Residues of ^{14}C-Carbofuran in Undisturbed Soil Cores in mg/g

days	0-5cm				5-15cm				15-25cm				total	
	rep1	rep2	rep3	mean	rep1	rep2	rep3	mean	rep1	rep2	rep3	mean		%
0	1.26	1.43	1.29	1.33	-	-	-	-	-	-	-	-	-	78.2
15	1.23	2.14	1.86	1.74	0.07	0.12	0.01	0.07	0.03	0.02	0.29	0.02	0.11	107.7
30	0.76	0.65	0.42	0.61	0.14	0.08	0.08	0.10 °	0.02	0.02	0.02	0.02	0.02	42.9
60	0.20	0.25	0.27	0.24	0.06	0.05	0.07	0.06	0.10	0.20	0.07	0.12	0.13	25.3
90	0.41	0.54	0.20	0.37	0.08	0.03	0.09	0.07	0.05	0.01	0.04	0.03	0.03	27.7
180	0.30	0.39	0.26	0.32	0.11	0.10	0.06	0.09	0.06	0.02	0.03	0.04	0.03	26.5

The initial concentration of Carbofuran was 1.7mg/Kg soil.

TABLE II. Total ^{14}C-Carbofuran residues in corn leaves.

Sample	Time		Residues	
	days after treatment	days after germination	Bq/g	mg/g
rep 21	15	8	188	0.97
rep 22			172	0.89
rep 23			100	0.52
rep 31	30	22	62	0.32
rep 32			55	0.29
rep 33			53	0.27
rep 41	60	52	48	0.25
rep 42			34	0.17
rep 43			20	0.10
rep 51	90	82	1.5	0.01
rep 52			1.5	0.01
rep 53			1.0	0.01

TABLE III: Percent distribution of 14 C-carbofuran in disturbed soil cores.

Time	C-14 Total Residues	
Days after treatment	mg/g	% of original added
0	14.7	94.3
8	6.1	44.1
15	5.2	36.1
30	5.7	45.9
60	4.9	38.6

The initial concentration of carbofuran was 15.6mg per gram soil.

Standard Deviation: +6-2.5 %

TABLE IV: Extractable ^{14}C-Residues from disturbed soil cores

Days after treatment	Soil planted with maize		Soil without plants	
	mg/g	% of original added	mg/g	% of original added
0	9.8	82.3	1.9	14.9
8	2.2	13.3	4.8	34.4
15	0.8	5.5	5.1	35.5
30	0.5	3.6	4.0	31.8
60	0.4	2.5	3.4	24.4

TABLE V: Residues of total ^{14}C-carbofuran leached through disturbed soil

Days post-treatment	^{14}C-Extractable		Non-extractable ^{14}C	
	mg/g	% of original added	mg/g	% of original added
8	3.0	19.2	3.6	22.8
15	1.3	8.6	1.6	10.1
30	0.5	3.2	0.5	3.3
60	0.2	1.1	0.1	0.6

TABLE VI : Degradation products of 14C-Carbofuran 30 Days after treatment of Soil.

compound	Rf-value	in soil mg/Kg	in leachate mg/L
3-hydroxycarbofuran	0.10	0.90	0.60
3-ketocarbofuran	0.25	<0.05	0.52
carbofuran	0.42	0.20	0.12
3-keto-7-phenol-carbofuran	0.55	<0.05	0.19

TABLE VII : Temperature and rainfall data during the experiment.

month	T max	T min	Tmean	Rainfall	
	°C	°C	°C	ml/cm^2	events
10-1994	30.7	23.0	26.9	22.1	8
11-1994	32.3	21.5	26.9	44.8	8
12-1994	31.9	22.2	27.1	0.0	0
1/2-1995	32.8/33.3	21.5/20.8	27.2/27.1	13.8	2
3-1995	33.3	21.6	27.5	0.0	0
subtotal	-	-	-	80.7	18
12-1995	31.0	22.2	26.6	2.1	1
1-1996	30.8	21.4	26.1	1.3	1
2-1996	32.0	21.7	26.8	0.1	1
3-1996	32.7	21.5	27.1	0.6	1
4-1996	33.1	22.2	27.6	0.6	1
subtotal	-	-	-	4.7	5
grand total				85.4	23

TABLE VIII: Leaching volumes through undisturbed soil cores in ml/cm^2

time [days]	Rainfall Gauge	Lysimeter 1	Lysimeter 2	Lysimeter 3
0	0.00	0.00	0.00	0.00
2	0.36	0.00	0.00	0.00
5	9.93	6.11	5.97	5.28
7	0.42	0.36	0.27	0.28
12	1.27	0.00	0.00	0.02
14	0.95	0.00	0.00	0.00
19	2.24	0.00	0.34	0.00
21	7.07	4.33	5.09	5.22
22	1.91	1.37	1.50	1.63
32	3.26	2.34	2.66	2.72
27	14.77	4.15	1.57	5.35
28	2.92	4.81	3.82	6.36
33	11.46	7.19	7.19	7.19
34	1.13	1.15	4.21	1.19
49	10.19	2.54	2.54	5.41
55	14.90	7.19	2.54	5.41
180	0.00	0.00	0.00	0.00
Total	82.74	41.53	42.35	47.84

4. CONCLUSIONS

- Carbofuran and degradation products move easily up and downwards in the soil-plant system.
- At 15 days after the treatment the non-extractable residues were around 10% of the total readioactivity.
- Plants rapidly absorb carbofuran residues and store them in the leaves where they can be found during the next 15-30 days.
- Carbofuran residues in soil decreased steadly with time and a half-life of 30 days was determined.
- Carbofuran residues dissipate more or less similarly in disturbed and undisturbed soil.
- Non extactable residues increased with timeto as much as 35.5% of original amount.
- Extractable residues after 30 days of treatment included the parent compound as well as the metabolites 3-hydroxy-3ketocarbofuran and 3-keto-7-phenolcarbofuran.

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PERSISTENCE OF ENDOSULFAN AND ITS METABOLITES IN TOMATO PLANTS AND SOIL

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Abstract

Tests were conducted to study the persistence of ^{14}C-labelled α and β -endosulfan in tomato plants and soil under the greenhouse conditions when applied at the rate and number of applications used by tomato growers in Costa Rica. Two applications, at 30 and 55 days after planting were made. Plant and soil samples were extracted 37, 49, 71 and 125 days after planting and analyzed by LSC, TLC and GC-ECD. At 37 days after planting the compounds identified were α-endosulfan, β-endosulfan and endosulfan sulphate with a combined concentration of 3.6 mg/kg in plant and 0.6 mg/kg in the soil. At 49 days after planting the same three compounds were found at the combined concentration of 1.51 mg/kg in the plant and at 0.34 mg/kg in the soil. After 71 days low levels of α-endosulfan, β-endosulfan, endosulfan sulphate and endosulfan lactone were found in plants and soil. Similarly, at 125 days low levels of these compounds as well as low levels of two other metabolites, endosulfan alcohol and endosulfan ether were detected. Under the conditions of the experiment endosulfan residues do not seem to be significant or persistant.

1. INTRODUCTION

Endosulfan is an broad spectrum organochlorine insecticide and comes in α and β isomers, both of which are found in commercial formulations. It is practically insoluble in water, but is soluble in organic solvents. In the biological tissues and the environment it is known to degrade into various metabolites, which include endosulfan sulphate, endosulfan alcohol, endosulfan ether and endosulfan lactone. The only metabolite toxicologically important is the endosulfan sulphate.

In Costa Rica endosulfan is widely used on tomato to control lepidopterous larvae and whiteflies. The most common formulation used on tomato crop is 0.1 to 0.2% suspension of Thiodan® 35 EC. The present work was intended to study the persistence, degradation and residues of endosulfan in tomato plants and soil after applications made similar to those used by tomato growers in Costa Rica.

2. MATERIAL AND METHODS

A seedbed with tomato var. Hayslip (Asgrow Seed Co., Michigan, USA) was planted under greenhouse conditions (25 ± 5°C); fertilized with 14-14-14 (N-P-K), fungicides were applied to protect the seedlings. On emergence each seeding was tranplanted into a plastic cup with soil treated with organic fertilizer and fungicides. The plants were covered with a special cloth

to protect them from an early whiteflies attack. After transplanting at 30 days to bigger pots (10 L) with loam soil (7.8% OM, 4.7 pH), the plants were fertilized with 10-30-10 (N-P-K). The insecticide treatment was a 0.1% suspension of Thiodan 35 EC (AgrEvo-Hoecht) fortified with 120 KBq of ^{14}C-α-endosulfan (Sp. act. 2962.3 MBq/g) and 90 KBq of β-endosulfan (Sp. act. 2921.4 MBq/g). The isomers were labelled in the 6,7,8,9,10 carbon positions with ^{14}C. Each plant was treated with an average of 1.2 mL suspension using a De VilBiss applicator. At 7 and 19 days after the first application, six plants were sampled to give three replicate of a composite sample of leaf tissue from two pots. Soil sampling was done after pulling the plants out and removing visible roots with tweezers; the soil was collected from the top 15 cm of the two pots and combined to get 1 kg of composite sample, which was sieved through a 2 mm sieve.

The second application of the insecticide solution was made 55 days after planting. As describe before, a 0.1 % suspension of the insecticide, containing 86 KBq of α-^{14}C-endosulfan and 150 KBq β-^{14}C-endosulfan was applied per plant. Sixteen and 70 days after the second application, six plants and pots were sampled, each time.

A US FDA method based on the method 301 from the Pesticide Analytical Manual [1] validated by Mejías *et al.*[2] was used to analyze the residues in soil and plant by GC-ECD. A Shimadzu Model 14A gas chromatograph (GC) equipped with an electron capture detector (ECD) was used and the operational conditions were as following:
Carrier and make up gas : Nitrogen at a rate of 40 mL/min
Injector Temperature: 275 °C
Detector Temperature: 235 °C
Column: SPB-1 30 m x 0.32 cm x 0.25 μm
Injection volume: 1 μL
T° program: T_1= 200°C t_1= 2 min R_1= 2°C/min
T_2= 250°C t_2= 1 min R_2= 0°C/min

One sample of the extract was counted by liquid scintillation counter (LSC) and another was analysed on a Silica gel G_{F254} TLC plate. The plates were developed in acetone : hexane (1 + 4, v/v) solvent mixture. The plates were autoradiographed on X-OMAT Kodak XRay film for identification of the metabolites.

3. RESULTS

The GC retention times for the compounds were: 6.8 min for α-endosulfan, 8.3 min for β-endosulfan, 9.9 min for endosulfan sulphate, 5.9 min for endosulfan alcohol, 5.3 min for endosulfan ether and 3.7 min for endosulfan lactone

The detection and quantification limits for analysis of endosulfan and metabolites in tomato plant are shown in Table I, and the detection and quantitation limits for analysis of these compounds in soil are shown in Table II.

The R_f's on the Silica gel G_{F254} plates for the α-endosulfan, β-endosulfan, endosulfan sulfate, endosulfan lactone, endosulfan alcohol and endosulfan ether were 0.98, 0.89, 0.62, 0.60, 0.43 and 0.95, respectively.

The amounts of residues (mg/kg) found for all the compounds are shown on Table III.

Table I. Detection Limit and Quantification Limit of endosulfan and metabolites analyzed by CG-ECD in plant tissue.

	Endosulfan α	Endosulfan β	Endosulfan Sulfate	Endosulfan Alcohol	Endosulfa n Ether	Endosulfan Lactone
Detection Limit (mg/kg)	0.002	0.001	0.001	0.01	0.002	0.001
Quantif. Limit (mg/kg)	0.006	0.003	0.003	0.03	0.006	0.003

Table II. Detection Limit and Quantification Limit of endosulfan and metabolites analyzed by CG-ECD in soil.

	End. Alpha	End. Beta	End. Sulfate	End Alcohol	End. Ether	End. Lactone
Detection Limit (mg/kg)	0.0004	0.0003	0.0003	0.0027	0.0004	0.0003
Quantif. Limit (mg/kg)	0.0012	0.0009	0.0009	0.0080	0.006	0.003

Table III. Residues of endosulfan and its metabolites (mg/kg) in soil and plant samples[1].

Time	α-Endosulfan	β-Endosulfan	Endosulfan Sulfate	Endosulfan Lactone	Endosulfan Ether	Endosulfan Alcohol
$t_{1\text{-Plant}}$	0.704 ± 0.380	1.921 ± 0.266	0.968 ±	N.D.	N.D.	N.D.
$t_{1\text{- Soil}}$	0.236 ± 0.134	0.267 ± 0.202	0.296	N.D.	N.D.	N.D.
			0.106 ± 0.072			
$t_{2\text{-Plant}}$	0.114 ± 0.028	0.537 ±	0.915 ±	N.D.	N.D.	N.D.
$t_{2\text{-Soil}}$ •	0.110 ± 0.084	0.141	0.175	*	*	N.D.
		0.128 ± 0.045	0.010 ± 0.061			
$t_{3\text{-Plant}}$	0.086 ±	0.370 ±	0.430 ±	**	N.D.	N.D.
$t_{3\text{-Soil}}$	0.058	0.364	0.402	0.291 ± 0.119	N.D.	N.D.
	0.281 ± 0.052	0.334 ± 0.066	0.127 ± 0.036			
$t_{4\text{-Plant}}$ ••	***	0.042 ± 0.038	0.093 ±	***	***	***
$t_{4\text{-Soil}}$ •••	0.064 ± 0.010	0.213 ± 0.245	0.047	****	0.248 ±	****
			0.080 ± 0.043		0.042	

[1] Average of three composite samples

* One of the samples showed residue of 0 24 mg/kg endosulfan ether and 0 01 mg/kg endosulfan lactone

** One of the samples showed residue of 0 05 mg/kg endosulfan lactone

*** One of the samples showed residue of 0 03 mg/kg endosulfan lactone, another showed 0 02 mg/kg endosulfan ether, 0.17 mg/kg endosulfan alcohol and 0 01 mg/kg α endosulfan

****One of the samples showed residue of 0 33 mg/kg endosulfan lactone and 0 10 mg/kg endosulfan alcohol

t_1= time 1, 37 days after planting, 7 days after first application

t_2= time 2, 49 days after planting, 19 days after first application

t_3= time 3, 55 days after planting, 16 days after second application

t_4= time 4, 125 days after planting, 70 days after second application

4. DISCUSSION

After 7 days the first application (t_1), the total residue found was 3.6 mg/kg in plants and 0.6 mg/kg in soil. The residue in plants was mainly of β-endosulfan, but α-endosulfan and endosulfan sulphate were present, no other endosulfan metabolites were found.

At time 19 days after the first application (t_2) of the total residues in plants were half of the level found at 7 days after application in plants and soils; in plants, mainly, endosulfan sulphate was found; endosulfan α and endosulfan β were present at low levels. In soil the levels of the α and β endosulfan and endosulfan sulphate were low.

At time 16 days after the second application (t_3), in plants, residues of endosulfan β and endosulfan sulphate were similar, the residues of α endosulfan were lower. In soil, at this time, residues of α and β endosulfan were similar and the residues of endosulfan sulphate were lower; residues of endosulfan lactone were found only in one of the replicates.

After 70 days the second application (t_4) residues on plants, were low, (endosulfan α and β as well as endosulfan sulphate were the compounds identified). Very low concentrations of endosulfan lactone, endosulfan ether and endosulfan alcohol were found in one of the samples. In soil small amounts of α and β endosulfan and endosulfan sulphate were found but endosulfan ether was found at higher levels in one of the sample concentrations of endosulfan lactone (0.3 mg/kg) and endosulfan alcohol (0.10 mg/kg) were found.

The residues in plants and soils disappear quickly as was mentioned before elsewhere [3, 4, 5, 6, 7] due in part specially to the greenhouse conditions where the temperature is relatively high (25 ± 5°C) and the appreciably volatility of the main compound (endosulfan) seems to be important on the behavior of the residues. The same volatility property, according to Gorbach [8], is responsible for the composition of the terminal residue that shifts first to β endosulfan and at the end, towards endosulfan sulfate that is less volatile.

We conclude that under the experimental conditions endosulfan residues do not seem to be important, due to the low concentration found and low persistence.

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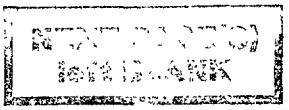

A STUDY OF DISSIPATION, DEGRADATION AND BINDING OF ^{14}C- LABELED ENDOSULFAN TO SOIL IN MODEL LYSIMETER

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Abstract

The degradation, dissipation and binding of α-endosulfan in two agricultural soils and sand was studied in lysimeter system under outdoor conditions, using ^{14}C labeled insecticide. Dissipation was rapid during the first few weeks after application. The half life of disappearance was 38 to 61 days for the soils from Cerro Punta and El Ejido, whereas, in sand it was 91 days. The insecticide degraded by oxidation at the sulfite group to the sulfate. The resultant product underwent further degradation to form $^{14}CO_2$ and bound residues. Although a significant amount of ^{14}C leached through the sand, which contained less that 0.1 % organic matter, there was no leaching of endusolfan through the other two types of soil, when leaching was started immediately after treatment.

1. INTRODUCTION

Endosulfan (1, 4, 5, 6, 7, 7-hexachloro-8,9,10-trinorborn-5-en-2, 3- ylenebismethylene sulfite) is a non-systemic contact and stomach insecticide effective against numerous arthropod pests that attack economically important crops [1].

It was first developed in Germany by Hoechst for use on agriculture in 1955; thereafter, registration for uses on many agronomic crops have been accepted in many countries, including Central America. Endosulfan as Thiodan was first registered in Panama for use in agricultural crops in 1980; it is one of the organochlorine pesticides which is not banned for use under the Panamanian law of 1986 [2], as it is needed for the control of *Hypothenemus hampei-Ferrari* on coffee trees. Presently, its use is recommended for crops such as fruits, grains (cereals), potatoes and coffee.

Most of the research on the fate of pesticides in soil has been conducted under the temperate conditions [3], and little work has been done in the tropical countries of Central America where substantial quantities of pesticides are used. Despite efficient performance of pesticides in the control of pests and plant disease organisms, there is great concern regarding their potential for adverse effects on human health and the environment.
In general, the loss of endosulfan from soil is considered to be due to microbial, chemical and photo-degradation as well as dissipation by volatilization, leaching and adsorption on fine soil particles. Therefore, there is need to monitor the dissipation, degradation and binding to soil of the insecticide to reduce the potential for environmental contamination from this pesticide.

The purpose of these experiments was to provide information on the dissipation, degradation and binding of ^{14}C α-endosulfan to soil in a model lysimeter system. The experimental protocol established by FAO/IAEA [4,5] was followed in conducting this study in order to evaluate the magnitude and significance of endosulfan residues which may be present in the soil in the crop route zone following application of the insecticide under tropical outdoor conditions typical of commercial farming in Panama and Central America.

2. MATERIAL AND METHODS

2.1. Chemicals.

Thiodan (alfa + beta endosulfan, 35%) emulsifiable formulation marketed by Hoechst, was obtained locally from agrochemical store. Analytical grade alpha and beta endosulfan and endosulfan sulfate, alcohol, ether and lactone were obtained from Chem Service, West Chester, Pa, U.S.A. Radiolabeled α-endosulfan was a gift from IAEA, Vienna. All other chemicals and solvents used in these experiments were of analytical grade and available commercially.
A working solution of ^{14}C-labelled α-endosulfan, with an activity of 66,387 Bq/ml, in toluene was prepared for the treatment of soil. Radiochemical assay by TLC on silica gel and n-hexane /acetone (9/1) showed the chemical to be 97% pure. For this purpose, 26.1 mg commercial endosulfan (alpha+beta) dissolved in toluene were spiked with 20 mg ^{14}C-labelled α-endosulfan and volume was adjusted to 100ml with toluene.

2.2. Field site and experimental set-up

The experiments were conducted in an outdoor field site near the Agroecotoxicology Laboratory, at Río Tapia, Tocumen. Environmental data is shown in Fig. 1.. The Soils and sand equivalent to 1 kg (dry weight) were taken into microlysimeters using PVC cylinders (17 cm long, 10 cm i.d.) The PVC cylinders were driven into the ground with 3 cm left protruding above the soil surface. After a 2 weeks pre-incubation period at outdoor conditions (temperature: 23-34°C and a soil moisture of 40%; 10-12 hours daylight) the radiolabeled α-endosulfan was applied on the surface at the rate of 0.6 kg a.i./ha. The radioactivity applied initially to each lysimeter was 66,387 Bq and it was applied by pipetting 2 ml of the working solution to each lysimeter to give an initial concentration of 522 ug endosulfan/kg soil.

2.3. Soil

Soil (0-10 cm depth) was collected from experimental field sites at Cerro Punta in Chiriquí Province and at El Ejido in Los Santos Province. Soil characteristics are summarized in Table I. The soil samples were air dried and passed through a 2 mm sieve, then conditioned for two weeks under outdoors. Sand was obtained from the pacific coast and washed (5 times) with water. It was air dried, passed through a 2 mm sieve and conditioned for two weeks outdoors.

2.4. . Sampling

Three replicates for each soil and sand were taken for analysis at 0, 14, 30, 60, 90, 180 and 332 days after endosulfan application. Soil cores were pushed out from the PVC-cylinders and the fresh weight was recorded. Soil was homogenised for 10 minutes, samples were wrapped separately in aluminum foil an packed in polyethylene bags. The bags had been pre-labelled with a code number and maintained frozen at -20°C until the extraction and combustion for the determination of insecticide residues.

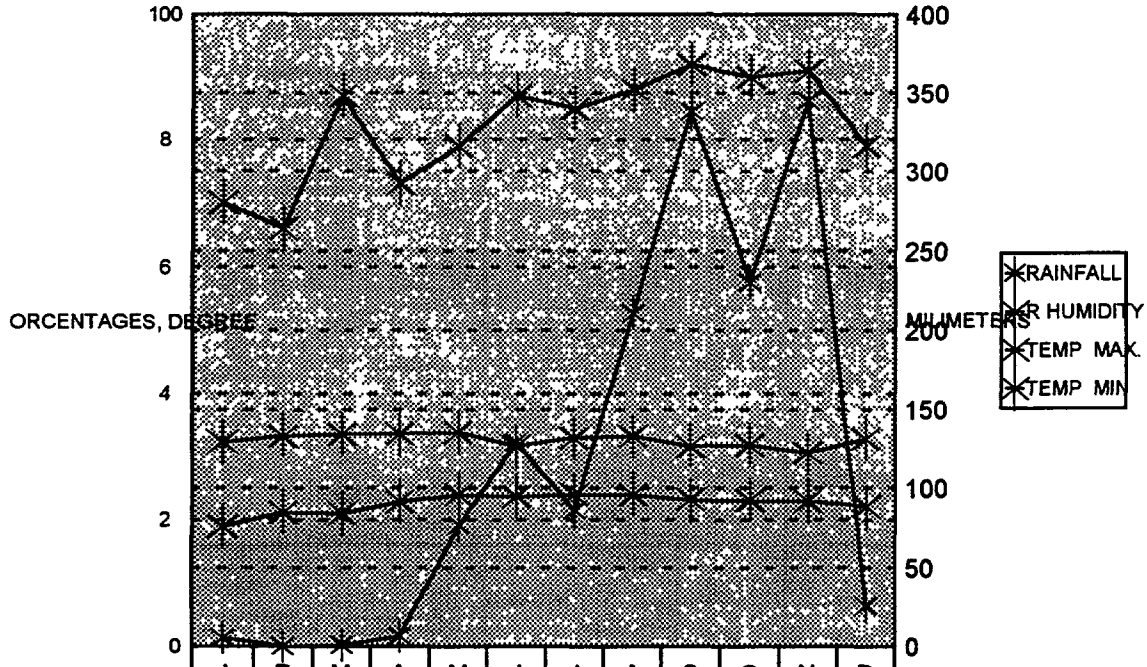
2.5. Analytical procedures

The ^{14}C-activity (total residues) of soil samples was determined by combustion of triplicate 1 0 g samples in a Harvey OX-600 Biological Oxydizer The radioactivity was measured in a Packard Tricarb Model 1000 liquid scintillation counter (LSC) using external calibration Moisture was determined by weighing 5.0g of sample and drying in a convection oven at 120° for 12 hours, and cooling to room temperature. After measuring the weight loss from soil, moisture was calculated in % of total weight Extractable ^{14}C-activity was determined in 50g soil samples by ultrasonic extraction with acetonitrile.

Liquid scientillation counting (LSC), combustion analysis and autoradiography were carried out according to standard operation procedures. Liquid samples (1 ml) were mixed with 10 ml Instagel and counted for 5 minutes (3 times). Solid samples (1 g) were combusted in triplicate in a Harvey Biological Oxydizer Ox-600. The trapped carbon dioxide in (10 ml) LSC cocktail was analyzed in a Packard Tricarb Model 1000 liquid scintillation counter The external standarization technique was used for corrections.

2.6. Thin-Layer Chromatography (TLC)

The precoated silica Gel 60 F-254 chromatoplates (20 x 20 cm, 0.25 mm thickness, glass, E-Merck) were used for analytical purposes. The concentrated solution was directly applied at the bottom of the central linear region of the TLC plate while vertical channels on the two



	J	F	M	A	M	J	J	A	S	O	N	D
RAINFALL	5.4	0	0.	6.4	77	29.3	86.2	11.3	37.8	30.7	44.9	25.
R.HUMIDITY	70	66	87	73	79	87	85	88	92	90	91	79
TEMP. MAX.	32.4	33.3	33.	33.	33.9	31.	33	33.3	31.7	31.9	30.6	32.
TEMP. MIN.	19	21.2	21	22.	24	23.	24	24	23.2	23.1	23	22

FIGURE 1. RAINFALL, AIR TEMPERATURE AND RELATIVE DURING THE EXPERIMENTAL PERIOD.

sides were used for reference authentic compounds. After developing the plate using n-hexane/acetone (9/1, v/v), it was autoradiographed using X-ray film to detect radioactive regions. The radioactive spots were scraped off the plate and radioassayed in the LSC after mixing with 10 ml Instagel.

2.7. Leaching

In order to study the leaching of endosulfan in the soil, radiolabelled insecticide was applied to the top of 30 cm long (2 cm i.d) columns packed with the test soils. Water (15 mL) was applied to the top of each column and the leachate collected at the bottom. Water application was repeated 40 time to each column in such a way that the water accumulated on top of the the soil, as it occures in the field in the tropics after heavy rains. During the percolation the column was covered with aluminum foil and kept at 24-27°C. The radioactivity in the leachate determined by mixing 1 ml with 10ml Instagel liquid scintillation cocktail and counting in the LSC. At the end of the leaching the column was allowed to drain off and the the soils were removed. The air dried soil samples were homogenized and subjected to combustion analysis to examine the amount of radiocarbon in the soil columns.

2.8. Volatility

The loss of endosulfan from the surface of glass plates (2cm x 5cm x 0.2 cm) was examined after the application of commercial endusolfan formulation (aprox. 800 ug/plate) by pippeting

TABLE I. CHARACTERISTICS OF SOIL AND SAND

Soil property	El Ejido soil	Cerro Punta soil	Sand
pH (0.1M KCL %)	5.7	5.71	5.4
C%	1.7	4.1	<0.1
Max water holding capacity (%) 1/3 bar	31.4	65.8	11.2
Sand (%)	16.9	66.7	100
Silt (%)	28.2	28.3	----
Clay (%)	54.9	5.0	----
Soil texture	silty clay	sandy loam	sand, pure
Taxonomy	Isohiperthermic, udic Haplustalf/Alfisol	medial, Isohiperthermic Umbric vitandept/Inceptisol volcanic	----
Main crops	Corns, vegetables, cucurbits	vegetables, potatoes	----

0.2ml of the acetone solution of the insecticide on eight plates. Four of these plates were sampled at 0 day after treatment. The other four plates were exposed in the laboratory under darkness at a temperature of 29-30° for 15 days.

The plates were extracted with toluene and the extracts were analyzed by gas chromatograph (GC) The GC was equipped with an electron capture detector (ECD) and a DB-5 capillary column of 30 m length, 0.25 mm i.d. and 0.25 um film thickness.

3. RESULTS AND DISCUSSION

The dissipation data and for alfa endosulfan in two soils and sand are shown in Tables II, III and IV. The half-life (t ½) of disappearance was determined by a regression analysis. The

TABLE II. VARIATION IN TOTAL RESIDUES WITH TIME IN EL EJIDO SOIL FROM LYSIMETERS TREATED WITH C-14 ALPHA ENDOSULFAN.

TIME (Days after treatment)		C-14 ACTIVITY			% ORIGINAL
		Bq/g	Bq (total) ± sd	Mean Bq	
0	LS1	94	86,479 ± 3.5	83,348	100
	LS2	89	80,264 ± 4.1		100
	LS3	90	83,288 ± 4.7		100
14	LS11	56	53,647 ± 2.1	55,614	62.03
	LS12	67	63,385 ± 3.5		78.97
	LS13	51	49,809 ± 2.5		59.80
30	LS21	58	53,636 ± 6.5	49,967	62.02
	LS22	53	48,641 ± 4-3		60.60
	LS32	51	47,623 ± 4-7		57.18
60	LS31	44	38,064 ± 4.1	38,893	44.02
	LS32	45	41,848 ± 4.3		52.14
	LS33	40	36,768 ±5.7		44.15
90	LS41	37	32,813 ± 7.4	33,448	37.94
	LS42	43	32,600 ± 5.3		40.62
	LS43	39	34,932 ± 4.7		41.94
180	LS51	27	23,259 ± 5.1	23,184	26.90
	LS52	30	24,043 ± 7.8		29.95
	LS53	25	22,249 ± 8.5		26.71

TABLE III. VARIATION IN TOTAL RESIDUES WITH TIME IN CERRO PUNTA SOIL FROM LYSIMETERS TREATED WITH C-14 ALPHA ENDOSULFAN.

TIME (Days after treatment)	C-14 ACTIVITY			% ORIGINAL
	Bq/g	Bq (total)	Mean Bq	
0 cp-1	78	67,368 ± 0.22	76,512	100
cp-2	82	75,866 ± 0.12		100
cp-3	84	83,303 ± 0.04		100
30 cp-11	61	14,923 ± 0.02	15,242	22.15
cp-12	58	15,716 ± 0.12		20.72
cp-13	64	15,087 ± 0.13		18.11
90 cp-21	47	10,808 ± 0.11	9,718	16.04
cp-22	46	9,100 ± 0.11		11.99
cp-23	44	9,245 ± 0-10		11.10
180 cp 31	26	7,008 ± 0.06	6,347	10.40
cp32	33	5,977 ±0.12		7.88
cp 33	26	6,056 ± 0.07		7.27

calculation was based on the residue data of 0-180 days in all Lysimeters. The half-life was estimated to be 61 and 38 days in El Ejido and Cerro Punta soil, respectively. A half-life of 91 days was estimated for the insecticide in sand. The correlation coefficients (r2) were more than 0.73 with a probability of 0.03 in each case. After 90 days the radioactivity decreased to 40.17% and to 13.04% in El Ejido and Cerro Punta soils. At this time the C-14 activity found in sand was 51.88% of original added. On 180th day, soils from El Ejido and Cerro Punta contained 26.85% and 8.52% of its respectively original added. Radioactivity at this period (residues of endusulfan) in sand was 33.99% of original added radioactivity. On 332nd day, residues of endosulfan were in all lysimeters in the range of values less than 10% of original added C14 activity.

The residues of alfa endosulfan declined with time and dissipation on 30th day was more rapid in the soil from Cerro Punta than in soil from El Ejido (3 times) and sand (4 times) respectively. About 80% of the applied endosulfan was lost during the 14 days of application

TABLE IV. VARIATION IN TOTAL RESIDUES WITH TIME IN SAND FROM LYSIMETERS TREATED WITH C-14 ALPHA ENDOSULFAN.

TIME (Days after treatment)		C-14 ACTIVITY		% ORIGINAL
		Bq/g	Bq (total)	
0	Ar-1	65	73,620 ± 0.16	100
	Ar-2	79	76,807 ± 0..22	100
	Ar-3	90	75,825 ± 0.08	100
30	Ar 11	13	56,988 ± 0.26	77.41
	Ar-12	15	53,217 ± 0.16	69.29
	Ar-13	15	58,565 ± 0.07	77.24
90	Ar 21	12	34,552 ± 0.03	46.93
	Ar-22	9	42,458 ±0.10	55.28
	Ar-23	10	40,513 ± 0.08	53.43
180	Ar 31	8	23,376 ± 0.63	31.75
	Ar-32	6	29,776 ±0.02	38.77
	Ar-33	7	23,852 ± 0.06	31.46

SD= standard deviation

to the Cerro Punta soil. The results were not at all in accord with previous observations in which 94% of the endosulfan applied to soil were degraded to $^{14}CO_2$ in 120 days [6]. Since the compound is considered immobile and multiple pathways exist for dissipation of this insecticide including volatilization, chemical and microbial degradation and adsorption to soil, more than one process for dissipation would be expected to take place; volatilization and microbial degradation appear to be the most important route of dissipation.

The half-lives of endosulfan in the soils studied are clearly of shorter duration than those reported for persistent organochlorinated pesticides like DDT which shows under similar conditions a half-live of 100 days and more [7]. The levels of residues found in Cerro Punta at 180 days after application seems to be negligible and consequently, the risk potential for the environment by an application of endosulfan to this soil at a rate of 0.6 kg a.i. is low. In the case of El Ejido soil there are residues in the range of 30% of applied amount.

The extractable ^{14}C decreased steadily, a large proportion of the ^{14}C was on soil as non extractable residues (Tables V, VI and VII). The bound ^{14}C in El Ejido and Cerro Punta soils amounted to 24.8-58.0% and 30.5-63.3% of the applied ^{14}C activity. The bound ^{14}C in both soils gradually decreased after 180 days treatment to 24.8-31.6% and to 30.5-33.8% in El Ejido and Cerro Punta soils, respectively. At this time extractable ^{14}C residues were low 1.3% in El Ejido soil and 3.7% in Cerro Punta soil.

TABLE V. DISTRIBUTION OF RADIOACTIVITY IN EL EJIDO SOIL

TIME (DAYS AFTER TREATMENT)		NON EXTRACTABLE RESIDUES (C-14 BOUND) Bq total	EXTRACTABLE RESIDUES Bq total	TOTAL RESIDUES	
				BY ADDITION OF EXTRACTABLE +BOUND Bq total	BY COMBUSTION Bq total
	LS 1	50,147	4155	54,302	86,479
0	LS 2	45,426	4308	49,734	80,264
	LS 3	45,097	4308	49,405	83,288
60	LS 11	33,105	1542	34,647	38,064
	LS 12	29,511	1444	30,955	41,848
	LS 13	36,831	1535	38,366	36,768
90	LS 21	29,699	1437	31,136	32,813
	LS 22	29,644	1145	30,789	32,600
	LS 13	31,206	1519	32,725	34,932
180	LS 31	24,587	1124	25,711	23,259
	LS 32	21,417	938	22,355	23,043
	LS 33	27,339	1058	28,397	22,249

The analysis by TLC showed that there were at least 3 degradation products in the extracts of the soils treated with endosulfan C-14. The products were identified as endosulfan sulfate, alcohol and the endosulfan. The Rf values for α-endosulfan, β-endosulfan, endosulfan sulfate, endosulfan alcohol, endosulfan ether and endosulfan lactone were 0.52, 0.26, 0.16, 0.05, 0.40 and 0.15, respectively. There was a considerable variation in amount of degradation products between the soils. The degradation products by oxidation appear to be predominantly formed, and then gradually decreased. Thus, these degradation products were not persistent in soil for a longer period of time.

The distribution of radiocarbon in the soil columns and the eluates is shown in Table VIII. C14 was eluted in larger amounts from the sand columns (73.38%) than from El Ejido

TABLE VI. DISTRIBUTION OF REDIOACTIVITY IN CERRO PUNTA SOIL

				EXTRACTABLE AND BOUND RESIDUES	
TIME (DAYS AFTER TREATMENT)		NON EXTRACTABLE RESIDUES (C-14 BOUND) Bq total	EXTRACTABLE RESIDUES Bq total	BY ADDITION: EXTRACTABLE +BOUND Bq total	BY COMBUSTION Bq total
	CP 1	52,563	6728	59,291	73,620
0	CP 2	44,135	6508	50,643	76,807
	CP 3	52,720	6358	59,078	75,825
90	CP 1	37,378	2403	39,781	34,552
	CP 2	36,161	2383	38,544	42,458
	CP 3	41,732	2456	44,188	40,513
180	CP 1	25,427	2874	28,301	23,376
	CP 2	26,895	3114	30,009	29,776
	CP 3	28,128	2838	30,966	23,852

(11.52%) and Cerro Punta (22.26%) soils. The major part of the radiocarbon resided in the treated portion of each soil columns.

The loss of the insecticide from the surface through volatility is influenced by the physico-chemical properties of the chemical (vapor pressure) and the properties of the surface including environmental conditions (e.g. temperature, moisture, etc.) From the results in Table IX is clearly evident that endosulfan volatilized easily from the glass surface. After 15 days 69.1% of the applied endosulfan disappered with amount was lost. Endosulfan disappeared with a half life of 11 days. This compound although was found to disappear rapidly from tomatoe leaf surface with a half-life of 6-7 days. 8).

TABLE VII. DISTRIBUTION OF RADIOACTIVITY IN SAND

				EXTRACTABLE AND BOUND RESIDUES (TOTAL)	
TIME (DAYS AFTER TREATMENT)		NON EXTRACTABLE RESIDUES (C-14 BOUND) Bq total	EXTRACTABLE RESIDUES Bq total	BY ADDITION: EXTRACTABLE +BOUND Bq total	BY COMBUSTION - Bq total
	Ar 1	27,961	4293	32,254	67,368
0	Ar 2	35,057	4624	39,681	75,866
	Ar 3	30,566	4477	35,043	83,303
90	Ar 1	7,533	603	8,137	10,808
	Ar 2	7,0311	881	7,912	9,100
	Ar 3	6,754	682	7,436	9,245
180	Ar 1	4,583	356	4,939	7,008
	Ar 2	5,258	475	5,733	5,977
	Ar 3	4,329	362	4,6916	6,056

TABLE VIII. LEACHING OF ALFA ENDUSOLFAN FROM SOIL COLUMNS TREATED WITH C-14 ENDOSULFAN

SOIL	TOTAL RADIOACTIVITY IN LEACHATE	% OF ORIGINAL	ADSORBED RADIOACTIVITY ON SOIL	% OF ORIGINAL
El Ejido, Los Santos	992 Bq/50ml	11.52	4283 Bq	49.71
Cerro Punta, Chiriquí	1917 Bq/570ml	22.26	4846 Bq	56.25
Sand	632 Bq/390ml	73.38	603 Bq	7.0

SD: ± 4.7 - 7.0%

TABLE IX. VOLATILITY OF ENDOSULFAN FROM GLASS SURFACE.

TIME (DAYS AFTER TREATMENT)		AMOUNT OF ALPHA ENDOSULFAN UG	% REC	AMOUNT OF BETA ENDOSULFAN UG	% REC
0	REP 1	299	147.3	632	99.8
	REP 2	103	50.7	378	59.7
	REP 3	234	115.3	893	141.1
	REP 4	176	86.7	632	99.8
		203	100	633	100
15	REP11	22.4	11.0	73.8	11.7
15	REP12	36.2	17.8	99.6	15.7
15	REP13	40.5	20.0	110.4	17.4
15	REP14	32.6	16.1	87.1	13.8
		32.9	16.2	92.7	14.7

CONCLUSIONS

Alpha endosulfan disappeared from soils with half lives of 61 days (El Ejido soil) and 38 days(Cerro Punta soil). A half life of 91 days was calculated for the insecticide in sand. The half life in soil is shorter than that for the other organochlorine pesticides. Dissipation was faster in the volcanic soil from Cerro Punta than in the soil from El Ejido and in sand. At least 3 degradation products were detected in the soils. Main products were the sulfate , the alcohol and the ether. Residues leached in larger amounts from sand and from El Ejido soil than from the volcanic soil..

ACKNOWLEDGEMENT

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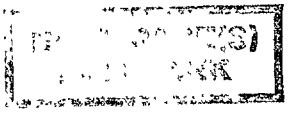
Part III

STUDY OF THE EFFECT OF PESTICIDES ON PEST INSECTS AND BENEFICIAL ARTHROPODS



IMPACT OF TERBUFOS ON *Cotesia flavipes*, A PARASITOID OF *Diatraea saccharalis*

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Abstract

The effect of terbufos on larvae of *Diatraea saccharalis* and its parasitoid, *Cotesia flavipes* was evaluated in the laboratory. Bioassays were conducted to determine the dose response of non-parasitized larvae of *D. saccharalis* feeding on artificial diet contaminated with terbufos. From the dose-response curve based on larval fresh weight, sublethal doses ranging from 1.32 ppm to 108 ppm of terbufos were selected for further studying the effect of the insecticide on both species. Both parasitized and non-parasitized larvae were exposed to the selected sublethal doses of terbufos in the diet. Consumption of the insecticide by the host resulted in mortality of the parasitoid, increased length of its larval and pupal periods, decreased adult fresh weight and changes in sex proportions. These negative effects were more severe as the dose of the insecticide increased. *D. saccharalis* was also affected by terbufos; larvae showed abnormalities, the length of the larval and pupal periods increased and the proportion of the females was reduced. In a preliminary greenhouse bioassay, only traces of terbufos or its metabolites were found in treated maize plants and in tissue of *D. saccharalis* larvae feeding on them.

1. INTRODUCTION

Diatraea saccharalis is an important pest of sugar cane and maize. The larvae of this stemborer can attack any part of the plant, except the fibrous root system and the central nerve of the leaf. Most often they bore the stem from the base up to the fourth internode. Affected plants can easily become lodged; the abnormal flow of nutrients in affected plants will decreased yields. Early attack may result in death of the seedlings. The adult moth lives 5-6 days, copulate after the second day and the female lays the eggs on the foliage. Eggs hatch within 4-9 days. Each female can lay several masses containing 25 or more eggs, depending on the surface area available to it. The larval stage lasts 28-35 days and goes through seven instars depending on weather conditions. The pupal stage lasts 8-10 days.

Biological control of *Diatraea saccharalis* is very successful in sugarcane plantations but small maize growers lack the knowledge and resources to implement it. Insecticides are not efficient because the pest remains inside the stem most of the time. However, growers use organophosphate and carbamate insecticides to control this pest as well as fall armyworm, *Spodoptera frugiperda*.

The parasitoid, *Cotesia flavipes* is a braconidae whose main host is *Diatraea*. Only in Costa Rica there are more than 2000 species of braconidae, most of them are parasitoids [1]. *C. flavipes*, native to South East Asia, was introduced in America initially in Florida against *Diatraea* [2] and later to other countries in the continent. In Central America it was

introduced in the mid 1980s [3]. The female of the endoparasitoids enter the tunnel made by the host in the plant to parasitize larvae of the third to sixth instar.

C. flavipes can be easily reared in the laboratory, making it an ideal species for innundative biological control. There are laboratories supplying parasitoids to farmers in Brazil [4], Costa Rica [1,5]and Venezuela [6].

Terbufos is the most widely used soil insecticide in Costa Rica. In the soil it is oxidized to terbufos sulfoxide and terbufos sulfone, which are more soluble and move more readily than the parent compound. Soil colloids adsorb terbufos which has a half life of 15-22 days [7]. Terbufos is not considered a systemic insecticide but both terbufos and its metabolites, except terbufos-oxon, penetrate the plant [7].

Direct and indirect effects of insecticides on parasitoids are well documented in the literature [8], however very little is known about the interactions between terbufos, *D. saccharalis* and its parasitoid *C. flavipes*, especially under tropical conditions. The objectives of the research reported here were to determine the dose response of *D. saccharalis* to terbufos incorporated in the diet under laboratory conditions and to characterize the effect of sublethal doses on terbufos on the parasitoid. Additionally, a preliminary bioassay determined the fate of terbufos in the soil, the plant and *D. saccharalis* under greenhouse conditions.

2. MATERIALS AND METHODS

Laboratory bioassays were conducted in collaboration with the Biological Control Laboratory of the Sugarcane Research and Extension Institute in Costa Rica.

2.1 Response of *D. saccharalis* to increasing doses of terbufos.

Larvae of *D. saccharalis* (14 days old, instar L_3) were individually placed in petri dishes containing approximately 1 cm^2 of solidified diet containing increasing concentrations of terbufos (0, 2, 4, 8, 16, 32, 64, and 128 ppm). Terbufos was dissolved in 2% ethanol and added to each batch of 200 ml of liquid diet and mixed for 1 min with a regular kitchen mixer. The diet was poured in plastic trays until solidified before cutting it in 1 cm^2 blocks. Each larva was weighed before it began feeding and every day thereafter until completion of the bioassay. Each treatment consisted of 50 larvae (each one considered a replication). Additionally, 15 larvae were included in each treatment to be sacrificed (3 daily), weighed and lyophilized to estimate dry weight. Treatments were arranged according to a completely randomized design. Mortality was also recorded daily. The bioassay was repeated and data from both was pooled after corroboration of homogeneity of variances.

2.2 Effect of terbufos on *D. saccharalis* and *C. flavipes*

Based on the dose response obtained in the initial bioassays, terbufos doses of 1.3, 9.0, 18.8, 32.1, 53.2, and 108.0 ppm, corresponding to the I_{10}, I_{20}, I_{30}, I_{40}, I_{50}, and I_{60} values, respectively, plus an untreated control, were used to determine the effect of the insecticide on *C. flavipes* parasitizing *D. saccharalis.*

Contaminated diets were prepared as before and 50 *D. saccharalis* larvae previously parasitized by *C. flavipes* were individually placed in petri dishes with the artificial diet. A parallel bioassay was conducted simultaneously using non-parasitized larvae. All larvae were weighed before starting the experiment and daily after feeding initiated until the larvae of the parasitoid emerged from the host or when the first adult was obtained in the case of non-parasitized *D. saccharalis*. The experiment was laid out as a completely randomized design and conducted twice. The following variables were used to assess the effect of the insecticide on the parasitoid: time required for *C. flavipes* larvae to leave the host, mortality, number of pupae, time required for adults to emerge, sex ratio, and fresh weight. When *D. saccharalis* larvae pupated, parasitism was considered failed and the data was omitted for the analysis. The effect of terbufos on the host was assessed in the parallel experiment by determining the fresh weight of the larvae, time required for pupating, eclosion time, and sex proportion. When statistically appropriate, data from repeated bioassays was combined.

2.3 Determination of terbufos in treated plants and *D. saccharalis*

A preliminary greenhouse study was conducted at the Environmental Pollution Research Center, University of Costa Rica, to quantify the presence of terbufos in maize plants growing in pots treated with ^{14}C terbufos and on *D. saccharalis* larvae feeding on these plants. Pots containing 750 g of soil were treated with terbufos at concentrations 0, 6, 9 and 12 ppm w/w using a commercial granular formulation containing 10% terbufos. ^{14}C terbufos (6 Mbq in 200 µl of acetone and hexane) were added to the commercial granules before soil incorporation. After allowing time for solvent evaporation, a seed of maize (cv Pioneer) was planted in each pot. Experiment was laid out as a complete randomized block design with 5 replications. When maize seedlings reached the 3-4 leaf growth stage (22 days after planting) a 14-day larvae of *D. saccharalis* previously parasitized by *C. flavipes* was placed on each plant and allowed to feed for 24 h. Larvae were collected and plants were harvested, washed, and divided into foliage and roots and stored at -22C until extraction. ^{14}C terbufos was extracted according to standard procedures [9] and analyzed by LCC.

3. RESULTS AND DISCUSSION

3.1 Response of *D. saccharalis* to increasing doses of terbufos.

In preliminary bioassays (data not shown) using tebufos doses ranging from 0.46 to 300 ppm, it was not possible to obtain a clear dose response based on mortality that would allow determining the LC_{50} value and deciding on sublethal doses for the succeeding experiments. In the definite bioassays, mortality was observed a the highest rates (64 and 128 ppm) four days after began feeding on contaminated diet. To obtain the dose response curve, fresh weights of larvae feeding for three days on contaminated artificial diet were used. Fresh weight of larvae decreased as terbufos dose increased (Figure 1); untreated larvae increased 198% in fresh weight before reaching the pupal stage on the sixth day. A similar response was obtained using estimated dry weights (data not shown).

Feeding decreased in larvae placed on contaminated diet compared to the untreated controls, probably because of sublethal toxicity or antifeeding properties of the insecticide. Excretes decreased up to 86% as terbufos dose increased compared to those of healthy larvae feeding normally (untreated control). If this would occur under field conditions, it could have

biological implications as the females of *C. flavipes* depend on the excretions (kairomones) to find its host [10].

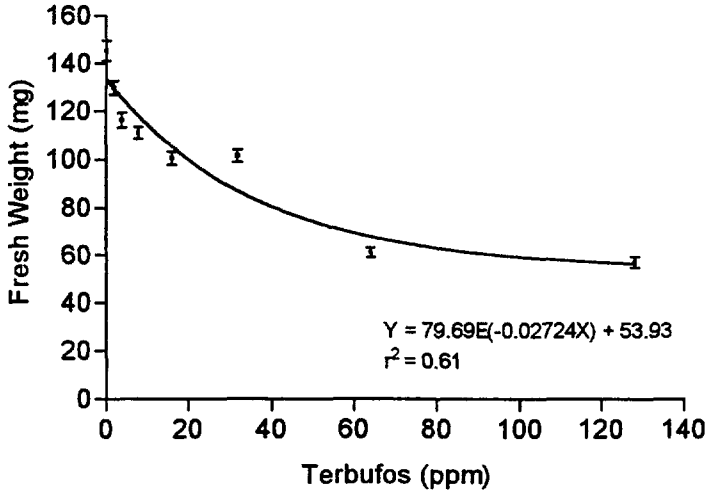

Figure 1. Fresh weight of D. saccharalis larvae feeding for three days on artificial diet treated with terbufos. Data are averages of two bioassays using 50 larvae per treatment each.

3.2 Effect of terbufos on *D. saccharalis* and *C. flavipes*

The fresh weight reduction produced by terbufos on non-parasitized larvae of *D. saccharalis* was similar to that observed in the initial dose response bioassay (data not shown). Larvae affected by terbufos were irritable and suffered of convulsions, probably as a result of the insecticide interference with the insect nervous system. Larvae sometimes remained away from the diet and then resumed feeding; under normal conditions, larvae feed continuously. Fresh weight of parasitized larvae increased more over time that that of non-parasitized larvae but was affected similarly by terbufos. At the end of the larval period, fresh weight decreased up to 51% with the highest dose (108 ppm terbufos) compared to the untreated control (data not shown).

The time required by the non-parasitized larvae of *D. saccharalis* to pupate increased in proportion to the dose of terbufos in the diet (Figure 2), from 6.6 days for the untreated larvae to 11.8 days for larvae exposed to the highest dose in the diet. Under field conditions such an extension of the larval period could have negative consequences on the crop if feeding is not severely affected by the insecticide.

D. saccharalis emerged on average 7.6 days after pupating in the untreated controls. Terbufos slightly delayed this period but there was no clear response to increasing doses. Most of the larvae (ca. 62%) feeding on contaminated diet did not pupate or exhibit growth abnormalities and died within two days. With the sublethal terbufos concentrations used in the bioassays,

larval mortality increased with the amount of the insecticide in the diet to a maximum of 13% (Figure 3).

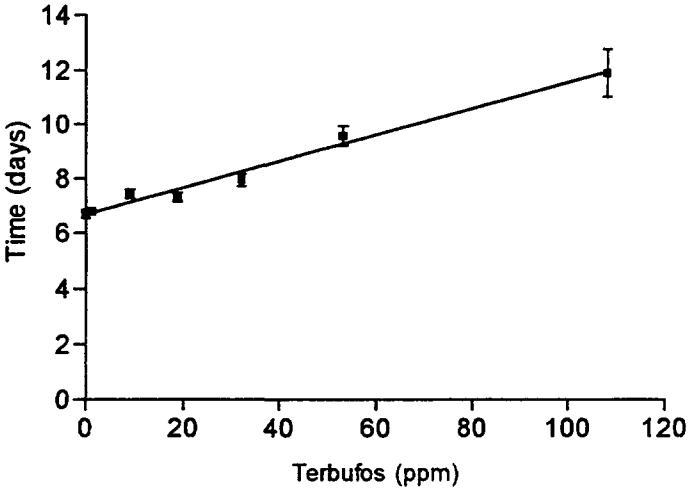

Figure 2. Time required for formation of pupae for larvae of D. saccharalis feeding on artificial diet containing sublethal concentrations of terbufos.

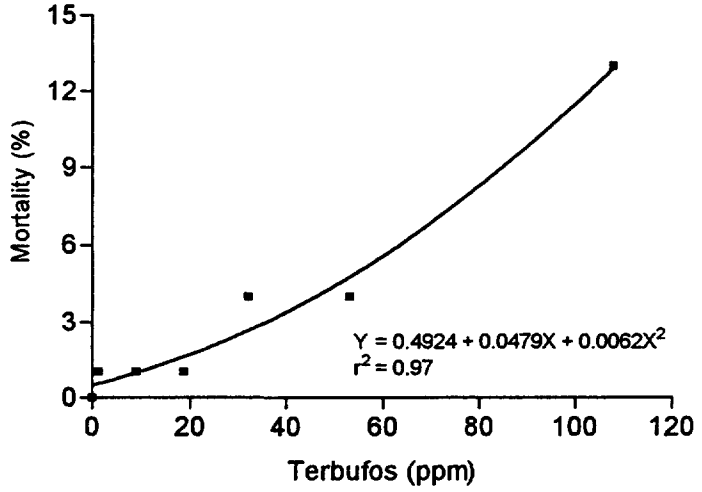

Figure 3. Mortality of non-parasitized larvae of D. saccharalis feeding in artificial diet containing increasing concentrations of terbufos.

Because of the negative effects of terbufos on larvae and pupae of *D. saccharalis*, the number of adults that emerged was also reduced (Figure 4).

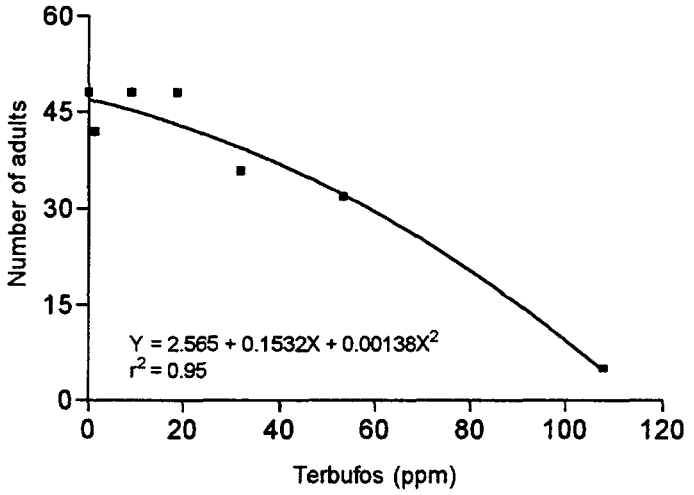

Figure 4. Number of adults of D. saccharalis emerging after larvae fed on artificial diet containing increasing concentration of terbufos. Data are the average of two bioassays.

The sex proportion of the emerged adults was also altered by the insecticide; in the controls the female:male ratio was 3:1 and decreased to less than 0.5:1 when larvae fed on terbufos-treated diet. The sex proportion is an important component that characterizes insect populations since it can determine its reproductive opportunities.

Terbufos had only a slight effect on the time required by *C. flavipes* to emerge from the host. *C. flavipes* larvae that developed in *D. saccharalis* larvae feeding on untreated diet emerged 10.1 days after oviposition; those developed in *D. saccharalis* larvae feeding on diet containing 53.2 ppm terbufos required 10.4 days to emerge. However, larvae of the parasitoid did not emerged from hosts feeding on diet containing the highest concentration of terbufos.

Mortality of emerged larvae of *C. flavipes* increased with the concentration of terbufos in the diet of the host (Figure 5). On average, only 0.15 larvae died in the control treatment and 13.5 when the larvae developed in host feeding on diet containing 53.2 ppm terbufos. It is possible that both the weight reduction of the host caused by the insecticide, which would limit the availability of food for the parasitoid, and the insecticide itself or its toxic metabolites are responsible for parasitoid mortality. Under the experimental conditions, the total number of larvae that developed inside the host could not be determined.

As a result of the effects of terbufos on the larvae, the number of pupae of *C. flavipes* was also reduced by the insecticide at increasing doses (Figure 6) but additionally there was a reduction in the number of pupae that completed their development and an extension in the time required for adults to emerge (data not shown). The number of adults of the parasitoid decreased substantially, especially at the higher concentrations of terbufos; less than 4% adults (of the

total 4796 obtained in the controls) emerged when *D. saccharalis* larvae were exposed to 32.1 and 53.2 ppm of terbufos.

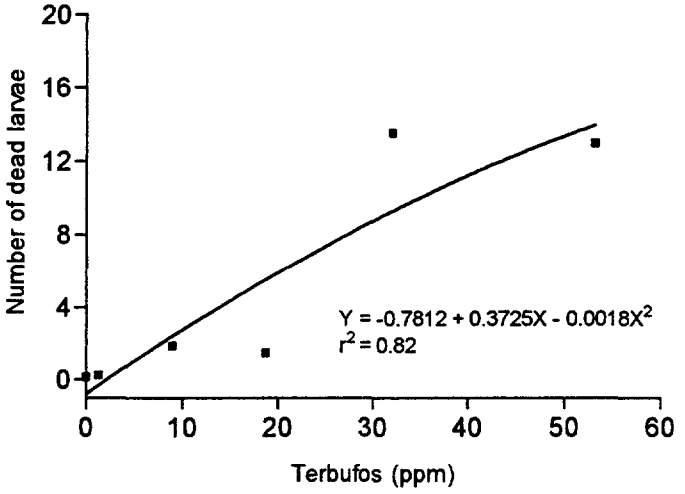

Figure 5. Mortality of C. flavipes larvae emerged from hosts feeding on artificial diet containing increasing concentrations of terbufos.

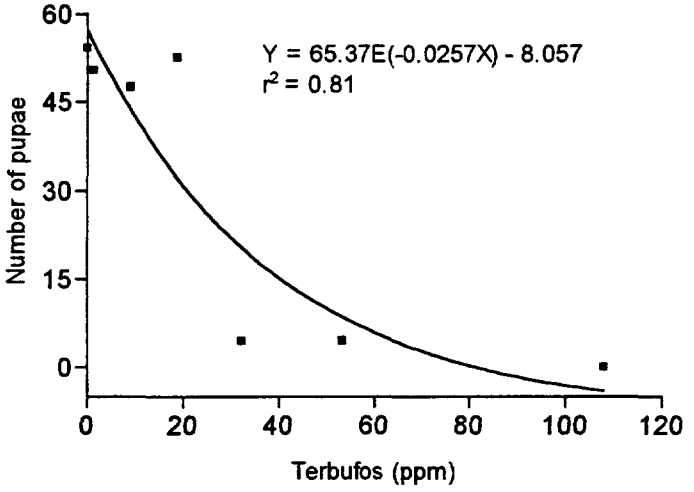

Figure 6. Number of pupae of C. flavipes developed on larvae of D. saccharalis feeding on artificial diet containing increasing concentrations of terbufos.

The fresh weight of the adults of *C. flavipes* at 24 h after emergence, especially that of females, decreased with increasing concentrations of terbufos in the diet of the host (Figure 7). This secondary effect of terbufos is important as healthy adults are expected to be able to reproduce, better locate its host, and parasitize it. The increased susceptibility of females

should also be stressed since the females are responsible for controlling the pest. Furthermore, the ratio of females:males also decreased with increasing concentrations of terbufos in the diet of the host (Figure 8).

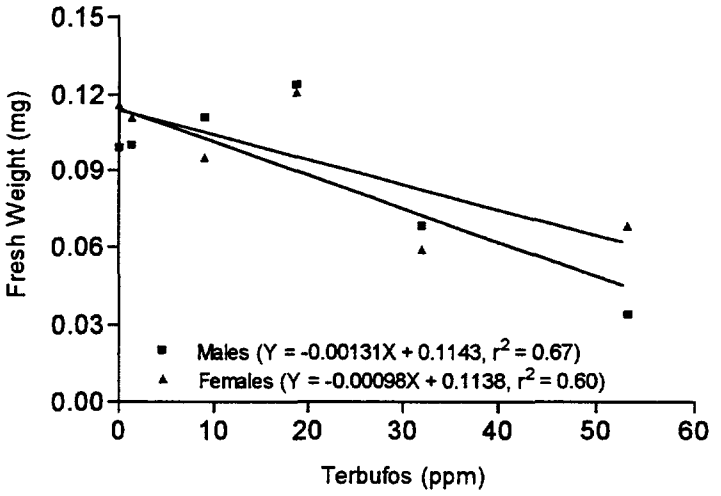

Figure 7. Fresh weight of adults of C. flavipes emerged from D. saccharalis larvae feeding on artificial diet containing increasing concentrations of terbufos.

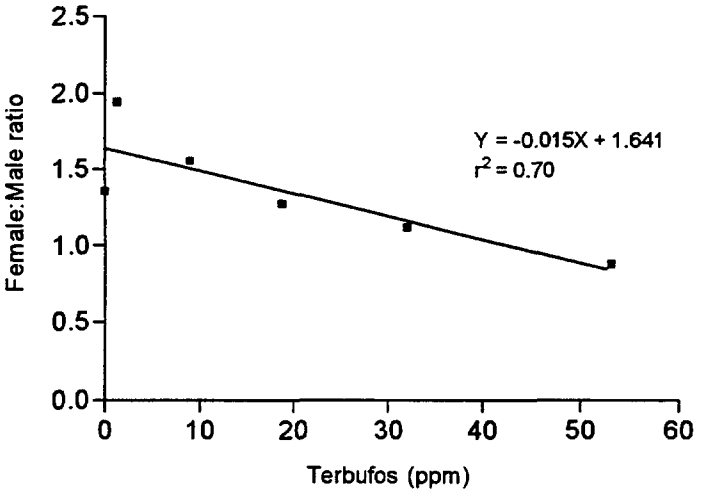

Figure 8. Proportion of adult females and males of C. flavipes obtained from D. saccharalis larvae feeding on artificial diet containing increasing concentrations of terbufos.

3.3 Determination of terbufos in treated plants and *D. saccharalis*

Only trace amounts of ^{14}C material were found in plant tissue and larvae of *D. saccharalis* (Table 1), without differences among treatments. Recovery in the laboratory was 84.5%. Therefore, plant uptake and translocation of terbufos was minimal. Thin layer

chromatographic analyses indicated that primarily terbufos metabolites (terbufos oxon sulfone, terbufos sulfone and terbufos sulfoxide) were present in the plant tissues.

Results obtained in these bioassays clearly demonstrate negative indirect effects of terbufos at sub-lethal doses on the development of the parasitoid, *C. flavipes*. If these sub-lethal doses represent levels of terbufos that *D. saccharalis* can obtain through feeding from plant tissue under field conditions, it is possible that the efficacy of the parasitoid as a biological control agent be diminished in terbufos-treated fields. Preliminary greenhouse bioassays showed that minute amounts of terbufos and its metabolites are moved to the foliage and ultimately reach the parasitoid host. However, it is unlikely that these amounts would pose any harm to *C. flavipes* under field condition.

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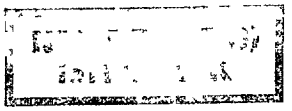

MANAGEMENT OF WHITE FLIES (*Bemisia tabaci*) IN TOMATO WITH A COMBINATION OF PLASTIC MULCH AND INSECTICIDES IN COSTA RICA

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Abstract

A combination of silver plastic soil cover and application of endosulfan or imidacloprid for the control of whiteflies (*Bemisia tabaci*) in tomato was compared to farmer's practice in Guayabo, Turrialba, Costa Rica. None of the treatments affected the whitefly infestation which was very low, the highest value being 2.25 adults/10 plants. Viral disease incidence amounted only 2.4 to 4.4% by the end of the crop season, the highest incidence levels were consistently observed in control treatments. Such negligible incidence values precluded evaluation of disease severity. Fruit number or weight and total yield were not affected by the treatments. No detectable residues of endosulfan or its metabolites were present in either foliage or fruit tissues. In soil samples, minute amounts of α- and β–endosulfan were found as well as endosulfan sulphate, the most important metabolite. Insecticide residues were always higher in the plots that were treated twice compared to those that were sprayed only once with endosulfan. No endosulfan or metabolites were detected in control plots. The lack of residues in plant tissues and the minute amounts found in soils indicate that endosulfan can be used as part as an integrated program for whitefly control in tomato.

1. INTRODUCTION

Several geminiviruses affect tomatoes in the Americas [1], whose impact on tomato yields depends on plant age at time of infection. The critical period of susceptibility for several tomato geminiviruses encompasses the first 50-60 days after emergence [2,3,4], thus management should focus on this period to minimize contact between the vector (*Bemisia tabaci*) and the host plant [5]. A sound possibility for the integrated management (IPM) of the whitefly-geminiviruses complex is the production of virus-free seedlings for 30 days under tunnels covered by fine nets [6,7], complemented with post-transplant protection by combinations of ground covers and timely application of selected insecticides.

Both ground covers (silver plastic mulches) and two insecticides (endosulfan and imidacloprid) have proven effective in decimating whitefly populations, thus reducing virus disease incidence and severity, and providing satisfactory yields [8,9,10,11]. Therefore, the objective of this experiment was to evaluate several combinations of the best methods so far available to control whiteflies, within an IPM approach.

2. MATERIALS AND METHODS

The experiment was established in a farmer's field in Guayabo (Turrialba), which is located in the Caribbean watershed of Costa Rica. Tomato seedlings (c.v. Hayslip) were grown according to standard procedures, in newspaper cups placed under field tunnels covered with Tildenet IN50. Seedlings (22 days old) were transplanted at 1.2 m between rows and 0.4 m between plants. Before transplanting, soil was prepared according to local practices.

A randomized complete block design with four replications was used, with plot size of 7.2 X 5 m; the effective plot consisted of the two central rows less a 1-m border on each end, for a total of 10 m. Treatments were combinations of a silver plastic mulch (SP) and the insecticides endosulfan (END) and imidacloprid (IMI) applied before or after transplanting or both.

Endosulfan (Thiodan 35% SC) was applied as a foliar spray at the rate of 525 g a.i./ha to tomato seedlings 5 days before transplanting (dbt) or 20 days after transplanting (dat) and imidacloprid (Confidor 350 SC) as a drench using 25 ml/plant of a suspension containing 0.35 g i.a./l. There were six treatments, as follows: SP + END (5 dbt) + END (20 dat), SP + END (5 dbt), SP + IMI (5 dbt), SP, AC (absolute control: bare soil), and RC (relative control: conventional grower practices.

Whitefly adult numbers (in 10 plants) as well as disease incidence and severity (in all the plants within the effective plot) were recorded weekly. Fruit yield was determined at crop maturity, and the number and weight of fruits were recorded according to local quality standards. Endosulfan residues were determined on both foliage and fruits after the last application of such insecticide. For this purpose, three replicates of a composite sample of about 50 g of leaf tissue was collected from each treatment.

For analysis of residues of the pesticides and their metabolites in fruits, three tomato fruits were picked from each of three individual plants from each plot at maturity. Soil samples were also taken from each effective plot (one core each), mixed and three 50-g subsamples were analyzed in the laboratory.

Residues of α- and β–Endosulfan and its metabolites (sulfate, alcohol, ether, and lactone) were analyzed at the Environmental Pollution Research Center, University of Costa Rica, according to United States Food and Drug Administration method [12]. Detection was carried out using a GC-EDC equipped with a SPB-1 column (30 m * 0.32 cm * 0.25 μm). IMI residues were not determined.

3. RESULTS AND DISCUSSION

Whitefly infestation was very low throughout the experiment and was not affected by the treatments ($p > 0.05$) (Table 1); the highest value was 2.25 adults/10 plants (0.225 adults/plant). The heavy rains that occurred during the experimental period explain these low values.

Disease incidence also was very low, ranging from 2.4 to 4.4% by the end of the crop season. The highest incidence levels were consistently observed in both controls (Table 2). Because of such negligible incidence values, severity was not estimated.

Data in Table 3 indicate that the pesticide treatments had no effect ($p > 0.05$) on the number of fruit or weight and the average yield was 22 to 28 t/ha.

TABLE 1. AVERAGE NUMBER OF ADULT WHITEFLIES ON TOMATO PLANTS

Treatments	Weeks after transplanting										
	1	2	3	4	5	6	7	8	9	10	11
SP+END+END	0 b[1]	0 b	0 a	0.25 a	0.25 a	0 b	0 b	0.5 a	0 a	0 a	0.75 a
SP+END	0 b	0 b	0 a	0.25 a	0 a	0 b	0 b	0 a	0.5 a	0.5 a	1.5 a
SP+IMI	0 b	0 b	0 a	0 a	0 a	0.25 ab	0.25 ab	0 a	0 a	0 a	0.5 a
SP	0.25 b	0 b	0 a	0 a	0.5 a	1.0 ab	1 ab	0 a	0 a	0 a	0.5 a
AC	1.5 a	0.75 a	0.75 a	0.75 a	0.25 a	2.25 a	2.25 a	0.5 a	0 a	1.75 a	0 a
RC	0.75 ab	0.25 ab	1.0 a	1.0 a	0 a	1.75 ab	1.75 ab	0.75 a	0.5 a	0 a	1.5 a

[1] Averages followed by the same letter do not differ according to the LSD test ($p = 0.05$).

TABLE 2. AVERAGE PERCENTAGE OF DISEASED TOMATO PLANTS

Treatments	Weeks after transplanting				
	7	8	9	10	11
SP+END+END	0	0.58 b[1]	0.69 b	2.45 a	2.73 ab
SP+END	0	0.29 b	0.88 b	2.52 a	2.73 ab
SP+ IMI	0.28	0 b	0.57 b	2.38 a	2.51 ab
SP	0	0.4 b	0.68 b	2.11 a	2.4 b
AC	0.28	2.33 a	2.91 a	3.94 a	4.4 a
RC	0.6 ns	1.81 a	2.14 ab	3.08 a	3.21 ab

[1] Averages followed by the same letter do not differ according to the LSD test ($p = 0.05$).

TABLE 3. AVERAGE NUMBER OF TOMATO FRUITS AND YIELD ACCORDING TO COMMERCIAL CATEGORIES

Treatments	Fruit quality categories			
	Fruits.	First	Second	Total fruit yield
	No./10 plants	kg/ha		
SP+END+END	134.00 a[1]	24.658 a	2.515 a	27.173 a
SP+END	140.00a	26.120 a	1.965 a	28.085 a
SP+ IMI	136.75 a	25.795 a	1.543 a	27.338 a
SP	140.75 a	26.108 a	1.715 a	27.823 a
AC	133.25 a	24.095 a	1.205 a	25.300 a
RC	132.00 a	24.320 a	1.690 a	26.010 a

[1] Averages followed by the same letter do not differ according to the LSD test ($p = 0.05$).

Normally, whitefly infestation of tomato fields in Guayabo is very low, with averages of one to four adults per plant. However, incidence of up to 100% diseased plants is commonly found, sometimes causing serious yield losses [13]. In this experiment, because of such low adult numbers, disease incidence was quite low and did not affect yields, which fell within the normal range for Guayabo (20-35 t/ha).

The detection and quantification limits for endosulfan and its metabolites in tomato leaves and fruits and in the soil were obtained using the Program A, Release 1.0 of Statistical Methods on Analytical Chemistry [14]. These limits are presented in Table 4.

TABLE 4. DETECTION AND QUANTIFICATION LIMITS FOR ENDOSULFAN AND ITS METABOLITES IN TOMATO LEAVEST, FRUITS AND SOIL

Analytical limits	α-Endosulfan	β-Endosulfan	Endosulfan sulfate	Endosulfan alcohol	Endosulfan ether	Endosulfan lactone
	(mg/kg)					
Plant tissue						
Detection	0.002	0.001	0.001	0.01	0.002	0.001
Quantification	0.006	0.003	0.003	0.03	0.006	0.003
Soil						
Detection	0.0004	0.0003	0.0003	0.0027	0.0004	0.0003
Quantification	0.0012	0.0009	0.0009	0.0080	0.006	0.003

No detectable residues were present in either foliage or fruit tissues. In soil samples, only minute amounts of α- and β–endosulfan were found (Table 5).

TABLE 5. RESIDUES OF ENDOSULFAN AND METABOLITES FOUND IN SOIL SAMPLES FROM PLOTS TREATED WITH ENDOSULFAN

Treatment	α-Endosulfan	β-Endosulfan	Endosulfan sulfate	Endosulfan alcohol	Endosulfan ether	Endosulfan lactone
	(mg/kg of soil)					
SP+END+END	0.014 ± 0.001	0.017 ± 0.002	0.044 ± 0.004	0.009 ± 0.001	0.0031 ± 0.0003	ND
SP+END	0.022 ± 0.002	0.19 ± 0.02	0.27 ± 0.02	0.005 ± 0.001	0.012 ± 0.001	ND
AC	ND	ND	ND	ND	ND	ND

The most important metabolite in soil was endosulfan sulfate; no endosulfan alcohol was detected. Concentration of endosulfan and its metabolites was always higher in the plots that were treated twice with the insecticide compared to those that were sprayed only once with endosulfan. No endosulfan or metabolites were detected in absolute control plots. The lack of residues in plant tissues and the minute amounts found in soils indicate that endosulfan can be used as part as an integrated program for whitefly control in tomato.

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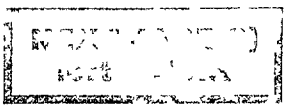

EFFECT OF THE APPLICATION OF CHLORPYRIFOS TO MAIZE ON PESTS AND BENEFICIAL ARTHROPODS IN NICARAGUA

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Abstract

Field experiments were performed between 1994 and 1997 to evaluate the effect of chlorpyrifos insecticide on arthropods in maize agroecosystem . The experiments were carried out in Boaco (Central zone) and Managua (Pacific zone) areas. Experiments were set up according to randomized block design, with large plots (750 m^2) and four replications. The treatments were 1L/ha Lorsban 4E (containing 480 g a.i./L) and control. Visual sampling, pitfall traps and yellow traps were used to estimate numbers of pest insects and beneficial arthropods. Chlorpiryfos had a measureable affect on fall armyworm (*Spodoptera frugiperda*) and *Dalbulus maidis*. The plots sprayed with the insecticide had the lowest population of *S. frugiperda* and the highest population of *D. maidis*. Beneficials insects, mainly parasitoids were more affected than pests by the insecticide sprays. The highest parasitism was found in the unsprayed plots. Overall, the lowest population of arthropods was found in the sprayed plots, except that in Managua the highest number of *D. maidis* were found in the sprayed plots.

1. INTRODUCTION

Maize is an important food crop of the small farmer in Central America. However, the insect pest attacks considerably reduce the yields. The most commonly practiced method for the control of insect pests is the use of insecticides [1]. The insecticides most commonly used in maize cultivation in Nicaragua are chlorpyrifos and carbofuran. Chlorpyrifos is used to control the fall armyworm and carbofuran for the control of several other pests, such as *D. maidis as well as* soil dwelling pests. Even though these insecticides are considered non-persistent, they are implicated in several problems including adverse effect on beneficial arthropods. There is no information on the impact of these insecticides on the non target species, inparticular beneficial arthropods in Nicaragua. The objective of this study was to evaluate the impact of chlorpyrifos on the populations of pest insects and on the parasitism of *Spodoptera frugiperda* in maize agroecosystem.

2. METHODS AND MATERIALS

2.1. Field plots

Field experiments were carried out from 1994 to 1997 in Santa Rosa in the Pacific zone and Santa Lucia in the Central zone of Nicaragua. Santa Rosa is located in Managua at an altitude of 56m above sea level and has an annual mean temperature of 27 C and average annual precipitation of 1,102 mm. Santa Lucía is located in Boaco at an altitude of 660 meters above sea level and has an annual mean temperature of 24.7 C and annual precipitation of 873 to 1543 mm. All experiment were carried out during the first growing season of each year.

The size of plots was of 25x30 m and 40x40m in Santa Rosa and 37x35m in Santa Lucía. The NB-6 variety of maize was sown. The soil was prepared in Santa Rosa by using conventional tillage and mechanical sowing. In Santa Lucía no tillage was used and the sowing method was by hand (using "espeque") . The density of established crop:was 6x20 cm. (approximately 60,000 plants/ha) in Santa Rosa, and 85x40cm (approximately 30 000 plants/ha) in Santa Lucía. The total area of each

experiment was around 6000 and 12800 squared meters. Fertilizer was used according to the recomendations and it consisted of 90.7 kg/ha NPK (10-30-12) applied at sowing time, and 46% urea. Urea was applied 45.4 kg/ha one month after the sowing time and 45.4 kg at the beginning of flowering. The previous crops grown in the experimental plots was also maize. The crops grown in the neighbouring fields were tomato and maize in Santa Lucía and maize in Santa Rosa.

2.2. Experimental design

The experiment was carried out on a randomized block design, with four blocks and two treatments which were: (1) treated woth chlorpiryfos (1 L/ha)and (2) untreated control. The number of applications of chlorpyrifos were three in Santa Lucía (15, 35 and 70 days after emergence) and two in Santa Rosa (25 and 40 days after emergence). The dosage was of 1 L/ha in each application. The insecticide applications were made using a high volume application with a Matabi knapsack sprayer Matabi with a capacity of 20 litres and a hollow cone nozzle. The insecticide used was Lorsban 4E emulsifiable formulation containing chlorpyrifos as active ingredient at a concentration of 480 g/L.

2.3. Artificial infestation

To enhance the incidence of *S. frugiperda*, in the field, the plants were artificially infested with the larvae of the pest insect just before spraying. One hundred plants per plot were infested with small, medium and large larvae by placing the larvae in the whorls. In some cases egg masses were also used. The artificially plants were then covered with plastic bags in order to protect them against the insecticide deposit. In order to protect the larvae from the predators (ants) a plastic barrier (cover) was put on the base of the plant.

2.4. Sampling

2.4.1. Visual Sampling

Visual sampling was carried out to estimate the populations of *Spodoptera frugiperda, Dalbulus maidis* and beneficials insects mainly earwigs, spiders an wasps. One subplot of 10x 10 m area was marked out in the centre of each plot and twelve 10 m long rows were selected. In this subplot 60 plants were sampled by sampling in alternate rows. Two plants were sampled in each row leaving a plant without sampling until a total of 60 plants in the subplot had been sampled. Fifteen samplings were done during the crop season. These were done one day before the first application, 1, 3 and 6 days after the first application, and 1, 3, 6, 13, 20, 27, 34, 41, 48, 56 and 62 days after the second application.

2.4.2. Sampling by use of pitfall traps and yellow traps

Pitfall traps were used to estimate the populations of general predators and yellow traps were used to estimate the incidence of Hymenopteran parasitoids. Sampling by traps were carried out 2 days before, and 3 and 6 days after the first application and 3, 6, 16 and 23 days after the second application. All sampling activities were organized in two groups, during the applications and after the applications time.

2.4.3. Parasitism of *Spodoptera frugiperda*.

The artificially infested plants were collected four days after the infestation to evaluate parasitism of *Spodoptera frugiperda*. These plants were taken to the laboratory where the larvae were extracted from them and placed individually in glass jars containing diet. Data on the emergence of parasitoids were collected.

3. RESULTS AND DISCUSSION

3.1. The effect of insecticide sprays on insect populations in general

In general the populations of arthropods was higher in Santa Lucía than in Santa Rosa, this may be attributed to more favourable climatic conditions and plant diversity in Santa Lucia.

Populations of arthropods in the unsprayed fields were higher than those in the sprayed fields. The differences were significant in Santa Lucía but not in Santa Rosa, where the population was similar in the sprayed and unsprayed plots. The most common insect found was *Dalbulus maidis*. In Santa Rosa the lowest *D. maidis* population was found in the unsprayed plots.

During the sampling before each application a higher number of insects was found, but immediatly after the application the population was reduced. After each application the unsprayed plots showed a higher number of insects than the sprayed plots. Insects found in the different sampling methods are showed in the Table 1.

It was observed that immediatly after the application, the arthropods population declined in both sprayed and unsprayed plots. The arthropods population, mainly *S. frugiperda* and *D. maidis*, also declined 60 days after the emergence of the crop; however, some of the beneficials insects, mainly earwigs (*Doru* sp), tended to remain during that period. The decrease in arthropods population is mainly because of the crop maturity.

In general the unsprayed plot showed a higher number of insects. The higher number of pests was found in the unsprayed plot and the higher number of beneficials insects was found in the sprayed treatment. The most common pest insects found were *Spodptera frugiperda* and *Dalbulus maidis*. However the population of *D. maidis*.was high and that of *S. frugiperda* very low.

TABLE 1. LIST OF ARTHROPODS FOUND DURING SAMPLING AND IN TRAPS

Visual Sampling	Pitfall Traps	Yellow Traps
Spodoptera frugiperda	Ants	Mimaridae
Dalbulus maidis	Spiders	*Chelonus* sp
Cicloneda		Scelionidae
Doru sp		Ceraphronidae
Polibia sp		Eucoilidae
		Ichneumonidae
		Torymidae
		Pteromalidae
		Eurytomidae
		Platygasteridae
		Eucharytidae
		Eulophidae
		Chalcididae
		Eupelmidae
		Diapriidae
		Bethylidae
		Encyrtidae

The study suggests that there was no significant effect from insecticide sprays on the total pest populations, which were statistically similar in both treatments, although the populations in the unsprayed plots were somewhat higher than those in the sprayed plots.

Before the application of the pesticide there was no significant difference between the insect population in the treated and untreated plots. However, after the applications insect populations were higher in the sprayed plots. It could be due to adverse effect of the insecticide on the natural enemies of the pest. The pest populations fluctuated between sampling intervals, but the highest populations were found during the first four samplings after the pesticide application.

3.2. The effect of insecticide sprays on *Spodoptera frugiperda*.
In general the highest number of this insect was found in unsprayed plots. It means the insecticide was effective against the pest. These results are similar to those reported by Van Huis[1] who found that chlorpyrifos was effective against *S. frugiperda* on maize plants. The largest number of fall armyworms were found 34 days after the second application, which was 60 days after germination. At this stage *S. frugiperda* fed on the maize ears as an earworm.

During the application period there was significative difference between treatments, but the sampling dates were similar. After the application period the treatments were statistically similar. This was because the effect of insecticide disappeared for this time. At the end of this period the population of *S. frugiperda* was decreasing because of the age of the crop and incidence of natural enemies (*Chelonus* and *Doru* spp).

3.3. The effect of insecticide sprays on *Dalbulus maidis*.
This insect was found in higher populations than any other species. During and after the applications the highest number of *D. maidis* was found in the sprayed plots. Significant differences between treatments were found in both periods. The population of this pest increased at the beginning of vegetative stage of the crop until after the flowering of the crop because the pest trend to disappear at the end of the crop season because of the age of maize plant.

Is important to note that the highest population of dalbulus was found in the sprayed plots because chlorpyrifos do not have effect on this insect, because the pest feed sucking of the plant and the penetration way of insecticide which is a not sistemic insecticide. Beside the insecticide affected the natural enemies of the pest, mainly parasitoids, reducing of this way the natural control of the pest.

In former investigations high level of parasitism on dalbulus has been found when no insecticide has been applied. In the present work we assume the parasitism was affected, because in the unsprayed plots the dalbulus population was lower because of the natural control was no affected.

3.4. The effect of insecticide sprays on the beneficial arthropods (insect predators)
In general the incidence of predators was low, however there was a highest incidence of beneficial arthropods in Santa Lucía than in Santa Rosa. Is important to mention all the beneficial insects found in these experiments are reported by others authors as important agents of natural control on *S. frugiperda*. The highest population of these insects was found during the period after applications in Santa Rosa but there were not significative differences between treatments, however during the applications period in the unsprayed plots was found a higher population. In Santa Lucía the results indicate the significative effect of the insecticide on beneficial insects, thus the highest population was always found in the unsprayed plots. Beside significative differences between treatments were found.

A greater number of *Cycloneda sp. were* found in the unsprayed plots, although they were not significantly different than the sprayed plots. . During the sampling after applications time significative difference was found between sampling dates, because 34 days after the second application the population began to disappear. May be the there was a low population of this insect in both treatments because this insect was affected in both plots because of the movement of the insects.

The population of earwigs (*Doru sp)* was low at the beginning of the vegetative stage of the maize plant, but it later it increased and higher populations were present at the end of the crop season. During the applications time the population of earwigs was very low and there was not difference between treatments nor between sampling dates. During this period the raining season had not began which could have effect on the population. According to Marenco [2] rainfall helps increase population of earwigs. After applications there was not significative difference between treatments, however a higher number of insects was found in most of cases in the unsprayed plots. The highest population was found during the last two sampling dates. The results found in Santa Lucía indicate the significative effect of the insecticide on earwigs population, thus the highest population was always found in the unsprayed plots. Beside significative differences between treatments were found.

The populations of spiders were greater in Santa Lucia than in Santa Rosa. In Santa Lucia their populations were significantly greater in unsprayed plots than sprayed plots, but in Santa Rosa there was no significat difference. The populations of ants were also greater in Santa Lucia than in Santa Rosa. Therefore, the effect of insecticide sprays on theie populations was monitored only in Santa Lucia where highest populations were found in the unsprayed plots. The greatest effect of the insecticide is observed during the time of applications. During this time significant differences between treatments were found.

The population of *Polybia* sp was in general quite low, but higher numbers were found during the period after the insecticide applications. During the applications period no significant differences were found; however, higher numbers were found in the unsprayed plots. In this period significant difference between sampling dates was found because one day after the first application this insect was no found in any plot. During the period after application no significative difference was found between treatments nor between sampling dates. Even though no significative difference between treatments was found, a higher number of this insect was found in the sprayed plots. Is important to mention this insect is flier and could move from sprayed to unsprayed plots and viceversa, also to this time the effect of insecticide has disappeared.

3.5. The effect of insecticide sprays on beneficial insect parasites.

The Hymenopterous parasitoids were sampled using yellow traps. In general a high diversity of parasitoids insects were found, the most common families are showed in Table 1. Although the population of these insects was not too high, the effect of insecticide was measurable. During the application time significant difference between treatments were found, the highest number of insects was found in the unsprayed plots. Also significant differences between sampling dates were found. The highest population was found 6 days after the second application. Although the population of Braconids was not significantly different in the treated and control plots, highest population of these parasitoids was found in the unsprayed plots, except during the second and fifth sampling.

The parasitoid belonging to *Chelonus* group of Braconids is the main and most important parasitoid of the fall armyworm. During the application period, even though significant difference was not found a higher number of insects was found in the unsprayed plots. The highest number of

Chelonus sp was found in unsprayed plots, because in these plots there was a higher population of fall armyworm and also because the parasitoid was less affected than in the sprayed plots . The highest population was found during the first sampling, 2 days before the application of clorpyriphos and during the last sampling 23 days after the second application, when the activity of insecticide had disappeared.

3.6. The effect of insecticide sprays on the parasitism of *Spodoptera frugiperda*

Although the level of parasitism was low, during the experiments was possibble to observe that insecticide application affect the activity of parasitoids. The parasitism on *Spodoptera frugiperda* was higher in untreated plots than in the treated.

The most effect of chlorpyrifos on parasitoids is because the insecticide is not applied directly on them, so the parasitoids do not have any mechanism to escape to the insecticide and of this way they are more susceptible than pests. The number of larvae collected was very low compared with the total number of larvae that were used in the artificial infestation. It appears the larvae were affected by natural conditions and natural enemies, possibly ants.

3.7. The effect of insecticide sprays on the yield of maize

Although the yield was low, because of affect by others factors, it was possible to determine that the best yield was always obtained in the treated plots; however, the difference was not significant between the yield from the treated and untreated plots.

CONCLUSIONS

In general Chlorpyrifos had effect on population of insects, however this effect is not yet too clear, because in some cases the unsprayed treatment showed lower amount of insects. The effect of the insecticide on parasitism of *Spodoptera frugiperda* was highest in the sprayed plots, and the highest percentage of parasitism was found in the unsprayed plots. The population of *D. maidis* was favored by application of chlorpyriphos, because the insecticide seems to have an adverse effect on the natural enemies of this pest. The insecticide was found to be very effective against *Spodoptera frugiperda*, as the lowest population was found in the sprayed plots.

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SUSCEPTIBILITY OF NATURAL ENEMIES OF PESTS OF AGRICULTURE TO COMMONLY APPLIED INSECTICIDES IN HONDURAS

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Abstract

Insecticides are commonly used by Honduran farmers to control pest insects in agricultural crops such as corn, melons and tomatoes. However, the insecticides have the potential for toxicity to the natural enemies of the pest insects also. Therefore, efforts are being made to identify inseciticides which, when used within the Inegerated Pest Management (IPM) programme, are selectively more toxic to the pest insects than their natural enemies. A number of selected chemical insecticides and a biological insecticide (NPV) were tested in three different tests to determine toxicity to two beneficial insects: *Telenomus remus* Nixon (Hymenoptera: Scelionidae) and *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae). All insecticides were toxic to *T. remus* which suffered high mortality. There was no significant difference in mortality of the insect due to the method of exposure to the insecticides. There were some differences in the toxicity of the insecticides to *C. carnea,* and abamectin, bifenthrin, cypermethrin, diafenthiuron, imidacloprid and fenpropathrin were relatively less toxic and could be used in IPM for the control of pest insects.

1. INTRODUCTION

Biological control is an important tool in Integrated Pest Management (IPM) programme because it can maintain pest populations under economic threshold level. By reducing the number of pest insects biological control reduces crop damage and future population explosions of the pest, and unlike insecticides, it does not have the potential for adverse effects on human health and the environment. IPM programs include the use of insecticides, but they are used when absolutely necessary. However, in Honduras the farmers almost entirely rely on the use of insecticides for pest control, applying them every week or 2 to 3 times a week, endangering natural control agents as well as human health and the environment.

Biological control technology includes mass rearing and release of parasitoids and predators of pests. The application of broad spectrum insecticides carry the risk to these beneficial insects. It is, therefore, necessary that if the insecticides have to be used, they should be selectively more toxic to the pest insects than their natural enemies.

The present study was initiated in 1994 to evaluate the toxicity to three beneficial insects *Telenomus remus* Nixon, *Chrysoperla carnea* Stephens and *Diadegma insulare* Cresson of insecticides commonly used by farmers for the control of insect pests in tomato, melon, corn and cabbage crops in Honduras. Mortality of the insects exposed to the insecticides indicated their relative susceptibility to the insecticides.

2. METHODS AND MATERIALS

2.1. Sources of parasitoids and predators

Adult parasitoids ***Telenomus remus*** Nixon were available in this laboratory, C***hrysoperla carnea*** Stephens eggs were procured from ARBICO company, Tucson, Arizona, USA, and ***Diadegma insulare*** Cresson pupae were collected from local fields.

2.2. Insecticides

A biological insecticide, NPV, and a number of synthetic insecticides from different chemical classes were used at dosage recommended in the label. The insecticides were available in this institution and NPV 80 (1.6% of NPV of *Autographa californica*) was obtained from Agrícola El Sol, Guatemala. Table I shows the beneficial insects and the insecticides, methods of exposure and application rates tested on them

TABLE I. LIST OF BENEFICIAL INSECTS AND THE INSECTICIDES, METHODS OF EXPOSURE AND APPLICATION RATES TESTED ON THEM.

Beneficial insect	Insecticide	Insecticide class	Dose g (a.i.)/ha	Concentration µl/200 mL water
Telenomus remus Nixon laboratory/ semifield/ field applications	Phoxim	Ophosphate	250	500
	Chlorpyrifos	Ophosphate	140	280
	Cypermethrin	Pyrethroid	30	150
	NPV	Biological	1*1012 C.I.2	1.2*109 C.I.3
	Cypermethrin	Pyrethroid	0.5 l/ha	
	Methomil	Carbamate	0.3 l/ha	
	Clhorpyrifos	Ophosphate	1.5 l/ha	
	Phoxim	Ophosphate	1.4 l/ha	
	NPV	Biological	1.0 l/ha	
Chrysoperla carnea Stephens	Cypermethrin	Pyrethroid	30	2500
	Imidacloprid	Nitroguanidin	12.5	50
	Phenpropathrin	Pyrethoid	75	500
	Oxydemeton metil	Organophos-		
	Methamidophos	Phate	250	500
	Diafenthiuron	Ophosphate	600	1000
	Bifenthrin	Thiourea	42	280
	Endosulfan	Pyrethroid	50	750
	Abamectin	Chlorinated	54	1400
	Oxamyl	Avermectin	2.7	150
		Carbamate	500	2000
Diadegma Insularis Cresson	Cypermethrin	Pyrethroid	30	200
	B. thuringiensis	Biologic	0.9 l/ha	600
	Tyociclan		150	0.64 g.
	Methamidophos	Ophosphate	450	500
	Cypermethrin +	Pyrethroid +		
	Prophenophos	Ophosphate	0.75 l/ha	500
	Endosulfan	Chlorinated	420	800
	Methomil	Carbamate	162	0.50 g.

2.3. Test of the toxicity of insecticides to beneficial insects

The toxicity of the insecticides to the three species of beneficial insects was studied in laboratory, minigreenhouse and semi-field tests. For these tests procedures recommended by International Organization for Biological Control (IOBC) were followed. These procedures recommend that toxicity tests be carried out in stages from laboratory to greenhouse to field.

For classification of the degree of toxicity of the insecticides to the test insects, the procedure suggested by Hassan (1985) in the IOBC guide book was used. These categories are as following:

- little toxicity, where the mortality was less than 50%,;
- lightly toxic, where the mortality was between 50 and 79%;
- moderately toxic, where the mortality was between 80 and 90%; and
- toxic, if the mortality was greater than 90%

In the laboratory test leaves of common bean plant were dipped for 10 seconds in the aqueous solution/suspension of each insecticide. After allowing the excess moisture to evaporate from the leaf surface, it was transferred to a petri dish. Leaves dipped in distilled water were used as control. Five *C. carnea or 30-40 T. remus* were placed on the treated leaf in each petri dish. Cotton soaked in honey-water was placed in each dish to provide diet for *T. remus* and 4 egg masses of *Spodoptera frugiperda* per petri dish were provided for *C. carnea.*. The insects were allowed to remain in the petri dishes for 2, 4, 12 and 24 h The mortality was estimated.at the end of each exposure period.

In micro-greenhouse tests potted 2-week old bean plants were covered with bottomless plastic bottles. Opening was cut on the side of the plastic enclosure and the plant was sprayed through the opening with the insecticide solution/suspension using a hand sprayer. The side opening was covered with a cloth after the spray and the excess moisture from the plant was allowed to evaporate. A cotton ball soaked with honey-water was inserted in the top opening of the enclosure to provide food for *T. remus* and 10 egg masses of *S. fugiperda* were placed inside the enclosure as food for *C. carnea.* The plants were placed in the greenhouse and ten *C. carnea* or 30 - 40 *T. remus* were transferred onto each of the treated plants The insects were exposed to the treated plant for 2, 4, 12 and 24 hours. After each period of time mortality was counted.

In the third test the set up was similar to the second test except that the bean plants were sprayed with a knap sack sprayer, which delivered smaller droplets in the spray. The treated plants were placed outdoors and ten *C. carnea* or 30-40 *T. remus* were transferred onto each treated plant. Mortality was assessed after 2, 4, 12 and 24 hours. The insecticide treated plants placed outdoors were exposed to more severe changes in climatic conditions as compared to the plants placed in the greenhouse.

3. RESULTS AND DISCUSSION

3.1. Toxicity of the insecticides to *Telenomus remus*

The results of the tests in the laboratory and under mini-greenhouse and semi-field conditions are shown in Tables II, III and IV.

Chlorpyrifos, phoxim and cypermethrin were toxic to *T. remus* in all three tests, and there was no significant difference in the toxicity when the duration of exposure ranged between 2 and 24 hours. In all cases the mortality was higher than 93%.

There was also high dergee of mortality from exposure to NPV, and the exposed insects died quickly. It was unanticipated. Death from NPV is usually slow, but in this case the insects died in a short period of time. The fast mortality on exposure to NPV was not typical of the effect

TABLE II. MORTALITY (%) OF *Telenomus remus* FROM THE EFFECT OF INSECTICIDES IN THE LABORATORY TESTS.

Insecticides	2 h	4 h	12 h	24 h
Chlorpyrifos	100a	100a	100a	100a
Phoxim	98a	100a	100a	100a
Cypermethrin	93a	97a	100a	100a
NPV (VPN)	56b	95a	100a	100a
Control	27c	38b	47b	84b

a,b,c Figures followed by the same letter are not significantly different (P>F=0.0001)

TABLE III. MORTALITY (%) OF *Telenomus remus* FROM THE EFFECT OF INSECTICIDES IN MICRO-GREENHOUSE TESTS.

Insecticides	2 h	4 h	12 h	24 h
Chlorpyrifos	100a	100a	100a	100a
Phoxim	100a	100a	100a	100a
Cipermetrina	97a	100a	100a	100a
V.P.N.	60b	55b	82b	96a
Control	50b	42c	60c	69b

a,b,c Figures followed by the same letter are not significantly different (P>F=0.0001)

TABLE IV. MORTALITY (%) OF *Telenomus remus* FROM THE EFFECT OF INSECTICDES IN SEMIFIELD TESTS.

Insecticides	2 Hours	4 Hours	12hours	24 hours
Chlorpyrifos	98a	100a	100a	100a
Phoxin	94a	100a	100a	100a
Cipermetrina	94a	95a	98ab	99a
V.P.N.	79b	84b	94b	99a
Control	20c	27c	87c	92b

a,b,c Figures followed by the same letter are not significantly different (P>F=0.0001)

of virus, it was more like the result of insecticide toxicity. It is possible that the artificial diet fed to the test insects was contaminated with toxic chemicals. Same may be true for the mortality in the control. It is also possible that some of the mortality was from the effect of unfavourable environmental conditions in the confinement. The tests were carried out under uncontrolled environmental conditions during different times of the year. Some tests were carried out in cold and dry months and others under warm and humid conditions. Such variation in the environmental conditions would be expected to affect the results. Some of this affect can be observed in the control, which shows mortality which can not be explained otherwise.

3.2. Toxicity of the insecticides to *Chrysoperla carnea*

The insecticides tested for toxicity to *C. carnea* included abamectin, bifenthrin, cypermethrin, diafenthiuron, endosulfan, fenpropathrin, imidacloprid, methamidophos, oxydemeton methyl and oxamyl. The results are shown in Tables V to VII.

In laboratory tests endosulfan, fenpropathrin, oxydemeton methyl and oxamyl were toxic to *C carnea* as 24 h exposure resulted in 100% mortality. Fenpropathrin and diafenthiuron were moderately toxic; and bifenthrin, cypermethrin, diafenthiuron, imidacloprid were non toxic to this insect.

In micro-greenhouse tests methamidophos, oxamyl and oxydemeton methyl were toxic, but endosulfan was moderately toxic. Bifenthrin, cypermethrin, diafenthiuron, fenpropathrin and Imidacloprid were also moderately toxic. However, abamectin was non toxic to *C. carnea.*

In semi-field tests methamidophos, oxamyl and oxydemeton methyl were toxic; bifenthrin, cypermethrin, diafenthiuron, endosulfan, fenpropathrin, and imidacloprid were moderately toxic; whereas, abamectin was slightly toxic.

TABLE V. MORTALITY (%) OF *Chysoperla carnea* FROM EXPOSURE TO INSECTICIDES IN LABORATORY TESTS.

InsecticidE	2 Hours	4 Hours	12 Hours	24 Hours
Endosulfan	72a	100a	82	100a
Oxidemeton-Metil	62a	100a	73ab	100a
Oxamil	37b	71ab	86a	100a
Fempropatrin	26bc	54bc	51abc	70ab
Metamidofos	25bc	47bc	47abc	100a
Cipermetrina	17bc	29bc	37bc	47bc
Imidacloprid	16bc	20c	30bc	49bc
Diafentiuron	15bc	16c	27bc	52bc
Bifentrin	11bc	16c	30bc	44bc
Abamectina	0c	11c	7c	20c
Control	0c	7c	15c	19c

a,b,c Figures followed by the same letter are not significantly different (P>F=0.0001)

TABLE VI. MORTALITY (%) of *Chysoperla carnea* FROM EXPOSURE TO INSECTICIDES IN THE MICROGREENHOUSE.

Insecticide	% of mortality 24 hours
Methamidophos	97a
Oxidemeton metil	92a
Oxamil	88a
Endosulfan	63b
Imidacloprid	61b
Fenpropatrin	52b
Diafenthiuron	45b
Bifentrin	42b
Cypermetrin	54b
Abamectin	18c
Control	18c

a,b,c Figures followed by the same letter are not significantly different (P>F=0.0001)

TABLE VII. MORTALITY (%) OF *Chysoperla carnea* ON EXPOSURE TO INSECTICIDES IN SEMIFIELD TESTS

Insecticide	Mortality (%) after 24 h
Methamidophos	97a
Oxidemeton metil	84ab
Oxamil	83ab
Endosulfan	75abc
Imidacloprid	68bcd
Fenpropatrin	66bcd
Diafenthiuron	58cde
Bifentrin	56cde
Cypermetrin	53cde
Abamectin	46de
Control	38e

a,b,c,d,e Figures followed by the same letter are not significantly different (P>F=0.0001)

These data indicate that of the tested insecticides the two organophosphorus insecticides, methamidophos and oxydemeton methyl, were the most toxic. These were followed by oxamyl, the only carbamate insecticide tested; and endosulfan, the only organochlorine insecticide tested. The other insecticides, especially the three organopyrethroid insecticides, bifenthrin, cypermethrin and fenpropathrin , were less toxic. This information can be used in Integrated Pest Management programmme to reduce harm to beneficail insects from insecticide applications.

CONCLUSIONS

Under the conditions used in these evaluations all tested pesticides were toxic to *Telenomus remus* Nixon. The environmental conditions seem to influence the toxic effects of the insecticides on this insect. The tested insecticides were also toxic to *Chrysoperla carnea,* but some were more toxic than the others. Thus, in general the two organophosphorus insecticides, methamidophos and oxydemeton methyl as well as the carbamate insecticide, oxamyl, caused the highest mortality of this insect, whereas, abamectin, bifenthrin, cypermethrin, diafenthiuron, fenpropathrin and imidacloprid were less toxic. This information can be used in the development and improvement of IPM programmes.

THE EFFECT OF INSECTICIDE APPLICATIONS TO MELON CROP ON MELON APHID AND ITS NATURAL ENEMIES

(Abstract)

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Melons are an important export crop for Panama and are cultivated on more than 1000 ha of land. Long growing season, extending well into January, allows several generations and build up of heavy populations of an important insect pest, *Aphis gossypii,* the melon aphid. Growers find it difficult to cultivate melons without several applications of insecticides. Although the insecticide applications control the aphids, they may also have adverse effects on the natural enemies of the aphid, in particular the two predatory insects *Cycloneda sanguinea* and *Chrysoperla carnea.* The purpose of this research was to evaluate the impact of insecticide applications on these insects and on the yield of melons, and to estimate residues of the applied insecticides in soil. The insecticides were applied as four different type of treatments to melon crop. The treatments were (i) three periodic applications of endosulfan (Thiodan 35EC), each at 0.52 kg a.i./ha, (ii) three applications of fenitrothion (Sumithion 50WP), each at 0.35 kg a.i./ha, (iii) two applications of fenitrothion and one of endosulfan, and (iv) grower's treatment, which included applications of six different insecticides. .The effect of the insecticide applications was evaluated by estimating numbers of each of the three type of insects before and within 72 hours after the applications and estimating yield of melons. All insecticide treatments reduced the populations of *Aphis gossypii,* but they also reduced the numbers of the benificial insects. Endosulfan was somewhat less toxic to *C. carnea* than the other insecticides were, since greater number of *C. carnea* were recorded from the plots treated with endosulfan than the other treated plots. The best yield of melons was recorded in the plots which were sprayed with fenitrothion, followed by the plots sprayed with endosulfan. and then those with grower's insecticides. Soon after the application of endosulfan the residue in the soil was 0.2 mg/kg , but it declined to less than 0.1 mg/kg in 10 days.

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