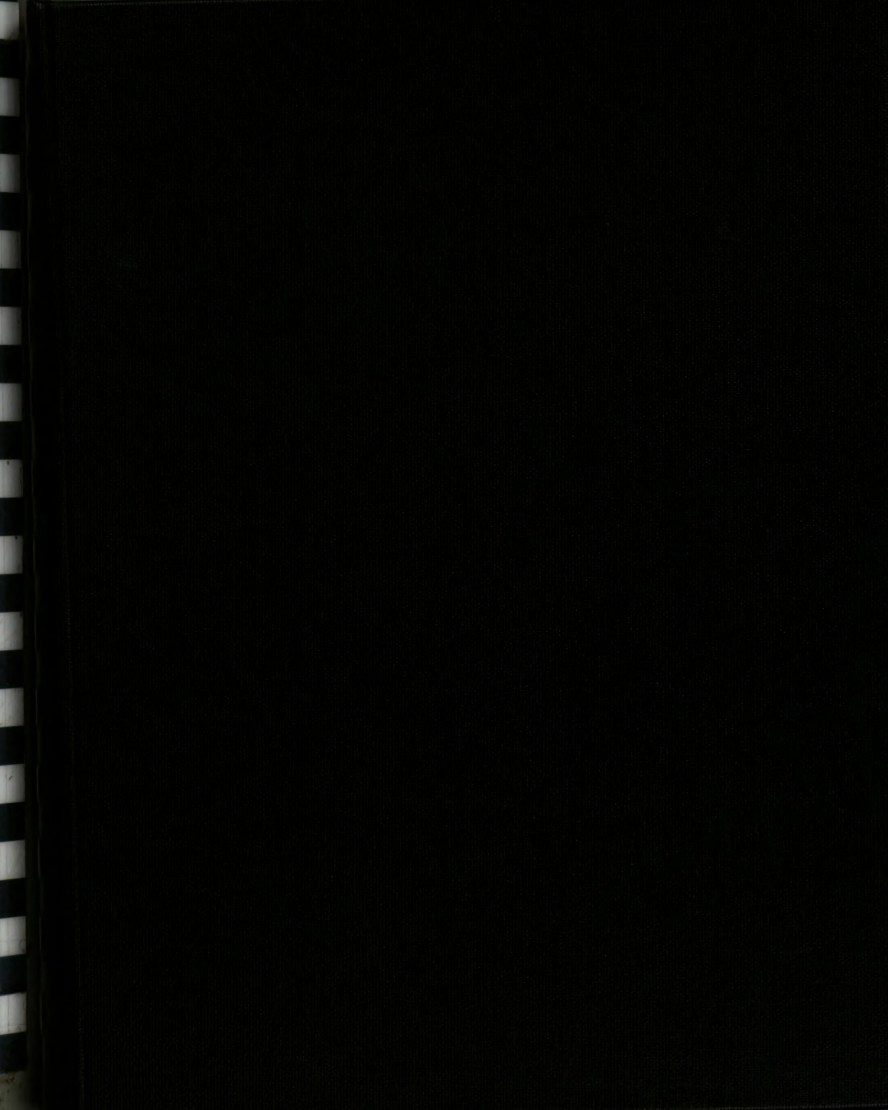






LIBRARY
Michigan State
University

This is to certify that the

dissertation entitled

*Topics in Pesticide Residue Analysis: Assessment
of Contamination of Surface Water and Fish from Côte
d'Ivoire and The Evaluation of Immunoassays for the
Detection of Pesticide in ^{plant and fish} samples.*
presented by

EBOUA N. L'WANDAN

has been accepted towards fulfillment
of the requirements for

Ph. D degree in *Entomology / Env. Toxic*

Date *6/12/96*


Major professor
for M. Zubik

**PLACE IN RETURN BOX to remove this checkout from your record.
TO AVOID FINES return on or before date due.**

DATE DUE	DATE DUE	DATE DUE
JAN 11 2000	_____	_____
04 22 03 APR 25 2003	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

MSU Is An Affirmative Action/Equal Opportunity Institution

c:\cric\datedue.pm3-p.1

TOPIC
CONTAINING
AND THE

Deputy

**TOPICS IN PESTICIDE RESIDUE ANALYSIS: ASSESSMENT OF
CONTAMINATION OF SURFACE WATER AND FISH FROM CÔTE D'IVOIRE
AND THE EVALUATION OF IMMUNOASSAYS FOR DETECTION OF
PESTICIDES IN PLANT AND FISH SAMPLES**

by

Eboua Narcisse Wandan

A DISSERTATION

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

DOCTOR OF PHILOSOPHY

Department of Entomology and the Program of Environmental Toxicology

1996

TOPIC
CONTAMINANTS
AND TR

The d

pollution

to also on

contaminant

reason, water

of these an

characteris

zinc and c

organochlor

residue in

alimentary

agriculture

These res

source of

ABSTRACT

TOPICS IN PESTICIDE RESIDUE ANALYSIS: ASSESSMENT OF CONTAMINATION OF SURFACE WATER AND FISH FROM CÔTE D'IVOIRE AND THE EVALUATION OF IMMUNOASSAY FOR DETECTION OF PESTICIDES IN PLANT AND FISH SAMPLES

BY

Eboua Narcisse Wandan

The developing world, has focused on producing more food to feed the growing population. To do so, farmers must not only rely on improved seeds and mechanization but also on fertilizer and pesticides. As in the developed world, the assessment of contamination of the environment by these chemicals should be of great concern. For this reason, water and fish samples were collected from selected rivers and lagoons from Côte d'Ivoire and were brought to the USA to be analyzed. The physico-chemical characteristics values indicate that these water are suitable for drinking. Only two metals, zinc and copper were detected at very low levels (range and values). The levels of organochlorine pesticides detected in water and fish samples were below the extraneous residue limits (ERL) and the acceptable daily intake set by the FAO/WHO codex alimentarius commission. The levels were higher in the south of the country where agriculture is more intensified and in urban areas compared to the north and rural areas. These results indicate that agricultural and industrial activities are the most important source of surface water contamination by xenobiotics. Three commercially available

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

100-100000

immunoassay kits from different manufacturers were evaluated for the determination of pesticides, as an alternative to gas chromatographic (GC) methods used for the determination of organochlorine pesticides. Interference due to fish and corn leaf coextracts was corrected with dilution using distillate water. The resulting assays showed good reproducibility and accuracy and had an estimated limit of pesticide detection of 0.25 ppb in fish and plant material. The results of the study indicated that the two types of immunoassay kits gave similar results in the detection of alachlor, atrazine, and carbofuran in corn leaf and fish fillet but the Ohmicron RaPID Assays® kit was more accurate and sensitive and less expensive compared to the Millipore Envirogard™ kit. The analysis of incurred corn leaf and fish samples show that the ELISA compares favorably with GC measurements. The ELISA was found to be less expensive and easy to use compared to gas chromatography and could be a good analytical tool for developing countries where financial resources are scarce.

This dissertation is dedicated to the memories of my mother in law Ekora and my sister Andeh who passed away while I was here. May they rest in peace.

ACKNOWLEDGMENTS

I would first like to express my gratitude and my sincere appreciation to Dr. Matt Zabik, my major professor for the opportunity to work in his laboratory and for his guidance during the past six years. I would like also to thank Drs. R. Leavitt, P. Hart, Geo Bird, and specially Don Penner for his help in all the aspects involving this work.

I would like also to express my heartfelt appreciation to my lab mates: Gamal, Glenn, Melvin, Matt. and the team of IR4.

I would like to deeply thank the government of Côte d'Ivoire and the College of Agriculture (ENSA), the African-American Institute (AFGRAD), together with Michigan State University for allowing me to follow this program and providing me with their financial support. Grateful acknowledgment is extended to Ms. Elizabeth Ward, Academic advisor at the AAI, for her counseling and support during my stay here.

Special thanks to everyone of my family, particularly my father and mother for providing me with their love and support and for teaching me that success comes with hard work and perseverance.

Finally and most importantly, I would like to thank my lovely wife Brigitte and my two sons Miessan-Aka and Kadjo Wandan, Jr. for their love and support

ASSESSMENT

LIST OF TABLES

LIST OF FIGURES

A INTRODUCTION

B OVERVIEW

1. G

2. S

3. M

4. P

5. E

C EXPERIMENTAL

1

2

TABLE OF CONTENTS

PART I

ASSESSMENT OF CONTAMINATION OF SURFACE WATER AND FISH FROM CÔTE D'IVOIRE

	<u>Page</u>
LIST OF TABLES	xi
LIST OF FIGURES	xv
A. INTRODUCTION	1
B. OVERVIEW OF THE COUNTRY	15
1. GENERAL DESCRIPTION	15
2. SURFACE WATER	17
3. ICULTURE	22
4. PEST PROBLEMS	25
a. <u>CASH CROPS</u>	26
b. <u>FOOD CROPS</u>	27
c. <u>MAIN CEREALS</u>	27
d. <u>FUIT AND VEGETABLES</u>	28
e. <u>POSTHARVEST PESTS</u>	28
f. <u>INSECTS VECTOR OF DISEASES</u>	29
5. PEST CONTROL MEASURES AND PRESENT USE OF PESTICIDE	30
a. AGRICULTURE	30
b. HUMAN HEALTH	32
C. EXPERIMENTAL PROCEDURE	34
1. STUDY DESIGN AND SAMPLE COLLECTION	34
a. <u>SAMPLING SITES</u>	34
b. <u>SAMPLE COLLECTION AND PROCESSING</u>	37
2. MATERIALS	37
a. <u>EQUIPMENT</u>	37
b. <u>SOLVENTS, REAGENTS AND SOLUTIONS</u>	39

3 A

D RESULT

1 P

2 H

3 C

4 C

E CONCL

APPENDIX

APPENDIX

LIST OF P

THE EVA

A INTRO

B ENZY

1

2

3

3. ANALYTICAL METHODS	40
a. <u>DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES</u> ..	40
b. <u>HEAVY METALS DETERMINATION IN WATER</u>	40
c. <u>ORGANOCHLORINE PESTICIDES DETERMINATION IN FISH</u>	41
d. <u>CHROMATOGRAPHIC DETERMINATION</u>	42
D. RESULTS AND DISCUSSION	44
1. PHYSICOCHEMICAL PROPERTIES	44
2. HEAVY METALS CONCENTRATION IN WATER SAMPLES	46
3. ORGANOCHLORINE PESTICIDES IN WATER	47
4. ORGANOCHLORINE PESTICIDES IN FISH	51
E. CONCLUSIONS	54
APPENDIX A: LIST OF THE PESTICIDES DISTRIBUTED IN COTE D'IVOIRE	59
APPENDIX B: CHROMATOGRAMS OF THE ANALYSIS	65
LIST OF REFERENCES..	70

PART II

THE EVALUATION OF IMMUNOASSAY FOR DETECTION OF PESTICIDES IN PLANT AND FISH SAMPLES

	<u>Page</u>
A. INTRODUCTION	76
B. ENZYME IMMUNOASSAYS AND ITS APPLICATIONS	80
1. THE IMMUNE RESPONSE	80
2. ANTIBODY STRUCTURE AND FUNCTION	82
3. ANTIGEN STRUCTURE	82

4. PRODUCTION OF ANTIBODIES FOR LABORATORY USE	85
a. MONOCLONAL ANTIBODIES	85
b. ANTIBODY-ANTIGEN INTERACTIONS	87
5. IMMUNOASSAYS	88
a. ENZYME IMMUNOASSAYS	88
b. TYPE OF ENZYME IMMUNOASSAYS	90
6. APPLICATION OF IMMUNOASSAYS	91
a. HEALTH AND AND CLINICAL MEDECINE	91
b. AGRICULTURAL USES	92
c. TOXINS AND CONTAMINANTS SCREENING IN FOOD . . .	93
d. ENVIRONMENTAL ANALYSIS	93
C. CHARACTERISTICS OF THE PESTICIDES	95
1. ALACHLOR	95
a. USES	95
b. BEHAVIOR IN PLANTS	97
c. BEHAVIOR OF ALACHLOR IN SOIL	97
d. BEHAVIOR IN AQUATIC ENVIRONMENT	97
e. TOXICOLOGICAL PROPERTIES	98
2. ATRAZINE	99
a. USES	99
b. BEHAVIOR IN PLANTS	101
c. BEHAVIOR IN SOIL	101
d. BEHAVIOR IN THE AQUATIC ENVIRONMENT	102
e. TOXICOLOGICAL PROPERTIES	103
3. CARBOFURAN	105
a. USES	105
b. BEHAVIOR IN PLANTS	107
c. BEHAVIOR IN SOILS	107
d. BEHAVIOR IN AQUATIC ENVIRONMENT	108
e. TOXICOLOGICAL PROPERTIES	108
D. MATERIALS AND METHODS	112
1. STUDY DESIGN AND SAMPLE COLLECTION	112
a. FISH REARING	112
b. CORN RAISING	113

2 M

3 AN

E RESC

2. MATERIALS	114
a. REAGENTS	114
b. EQUIPMENT	114
a1. ELISA	114
a2. CHROMATOGRAPHY	115
3. ANALYTICAL METHODS	116
a. EXTRACTION OF FISH FOR ELISA DETECTION	116
b. EXTRACTION OF CORN LEAF FOR ELISA DETECTION ..	116
c. EXTRACTION OF CORN LEAF FOR CHROMATOGRAPHIC	
d. ANALYSIS	117
e. EXTRACTION OF FISH FOR CHROMATOGRAPHY	119
f. DETECTION AND QUANTIFICATION	119
f1. <u>ELISA ASSAY PROCEDURES</u>	119
f2. <u>CHROMATOGRAPHIC PROCEDURES</u>	120
f3. <u>CALCULATION OF THE PESTICIDE</u>	
f4. <u>CONCENTRATION</u>	121
f5. <u>CONFIRMATION</u>	122
E. RESULTS AND DISCUSSION	123
1. ALACHLOR	123
a. ACCURACY	125
a1. <u>FISH FILLET</u>	125
a2. <u>CORN LEAF</u>	125
b. REPRODUCIBILITY	128
c. CROSS-REACTIVITY	130
d. SENSITIVITY	132
e. INCURRED SAMPLES	133
e1. <u>CORN LEAF</u>	133
e2. <u>FISH FILET</u>	
2. ATRAZINE	136
a. ACCURACY	137
a1. <u>CORN LEAF</u>	137
a2. <u>FISH FILLET</u>	140
b. REPRODUCIBILITY	141
c. CROSS-REACTIVITY	142
d. SENSITIVITY	145
e. INCURRED SAMPLES	146

3 CARBOR

F CONCL

APPENDIX

APPENDIX

APPENDIX

REFEREN

e1. <u>CORN LEAF</u>	146
e2. <u>FISH SAMPLES</u>	147
3. CARBOFURAN	149
a. ACCURACY	150
a1. <u>FISH FILLET</u>	150
a2. <u>CORN LEAF</u>	153
b. REAPITABILITY	154
c. SENSITIVITY	156
d. CROSS-REACTIVITY	157
e. INCURRED SAMPLES	159
e1. <u>CORN LEAF</u>	159
e2. <u>FISH FILLET</u>	161
F. CONCLUSIONS	163
APPENDIX A: RESULTS OF ALACHLOR DETERMINATION	178
APPENDIX B: RESULTS OF ATRAZINE DETERMINATION	179
APPENDIX C: RESULTS OF CARBOFURAN DETERMINATION	187
REFERENCES CITED	199

Title Title

1 Orga
from
Publ

2 Occ
from

3 May

4 Day

5 Hea
five

8 Me
him

9 Me
nin

A1 Lis
Se
25

A2 L
S
25

A3 L
S
25

A4 L
C

LIST OF TABLES

<u>Table</u>	<u>Title</u>	<u>Page</u>
1.	Organochlorine pesticides commonly encountered in natural waters. Extracted from Environmental Chemistry; 4th ed. Stanley E. Manahan, Lewis Publishers. 1991.	7
2.	Occurrence and significance of trace elements in natural waters. Adapted from Environmental chemistry. Stanley E. Manahand 5th Ed, 1991.	10
3.	Major crops grown in Côte d'Ivoire	23
4.	Physico-chemical characteristics of water samples from the collection sites	45
5.	Heavy metals contaminant levels ($\mu\text{g/L}$) in water samples. Mean from five determinations	46
8.	Mean recovery (%) $\pm$ RSD (n = 3) for the pesticides in fish and detection limits ($\mu\text{g/kg}$).	
9.	Mean (n = 5) pesticide residue levels (mg/kg) in fillet of Tilapia from the nine collection sites	52
A1.	List of the insecticides used in crop protection in Côte d'Ivoire. Source: Rapport annual sur la vente des pesticides pour utilisation agricole. UNIPHYTO (1991).	60
A2.	List of the fungicides used in crop protection in Cote d'Ivoire Source: Rapport annual sur la vente des pesticides pour utilisation agricole. UNIPHYTO (1991).	61
A3.	List of herbicides used in crop protection in Cote d'Ivoire. Source: Rapport annual sur la vente des pesticides pour utilisation agricole, UNIPHYTO (1991)	62
A4.	List of nematicides and miscellaneous pesticides used in crop protection in Côte d'Ivoire. Source: Rapport annual sur la vente des pesticides pour	

Page

1

2

4

6

7

8

9

11

12

14

15

16

17

utilisation agricole, UNIPHYTO (1991).	63
--	----

PART II

<u>Table</u>	<u>Title</u>	<u>Page</u>
1.	Chemical and physical properties of the technical alachlor	96
2.	Chemical and physical properties of the pure atrazine	100
4.	Chromatographic conditions for the determinations of the pesticide	121
6.	Accuracy of alachlor determination in spiked corn leaf (2 replications per assay). A: Ohmicron kit; B: Millipore kit.	127
7.	Assay reproducibility (%CV) for the EIA for alachlor in corn leaf extract	129
8.	Method determination limit (MDL) calculated from standard deviation (σ) of 6 replicate assays of corn leaf (A) and fish tissue (B) spiked with alachlor. . . .	132
9.	Alachlor concentration in incurred fish fillet samples by ELISA. 2 assays per samples.	136
11.	Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination alachlor in spiked fish fillets (2 replications per assay).	140
12.	Assay reproducibility for of the EIA for alachlor in corn leaf extract	141
14.	Alachlor concentration in incurred fish fillet samples by ELISA. 2 assays per samples.	148
15.	Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination carbofuran in spiked fish fillets (2 replications per assay). . .	152
16.	Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination carbofuran in spiked fish fillets (2 replications per assay). . .	154
17.	Assay reproducibility for of the EIA for carbofuran in corn leaf extract . .	155

18 M.
6

19 Ca
pe

A1 St

A2 Re

A3 Re

A4 Se

A5 C

A6 In

A8 In

B1 St

B3 R

B4 R

B5 S

B6 C

B7 In

B8 In

C1 S

C4

C5

18.	Method determination limit (MDL) calculated from standard deviation (σ) of 6 replicates assays of the samples spiked with carbofuran	156
19.	Carbofuran concentration in incurred corn leaf samples by ELISA. 2 assays per samples.	161
A1.	Standard Curve Data	170
A2.	Recovery Study Data for Fish Samples	172
A3.	Recovery Study Data for Corn Leaf data	173
A5.	Sensitivity Study Data	175
A6.	Cross-Reactivity Study Data	176
A7.	Incurred Leaf Study Data	177
A8.	Incurred Fish Study Data	178
B1.	Standard Curve Data	179
B3.	Recovery Study Data for Corn Leaf data	181
B4.	Repeatability Study Data	182
B5.	Sensitivity Study Data	183
B6.	Cross-Reactivity Study Data	184
B7.	Incurred Leaf Study Data	185
B8.	Incurred Fish Study Data	186
C1.	Standard Curve Data	187
C4.	Repeatability Study Data	190
C5.	Sensitivity Study Data	191

C6.	Cross-Reactivity Study Data	192
C7.	Incurred Leaf Study Data	193
C8.	Incurred Fish Study Data	194
D1.	Tank Water Characteristics for Alachlor	196
D2.	Tank Water Characteristics for Atrazine	197
D3.	Tank Water Characteristics for Carbofuran	198

Figure 1

1 P

2 V

3 V

4 S

5 V

B1 C

B2 C

B3 C

B4 C

B5 C

B6 C

B7 C

B8 C

B9 C

B10 C

B11 C

B12 C

B13 C

B14 C

B15 C

B16 C

B17 C

B18 C

B19 C

B20 C

B21 C

B22 C

B23 C

B24 C

B25 C

B26 C

B27 C

B28 C

B29 C

B30 C

B31 C

B32 C

B33 C

B34 C

B35 C

B36 C

B37 C

B38 C

B39 C

B40 C

B41 C

B42 C

B43 C

B44 C

B45 C

B46 C

B47 C

B48 C

B49 C

B50 C

B51 C

B52 C

B53 C

B54 C

B55 C

B56 C

B57 C

B58 C

B59 C

B60 C

B61 C

B62 C

B63 C

B64 C

B65 C

B66 C

B67 C

B68 C

B69 C

B70 C

B71 C

B72 C

B73 C

B74 C

B75 C

B76 C

B77 C

B78 C

B79 C

B80 C

B81 C

B82 C

B83 C

B84 C

B85 C

B86 C

B87 C

B88 C

B89 C

B90 C

B91 C

B92 C

B93 C

B94 C

B95 C

B96 C

B97 C

B98 C

B99 C

B100 C

B101 C

B102 C

B103 C

B104 C

B105 C

B106 C

B107 C

B108 C

B109 C

B110 C

B111 C

B112 C

B113 C

B114 C

B115 C

B116 C

B117 C

B118 C

B119 C

B120 C

B121 C

B122 C

B123 C

B124 C

B125 C

B126 C

B127 C

B128 C

B129 C

LIST OF FIGURES

PART I

<u>Figure</u>	<u>Title</u>	<u>Page</u>
1.	Processes influencing the behavior and fate of xenobiotics in the environment (1973)	3
2.	Map of physical characteristics of Côte d'Ivoire	16
3.	Map of the water systems illustrating the lagoons and rivers in Cote d'Ivoire.19	
4.	Structure of pesticide market in Cote d'Ivoire	32
5.	Map of Côte D'Ivoire illustrating the sample collection sites	36
B1.	Chromatogram of 00.1 ppm of standard solution of organochlorine pesticide mixture	65
B2.	Chromatogram of water sample from	66
B3.	Chromatogram of water sample from	67
B4.	Chromatogram of fish sample from	68
B5.	Chromatogram of fish sample from	69

PART II

<u>Figure</u>	<u>Title</u>	<u>Page</u>
1.	Cellular events leading to antibody production following B-cells activation by antigen molecules.	81
2.	The Structure of an Antibody Molecule. Four protein chains combine to form an antibody molecule.	82

3.	Illustration of the binding of the antibodies to the antigen specific binding sites called epitopes. Each epitope can bind to a different antibody containing a specific antigen-binding site.	84
4.	Illustration of a synthetic antigen indicating the hapten (small organic chemical) covalently attached to immunogenic carrier molecule.	85
5.	Polyclonal antiserum containing a mixture of antibodies produced by multiple B cells.	86
6.	Illustration of Monoclonal Antibodies Production. One B cell is fused in the laboratory with a tumor cell. The resulting hybridoma produces multiple copies of specific type of antibody (monoclonal antibody).	87
7.	Enzyme conjugate. Enzyme are physically linked to antibodies or antigens to form an indicator system. The enzyme, the antibody, and the antigen must retain their activities and binding capacities to be useful.	89
8.	Color reaction catalyzed enzymes used as indicators or tags in immunoassay.	89
9	Plot of the standard curves of the Ohmicron and Millipore test kits (average of 4 determinations). %Bo = % (absorbance of the sample/absorbance of the zero control).	124
10.	Response of the ELISA kits to alachlor and metolachlor. (□) alachlor; (■) metolachlor; (♦) mixture.	131
11.	Alachlor concentration in incurred corn leaf samples by ELISA. 2 assays per samples.	134
12.	Correlation of alachlor concentrations as determined by ELISA and gc methods, n=4, $r = 0.996$, $y = 1.017 X + 0.0005$	136
13.	Confirmation of the ELISA results by GC/MS.	136
14.	Plot of the standard curves of the two test kits (average of 4 determinations). %Bo = % (absorbance of the sample/absorbance of the zero control).	

15.	Response of the ELISA kits to alachlor and metolachlor. (□) alachlor; (■) metolachlor; (♦) mixture.	143
16.	Alachlor concentration in incurred corn leaf samples by ELISA. 2 assays per samples.	146
17.	Correlation of alachlor concentrations as determined by ELISA and gc methods, $n = 4$, $r = 0.996$, $y = 1.017 X + 0.0005$	147
18.	Confirmation of the ELISA results by GC/MS.	149
19.	Plot of the standard curves of the two test kits (average of 4 determinations). %Bo = % (absorbance of the sample/absorbance of the zero control). . . .	150
20.	Response of the ELISA kits to carbofuran; 3-ketocarbofuran and mixture	158
21.	Carbofuran concentration in incurred corn leaf samples by Ohmicron EIA kit. 2 assays per samples.	160
22.	Correlation of carbofuran concentrations as determined by ELISA and gc methods, $n = 4$, $r = 0.996$, $y = 1.017 X + 0.0005$	160
23.	Mass spectra of GC peaks of incurred corn leaf and fish fillet. (A) carbofuran standard solution; (B) non treated corn leaf, (C) treated corn leaf; (D) non-treated fish fillet, (E) treated fish fillet.	163

PART I

**ASSESSMENT OF CONTAMINATION OF SURFACE WATER AND FISH FROM
CÔTE D'IVOIRE**

of the

W. and

year 18

supply

pipel

develop

Sahara

destruct

pests

mechan

in ord

Most

preva

of the

been

CHAPTER I

INTRODUCTION

In 1988, the world population amounted to 5 billion, and it is estimated to reach 6.5 billion in the year 2000. While the increase has stabilized in the developed world (0.6 % annual growth rate), it is increasing in the developing world at the rate of 1.6% per year (IBRD, 1989). As result of this steep increase in population there is insufficient food supply leading to malnourishment and a shorter life span. To feed this growing population, the world food production must increase. This has been the case in the developed world and in some underdeveloped countries. But many of the nations of sub-Saharan Africa still suffer from low productivity in part due to insects and other destructive pests. In all of these countries pesticides use will help prevent loss due to pests. The use of pesticides, together with other means like irrigation, fertilization, and mechanization would help reduce damage to crops and maintain adequate food supplies in order to feed the growing population.

Most of the developing countries are located in areas where endemic diseases are still prevalent. Reports of the World Health Organization (1985, 1987) indicate that a third of the total population is threatened by vector-born diseases. Drugs and vaccinations have been used to reduced the impact of these diseases. Although some success have been

and the

has been

million

in Africa

pesticide

the area

and growth

hypera

environment

The atmosphere

in aqua

during

away from

Côte d'Ivoire

banana

The export

conduct

reduce

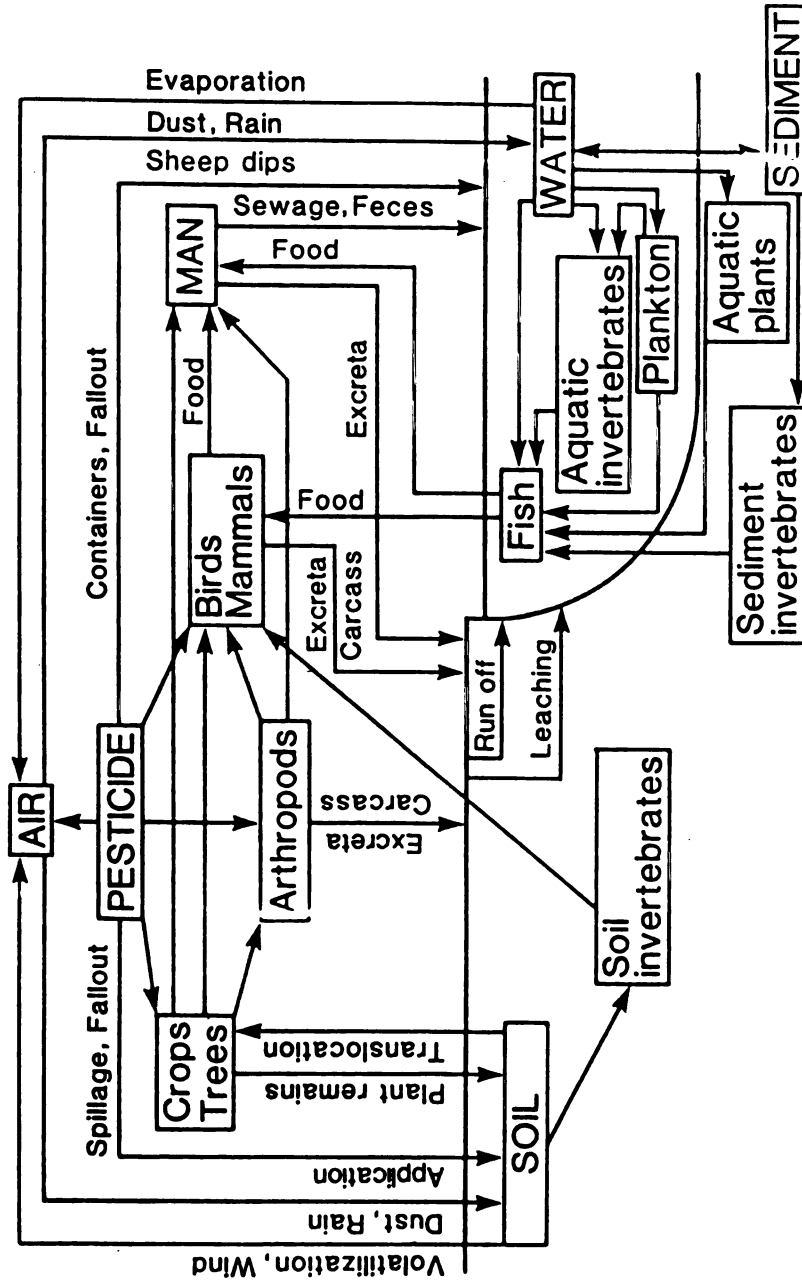
and create

of drinking

achieved by these means, the most efficient way to reduce the impact of these diseases has been the control of the insect vectors by continuous application of insecticides and molluscides.

From the economic standpoint as well as human and animal health, pesticides use in Africa is vital in the production of food and for the protection of man and animal. But pesticides applied to croplands or in localized areas have been shown to move through the environment affecting not only non-target organisms but also contaminate soil, surface and groundwater, and air creating a great concern among general public. It is therefore imperative to ascertain the extent of environmental contamination.

Pesticides applied in the lithosphere for pest control are transported to the aquatic environment through atmospheric transport, soil runoff, erosion, and leaching (Figure 1). The atmosphere is a mobile medium and serve as major transport route to move pesticides to aquatic environments. The atmosphere becomes contaminated with pesticides by drift during application, volatilization, and wind erosion. Drift is the portion that is moved away from the target area by wind. Aerial application contributes to more drift but in Côte d'Ivoire this method is used only in banana production because of the size of the banana plant and for insect vectors control; all others crops receive ground application. The extent of pesticide dispersal in the air due to drift is governed primarily by prevailing conditions, formulation of the pesticides, and the method of application. Drift losses reduce application efficiency, necessitating more frequent applications, increases costs, and create hazards to non-target organisms and the environment. The quantity and extent of drift can be reduced by considering the appropriate spray formulation, the spray



Movement of Pesticide between Environmental Compartments

20

has

sum

more

press

en. n

at m

cont

signif

Losse

such a

petion

Howe

because

Perme

and tra

where

in coa

(Ruse

receiv

equipment used such as ultra-low volume (ULV) and, weather conditions. Volatilization has been recognized as a major pathway for loss of pesticides from soil, plant, and water surfaces (Spencer et al., 1973).

Volatilization from the soil is evident for both surface-applied and soil incorporated pesticides. The vaporization rate of a pesticide is function of its vapor pressure, but once it is in contact with the soil its vapor pressure is modified by environmental variables. Climatic and soil factors regulating volatilization rate include, air movement, temperature, relative humidity, soil moisture content, soil organic matter content, and pesticide concentration in the soil. Field measurements indicate that significant volatilization loss may occur if pesticides are not incorporated in the soil. Losses have been observed for DDT (Spencer and Cliath, 1972) and related compounds such as lindane (Spencer and Cliath, 1970). To prevent excessive loss of more volatile pesticides, it is necessary to incorporate them into the soil immediately after application. However, under normal farming practices, pesticides incorporation is not always possible because many of the insecticides and fungicides are applied on soil and plant surfaces. Pesticides dispersed in the atmosphere may be associated with airborne particulate matter and transported. Wind erosion may provide an important atmospheric source of pesticides whereby they are redispersed on aquatic ecosystems. Data indicates that DDT distribution in coastal and oceanic waters results from fallout of airborne particulate material (Risebrough, 1969). This implies that a vast area of water surface, such as lagoons may receive significant inputs of pesticides from the atmosphere.

Pesticide contamination of groundwater and surface water can occur through

leaching

The leach

in soil co

textured

pesticide

concentra

through

water

organop

Bailey a

basic con

controls

colloids

are often

compou

Studies

solubili

soluble

phenyl

in gene

upper

mover

leaching. This downward movement is controlled by soil, pesticides, and climatic factors. The leachability of a compound depends primarily to the degree to which it is adsorbed to soil colloids. Pesticides are leached more readily in coarse-textured soils than in fine-textured soils as the latter contain more clay and organic matter. The solubility of pesticides plays an important role in their movement in the soil since solubility limits the concentration of the compound in the soil-water phase. The transport of pesticides through the soil is conditioned by the amount, intensity, and frequency of percolating water. Adsorption of non-ionic pesticides, which includes the organochlorine and organophosphate insecticides, is correlated primarily with soil organic matter content (Bailey and White, 1970) and to a lesser extent with clay content. Retention of acidic and basic compounds is affected markedly by soil pH (Donaldson and Foy, 1965). Soil pH controls the overall charge of the molecule and hence its adsorptivity to clay and organic colloids. The organic cations; diquat and paraquat, are held strongly by clay minerals and are often adsorbed irreversibly (Weed and Weber, 1969). Weakly adsorbed water-soluble compounds are desorbed readily by water and hence pose a greater potential for leaching. Studies have shown that the organochlorine (OC) insecticides, which have limited water solubility are the least mobile, followed by the organophosphate insecticides. The water-soluble acidic herbicides are most mobile. Most of the pesticides, such as the triazines, phenylureas and carbamates, have intermediate mobility. Organochlorine insecticides are, in general, non leachable. Field trials have shown that they are retained largely in the upper 15-20 cm layer of most agricultural soils (Cliath and Spencer, 1971.). Any movement to lower depths and subsequently to water tables may be attributed to physical

transport

Wills

the

either in

to erosion

drainage

nature of

of most

insects

and are

Pesticide

steady

reservoir

microbe

the food

result in

represent

and mu

Table

pests

insect

transport of adsorbed compounds through vertical cracks formed during dry periods (Willis and Hamilton, 1973).

Pesticides present on agricultural land may be transported through surface runoff either in solution and/or as adsorbed on soil particles. Since surface soils are susceptible to erosion, pesticides retained in subsurface layer are potentially transportable by surface drainage. The degree of pesticide loss by runoff depends principally on soil properties, nature of the pesticide, and climatic factors (Bailey , 1966). Studies revealed that losses of most OC insecticides relative to the amount applied are low even for surface-applied insecticides. Once OC are present in the soil, they may persist for a long period of time and are capable of being carried from one season to the next (Hindin and Bennett, 1970). Pesticides associated with soil may be subjected to continual runoff, thereby providing a steady, low level residue to aquatic systems. Since aquatic ecosystems may serve as reservoirs or sinks for various chemicals, the indigenous species of animals, plants, and microorganisms immersed in the water medium may incorporated these chemicals into the food chain and pass them along to the highest predators in the food chain which may result in higher concentration. Therefore, aquatic animals consumed as foodstuff may represent a potential source of human exposure to toxic chemicals, including carcinogens and mutagens.

Organochlorine pesticides such as DDT, aldrin, lindane, dieldrin, and heptachlor (Table 1) were introduced in the early 40's and were very successful for the control of pests. They have a long persistence in soils and provide a greater potential than the other insecticides for contaminating aquatic systems. This may result in their deposition in

Table 1. Organochlorine pesticides commonly encountered in natural waters. Extracted from Environmental Chemistry, 4th ed. Stanley E. Manahan, Lewis Publishers. 1991.

<i>Pesticide</i>	<i>Formula</i>	<i>*Fresh-water quality criteria</i>	<i>Uses and characteristics</i>
Aldrin-Dieldrin		0.003 µg/L	Persistent and stable in soil, effective against insect in soil. Organisms convert aldrin to dieldrin, known to be carcinogenic to mice. Banned in the USA for most uses in 1975.
DDT		0.00 µg/L	Low acute toxicity to mammals; persistent; accumulate in food chain. Some evidence of carcinogenicity, use banned in the U.S. in 1972.
Endosulfan		0.003 µg/L	Used in fruit to control aphids, beetles, and caterpillars. Lower chronic toxicity to mammals
Endrin		0.004 µg/L	Special precautions must be used to avoid skin contact during application; readily photolyzes to non-toxic ketone form.
Heptachlor		0.001 µg/L	Used to control pests in soil; insecticide in feed. Change to more the more toxic epoxide which persists for a long time in the soil; use restricted in U.S. in 1978.
Lindane		0.01 µg/L	Used to control insects, plant pests, animal parasites; widely manufactures because of convenience, lack of odor, minimal residue

* Public Health Service Drinking Water Standard, U.S. Public Health Service, 1962

as

co

re

gP

cc

re

in

in

in A

were

Zerg

total

184 n

intend

lake

in be

mg

and

res

aquatic organisms, such as fish resulting in their accumulation in the food chain. These compounds may be also detrimental to fish by interfering with their metabolism and/or reduce egg hatchability. Because of their adverse environmental impacts, the OC have generated considerable public concern and were banned in most industrial world.

OC were introduced in Côte d'Ivoire in the 50's for the control of pests in commercial agriculture (coffee, cocoa, cotton, etc.) and to protect human health against vectors of diseases. Although some of them were commercially banned, many are still in use because of their efficiency (e.g. lindane, endosulfan) or through their illegal entry in the country.

A number of studies have been conducted on the presence of OC in water system in Africa. In Sudan, fish collected from the Gwydir River in the cotton growing area were found to contain endosulfan residues. In the same country, fish from Gezira (El Zorgha.G.A., 1980), Lake Nubia (Novak and N. Ahmad, 1989), were found to contain total residues concentration of endosulfan ranging from 0.27 to 16 mg/kg and from 2 to 184 mg/kg respectively.

Kenya is the only country in Africa where inland waters and fish have been intensively investigated. The first studies done by Koeman et al. (1972) in the Rift Valley lake found very low to undetectable residue of DDE in Tilapia and high levels of DDE in bottom-feeding fish. Lincer et al. (1981) reported extremely low levels (below 0.007 mg/kg) of dieldrin, p,p'-DDT and undetectable to low levels of DDE in fish from lake Nakuru and lake Naivasha respectively. Studies done in the 1980's reported levels of DDT ranging from 0.004 to

110

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

recess

0.367 mg/kg in fish from Lake Victoria (Kenja, 1989; Mitema and Gitau, 1989). More recent studies reported finding DDT in all fish samples. Mugachia et al. (1992) found that 73% of estuarine fish from the Athi River were positive for one or more of the OC. Mitema and Gitau (1990) reported mean DDT levels of 0.45 mg/kg in fresh Nile perch fillet.

In Côte d'Ivoire, only the Ebrié lagoon has been investigated for the presence of organochlorine pesticides (OC). The results indicated levels of DDT and its metabolites ranging from 60-200 mg/kg in the sediment (Marchand and Martin, 1985). In analyzing for residues in fresh water fish from Main lake in Nigeria, Koffi Kobenan (1986) found residues of OC in all the samples at concentration ranging from trace to 0.593 ppm. He also found traces of some organophosphates such as malaoxon (0.220 ppm).

Heavy metal contamination of aquatic environment is a continuing global concern and seems to be more pronounced in countries where environmental regulation and monitoring is not routine practice. The most problematic heavy metals are lead, mercury, arsenic cadmium, tin, chromium, zinc, and copper. These metals are widely used in industry, particularly in metal-working or metal plating-shops and in such products as batteries and electronic (Table 2). Because heavy metals have brilliant color; they are used in paint pigments, glazes, inks, and dyes. They are also used in some pesticides and medicines. Thus heavy metals may enter the environment, wherever any of these products are produced, used, and ultimately discarded. Heavy metals are extremely toxic (Table 2) because, as ions or in certain compounds, they are soluble in water and may be readily absorbed into the body, where they tend to combine with and inhibit the

fact

phys.

water

ppm

contam

para

source

and so

water

water

all

They

invest

much

In we

in N

entire

long

same

river

functioning of particular vital enzymes. Very small amounts can have severe physiological or neurological consequences.

Because of their greater solubility and volatility, the mercury find their way into water. In Sweden, fresh water fish such as perch and pike were found to contain 0.5-3.5 ppm Hg in their axial muscle. In Japan, the consumption of fish and shellfish contaminated with mercury wastes from chloroalkali plants caused an epidemic of paralysis among human populations at Minamata (Goldwater, L.J., 1972).

Information on the presence of heavy metals in African waters is scattered and scarce. Recently several monitoring programs have been initiated at various universities and scientific institutions but most of them deal with marine pollution. Concerning fresh waters, studies done in Northern Africa have been concentrating on Egyptian inland waters and coastal zones, particularly on the River Nile and the delta lagoons. Toga et al. (1981) found high concentrations of heavy metals in the western part of the Nile. They attributed these findings to contaminated drainage waters. Saad et al. (1981c) investigating Lake Mariut, found the levels of metals (Zn, Cu, Fe, Mn, and Cd) to be much higher in fish than in water.

In western Africa, the occurrence of heavy metals in fresh water have been investigated in Nigeria, Ghana, and Côte d'Ivoire. In Nigeria, Okoye et al.(1991) reported heavy metal enrichment of Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, and Zn in the Lagos lagoon. He implicated land base urban and industrial waste as sources of this contamination. In the same country, the studies of pollution in 26 rivers (Ayayi and Osibanjo, 1981), in the rivers in the Niger delta (Kakulu and Osibanjo, 1992), in the cocoa growing area of Ondo

Table 2. Sources and significance of heavy metals in natural waters. Extracted from Environmental Chemistry, 4th ed. Stanley E. Manahan, Lewis Publishers. 1991.

<i>Element</i>	<i>Sources</i>	<i>Effects and Significances</i>	<i>*U.S. Public health Service Limit, mg/L</i>
Arsenic	Mining by-product, pesticides, chemical waste	Toxic, possible carcinogenic	0.05
Cadmium	Industrial discharge, mining waste, metal plating, water pipes	Replaces zinc biochemically, causes high blood pressure and kidney damage, destroy testicular tissue and red blood cells, toxic to aquatic biota	0.01
Copper	Metal plating, industrial and domestic wastes, mining, mineral leaching	Essential trace element, not very toxic to animals, toxic to plants and algae at moderate levels	1.0
Lead	Industry, mining, plumbing, coal, gasoline	Toxicity (anemia, kidney disease, nervous system), wildlife destruction	0.05
Chromium	Metal plating, cooling-tower, water additive (chromate), normally found as Cr(VI) in polluted water	Essential trace element (glucose tolerance factor), possibly carcinogenic as Cr(VI)	0.05
Mercury	Industrial waste, mining, pesticides, coal	Acute and chronic toxicity	Not given
Zinc	Industrial waste, metal plating, plumbing	Essential element in many metallo-enzymes, component of sewage sludge	5.0
Selenium	Natural, geological sources, sulfur, coal	Essential at low levels, toxic at high levels, possible carcinogen	0.01

* Public Health Service Drinking Water Standard, U.S. Public Health Service, 1962

2

3

•

4

3.

22

3.

22.

4.

21

2.

ix

५३

۶۲۰

•

1

(1991b) demonstrated that with the exception of iron, the concentrations of most trace metals in the surface waters are generally lower than global average levels for surface waters and the international drinking water standards. In Ghana, Amasse (1975) found concentrations of As above normal values in the area of Obuasi gold mining. A survey conducted on fish and sediments from the river Wiwi in Kumasi demonstrated higher levels of Cd and Hg in fish compared to sediment. In Côte d'Ivoire, Marchand and Martin (1985), and Kouadio and Tefry (1987) have studied the sediment of the Ebrie lagoon and reported metal concentrations in excess of background levels; they attributed their findings to the disposal of untreated sewage and industrial effluents. A comparative study by Metongo (1991) of Cd, Cu, Hg, and Zn in samples of oysters (*Crassostrea gasar*) from urban and rural lagoons of Côte d'Ivoire revealed higher but background levels of the metals in the urban area.

In Eastern Africa, most of the studies of heavy metals contamination of fresh waters have been done in Kenya. In 1972, Koeman et al, concluded that metals (As, Cu, Zn, Cd, and Hg) concentrations did not constitute a hazard to the biota of Lake Nakuru. Six years later, Greichus, found slightly elevated levels of the same metals in the same lake. In lake Victoria, earlier studies (Ochieng, 1987) indicated no significant heavy metal pollution. However, more recent studies in the same area revealed increased lead levels largely due to increased shipping traffic and associated problems from car washing, and discharge from local industries (Onyari and Wandiga, 1989).

In southern Africa, higher levels of heavy metals were found in birds compared to fish and sediment in Hartbeespoort dam (South Africa) (Greichus, 1977). Greichus et

al (1978b) investigating metals in lake Mclwaine (Harare, Zimbabwe) and found intermediate levels of heavy metals than those found in South Africa.

Surface water in Côte d'Ivoire is composed of rivers and lagoons. Pesticides contamination of surface water may come from agricultural, industrial activities. Agrochemicals (fertilizers and pesticides) sprayed on farmland or in the cities for insect vectors control may be transported into the rivers and lagoons. Industrial activities generate wastewater that is discharged untreated or partially treated into the waters. Thus, chemical plants particularly those formulating pesticides may discharge pesticides into the aquatic ecosystem. Finally fishermen have been found to use pesticides to kill fish resulting in possible contamination of surface water.

Heavy metals contamination of surface waters in Côte d'Ivoire many come, as illustrated in Table 2, from chemical and metallurgical plants and a number of small factories (food , mechanic, tanneries, building, woodpulp, plastic, etc.) that have been built in cities. Many of these cities are located along side rivers and lagoons and waste waters from these factories and from the cities which may contain heavy metals are dumped without any treatment, into the lagoons or rejected into the rivers which flow into the lagoons. Heavy metals may also originate from agricultural activities; mainly from pesticides. The mercurials used as foliar fungicides or seed protectant (ethylmercuric chloride) can be source of general contamination. Until the 70's the timber industry in Côte d'Ivoire was the third exportation product after coffee and cocoa. Thus organomercuric compounds may have been used in the country as fungicides in pulp industries and as slimicide or mold retardant in paper industry. Finally transportation and

tourism may also generate heavy metals in the water systems.

Apart from the few studies reported above, no comprehensive study of the contamination of the aquatic system by pesticides and heavy metals have been conducted in Côte d'Ivoire. Moreover, rivers and lagoons represent an important source of protein in form of fish, water supplies for both human needs and agricultural activities, and waterway for transportation. Therefore to protect global health and productivity of the population, it is important that this fragile ecosystem be analyzed for the presence of pollutants.

We propose in the present study, to assess the contamination of surface water and fish from Côte d'Ivoire. The first objective was to identify and quantify the levels of organochlorine pesticide residues in surface water and fish samples from Côte d'Ivoire and to assess the extend of environmental contamination by these pesticides and also to discuss the toxicological significance of the findings to the health of people. The second objective was to assess the physico-chemical characteristics of the surface water as well as their contamination by heavy metals.

GENE

area of

referred

has the

average

mosaic

season

are of

2.4%

to 3%

season

pop-

199

to 1%

CHAPTER II

OVERVIEW OF THE COUNTRY

GENERAL DESCRIPTION

Côte d'Ivoire is located on the south side of the West African bulge. It covers an area of 322,462 km² (124,503 sq miles) (Figure 2). Except the west where the altitude reaches above 1,300 m, the land is almost flat and does not exceed 800 m. The country has three main types of vegetation; in the south reigns the tropical closed forest (humid evergreen and semideciduous forest), and then there is a transition zone (forest-savannah mosaic) in the center. The north is covered with vast woodlands or savannah.

The climate in the southern forest is tropical with two rainy seasons. The dry seasons are from December to April and from August to September. The rainy seasons are from May to July and from October to November. the rainfall may reach 1,250 to 2,400 mm annually. The temperatures are generally constant throughout the year (22 °C to 33 °C) with high humidity. Toward the north, the rainfall diminishes to one rainy season (May to October) and one dry season (November-April).

In 1960, the population was about 3.8 million. The 1975 census recorded a population of 6.67 million. The population was estimated to be 13.03 millions as of mid 1991, more than tripling in three decades. The high population growth rate is attributed to immigration, high fertility rate (7.4 births per woman), and improvements in healthcare.

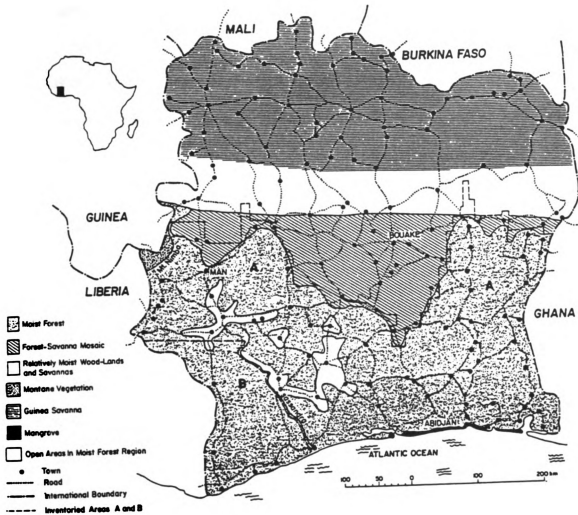


Figure 2. Map of physical characteristics of Côte d'Ivoire

Life e

Intelli

the at

1987

about

there

back

SURE

equal

between

kind

mm

also

by a

exc

wa

ac

and

Life expectancy at birth, rose from 44 years in 1965 to 52 years in 1987 (Economic Intelligent Unit, 1991).

An increased proportion of the population live in urban areas, for example, in 1960 the urban population as a proportion of the total population was estimated to be about 19.3 percent. It has increased to 32.2 percent by 1975, and by 1990 it was estimated to about 46.6 percent. Between 1960 and 1990, the urban population grew at an annual average of 7.2 percent, whereas the rural population grew at only 2.7 percent (FAO year book, 1993).

SURFACE WATER

The lagoon systems are composed of three main lagoons which borders the eastern equatorial Atlantic Ocean. They are situated along the north coast of the gulf of Guinea, between 2°50' W and 5°25' W (Figure 3). The lagoons cover a total surface area of 1200 km² with a climate similar to the equatorial climate; the annual rainfall is about 2000 mm. These lagoons, initially separated, were connected by the construction of canals that allow transportation across all of them. Each of the three main lagoons has a different hydrological system which is influenced by continental fresh water from rivers and an exchange with the marine environment. The lagoons are the reservoirs of the in-flow water from the rivers; they are also subjected to intense transportation as well as tourism activities. Fishing is the most important activity for people living around these lagoons and its provides protein to these people as well as those living in cities. Among the

likely

types of

and

may be

catch

should

product

of the

with

in the

consider

Ivory

has a

Skull

km

the

man

see

will

ichthyologic fauna, there are many types of biological cycles depending on the various types of salinity tolerances and on the conditions of the reproduction cycle.

The lagoon stocks are exploited by means of various types of artisanal fishing gear (individual or collective). The estimation of catches for 1977 was about 7000 tons and may have triple since the introduction of commercial fishing. Seventy percent of the total catch is made by purse seines and beach seines. For the three lagoons, the production should probably be 15-20000 metric tons.

The Aby lagoon located in the east of the coast is surrounded by some of the most productive farmland of the country; coffee, cocoa, palm trees and coconut trees are some of the crops grown in this area. It covers a surface area of 424 km² and has an exchange with the atlantic ocean by a natural opening. One big city, Adiaké and many villages inhabited by farmers and fishermen are located alongside this lagoon. The lagoon is crossed by many boats for public as well as tourists transportation.

The Ebrié lagoon system is the largest of the three main lagoons that form the Ivorian lagoonal system. This lagoon, located in the east-central part of the coastal zone, has a length of 120 km, is 5 to 10 km wide and covers an area of 550 km² (Dura and Skubish, 1982; Dufour, 1982). It is composed of the main lagoon "Ebrié lagoon", 523 km² and the lagoons of Aghien and Potou, 43 km². Many creeks and rivers empty into the lagoon. The Ebrié Lagoon exchanges water with the Atlantic Ocean via the man-made Vridi canal (300 m wide and 20 m deep) dug in 1950 for the construction of the sea port of Abidjan. Five cities: Abidjan, Dabou, Bingerville and Bassam and many villages where farmers and fisherman live are located around the lagoon.

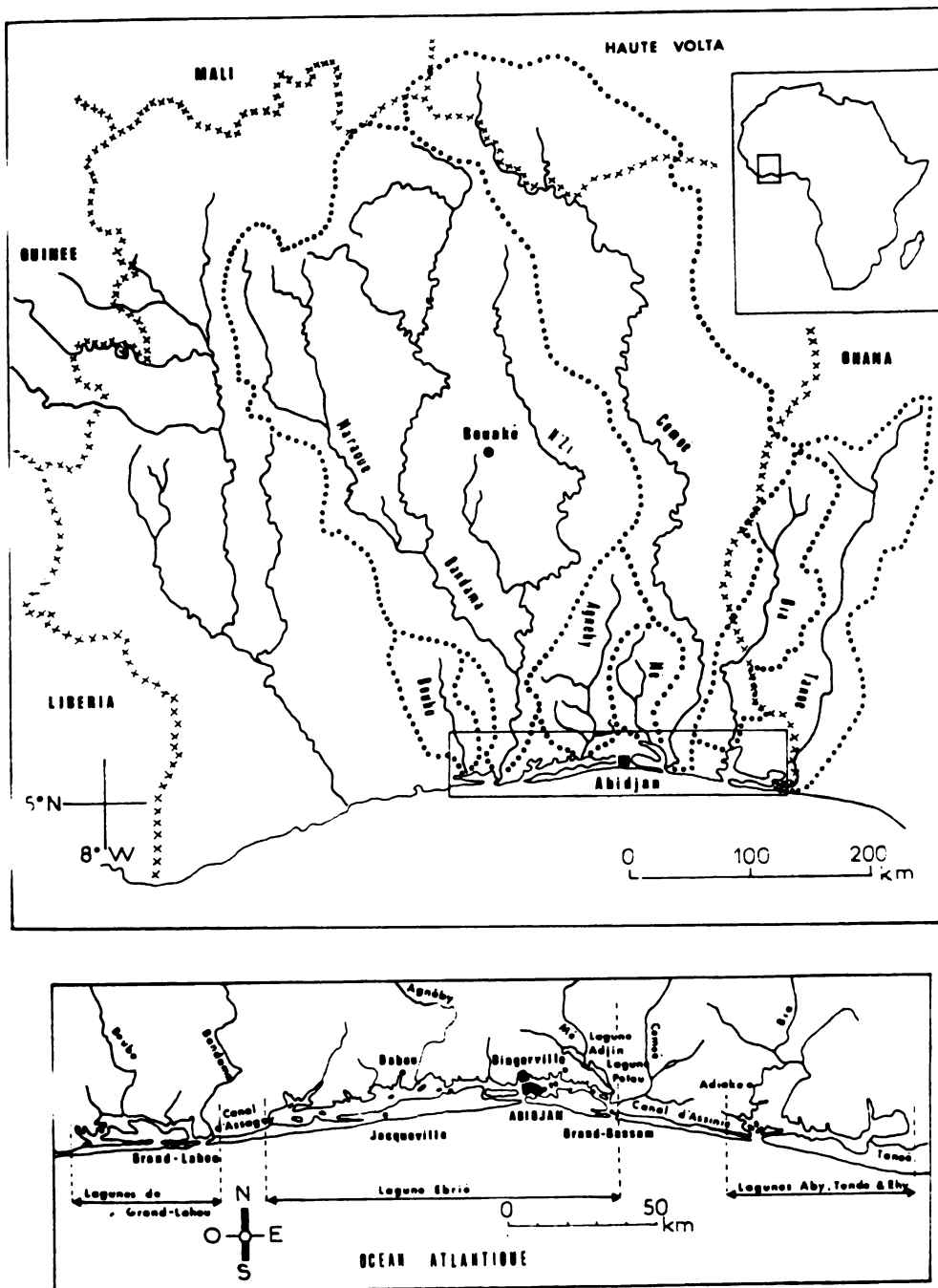


Figure 3. Map of the water systems illustrating the lagoons and rivers in Cote d'Ivoire.

Dea 10

Mar 1

St. 12

Ar. 10

Gr. 10

Zone

25 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

10 10

Due to the presence of the international airport and the sea port, Abidjan contains more than 75% of the industrial and commercial activities (Dufour, 1982). Besides that, it shelters about 3 millions inhabitants. Agricultural activity is also important in the land around the lagoon; rubber trees, pineapple, vegetable, and floriculture are among the crops grown in this area. The Grand Lahou lagoon is located in the midwest of the coastal zone. It is the smallest and shallowest of the lagoons. The city of Grand Lahou as well as small villages inhabited by fishermen are located alongside this lagoon. Palm oil and rubber trees are among the crops grown in this area.

There is a pressure on the lagoons resulting from the increased demography of the cities around them. For example, since 1960, the city of Abidjan located on the Ebrié lagoon has had an annual population increase of 11%. Its population was 1,625,000 in 1980 and was estimated to have reached 3,000,000 by 1995. The development of cities has resulted in the rejection of wastewater and untreated septic tank contents into the lagoons. Second, the creation of industries, in many of the cities has resulted in the generation of industrial wastewater that is dumped into the lagoons. Transportation is also a source of pollution. Washoff of soil and floods of rivers during the rainy season in May-June carries pesticides and fertilizers sprayed on farmland around the lagoons or pesticide sprayed for the control of vectors of diseases. Although officially banned, certain chemicals such as pesticides are used as ichthyotoxic for fishing purposes.

The river system is composed of three large rivers and 6 small rivers (Figure 3). Among these rivers, seven outflow in the lagoons. The Bandaman and the Boubo outflow in the Grand Lahou lagoon, the Rivers Agneby, Mé and Comoé into the Ebrié lagoon and

i

4

5

c.

22

51

13

1

2

1

1

finely the Rivers Bia and Tanoé into the Aby lagoon. Besides the Bandaman River and Comoé that flow from the north of the country to the south, the others rivers are mostly located in the southern part of the country in the forest land.

The river Comoé, the second largest rivers, flows from the savannah of the neighboring country (Burkina Fasso), crosses through the northeast and east of the country and outflows into the Ebrié lagoon in the south. In the northeast the River Comoé crosses the cotton land around Bouna and Bondoukou. In the east it cross the Indenié where coffee and cocoa are grown. In the south before the River Comoé reaches the Ebrié lagoon, it crosses pineapple, rubber trees and banana plantations.

The Bandaman River is the largest and longest river in the country. It flows from the north of the country, goes through the center of the country in the transition zone between forest and savannah, and reaches the Ebrié lagoon in the south. Different parts of the river have been enlarged for the construction of electrical as well as irrigation dams. The bed of the river is surrounded by the cotton land in the north and center. In the south the river cross rubber tree, palm tree, cocoa and coffee farms before reaching the Grand Lahou lagoon.

The River Agneby is a small river flowing in the coastal zone. It is surrounded by banana, pineapple, palm tree and rubber tree farms. It reaches the Ebrié lagoon around the city of Dabou.

The river Bia is a little longer than the Agneby River and flows along the eastern part of the country. Two hydroelectrical dams have been constructed on this river in the area of the city of Ayamé. This river crosses farm land composed of cocoa, coffee,

banana, pineapple, palm and coconut trees before reaching the Aby lagoon.

Agrochemicals (fertilizers and pesticides) used in agriculture may be carried by erosion and reach many of these rivers. Besides, many cities are located alongside these rivers and their waste may be discharged into these rivers. Finally, within the lagoons pesticides may be illegally used for killing fish in these rivers. For all these reasons, the investigation of these chemicals in surface water and fish is necessary to evaluate the health threat to consumers as well as to ascertain the pollution status of these ecosystems.

AGRICULTURE

Agriculture is the keystone of the Côte d'Ivoire economy, with a consistent annual growth of nearly 7% from 1960 to 1980. It contributes about 33 percent of the gross domestic product (GDP), provides between 50 to 75 percent of the nation's total export earnings, and employs an estimated 79 percent of the labor force (Simeon K. Ehui).

In 1990, there were 1,240,000 ha of cropland; 13,000,000 ha of permanent pasture; 7,630,000 ha of forest and woodland; and 7,510,000 ha of other land including urban and built-up areas (Agricultural Production Yearbook, 1991).

A wide variety of crops are grown in the country (Table 3) including small grains, tubers and roots, cash crops, vegetables and fruits. Estimates indicate that crop production apart from rice and wheat is sufficient to supply the food requirement of the population. Although there are no statistics, one can assume that over 45% of cultivated cropland is devoted to cash crops, 20% to small grain, 25% to tubers and roots, and the

remaining to others.

Table 3. Major crops grown in Côte d'Ivoire

Source: Sustainable Agriculture and the Environment in the Humid Tropics (NRC, 1993)

Table 1. Major crops grown in Côte d'Ivoire

Principal crops	1988	1989	1990
Maize.....	460	480	484
Millet.....	43	45	44
Sorghum.....	24	25	24
Rice (paddy).....	610	635	687
Potatoes*.....	24	24	24
Sweet potatoes*.....	12	18	18
Cassava (manioc).....	1,400	1460	1,393
Yams.....	2,500	2,600	2,528
Taro (Coco yam).....	290	302	282
Pulses*.....	8	8	8
Tree nuts*.....	11	11	11
Sugar cane*.....	1,500	1,500	1,550
Palm kernels.....	35.8	20.2	36.8
Groundnuts (in shell).....	121	126	134
Cottonseed.....	136	148	134
Coconuts*.....	470	470	470
Copra*.....	75	75	75
Tomatoes.....	20	21	22
Aubergines (Eggplants).....	25	27	30
Chilies, peppers*.....	23	23	23
Other vegetables*.....	329	368	372
Oranges*.....	28	28	28
Other citrus fruit*.....	30	30	30
Bananas.....	133	133	97
Plantains.....	1,100	1,145	1,087
Mangoes*.....	14	14	14
Pineapples.....	196	209	136
Other fruit*.....	12	12	13
Coffee (green).....	187	239	219
Cocoa beans.....	849	725	700
Tobacco (leaves)*.....	2	2	2
Cotton lint).....	114	128	108
Natural rubber (dry weight)....	61	60	74

* FAO estimate(s).

Source: FAO, Production Yearbook (1991).

Cas

for

ICo

Tr

wh

com

St

exp

pre

Se

val

Cas

com

sw

com

in ca

veg

sug

pre

Cash crops consist of cultivated plants that are usually grown in monocropping plantations for export or for use in local manufacturing industries. The major cash crops are coffee (*Coffea arabica*), of which the country is the world's third largest producer; cocoa (*Theobroma cacao*), of which the country is the world largest producer; and cotton for which the country is becoming the second largest producer in Africa. Together these commodities account for more than 60 percent of the area under cultivation. Since the 80's, the country has diversified its agriculture and today agricultural commodities have expanded to banana (*Musa* Sp.), palm tree (*Elaeis guineensis*), coconut (*Cocos nucifera*), pineapple (*Ananas chemises*), rubber tree (*Hevea brasiliensis*), and sugarcane (*Saccharum* Sp.).

Food crops are divided in two categories: (1) roots and tubers represent 76% in value and 60% of the bulk of staple food output (4.5 million tons/year); and (2) cereals. Cassava (*Manihot esculenta* Crantz), yams (*Dioscorea* spp.), cocoyam (*Xanthosoma sagittifolium* (L.) Schott) are the main root and tubers consumed by the population. The swollen tubers (storage roots in the case of cassava) and the leaves (except yams) are commonly consumed in a wide variety of fresh and processed forms. The tubers are rich in carbohydrates, while the leaves contain proteins, vitamins, and minerals.

Farming activities are distributed all over the country according to the climate and vegetation. Most of the cash crops are located in the south of the country, only cotton, sugarcane and tobacco are grown in the north. With food crops, small grains are predominantly grown in the north while roots and tubers are grown in the south

Traditionally, land cultivation was done by shifting (slash-and-burn). The creation

of

the

at

St

at

the

St

at

the

the

to

St

the

the

the

the

the

the

of a farm was done by cutting and burning the forest or woodland (slash and burn). The cleared area was cultivated for a few years (1 to 2 years). After that the land was abandoned and allowed to return to forest or bush (fallow) for a period of 4 to 20 years. Soils in the tropics have low nutrient content; thus clear and burn techniques make available to the soil the nutrients in living plants in form of nutrient-rich ash fertilizer.

From biological point of view, annual food crops such as rice, maize, cassava, and yams demand substantial quantities of nutrients for satisfactory yields, but many of the soils in the tropics are dystrophic. Improved adapted varieties and cultural practices that include minimum amounts of agricultural inputs (mainly fertilizers and herbicides) are needed to improve agronomic sustainability.

PEST PROBLEMS

As in most of tropical Africa, climatic conditions in Côte d'Ivoire are conducive to the rapid multiplication of insects, many of them are vectors of diseases. For example, 407 insects species of major importance and 778 species of minor importance are listed in Africa (Hill, 1975). Besides climatic conditions, recent changes in farming have increased pest pressure. For example, enlargement and aggregation of fields in case of cash crops has favored the rapid spread of pests and hampered natural enemies of these pests. Genotype uniformity has created extreme vulnerability to pests. Specialization in case of corporate or state own plantations has helped increase pest pressure. Finally, free international exchange and transboundry transfer of infected or infested plant

ma

CA

He

the

for

on

ca

to

see

and

the

the

the

per

imp

note

materials has greatly contributed to pest invasion.

CASH CROPS

Cotton is attacked by many pests but the most important are the bollworms (*Heliothis armigera*, *Pectinophera Gossypiella* (Saund.), and *Earias insulana* (Boisd.)); thrips (*Thrips tabaci*); aphids (*Aphis gossypii* Glov.); cotton leaf worm (*Spodoptera littoralis* (Boisd.)); and jassids (*Empoasca lybica* (De Berg)).

Coffee is also attacked by many pests; among them, the antestia bug (*Antestiopsis orbitalis* (Westw.)), leaf miners (*Leucoptera meyricki* Ghesq.), mealybugs, looper caterpillars (*Epicampoptera strandi glauca* Hmps. and *E. ivoirensis*), stem borer (*Xyleborus morstatti* Hagdn.), berry borer (*Hypothenemus hampei* (Ferr.)), and the green scale (*Coccus viridis* (Green)).

The major pest problems of cocoa are the mirids, *Sahlbergella singularis* Hagland and *Distantiella theobroma* (Dist.). Mirid feeding lesions on cocoa stems are invaded by the weakly pathogenic fungus *Calonectria rigidiuscula*, resulting in extensive die-back of branches and canopy degeneration. *Earias biplaga* Wlk. attacks the apical buds of young cocoa plants and adversely affects establishment. Caterpillars of *Earias* also eat the pericarp of green cocoa pods, *Bothcoelia thalassina* H.&S. The shield bug, is also important on cocoa. Mealybugs, especially *Planococcoides njalensis* (Laing), are notorious in spreading the cocoa swollen shot virus.

FOO

1914

ma

date

800-

ca

Pr

are n

MA

1800

est

inc

int

na

Op

fee

Es

Ad

FOOD CROPS

It is estimated that more than 30% of cassava is lost annually (Herren and Bennett, 1984). This loss is caused by the combined actions of cassava green mite and the cassava mealybug, *phenacoccus manihoti*. The variegated grasshopper, *Zonocerus variegatus* ^I. defoliates the cassava plant, strips the bark, and sometimes eats the stems almost to ground level. The whitefly, *Bemissia tabaci* (Genn.) is responsible for transmitting the cassava mosaic virus disease (CMVD), which causes malformations in cassava leaves. Preharvest damage to yam by insects pests (*heteroligus* spp.), nematodes, and pathogens are responsible for 15-20% crop loss .

MAIN CEREALS

Cereals comprise paddy rice (*Oryza* spp.), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), and millet (*Pennisetum glaucum* L.). The production of cereals is estimated at 1 million tons per year. The increase of these food crops is bellow the increase in population rate resulting in the importation of rice and bread. In 1983, imports of rice and wheat amounted to 590,000 tons representing nearly 40% of the national cereal consumption.

The most important pests are Lepidopterous borers: *Eldana saccharina* Wilk., dipterous: *Diopsis thoracica* Westw. Generally, stem borers cause damage to cereals by feeding on the leaves and in the leaf whorls and boring into the stems and fruit heads. Estimates of grain yield losses caused by stem borers damage in Africa vary considerably. About 14% of the rice cultivated in Africa is lost to insect pests. In Côte d'Ivoire. it is

25.

26.

27.

28.

29.

30.

31.

32.

33.

34.

35.

36.

37.

38.

39.

40.

41.

42.

43.

44.

45.

46.

47.

48.

estimated that insect pests damage to rice results in loss of up to 1 ton of paddy/ha.

FRUIT AND VEGETABLES

Fruits and vegetables are important components of farming in Africa. They provide essential vitamins and minerals in the diet. Many subsistence farmers intercrop fruit and leafy vegetables with roots and tubers. The principal fruits grown in Côte d'Ivoire are citrus, papaya, guava, mango, banana, pineapple, cashew, passion fruit. Among the fruits, pineapple, banana, citrus represent cash crops. Vegetables include tomato, onions, okra, cabbage, cucurbits, chili, eggplant, and a wide variety of leafy vegetables.

Citrus are attacked by a variety of red scale that attack the young citrus seedling and affect establishment. Citrus are also attacked by fruit flies that pierce the citrus fruit and can cause severe crop loss. The most important damage on mango is done by mealybugs. Bananas (fruit and plantain) are attacked by the weevil *Cosmopolites sordidus* (Germ.) and pineapple suffer the pineapple mealybug, *Dysmicoccus brevipes* (Ckll.).

In general, vegetable crops are attacked by a wide range of insects pests including *heliiothis armigera* (Hb.) on cucurbits, *Bemissia tabaci*, *Heliothis* Sp. and *Agrotis* sp. on tomato and cabbage, *Thrips tabaci* Lind, on onions, *Dysdercus* spp. (F), and the leaf roller *Sylepta derogata* (F.) on okra.

POSTHARVEST PESTS

Stored grain and other food items are also attacked and damaged by a large number of insect, causing serious losses at a time when the production system cannot compensate for such losses. In Africa, where farm storage systems at the subsistence farmer level are poor, average losses of stored grain have been estimated to exceed 30% (Ezueh, 1983) and may be estimated at millions of dollars annually.

The major storage pests of grain include *Sitophilus zeamais* Motschulsky, *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *Sitroga cerealella* (Olivier), *Trogoderma granarium* Everts., *Callosobruchus maculatue* (F.), and the larger borer, *Prostephanus truncatus* (Horn). Some of these pests actually infest the crops in the field and are subsequently carried into storage, where they develop under favorable conditions.

The economic impact of these storage losses extends well beyond reduction in grain weights. Grain damaged by storage pests is very much reduced in market value and consumer acceptance, especially in urban communities. Grain lose their viability, resulting in low germination potential and thus reducing the availability of planting materials for subsequent crop production.

INSECTS VECTOR OF DISEASES

To the above losses should be added the economic damage caused by insects which act as vectors of debilitating diseases of man and animals. Some representative of man vector-born diseases in Côte d'Ivoire are malaria (*Anopheles* spp), filariasis (*Culex* spp, *Mansonia* spp, *Anopheles* spp), onchocerciasis (*Simulium* spp), and Schistosomiasis

187

188

189

PE

A

20

21

F

22

23

24

25

26

27

28

29

30

31

32

33

34

(shellfish). Southwood (1977), pointed out that about one in six of mankind is suffering from insect-borne diseases. The full costs borne by individuals and families are largely unknown and the cost in term of loss in productivity from these disease are enormous.

PEST CONTROL MEASURES AND PRESENT USE OF PESTICIDE

AGRICULTURE

Traditionally, farmers relied on their own knowledge and understanding of the ecosystem and made decisions relating to farm practice independently of government control. They relied on a variety of management practices to deal with pest problems. Farmers used two main strategies for the control of pests. The first consists of direct, non-chemical methods (i.e., cultural, mechanical, physical and biological practices). The second one consists of a built-in pest control mechanisms inherent to the biotic and structural diversity of complex farming system. The farmers also use a variety of other management practices that, although targeted for other farm purposes, significantly impact pest dynamics.

With the introduction of modern agriculture, the accent has been placed on integrated pest control when possible. The drawback is the fact that integrated schemes of pest control are most easily implemented on large plantation like corporate owned or state owned plantations of rubber trees, pineapple, banana, palm and coconut trees. Because of traditional and hereditary land fragmentation, individual farmer holdings tend to be small (often only around 2 ha). Thus, they may be less easily amenable to the

integrated approach. The best avenue to this problem has been via farmer cooperatives.

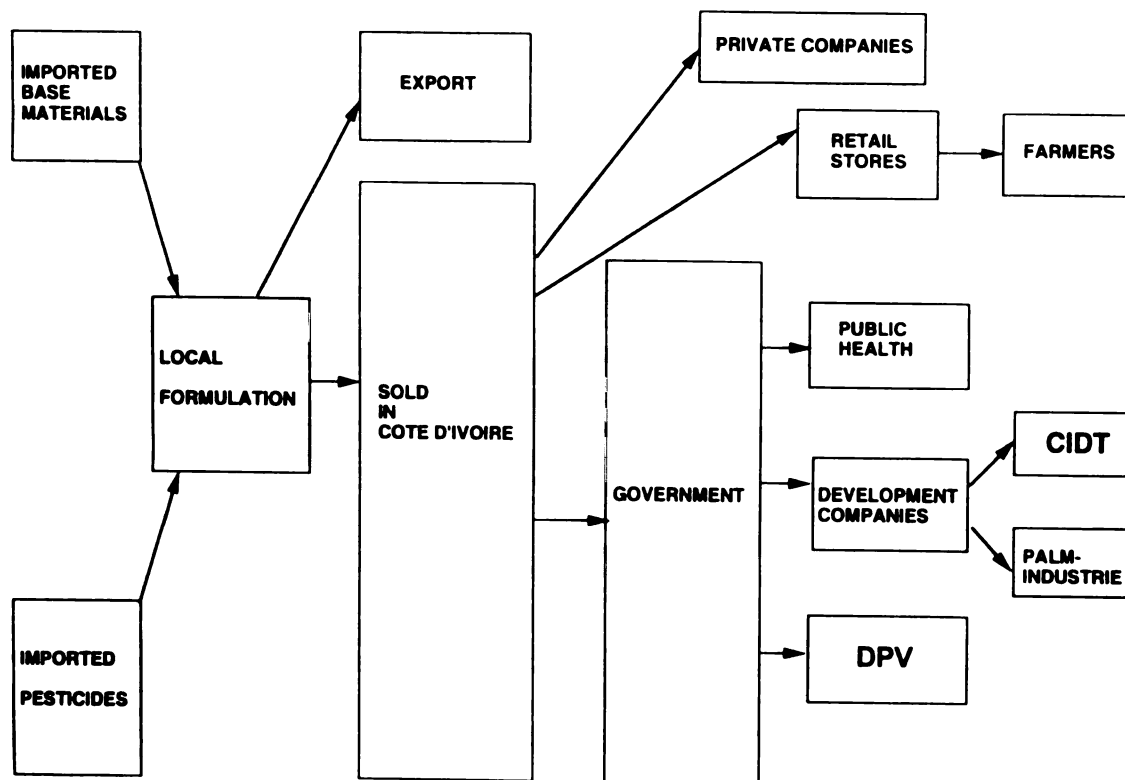


Figure 4. Structure of pesticide market in Cote d'Ivoire

Besides the fact that farmers are able to purchase inputs and application equipments together, it is also easy to allocate them an extension agent.

Over the past few decades, the use of pesticides has shown an upward trend which is a logical consequence of the changes in land use and agricultural practice in general. A number of pesticides are used in Côte d'Ivoire, including insecticides, fungicides and herbicides as listed in appendix 1. Pesticides used in the country are in part imported already formulated or formulated by local companies from imported base materials. The structure of pesticide market is shown in figure 2. Pesticides are either supplied to the farmers through state own companies or are purchased directly from retail stores by farmers. Farmers producing cotton or palm/coconut oil producers are supplied through two developmental companies, CIDE (cotton) and PALMINDUSTRIE (palm and coconut oil) which assist farmers through extension service and also by buying their products. The state is reimbursed for the supply of the equipment and materials, including seed, fertilizers and pesticides after the sale of their production.

Insecticides account for most of the pesticides applied to the cultures (Figure 3), they represent 60% of the total pesticides; followed by herbicides (27%) (Anonymous, 1995). Herbicide use is increasing because of the departure of young people to the cities and decreasing foreign labor. Among crops, cash crops receive the major treatment of pesticides, with cotton receiving 46%, followed by cocoa (15%) and banana (14%). Recently, pesticide use in food crops and vegetables has increased.

H

E

P

V

S

C

M

A

F

E

T

G

M

E

V

M

T

F

HUMAN HEALTH PROTECTION

In many of the developing countries, the amount of pesticides used in public health programs may currently exceed the amount used for the control of agricultural pests and diseases. In Côte d'Ivoire one may think of the control of mosquitoes (Malaria, yellow fever, Simulium larvae (Onchocerciasis), tsetse flies (Trypanosomiasis), and snails (Schistosomiasis). Apart from the simulium larvae control program, accurate quantitative data concerning the type of pesticide used and the amount have not yet been summarized in the literature. Onchocerciasis is endemic to the savannah area of the Volta River basin in west Africa covering part of the center-north of Côte d'Ivoire. In 1974, a control program was initiated in the region by WHO. The objective of the program was to eliminate onchocerciasis as a disease of public health and socioeconomic importance throughout the area covered by the program and to ensure that there is no outbreak of the disease in the future. Abate, temephos, and chlorphoxim were the first insecticides used in the program. Bt (H-14), permethrin, and carbosulfan were later added. From an environmental point of view it is important to stress the fact that chemical control of these vectors takes place in more or less natural habitats. Thus, non-target organisms are more liable to get exposed to the pesticides than is the case in various agricultural applications. The same applies to other uses such as in forestry and livestock protection. The use of pesticides in forestry is not widely practiced in the country; however this may change with the recent intensive forest management program financed by the World bank.

STC

SAY

for

lag

1

2

CHAPTER IV

EXPERIMENTAL PROCEDURE

STUDY DESIGN AND SAMPLE COLLECTION

SAMPLING SITES

Nine collection sites located in areas of intense agricultural practice were selected for the study; three sites were selected on lagoons and 7 on rivers (Figure 5). The lagoons samples were:

1. The lagoon Aby located in the southeast of the country. This lagoon is surrounded by one of the most productive farmland of the country; coffee, cocoa palm tree and coconut tree are some of the crops grown in this area.
2. The lagoon Ebrié border the capital city, Abidjan. Due to the presence of the international airport and the port, most of the industrial and commercial activities are located in this area. The agricultural activity is also important; Hevea, pineapple, vegetable, and floriculture are among the crops grown in this area.
3. The lagoon of Azagny is in the south midwest of the country. Palm oil, rubber tree are among the crops grown in this area.

All these lagoons are the reservoir of the in-flow water from the rivers. They are also subjected to intense transportation as well as tourism activities. Fishing is the most important activity for people living around these lagoons which provides protein to these

2007

Seve

one

At...

1

2

3

27

people as well as those living in cities.

Seven collection sites were selected on rivers. Two were located on the River Comoé, one in the south near the city of Moossou and the second in the east in the village of Aniassué.

1. The river Comoé flows from the savannah of the north of the country (cotton) through the east of the country (coffee, cocoa) to the lagoon Ebrié in the south (pineapple, rubber tree, banana).
2. The river Agneby flows in the central part of the country from the center to the south. It is surrounded by banana, pineapple, palm oil production. The sampling site was located near the city of Dabou where the river reaches the Ebrié lagoon.
3. The river Bandaman flows from the north of the country through the center to the south into the lagoon of Dabou. Two sampling were chosen on this river; the first site was located at five kilometers from the city of Ferkéssédougou and the second in the Lake Kossou. In the north, the Bandaman river is surrounded by the cotton land. In the center, the river is enlarged (Lake Kossou) for the building of the third electrical dam of the country.

SAMPLE COECTION AND PROCESSING

The samples for analysis were collected from October to November 1994. Water samples. were collected midstream at depths of 15-20 cm by dipping the glass containers into the river or lagoon from a row boat. Five samples were collected from each sampling site for the measurement of the physicochemical characteristics (temperature,

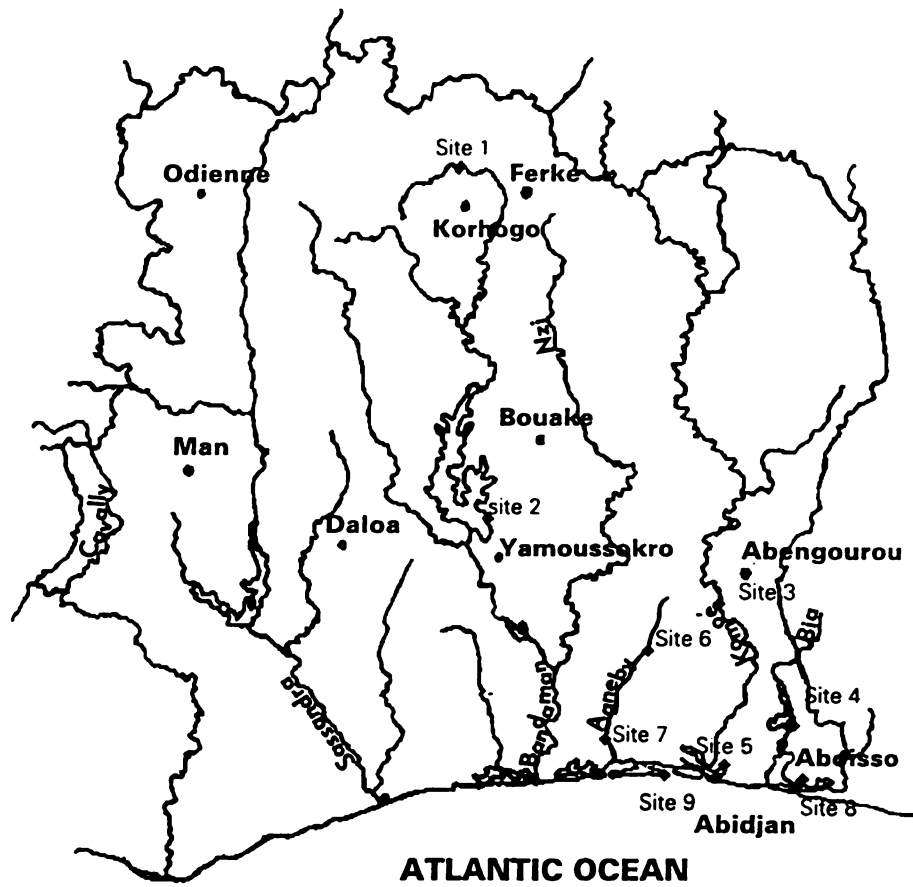


Figure 5. Map of Côte d'Ivoire illustrating the sampling collection sites

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

pH, color, total hardness, alkalinity, suspended solid, dissolved oxygen (DO), and chemical oxygen demand (COD), heavy metals (Cr, Se, As, Zn, Cd, Cu, Hg, and Pb), and organochlorine pesticides.

Tilapia (*Oreochromis niloticus*) was the fish selected for this study because they are found in most warm water (rivers and lagoons). Tilapia are resistant to disease, very hardy, and tolerant to low levels of dissolved oxygen allowing them to overcome overcrowded conditions. Because of their high yield potential and mild flavor, they are appreciated by the population. They were caught either by gillnets or by line and were transported from the fishing sites to the laboratory in a freezer. All the fish were processed within 24 hours after harvest. All the fish were processed as followed; each fish was scaled, deheaded, deguted and cleaned. The fish were then filleted and samples from each fish were wrapped in aluminum foil and packed with labelled plastic bags and stored at -20 °C until transportation to the USA.

MATERIALS

EQUIPMENT

- pH meter (Beckman PHY 72)
- cm x 1 cm i.d. chromatographic column fitted with a 200 ml reservoir.
- 250 ml round-bottom flask
- 500 ml separatory flask
- Graduate cylinders 25-1000 ml

- Column for resin (Econo pack, Millipore)
- Rotary evaporator (Buchler Instruments)
- Zymark Turbo-Vap® evaporator
- HPLC pump (Waters model 510)
- Programmable HPLC pump (waters 590)
- Automatic injector for HPLC (Waters WISP 712)
- Fraction collector (Waters)
- Ultrastyrigel 500 Å resin column (Waters, Millipore) cat No 20574, 19 x 300 mm
- Gas chromatograph (Hewlett-Packard 5890 Series II) equipped with a ⁶³Ni electron capture detector (ECD).
- Automatic injector for GC (HP 7673)
- Hewlett-Packard computer and Laserjet IIIp printer for GC data handling
- GLC column. J&W fused silica capillary column (Durabond); ID #122-5042; Liquid phase: DB-5 (non-extractable bonded phase); Film thickness, 0.25 mm; Column dimensions, (30 M x 0.333 mm id).
- Glass chromatographic column: 1.2 x 22 cm (1.0 cm i.d.), topped with a 3 x 10 cm reservoir and fitted a teflon stopcock with a 1.5 cm delivery tip.

All glassware (separatory funnels, beakers, funnels, teflon seals, and chromatographic columns) were thoroughly washed in hot water with detergent, rinsed with tap water, and distillate water, then with acetone, and finally with hexane when necessary. The cleaned glassware was dried in an oven.

SOLVENTS, REAGENTS AND SOLUTIONS

- SPE C₁₈ cartridges (Alltech Associates)
- Chelex 100 resin (hydrogen form, 200-400 mesh, 3 nm pore diameter) from BioRad Laboratories,
- Florisil; 60-80 mesh. Activate the florisil by placing in an oven ~130 °C for 16 hours. Cool before using
- Glass fiber filter paper (Whatman Gf 0.5 mm).
- Silane treated glass wool (Anspec; Ann Arbor, MI)
- Hexane (96% n-hexane), distilled in glass JT baker analyzed HPLC
- Ethyl acetate, distilled in glass Malinckrodt ChroAR HPLC
- Methanol, distilled in glass JT baker analyzed HPLC
- Dichloromethane, distilled in glass JT baker analyzed HPLC
- Sodium sulfate (Na₂SO₄)-granular anhydrous
- Sulfuric acid 95-98%, ABCS reagent (BASE, C)
- phenolphthalein , ABCS reagent (BASF, C)
- bromocresol green /methyl red indicator (Aldrich).
- EDTA (BASE, C)
- Eriochrome black T indicator (BASE, C).
- Nitric acid 90% ABCS reagent (BASE, C)
- Ethyl acetate in hexane (v/v) was prepared by measuring 2,100 mls hexane into a suitable container (empty solvent jug), add to the hexane 900 mls ethyl acetate, shake well to ensure complete mixing.

ANALYTICAL METHODS

DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES

The physicochemical properties of water samples were determined according to the Standard Methods for the Examination of Water and Wastewater (Clesceri et al., 1989). DO was determined by the azide modification of the Winkler's method on dilute samples (Hanson 1973). Suspended solids were determined by filtering a known volume of water through a glass fiber membrane filter (GF/C, 0.25 μ m), drying and weighing. pH was determined with a direct reading pH meter (Beckman PHY 72), standardized with acetate and phosphate buffers at pH 4.0 and 9.2 respectively. Alkalinity was determined by titration of 50 ml sample with 0.01 M H_2SO_4 using phenolphthalein and a mixed indicator bromocresol green-methyl red. Total hardness was determined by means of EDTA titration using eriochrome black T indicator.

HEAVY METALS DETERMINATION IN WATER

One hundred ml of the water sample was filtered through a glass fiber (Whatman Gf 0.5 μ m). The filtrate was acid digested and passed through a column (Econo pack, Millipore) filled with chelex 100 resin (hydrogen form, 200-400 mesh, 3 μ m pore diameter) obtained from BioRad Laboratories, CA. The resin columns were kept frozen until their transportation to the USA. In the USA, the heavy metals retained in the columns were eluted with strong nitric acid and analyzed by ICP (Termo Jarrell Ash, Polyscan 61E).

ORGANOCHLORINE PESTICIDES DETERMINATION IN WATER

The solid phase extraction (SPE) procedure was adapted from the method provided by Alltech Associates, Inc (Anonymous). The solvents used were "pesticide residue grade". Sep-Pack cartridges (Alltech Associates, INC) containing 1000 mg of packing material (C_{18} -bonded silica) were used for sample collection. The cartridges were coupled to a vacuum glass. The cartridges were washed with 10 ml hexane followed by 10 ml ethyl acetate. The cartridges were dried briefly, under vacuum to remove excess solvent and were conditioned with 10 ml methanol (Meow) then 10 ml deionized water. Five hundred ml separatory funnel containing 250 ml of the water sample was connected to the cartridge and water was passed through the column by hand suction at a rate of approximately 15 ml/min. After passing the samples, the cartridge were stored at 4 °C until transportation to the USA.

In the USA, the cartridges were inserted into a vacuum manifold and dried at a pressure of 500 mbar. The columns were then washed with 10 ml deionized water followed by 10 ml Meow:deionized water (20:80 v/v). The absorbed pesticides were eluted with a solution of hexane:ethyl acetate (70:30) at a flow rate of approximately 2-3 ml/min until 4 ml were collected.

ORGANOCHLORINE PESTICIDES DETERMINATION IN FISH

Ten g of fish tissue was homogenized with 40 g anhydrous Na_2SO_4 . The dried mixture was ground to a fine powder and packed into a 30 cm x 1 cm i.d. chromatographic column fitted with a 200 ml reservoir. The samples were extracted with

200 ml of dichloromethane at a flow rate of 3-4 mL/min. The lipid extracts were collected in a 250 ml round bottomed flask, and the solvent was reduced to approximately 1 ml by rotary evaporation. The concentrated extracts were then diluted to 10 ml with hexane and used for gel permeation (GPC) fractionation .

Automated GPC consisting of a 60 g bed Ultrastyrigel 500 Å resin (Waters, Millipore) attached to waters programmable HPLC pump (Waters 590) and waters fraction collector was used to separate the OC from lipids and other pesticides. An aliquot of the lipid extract (200 ml) was injected into the GPC column. The first 100 ml of eluate were dumped and the next 50 ml containing the OC was collected in a flask and turbo-vaped to approximately 5 ml.

The concentrated lipid extract was transferred into a column filled with a small plug of glass wool at the base followed by 7 grams of florisil and topped with 1-2 grams of anhydrous sodium sulfate. After addition of the GPC concentrate, the column was eluted with 40 ml of elution solvent (dichloromethane). The eluate was concentrated by turbo-evaporation (Zymark) to 0.5 ml, and then redissolved with 2 ml hexane prior to GC/ECD analysis.

CHROMATOGRAPHIC DETERMINATION

The detection and quantification of the pesticides in water and fish samples was performed by using a Hewlett-Packard 5890 Series II gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD). The conditions of analysis were the following:

- Column: DB-5 fused capillary column (30 m x 0.333 mm id) with

0.25 μm phase thickness.

- **Oven:** isothermal temperature 275 OC
- **Injector :** temperature 270 °C
- **Detector:** temperature 250 oC.
- **Carrier gas :** Helium at the pressure of 150 Kpa
- **Make-up gas:** Nitrogen

The acquisition of the data was done using a Hewlett-Packard Bell computer equipped with HPCHEM software (Hewlett-Packard, Palo Alto, CA).

1
V
n
S
a
in
to
in

fer

CHAPTER V

RESULTS AND DISCUSSION

PHYSICOCHEMICAL PROPERTIES

Physicochemical properties for the selected rivers and lagoons are given in Table 3. The temperature of water of the lagoons was higher than the temperature of water from the rivers. The lagoons are large bodies of water with more evapotranspiration than rivers. The mean pH of 7.18 was within the range of the WHO recommendation of 7.0 - 8.5 for drinking water. The high value observed at site 9 may be attributed to the industrial activities surrounding the area.

Total hardness ranged between 21.2 and 41.5. Most of the values were below 40 mg/L indicating that these waters are not too soft. As the color index indicated, most of the water samples were turbid and colored. It did rain at the collection sites 1, 3, and 6 the morning before the collection of the samples. the transport of organic matter, clay and surface litter to the rivers is origin of this brownish color observed. The turbidity as well as the brown color observed at sites 5 and 9 may be attributed to agricultural and industrial activities surrounding these sites. The COD observed increased from the north to the south of the country, principally in the area around the capital city. The increase in COD may be attributed to the increase of organic matter due to agriculture (pesticides, fertilizers), urbanization (waste water) and industrialization (oil refineries, food processing

plants, etc..).

Table 4. Physico-chemical characteristics of water samples from the nine collection sites

	Collection Sites								
Mean Values	S.1	S.2	S.3	S.4	S.5	S.6	S.7	S.8	S.9
Temperature (C)	27.4	28.0	27.7	27.2	26.7	27.2	31.3	30.5	31.7
pH	7.6	7.2	7.1	6.8	6.5	6.2	6.9	7.5	8.9
Color	85.5	15.3	87.2	25.2	88.5	75.7	15.4	13.2	89.3
Total hardness (mg/L)	39.2	29.4	28.4	31.0	37.0	35.2	41.5	42.0	21.2
Total solid (mg/L)	45.2	15.2	50.0	22.5	32.1	44.4	20.3	15.2	19.6
DO*	5.4	2.3	8.2	3.5	10.1	10.2	11.2	10.1	12.2
Total alkalinity	15.0	5.5	17.2	4.2	17.0	16.5	20.1	21.2	30.2
COD**	87.0	102.0	145.0	105.0	125.0	130.0	121.0	103.0	254.0

* Dissolved oxygen

** Chemical oxygen demand

Table 5. Heavy metals contaminant levels (µg/L) in water samples. Mean from five determinations

[illegible]

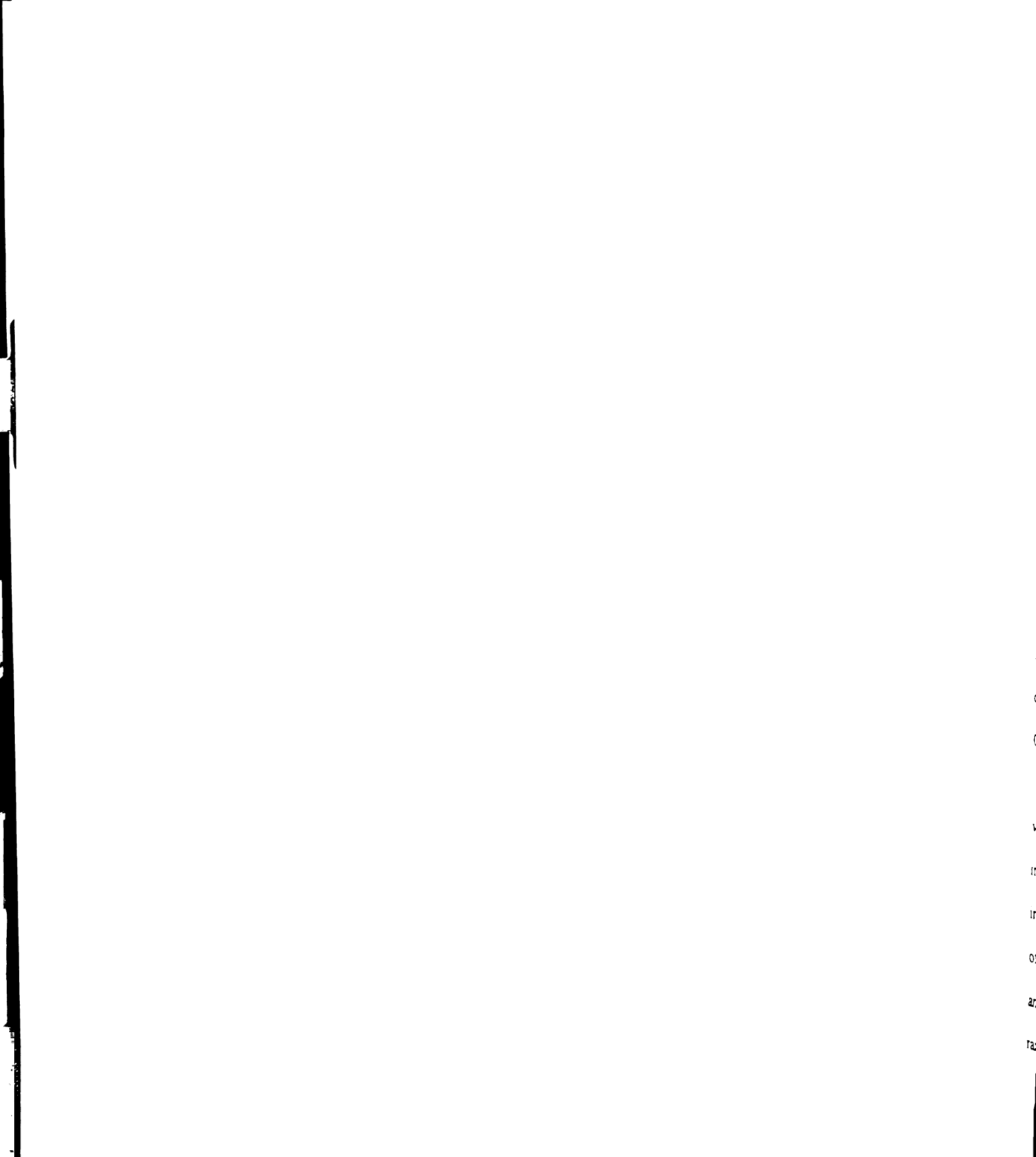
is lower than the limit of 5.0 mg/L set for this metal in drinking water (U.S. Public Health Services, 1962). In general as indicated by Table 2, the sources of zinc is industrial waste, metal plating or plumbing. Zinc inputs at sites: 5, 6, 7 where the levels are high, are most likely related to effluent discharge. In others area the presence of zinc might be related to background.

ORGANOCHLORINE PESTICIDES IN WATER

For statistically evaluating the extraction efficiency of the targeted pesticides in water by solid phase extraction (SPE) techniques, 500 ml of distillate water from our laboratory was fortified with the compounds at three concentration levels (35.0, 55.0, 250.0 µg/L).

Table 6. Recovery (%) $\pm$ RSD (n = 3) for the organochlorine pesticides in water samples and detection limits (µg/L).

Pesticide	Spiking Levels (µg/L)			Detection Limits
	35.0	55.0	250.0	
Aldrin	41 $\pm$ 1.5	45 $\pm$ 5.6	50 $\pm$ 4.1	0.002
DDD	98 $\pm$ 9.0	88 $\pm$ 7.5	102 $\pm$ 5.6	0.003
DDE	101 $\pm$ 7.0	99 $\pm$ 6.2	77 $\pm$ 4.7	0.002
DDT	97 $\pm$ 5.2	102 $\pm$ 4.0	78 $\pm$ 3.3	0.003
Dieldrin	105 $\pm$ 6.6	77 $\pm$ 6.1	72 $\pm$ 4.4	0.004
Endosulfan	55 $\pm$ 3.1	46 $\pm$ 4.5	49 $\pm$ 6.1	0.003
Endrin	96 $\pm$ 9.0	97 $\pm$ 8.2	92 $\pm$ 6.5	0.005
Heptachlor	92 $\pm$ 9.7	95 $\pm$ 9.0	105 $\pm$ 8.7	0.002
Lindane	92 $\pm$ 3.2	102 $\pm$ 5.1	99 $\pm$ 4.5	0.001



The average recoveries obtained were between 72 and 105% except for aldrin and endosulfan with 41 and 50% (Table 6). The relative standard deviation was between 2 and 22% (in accord with residue analysis, Greve 1984).

Identification and quantitation of compounds in water and fish samples was accomplished using reference solutions of a mixture containing the targeted pesticides. One μl of 0.01 to 0.08 ppm of the mixture solutions were injected into the GC and a standard curve was determined and used to quantitate the solutes in the samples. Results given in table 6 present the concentration levels of organochlorine pesticides in water samples from the nine collection sites. Among the targeted pesticides, five were found in the water samples. Dieldrin and lindane occurred in all the samples except in the samples from site 1. p,p'-DDT and endosulfan were detected in samples from 6 sites whereas aldrin was found only at three locations. The mean concentration of aldrin, p,p'-DDT and dieldrin were 0.3 mg/l, 0.5, and 0.4 mg/l respectively. The mean concentration of endosulfan was 1.7 (range 1.3-1.9). The mean concentration of lindane was 2.6 (range 0.3-3.9).

The results of the study (Table 7) show that among the targeted pesticides, five were found in the water samples. Dieldrin and lindane occurred in all the samples except in the sample from site 1 (north of the country). p,p'-DDT and endosulfan were detected in samples from 6 sites whereas aldrin was found only at 3 locations. The metabolites of DDT (DDD and DDD) were not found. The mean concentration of aldrin, p,p'-DDT and dieldrin were 0.3, 0.5, and 0.4 $\mu\text{g/L}$ respectively. The mean concentrations and ranges of endosulfan and lindane were 1.7 $\mu\text{g/L}$ (1.3-1.9 $\mu\text{g/L}$) and 2.6 $\mu\text{g/L}$ (0.3-3.9

µg/L) respectively.

Table 7. Mean (n = 5) pesticide residue levels (µg/L) in water samples from the nine collection sites. nd = non detected at the detection limit.

Compound	Collection Sites								
	S.1	S.2	S.3	S.4	S.5	S.6	S.7	S.8	S.9
Aldrin	nd	nd	nd	nd	nd	nd	0.3	0.3	0.5
p,p'-DDE	nd	nd	nd	nd	nd	nd	nd	nd	nd
p,p'-DDD	nd	nd	nd	nd	nd	nd	nd	nd	nd
p,p'-DDT	nd	nd	nd	0.4	0.5	0.3	0.5	0.5	0.6
Dieldrin	0.1	0.2	0.3	0.5	0.4	0.4	0.5	0.5	0.6
Endosulfan	nd	nd	nd	1.3	1.7	1.7	1.9	1.9	1.9
Endrin	nd	nd	nd	nd	nd	nd	nd	nd	nd
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lindane	nd	0.3	1.1	3.1	2.9	3.1	3.2	3.3	3.9

* nd = non detected at the detection limit

The results of the study show that the levels of OC found in the rivers were higher in the south than in the north of the country. For example the mean concentration of lindane in the river Comoé was 1.1 mg/L at site 3 (upper Comoé) while in the lower Comoé (site 5) it was 2.9 mg/L. The rivers flow from the north to the south of the

country and they carry with them run-off water from farmlands that may contain dissolved pesticides and/or pesticides attached to soil particles. The findings can also be explained by the difference in agricultural activities; in the north of the country only cotton, and recently sugar cane are grown whereas most of the commercial crops are grown in the south.

The results indicate also that the levels of OC in the lagoons were higher than those found in the rivers. This finding can be explained by a biomagnification process because the rivers flow from the north of the country, crossing all the farmlands and outflow in the lagoons in the south. Thus any chemical (pesticide) transported by the rivers reaches the lagoons.

The residue levels of lindane (range 0.3-3.9) and endosulfan (range 1.3-1.9) were higher than those of the other compounds. This finding can be explained by the fact that lindane and endosulfan are still extensively used in the country and therefore are carried by run-off to surface water. Endosulfan is used in crop protection as an insecticide against termites and variegated grasshopper and as a nematicide in banana production. Endosulfan is also used against vectors of diseases. Lindane is extensively used against cocoa mirids and other pests (Anonymous). The level of lindane found in water samples from the Ebrié lagoon (3.2-3.9) at the collection site 7 and 9 is higher than the levels found in the sediments from the same site (0.6-1.7 ppb) as reported by Marchand and martin (1985). This finding is perhaps related to the possible use of lindane as ichthyotoxic compound for the fishing activities in these areas (Colconap and Dufour, 1982). The fact that DDT was found in water samples but not its metabolites can be only

v

z

(

T

c

S

b

w

fi

m

ba

explained by recent use of this pesticide

The concentration of organochlorine insecticides in water samples were considerably lower than that reported in River Nile by El-Dib and Badawy (1985) and in several African lakes (Greichus, 1978). The residue levels of the studied compounds were still low compared with the permissible levels for drinking waters (Train, 1979, WHO, 1982).

ORGANOCHLORINE PESTICIDES IN FISH

To evaluate the performance of the extraction and cleanup procedures, fish samples were fortified at three levels with known amounts of the pesticides (0.5, 1.5, 5.0 mg/kg) and then analyzed. The recoveries for the selected pesticides were between 75-110% (Table 8).

Table 9 shows the results of the fish analysis expressed as mg/kg wet weight. The chromatograms showed no indication of polychlorinated biphenyl (PCB) contamination. Out of the forty five samples of fish tissue analyzed, 47% contain aldrin, 56% DDE, 11% endrin, 76% lindane, and 69% endosulfan. DDD, DDT, dieldrin and heptachlor were not detected. The results show that the levels of OC found in fish tissue were higher in fish from the south than from the north. This was in concordance with the findings for water. DDT was found in water samples but not in fish samples while its metabolite DDE was not found in water but was found in fish samples. Since DDT is banned in the country, its presence in fish samples suggests a possible uptake from the

Table 8. Mean recovery (%) $\pm$ RSD (n = 3) for the pesticides in fish and detection limits ($\mu\text{g/kg}$).

Pesticide	Spiking Levels			Detection Limits
	0.5	1.5	5.0	
Aldrin	75 $\pm$ 4.4	80 $\pm$ 6.0	82 $\pm$ 6.1	0.002
DDD	110 $\pm$ 5.1	95 $\pm$ 5.0	100 $\pm$ 7.2	0.003
DDE	90 $\pm$ 6.6	89 $\pm$ 6.5	104 $\pm$ 4.0	0.002
DDT	86 $\pm$ 7.7	100 $\pm$ 7.7	93 $\pm$ 10.1	0.003
Dieldrin	84 $\pm$ 7.3	87 $\pm$ 7.1	87 $\pm$ 8.5	0.003
Endosulfan	67 $\pm$ 6.6	65 $\pm$ 8.2	81 $\pm$ 9.2	0.005
Endrin	102 $\pm$ 9.0	98 $\pm$ 7.0	88 $\pm$ 9.5	0.005
Heptachlor	101 $\pm$ 5.5	97 $\pm$ 3.6	93 $\pm$ 6.3	0.002
Lindane	107 $\pm$ 7.1	95 $\pm$ 5.6	92 $\pm$ 7.3	0.001

water column. The OC adsorbed on sediment or plants, may be released in water and be absorbed by fish. Fish are able to uptake DDT adsorbed on the surface of particles (silt), and plants such as algae. The BHC isomers are relatively short-lived compounds and have a bioconcentration factor (BCF) of 50-900 and therefore they should not normally accumulate.

Table 9. Mean (n = 5) pesticide residue levels (mg/kg) in fillet of Tilapia from the nine collection sites. nd = non detected at the detection limit.

Compound	Collection Sites								
	S.1	S.2	S.3	S.4	S.5	S.6	S.7	S.8	S.9
Aldrin	nd	0.018	0.005	0.004	0.029	0.026	0.015	0.010	0.027
p,p'-DDE	0.005	0.034	0.107	0.057	0.249	0.184	0.048	0.208	0.493
p,p'-DDD	nd	nd	nd	nd	nd	nd	nd	nd	nd
p,p'-DDT	nd	nd	nd	nd	nd	nd	nd	nd	nd
Dieldrin	nd	nd	nd	nd	nd	nd	nd	nd	nd
Endosulfan	nd	0.006	0.997	1.300	1.700	1.700	1.900	1.900	1.900
Endrin	nd	nd	nd	nd	0.013	0.017	nd	nd	0.061
Heptachlor	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lindane	0.045	0.080	0.086	0.073	0.155	0.142	0.056	0.114	0.220

The fact that lindane was found at high concentration compared to the others OC indicates a build-up or a continuing input into the aquatic habitat. These findings can be explained by the fact that lindane is still in use in the country. The presence of aldrin in the majority of the samples may be explained by its relative high BCF's and long half-lives

in fish samples (Clark et al., 1983). Endosulfan presence in the samples can be explained by the fact that it is still in used in the country.

The results show that none of the samples analyzed had residue levels above the extraneous residue limits (ERL) and acceptable daily intake (ADI) for the respective pesticides set by the FAO/WHO codex alimentarius commission (1986). This indicates that the residue levels of OC in fish were within the acceptable limits for human consumption.

The levels of OC found in this study are lower than those reported in fish elsewhere in Africa. El Zorgani (1980) reported a sum of DDT ranging from 0.38 to 1.31 mg/kg in *Tilapia niloticus* from Lake Nuba in Sudan; Mugachia and al. (1992) reported levels of DDT and lindane ranging from 0.102-1.185 to 0.033-0.295 respectively.

CHAPTER VI

CONCLUSIONS

The present study shows that the rivers and lagoons investigated do not appear, at present, to have serious pesticide and heavy metal pollution problem. Physicochemical characteristics of the rivers and lagoons sampled are in general in the normal range. The organochlorine pesticides when found were at a very low levels. This was true for metals although it is difficult to distinguish between naturally occurring metals (background from the crust) and those due to human activities.

Recently, blooming of aquatic plants has been observed in many of the rivers and lagoons indicating nutrient enrichment of the water systems. For example the lagoon Ebrié in the Capitol city Abidjan has been periodically invaded in the last five years by aquatic plants. This has caused serious difficulties for urban transportation by boat. Furthermore, the Ebrié lagoon is now less than appealing for swimming, boating, and sport fishing. Even the lagoon has foul odors and fish have been found dead. This eutrophication is seen in other lagoons although very minor. The major sources of this eutrophication-causing nutrient enrichment are: (1). agriculture (eutrophication from the croplands, leaching of fertilizer applied to crops, runoff from animal feedlots, dairy barns); (2). urban/suburban runoff; (3). sewage effluents (discharge from treated and untreated sewage, usage of detergent containing phosphate, sewage from individual septic systems). Urbanization is rapidly growing in the country, the population of Abidjan, the capitol city has triple in

v

a

E

f

w

d:

re

pr

ra

P

l

s

less than ten years and the population of other cities is also increasing. In order to prevent future environmental disaster, routine monitoring of the aquatic systems (water and sediments) and stricter regulations in waste discharge must be invoked.

Concerning the organochlorine pesticides, their use in the country is expected to increase although many of them have been replaced by less persistent pesticides such as carbamates and organophosphates. Until now, the country has been able to feed its population but the situation is changing because of the rapid growing of the population. Agriculture must produce more food but the young people able to farm have fled the villages to the cities for an illusive better life. In order to produce more with less people, agriculture must be intensified; one the components of this intensification is pesticides. By the use of pesticides, farmers will be able to reduce the damage to their crops in the field and in the storage rooms. Because of the reduction in manpower, more herbicides will be needed for weed control.

Côte d'Ivoire relies on importation of meat from neighboring countries but recent drought in many of these countries have severely impaired animal husbandry. For this reasons, programs have been implemented in the country to increase cattle and sheep production in order to meet the demand in meat. The major constraint to cattle and sheep raising in Côte d'Ivoire has been diseases. For animal raising, there is a necessity to use pesticides to control insect vectors such as trypanosomiasis.

Everything indicates that pesticides will be an important component of pest control in Côte d'Ivoire. But the goal here should be to rationalize their use in order to reduce ecological disruptions, which may threaten long-term sustainability, and to reduce

en

wa

da

pr

as

le

pe

co

na

bu

Tr

un

se

hu

hu

po

Pe

or

ti

bi

ti

ti

environmental and health hazards. Pesticide usage must be rationalized, and means and ways have to be found to attain this objective. This is not possible unless a groundtruth data-base is available on pesticides at the grassroots level. It should be mentioned that proper and solid legislation with regards to pesticide sale, handling, storage and disposal, as well as worker protection from occupational hazards does exist but its implementation leaves much to be desired. One of my future studies will be to survey for pests, pesticides, pesticide legislation and management in the country. This work will allow the identification of the various factors necessary for good pesticide use practice so that necessary corrections may be made. Pesticide use on vegetables has increased recently but there is no information regarding the use of pesticides by the small vegetable growers. There is no extension service for these farmers, and therefore, such a study may help understand where and how they get their advice and what kind of records they keep. My second subject of concern will be to assess the impact of pesticide use on animal husbandry. In rural areas, small scale farmers associate crop production with animal husbandry, the latter feeding on fallen grains, insects and worms. In case of aerial spray, poultry may ingest sprayed insects that may have ingested or adsorbed the insecticide. Poultry may also ingested granular insecticides. No data exist in the codex alimentarius on the ADI of pesticides in Africa because of the lack of diet determination. I would like to conduct research in this area. Concerning the heavy metals, one possible study would be to determine the sources of contamination by monitoring points of injection. This can be done only if there is a strong support from the authorities. One feasible study would be to monitor these chemicals in air due to the emission from the refineries by using

passive detection devices.

APPENDICES

APPENDIX A

LIST OF THE PESTICIDES DISTRIBUTED IN COTE D'IVOIRE

T2

Sc

UN

C

F

S

D

G

D

T

S

E

E

T

S

S

U

C

S

F

T

S

E

T

S

E

T

S

E

T

S

E

T

S

E

APPENDIX A

LIST OF THE PESTICIDES DISTRIBUTED IN COTE D'IVOIRE

Table A1. List of the insecticides used in crop protection in Côte d'Ivoire.

Source: Rapport annuel sur la vente des pesticides pour utilisation agricole. UNIPHYTO (1991).

Commercial Speciality	Active Ingredient	Distributor
Typhon 50 EC	500 g/l Ethylparathion	Sofaco
Systhoate 40	400 g/l Dimethoate	Sofaco
Dyfonate 5G	5% Fonofos	Sofaco
Gammatif 5	5% lindane	Sofaco
Decis D5+150 ULV	5 g/l Deltamethrine	Sofaco
Thioral 25/25	25% Thirame + 25%	Sofaco
Malathion CE 50	500 g/l Malathion	Shell
Ekalux Forte	480 g/l Quinalphos	Shell
Basudine 600	600 g/l Diazinon	Ciba-
Thiodan 50 EC	500 g/l Endosulfan	Hoechst
Sumithion CE 60	600 g/l Fenitrothion	shell
Sumicidin 100 CE	100 g/l Fenvalerate	Shell-Chimie-CI
Undene 75 PM	75% Propoxur	Bayer
Callifan 50 EC	50 g/l Endosulfan	Callivoire
Marshall 25 ST	25% Carbosulfan	Callivoire
Karate CE	50 g/l Lamdacyhalothrine	Rhone-Poulenc
Nurelle D 12/100 ULV & ULV P	12 g/l Cyperm + 50 g/l	STEP C
Dursban 100 ULV	100 g/l Chlorpyrophos ethyl	Shell-Chimie-CI
Sevin	480 g/l Carbaryl	Shell-Chimie-CI
Fastac 40 EC	400 g/l Alphacypermethrine	Shell-Chimie-CI
Lindal 90	90 g/l Lindane	Callivoire
Teknar	1 Kg/l unit Aedes Aegypti	Sandoz
Caid Procida	2.5 g/l Chlorophacinone	Sofaco
Folithion EC 500	500 g/l Fenitrothion	Bayer
Solfac EC 050	50 g/l Cyfluthrine	Bayer
Marshall 2% PP	20 g/kg Carbosulfan	Rhone-Poulenc
Thionex 50 EC	500 g/l Endosulfan	Rhone -Poulenc

Table A2. List of the fungicides used in crop protection in Cote d'Ivoire
Source: Rapport annuel sur la vente des pesticides pour utilisation agricole.
UNIPHYTO (1991).

Commercial Speciality	Active Ingredient	Distributor
Ridomil 25	25 % Metalaxyl	Ciba-Geigy/SOCHIM
Caocobre	50 % Oxyde of cupper	Agro Business
BBS Procida	25 % Sulphate of cupper	SOFACO
Difolatan 80	80 % captafol	STEPC/Rhone-Poulenc
Kou-Foura	1.6 % Thiabendazole/1.5 % Permethrine	Callivoire
Alto 100 SL	100 g/l Cyproconazole	Sandoz
Tilt 250	100 g/l Propiconazole	Ciba-Geigy/SOCHIM
Sandofan	20 % Oxadixil	Sandoz
Fungasil 100	100 g/l Imazalil	SOFACO
manate 80	80 % Manebe	SOFACO
Alliette	800 g/l Phosethyl-Al	Rhone-poulenc
Benlate	50 % Benomyl	Rhone-Poulenc
Punch 40 EC	400 g/l Flusilazol	SOFACO
Manesan	80 % Manebe	Rhone-Poulenc

Table A3. List of herbicides used in crop protection in Cote d'Ivoire.
Source: Rapport annuel sur la vente des pesticides pour utilisation agricole, UNIPHYTO (1991)

Commercial Speciality	Active Ingredient	Distributor
Cotodon 400	240 g/l Dipropetryne/160 g/l metolachlore	Ciba-Geigy/SOCHIM
Cotoran 500	500 G/L Fluometuron	Ciba-Geigy/SOCHIM
Velpar L	240 g/l	Shell Chimie-CI
Ronstar 25 CE	250 g/l Oxadiazon	Rhone-Poulenc
Ronstar 12 L	120 g/l Oxadiazon	Rhone-Poulenc
Ronstar PL	100 g/l Oxadiazon/300 g/l Propanil	Rhone-Poulenc
Tazon 80 PL	500 g/l Atrazine	SOFACO
Tazon 80 PM	80 % Atrazine	SOFACO
Basagran	480 g/l Bentazone	BASF
Iaddok	200 g/l bentazone/200 g/l Atrazine	BASF
Amex 820	480 g/l Butraline	Ciba-Geigy/SOCHIM
Sofacox	100 g/l Paraquat/300 g/l Diuron	SOFACO
Sofacox	200 g/l Paraquat	SOFACO
Canazol Procida	80 % Diuron	SOFACO
Herbazol	2,4-D 720 g/l	SOFACO
Gramuron	100 g/l Paraquat/300 g/l Diuron	SOFACO
Gramoxone	200 G/L Paraquat	SOFACO
Callifor	250 g/l Promethrine/250 g/l Fluometrion	Callivoire
starane 200	200 g/l Fluroxypyr	Callivoire
Roundup	360 g/l Glyphosate	Rhone-Poulenc
Gallant 125	104 g/l Haloxyp acid	Callivoire
Bastas LS	200 g/l Glufosinate	SOFACO
Hyvar XL	200 g/l bromacil	Dupont/Rhone-Poulenc
Hyvar X	80% Bromacil	Rhone-Poulenc
Tordon 101 CE	64 g/l Pichlorane/240 g/l 2,4-D	Shell Chimie-CI
Tordon 225 E	120 Pichlorane/120 g/l 2,4-D	Shell Chimie-CI
Tordon 155	120 g/l Pichlorane/480 g/l 2,4-D	Shell Chimie-CI
Gespax 500	500 g/l Ametrine	Ciba-Geigy/SOCHIM
Gespax Combi-500	250 g/l Ametrine/250 g/l Atrazine	Ciba-Geigy/SOCHIM
Gespax 80	80 g/l Ametrine	Ciba-Geigy/SOCHIM
Lasso	480 G/L Alachlore	Rhone-Poulenc
Lasso GD	350 g/l Alachlore/200 g/l atrazine	Rhone-Poulenc
Primagram	250 g/l Metolachlore/235 g/l Atrazine	Ciba-Geigy/SOCHIM
Garlon 4	61.5 % Butoxy ethylic ester of Triclopyr	SOFACO
Spica 30	57 % Diuron/23 % Bromacil	SOFACO
Atral 80	80 % Atrazine	Callivoire
Atral 50	500 g/l atrazine	Callivoire
Atrazine 4L	480 g/l Atrazine	Callivoire
Ametral 80 PM	80 % Ametryne	Callivoire
Ametral 50 FW	500 g/l Ametryne	Callivoire
Ametral Mixte-PM	250 g/l Atrazine/40 % Ametryne	Callivoire
Ametral Mixte-L	250 g/l Atrazine/250 g/l Ametryne	Callivoire
Garil EF	72 g/l Triclopyr ester butoxy ethyl	Callivoire
Armada TM	90 g/l Glyphosate	STPC
Asulox 40	400 g/l Asulame	Rhone-Poulenc
Benazol	400 g/l MSMA/200 g/l Diuron	SOFACO
Ronstar 38 FLO	380 g/l Oxadiazon	Rhone-Poulenc

Table A4. List of nematicides and miscellaneous pesticides used in crop protection in Côte d'Ivoire.

Source: Rapport annuel sur la vente des pesticides pour utilisation agricole, UNIPHYTO (1991).

Commercial Speciality	Active Ingredient	Distributor
Nemacure 5G	5 % Phenamiphos	Bayer
Miral 10 G	10 % Isazophos	Ciba-Geigy/SOCHIM
Nemacure 400 EC	400 g/l Phenamiphos	BAYER
Mocap 10 G	10 % Ethophos	SOFACO
Temik 10	10 % Aldicarbe	Rhone-Poulenc
Furadan 4F	480 g/l Carbofuran	STEP C
Furadan 5G	5 % Carbofuran	STEP C
Furadan 10 G	10 % Carbofuran	STEP C
Telone II EC	90 % Dichloropropene	SOFACO
Spic 5G	5 % Metahaldehyde	SOFACO
Carel 480	480 g/l Etephon	Callivoire
Ethrel Stimulatex	480 g/l Etephon	Ciba-Geigy/SOCHIM
Ethrel Special ananas	480 g/l Etephon	Ciba-Geigy/SOCHIM
Klerat Blocs	0.05 % Brodifacoum	SOFACO
Klerat Granules	0.05 % Brodifacoum	SOFACO

APPENDIX B
CHROMATOGRAMS OF THE ANALYSIS

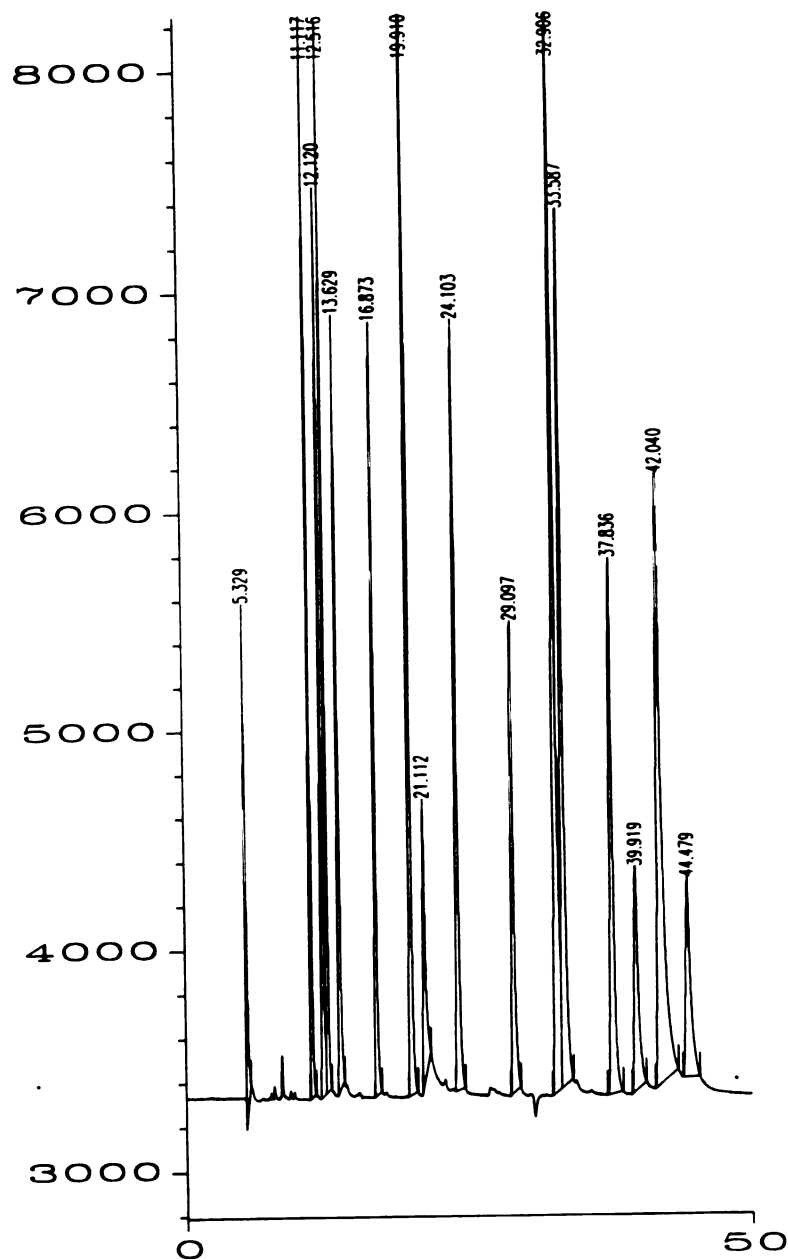


Figure B1. Chromatogram of 00.1 ppm of standard solution of organochlorine pesticide mixture

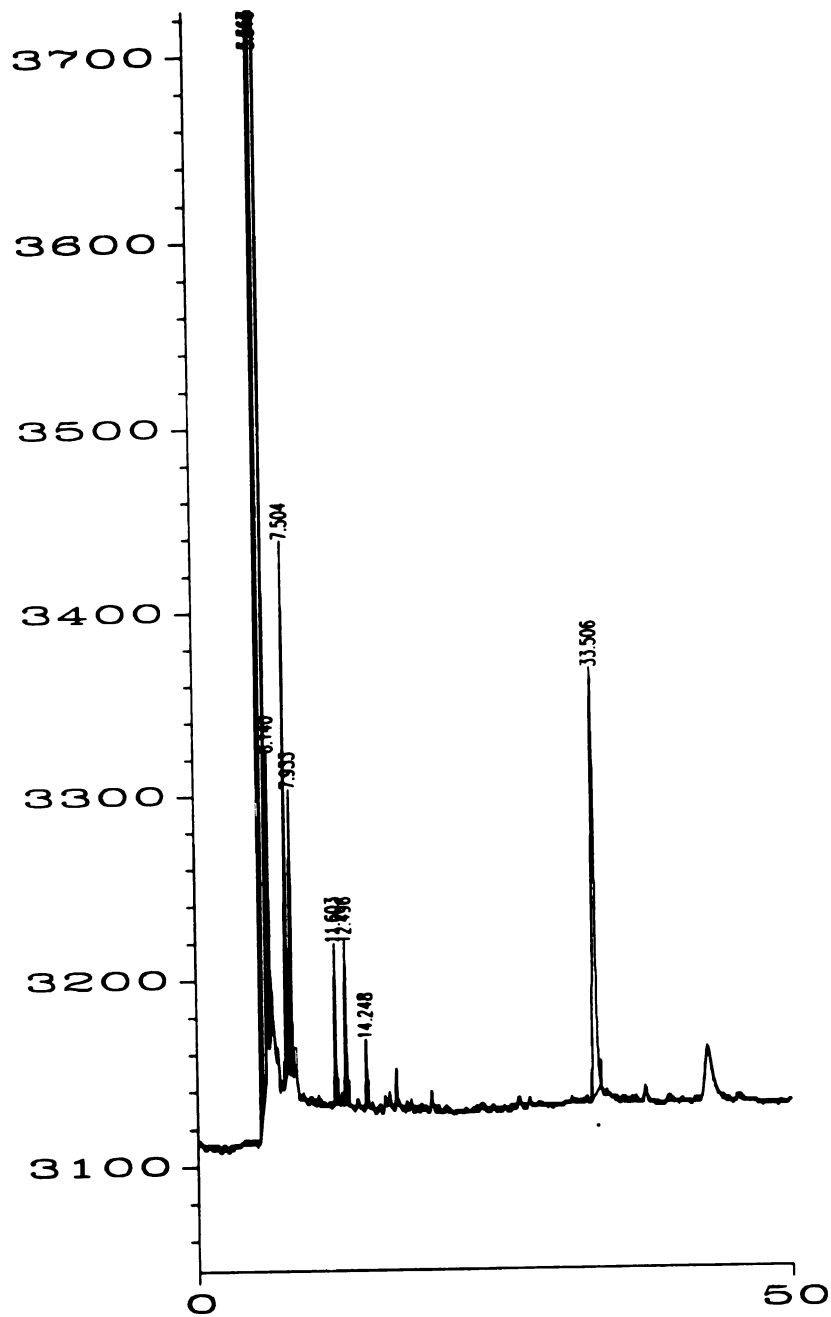


Figure B2. Chromatogram of water sample from site 1 (Ferké)

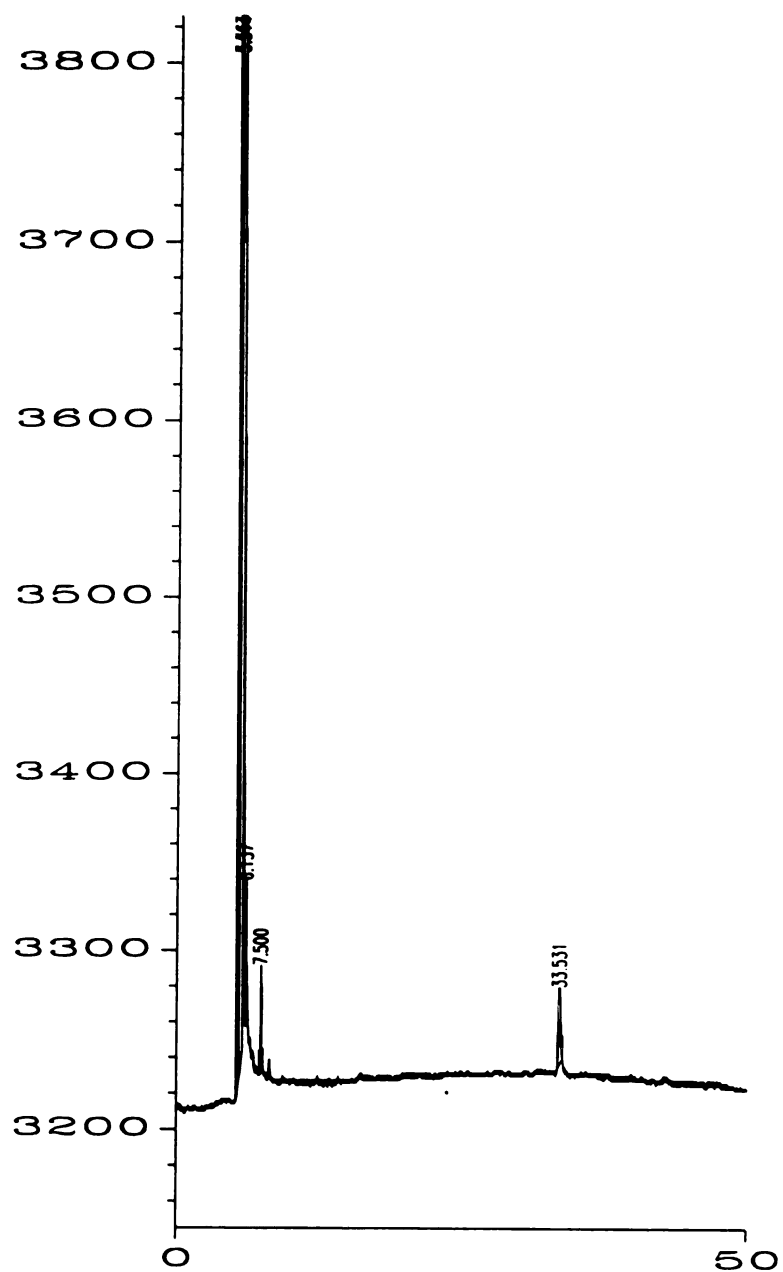


Figure B3. Chromatogram of water sample from site 3 (Abengourou)

Figure B4. Chromatogram of fish sample from site 5 (Moossou)

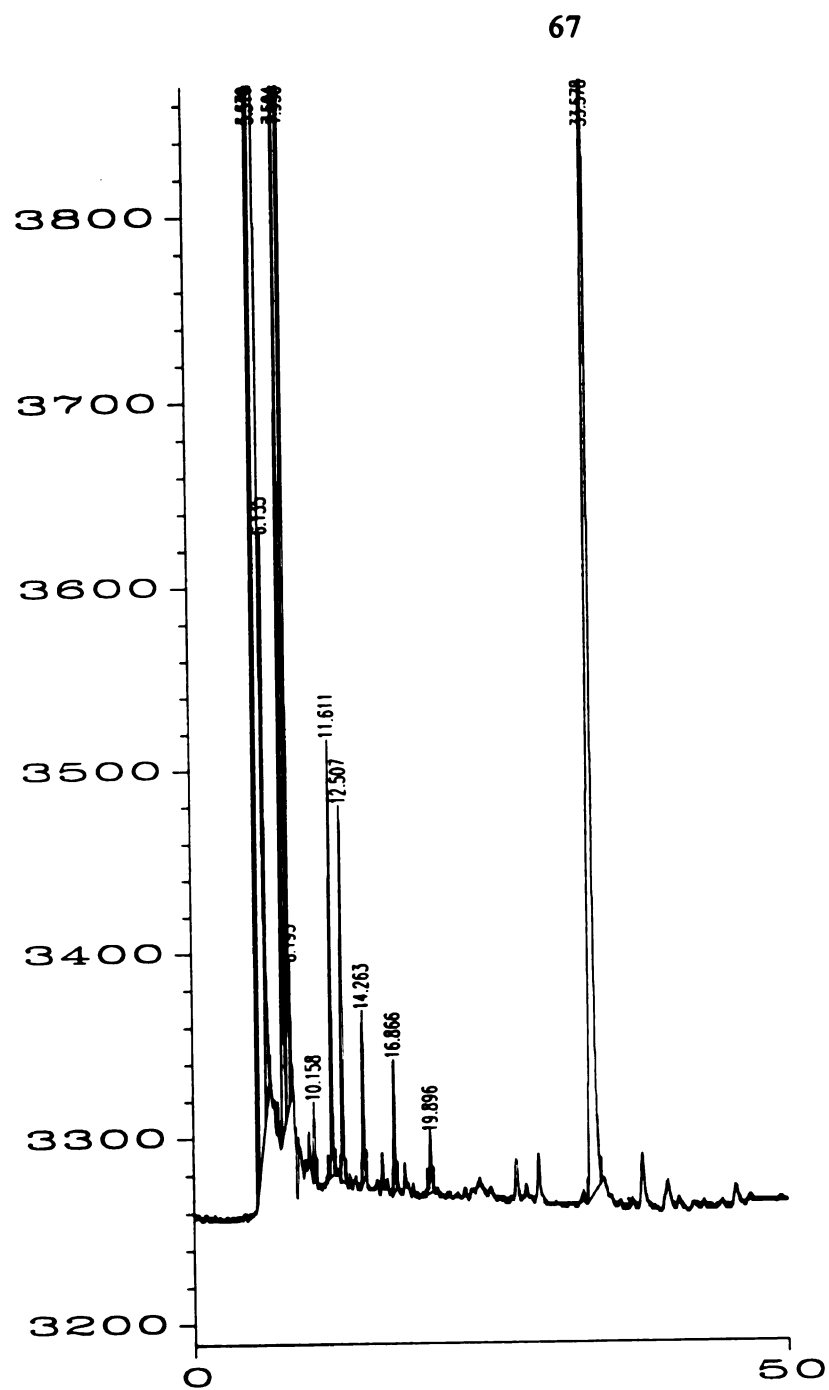


Figure B5. Chromatogram of fish sample from site 9 (Abidjan)

LIST OF REFERENCES

LIST OF REFERENCES

Abedi, Z.H. and D.E. Turton. 1968. Note on the response of zebrafish larvae to folpet and difolatan. J. Assoc. Off. Anal. Chem. 51:1108-1109.

Alexander, M. Microbial degradation of pesticides. In: F Matsumura, G.M. Boush, and T. Misato (eds.). Environmental Toxicology of Pesticides, Academic Press, New York, N.Y. pp. 365-395, 1972

Anonymous. 1984. Codex Alimentarius. Ambrus A. and Grenhalgh, R. Eds. WHO/FAO. Rome. pp. 28.

Ayayi, S.O. and Osibanjo, O. Environ. 1981. Pollution Studies on Nigerian Rivers: II. Water Quality of Some Nigerian Rivers. Pollut. (B) 2:87-95

Bailey G.W. 1966. Entry of biocides into water courses. Proc. Symp. Agr. Waste Waters. California Water Resource center Report 10:94.

Cliath, M.M. and Spencer, W.F 1971. Movement and persistence of dieldrin and lindane in soil as influenced by placement and irrigation. Soil Sci. Soc. Amer. Proc. 35:791-785.

Bailey G.W. and White, J.L. 1970. Factors influencing the adsorption and movement of pesticides in soil. Residue Revs. 32:29-92

D'Itri, F, 1986. Impact of Toxic Contaminants on Fisheries Management. In "Toxic Contaminants Management". Proc. Technical Session of the World Conference on Large Lakes, May 18-21, Mackinac Island, Mich. Ed. Norbert W. Schmidtke.

Donaldson, T.W. and Foy C.L., 1965. The phytotoxicity and persistence in soils of benzoic acid herbicides. Weeds 13:195-199.

Durand, J.R. and Skubich, M. 1982 Les Lagunes Ivoiriennes, Aquaculture, 27. 211-250.

Clesceri, S.S.L., Greenberg, A.E., and Trussell, R.R. (1989). Standard Methods for the Examination of Water and Wastewater (17th Edition).

Crop protection Strategies for Subsistence Farmers. Edited by Miguel A. Altieri. Westview Press, Inc., 1993.

El-Dib M.A. and Badawi M.I. (1985) Organochlorine insecticides and PCBs in River Nile water, Egypt. Bull Environ Contam. Toxicol 40:86-93.

Ehui, S.K. 1993. Côte d'Ivoire in "Sustainable Agriculture and the Environment in the Humid Tropics". National Academy Press, Washington, D.C.

El Zorgani, G.A. (1980). Residues of organochlorine Pesticides in fish in Sudan. J. Environ. Sci health, B15(6), 1091-1098.

Goldwater, L.J. 1972. Human toxicology of mercury. In Environmental Toxicology of Pesticides. Eds. F. Matsumura, G.M. Boush, and T. Misato. Academic pp. 165-175.

Greve, P.A. (1984). Good Laboratory practice in pesticide residue analysis. In "Pesticide residue analysis" (Ambrus A. and Greenhalgh R., eds.), WHO/FAO, Rome, p.281.

Greichus Y.A., Greichus A., Aman B.D., and Hopcraft J. (1978). Insecticides, polychlorinated biphenyls and metals in African lake ecosystems III, Lake Nakuru, Kenya. Bull. Environ Contam Toxicol 19:455.

Hindin, E. and Bennett P.J. 1970. Transport of organic insecticides to the aquatic environment. Intern. Water Poll. Res. Conf. Proc. 5th. III: 19/1-19/16. San Francisco, Ca.

Hill, D. (1975). Agricultural insect pests of the tropics and their control. Cambridge University Press, London. 516 pp.

I.ATA. Rapport of the Biological Control program center for Africa, 5-9 December 1988. Cotonou, Benin.

Kenaga, E.E., 1972. Guideline for environmental study of pesticides: Determination of bioconcentration potentials. Residue Revs. 44:73-113.

Koeman J.H., Pennings J.H., De Goeij, J.J.M., Tjioe P.S., Olindo P.M., and Hopcraft, J. (1972). A preliminary survey of the possible contamination of Lake Nakuru in Kenya with some metals and Chlorinated hydrocarbons. J. Appl. Ecol., 9:411.

Kouadio, I. and Trefry, J.H. 1987. Sediment Trace Metal Contamination in the Ivory Coast, West Africa. Water, Air, and Soil Pollution, 32:145-154.

Manahan, S.E. 1991. Environmental Chemistry. 5th Ed. Lewis Publishers, Inc.

Marchand, M. and Martin, J-L. 1985. Détermination de la pollution chimiques (hydrocarbures, organochlorés, métaux) dans les lagunes d'Abidjan (Côte d'Ivoire) par l'étude des sédiments. Océanogr. Trop. 20:25-39.

Mugachia, J.C, Kanja, L, and Maitho, T.E. (1992). Organochlorine Pesticides in estuarine Fish from the Athi River. Kenya. Bull. Environ. Contam. Toxicol., 49:199-206.

Okeye, B.C.O., Afolabi, O.A., and Ajao, E.A. 1991. Heavy metals in the Lagos lagoon sediments. Int. J. Environ. Stud. 37:35-41.

Risebrough, R.W. 1969. Chlorinated hydrocarbons in marine ecosystems. In "Chemical fallout: Current research on persistent pesticides". Eds M.W Miller and G.G, Berg. Charles C. Thomas, Springfield, Ill., pp.5-23.

Saad, M.H., Ezzat, A.A., El-Rayis, O.A., and Hafez, H. 1981c. Occurrence and Distribution of Chemical Pollutants in Lake Mariut, Egypt: II. Heavy Metals. Water Air Soil Pollut., 16:401-407

Sinha, A.P., Kishnan Singh, and A.N. Mukhopadhyay. Soil Fungicides. Vol II. CRC Press, Inc. Boca raton, Florida.

Spencer W.F. and Cliath, M.M. 1970. Vapor density and apparent vapor pressure of lindane. J. Agr. Food Chem. 18:529-530.

Spencer W.F. and Cliath, M. 1972. Volatility of DDT and related compounds. J. Agr. Food Chem. 20:645-649.

Spencer, W.F, Farmer, W.J., and Cliath, M.M. 1973. Pesticide volatilization. Residue Revs. 49:1-47.

Southwood, T.R.E. 1977. Entomology and mankind. Proc. XV. International Congr. Ent. washington., 36-51.

Toma, S.A., Saad, M.A.H., Salama, M.S., and Halim, Y.J. 1981. The distribution of some absorbed elements on the Nile continental shelf sediments. J Etud. Pollut. CIESM., 5:377-382.

UNIPHYTO (1991). Rapport annuel sur la vente des pesticides pour utilisation agricole.

U.S.E.P.A. 1976. Quality Criteria for water. Washington, D.C.

Weed, S.B. and Weber, J.B. 1969. The effect of cation exchange capacity on the retention of diquat²⁺ and paraquat²⁺ by three layers type clay minerals. I. Adsorption and

release. Soil Sci. Soc. Amer. Proc. 33:379-382

Willis, G.H. and Hamilton, R.A. 1973. Agricultural chemicals in surface runoff, groundwater, and soil: I. endrin. J. Environ. Qual. 2:463-466.

World Bank Technical paper N0. 142. Africa Technical Department Series. Integrated Pest management and African Agriculture. Agnes Kiss and Frans Meerman. The World bank, Washington DC.

World Health Organization (1971). International Standard for drinking water, 3rd Edition, WHO, Geneva.

Yaninek, J.S. and H.R. Herren. Biological control: A sustainable solution to crop pest problems in Africa. Proceedings of the inaugural conference and workshop of the IITA

PART II

EVALUATION OF COMMERCIAL IMMUNOASSAY FOR THE DETECTION OF PESTICIDES IN PLANT AND FISH

CHAPTER I

INTRODUCTION

Today's farm practices are being scrutinized for their contributions to water pollution, water shortages and soil erosion. There is public perception that food is unsafe because of the presence of pesticides and other chemical residues in food. There is also a concern by farmers for their own health and for the quality of the environment. Nearly half of the farmers in 1989 nationwide survey by Jefferson Davis Associates in Iowa were worried that their use of chemicals poses a danger to themselves and to the environment (Anonymous, 1990). As public concern about the pesticide issue increases, the pressure to provide new information and guidelines on the fate of pesticides in the environment has become important for regulatory agencies and governments. Many countries have not only introduced rigid legislation requiring detailed examination of all aspects of the potential hazard before a new chemical can be approved for specific usages but also surveillance of the food supply for the presence pesticides. Furthermore research must provide critical evaluations on the fate of pesticides in the soil, water, and food. At the stage of inquiry and for purposes of implementing legislation, analytical methods are required to locate and quantify contamination, to determine the risks that pollutants pose to human and ecological health, and to actively remediate polluted sites when necessary.

This presents an imposing analytical challenge when one considers the total number of analyses needed, the broad spectrum of analytes which must be determined, the multitude of matrices in which these analytes must be quantified, and the economic constraint in carrying out these measurements. Various analytical methods can be used for the determination of pesticides in different matrices but up today chromatographic techniques (gas chromatography, high-performance liquid chromatography, and thin layer chromatography) alone or coupled with mass spectrometry are the most commonly used. However monitoring the supply or the environment for residue by these analytical methods is expensive, time consuming, complicated, potentially unsafe, and require the use of polluting solvents.

For example, the general procedure for the determination of most pesticides and their metabolites in plant materials involves the following steps: 1. collection of sample materials; 2. extraction of the sample with an organic solvent 3. decoloration with activated carbon/Attaclay mixture; 4. partition with an organic solvent followed by a second partition with a mixture of organic solvent; 5. column chromatography clean-up; and 6. analysis by chromatography with a specific detector. This method may take several hours to analyze a dozen samples and generates appreciable amount of hazardous waste.

More recently enzyme-linked immunosorbent assays (ELISAs) have gained interest for pesticide residue analysis. Several books, articles and reviews have discussed the theory and applications of these techniques (Newsome, 1986; Van Emon and Mumma, 1990; Van Emmon et al., 1989; Van Emmon and Lopez-Avilila, 1992). Commercial

enzyme-linked immunosorbent assays (ELISA) are becoming increasingly available that a section was totally dedicated to this technique during the ACS 211th meeting in New Orleans. These techniques offer several advantages over chromatographic techniques; relatively rapid analysis times, high samples throughput and sensitivity at a relatively low cost. Thus these techniques are appealing for developing countries such as Côte d'Ivoire where large scale of pesticide analysis is often difficult as instrumentations such as GC or HPLC are often unavailable or when they are available, the cost associated with their maintenance and the purchasing of solvent is often too high.

Most of the commercially available ELISA kits have been marketed for the analysis of water samples because of the absence of matrix interference. For example, ELISA kits have been used for the determination of atrazine (Bushway et al., 1988; Schaleppi et al., 1989), alachlor (Feng et al., 1990; Rittenberg et al., 1991; Lawruk et al., 1992), and carbofuran (Bushway et al., 1992) in water. Recently works have been done to extend the use of these kits to more complicated matrix such as soil samples, food. The present study investigate a broader use of commercial kits as rapid detection systems for the screening of three commonly use pesticides in two complex matrices: corn leaves and fish. The first objective is to verify the method precision and accuracy by comparing it to established chromatographic methods and establish the method sensitivity by determining its limit of quantitation (LOQ) and its limit of detection (LOD). LOQ is the level above which quantitative results may be obtained with a specified degree of confidence while (LOD) represents the lowest concentration that can be determined to be statistically different from blank.

Because of the binding of the pesticide to the matrix (soil) or its conjugation to matrix components (plant or animal tissue), immunoassay may give different results with environmental samples compared to fortified samples. Samples components (proteins, fat, pigments), pH and ionic strength, viscosity, solubility of chemicals and extraction solvents may interfere with the reading and give false results. The second objective of this study was to analyze environmental plant material and fish samples and to determine the effect of matrices on the accuracy and efficacy of the ELISA.

The commercial immunoassay kits may performed differently depending on the type of solid phase by the manufacturer. The solid phase employed may be polystyrene wells, balls or tubes, on which antibody or hapten-protein conjugate are passively adsorbed or it can be magnetic particles coupled to the antibody. The third objective in this study was to compare the sensitivity and precision of two types of these solid phases for the analysis of sample extracts. The first kit Enviro-guard® (Millipore) has the antibodies coated on the bottom of test tubes. The second type of kit is RAPD™ (Ohmicron corp.) has the antibodies adsorbed on fine magnetic particles suspended in solution. The sensitivity and precision of these kits was compared. The last objective of this study was to analyze the usefulness of ELISA compared to traditional techniques by evaluating the cost of equipments and reagents; cost associated with the training of technicians, cost associated with quality control, and the availability and stability of the kits.

CHAPTER II

ENZYME IMMUNOASSAYS AND ITS APPLICATIONS

THE IMMUNE RESPONSE

The immune system protects animals from infectious organisms. It comprises several different types of cells, each with a variety of functions. One group of white blood cells, lymphocytes, secrete proteins that bind in a highly specific manner to foreign molecules (Benjamin and Leskowitz, 1988). These proteins are called antibodies while the foreign molecules are called antigens. Lymphocytes that produce antibodies are called B lymphocytes or B cells (Benjamin and Leskowitz, 1988). B cells specifically bind to a particular antigen (Figure 1). Once binding occurs, these cells are activated and divide, producing identical copies of themselves (clones). Each new B cell secretes antibody molecules that bind specifically to the antigen. B cells release antibodies which then circulate throughout the body in the blood stream. When the antibody encounters their specific antigen they bind to it, and the antigen is marked for destruction by other components of the immune system such as the macrophages (scavengers cells that engulf and destroy). Most of the antibody structure is relatively constant, except for the antigen-binding site (variable region). There are at least two antigen binding sites per antibody structure.

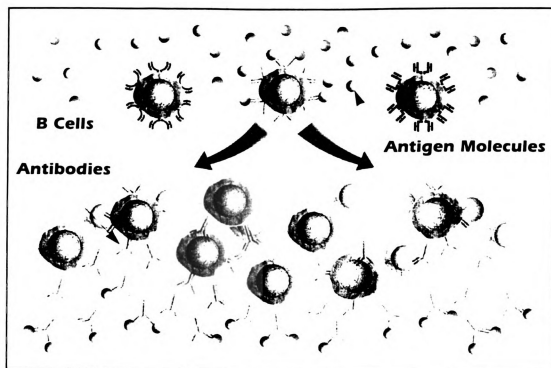


Figure 1. Cellular events leading to antibody production following B-cells activation by antigen molecules. Source

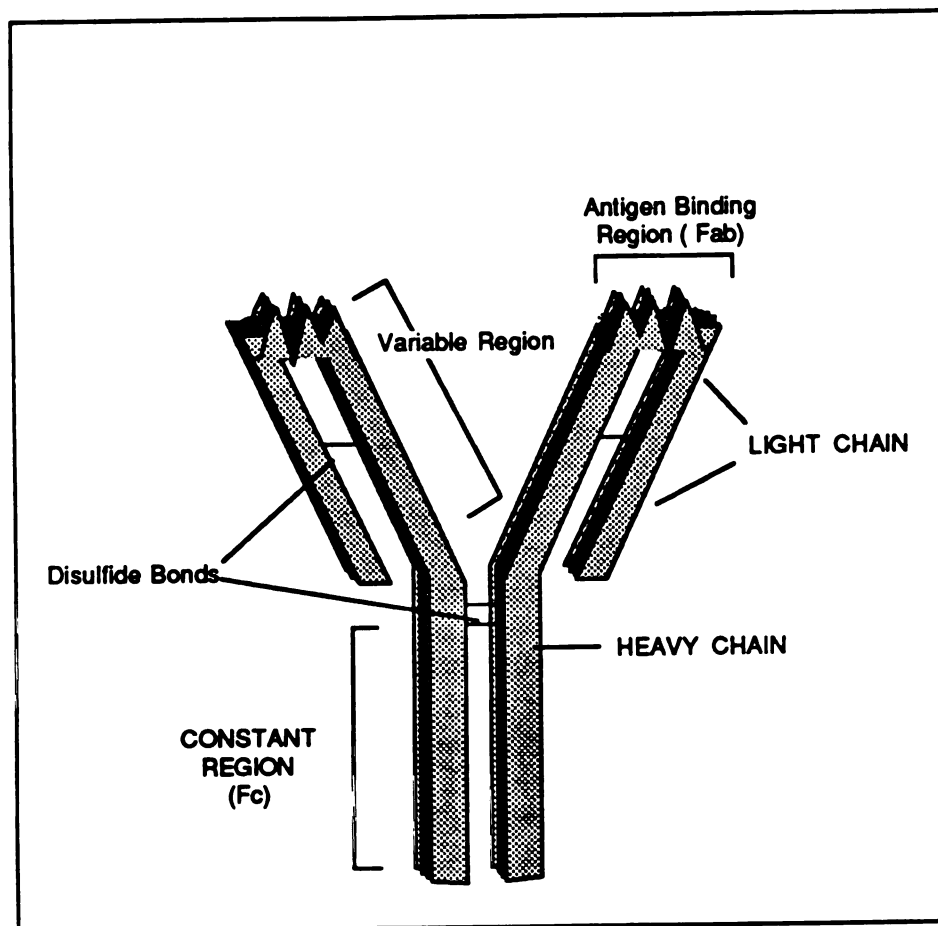


Figure 2. The Structure of an Antibody Molecule. Four protein chains combine to form an antibody molecule.

ANTIBODY STRUCTURE AND FUNCTION

Antibodies also called immunoglobulins (Ig) are glycoproteins (Goodman J.W., 1991). They are grouped in five classes (IgA, IgD, IgE, IgG, IgM). Antibodies of different class ranged from 150 to 900 Kilodaltons (Kd) in molecular weight and mediate different immunological functions. Some other vertebrate animals produce fewer classes, but most produce IgG and IgM.

An antibody molecule consists of two heavy chains and two light polypeptide chains connected through disulfide bound (Fig 2). The Fab portion (light chain) of the antibody contains the variable region that is responsible for the specificity of the molecule. Variation of the polypeptide sequence of this region are complimentary to the antigenic determinant, thus providing the basis for antigen/antibody binding. The Fc region (heavy chain) of an antibody molecule is constant within a particular antibody class. The Fc region mediates secondary immunological functions such as complement fixation. Labels or "tags" which are used for visualization of immunoassays are generally attached to the Fc region so the antibody retains the antibody/antigen capacity of the Fab region.

ANTIGEN STRUCTURE

An antigen is any molecule which can bind to an antibody (Benjamin and Leskowitz, 1988). Antigens can be biological molecules or synthetic compounds. Immunogens are molecules or part of a molecule that stimulates a B cell to produce

antibodies. To be immunogenic a substance must:

(1) contain a region B cells recognize as foreign; (2) contain sufficient complexity; and (3) have sufficient molecular weight (usually $> 3,000$ daltons). Large antigens may contain a number of recognition sites called epitopes (Benjamin and Leskowitz, 1988); each epitope activates a different B cell which in turn produces antibody with a unique binding specificity. An immune response in which many different lymphocytes produces antibodies to a complex immunogen is said to be polyclonal, and the resulting antibodies are called polyclonal antibodies (Figure 3).

Some molecules are too small ($MW < 1,000$ daltons) to elicit an effective humoral response. These molecules, defined as haptens, must be physically coupled to a larger immunogenic molecule in order to elicit an antibody response to the hapten. The large molecule, known as carrier, helps produce a necessary recognition signals for activation of the immune response. This type of system is employed in immunoassay for analytical purposes to produce antibodies which can then be bind to certain chemicals (e.g. pesticides) and to small polypeptides (Van Emon and Lopez-Avila, 1992; Rittenburg et al., 1989) (Figure 4).

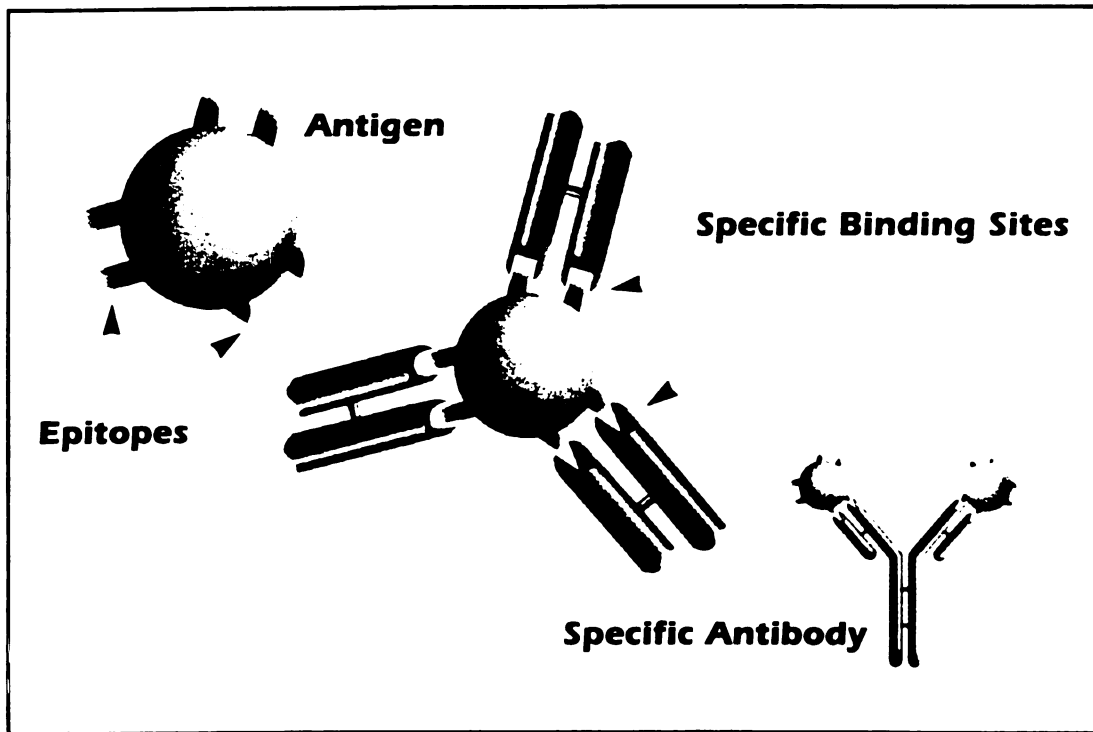


Figure 3. Illustration of the binding of the antibodies to the antigen specific binding sites called epitopes. Each epitope can bind to a different antibody containing a specific antigen-binding site.

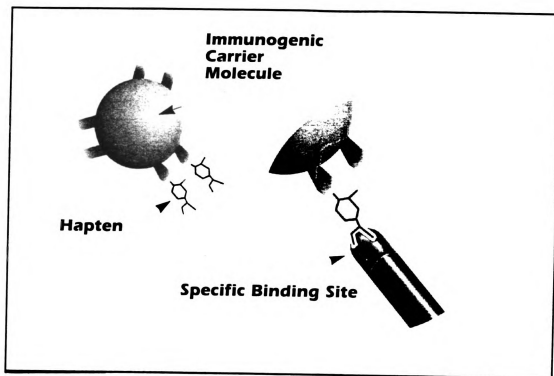


Figure 4. Illustration of a synthetic antigen indicating the hapten (small organic chemical) covalently attached to immunogenic carrier molecule.

PRODUCTION OF ANTIBODIES FOR LABORATORY USE

The humoral response, which involves production of antibodies to foreign substances (antigens), is the arm of the immune system which provides the basis for immunoassay systems. Immunoassays are tests in which antibodies are used as analytical chemistry reagents. Antibodies are produced for use in an immunoassay by exposing an animal or specialized cells from an animal to a target substrate. For example, a laboratory animal such as a rabbit may be immunized with a preparation of the target substance (i.e. pesticide) to stimulate the production of antibodies. Serum from the blood of the animal, which contains the antibodies to a wide variety of substances, is then collected at the appropriate time. This is called polyclonal serum (Figure 5). The antibodies are purified and incorporated into a detection system.

MONOCLONAL ANTIBODIES

The production of monoclonal antibodies is one of the most useful recent scientific advances applied to immunoassay technology. They have the ability to produce a single type of antibody from the B-cell partner and the ability to survive and proliferate outside the body of the animal for an extended period of time from the myeloma partner.

Monoclonal antibodies are produced in a series of steps as illustrated by figure 6. It begins with the immunization of a mouse and then the removal of its spleen after an appropriate period of time. Antibody-producing cells are isolated from the spleen and

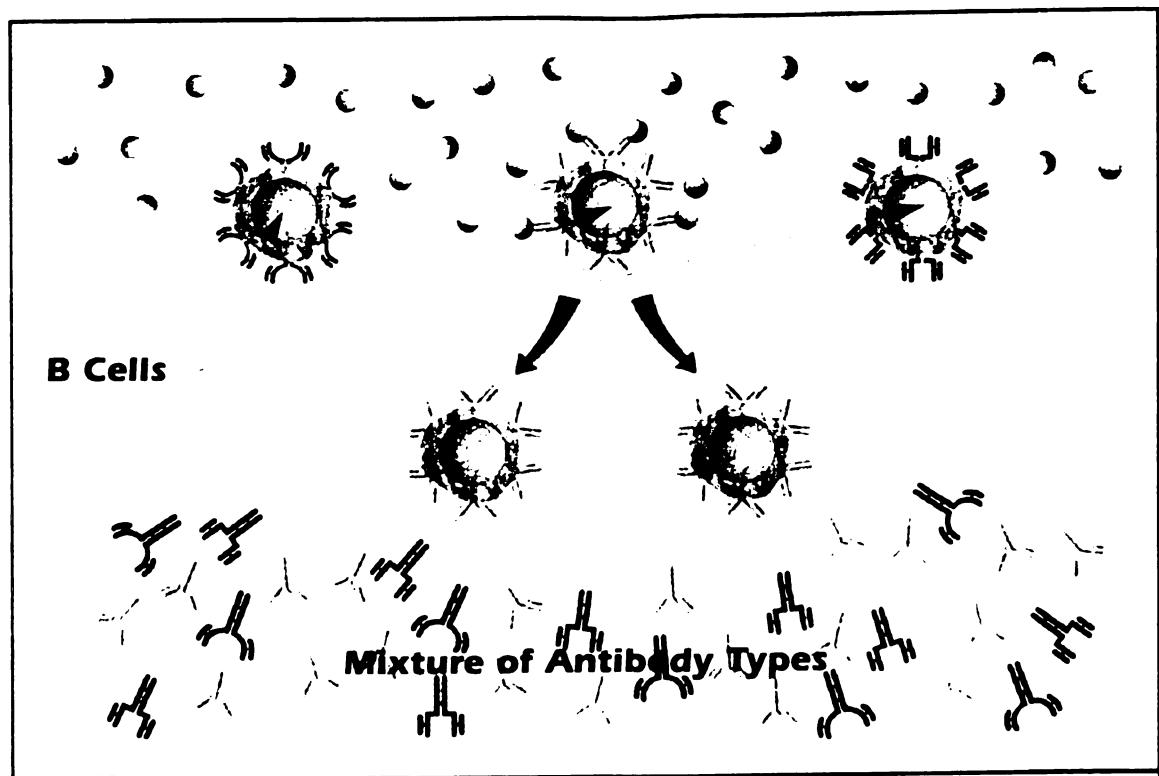


Figure 5. Polyclonal antiserum containing a mixture of antibodies produced by multiple B cells.

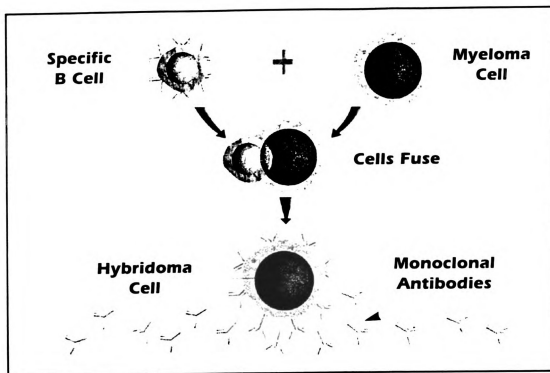


Figure 6. Illustration of Monoclonal Antibodies Production. One B cell is fused in the laboratory with a tumor cell. The resulting hybridoma produces multiple copies of one specific type of antibody (monoclonal antibody).

fused with "immortal" myeloma cells from tissue culture through the use of polyethylene glycol. Cells resulting from the fusion of a B-cell and myeloma cells are called hybridomas. Through a series of manipulations in tissue culture, individual hybridomas are isolated and allow to produce antibodies that are then tested for desirable antigen-binding characteristics.

ANTIBODY-ANTIGEN INTERACTIONS

The binding of an antibody to an epitope on an antigen depends on non covalent interaction such as electrostatic, hydrophobic, and Van der Waal forces (Benjamin, E. and Leskowitz, S., 1988). The antigen-binding site and its epitope must be in close proximity before optimal binding can occur. Hapten-carrier combinations can be designed with various orientations, allowing an induction of an array of antibodies with different ability to distinguish among closely related compounds. Relatively small changes in the structure of the epitope itself or even its stereochemistry, can affect antibody binding.

Affinity is a measure of the strength of an individual antibody-antigen binding interaction. Affinity is the most important consideration for the usefulness of a particular antibody in an analytical assay. High affinity antibodies will bind larger amounts of antigens in a shorter period of time than low affinity antibodies and produce a stable complex. Monoclonal antibodies are homogeneous and their affinities can be determined precisely. Polyclonal antibody mixtures have a variety of affinities so the overall binding energy of the polyclonal mixture is referred to as avidity (Benjamin and Leskowitz, 1988).

IMMUNOASSAYS

Immunoassays are powerful techniques that rely on the specific interactions between antibodies and antigen to detect and quantify a wide variety of substances (microorganisms, environmental contaminants, etc). In a typical immunoassay either antibodies or antigens are immobilized on a solid phase. Example of solid supports include nitrocellulose or nylon membrane, test tubes, microscopic particles, or microtiter plates (Van Emmon and Lopez-Avila, 1992).

The binding of the antigen to the antibody is detected by using markers. A number of markers have been used in the detection system; among them radioactivity, fluorescence, polarization of light, visible or ultraviolet absorbance, phosphorescence, chemiluminescence, bioluminescence or electron spin luminescence (Jung et al., 1989; Kaufman et al. 1982; Anonymous 1990). Radioactive markers (tags) were used widely for many years, but the inherent problems in handling radioactive compounds has driven most immunoassays to employ non radioactive markers. Fluorescence tags are used in many immunoassay procedures and can be detected with special instruments or microscopes.

ENZYME IMMUNOASSAYS

The most widespread class of immunoassays employ enzymes as markers (Figure 7). These assays are called enzyme immunoassay (EIA) or Enzyme-linked immunosorbent assay (ELISA). EIA produces a color reaction (Figure 8) that is proportional to the level of the antigen being measured. The enzymes commonly use as

markers catalyze reactions that yield colored end-products. The enzyme must be stable,

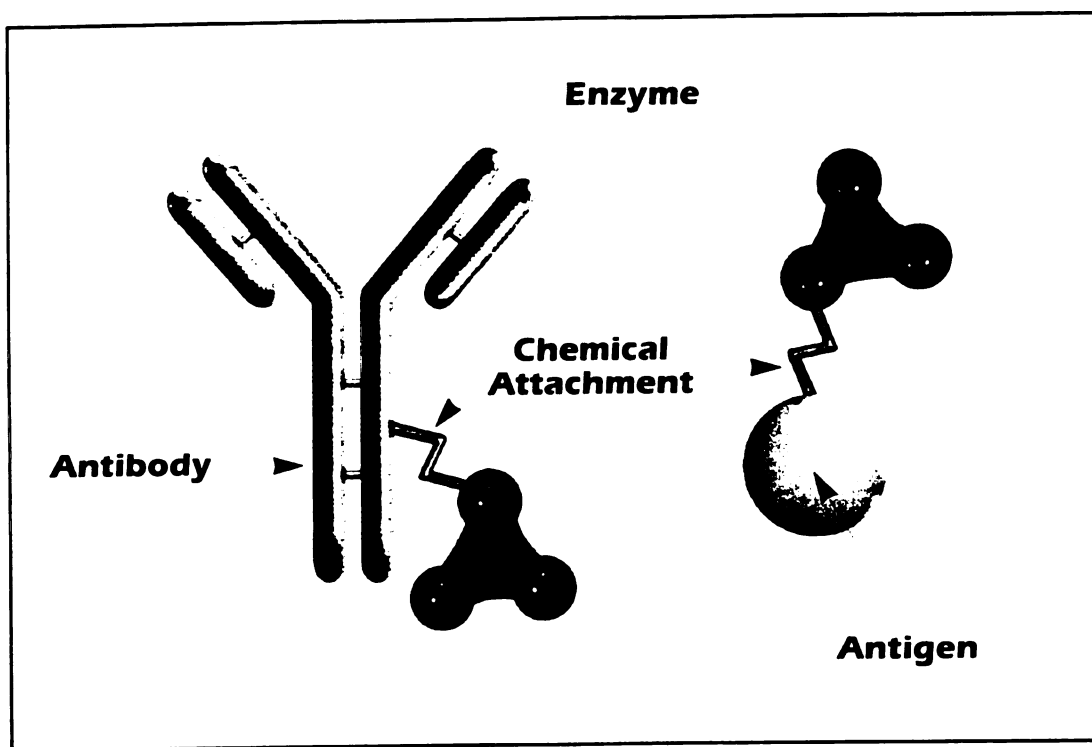


Figure 7. Enzyme conjugate. Enzyme are physically linked to antibodies or antigens to form an indicator system. The enzyme, the antibody, and the antigen must retain their activities and binding capacities to be useful.

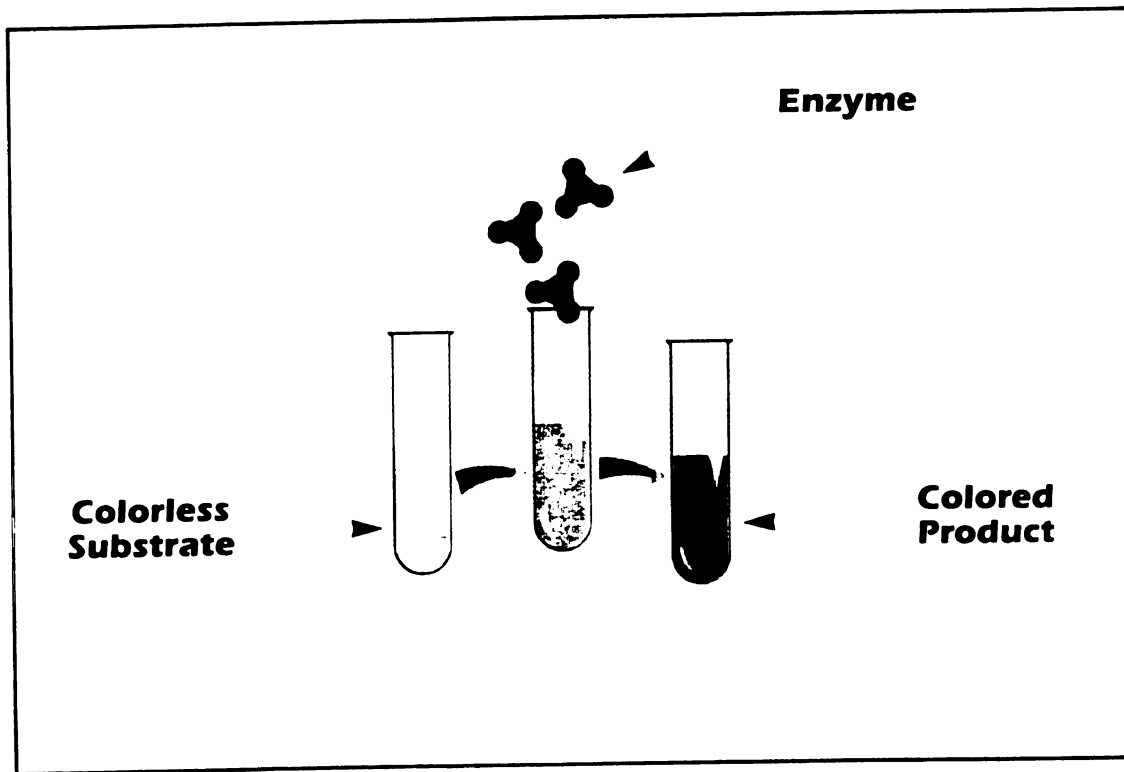


Figure 8. Color reaction catalyzed enzymes used as indicators or tags in immunoassay.

operate under a wide range of conditions, and deliver a high reaction rate. The enzymes most frequently used include horseradish peroxidase, alkaline phosphatase, galactosidase, and urease.

The results of immunoassay are determined with instruments that measure the amount of color, fluorescence or radioactivity of the assay. The level of signal detected is either directly or inversely proportional to the concentration of the antigen of interest.

TYPE OF ENZYME IMMUNOASSAYS

Immunoassay product designs vary and certain formats may be appropriate for specific applications. Immunoassays are categorized according to the way the antigen-antibody complex forms. Competitive ELISA is the immunoassay format in which the target analyte and an enzyme tagged with the target analyte compete to bind to an antibody specific for the target. In this assay, the detecting reagent (antibody), is attached to a solid support. Free antigen in the sample and a test antigen linked to an enzyme compete to bind to the immobilized antibody. The ratio of free analyte to enzyme conjugate determines the amount of enzyme conjugate that will bind to the immobilized antibody. After the unreacted material is removed from the reaction solution, the enzyme converts a substrate to a color product. The level of color is inversely proportional to the amount of antigen in the sample since the free antigen prevents the enzyme-antigen conjugate from binding. Competitive ELISAs are most often used to detect small organic molecules such as pesticides or drugs and will be the format used in this study.

Double-antibody sandwich ELISA. Double-antibody sandwich assays are used to

determine the concentration of large antigens such as proteins, viruses, and bacteria. Antibody is bound to a solid matrix, the sample antigen is allowed to bind, and unbound antigens are removed by washing. Then a second, labelled antibody is added which binds to the immobilized antibody-antigen complex. the assay is quantified by measuring the color produced by the labeled second antibody.

APPLICATION OF IMMUNOASSAYS

HEALTH AND CLINICAL MEDICINE

Immunoassays are used routinely in a variety of applications in the medical field. The first widespread applications were in biomedical research and human diagnostics. Uses include the diagnosis of virus and bacterial infections, cancer screening, drug monitoring, and pregnancy testing.

AGRICULTURAL USES

Immunoassays are used for the diagnosis of diseases and pregnancy in animals (Miller, S. et al., 1988; Lankow, R.K. et al, 1987). More recently immunoassays have been applied to the detection of crop diseases. They are simple, rapid, and sensitive detection methods of pathogens in crops, seeds, bulbs, and soil. Traditionally, the diagnosis of plant diseases was slow and inconsistent because plant pathogens are not easily cultured. Besides conventional methods relied on specialized techniques such as electron microscopy. In contrast detection by immunoassay methods do not require specialized training or equipment. Once limited to the laboratory, immunoassays now provide on-site testing by the growers, consultants and other agricultural professionals. The ready availability of accurate information allows the farmer to make more timely and informed management decisions regarding planting, pesticide use, and harvest timing.

TOXINS AND CONTAMINANTS SCREENING IN FOOD

Antibiotics can be found as residue in animal derived food if improperly used or if withdrawal times have not been observed for treated animals. This can have potential health hazard such as direct toxic effect on the consumer (sulfamethazine), transmission of antibiotic resistance (salmonella), and development of allergy due to drugs that have sensitized some individuals (penicillin). Mycotoxins are diverse family of poisonous fungal metabolites. Aflatoxins, one these toxins can cause edema and necrosis of hepatic and renal tissues. Cereals, bakery and oilseed products present high risk with regard to aflatoxin contamination. Pesticides are applied at the farm levels for the protection of crops against insects and diseases or on various foodstuffs after harvest for protection against various types of pests during extending periods of storage. These chemicals may be found at the levels above their tolerance levels in food and feed. Immunoassays techniques can replace the standard chromatographic methods for the determination of antibiotics, mycotoxins, and pesticides.

ENVIRONMENTAL ANALYSIS

During the past decade, immunoassays have been developed for the monitoring of environmental contaminants. Monoclonal antibody technology combined with new techniques to produce sensitive and specific assays have allow the detection and quantification of pesticides, PCB's, petroleum and other contaminants. Today more than 50 commercial and experimental immunoassays have been described for the detection of these types of compounds. Immunoassays can be used during site assessment;

remediation and post remediation monitoring; RCRA testing.

CHAPTER III

CHARACTERISTICS OF THE PESTICIDES

ALACHLOR

USES

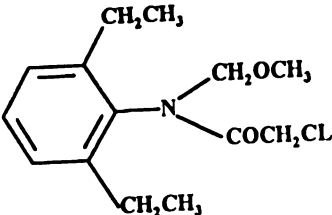
Alachlor [(chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetanilide], is a selective systemic herbicide used for pre- and post-emergence control of most annual grasses and broad-leaved weeds in such crops as corn (*Zea mays* L.), soybeans [*Glycine max* (L.) Merr.], peanuts (*Arachis hypogaeae*), cotton (*Gossypium* Spp.) and sugarcane (*Saccharum* Spp.) (Anonymous, 1988). Lasso® which contains of alachlor as the active ingredient is one of the most widely used herbicides in North America (Sun, 1986).

BEHAVIOR OF ALACHLOR IN PANTS

Alachlor is absorbed and translocated by tolerant and susceptible plants. The basis for the selective toxicity is the rapidity of metabolic deactivation. In tolerant plants, the herbicide is detoxified by rapid conjugation with glutathione (GSH) and/or homoglutathione (hGSH) (Breux et al., 1987). The GSH conjugate is subsequently metabolized to malonylcysteine conjugate (WSSA Herbicide handbook, 7th edition, 1994).

The site of action of alachlor is unknown but it may as most of the chloroacetamides, inhibit lipid and protein synthesis and interfere with respiration and photosynthesis and

Table 1. Chemical and physical properties of the pure alachlor

Structure:	
CAS No:	15972-60-8
Common name:	alachlor
Chemical name:	2-chloro
Trade name:	Lasso
Chemical family:	acetamide
Molecular formula:	$C_{14}H_{20}Cl NO_2$
Molecular weight:	269.77
Manufacturer:	Monsanto
Physical form:	colorless to yellow crystals
Melting point:	39.5 - 41.5 °C
Boiling point:	100 °C at 0.02 mm Hg or 135 °C at 0.3 mm Hg
Vapor pressure:	2.9 mPa at 25 °C
Specific gravity:	1.133 at 25 °C
Stability:	Hydrolyzed by strong acids and alkali. Stable in UV light. Decomposes at 105°C.
Solubility:	into water at 25 °C, 242 mg/L. Soluble in diethylether, acetone, chloroform, and ethyl acetate.

membrane phenomena (Ashton, F.M. and Craft, A.S., 1973. Mode of action of herbicides. John Wiley & Sons, New York, pp 127-146.).

BEHAVIOR OF ALACHLOR IN SOIL

Alachlor undergoes chemical and microbial degradation in soil. Chemical degradation of alachlor occurs under low humidity and high temperature conditions, resulting in formation of 2-chloro-2',6'-diethyl acetanilide. This intermediate decomposition product did not accumulate under natural soil conditions (Hargrove and Merkle, 1971). Microbial degradation was found to be the major route of alachlor degradation in soil with half-lives ranging between 2 to 14 days for several soils (Beetstman and Deming, 1972).

BEHAVIOR IN AQUATIC ENVIRONMENT

Little information is available on the degradation of alachlor in aquatic systems. Studies found mineralization in aquifer materials to be extremely slow (Novick et al., 1986). Under flooded soil conditions, eight metabolites were detected (Lee, 1984).

TOXICOLOGICAL PROPERTIES

Metabolism of alachlor in domestic animals is poorly understood but it is similar to that in plants. While plants retain metabolites, animal eliminate metabolites quickly and almost entirely. In rat, alachlor was rapidly metabolized and the metabolites in urine and feces were excreted as conjugates of mercapturic acid, glucuronic acid, sulfate and

products hydroxylated at the O-alkyl substituents (USEPA, 1984).

Alachlor has been classified in group B2 by the EPA, as a probable human carcinogen (U.S. EPA, 1986). The USEPA has established residues tolerances for food and feed expressed as DEA and HEEA. However, the proposed methods for tolerance enforcement may not measure all metabolites of toxicological concern (Kovacs, 1986). The wide use of alachlor suggests that the major pathways of human exposure are direct contact during application, dietary exposures from ingested residue-containing foods, and drinking contaminated water. Alachlor exhibits low mammalian acute toxicity, $LD_{50} = 0.93$ (rat) and a systemic NOEL of 30 mg/kg in food (1.5 mg/kg/d) was determined (USEPA, 1984).

Effects of alachlor on wildlife and aquatic organisms is of concern because the herbicides may reach surface waters inadvertently through runoff from terrestrial treated fields and spray drifts. Browsing animals may be exposed to residues that persist in terrestrial plants. The herbicide has low avian toxicity, is slightly toxic to aquatic invertebrates, and moderately toxic to fish. Dietary LC_{50} values of alachlor for mallard ducklings is $>5,000$ and the 96-hr LC_{50} for blue gill is 2.8 mg/L. Limited data show fish are unlikely to accumulate alachlor because of rapid elimination. Call et al.(1984) found that 85% of ^{14}C alachlor injected into rainbow trout is readily eliminated within 24 hr, 40% as metabolites. The same study showed that ^{14}C -Alachlor was absorbed rapidly by fathead minnows and the BCFs of total radioactivity from 1-21 d were 50 and 41 for exposures at 0.66 and 9.95 mg/L respectively. However, only 13% of total ^{14}C was extracted as parent compound, for a mean BCF of 6.0 as alachlor. In all the studies,

tissue residues declined to low levels after depuration, probably due to metabolism and excretion.

ATRAZINE

USES

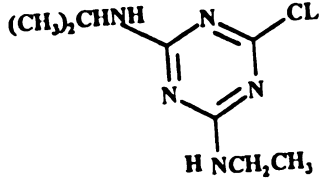
Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] is a triazine herbicide marketed under the trade names of Gesaprim® and Aatrex®. Atrazine is the second most widely used pesticide in the USA, mostly in corn production. It is also used in sorghum, sugarcane and a variety of other crops. Current annual sales are approximately 27.2 million kg (Regehr, 1992).

BEHAVIOR IN PLANTS

Atrazine is absorbed through roots from soil applications and translocates to the shoots via the apoplast. It is also adsorbed into leaves from post applications. Tolerant plants such as maize or millet possess efficient detoxification mechanisms. In these plants, atrazine is rapidly detoxified by conjugation with Glutathione (GSH). Benzoxazinone-catalyzed hydrolysis and N-dealkylation of side chains contributed significantly to the detoxification of atrazine (Anonymous, 1994).

Atrazine inhibits the photosynthetic transport of electrons and this inhibition affects processes dependent on photosynthesis such as the opening of stomata, transpiration, ion transport, which may lead to the disruption of overall metabolism including RNA, enzyme, and protein synthesis (Ebert, E. and Dunford, S.W., 1976).

Table 2. Chemical and physical properties of the pure atrazine

Structure:	
Common name:	Atrazine
CAS register No:	1912-24-9
Chemical name:	2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine
Trade name:	Gesaprim (Ciba-Geigy), Primatol (Ciba-Geigy), Aatrex (Ciba-Geigy)
Chemical family:	Triazine
Molecular formula:	C ₆ H ₁₄ Cl N ₆
Molecular weight:	215.69
Manufacture:	Ciba-geigy
Physical form:	colorless crystals
Melting point:	176 °C
Vapor pressure:	0.04 mPa at 20 °C
Stability:	stable in neutral, weekly acidic and weakly alkaline media. Hydrolyzed to the herbicidally-inactive hydroxy derivative in strong acid and alkalis, and at higher temperature, in neutral media.
Solubility:	in water at 20 °C, 28 mg/L; in dimethylsulfoxide 183, chloroform 52, ethyl acetate 28. methanol 18, diethyl ether

In the environment atrazine is metabolized in three ways (Knuesli et al., 1969): (1). By hydrolysis of the chlorine-carbon bonds yielding a non phytotoxic compound, hydroxyatrazine, which is one of the main metabolites in both soil and aquatic systems; (2). By N-dealkylation of carbon atom 4 and/or carbon atom 6. This gives rise to deethylatrazine, deisopropylatrazine, and diaminochloro-s-triazine (Schiavon, 1988;). (3). By splitting of the triazine ring, usually caused by microorganisms (Wolf and Marin, 1975). The products resulting from decomposition are less toxic to plants and animals than the original substances (Straton, 1984).

BEHAVIOR IN SOIL

Atrazine has come under close scrutiny due to its persistence in soil which can causes injury to succeeding sensitive plants during crop rotation. Its concentration and persistence in the soil depends on the amount applied, soil composition, and climate. The data for the persistence of atrazine in the soil are extremely variable. The half-life range from 20 to more than 385 days (Winkelmann and Klaine, 1991).

Atrazine is moderately adsorbed to soil and adsorption increases at lower pH. Biological degradation contributes to a moderate extent to field dissipation of atrazine. The products of biological degradation are N-deethylated atrazine and N-demethylated atrazine. Soil hydrolysis of atrazine is slow at high pH (7.5-8), but it contributes to degradation at lower pH (5.5-6.5) producing hydroxy atrazine.

Atrazine has recently been identify as a potential pollutant of both ground water and surface water. Surface water contamination occurs primarily as the results of runoff

processes following precipitation or irrigation. Loss due to runoff may reach up to 18% of the total volume of applied atrazine. The transport of atrazine in the soil is affected by structure and composition of the soil and by climatic conditions (Premazzi and Steechi, 1990). Sandy soils allow considerably more rapid translocation than humus soils. Under moderately moist conditions, over a period of one year, virtually no translocation occurs beyond a depth of 30 cm (Frank. and Sirons, 1979). In general, atrazine may be classified as a substance that is moderately mobile in soil.

BEHAVIOR IN THE AQUATIC ENVIRONMENT

In aquatic systems, the half-life of atrazine is reported to be between 3 and 300 days (Yoo and Solomon, 1981). Hydrolytic decomposition can be a factor if the medium is slightly acidic. The rate of decomposition is affected by the chemical composition of water. A higher saline content, such as may occur in the estuaries appears to accelerate the decomposition in water (Jones et al., 1982).

A large number of reports have documented the occurrence of atrazine in surface water. In the USA, atrazine concentration of 0-87 mg/L have been found, with the majority of the findings below 10 mg/L (Eisler, 1989). A study by Hoermann et al.(1979) investigating nine central European rivers found that 59% of the tested samples contained <0.4 mg/L; only 2% exceeded the 10 mg/L limit. n the case of atrazine-contaminated rivers, concentrations of between 1.4 and 95 mg/L were detected in the river silts (Waldron, 974).

In standing freshwater (ponds, natural lakes, and reservoirs), the levels of atrazine

was found to be considerably lower. In the USA, concentrations up to 2 mg/L have been recorded (Premazzi and Stecchi, 1990).

Many studies have reported on groundwater contamination by atrazine but many of these studies involved major uncertainty due to shortcomings such as non representative sampling selection, non standardized sampling, and non conformity of sampling container used. The EPA study " National Survey of Pesticides in Drinking Water Wells" (U.S.E.P.A, 1990) which seems to be more rigorous reported atrazine concentrations of between 0.18 and 1.04 mg/l with 50% of the tested wells showing concentrations below 0.28 mg/L.

TOXICOLOGICAL PROPERTIES

For most of the organisms, atrazine is taken up from water by adsorption or via the food chain. In fish, direct accumulation of atrazine from water takes place according to simple saturation kinetics; the saturation point is reached after 6 h. The concentration limit for atrazine in the case of whitefish ranges between two to five and does not change significantly even in cases of long-term exposure (Gunkel, 1981). Investigations of atrazine uptake via contaminated food show atrazine taken up from contaminated food is assimilated rapidly. Only 70% of the atrazine taken up can still be determined 30 min after the food has been ingested. After that the quantity of detectable atrazine in fish declines rapidly within 12 h. Effective elimination mechanisms prevent residual concentrations of atrazine in fish. (Gunkel, 1981).

Atrazine degradation is insignificant in most aquatic organisms. Elimination is the

most important pathway of decontamination. The rates of atrazine elimination vary widely depending on the organism. In the algae it is within one minute, hours in case of water fleas, molluscs, and leeches. By contrast, elimination periods reported for fish range from 1.5 to several days (Gunkel, 1981).

In summary, atrazine is taken up more or less easily by most aquatic organisms. However, a large portion of the adsorbed substance is eliminated quickly by the organisms when they reach non contaminated water.

Atrazine has not been shown to present serious adverse effect in wildlife. In fish there is some indications that atrazine has an impact on carp (*Cyprinus carpio*) at even low concentrations (100-1000 mg/L) and short exposure period. At these concentrations, the hydrocortisone and glucose levels increase which indicates a typical stress defense reaction (Eisler, R., 1989).

The LC₅₀ values based on 96 h of exposure are variable depending on the species. Values of 19,000 mg/L have been reported for common carp (*C. carpio*) and 42, 000 mg/L for bluegill sunfish (*Lepomis macrochirus*) (Premazzi and Stecchi, 1990; Eisler, 1989).

Various studies have reported with NOEC or LOEC values. These values vary between 100 and 2,100 mg/L in the case of trout (*S. gairdneri*) with an exposure time of 96 h. Values of 1,000 mg/L have been measured for Blue gill (*Lepomis macrochirus*). The NOEC values decline with longer exposure time (Premazzi and Stecchi, 1990; Eisler, 1989).

CARBOFURAN

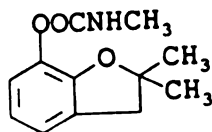
USES

Carbofuran, (2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate), is a broadspectrum insecticide-nematicide used in a variety of crops (Cox, 1966; Turnipseed, 1967). It is effective as both a contact and systemic insecticides (Shorey and Hale, 1967). It is used to control a wide range of agricultural pests (Caro et al., 1973). In the midwest of the USA, carbofuran has provided effective control of the corn root worm. Carbofuran is also used for the control of root weevils and a moth larva in small fruits. These include: the strawberry root weevil, *Brachyrhinus ovatus* (L.), and the bush weevil, *Nemocestes incauptus* (Horn) in strawberry; the bud weevil, *B. Singularis* (L.) in raspberry; the black vine weevil, *B. sulcatus* (Fab.), and in blueberry; the black-headed fireworm, *Rhopobota naevana* (Hub.). In Africa, carbofuran has been increasingly used as the most effective insecticide to control rice pests in paddy fields. Some of the pests controlled are the green leafhopper (*Nephotettix virescens*), the brown planthopper (*Nilaparvata lugens*) and the stem borers (*Tryphorhiza mcertulas*, *chilo suppressalis*).

BEHAVIOR IN PLANTS

Because of its systemic nature, carbofuran is taken up by the root system and distributes throughout the entire plant and could remain in grains or plant materials after harvesting. Carbofuran is metabolized by hydroxylation and hydrolysis in plants (Metcalf et al., 1968). Carbofuran deteriorates rapidly on vegetation sprayed with flowable and wettable powder formulation (e.g., half-life of less than 7 days on alfalfa and Bermuda

Table 3. Chemical and physical properties of the pure atrazine

Structure:

Common name:	Carbofuran
CAS register No.:	1563-66-2
Chemical name:	2,3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate
Trade name:	Furadan (FMC), Curater (Bayer), Bay 70143 (bayer)
Chemical family:	carbamate
Molecular formula:	C₁₂H₁₅NO₃
Molecular weight:	221.25
Manufacturer:	FMC, Bayer
Physical form:	colorless crystals
Melting point:	153-154 °C (pure), 150-152 °C
Vapor pressure:	2.7 mPa at 33 °C
Specific gravity:	1.18 at 20 °C
Stability:	unstable in alkaline media, stable in acidic and neutral media.
Solubility:	in water, 700 mg/L. In acetone 150, acetonitrile 140, dichloromethane 120, cyclohexanone 90, benzene 40,

grass) (Leuck et al., 1968; Fahey et al., 1970). In furrow application of granular formulation carbofuran is readily translocated through the roots and stems, with significant insecticidal activity continuing in foliage for 2 to 4 months. An appreciable amount of carbofuran and its major metabolite 3-hydroxycarbofuran remain in the leaves of corn at silage stage as well as at harvest (Turner and Caro, 1973).

BEHAVIOR IN SOILS

Carbofuran's fate in soil is affected by the pesticide formulation, the rate and method of application, soil type, pH, rainfall and irrigation, temperature, moisture content, and microbial population (Kuhr and Dorough, 1976). Carbofuran is stable at pH 5.5 but decomposes rapidly in alkaline soil. The hydrolytic half-life in soil at pH 7 is about 35 days (Finlayson et al, 1979). Temperature and moisture content are positively correlated with degradation; maximum degradation to hydrolytic metabolites occurs at 27 °C (Ou et al., 1982). The terrestrial dissipation half-life of carbofuran in irrigated soils is reported to be 4 to 11 days in sandy loam, and less than 5 months in silty loam (U.S. EPA, 1991). Carbofuran is mobile and likely to be found in streams, surface water, and runoff sediments from treated watersheds (U.S. EPA, 1991). This suggests that carbofuran may be quite stable in regions of significant acid precipitation

BEHAVIOR IN AQUATIC ENVIRONMENT

Carbofuran is soluble in water to 700 mg/l (25 °C) and in most organic solvents to 30%. It is essentially stable in acidic medium (Baron, 1991). The fate of carbofuran

in water is predominantly function of the pH, but is also influenced by photolysis, temperature, and trace impurities (Seiber et al., 1978). The half life of carbofuran in distilled water at 25 °C and pH 5.5 is 16.4; and decreases when pH increases. At pH 9 the half-life is 6 h (Finlayson et al., 1979). The rate of hydrolysis is positively correlated with ambient temperature.

TOXICOLOGICAL PROPERTIES

Aquatic invertebrates and fish do not tend to bioaccumulate carbofuran when studied in slightly alkaline model ecosystems (Isensee and Tayaputch, 1986). Crabs may be the exception, as one species (*Uca mimax*) is reported to bioaccumulate carbofuran (Yu et al., 1974).

Carbofuran is unstable in living animals and is readily excreted. Therefore significant bioaccumulation is not expected from sublethal exposure of either invertebrates or vertebrates (Finlayson et al., 1979). Nonetheless, a secondary hazard from carbofuran occurs in predatory vertebrates that feed on dead and struggling insects, earthworms, and small birds and mammals (US EPA, 1991). The source of poisoning is most likely to be from unabsorbed carbofuran on the cuticle of arthropods or in the gut of worms and small vertebrates.

Carbofuran is highly toxic to fish, but considerably less toxic to tubificid worms and marine shellfish. The 96-h acute toxicity tests of with seven species of juvenile fresh water fish indicate that LC_{50} s varied from 147 mg of carbofuran per liter for yellow perch (*Perch flavescens*) to 872 mg of carbofuran per liter for fathead minnow (*pimephales*

promelas) (Johnson and Finley, 1980). Two species of freshwater annelid worms and four species of salt water bivalve molluscs gave 96-h LC_{50} for carbofuran that varies from 3.75 to 125 mg/l (Eisler, 1985). The 96-h LC_{50} for the red crayfish (*Procambarus clarki*) is 2.26 mg/l (U.S. EPA, 1991). The 48-h EC_{50} for *Daphnia magna* and *Chironomus riparius* is 48 and 56 mg of carbofuran per liter (Johnson, 1986). Few adverse non lethal effects are reported for carbofuran in practical controlled studies of aquatic organisms. At current registered application rates, carbofuran has not proven accumulative in aquatic systems and poses little chronic hazard to fish and invertebrates. However, it has been observed that at the application rates in variety of formulations, carbofuran has been held responsible for sporadic fish kills (Flickinger et al., 1980).

Because carbofuran is sufficiently toxic to most aquatic organisms, a special warning is required on all use labels against application of carbofuran to water either directly or through drift or run-off from treated areas.

Carbofuran is highly toxic to most terrestrial animals and is non specific in its action on non beneficial non targeted invertebrates species (Finlayson et al., 1979). At recommended field application rates, losses of earthworms and springtails (Collembola) have occurred. Similarly, predatory and parasitic soil insects and parasites and predators of foliage pests are also vulnerable.

Birds and mammals are highly sensitive to acute or oral dosage of carbofuran and usually die within a few minutes of exposure, or recover with little evidence of toxicity within 0.5 to 2 h (Hill and camardese, 1984). The toxicity of carbofuran is a function of the formulation; the flowable concentrate, Ferritin® 4F is about four times as toxic as

granular Ferritin® 15G to northern bobwhite and the wettable powder Ferritin® 75 WP is nearly seven times as toxic as granular Ferritin® 10G to laboratory rats (Finlayson et al, 1979).

Experimental and operational agricultural applications of both foliar and soil-incorporated treatment with carbofuran in various formulations have consistently killed large number of birds of many species. Field studies have shown avian mortality and death of amphibians, reptiles, and mammals when carbofuran is applied in different formulation on corn, rice, and pine seed orchard, and alfalfa (U.S. EPA, 1991). Carbofuran is especially hazardous because of its extreme acute toxicity.

Carbofuran formulations and applications are highly toxic to most terrestrial wildlife, but the acute action is short-lived in survivors (Hill, 1992), and it is non-accumulative in most biological systems (Finlayson et al., 1979). Sublethal exposure to carbofuran is not usually expected to pose an important direct hazard to birds and mammals, but indirect effects due to reduced invertebrate food base and plant pollinators may be significant (Eisler, 1985). Also there is evidence that inclement weather may exarcebate the toxicity of low-grade exposure in young birds (Martin and Salmon, 1991).

Because of its high acute toxicity to fish and mammals (LD50 11 mg/Kg in rats), the fate of its residues in terms of its persistence, mobility, and dissipation pathways is of great concern.

CHAPTER IV

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLE COLLECTION

FISH REARING

The fish used in the experiment were bluegills (*Lepomis macrochirus*) size 4-5 inches, purchased from a commercial hatchery in Dexter, Michigan. The fish were first acclimated in the greenhouse by putting them in a big tank, and later they were transferred in 40 ml aquarium and exposed to the pesticides. The rearing conditions are summarized in table 1 (Appendix D). Stock solutions were prepared by diluting 1 ml of the commercial formulation in distilled water to reach 2.4 g/L for carbofuran (Furadan) and 1.2 g/L for atrazine (Aatrex) and alachlor (Lasso). For each pesticide the final concentration in the tanks were made in consideration of the 96h LC₅₀ for bluegill. The experiment consisted on a control (non treated) and two treatments as follow: for atrazine; for alachlor 1.2 and 2.4 mg/L; for carbofuran 0.12 and 0.24 mg/L. To avoid ammonia build-up in the tank, 25% of water in the tank was replaced each day. In order to keep constant exposure, 25% of the initial amount of pesticide was added each day. Each treatment was replicated and replication 1 was treated each day until the fourteen day by which the fish were removed from the tanks, and killed. For replication 2, the treatments were stopped 7 days before the fish were removed from the tanks. At the end of the

exposition, fish were removed from the tanks, killed, the scales removed, and effiletted. The fillets were put on plastic bags, marked and kept in the freezer until analysis.

CORN RAISING

Field corns (Great Lakes Signature hybrid GL 420) were grown in greenhouse and treated with the three pesticides. Atrazine (Aatrex 4L) and alachlor (Lasso 4EC) were applied by spray after corn plants have emerged. The experiment consisted on three treatments and one control. The treatments consist on half of the recommended rate (1 lb a.i./A); the recommended rate (2 lb a.i./A), and twice the recommended rate (4 lb a.i./A). These treatments correspond to 2.5 ml, 5.0, 10.0 of a.i. in 250 ml of water respectively. The characteristics of the sprayer were the following: 8001E/100 mesh screen, 10 inches high, 14 inches band width, and 30 gpa.

Because of its high vapor pressure that may cause intoxication, carbofuran was incorporated to the soil. The pots were first filled with soil and topped with 1 cm of the treated. The experiment consists on three treatments and a control (non treated). Treatment one was the recommended concentration (1 lb a.i./A), treatment two was twice the recommended rate (2 lb a.i./A) and treatment three was three times the recommended rate (3 lb a.i./A).

Corn leaves were sampled five times during the experiment as follows: one day before corn plants were treated, the day of treatment, five, seven, and eleven days after the treatment. The leaves were chopped using a food chopper and after thorough mixing, the samples were stored in refrigerator in deep freeze for further analysis.

MATERIALS

REAGENTS

- **Solvents:** methanol, acetone, and acetonitrile (Burdick and Jackson, Muskegon, MI) were pesticide grade (ChromAR-HPLC).
- **Analytical Reference:** Analytical reference of carbofuran (99.5%) and its metabolites 3-hydroxycarbofuran were provided free by FCC Corp., Agricultural Chemical Group (Princeton, NJ). Analytical standard stock solution of carbofuran was prepared in HPLC-grade methanol and stored in refrigerator. Formulated carbofuran (4F) used for fish exposure was provided by FCC Ag (Princeton, NJ). Atrazine and its metabolites were provided free by Ciba Geigy (Greensboro, NC). Formulated atrazine (Aatrex 4L) used for fish exposure and plant treatment was provided free by Ciba-Geigy. Alachlor and its analogs acetolachlor, metolachlor, and propachlor were purchased from AccuStandard (New Haven, CT). The formulated alachlor (Lasso) used for fish exposure and plant treatment was provided by Monsanto.

EQUIPMENT

ELISA

The RaPID™ Assays kits for alachlor, carbofuran, and atrazine; the magnetic separation rack; and the RAPID Photometric Analyzer™ were purchased from Ohmicron (Newton, PA). The kit contained: anti-pesticide antibody coupled to magnetic particles; pesticide

coupled to peroxidase; standard solutions of the pesticides in water; enzyme substrate solution; color generating products (peroxidase solution and chromogen solution); stopping solution (2M sulfuric acid solution); buffer saline diluent; washing solution; and test tubes.

EnviroGard™ assay kits for alachlor, carbofuran, and atrazine were purchased from Millipore (Bedford, MA). The kits contain antibody-coated test tubes; standard solutions of the pesticides; pesticide enzyme conjugate; enzyme substrate solution; Chromogen solution

CHROMATOGRAPHY

- Hewlett-Packard Model 5890 Series II GC (Palo Alto, CA) equipped with a ⁶³Ni electron capture detector (ECD) and a nitrogen specific detector (NPD).
- HP Model 7673 automatic injector
- J&W fused silica capillary column (Durabond); ID #122-5042; Liquid phase: DB-5 (non-extractable bonded phase); film thickness, 0.25 µm; column dimensions: 30 M x 0.333 mm id.
- J&W fused silica capillary column (Durabond); ID #122-5042; Liquid phase: DB-5 non-extractable bonded phase); film thickness, 0.25 µm; column dimensions: 15 x 0.333 mm id.

ANALYTICAL METHODS

EXTRACTION OF FISH FOR ELISA DETECTION

Five g of chopped fish fillet was spiked with standard solution of atrazine and let stand for 2 hr at room temperature (25 °C). The sample was grounded with twice its weight with Na_2SO_4 in a mortar and placed into an erlenmeyer. The grounded sample was homogenized for 10 min with 50 ml acetonitrile using a mechanical homogenizer. The homogenate let stand alone for 3-5 min, then an aliquot of the supernatant was collected and analyzed after dilution with the supplied buffered saline diluent (Ohmicron kits) or distillate water (Millipore kits). For carbofuran, the samples were also extracted with water and the results compared with acetonitrile. For water extraction, fish fillet was chopped in small pieces, grounded with 5 grams of Na_2SO_4 and blended in Sorvall Omni-Mixer with 50 ml distillate. The extract was analyzed according to the same procedure described above.

EXTRACTION OF CORN LEAF FOR ELISA DETECTION

Atrazine. 15 g of chopped corn leaves was blended for 2 min with 50 ml methanol and filtered with celite 455 under vacuum using whatman No. 1 filter.

Carbofuran. 15 gram chopped leave samples were placed into a round bottom flask. Seventy five ml of 0.25N HCl solution was added to the flask and heated for 1 hr (stirring). After cooling, the slurry was filtered under vacuum using Whatman No 1 filter paper and analyzed after dilution. When decoloration was needed, the slurry was mixed

with 2 grams of charcoal and celite 455 and filtered under vacuum using Whatman No 1 filter paper.

Alachlor. 15 grams of chopped leaves were placed into an erlenmeyer, 50 ml solution of 10% water in acetonitrile was added and homogenized and filtered under vacuum using whatman No 1 filter paper and analyzed after dilution.

EXTRACTION OF CORN LEAF FOR CHROMATOGRAPHIC ANALYSIS

Atrazine. 50 g of sample was blended with 100 ml of methanol for 2 min. The homogenate was filtered with celite 455 under vacuum using whatman No 1 filter paper. The filter cake was washed with 50 ml and an aliquot of 50 ml of extract was placed into a separatory funnel and extracted three times with 50 ml dichloromethane (DCM). The DCM extract was evaporated to dryness under vacuum, and diluted with 2 ml hexane and submitted to clean-up. For the clean-up, the glass column was filled with 10 g basic alumina topped with 2-3 g Na_2SO_4 . The column was washed with 40 ml 20% (v/v) ethyl ether (EE)/hexane. After transferring the extract into the column, the column was eluted with 40 ml 20% (v/v) EE/hexane which was discarded, then with 120 ml 20% EE/hexane which was collected and evaporated near dryness, redissolved in 2 ml hexane and injected into the GC.

Carbofuran. 25 g of sample of chopped sample was placed into a round bottom flask. One hundred fifty ml of 0.25N HCl solution was added to the flask and refluxed for 1 hr (stirring). After cooling,, the homogenate was filtered under vacuum using Whatman No 1 filter paper and the filter cake rinsed with 50 ml methylene chloride followed by 50

ml of 0.25N HCL. Each filtrate was transferred to a separatory funnel and after shaking, the methylene chloride layer was removed. Then the aqueous fraction was further extracted with 3 x 50 ml methylene chloride. The combined methylene layer was dried over anhydrous Na_2SO_4 and decolonized by adding 2 g of charcoal and filtered later by gravity. The filtrate was evaporated to dryness, then redissolved in 5 ml of 5% (v/v) acetone in hexane and subjected to a clean-up.

For the clean-up, the column was filled with 7 g of silica gel and topped with 2-3 g of anhydrous Na_2SO_4 . The column was washed with 50 ml hexane and the 5 ml extract was loaded into the column. Initially the column was eluted with 50 ml 5% (v/v) acetone in hexane, which was discarded. It was further eluted with 250 ml of 10% acetone in hexane, which was then evaporated to nearly dryness and redissolved in 5 ml hexane and analyzed by NPD-GC.

Alachlor. Twenty five grams of chopped leaves was blended for 3 minutes with 250 mL 10% acetone in water. The suspension was filtered under vacuum and the filtrate reduced to ~30 ml using a rotary evaporator. The evaporate residue was transferred into a separatory funnel with 2 x 50 ml 5% sodium sulfate solution and partitioned twice with 100 ml hexane. The combined hexane layers was dried over anhydrous Na_2SO_4 and evaporated to dryness using a rotary evaporator. The residue was taken to 10 ml with hexane and subjected to clean-up. For the clean-up, the column was filled with 5.5 grams of alumina/activated florisil and topped with 3-4 grams of anhydrous Na_2SO_4 . The column was pre-washed successively with 50 ml of 10% ethyl acetate in hexane and 50 ml of hexane. Then the extract was loaded into the column. Initially the column was

eluted with 50 ml 2% (v/v) ethyl acetate in hexane followed by 20 ml 5% ethyl acetate in hexane, which was discarded. It was further eluted with 50 ml 10% ethyl acetate in hexane, which was then evaporated to nearly dryness and redissolved in 5 ml hexane and analyzed by ECD-GC.

EXTRACTION OF FISH FOR CHROMATOGRAPHIC ANALYSIS

For all the three pesticides, 10 gram of sample were extracted according to the method of Martin and al. (12). Basically, the sample mixed with 50 gram Na_2SO_4 anhydrous, were grounded with a mortar and homogenized with acetonitrile. The homogenate was filtered, first partitioned with hexane, and second with 10% NaCl water and finely cleanup on silica gel column and analyzed by GC.

DETECTION AND QUANTIFICATION

ELISA ASSAY PROCEDURES

The assay, as applied to corn leaves and fish extract was carried out according to the procedure for water samples specified in the kit by the manufacturer, except all determinations were done in duplicate. Briefly, for Millipore kit, the samples and the enzyme conjugate were added into the test tubes and were allowed to incubate for a 20 minutes at room temperature. After washing the tubes four times with tap water, the substrate was added to the tubes followed by the chromogen and the tubes were allowed to incubate for 10 minutes. After that, the stop solution was added to the tubes. Absorbance readings were made at 450 nm with a portable tube photometer. For

Ohmicron, the standard, control or samples, carbofuran-enzyme conjugate and anti-carbofuran antibody coupled to magnetic particles were incubated in polystyrene tube held in a rack at room temperature for 30 minutes. After the incubation, a magnetic base was coupled to the rack, the tubes were rinsed twice with the washing solution. After having remove the rack from the separator, a freshly prepared chromogen solution was added to each tube and the tubes were incubated. After 20 minutes, an enzyme inhibitor (stop solution) was added to the tubes to stop the color development. The absorbance of the color in the sample and standard tubes was read at 450 nm using a portable tube photometer at 450 nm and the amount of the pesticides determined by reference to the standard curve

The concentration of the pesticide in fish or corn leaf extract is calculated by using the above equation:

$$Y = \text{Assay result (ppb)} \times \frac{\text{Extract vol. (mL)}}{\text{sample weight (g)}} \times \frac{\text{vol. extract (mL)} + \text{vol. diluent (mL)}}{\text{vol. extract (mL)}}$$

CHROMATOGRAPHIC PROCEDURES

A Hewlett packard 5890 Series II gas chromatograph equipped with a ⁶³Ni electron capture detector (ECD) and a nitrogen specific detector (NPD) was used for the detection of the pesticides in fish and corn leaf samples. The injection of the samples into the GC were done automatically using a HP Model 7673 automatic injector. A Hewlett packard computer and Laserjet IIIp printer for GC was used for data handling and processing.

Table 4. Chromatographic conditions for the determinations of the pesticide

Compound	Detector	Column Type	Temperature °C		
			Oven	Injector	Detector
Alachlor	ECD	DB-5 fused capillary 60 M x 25 mm id. 0.25 µm phase thickness. J7W # 122-5042	180	250	250
Atrazine	NPD	DB-5 fused capillary 30 M x 0.333 mm id. 0.25 µm phase thickness J7W 3 122-5043	200	250	250
Carbofuran	NPD	DB-5 fused capillary 30 M x 0.333 mm id. 0.25 µm phase thickness J7W 3 122-5043	200	250	250

CALCULATION OF THE PESTICIDE CONCENTRATION

Quantification of pesticides were based on peak areas. A series of pesticide standard solutions were injected and a standard curve was traced. calculation of the concentration levels for each pesticide in a sample on the wet weight of fish or volume of water was accomplished by the use of the above equations.

$$(1) \text{Standard curve: } y = ax + c \Leftrightarrow x = \frac{y - c}{a}; \quad x = g \text{ of pesticide, } y = \text{peak area}$$

$$(2) \text{Pesticide on final extract, ng/ml} = \frac{\text{ng of pesticide}}{\mu\text{l injection volume}} \times 1000 \mu\text{l/ml}$$

$$(3) \text{ppm} = \text{ng/ml}(\text{extract Vol.}) \times \text{final Vol.} \times (1 / \text{sample Wt}) \times 1 \mu\text{g} / 1000 \text{ng}$$

CONFIRMATION

Confirmation of the pesticide confirmation were performed on a tandem Hewlett-Packard Model 5890A GC (Palo Alto, CA) and a 5970A mass selective detector. Operating conditions were as followed: ionization voltage, 70 eV; ion source temperature 260 °C; electron multiplier 300 V above autotune; direct capillary interface at 300 °C. The filament and multiplier were not turn on until 5 min into the run. DB-5 30 M x 0.333 mm id fused capillary column, 0.25 µm phase thickness was used. The detection was made by using electron capture/negative ionization (ECNCl) in the scan mode. Initial column temperature was set at 80 °C for 5 min and programmed at 15 °C/min for 10 min. Confirmation was based upon presence of the molecular ion and two confirming ions.

APPENDIX C

RESULTS OF CARBOFURAN DETERMINATION

CHAPTER V

RESULTS AND DISCUSSION

The absorbance readings from ELISA are inversely proportional to the concentration of analytes in the sample extracts. The percentage control (total binding) was calculated as the absorbance of the sample (B) at 450 nm divided by the absorbance of the negative control (extract blank, Bo) at 450 nm and times 100. In this study B/Bo was referred as Bo, which plotted versus the log concentration is linear with a negative slope. These plots were used to approximate concentrations of the samples. Cross-reactivities (%CR) were calculated by dividing the metabolite concentration which produced 50% Bo by thealachlor concentration that gave 50% Bo. The least detectable dose (LDD) was the calculated analyte concentration yielding 90% Bo.

ALACHLOR

While performing the ELISA for the present study, blanks and calibration standards were always assayed with the samples. The results are summarized in appendix A. A compilation ofalachlor calibration curves generated with the two different test kits shows a linearity in the range of 0.1 ppb-5 ppb and 0.5-10 ppb for the Ohmicron and Millipore test kits respectively (Figure 9). The RaPID™ kit generated an equation with $R^2 = 0.99$ while the EnvriroGard® (Millipore) kits gave an equation with $R^2 = 0.97$.

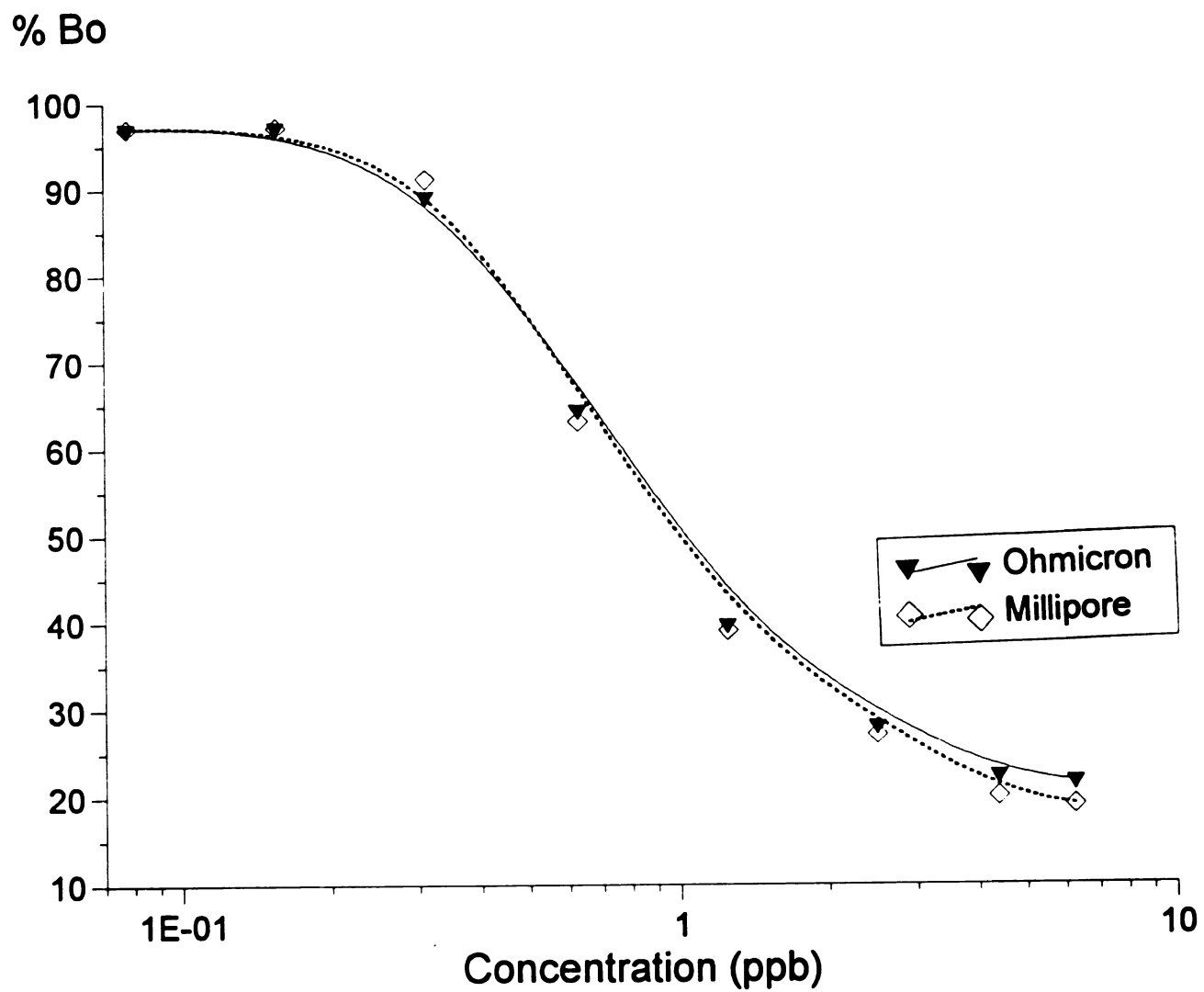


Figure 9. Plot of the standard curves of the Ohmicron and Millipore test kits (average of 4 determinations). %Bo = % (absorbance of the sample/absorbance of the zero control).

The Ohmicron kit has the lowest least detectable dose (LDD) of 0.07 ppb; the LDD for the Millipore kit was determined to be 1.3 ppb.

ACCURACY

The accuracy of the ELISA method was tested using fortified corn leaves and fish samples. Fish samples were spiked at the levels ranging from 5.5 ppb to 880 ppb and corn leaves samples were spiked at the levels ranging from 5.5 to 2200 ppb and assayed using the two ELISA kits.

A limiting factor in the use of ELISA kits for pesticide residue analysis is their requirement for water miscible sample extract. It was therefore necessary to use water miscible solvents for extraction. Acetonitrile and acetone were chosen because of their ability to efficiently extract alachlor from fish and corn leaves respectively. During this study the effects of corn leaves and fish coextracts as well extraction solvent on the determination by the kits were assessed.

FISH FILLET

Non-diluted acetonitrile (ACN) extract was found to give a positive response to the kit; the recovery was between 135 to 167% and 141 to 177% for the RaPID™ and EnviroGard® kits respectively (Table 5). This response enhancement must have been due to either sample matrix or ACN interference. To eliminate this enhancement of the readings, the samples were diluted 10 times. The recovery found ranged from 103 to 115% for the RaPID™ kit and 91 to 116% for EnviroGard® kit (Table 5). When the

Table 5. Accuracy of alachlor determination in spiked fish fillets (2 replications per assay). A: Ohmicron kit; B: Millipore kit.

Spiking Levels (ng/g)	*Assay 1	**Assay 2	***Assay 3
5.5	127	106	nd
11	122	120	108
22	126	113	98
44	118	102	92
110	nd	108	103
154	nd	116	95
Mean	112.75	97.25	99.20
SDV	7.68	4.35	6.40
%CV	7	4	6

Spiking Levels (ng/g)	*Assay 1	**Assay 2	***Assay 3
22	137	91	nd
55	128	111	nd
82.5	121	114	nd
165	119	86	101
275	129	111	96
550	114	102	106
880	112	115	90
1100	139	nd	nd
2200	89	nd	nd
Mean	120.89	99.40	98.25
SDV	15.20	5.46	6.80
%CV	13	5	7

***Dilution 1:10; no decoloration; provided standard**

****Dilution 1:10; decoloration; provided standard**

*****Dilution 1:50; decoloration; provided standard**

standard used in the assay was prepared in 10% ACN in water, the recovery found was in the range of 90.7-100.7% for The RaPID™ kit and 91 - 108% for EnvriroGard® kit.

Thus diluting the extract reduced the effect of matrix without eliminating the positive response due to the organic solvent. The solvent effect was eliminated by using a standard prepared in 10% ACN in water.

CORN LEAF

The assay of the undiluted sample extract gave a recovery between 137-187%. As in the previous case (Fish), this high enhancement of the response must be explained by sample matrices or solvent interference. Diluting the extract 1:10 with water fairly reduced the enhancement effect yielding a recovery of 118-127% as reported in Table 6. When the extract was decolonized by mixing the extract with decolonizing agent and diluted to times, the enhancement previously observed was markedly reduced (recovery 106-120%).

When the extract was diluted 1:50 after decoloration, the response enhancement was eliminated and the recovery was in the normal range (95-108%). The results showed the positive response due to the pigments (carotenoids and xanthophiles) which was eliminated by decolonizing the extract.

The recovery results ranged from 98 to 110% (fish) and 92 to 108% (corn leaves) for the Ohmicron kit and 91 to 117% (fish) and 90 to 106% (corn leaves) for Millipore kit (Table 6). In both fish sample and corn leaves extraction, the recoveries obtained are good. They seemed to be higher for fish samples compared to corn leaves sample. This enhancement may come from the fish matrix since the fish extract was diluted only 10x while corn leaves extracts were diluted 50x.

**Table 6. Accuracy of alachlor determination in spiked corn leaf (2 replications per assay).
A: Ohmicron kit; B: Millipore kit.**

Ohmicron

Spiked Levels (ng/g)	*Assay 1	**Assay 2	***Assay 3
5.5	127	106	nd
11	122	120	108
22	126	113	98
44	118	102	92
110	nd	108	103
154	nd	116	95
Mean	123.25	110.83	99.20
SDV	4.1	6.7	6.4
% CV	3%	6%	6%

* Dilution 1:10; No decoloration; Standard provided

** Dilution 1:10; Decoloration Standard provided

*** Dilution 1:50; Decoloration; Standard provided

Millipore

Spiked Levels (ng/g)	*Assay 1	**Assay 2	***Assay 3
22	137	91	nd
55	128	111	nd
82.5	121	114	nd
165	119	86	101
275	129	111	96
550	114	1021	106
880	112	115	90
1100	139	nd	nd
2200	89	nd	nd
Mean	120.89	235.57	98.25
SDV	15.2	346.5	6.8
% CV	13%	147%	7%

* Dilution 1:10; No decoloration; Standard provided

** Dilution 1:10; Decoloration Standard provided

*** Dilution 1:50; Decoloration; Standard provided

Table 7. Assay reproducibility (%CV) for of the EIA for alachlor in corn leaf extract.

Sample	*Intra-Assay	**Inter-Assay
Standard solutions (ppb)		
0.0	4.3	2.9
2.0	5.1	5.9
10.0	6.9	7.9
20.0	6.1	9.3
100.0	9.5	8.9
Mean	6.38	6.98
Spikes (ng/g)		
55.0	11.1	9.4
165.0	5.1	10.7
550.0	4.7	12.1
Mean	7.00	10.70

Sample	*Intra-Assay	**Inter-Assay
Standard solutions (ppb)		
0.0	3.0	2.9
0.1	7.9	5.9
1.0	6.4	7.9
5.0	5.9	9.3
Mean	5.8	6.98
Spikes (ng/g)		
5.5	3.0	6.7
11.0	7.9	7.9
44.0	6.4	9.0
110.0	5.9	6.7
154.0	5.7	5.6
Mean	5.80	7.20

*6 assays within 1 day

**1 assay per day during 6 days

As with any analytical technique, precision within and between days is important. The reproducibility of the extraction technique as well as the ELISA test were determined by performing 3 and 6 replicates assays were performed respectively on standard solutions provided with both ELISA kits and on spiked corn leaves samples. Within-day coefficient variation of 4.3-9.5% (standards) and 4.7-11.1 (spiked samples) were obtained for the Millipore kit and 3.0-7.9% (standards) and 3.0-7.9 (spiked samples) for Ohmicron kit (Table 7). In general the coefficient of variation of Millipore kit are higher than those of Ohmicron kit.

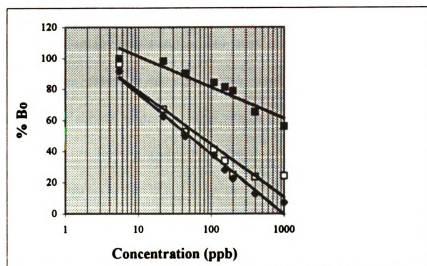
Between-day assays were performed at 3 days and 6 days successively for Millipore and Ohmicron kits respectively. The results showed a coefficient of variation of 2.9-12% for Millipore kit and 4.1-9.0% for Ohmicron kit (Table 7). As the results indicated there is no big difference between the two types of kits. Both kits showed slightly higher variation with inter-days tests compared to between-day assays, but in general the coefficients of variation were the ranges recommended by the manufacturers (%CV < 12%).

CROSS-REACTIVITY

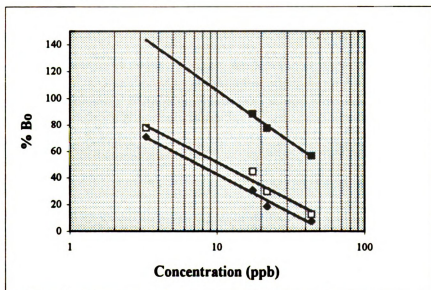
Alachlor belongs to a family of structurally similar chloroacetanilide herbicides. Specificity of the antibodies for alachlor was therefore crucial for the successful application of this assay to residue analysis. Studies were conducted to determine the specificity of the antibodies for alachlor. Both alachlor kits rely on antibodies which are

Figure 10. Response of the ELISA kits to alachlor and metolachlor. ($\square$) alachlor; ($\blacksquare$) metolachlor; ($\blacklozenge$) mixture.

A. Ohmicron test kit



B. Millipore test kit



specific to alachlor. In order to measure cross reactivity between alachlor and its related compounds, metolachlor, acetolachlor, and propachlor, corn leaf extracts were spiked at various levels of alachlor, acetolachlor, and propachlor separately. The preliminary results showed no detection of propachlor at reasonable levels. With both EIA test kits, the response of metolachlor and acetolachlor were significantly lower than that of alachlor (Figure 10). The presence of metolachlor and acetolachlor produced only a small enhancement to alachlor response. The response of metolachlor and acetolachlor were found to be respectively 7% and 1.7% of that of alachlor with Ohmicron kit, and 18% and 7.3 % with Millipore kit. Feng et al. (1990) have found cross reactivities lower than those found in this study but their study used antibodies developed by themselves which may differ in affinity. The cross-reactivities of the test kit for chemical analogs may due to how a chemical is conjugated to the IgG in the immunization antigen. The exact structure of the alachlor- immunogen used to produce the polyclonal antibodies used in this kit is proprietary, however, it must be similar in structure to those used by other investigators performing ELISA quantitation of alachlor residues (Feng et al., 1994). Only metolachlor, acetolachlor and propachlor were investigated in this study because their are the most widely used chloroacetanilide herbicides

Other chloroacetanilides, mainly those containing a thioether functional group may have greater cross-reactivity with the assay because of the thioether linkages in the immunogen antigen. These compounds were not investigated because they are not widely used. No metabolite was investigated in this study because they are not sold commercially and we were not able to obtain them from the manufacturer.

SENSITIVITY

Table 8. Method determination limit (MDL) calculated from standard deviation (σ) of 6 replicate assays of corn leaf (A) and fish tissue (B) spiked with alachlor.

A. Corn leaf

Ohmicron test kit						Millipore test kit				
Assay #	Abs 1	Abs 2	Mean	%Bo	Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo	Concentration (ppb)
Control	1.700	1.612	1.656			1.675	1.625	1.650		
1	1.040	1.117	1.079	65	0.530	1.432	1.412	1.422	86	1.700
2	0.995	1.126	1.061	64	0.563	1.343	1.377	1.360	82	2.150
3	1.107	1.034	1.071	65	0.544	1.266	1.234	1.250	76	3.240
4	1.180	0.989	1.085	65	0.519	1.382	1.355	1.369	83	2.690
5	1.223	1.040	1.132	68	0.443	1.523	1.544	1.534	93	1.120
6	0.986	1.050	1.018	61	0.592	1.243	1.320	1.282	78	2.880
Mean				65	1	Mean			83	2.297
Stdv				2.22	0.05	Stdv			6.17	0.79
3@				6.66	0.152	3@			18.50	2.380

B. Fish fillet

Ohmicron test kit						Millipore test kit				
Assay #	Abs 1	Abs 2	Mean	%Bo	Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo	Concentration (ppb)
Control	1.412	1.377	1.395			1.745	1.721	1.733		
1	0.876	0.856	0.866	62	0.542	1.465	1.444	1.455	84	2.330
2	0.912	0.897	0.905	65	0.460	1.432	1.387	1.410	81	2.730
3	0.854	0.866	0.860	62	0.556	1.435	1.476	1.456	84	2.320
4	0.843	0.900	0.872	62	0.530	1.378	1.427	1.403	81	4.040
5	0.835	0.921	0.878	63	0.515	1.487	1.466	1.477	85	2.150
6	0.900	0.872	0.886	64	0.498	1.366	1.354	1.360	78	3.260
Mean				63	0.517	Mean			82	2.805
Stdv				1.15	0.03	Stdv			2.50	0.72
3@				3.44	0.103	3@			7.51	2.175

Method detection limit for each kit was evaluated from standard deviation (σ) of 6 replicates of fish and corn leaves samples spiked with alachlor standard solution. With

both test kits, the minimum detectable levels (MDL) (Table 8) obtained were slightly higher than the lower limit of quantification (LOQ) which were 2 ppb and 0.1 ppb for EnviroGard® kit and RAPID™ kit respectively.

INCURRED SAMPLES

Fish fillets and corn leaves coming from fish and corn raised in the greenhouse and treated with alachlor were analyzed using the two ELISA kits and gas chromatography. Because of inhibition due to the extraction solvents and matrices interference, the extracts were diluted 1:10 and 1:50 for fish and corn leaves respectively prior to their analysis.

CORN LEAF

No alachlor was found in the non-treated samples as well as the samples collected the day before the herbicide application (Figure 11). Both kits detected alachlor at similar levels (0.02 µg/g - 0.6 µg/g) in the samples and the levels found were higher in the samples collected the day of treatment and decreased on subsequent days. The decrease in the amount found may be explained by the metabolism of alachlor in corn.

Because some of the metabolites of alachlor may cross react with the detection of alachlor by the ELISA kits, GC and GC/MS were used to evaluate results obtained by the kits. Gas chromatography equipped with electron capture detector (ECD) was used to analyze the samples. The conditions of analysis are described in the method section.

Figure 11. Alachlor concentration in incurred corn leaf samples by ELISA. 2 assays per samples.

DBT = Day before treatment; DOT = Day of treatment; DAT = Day of treatment

NT = Non treated; Trt = treatment

Ohm = Ohmicron; Mill = Millipore

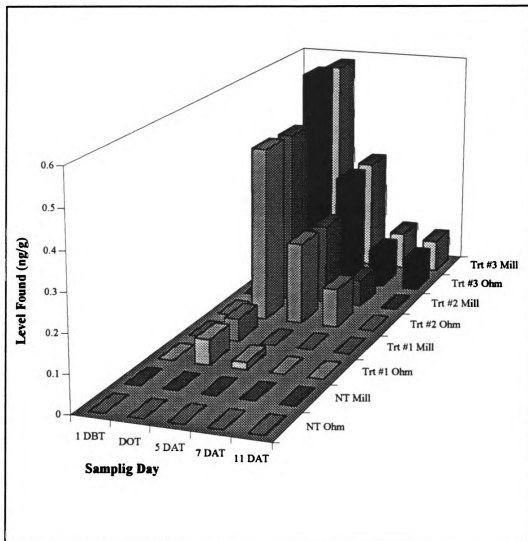


Figure 12 illustrates the correlation obtained when plotting the results of the ELISA tests against GC determination. The results obtained showed a good correlation between GC and the two kits with Ohmicron kit giving a slight better correlation coefficient ($R^2 = 0.997$) compared to the Ohmicron kit ($R^2 = 0.996$).

In plants, alachlor is first conjugated with glutathione (GSH) and/or homoglutathione (hGSH) and later metabolized to malonylcysteine conjugate (Breux et al., 1987). Therefore, there are no metabolites structurally close to alachlor. The chromatographs obtained with GC analysis contained different peaks beside alachlor peak which might correspond to coextracts from plant materials. The results obtained from the ELISA test kits correlated very well with the GC determination. From this I can assume that there is no cross reactivity at the levels obtained from any metabolite or any plant component and therefore the ELISA kits can be successfully used for the quantification of alachlor in plant material.

Confirmation of the incurred corn leaves was accomplished using GC/MS. As illustrated in Figure 13 the mass spectrum of cut 2 (Treatment #3) collected at 7 days after alachlor application yielded the molecular ion at 269 m/e and the fragments at 237, 146, and 160 m/e which were seen in the standard. The non treated sample did not yield the molecular ion nor the fragments seen in the standard. These results confirmed that alachlor was present in the samples for which the ELISA kits as well as GC determinations were positive.

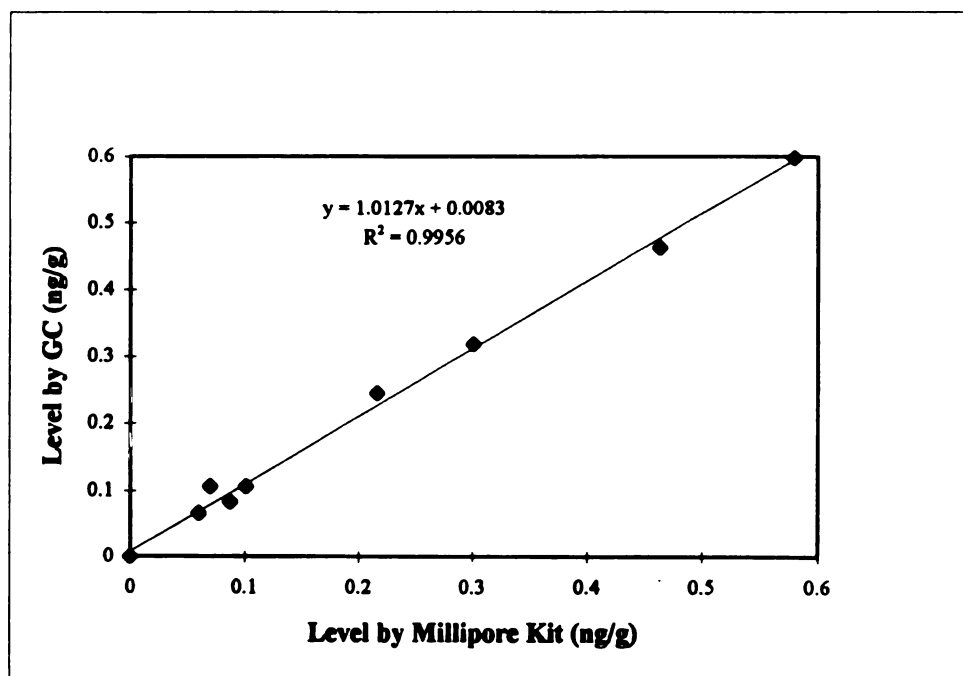
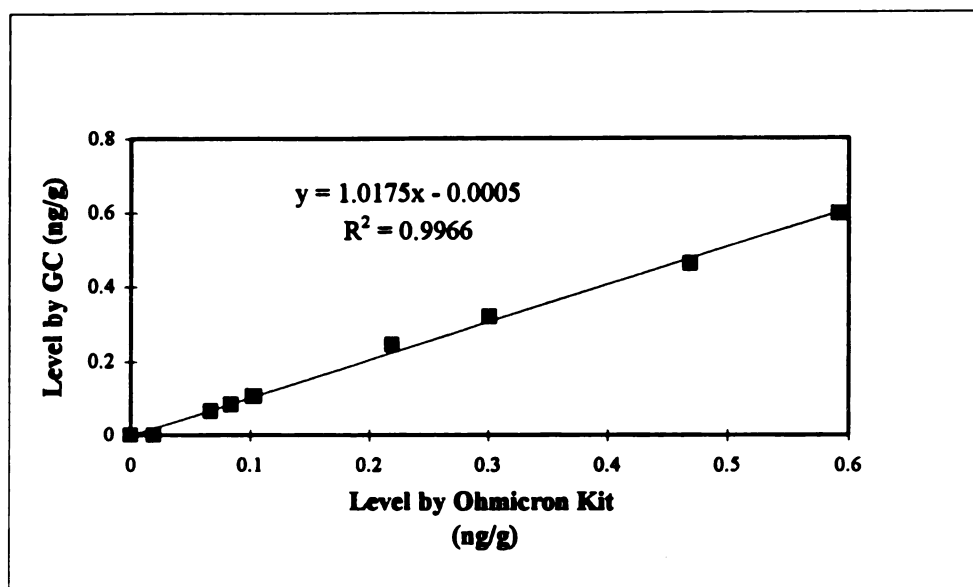


Figure 12. Correlation of alachlor concentrations as determined by ELISA and gc methods, $n=4$, $r = 0.996$, $y = 1.017 X + 0.0005$.

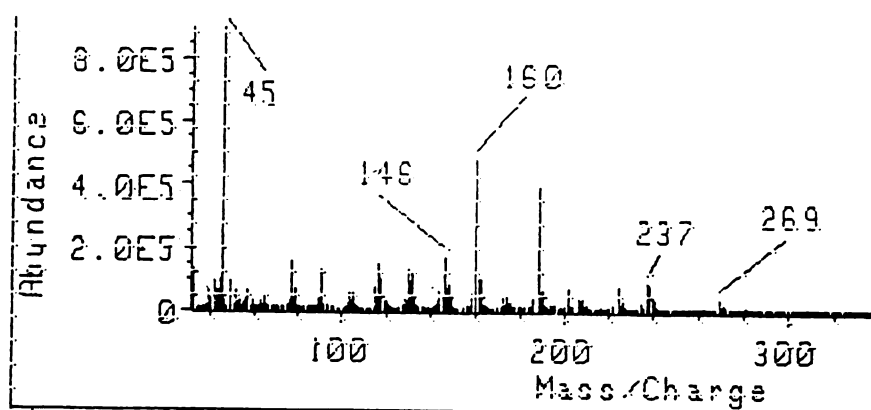
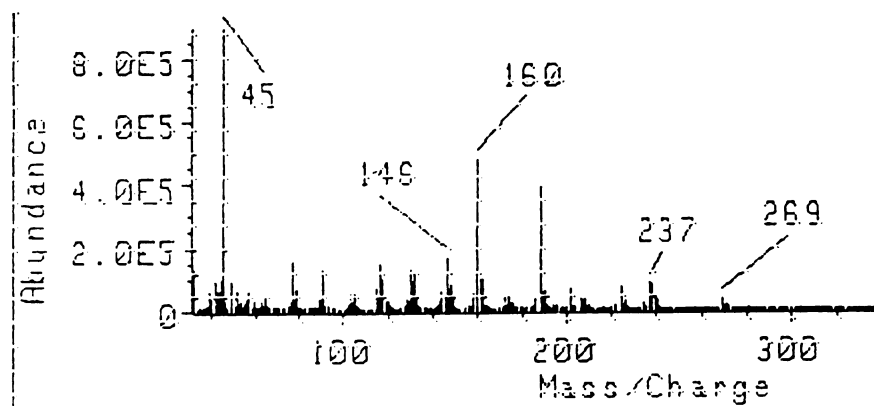


Figure 13. Confirmation of the ELISA results by GC/MS.

Table 9. Alachlor concentration in incurred fish fillet samples by ELISA. 2 assays per samples.

[illegible][illegible]

ATRAZINE

Blanks and calibration standard assays results are summarized in Appendix B. The linearity range of the Millipore and Ohmicron kits were 0.05-5 ppb and 0.25-10 ppb respectively (Figure 14). These results showed that Ohmicron kit was slightly more sensitive than Millipore kit. These results indicated also that the Ohmicron assay can detect very low concentrations (~ 0.05 ppb), while the Millipore kit can detect higher concentrations (~ 15 ppb).

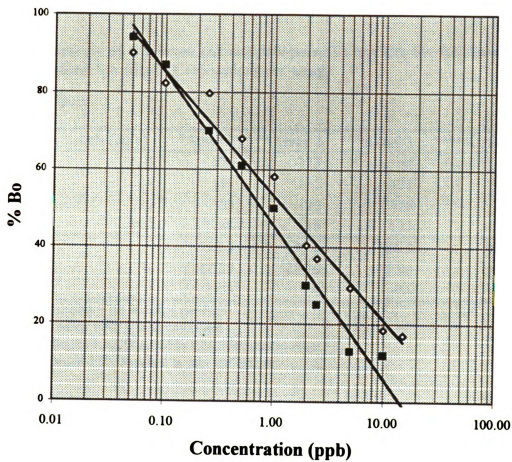
ACCURACY

Corn leaves and fish fillets were spiked with standard solutions, extracted and analyzed with the ELISA kits. The accuracy of the determinations were assessed by calculating the percent recoveries. The results are summarized in Appendix B. Strong matrices and solvent interferences were observed with the assays detections during the recovery study.

CORN LEAF

In the first two assays, the standard provided by the manufacturer was used. When the extract was diluted 1:10, the recoveries were very high indicating inhibition due to either coextracts or the extraction solvent. Diluting the extract 1:50 slightly reduced the enhancement observed in the first assay. A standard prepared in 10% methanol in water was used in the third assay.

Figure 14. Plot of the standard curves of the two test kits (average of 4 determinations).
 $\%B_0 = \% (\text{absorbance of the sample/absorbance of the zero control})$.



As the results (Table 10) indicated, the inhibition due to methanol as extraction solvent was reduced but still the recoveries were above the normal range indicating interferences due to sample matrix.

Table 10. Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination atrazine in spiked fish fillets (2 replications per assay).

A. Millipore

Spiking Levels (ng/g)	*Assay 1	**Assay 2	***Assay 3	****Assay 4
2.5	176	173	nd	nd
25.0	141	132	121	102
50.0	154	122	118	96
100.0	138	116	133	98
Mean	152.3	135.8	124.0	98.7
SDV	17.29	25.70	7.94	3.06
%CV	11%	19%	6%	3%

* Dilution 1:10; Standard provided

** Dilution 1:50; Standard provided

*** Dilution 1:50; Standard in 10% Methanol in water

**** Dilution 1:50; Standard in corn leaf extract

B. Ohmicron

Spiking Levels (ng/g)	*Assay 1	**Assay 2	***Assay 3	****Assay 4
0.7	123	108	98	104
1.0	125	119	91	91
1.5	151	109	116	99
2.0	136	129	120	93
4.0	117	98	100	97
7.0	122	123	123	89
Mean	129.0	114.3	108.0	95.5
SDV	12.47	11.38	13.31	5.58
%CV	10%	10%	12%	6%

* Dilution 1:10; Standard provided

** Dilution 1:50; Standard provided

*** Dilution 1:50; Standard in 10% Methanol in water

**** Dilution 1:50; Standard in corn leaf extract

In the fourth assay, 5 grams of untreated corn leaves were extracted with methanol. The extract diluted 100 times was kept in the refrigerator and used to prepare the standard solutions. The calibration standard solutions were prepared by adding the appropriate amount of atrazine standard solution solutions (10% methanol in water) to an aliquot of this extract. With this standard, the inhibition due to matrix interferences was eliminated.

FISH FILLET

The preliminary results indicated that when the extracts were diluted 1:10 and analyzed using the standards provided by the manufacturer, recoveries above 100% were obtained (Table 10).

Table 11. Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination atrazine in spiked fish fillets (2 replications per assay).

Spiking Levels (ng/g)	Millipore		Ohmicron	
	*Assay 1	**Assay 2	*Assay 1	**Assay 2
0.7	nd	nd	122	104
1.0	129	100	124	97
1.5	111	109	118	100
2.0	115	94	106	99
4.0	119	96	110	95
7.0	122	105	134	94
Mean	119.2	100.8	119.0	98.2
SDV	6.87	6.22	10.10	3.66
%CV	6%	6%	8%	4%

* Dilution 1:10; Standard provided

** Dilution 1:10; Standard in 10% ACN in water

When the standard used was prepared in 10% ACN in water, the mean recoveries were 101% and 98% for Millipore and Ohmicron respectively. These results suggested that acetonitrile can be used as extraction solvent for the ELISA determination but the standard used in the assay should contain a portion of acetonitrile in order to eliminate any inhibition of the detection which may result in false positives.

The overall results showed that in general more matrices interferences occurred in the assay with the Millipore kit compared to the Ohmicron kit. These results showed good recoveries for both kits ranging from 94-109% to 94-104% for the Millipore and Ohmicron kits respectively. The results indicate that there is no significant difference between the two kits.

REPRODUCIBILITY

The results of reproducibility studies are summarized in Appendix B. In this study, 6 replicate assays were performed in one day, on standard solutions provided with the kits for the determination of within-day coefficient of variation. For the determination of the between-day coefficient of variation, duplicate assays were performed on 6 successive days on the same standard solutions. The same assays were performed on corn leaf extracts spiked with standard solutions. Both within and between-assay coefficient of variation were good (Table 12). There was no difference between the two types of kit. The results indicated between-assay coefficient of variation slightly higher than within-assay coefficient of variation.

Table 12. Assay reproducibility for of the EIA for atrazine in corn leaf extract

A. Millipore

Sample	*Intra-Assay	**Inter-Assay
Standars Solutions (ppb)		
0.0	4.1	7.6
0.5	5.1	5.9
2.0	8.0	8.6
10.0	8.7	7.7
Mean	6.5	7.5
Spikes (ng/g)		
0.5	5.9	12.9
1.0	9.2	21.0
2.5	8.9	12.0
5.0	7.6	13.1
10.0	10.4	13.5
Mean	8.1	13.3

* 6 assays within 1 day

** 1 assay per day during 6 days

B. Ohmicron

Sample	*Intra-Assay	**Inter-Assay
Standars Solutions (ppb)		
0.0	4.8	5.6
0.1	3.5	6.1
1.0	4.1	5.2
5.0	8.3	9.9
Mean	5.175	6.7
Spikes (ng/g)		
6.56	7.7	13.3
32.80	4.2	8.3
65.60	3.0	10.4
31.20	6.1	16.7
Mean	5.3	12.2

* 6 assays within 1 day

** 1 assay per day during 6 days

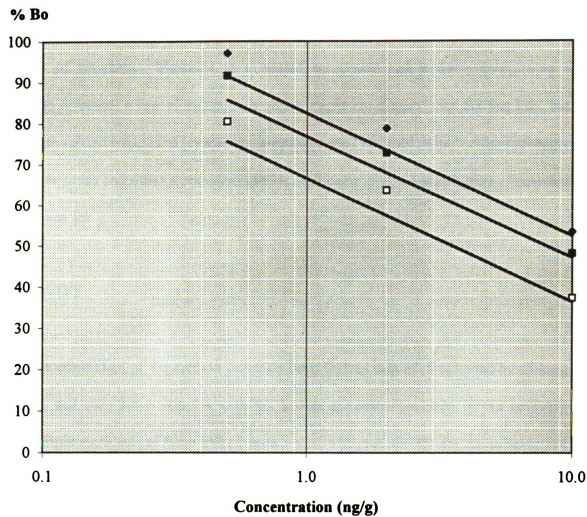
CROSS-REACTIVITY

Atrazine, molecular weight 269, is too small to be immunogenic in its own right. For this reason, atrazine hapten was obtained by derivatizing atrazine at the 2-chloro position which was later conjugated to bovine gamma globulin through a modified carbodiimide by cross-linking procedure (Bushway et al., 1988). The procedures used to prepare the kits used in this assay was not known for proprietary reasons but they must follow the same scheme. Because of the way the hapten is synthesized, the antibodies produced may cross react with compounds closely related to atrazine or metabolites of atrazine.

To assess the cross-reactivity of metabolite and related compound to the kits, assays of atrazine, simazine and hydroxyatrazine were carried out. The results are reported in Appendix B. The response of simazine and hydroxy-atrazine were significantly lower than the atrazine response (Figure 15). The results of Millipore kits were based on 4 assays of 3-point calibration curves, whereas the Ohmicron results were from 4 separate experiments. The reason that the LDD does not correlate with the cross-reactivities is due to the less steep slopes of the metabolite hydroxy-atrazine versus the greater atrazine sensitivity. The fact that hydroxy-atrazine concentration at 50% Bo was more removed from atrazine response than 90% Bo.

The results indicate that simazine as well as hydroxy-atrazine cross reacted more with Millipore kit than with Ohmicron kits; and the LDD's for Ohmicron kits are lower than those of Millipore kits. The overall results indicate the possibility of simazine to

Figure 15. Response of the ELISA kits to atrazine (□), hydroxyatrazine (■), and simazine (◆).



cross react with both kits; this might be a drawback for the kits when samples to be assayed contained simazine which is another s-triazine herbicide used extensively in agriculture. Using Ohmicron kits reduced to a certain extent the possibility of the cross-reactivity of simazine. Simazine as a related compound and hydroxy-atrazine as a metabolite represent a few of compounds which might cross-react with the test kits, but many more related compound (cyanazine, propazine, etc.) are used as herbicides sometime in the same crops and other metabolites (DEA, etc.) occur in plant which may cross-react with the test kit.

SENSITIVITY

Determination of the method detection limit (MDL) was accomplished by spiking fish fillet and corn leave samples with the lowest concentration used in the recovery studies. Eight replicates of these spiked extract were assayed; the results are summarized in Appendix B. The MDL for Ohmicron kits were 0.27 ng/g and 0.20 ng/g for corn leaves and fish fillet samples respectively, whereas the MDL for the Millipore kits were 1.97 ng/g and 0.55 ng/g respectively (Table 13). The MDL for the Ohmicron kits were lower than the MDL for Millipore kits and therefore more sensitive. This can be explained by the fact that corn extract interfered more with the kits detection compared to fish extracts.

INCURRED SAMPLES

CORN LEAF

Corn leaves samples obtained from corn plants grown in the greenhouse and sprayed with atrazine were assayed with both kits. The two test kits detect similar amounts of atrazine, but only the Ohmicron kits were able to detect very low levels (0.04-.06 ng/g) (Figure 16). This can be explained by the fact that Ohmicron kits (MDL = 0.27 ng/g) compared to Millipore kits (1.97 ng/g).

In order to verify the accuracy of the determination by both ELISA kits, corn leaves samples were extracted according to the procedure described in the method section, and analyzed by GC/NPD. The results are reported in Appendix B. The results showed that there is good correlation ($R^2 = 99$) between the ELISA kits and the GC determinations (Figure 17). These results indicate that the ELISA kits can be used for qualitative as well as quantitative determination of atrazine in plant materials.

FISH SAMPLES

Incurred fish samples were also analyzed by the ELISA kits. The results are reported in appendix B. No atrazine was found in any of the extracts by either the Millipore kit nor GC; only the Ohmicron kits, because of their high sensitivity, detected atrazine at the levels ranging from 0.042-0.118 $\mu\text{g/g}$ in two fish (Table 14).

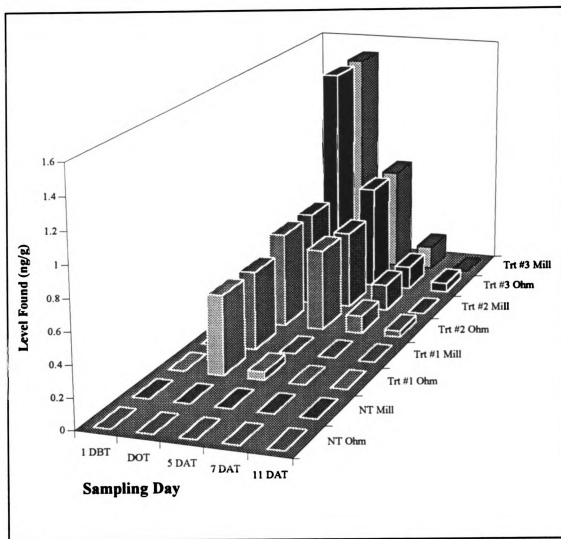


Figure 16. Atrazine concentration in incurred corn leaf samples by ELISA. 2 assays per samples.

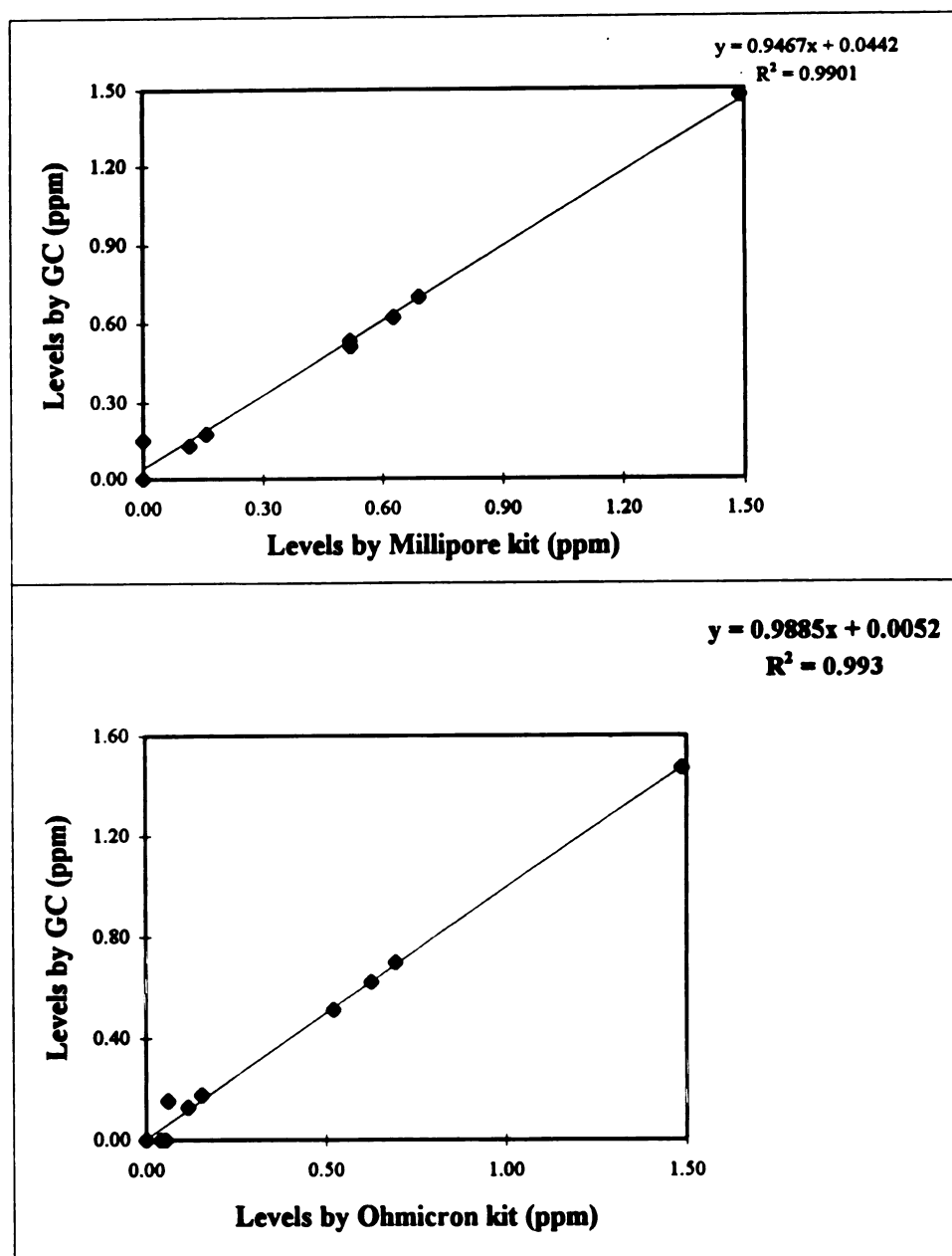


Figure 17. Correlation of atrazine concentrations as determined by ELISA and gc methods, $n = 4$, $r = 0.996$, $y = 1.017 X + 0.0005$.

Table 14. Atrazine concentration in incurred fish fillet samples by ELISA. 2 assays per samples.

ID	Non Treated #1			Treatment #1-1			Treatment #2-1		
	Ohm	Mill	GC	Ohm	Mill	GC	Ohm	Mill	GC
Fish 1	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 2	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 3	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 4	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 5	nd	nd	nd	nd	nd	nd	nd	nd	nd

ID	Non Treated #2			Treatment #1-2			Treatment #2-2		
	*Ohm	**Mill	***GC	Ohm	Mill	GC	Ohm	Mill	GC
Fish 1	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 2	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 3	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 4	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fish 5	nd	nd	nd	nd	nd	nd	nd	nd	nd

* Ohmicron test kit

** Millipore test kit

*** GC determination

Samples and standard solution of atrazine were run on GC/MS in order to confirm the results obtained by the ELISA kits. The mass spectrum of atrazine standard yielded the molecular ion at 215 m/e of atrazine and the fragmentation at 43, 93, and 200 characteristic of atrazine (S-triazine) (Figure 17). The same patterns were seen with the following samples: cut 2 (Trt #3) and F2 (Trt #2-1) which were found to be positive for atrazine by the kits and GC. The non-treated samples did not present the same pattern.

These results confirmed that the ELISA kits can successfully used for the determination of atrazine in weathered environmental samples and food provided that interference due to matrices and solvent inhibition be eliminated.

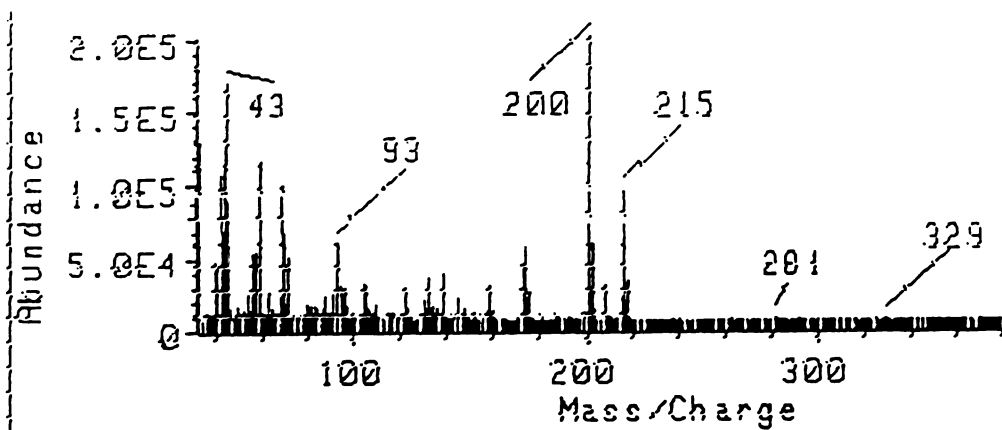
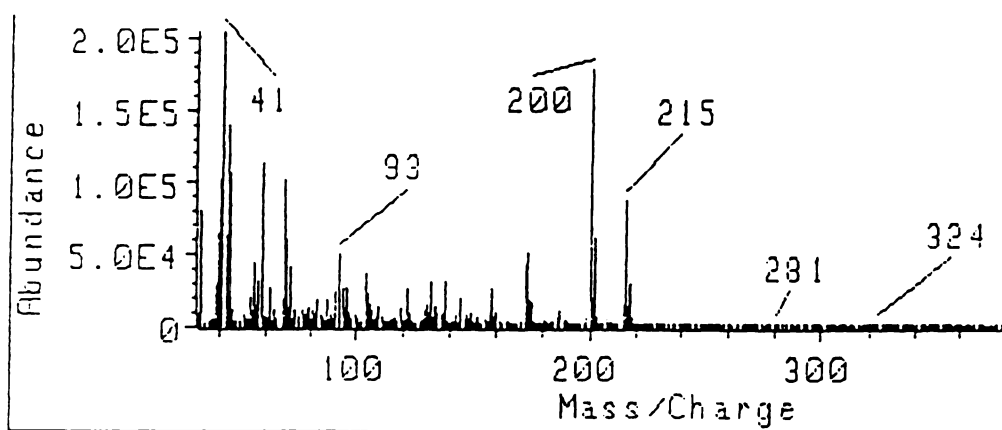
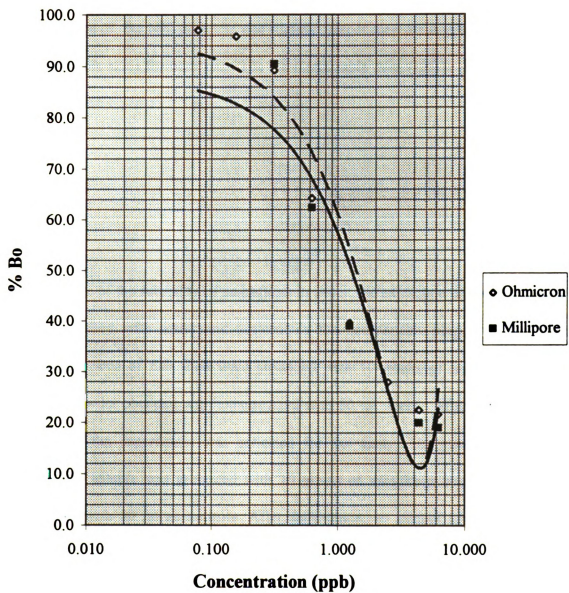


Figure 18. Confirmation of the ELISA results by GC/MS.

CARBOFURAN

Figure 19. Plot of the standard curves of the two test kits (average of 4 determinations).
%Bo = % (absorbance of the sample/absorbance of the zero control).



Standard solutions prepared in 10% methanol in water were assayed during the study. The concentrations ranged from 0.07 ppb to 6.24 ppb. Two replicate assays were performed (in duplicate) for the Millipore kits whereas 3 replicate assays (in duplicate) were performed with the Ohmicron kits. The results are reported in Appendix C. Both ELISA kits present a linear range between 0.312 ppb and 4 ppb (Figure 19). In this range, the slope of the Millipore kits was slightly steeper than the slope of the Ohmicron kits but R^2 is the same for both kits. The least detectable dose (LDD) calculated as 90% were similar and equal to 0.3 ppb.

ACCURACY

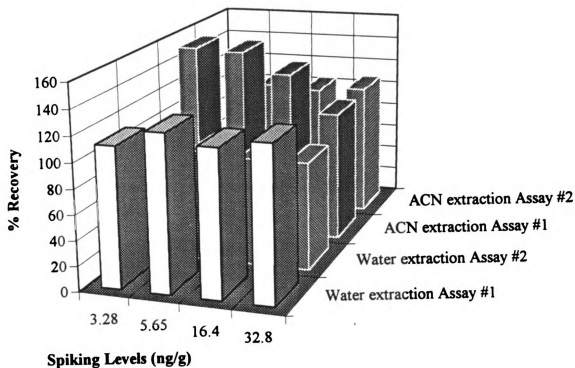
FISH FILLET

Because of the solubility of carbofuran in water, the extraction of the fish samples was accomplished with water and acetonitrile for comparison purpose. For water extraction, 5 grams of fish fillet were grounded with 10 grams of anhydrous sodium sulfate; the powder obtained was homogenized with 50 ml water. After 3 to 5 minutes, an aliquot of the supernatant was collected for the assay. In case of acetonitrile extraction, the sample was grounded with 10 grams of anhydrous sodium sulfate and homogenized with 50 ml acetonitrile. After decantation, an aliquot of the extract was collected for the assays.

The preliminaries assays, without dilution, gave very high recoveries (> 177%)

for both extraction solvents. This enhancement observed with the kits indicated

Table 15. Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination carbofuran in spiked fish fillets (2 replications per assay).



interference due to matrices and/or extraction solvent inhibition. Subsequently, the extracts were diluted for the assays. The results are reported in appendix C. As the results indicate (Table 9), when the extracts obtained were diluted 10 times with distillate water, the enhancement observed was markedly reduced for water extracts (112-122% recovery) compared to acetonitrile extract (104-152% recovery). Diluting the extracts 50 times eliminate the enhancement in both extracts but the recoveries with water extracts were low (70-90%) compared to acetonitrile extracts (104-107%).

These results can be explained by the fact that when the samples are extracted with water, fat and fish components which make the extracts cloudy, were allowed to settle out thereby reducing their interference with the assay. For this reason a 10x dilution resulted in a marked decrease of the enhancement seen when the sample was not diluted. The fact that 50 times dilution resulted in poor recoveries indicates possible loss of the pesticide during extraction. Water as polar solvent cannot successfully extract pesticides which tend to partition in the fat. Acetonitrile extracts have a high fat load which causes the high enhancement observed with 10 times dilution which was eliminated by diluting the extract 50 times.

CORN LEAF

Corn leaf samples free of any pesticide were spiked with a series of standard solution of carbofuran according to the procedure described in the method section. The extract were assayed directly using the Ohmicron test kits; the reading yielded recoveries in the rage of 170-180 indicating interference due to coextracts. The results are

summarized in appendix C.

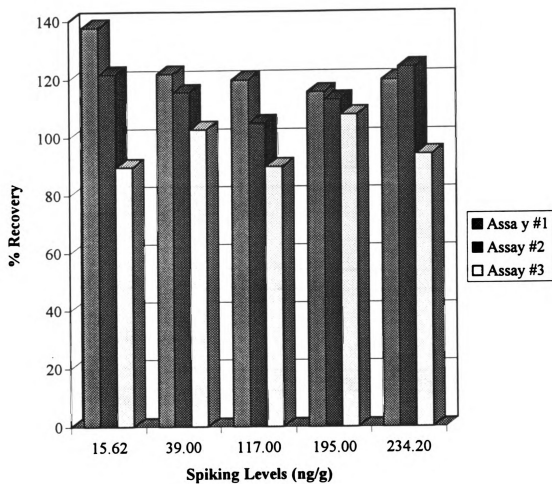
To eliminate the enhancement observed in the preliminary assay, the extracts were diluted 50x with distilled water and assayed. The recoveries obtained ranged from 115-137% (Table 16). This dilution markedly reduced the enhancement observed in the preliminary assay but did not totally eliminate the interference observed.

In the following two assays, the extract was decolorized to remove the green color observed and diluted 50x and 100x respectively. When the discolored extract was diluted 50 times, the enhancement was reduced but the recoveries were still high (105-124%). The enhancement was completely eliminated when the discolored extracts were diluted 100x; the recoveries obtained ranged from 90 to 108%.

The results of this study indicate that plant coextract (pigments and others) interfered with the ELISA detection. In order to eliminate this interference one must only decolor the extracts but dilute the extract 100 times. The extractions were done using 0.25N HCl solution which may have inhibited to a certain degree the detection contributing to the high enhancement observed during the preliminary assays. These results showed that the Ohmicron kits can quantitatively be used to determine carbofuran in plant material provided that matrix interference's and/or extraction solvent inhibition is eliminated.

The Millipore kits were not tested in this study because the kits were provided with only two standard solutions: 0.2 and 5 ppb. No 2-point calibration curve can be used for qualitative determination.

Table 16. Accuracy of Ohmicron (A) and Millipore (B) test kits for the determination carbofuran in corn leaf (2 replications per assay).



REAPITABILITY**Table 17. Assay reproducibility for of the EIA for carbofuran in corn leaf extract****A. Millipore test kit**

Sample	*Intra-Assay	**Inter-Assay
Standard Solutions (ppb)		
0.0	5.0	5.7
0.2	4.2	5.9
5.0	8.9	9.5
Mean	6.0	7.0
Spikes (ng/g)		
32.8	3.9	7.6
65.6	7.4	7.9
312.0	10.8	11.0
Mean	7.4	8.8

B. Ohmicron test kit

Sample	*Intra-Assay	**Inter-Assay
Standars Solutions (ppb)		
0.0	1.3	5.0
0.1	3.1	4.0
1.0	4.0	6.0
5.0	7.6	6.6
Mean	4	5.4
Spikes (ng/g)		
6.56	7.7	8.5
32.80	4.2	6.8
65.60	3.0	8.1
31.20	6.1	9.9
Mean	5.0	7.7

* 6 assays within 1 day

** 1 assay per day during 6 days

Standard solutions of carbofuran and spiked samples were assayed in order to determine how much variation are introduced in the determination from assay to assay or from day to day. For both test kits, 6 assays were conducted under the same conditions to determine the within-day variation, and one assay was conducted for six consecutive days for between-day variation determination. The results are reported in Appendix C.

The recoveries ranged from 3.9-10.8 (within-day) to 5.7-11% (between-day) for the Millipore test kits and 1.3-7.7% (within-day) to 4.0-9.9% (between-day) for the Ohmicron kits (Table 17). Within-day total coefficient of variation were determined to be 6.65 and 4.6% for the Millipore and Ohmicron kits respectively, whereas they were determined to be 8.1 and 6.9% respectively. The results showed also that variations were more pronounced with the spiked samples compared to the standard solutions. This can be explained by variations introduced during the different steps of extraction and principally in dilution steps.

The overall results showed coefficient of variation for the assays of less than 12% which is very good for qualitative determination.

SENSITIVITY

Sensitivity of the ELISA kits was assessed using Ohmicron test kits. Corn as well as fish samples were spiked at 3.28 ng/g and 15.62 ng/g respectively, extracted and assayed after dilution. Six replicate assays were done for both kits; the results of the study are reported in Appendix C. The method detection limit (MDL) of the test kits

Table 18. Method determination limit (MDL) calculated from standard deviation (σ) of 6 replicates assays of corn leaf (A) and fish tissue (B) spiked with carbofuran.

A. Corn leaf

Assay #	Abs 1	%Bo	Concentration (ppb)
Control	1.336		
1	1.321	99	0.067
2	1.242	93	0.108
3	1.117	84	0.176
4	0.967	72	0.317
5	1.105	83	0.185
6	0.845	63	0.512
Mean		82	0.228
Stdv		13.05	0.163
3@		39.16	0.5

B. Fish fillet

Assay #	Abs 1	%Bo	Concentration (ppb)
Control	1.346		
1	1.212	90	0.121
2	1.180	88	0.138
3	0.997	74	0.282
4	1.210	90	0.122
5	0.955	71	0.333
6	1.275	95	0.095
Mean		85	0.182
Stdv		9.66	0.100
3@		28.99	0.30

were 0.5 ng/g and 0.3 ng/g for corn leaves and fish sample respectively (Table 15). These values fall in the range of linearity of the standard solutions provided with the test kits and are well above the lowest standard solution (0.1 ppb).

CROSS-REACTIVITY

Carbofuran is metabolized by hydroxylation and hydrolysis in plants and the major metabolites are 3-hydroxy-carbofuran and 3-keto-carbofuran (Metcalf et al. 1968). These metabolites may cross react with the detection by the ELISA kits. For this reason, fish samples were spiked separately and in a mixture with standard solutions of carbofuran, 3-keto, and 3-hydroxy-carbofuran. The results of the assays are summarized in Appendix C. The response of the metabolites (3-keto-carbofuran and 3-hydroxy-carbofuran) were found to be significantly lower than the response for the parent compound carbofuran (Figure 20). With both kits, the presence of 3-keto and 3-hydroxy-carbofuran produced a slight enhancement to carbofuran responses with the 3-keto metabolite producing twice the enhancement due to the 3-hydroxy metabolite.

The overall results indicated that the metabolites particularly the 3-keto exhibit cross-reactivity with the Millipore kits (26%) whereas the cross reactivity was non significant with the Ohmicron kits. Practically 3-hydroxy-carbofuran was undetectable by the Ohmicron kits at the range of determination.

INCURRED SAMPLES

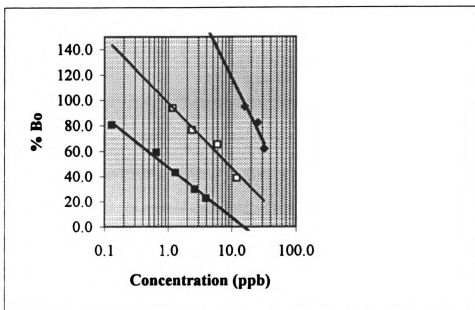
CORN LEAF

Ten g of sample were extracted according to the procedure described for the extraction of carbofuran in corn leaf. The extracts were diluted 1:100 in order to eliminate any interference or inhibition due to the matrix and the acid, and were assayed in replicate. Carbofuran was not detected by the Ohmicron EIA kit in the non-treated samples but was found in some of the treated samples (Figure 16). The concentrations found were higher in samples treated with high rate of carbofuran compared to samples treated with low rate carbofuran. The results indicate also that the concentrations found were high in samples collected the same day after the treatment and decreased with time.

The same samples were also subjected to gas chromatographic determination. The results are reported in Appendix C. All samples positive for carbofuran using Ohmicron kits were also positive by GC determinations. Plotting the ELISA results against GC results gave a regression line of 0.95 slope ($R^2 = 0.997$) (Figure 21). These results showed a good correlation between the ELISA and gas chromatography indicating that the Ohmicron kits can be successively used for quantitative determination of carbofuran in plant materials.

The Millipore kits were also used in this study, but because the kits contain only two standard solutions it was not possible to quantitatively determine the concentration of carbofuran in the samples. The samples positive for carbofuran by the Ohmicron kits were found to be positive with the Millipore kits. For the Millipore kits the results were

A. Ohmicron test kit



B. Millipore test kit

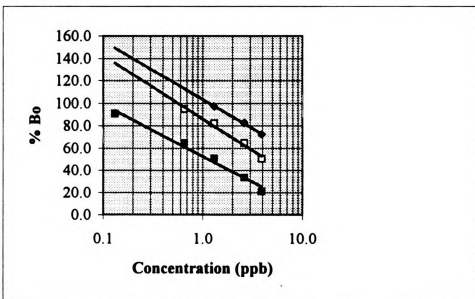


Figure 20. Response of the ELISA kits to carbofuran; 3-ketocarbofuran; and (♦) mixture.

expressed as contain carbofuran at a level above 0.2 ppb. This kind of kit can only be used for qualitative determination where one want to know yes or no as to whether the samples contain a certain pesticide.

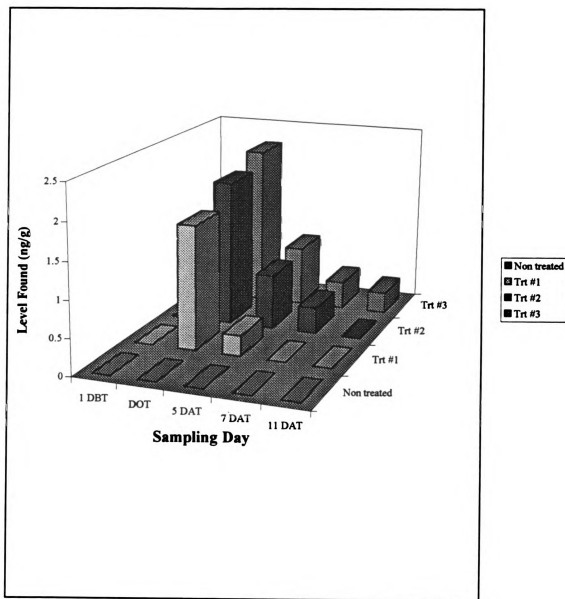


Figure 21. Carbofuran concentration in incurred corn leaf samples by Ohmicron EIA kit. 2 assays per samples.

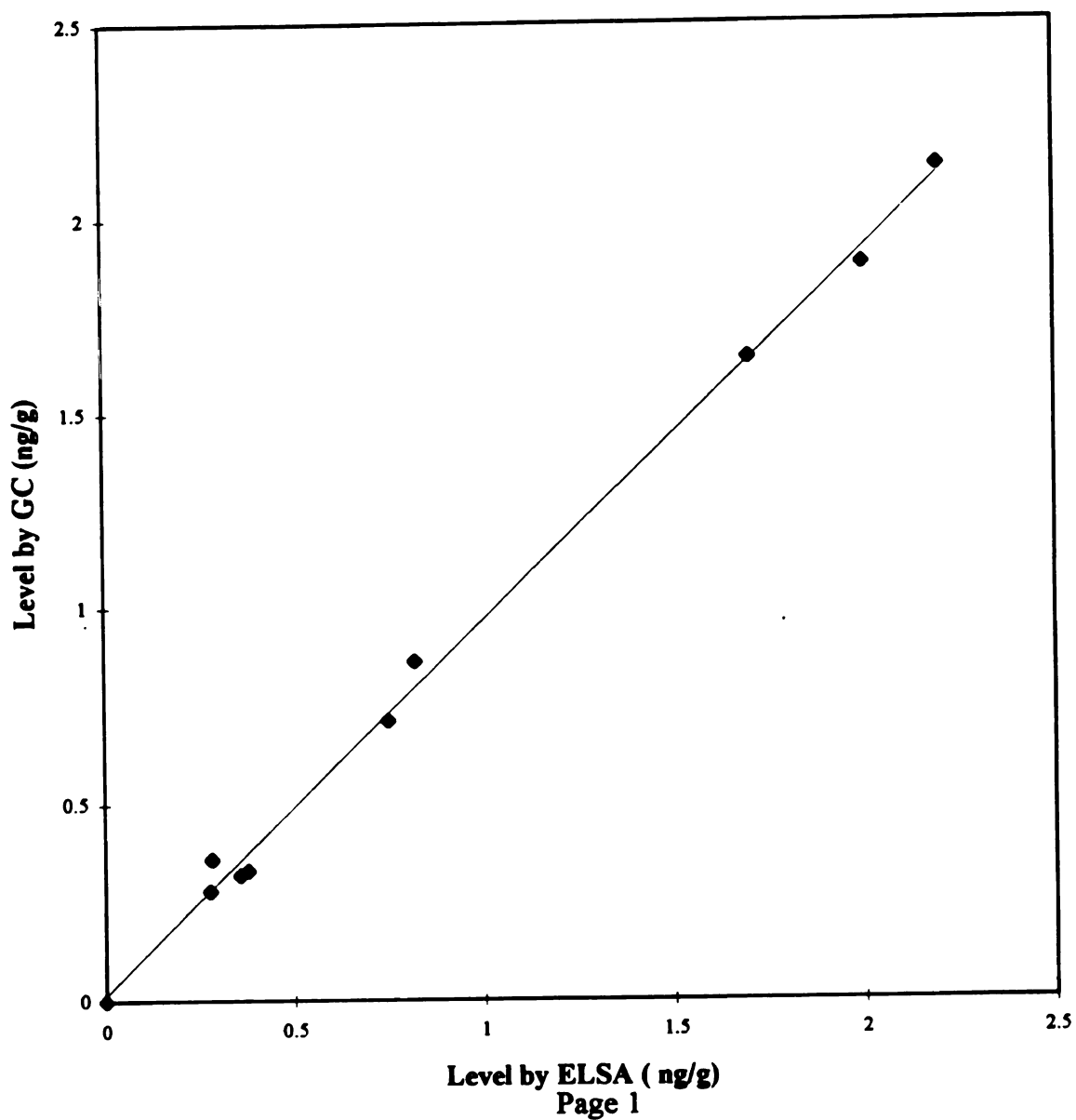


Figure 22. Correlation of carbofuran concentrations as determined by ELISA and gc methods, $n = 4$, $r = 0.996$, $y = 1.017 X + 0.0005$.

FISH FILLET

Fish samples were extracted with acetonitrile and assayed after 1:50 dilution. Carbofuran was detected in only 2 fish (Treatment 2-1) by the Ohmicron kits. The levels found using the Ohmicron kits ranged from 0.327 to 0.51 ng/g. The same fish were found to contained carbofuran at the levels above 0.2 ng/g by the Millipore test kits (Table 17). In order to assess the accuracy of the assays, the same samples were extracted according to the procedures described in the methods and analyzed by GC. The samples found to be positive for carbofuran by both kits were found to contain carbofuran by GC. The GC results were found to be 93-98% in concordance with the Ohmicron results.

Confirmation of the presence of carbofuran in corn leaf and fish fillet samples was performed using GC/MS on selected samples. In case of corn leaf samples, cut 1 and 2 (Treatment #3) samples and non treated sample were run. Cut 3 (Treatment #3) contained a molecular ion at 221 m/e in the negative ion mode with fragment ions at 43, 131, 148, and 164 which were expected for carbofuran (N-methylcarbamate). The control sample (non treated) did not show either the molecular ion at 221 m/e nor the fragments observed for the standard. With fish samples, the control sample did not also present the molecular ion at 221 m/e; the samples positive for carbofuran by the ELISA showed the molecular ion at 221 m/e and the fragments at 43, 131, 148, and 164. These results confirm the results obtained with the ELISA kits and the GC determinations.

Table 17. Carbofuran concentration in fish fillet by ELISA. 2 assays per samples.

[illegible][illegible]

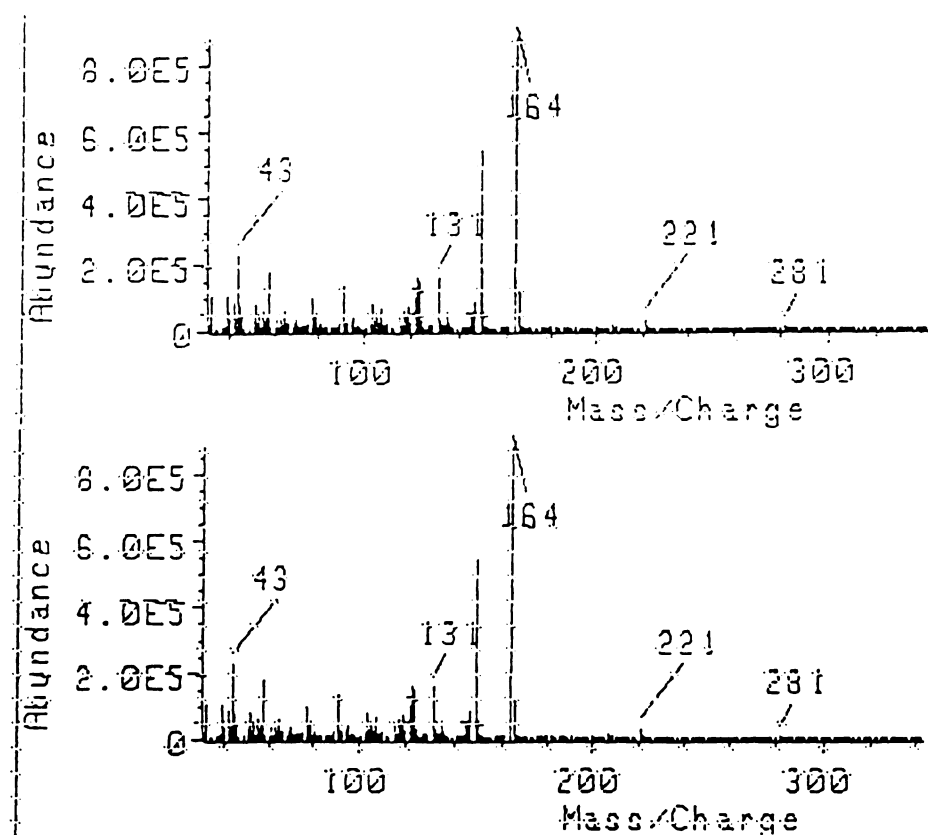


Figure 23. Mass spectra of GC peaks of incurred corn leaf and fish fillet. (A) carbofuran standard solution; (B) non treated corn leaf, (C) treated corn leaf; (D) non-treated fish fillet, (E) treated fish fillet.

CHAPTER VI

CONCLUSIONS

Commercial enzyme immunoassay kits (EIA) kits were successfully used to determine carbofuran (insecticides), and alachlor and atrazine (herbicide) in plant and fish samples. The comparison of the results showed good agreement between the immunoassays results and gas chromatographic determinations. No positive sample were found but significant matrices effects on the EIA assays were observed.

Most of the enzyme immunoassay kits were developed for the detection of pesticides in water samples and for this reason, the interferences from commonly found groundwater components were minimal. Our study showed that coextracts from fish (fat and proteins) and corn leaf (pigments, proteins, and cellulose) strongly interfered with the assay detection. Dilution of the samples with distillate water was used to reduce this interference but on the basis of recoveries, dilution was shown to be productive and counterproductive. In case of corn extracts, it helped reduce the effects of coextracts and the inhibition due to extraction solvents enabling the detection of the pesticides. But when the range of detection is of the test kit is very low 0.1-5 ppb such as the Ohmicron kits RaPID Assays® kit, the attempt to decrease the effects of background interference by diluting may be counterproductive because the kit can no longer detect the analytes at these level of dilution. In any case, as long as these interferences are relatively constant from one sample to another of the same matrix, the EIA can be applied for

screening analysis. It is therefore invalid to make quantitative assessments of the presence of a pesticide in matrices (e.g., fish) from calibration in another (e.g., corn leaf extract). For this reason, EIA appears more suitable as a qualitative technique in complex matrices rather than as quantitative method and will be suited for screening purpose. Using standard prepared in matrix extract helped counteract this problem and in this case EIA could be used for qualitative purpose.

The results of the study indicated that the two types of kits gave similar results in the detection of alachlor, atrazine, and carbofuran in corn leaf and fish fillet. The comparison study of the ELISA kits from the two manufacturers showed each to have separate advantages and disadvantages. The Millipore Envirogard™ kits showed more variability compared to the Ohmicron kits which seem to be more precise and reproducible. The solid phase employed in manufacturing the Millipore Envirogard™ kit is polystyrene tube on which antibodies are passively adsorbed. Studies have shown that the desorption or leaching off of the antibodies which have been passively adsorbed are major factors that adversely affect assay precision and reproducibility (Howell et al., 1981; Engvall, 1980; Lehtonen and Viljanen, 1980). The solid phase used in the manufacturing of the Ohmicron RaPID Assays® kits are small magnetic particles on which the antibodies are covalently bound. The dispersion of particles throughout the reaction mixture allows precise addition of antibody. The Ohmicron RaPID Assays® kit was more sensitive and had in general, a lower LDD compared to the Millipore Envirogard™ kits. This is explained by the fact that the lower limits of quantitation of the RaPID Assays® kit is always lower than the lower limit of quantification of the

Envirogard™ kit. For this reason, the Ohmicron RaPID Assays® kit is suited for the analysis of low level residue analysis while the Millipore Envirogard™ kit is suited for high level residue analysis. In many assays, samples are diluted in order to reduce interferences due to matrices; the dilution may lower considerably the levels of the pesticides to be detected. In such cases, the Ohmicron RaPID Assays® kit is the most suited for the assay. In case where there is no dilution to be done such as water samples both assays can be used but the Millipore Envirogard™ kit had a clear advantage on the Ohmicron RaPID Assays® kit because of the shorter time of this assay (30 min of incubation) compared to the Ohmicron RaPID Assays® kit (50 min incubation time). When considering the cost of the assay, the Ohmicron RaPID Assays® kit has an advantage over the Millipore Envirogard™ kit. The Millipore kit is sold in box of 40 tubes allowing the analysis of 32 samples which cost \$10 per sample. The Ohmicron RaPID Assays® kits is sold by batch of 30 or 100 test tubes. The cost per sample is \$8.63 and \$4.72 for the 30 tubes and 100 tubes batch respectively. With the Ohmicron RaPID Assays® kits (100 tubes), the samples can be analyzed in duplicate, and it will cost \$9.45 which is less than the cost of singlicate analysis by the Millipore assay (\$10.18). Besides its low cost, the Ohmicron RaPID Assays® kits has the edge over the Millipore Envirogard™ kits by the fact that up to 46 samples can be analyzed in duplicate or 96 samples in singlate with one batch whereas only up to 32 samples can be analyzed using the Millipore Envirogard™ kits.

The results of the study have shown that the ELISA compares favorably to GC determinations but the ELISA present several advantages over classical techniques. The

length of analysis is shorten with the ELISA compared to chromatographic techniques. The determination of the three pesticides in fish or corn leaf involved the extraction step, followed by two clean-up steps (liquid-liquid partition and liquid-solid partition whereas with the ELISA, the samples are assayed directly after the extraction. Besides, up to 90 samples can be assayed in less than one hour with the ELISA while the detection of the pesticide in one sample alone by GC takes 20 min. In ELISA, not only low volume of solvent were used for the extraction (~ 50 ml) but small size sample were also used for the assay whereas large volume of organic solvent (250-500 ml) and large sample size (15-25 g) were used in GC determination. Using low volume of solvent helps reduce the cost of analysis the cost related to the disposal of the organic solvents. In analysis where the size of the sample is a limiting factor, the ELISA has an edge over chromatographic techniques.

A cost analysis was done, the ELISA costs approximately \$9.7 to \$10.4 per sample whereas the GC determination costs \$13.2 to \$16.8. The cost included labor, depreciation of instrumentation, disposables (which include pipette tips, vials, test tubes), and reagents. The real advantage of the ELISA compared to chromatographic techniques for developing countries such as my country is the cost associated with instrumentation.; the GC instrumentation used in our study is composed of a Hewlett-Packard Model 5890 Series II with two detector (ECD and NPD) coupled with a HP Model 7673 automatic injector. This system costs more than \$ 35,000. Starting-up an EIA requires a photometer and different types of pipeters; the total costs less than \$3,000 (including the RaPID analyzer®). If one want to use a more sophisticated photometer such as the

EnviroQuant™ which can process the data, it costs \$3,675.00. For developing country one of the factor that might favor the ELISA over GC is the cost associated with installation, maintenance, and training associated with the GC. It is far less expensive to have a technician to install, maintain and train workers here in the USA than sending somebody in my country. Usually when a GC or HPLC is not working in my country, it takes six months, one to two years to be fixed. Because of the cost associated with sending somebody to fix the instrument, the company such as Hewlett-packard will wait to until it has several instrument to service before sending a technician over there to fix the problem.

Besides its low cost, the ELISA determination can be done in the field using a portable photometer. Example of on-site uses are remediation sites, in field after pesticide application for reentry checking purpose or to check the level of a given pesticide on vegetables before harvesting.

Using the EIA kits, it was possible of detecting 0.15 to 100 ppb of pesticide in complex matrices such as corn leaf and fish tissue. The disadvantage of these commercial kits include slow equilibration time and irreversible binding which prevent their re-use or continuous application. This has been solved by the development of immunosensors (biosensors) which may be regenerated several times.

As immunoassay for residue analysis becomes widely accepted and applied, new challenges involving more complex chemicals in more difficult matrix arise. The integration of traditional analytical methods of detection with immunoassay can provide new approaches useful in the field of trace analysis. Such approach will be to use thin

layer chromatography (TLC) in tandem with EIA. Compounds may be separated by TLC and the EIA used for determination. EIA can also be coupled with liquid chromatography (LC). In such a case, EIA may be used as immunoaffinity chromatography or for the quantitation of LC fractions.

APPENDICES

APPENDIX A

RESULTS OF ALACHLOR DETERMINATION

Table A1. Standard Curve Data

A. Millipore test kit assay

MILLIPORE KITS												
Assay #1							Assay #2					
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0	1.745	1.621	1.683	0.09	5%		1.767	1.945	1.856	0.13	7%	
1	1.456	1.436	1.446	0.01	1%	86	1.535	1.613	1.574	0.06	4%	85
2	1.345	1.447	1.396	0.07	5%	83	1.412	1.377	1.395	0.02	2%	75
5	1.21	1.25	1.230	0.03	2%	73	1.311	1.235	1.273	0.05	4%	69
10	1.05	0.925	0.988	0.09	9%	59	0.972	1.105	1.039	0.09	9%	56
20	0.654	0.67	0.662	0.01	2%	39	0.685	0.714	0.700	0.02	3%	38
50	0.423	0.476	0.450	0.04	8%	27	0.478	0.512	0.495	0.02	5%	27
100	0.212	0.244	0.228	0.02	10%	14	0.202	0.231	0.217	0.02	9%	12
150	0.205	0.248	0.227	0.03	13%	14	0.165	0.172	0.169	0.00	3%	9

Assay #3							Assay #4					
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0	1.561	1.465	1.513	0.07	4%		1.856	1.677	1.767	0.13	7%	
1	1.367	1.455	1.411	0.06	4%	93	1.612	1.587	1.600	0.02	1%	91
2	1.351	1.234	1.293	0.08	6%	85	1.550	1.421	1.486	0.09	6%	84
5	1.150	1.234	1.192	0.06	5%	79	1.337	1.211	1.274	0.09	7%	72
10	0.967	1.118	1.043	0.11	10%	69	1.123	0.987	1.055	0.10	9%	60
20	0.821	0.876	0.849	0.04	5%	56	0.876	0.921	0.899	0.03	4%	51
50	0.464	0.41	0.437	0.04	9%	29	0.525	0.467	0.496	0.04	8%	28
100	0.187	0.176	0.182	0.01	4%	12	0.211	0.177	0.194	0.02	12%	11
150	0.107	0.112	0.110	0.00	3%	7	0.184	0.207	0.196	0.02	8%	12

B. Ohmicron test kit assay

OHMICRON												
Assay #1						Assay #2						
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0.00	1.411	1.342	1.377	0.05	3.5		1.365	1.323	1.344	0.03	2.21	
0.05	1.326	1.255	1.291	0.05	3.9	0.94	1.245	1.325	1.285	0.06	4.40	0.96
0.10	1.233	1.201	1.217	0.02	1.9	0.88	1.125	1.178	1.152	0.04	3.25	0.86
0.15	1.050	0.987	1.019	0.04	4.4	0.74	0.945	1.115	1.030	0.12	11.67	0.77
1.00	0.643	0.585	0.614	0.04	6.7	0.45	0.545	0.587	0.566	0.03	5.25	0.42
3.00	0.455	0.421	0.438	0.02	5.5	0.32	0.423	0.475	0.449	0.04	8.19	0.33
5.00	0.356	0.341	0.349	0.01	3.0	0.25	0.332	0.312	0.322	0.01	4.39	0.24
10.00	0.323	0.344	0.334	0.01	4.5	0.24	0.311	0.321	0.316	0.01	2.24	0.24
Assay #3												
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0	1.521	1.434	1.478	0.06	4.16		0.935	0.956	0.946	0.01	1.57	
0.05	1.423	1.354	1.389	0.05	3.51	0.94	0.921	0.904	0.913	0.01	1.32	0.97
0.10	1.289	1.343	1.316	0.04	2.90	0.89	0.841	0.826	0.834	0.01	1.27	0.88
0.15	1.254	1.12	1.187	0.09	7.98	0.80	0.728	0.715	0.722	0.01	1.27	0.76
1.00	0.945	0.921	0.933	0.02	1.82	0.63	0.471	0.467	0.469	0.00	0.60	0.50
3.00	0.756	0.654	0.705	0.07	10.23	0.48	0.323	0.345	0.334	0.02	4.66	0.35
5.00	0.432	0.41	0.421	0.02	3.70	0.28	0.118	0.13	0.124	0.01	6.84	0.13
10.00	0.32	0.355	0.338	0.02	7.33	0.23	0.114	0.121	0.118	0.00	4.21	0.12
Assay #4												
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0	1.521	1.434	1.478	0.06	4.16		0.935	0.956	0.946	0.01	1.57	
0.05	1.423	1.354	1.389	0.05	3.51	0.94	0.921	0.904	0.913	0.01	1.32	0.97
0.10	1.289	1.343	1.316	0.04	2.90	0.89	0.841	0.826	0.834	0.01	1.27	0.88
0.15	1.254	1.12	1.187	0.09	7.98	0.80	0.728	0.715	0.722	0.01	1.27	0.76
1.00	0.945	0.921	0.933	0.02	1.82	0.63	0.471	0.467	0.469	0.00	0.60	0.50
3.00	0.756	0.654	0.705	0.07	10.23	0.48	0.323	0.345	0.334	0.02	4.66	0.35
5.00	0.432	0.41	0.421	0.02	3.70	0.28	0.118	0.13	0.124	0.01	6.84	0.13
10.00	0.32	0.355	0.338	0.02	7.33	0.23	0.114	0.121	0.118	0.00	4.21	0.12

Table A2. Recovery Study Data for Fish Samples

A. Ohmicron test kit assay

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean	% Bo
0.0	1.344	1.351	1.348	
0.1	1.212	1.231	1.222	90.6
1.0	0.786	0.736	0.761	56.5
5.0	0.400	0.400	0.400	29.7

Spiking Levels (ng/g)	*Abs 1	Abs 2	Mean	%Bo	LogConc	Conc	%Rec
0.0	1.542	1.54	1.541				
5.5	0.957	0.987	0.972	63	-0.22	6.08	110.5
11.0	0.812	0.832	0.822	53	0.06	11.38	103.4
22.0	0.635	0.623	0.629	41	0.41	25.48	115.8
44.0	0.466	0.476	0.471	31	0.69	49.31	112.1
110.0	0.213	0.234	0.224	15	nd	nd	nd

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean	% Bo
0.0	1.422	1.325	1.374	
0.1	1.244	1.231	1.238	90.1
1.0	0.634	0.664	0.649	47.3
5.0	0.404	0.385	0.386	28.1

Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	%Bo	logCon	Conc	%Rec
0.0	1.422	1.325	1.374				
5.5	0.865	0.821	0.843	61	-0.27	5.38	97.9
11.0	0.712	0.656	0.684	50	0.04	11.08	100.7
22.0	0.577	0.532	0.555	40	0.30	19.95	90.7
44.0	0.389	0.377	0.383	28	0.64	43.46	98.8
110.0	0.211	0.178	0.195	nd	nd	nd	nd

B. Millipore test kit assay

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.734	1.645	1.690
2.0	1.455	1.432	1.444
10.0	0.976	0.956	0.966
100.0	0.210	0.214	0.212

Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	LnCon	Conc	% Rec
0.0	1.734	1.645	1.690			
22.0	1.487	1.455	1.471	0.89	24.32	110.6
55.0	1.168	1.178	1.173	1.78	59.52	108.2
82.5	0.954	1.101	1.028	2.22	92.14	111.7
165.0	0.823	0.855	0.839	2.79	162.29	98.4
275.0	0.666	0.611	0.639	3.39	296.33	107.8
550.0	0.375	0.388	0.382	4.16	641.14	116.6
880.0	0.310	0.303	0.307	4.39	803.09	91.3

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.542	1.540	1.541
2.0	1.367	1.410	1.389
10.0	0.978	1.070	1.024
100.0	0.213	0.225	0.219

Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	LnCon	Conc	% Rec
0.0	1.542	1.54	1.541			
22.0	1.423	1.412	1.418	0.75	21.10	95.9
55.0	1.156	1.135	1.146	1.65	51.93	94.4
82.5	0.976	0.988	0.982	2.19	89.24	108.2
165.0	0.797	0.791	0.794	2.81	166.31	100.8
275.0	0.642	0.657	0.650	3.29	268.36	97.6
550.0	0.466	0.460	0.463	3.91	497.64	90.5
880.0	0.277	0.297	0.287	4.49	891.27	101.3

Table A3. Recovery Study for Corn Leaf

A. Ohmicron test kit

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo			
0.0	1.310	1.346	1.328				
0.1	1.120	1.213	1.167	87.8389			
1.0	0.723	0.724	0.724	54.4804			
5.0	0.278	0.305	0.292	21.9503			
Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	%Bo	LogCon	Conc	%Rec
0.0	1.310	1.346	1.328				
5.5	0.736	0.776	0.756	56.93	(0.16)	6.97	127
11.0	0.610	0.610	0.610	45.93	0.13	13.47	122
22.0	0.424	0.477	0.451	33.92	0.44	27.67	126
44.0	0.321	0.300	0.311	23.38	0.72	52.05	118
110.0	0.170	0.170	0.170	12.80	nd	nd	nd
154.0	0.167	0.161	0.164	12.35	nd	nd	nd

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo			
0.0	1.455	1.51	1.483				
0.1	1.258	1.289	1.274	85.9022			
1.0	0.817	0.788	0.803	54.1315			
5.0	0.331	0.322	0.327	22.0236			
Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	%Bo	LogCon	Conc	%Rec
0.0	1.310	1.346	1.328				
5.5	1.154	1.102	1.128	85	-0.93	5.85	106
11.0	0.976	0.932	0.954	72	-0.58	13.17	120
22.0	0.823	0.811	0.817	62	-0.30	24.94	113
44.0	0.676	0.705	0.691	52	-0.05	44.98	102
110.0	0.477	0.486	0.482	36	0.38	119.16	108
154.0	0.401	0.387	0.394	30	0.55	179.17	116

Standard

Conc	Abs 1	Abs 2	Mean	%Bo			
0.0	1.367	1.342	1.355				
0.1	1.223	1.201	1.212	89.4795			
1.0	0.767	0.753	0.760	56.1093			
5.0	0.367	0.381	0.374	27.6117			
Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	LogCon	Conc	%Rec
0.0	1.310	1.346	1.328				
5.5	1.365	1.324	1.345	101	nd	nd	nd
11.0	0.987	1.050	1.019	77	-0.62	11.88	108
22.0	0.900	0.887	0.894	67	-0.36	21.62	98
44.0	0.750	0.775	0.763	57	-0.09	40.49	92
110.0	0.564	0.532	0.548	41	0.35	113.11	103
154.0	0.487	0.501	0.494	37	0.47	146.49	95

Assay #1

Standard Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.936	1.930	1.933
2.0	1.486	1.502	1.494
10.0	1.030	1.112	1.071
100.0	0.216	0.189	0.203

Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	LnConc	*Conc	* **%Rec
0.0	1.936	1.930	1.933			
22.0	1.40	1.400	1.400	1.10	30.10	137
55.0	1.11	1.122	1.116	1.95	70.64	128
82.5	0.977	1.024	1.001	2.30	99.92	121
165.0	0.767	0.782	0.775	2.98	196.97	119
275.0	0.583	0.575	0.579	3.57	354.30	129
550.0	0.377	0.400	0.389	4.14	627.80	114
880.0	0.245	0.233	0.239	4.59	983.56	112
1100.0	0.097	0.087	0.092	5.03	1529.38	139
2200.0	0.007	0.012	0.010	5.28	1959.34	89

* concentration

** Percent recovery

Assay #2

Standard Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.755	1.682	1.719
2.0	1.464	1.453	1.459
10.0	1.030	1.117	1.074
100.0	0.210	0.231	0.221

Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	LnCon	Conc	%Rec
0.0	1.755	1.682	1.719			
22.0	1.512	1.503	1.508	0.69	19.98	91
55.0	1.178	1.124	1.151	1.81	60.88	111
82.5	0.997	1.025	1.011	2.24	94.29	114
165.0	0.876	0.883	0.880	2.65	142.21	86
275.0	0.645	0.623	0.634	3.42	306.27	111
550.0	0.432	0.455	0.444	4.02	555.45	101
880.0	0.267	0.235	0.251	4.62	1013.67	115
1100.0	0.210	0.187	0.199	nd	nd	nd
2200.0	0.191	0.169	0.180	nd	nd	nd

B. Millipore test kit assay. con't.

Assay #3

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.712	1.735	1.724
2.0	1.513	1.486	1.500
10.0	1.121	1.155	1.138
100.0	0.232	0.239	0.236

Spiking Levels (ng/g)	Abs 1	Abs 2	Mean	LnCon	Conc	%Rec
0.0	1.755	1.682	1.719			
82.5	1.578	1.582	1.580	nd	nd	nd
165.0	1.388	1.400	1.394	1.20	166.2	101
275.0	1.277	1.210	1.244	1.66	263.0	96
550.0	0.945	1.020	0.983	2.46	582.8	106
880.0	0.897	0.865	0.881	2.77	794.1	90
1100.0	0.203	0.211	0.207	nd	nd	nd
2200.0	0.103	0.111	0.107	nd	nd	nd

Table 4. Repeatability study data

A. Ohmicron test kit assay

Absorbance										
Standard (ppb)	Assay #1			Assay #2			Assay #3			Mean
	*Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	
0.0	1.453	1.440	1.447	1.512	1.463	1.488	1.355	1.350	1.353	
0.1	1.310	1.303	1.307	1.376	1.354	1.365	1.310	1.245	1.278	
1.0	0.812	0.800	0.806	0.854	0.823	0.839	0.821	0.812	0.817	
5.0	0.413	0.401	0.407	0.412	0.422	0.417	0.412	0.396	0.404	
Spike (ppb)										
5.5	1.364	1.342	1.353	1.324	1.288	1.306	1.287	1.385	1.336	
11.0	0.977	0.966	0.972	0.933	0.955	0.944	0.922	0.931	0.927	
44.0	0.743	0.800	0.772	0.812	0.832	0.822	0.767	0.812	0.790	
110.0	0.55	0.578	0.564	0.6	0.553	0.577	0.603	0.565	0.584	
154.0	0.337	0.310	0.324	0.344	0.312	0.328	0.341	0.321	0.331	
Standard (ppb)	Assay #4			Assay #5			Assay #6			Mean
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	
0.0	1.345	1.457	1.401	1.336	1.330	1.333	1.455	1.400	1.428	
0.1	1.178	1.196	1.187	1.145	1.151	1.148	1.340	1.340	1.340	
1.0	0.812	0.822	0.817	0.745	0.750	0.748	0.921	0.925	0.923	
5.0	0.400	0.398	0.399	0.368	0.360	0.364	0.475	0.468	0.472	
Spike (ppb)										
5.5	1.145	1.189	1.167	1.182	1.157	1.170	1.325	1.327	1.326	
11.0	0.936	0.93	0.933	0.886	0.875	0.881	1.105	1.1	1.103	
44.0	0.798	0.795	0.797	0.683	0.672	0.678	0.9	0.895	0.898	
110.0	0.632	0.654	0.643	0.612	0.6	0.606	0.7	0.635	0.668	
154.0	0.378	0.372	0.375	0.342	0.331	0.337	0.323	0.377	0.350	

B. Millipore test kit

Absorbance									
Standard (ppb)	Assay #1			Assay #2			Assay #3		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.445	1.462	1.454	1.402	1.432	1.417	1.521	1.465	1.493
0.1	1.312	1.301	1.307	1.310	1.287	1.299	1.343	1.376	1.360
1.0	0.782	0.805	0.794	0.777	0.782	0.780	0.845	0.834	0.840
5.0	0.345	0.365	0.355	0.387	0.375	0.381	0.345	0.410	0.378
Spike (ppb)									
5.5	1.364	1.342	1.300	1.289	1.365	1.327	1.356	1.32	1.338
11.0	1.012	0.98	0.996	1.121	0.967	1.044	1.11	0.976	1.043
44.0	0.912	0.877	0.895	0.88	0.870	0.875	0.87	0.900	0.885
110.0	0.543	0.512	0.528	0.535	0.587	0.561	0.532	0.534	0.533
154.0	0.345	0.434	0.390	0.435	0.430	0.433	0.432	0.456	0.444
Standard (ppb)	Assay #4			Assay #5			Assay #6		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.321	1.375	1.348	1.455	1.410	1.433	1.235	1.345	1.290
0.1	1.310	1.365	1.338	1.300	1.370	1.335	1.212	1.230	1.221
1.0	0.867	0.900	0.884	0.934	0.901	0.918	0.887	0.900	0.894
5.0	0.375	0.385	0.380	0.320	0.355	0.338	0.347	0.434	0.391
Spike (ppb)									
5.5	1.33	1.37	1.350	1.31	1.212	1.261	1.254	1.312	1.283
11.0	0.856	0.9	0.878	0.9	0.878	0.889	0.921	0.856	0.889
44.0	0.805	0.835	0.820	0.745	0.756	0.751	0.84	0.81	0.825
110.0	0.525	0.477	0.501	0.478	0.521	0.500	0.476	0.52	0.498
154.0	0.420	0.4	0.410	0.404	0.375	0.390	0.41	0.387	0.399

B. Millipore test kit assay

Standard	Absorbance					
	Assay #1	Assay #2	Assay #3	Mean	Stdv	%CV
0	1.725	1.761	1.621	1.698	0.073	4.3
2	1.464	1.356	1.332	1.382	0.070	5.1
10	0.932	0.845	0.967	0.910	0.063	6.9
20	0.612	0.66	0.69	0.650	0.039	6.1
100	0.234	0.243	0.202	0.227	0.022	9.5
Spike (ng/g)						
55	1.156	1.23	0.997	1.070	0.119	11.1
165	0.857	0.925	0.842	0.870	0.044	5.1
550	0.375	0.342	0.365	0.357	0.017	4.7

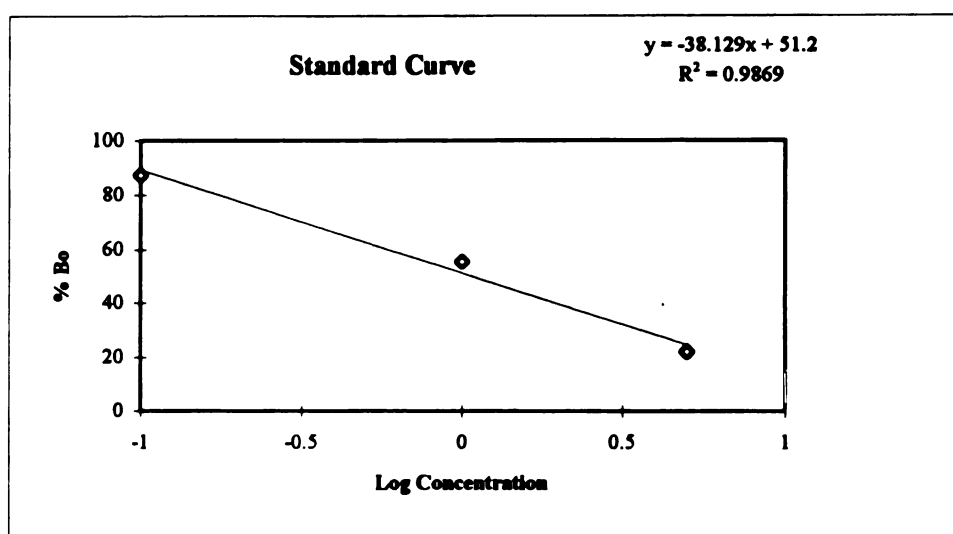
Standard	Absorbance					
	Assay #1	Assay #2	Assay #3	Mean	Stdv	%CV
0	1.656	1.684	1.756	1.778	0.052	2.9
2	1.276	1.367	1.456	1.532	0.090	5.9
10	0.765	0.945	0.912	1.210	0.096	7.9
20	0.545	0.633	0.66	0.650	0.060	9.3
100	0.225	0.24	0.2	0.227	0.020	8.9
Spike (ppb)						
55	1.21	1.17	1.02	1.070	0.100	9.4
165	0.812	0.956	0.987	0.870	0.093	10.7
550	0.423	0.34	0.36	0.357	0.043	12.1

Table A5. Sensitivity Study Data.

A. Ohmicron test kit assay

Standard (ppb)	*Abs 1	Abs 2	Mean	%Bo
0.0	1.412	1.377	1.395	
0.1	1.232	1.210	1.221	88
1.0	0.766	0.782	0.774	56
5.0	0.312	0.302	0.307	22

Absorbance at 450 nm



Assay #	*Abs 1	Abs 2	Mean	%Bo	Log Conc	**Conc
Blk	1.412	1.377	1.395			
1	0.876	0.856	0.866	61	-0.266	0.542
2	0.912	0.897	0.905	64	-0.337	0.460
3	0.854	0.866	0.860	61	-0.255	0.556
4	0.843	0.900	0.872	62	-0.276	0.530
5	0.835	0.921	0.878	62	-0.288	0.515
6	0.900	0.872	0.886	63	-0.303	0.498
Mean			0.878	62.158	-0.287	0.517
Stdv			0.016	1.131	0.030	0.035
3@			0.048	3.394	0.089	0.104

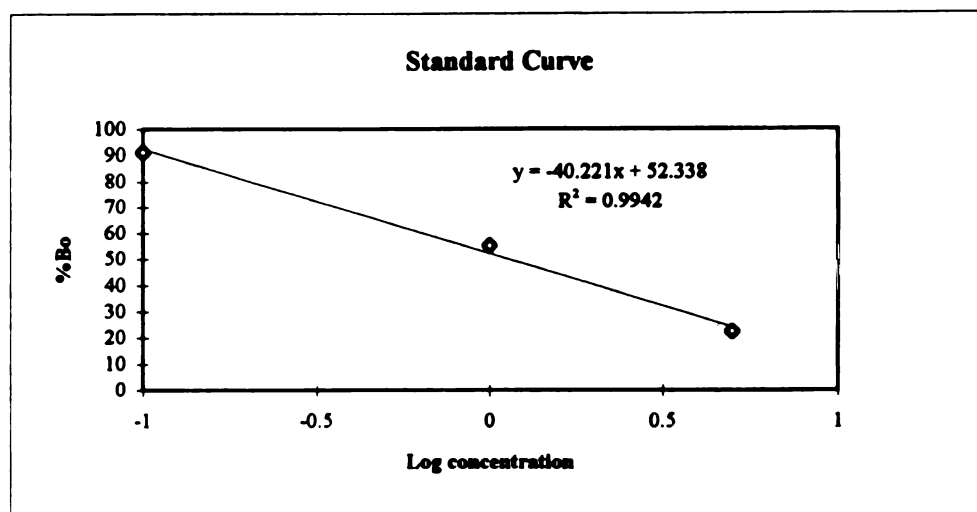
*Absorbance at 450 nm

**Concentration

A. Ohmicron test kit assay. con't

Standard (ppb)	*Abs 1	Abs 2	Mean	%Bo
0.0	1.343	1.400	1.372	
0.1	1.240	1.265	1.253	91
1.0	0.754	0.764	0.759	55
5.0	0.312	0.304	0.308	22

* Absorbance at 450 nm

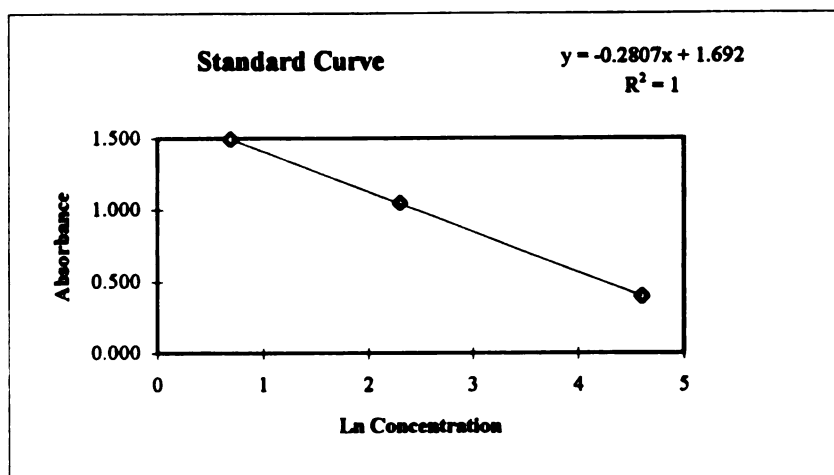


Assay #	Abs 1	Abs 2	Mean	%Bo	LogConc	Conc	Conc
Blk	1.700	1.612	1.656				
1	1.040	1.117	1.079	63	-0.276	0.5296	5.296
2	0.995	1.126	1.061	62	-0.250	0.5627	5.627
3	1.107	1.034	1.071	63	-0.264	0.5441	5.441
4	1.180	0.989	1.085	64	-0.285	0.5190	5.190
5	1.223	1.040	1.132	67	-0.354	0.4430	4.430
6	0.986	1.105	1.046	62	-0.228	0.5918	5.918
Mean			1.079	63.4	-0.276	0.532	5.317
Stdv			0.029	1.7	0.043	0.051	0.505
3@			0.088	5.2	0.129	0.152	1.515

B. Millipore test kit

Standard (ppb)	*Abs 1	Abs 2	Mean
0.0	1.745	1.721	1.733
2.0	1.523	1.468	1.496
10.0	1.120	0.978	1.049
100.0	0.386	0.410	0.398

* Absorbance at 450 nm



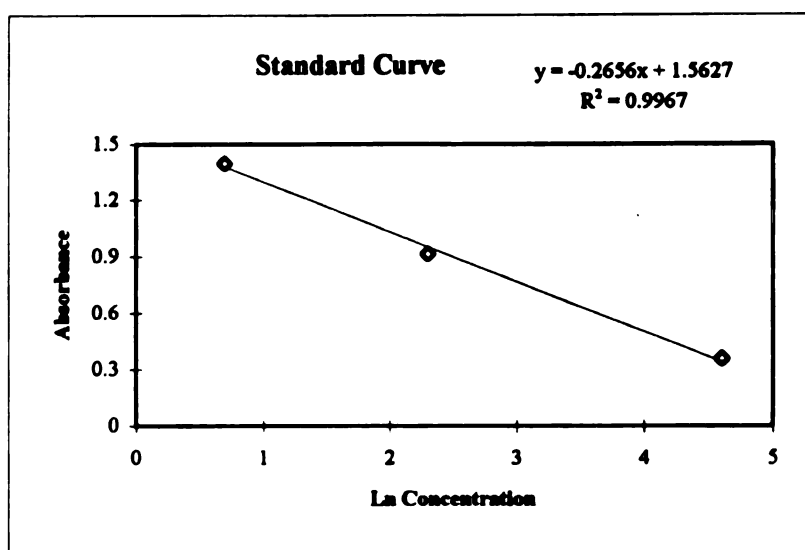
Assay #	*Abs 1	Abs 2	Mean	Ln Con	**Con
Blk	1.745	1.721	1.733		
1	1.465	1.444	1.455	0.8	2.33
2	1.432	1.387	1.410	1.0	2.73
3	1.435	1.476	1.456	0.8	2.32
4	1.378	1.427	1.300	1.4	4.04
5	1.487	1.466	1.477	0.8	2.15
6	1.366	1.354	1.360	1.2	3.26
Mean	1.427	1.426	1.409	1.0	2.80
Stdv	0.047	0.047	0.068	0.2	0.72
3@	0.142	0.142	0.204	0.7	2.17

* Absorption at 450 nm

** Concentration (ppb)

B. Millipore test kit assay; con't.

Standard	Abs 1	Abs 2	Mean
0.0	1.675	1.625	1.650
2.0	1.423	1.375	1.399
10.0	0.912	0.921	0.917
100.0	0.347	0.361	0.354



Assay #	*Abs 1	Abs 2	Mean	Lncon	Con	Conc
Blk	1.675	1.625	1.650			
1	1.432	1.412	1.422	0.5	1.70	84.95
2	1.343	1.377	1.360	0.8	2.15	107.25
3	1.266	1.234	1.250	1.2	3.24	162.18
4	1.382	1.355	1.300	1.0	2.69	134.39
5	1.523	1.544	1.534	0.1	1.12	55.86
6	1.243	1.32	1.282	1.1	2.88	144.07
Mean	1.365	1.374	1.358	0.8	2.3	114.78
Stdv	0.105	0.103	0.106	0.4	0.8	39.77
3@	0.314	0.310	0.317	1.2	2.4	119.3

* Absorbance at 450 nm

Table A6. Cross-Reactivity Study Data

A. Ohmicron test kit assay

Spiking Levels(ppb)	Alachlor				Metolachlor				Mixtute			
	Abs 1	Abs 2	Mean	%Bo	Abs 1	Abs 2	Mean	%Bo	Abs 1	Abs 2	Mean	%Bo
0.0	1.356	1.375	1.366		1.356	1.375	1.366		1.356	1.375	1.366	
5.5	1.323	1.304	1.314	96	1.357	1.364	1.361	100	1.255	1.245	1.250	92
22.0	0.877	0.943	0.910	67	1.345	1.333	1.339	98	0.866	0.835	0.851	62
44.0	0.710	0.723	0.717	52	1.265	1.2	1.233	90	0.680	0.677	0.679	50
110.0	0.560	0.563	0.562	41	1.152	1.137	1.145	84	0.521	0.503	0.512	37
154.0	0.450	0.47	0.460	34	1.111	1.101	1.106	81	0.391	0.377	0.384	28
200.0	0.315	0.365	0.34	25	1.131	1.012	1.072	78.47	0.307	0.302	0.305	22.3
400.0	0.315	0.325	0.32	23	0.878	0.9	0.889	65.1	0.167	0.182	0.175	13
1000.0	0.321	0.333	0.327	24	0.773	0.759	0.766	56.1	0.102	0.095	0.099	7

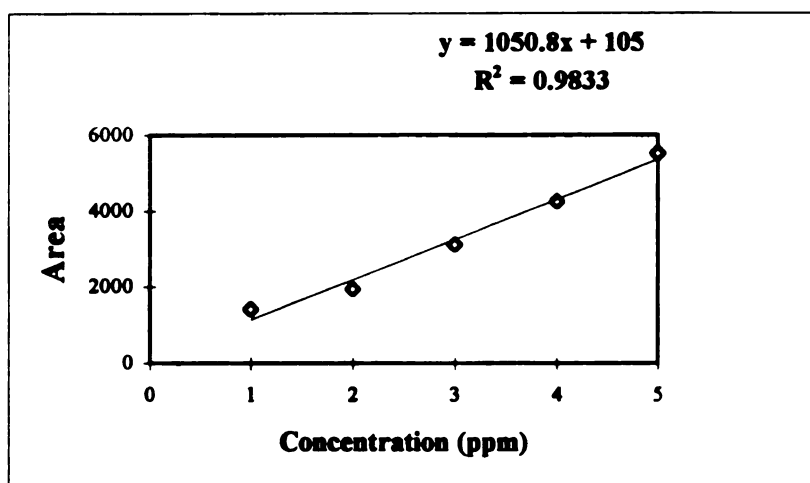
B. Millipore test kit assay

Spiking Levels (ppb)	Absorption		
	Ala	Meto	Mixt
0	1.645		
165	1.278	nd	1.167
880	0.734	1.456	0.510
1100	0.489	1.277	0.311
2200	0.210	0.931	0.127

Table A7. Incurred Corn Leaf Study

A. Gas Chromatographic determination

Concentration (ppb)	Area 1	Area 2	Mean
0.0944	1421	1432	1427
0.1888	1943	1956	1950
0.2360	3123	3131	3127
0.4720	4254	4261	4258
0.9440	5532	5521	5527

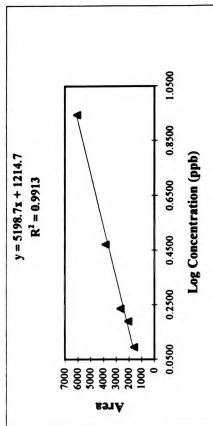


Spiking Levels (ppb)	Area1	Area 2	Area 3	Mean	Conc	*Concf	**%Rec
0.236	146	158	168	157	0.050	0.249	105.5
0.472	195	203	205	201	0.091	0.457	96.8
0.944	288	300	291	293	0.179	0.895	94.8
1.180	367	354	336	352	0.235	1.177	99.7
2.360	523	573	555	550	0.424	2.119	89.8

Gas chromatographic determination; con't

Standard

Concentration (ppb)	Area 1	Area 2	Mean
0.0944	1579	1602	1591
0.1888	2043	2032	2038
0.2360	2654	2643	2649
0.4720	3812	3826	3819
0.9440	6035	6042	6039



ID	Treatment #1			Non Treated		
	Area 1	Area 2	Mean	Area 1	Area 2	Mean
cut 1	np	np	np	*np	np	np
cut 2	2925	2947	2936	np	np	np
cut 3	1712	1735	1724	np	np	np
cut 4	np	np	nd	np	np	np
cut 5	np	np	nd	np	np	np

ID	Treatment #3			Treatment #2		
	Area 1	Area 2	Mean	Area 1	Area 2	Mean
cut 1	np	np	np	np	np	np
cut 2	16821	16710	16766	13242	13256	13249
cut 3	9518	9503	9511	7578	7583	7581
cut 4	3981	3965	3973	3981	3965	3973
cut 5	3345	3378	3362	np	np	nd

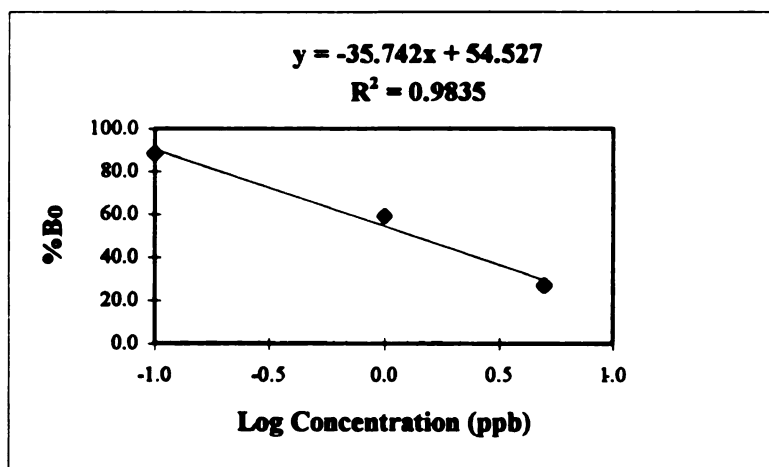
Table A7. Incurred Corn Leaf Study

B. Ohmicron test kit assay data

Standard

Conc	Abs1	Abs2	Mean	%Bo
0.0	1.395	1.415	1.405	
0.1	1.247	1.237	1.242	88
1.0	0.825	0.835	0.83	59
5.0	0.367	0.388	0.378	27

Conc = concentration (ppb)



Ohmicron test kit assay; con't

ID	Non Treated							Treatment #1						
	ABS1	ABS2	Mean	%Bo	Log Con	Con	Conf	ABS1	ABS2	Mean	%Bo	Log Con	Con	Conf
control	1.540	1.480	1.510					1.540	1.480	1.510				
IDBT	1.523	1.435	1.479	97.95	nd	nd	nd	1.43	1.345	1.388	92	-1.05	0.09	nd
IDOT	1.569	1.510	1.540	101.95	nd	nd	nd	0.646	0.655	0.651	43	0.32	2.09	69.64
5DAT	1.340	1.390	1.365	90.40	nd	nd	nd	0.956	0.96	0.958	63	-0.25	0.56	18.75
7DAT	1.400	1.450	1.425	94.37	nd	nd	nd	1.377	1.389	1.383	92	-1.04	0.09	nd
11DAT	1.424	1.430	1.427	94.50	nd	nd	nd	1.36	1.346	1.353	90	-0.98	0.10	nd

	Treatment #2							Treatment #3						
	ABS1	ABS2	Mean	%Bo	LogCon	Con	Conf	ABS1	ABS2	Mean	%Bo	LogCon	Con	Conf
ID control	1.540	1.480	1.510					1.540	1.480	1.510				
IDBT	1.544	1.534	1.539	102	-1.33	nd	nd	1.534	1.542	1.538	102	-1.32	0.05	nd
IDOT	0.211	0.196	0.204	13	1.15	14.08	468.93	0.156	0.142	0.149	10	1.25	17.77	591.69
ID5DAT	0.379	0.384	0.382	25	0.82	6.59	219.42	0.304	0.311	0.308	20	0.96	9.04	300.88
ID7DAT	0.54	0.572	0.556	37	0.50	3.13	104.22	0.567	0.553	0.560	37	0.49	3.08	102.45
ID11DAT	1.43	1.452	1.441	95	-1.14	nd	nd	0.612	0.601	0.607	40	0.40	2.52	84.02

Con = concentration

Conf = final concentration

* Day before treatment

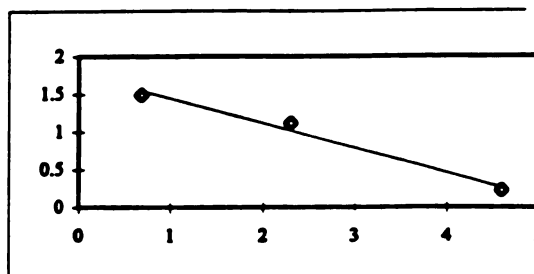
**Day of treatment

***Day after treatment

B. Millipore test kit assay

Standard 1

Concentration (ppb)	Abs1	Abs2	Mean
0.0	1.656	1.651	1.654
2.0	1.477	1.500	1.489
10.0	1.110	1.123	1.117
100.0	0.224	0.227	0.226



Non Treated					Treatment #1			
ID	ABS	Ln Con	Con	Conf	ABS	Ln Con	Con	Conf
control	1.756				1.756			
1DBT	1.654	0.35	1.42	nd	1.577	0.58	1.79	*nd
DOT	1.560	0.64	1.89	nd	1.540	0.70	2.01	66.25
5DAT	1.670	0.30	1.35	nd	1.567	0.62	1.85	nd
7DAT	1.575	0.59	1.81	nd	1.557	0.65	1.91	nd
11DAT	1.611	0.48	1.62	nd	1.555	0.65	1.92	nd

Non determined

Treatment #2					Treatment #3			
ID	ABS	Ln Con	*Con	**Conf	ABS	Ln Con	Con	Conf
control	1.756				1.756			
1DBT	1.567	0.62	1.85	nd	1.650	0.36	1.44	nd
DOT	0.898	2.64	14.05	463.56	0.819	2.88	17.85	588.94
5DAT	1.150	1.88	6.55	216.00	1.040	2.21	9.14	301.46
7DAT	1.523	0.75	2.11	69.76	1.401	1.12	3.06	100.96
11DAT	1.643	0.38	1.47	nd	1.452	0.96	2.62	86.50

*Concentration

**Final concentration

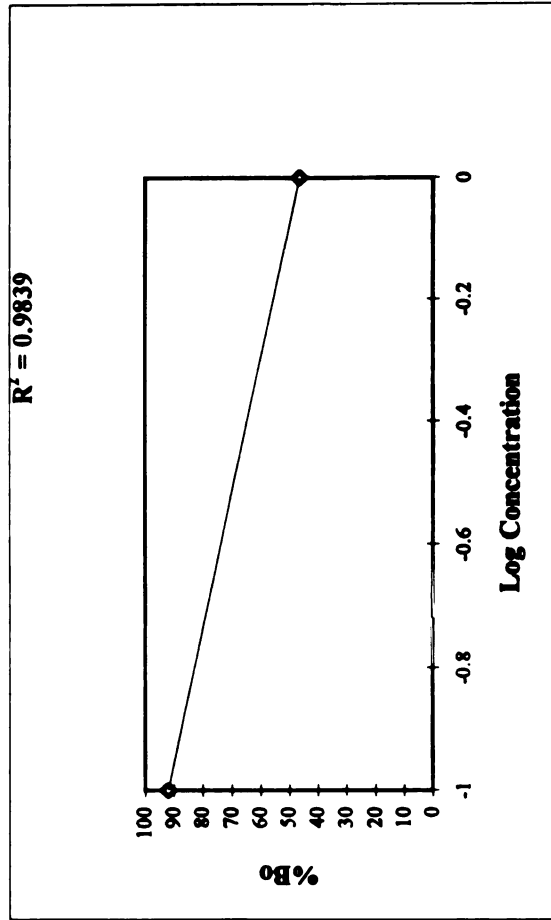
Table A8. Incurred Fish Study Data A. Gas chromatographic determination

Sample	Non treated #1				Non treated #2			
	Area 1	Area 2	Mean	*Conc	**Concl	Area 1	Area 2	Mean
Fish1	***np	np	np	****nd	nd	np	np	np
Fish2	np	np	np	nd	nd	np	np	np
Fish3	np	np	np	nd	nd	np	np	np
Fish4	np	np	np	nd	nd	np	np	np
Fish5	np	np	np	nd	nd	np	np	np
Mean	np	np	np	nd	nd	np	np	np
*** no peak								
** final concentration								
**** not detected								
ID	Treatment #1-1				Treatment #1-2			
	Area 1	Area 2	Mean	Con	Conf	Area 1	Area 2	Mean
Fish1	np	np	np	nd	nd	np	np	np
Fish2	np	np	np	nd	nd	np	np	np
Fish3	np	np	np	nd	nd	np	np	np
Fish4	np	np	np	nd	nd	np	np	np
Fish5	np	np	np	nd	nd	np	np	np
Mean	np	np	np	nd	nd	np	np	np
ID	Treatment #2-1				Treatment #2-2			
	Area 1	Area 2	Mean	Con	Conf	Area 1	Area 2	Mean
Fish1	np	np	np	nd	nd	np	np	np
Fish2	np	np	np	nd	nd	np	np	np
Fish3	np	np	np	nd	nd	np	np	np
Fish4	np	np	np	nd	nd	np	np	np
Fish5	np	np	np	nd	nd	np	np	np
Mean	np	np	np	nd	nd	np	np	np

B. Ohmicron test kit assay

Standard

Conc (ppb)	Abs1	Abs2	Mean	B/Bo
0.0	1.345	1.34	1.343	
0.1	1.221	1.252	1.237	92
1.0	0.622	0.63	0.626	47
5.0	0.403	0.352	0.367	27



Control 1

ID	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf
control	1.450	1.455	1.453				
Fish1	1.300	1.276	1.288	89	-1.297	0.05	nd
Fish2	1.210	1.250	1.230	85	-1.186	0.07	nd
Fish3	1.230	1.420	1.325	91	-1.367	0.04	nd
Fish4	1.456	1.214	1.335	92	-1.386	0.04	nd
Fish5	1.400	1.450	1.425	98	-1.559	0.03	nd
Mean	1.230	1.322	1.321	91	-1.359	0.04	nd

Conc = concentration

Concf = final concentration

Control 2

ID	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf
control	1.450	1.455	1.453				
Fish1	1.232	1.243	1.238	85.20	-1.200	0.06	nd
Fish2	1.310	1.300	1.305	89.85	-1.329	0.05	nd
Fish3	1.277	1.270	1.274	87.68	-1.269	0.05	nd
Fish4	1.345	1.352	1.349	92.84	-1.412	0.04	nd
Fish5	1.252	1.273	1.263	86.92	-1.248	0.06	nd
Mean	1.255	1.250	1.285	88.50	-1.292	0.05	nd

Ohmicron test kit assay; con't

ID	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf
control	1.450	1.455	1.453					1.450	1.455	1.453				
Fish1	1.230	1.300	1.265	87.09	-1.253	0.06	nd	1.500	1.520	1.510	104	-1.721	0.02	nd
Fish2	1.320	1.400	1.360	93.63	-1.434	0.04	nd	1.420	1.356	1.388	95.56	-1.488	0.03	nd
Fish3	1.250	1.260	1.255	86.40	-1.233	0.06	nd	1.256	1.250	1.253	86.27	-1.230	0.06	nd
Fish4	1.323	1.400	1.362	93.73	-1.437	0.04	nd	1.232	1.245	1.239	85.27	-1.202	0.06	nd
Fish5	1.354	1.323	1.339	92.15	-1.393	0.04	nd	1.310	1.344	1.327	91.36	-1.371	0.04	nd
Mean	1.295	1.337	1.316	90.60	-1.350	0.04	nd	1.344	1.343	1.343	92.48	-1.402	0.04	nd

200

TRT #2-1

TRT #2-2

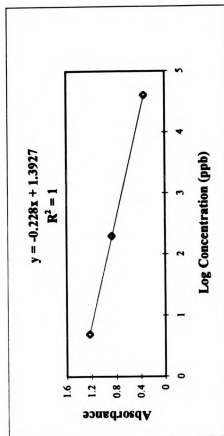
ID	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf	Abs1	Abs2	Mean	%Bo	LogConc	Conc	*Concf
control	1.450	1.455	1.453					1.450	1.455	1.453				
Fish1	1.340	1.450	1.395	96.04	-1.154	0.07	nd	1.340	1.350	1.345	92.60	-1.406	0.039	nd
Fish2	1.440	1.470	1.455	100.17	-1.260	0.05	nd	1.400	1.400	1.400	96.39	-1.511	0.031	nd
Fish3	1.250	1.650	1.450	99.83	-1.252	0.06	5.60	1.277	1.256	1.267	87.19	-1.255	0.056	nd
Fish4	1.232	1.210	1.221	84.06	-0.843	0.14	nd	1.247	1.490	1.369	94.22	-1.450	0.035	nd
Fish5	1.120	1.356	1.238	85.23	-0.874	0.13	nd	1.412	1.378	1.395	96.04	-1.501	0.032	nd
Mean	1.276	1.427	1.352	93.07	-1.077	0.08	nd	1.335	1.375	1.355	93.29	-1.425	0.038	nd

C. Millipore test kit assay

Standard #1

Concentration (ppb)	*Abs1	Abs2	Mean
0.0	1.743	1.756	1.750
2.0	1.232	1.234	1.233
10.0	0.876	0.865	0.871
100.0	0.345	0.338	0.342

*Absorbance at 450 nm



201

ID	Control				Control 2			
	Abs 1	Log Conc	**Conc	***Concf (ppb)	Abs1	LogConc	Conc	Concf (ppb)
BLK	1.634				1.634			
Fish1	1.532	nd	nd	nd	1.481	*nd	nd	nd
Fish2	1.424	nd	nd	nd	1.411	nd	nd	nd
Fish3	1.455	nd	nd	nd	1.302	nd	nd	nd
Fish4	1.456	nd	nd	nd	1.382	nd	nd	nd
Fish5	1.439	nd	nd	nd	1.520	nd	nd	nd
Mean	1.461	nd	nd	nd	1.419	nd	nd	nd

*Non-detected

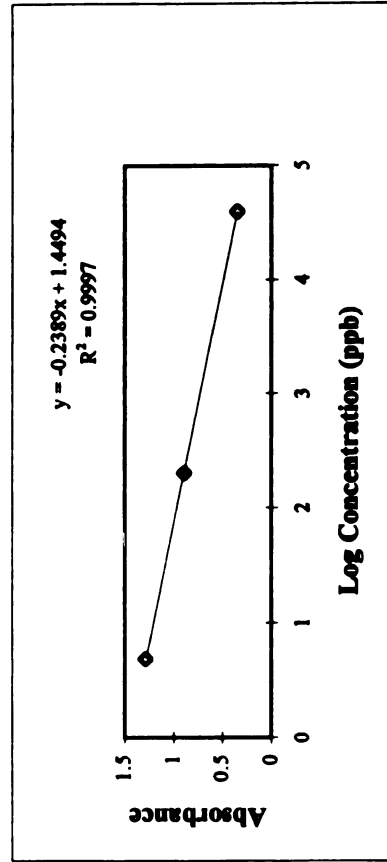
**Concentration

***Final concentration

Millipore test kit assay; con't

Standard #2

Concentration (ppb)	Abs1	Abs2	Mean
0.0	1.645	1.640	1.643
2.0	1.278	1.300	1.289
10.0	0.880	0.901	0.891
100.0	0.356	0.350	0.353

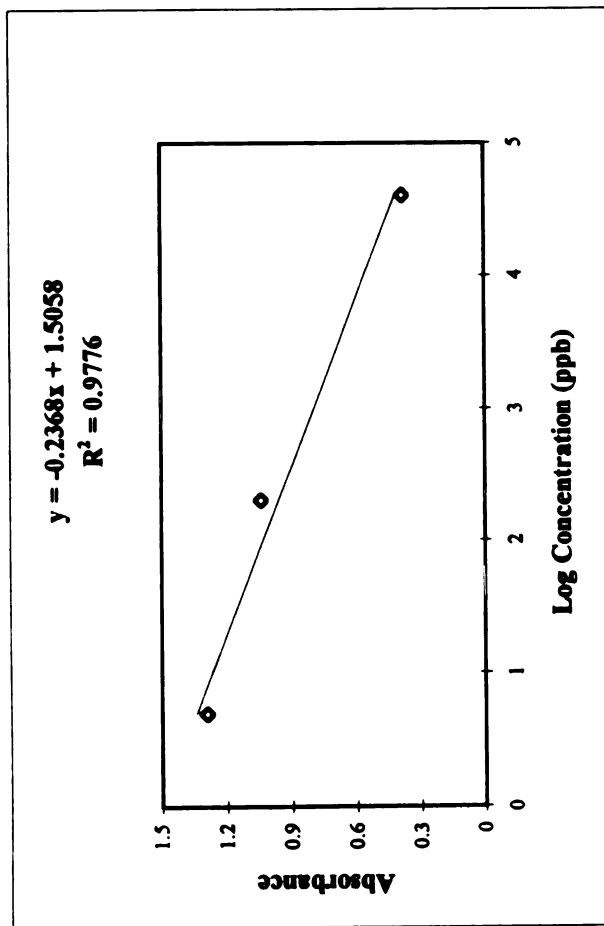


ID	Treatment #1-1				Treatment #1-2			
	Abs1	LnConc	Conc	Concf (ppb)	Abs1	Ln Conc	Conc	Concf (ppb)
BLK	1.645				1.645			
Fish1	1.423	nd	nd	nd	1.454	nd	nd	nd
Fish2	1.400	nd	nd	nd	1.377	nd	nd	nd
Fish3	1.432	nd	nd	nd	1.377	nd	nd	nd
Fish4	1.415	nd	nd	nd	1.540	nd	nd	nd
Fish5	1.650	nd	nd	nd	1.546	nd	nd	nd
Mean	1.464	nd	nd	nd	1.459	nd	nd	nd

Millipore test kit assay; can't

Standard #3

Concentration (ppb)	Abs1	Abs2	Mean
0.0	1.550	1.600	1.575
2.0	1.300	1.288	1.294
10.0	1.107	0.976	1.042
100.0	0.377	0.387	0.382



ID	Treatment #2-1				Treatment #2-2			
	Abs1	LnConc	Conc	Concf (ppb)	Abs1	LogConc	Conc	Concf (ppb)
control	18.000				1.800			
Fish1	1.455	nd	nd	nd	1.400	nd	nd	nd
Fish2	1.372	nd	nd	nd	1.432	nd	nd	nd
Fish3	1.430	nd	nd	nd	1.327	nd	nd	nd
Fish4	1.431	nd	nd	nd	1.315	nd	nd	nd
Fish5	1.612	nd	nd	nd	1.540	nd	nd	nd
Mean		nd	nd	nd	1.403	nd	nd	nd

APPENDIX B
RESULTS OF ATRAZINE DETERMINATION

APPENDIX B

RESULTS OF ATRAZINE

Table A1. Standard Curve Data
Ohmicron test kit

OHMICRON KIT												
			Assay #1				Assay #2					
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0.00	0.917	0.963	0.940	0.03	0.03		0.912	0.9	0.906	0.01	0.01	
0.05	0.857	0.876	0.867	0.01	0.02	92	0.840	0.85	0.845	0.01	0.01	93
0.10	0.812	0.833	0.823	0.01	0.02	88	0.777	0.758	0.768	0.01	0.02	85
1.00	0.447	0.484	0.466	0.03	0.06	50	0.455	0.477	0.466	0.02	0.03	51
2.50	0.224	0.243	0.234	0.01	0.06	25	0.237	0.235	0.236	0.00	0.01	26
5.00	0.115	0.123	0.119	0.01	0.05	13	0.113	0.117	0.115	0.00	0.02	13
10.00	0.096	0.105	0.101	0.01	0.06	11	0.113	0.111	0.112	0.00	0.01	12
			Assay #3				Assay #4					
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0.00	0.925	0.937	0.931	0.01	0.01		0.945	0.935	0.940	0.01	0.01	
0.05	0.817	0.907	0.862	0.06	0.07	93	0.911	0.924	0.918	0.01	0.01	98
0.10	0.805	0.814	0.810	0.01	0.01	87	0.840	0.806	0.823	0.02	0.03	88
1.00	0.456	0.514	0.485	0.04	0.08	52	0.451	0.417	0.434	0.02	0.06	46
2.50	0.244	0.213	0.229	0.02	0.10	25	0.233	0.225	0.229	0.01	0.02	24
5.00	0.130	0.122	0.126	0.01	0.04	14	0.105	0.124	0.115	0.01	0.12	12
10.00	0.105	0.113	0.109	0.01	0.05	12	0.104	0.127	0.116	0.02	0.14	12

B. Millipore test kit assay

MILLIPORE KITS												
Assay #1						Assay #2						
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0.00	1.579	1.529	1.554	0.04	2%		1.489	1.512	1.5005	0.02	1%	
0.05	1.329	1.315	1.322	0.01	1%	85	1.309	1.325	1.317	0.01	1%	88
0.10	1.285	1.297	1.291	0.01	1%	83	1.249	1.235	1.242	0.01	1%	83
0.25	1.21	1.197	1.204	0.01	1%	77	1.215	1.207	1.211	0.01	0%	81
0.50	0.998	1.036	1.017	0.03	3%	65	0.992	1.005	0.999	0.01	1%	67
1.00	0.807	0.817	0.812	0.01	1%	52	0.817	0.807	0.812	0.01	1%	54
2.00	0.575	0.57	0.573	0.00	1%	37	0.607	0.5	0.554	0.08	14%	37
5.00	0.407	0.418	0.413	0.01	2%	27	0.387	0.352	0.370	0.02	7%	25
10.00	0.207	0.217	0.212	0.01	3%	14	0.283	0.276	0.280	0.00	2%	19
15.00	0.195	0.208	0.202	0.01	5%	13	0.245	0.256	0.251	0.01	3%	17
Assay #3						Assay #4						
Concentration (ppb)	Abs 1	Abs 2	Mean	SDV	%CV	%Bo	Abs 1	Abs 2	Mean	SDV	%CV	%Bo
0.00	1.601	1.575	1.588	0.02	1%		1.502	1.476	1.489	0.02	1%	
0.05	1.429	1.415	1.422	0.01	1%	90	1.443	1.467	1.455	0.02	1%	98
0.10	1.377	1.237	1.307	0.10	8%	82	1.200	1.215	1.208	0.01	1%	81
0.25	1.285	1.295	1.290	0.01	1%	81	1.176	1.192	1.184	0.01	1%	80
0.50	1.105	1.121	1.113	0.01	1%	70	1.025	1.056	1.041	0.02	2%	70
1.00	0.967	0.947	0.957	0.01	1%	60	0.978	0.995	0.987	0.01	1%	66
2.00	0.79	0.715	0.753	0.05	7%	47	0.6	0.607	0.604	0.00	1%	41
5.00	0.515	0.506	0.511	0.01	1%	32	0.511	0.512	0.512	0.00	0%	34
10.00	0.43	0.321	0.376	0.08	21%	24	0.264	0.27	0.267	0.00	2%	18
15.00	0.357	0.325	0.341	0.02	7%	21	0.243	0.262	0.2525	0.01	5%	17

Table B2. Recovery Study Data for Fish Samples**A. Ohmicron test kit assay****Standard**

Concentration (ppb)	Abs1	Abs 2	Mean	% Bo
0.0	0.905	1.11	1.008	
0.1	0.783	0.791	0.787	78
1.0	0.427	0.445	0.436	43
5.0	0.185	0.2	0.193	19

ASSAY #1: Dilution 1:10; Standard provided

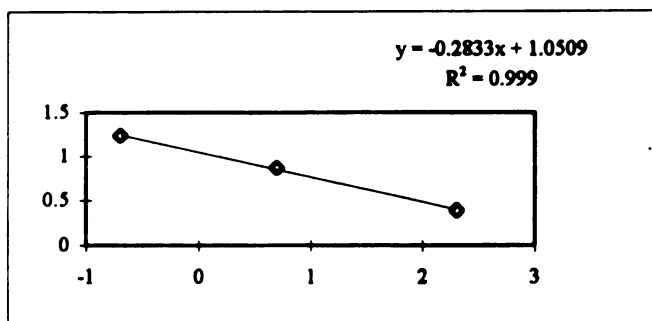
Spiking Levels ng/g)	Abs1	Abs 2	Mean	% Bo	Log Con	Conc	%Rec
control	0.905	0.915	0.910				
0.7	0.727	0.738	0.733	80	-1.070	0.85	122
1.0	0.687	0.676	0.682	75	-0.908	1.24	124
1.5	0.637	0.627	0.632	69	-0.752	1.77	118
2.0	0.612	0.603	0.608	67	-0.674	2.12	106
4.0	0.502	0.512	0.507	56	-0.356	4.40	110
7.0	0.402	0.405	0.404	44	-0.029	9.36	134

ASSAY #2: Dilution 1:50; Standard prepared in 10% ACN in water

Spiking Levels	Abs1	Abs 2	Mean	% Bo	Log Con	Conc	%Rec
control	0.925	0.915	0.920				
0.7	0.757	0.768	0.763	83	-1.14	0.73	103.93
1.0	0.720	0.725	0.723	79	-1.01	0.97	97.04
1.5	0.667	0.657	0.662	72	-0.82	1.50	100.04
2.0	0.620	0.628	0.624	68	-0.70	1.97	98.66
4.0	0.533	0.532	0.533	58	-0.42	3.81	95.37
7.0	0.450	0.465	0.458	50	-0.18	6.55	93.54

B. Millipore test kit assay

Concentration (ppb)	Abs 2	Abs 2	Mean
0.0	1.512	1.500	1.506
0.5	1.240	1.238	1.239
2.0	0.875	0.865	0.870
10.0	0.397	0.386	0.392



ASSAY #1 Dilution 1:10, Standard provided

Spiking Levels (ppb)	Abs1	Log Con	Conc	%Rec
control	1.457			
0.7	1.210	nd	nd	nd
1.0	0.978	0.2541	1.29	128.94
1.5	0.905	0.5118	1.67	111.22
2.0	0.813	0.8366	2.31	115.42
4.0	0.608	1.5602	4.76	118.99
7.0	0.442	2.1461	8.55	122.17

ASSAY #2: Dilution 1:10; Standard prepared in 10% ACN in water

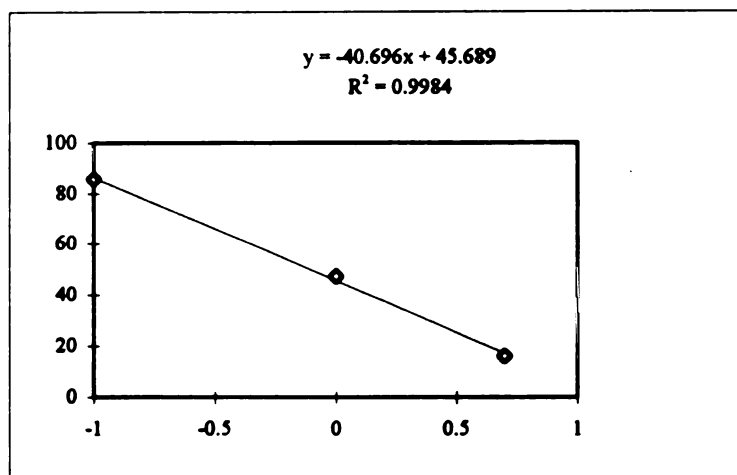
Spiking Levels (ng/g)	Abs1	Log Con	Conc	%Rec
control	1.457			
0.7	1.220	nd	nd	nd
1.0	1.050	0.000	1.00	100.00
1.5	0.910	0.494	1.64	109.28
2.0	0.870	0.635	1.89	94.39
4.0	0.670	1.341	3.82	95.60
7.0	0.485	1.994	7.35	104.96

Table B3. Recovery Study Data for Corn Leaf

A. Ohmicron test kit assay

Standard

Concentration (ppb)	Abs 2	Abs 2	Mean	% Bo
Blank	0.912	0.923	0.918	
0.1	0.782	0.791	0.787	85.7
1.0	0.431	0.437	0.434	47.3
5.0	0.147	0.152	0.150	16.3



ASSAY #1: Dilution 1:10; standard provided

Spiking Levels (ng/g)	Abs1	Abs 1	Mean	% Bo	Log Con	Conc	%Rec
control	0.942	0.951	0.947				
0.7	0.830	0.856	0.843	89	-1.065	0.86	123
1.0	0.770	0.79	0.780	82	-0.902	1.25	125
1.5	0.677	0.684	0.681	72	-0.644	2.27	151
2.0	0.645	0.655	0.650	69	-0.564	2.73	136
4.0	0.550	0.568	0.559	59	-0.328	4.70	117
7.0	0.462	0.456	0.459	48	-0.069	8.54	122

Ohmicron test kit assay; con't

ASSAY #2: Dilution 1:50

Spiking Levels (ng/g)	Abs1	Abs 1	Mean	% Bo	Log Con	Conc	%Rec
control	0.915	0.957	0.936				
0.7	0.855	0.856	0.856	91	-1.123	0.75	107.66
1.0	0.771	0.79	0.781	83	-0.926	1.19	118.59
1.5	0.739	0.715	0.727	78	-0.786	1.64	109.24
2.0	0.649	0.655	0.652	70	-0.589	2.58	128.92
4.0	0.588	0.578	0.583	62	-0.408	3.91	97.82
7.0	0.449	0.456	0.453	48	-0.065	8.61	123.01

ASSAY #3: Dilution 1:50; Standard prepared in 10% methanol in water

Spiking Levels	Abs1	Abs 1	Mean	% Bo	Log Con	Conc	%Rec
control	0.915	0.957	0.936				
0.7	0.865	0.876	0.871	93	-1.162	0.69	98.33
1.0	0.779	0.869	0.824	88	-1.040	0.91	91.17
1.5	0.729	0.705	0.717	77	-0.759	1.74	116.05
2.0	0.679	0.648	0.664	71	-0.619	2.41	120.26
4.0	0.566	0.594	0.580	62	-0.400	3.98	99.61
7.0	0.455	0.451	0.453	48	-0.066	8.58	122.64

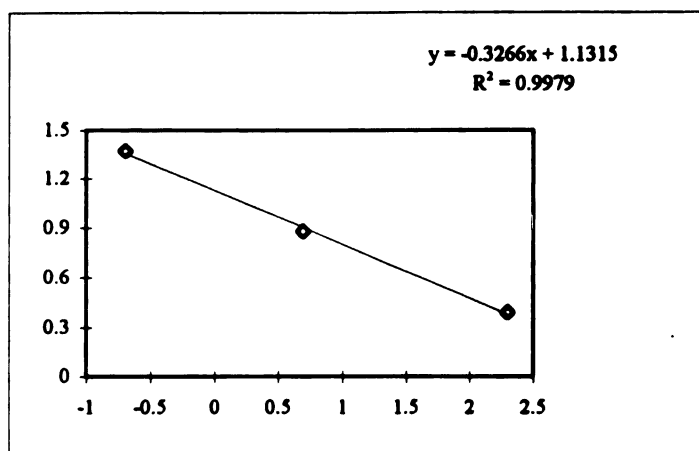
ASSAY #3: Dilution 1:50; Standard prepared in corn leave extract

Spiking Levels (ng/g)	Abs1	Abs 1	Mean	% Bo	Log Con	Conc	%Rec
control	0.915	0.957	0.936				
0.7	0.877	0.844	0.861	92	-1.136	0.73	104.46
1.0	0.788	0.861	0.825	88	-1.041	0.91	90.89
1.5	0.736	0.749	0.743	79	-0.826	1.49	99.47
2.0	0.700	0.712	0.706	75	-0.730	1.86	93.02
4.0	0.576	0.594	0.585	63	-0.413	3.87	96.64
7.0	0.497	0.515	0.506	54	-0.205	6.23	89.02

B. Millipore test kit assay

Standard 1

Concentration (ppb)	Abs1	Abs2	Mean
0.00	1.460	1.455	1.458
0.50	1.366	1.378	1.372
2.00	0.881	0.877	0.879
10.00	0.388	0.395	0.392



ASSAY #1: 1:10 Dilution; standard provided

Spiking Levels (ng/g)	Abs1	Log Conc	*Conc	**%Rec
control	1.460			
2.50	1.400	-0.8182	4.41	176.49
25.0	0.715	1.2576	35.17	140.68
50.0	0.456	2.0424	77.09	154.19
100.0	0.264	2.6242	137.94	137.94

*Concentration

**% recovery

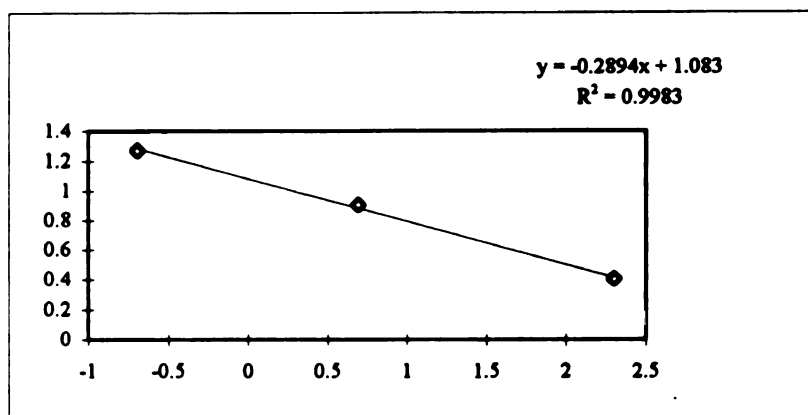
ASSAY #2 Dilution 1:50; standard provided

Spiking Levels (ng/g)	Abs1	Log Con	Conc	%Rec
control	1.460			
2.50	1.407	-0.8394	21.60	172.79
25.0	0.735	1.1970	165.50	132.40
50.0	0.533	1.8091	305.24	122.10
100.0	0.321	2.4515	580.30	116.06

Millipore test kit assay con't

Standard 2

Concentration (ppb)	Abs1	Abs2	Mean
0.0	1.475	1.521	1.498
0.5	1.263	1.282	1.273
2.0	0.910	0.896	0.903
10.0	0.410	0.404	0.407



Assay #3 Dilution 1:50; standard prepared in 10% water in methanol

Spiking Levels (ng/g)	Abs1	Log Con	Conc	%Rec
control	1.475			
2.50	1.458	nd	nd	nd
25.0	0.765	1.1061	151.12	120.90
50.0	0.545	1.7727	294.34	117.74
100.0	0.275	2.5909	667.09	133.42

Assay #4: Dilution 1:50; Standard prepared with corn extract

Spiking Levels (ng/g)	Abs1	Log Con	Conc	%Rec
control	1.458			
2.50	1.462	nd	nd	nd
25.0	0.821	0.9364	127.53	102.03
50.0	0.612	1.5697	240.26	96.10
100.0	0.376	2.2848	491.21	98.24

Table B4. Repeatability Study Data

A. Ohmicron test kit assay

Standard (ppb)	Assay #1			Assay #2			Assay #3		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.300	1.312	1.306	1.431	1.356	1.394	1.421	1.402	1.412
0.1	1.225	1.219	1.222	1.125	1.232	1.179	1.279	1.312	1.296
1.0	0.712	0.700	0.706	0.767	0.732	0.750	0.776	0.800	0.788
5.0	0.260	0.257	0.259	0.260	0.300	0.280	0.300	0.296	0.298
Spike (ppb)									
0.5	1.1	1.14	1.120	1.21	1.104	1.157	1.45	1.67	1.560
1.0	0.721	0.699	0.710	0.677	0.682	0.680	0.8	0.787	0.794
2.5	0.45	0.461	0.456	0.45	0.41	0.430	0.488	0.5	0.494
5.0	0.243	0.251	0.247	0.243	0.267	0.255	0.277	0.274	0.276

Standard (ppb)	Assay #4			Assay #5			Assay #6		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.310	1.321	1.316	1.327	1.317	1.322	1.231	1.237	1.234
0.1	1.210	1.200	1.205	1.210	1.208	1.209	1.178	1.185	1.182
1.0	0.744	0.742	0.743	0.777	0.795	0.786	0.765	0.721	0.743
5.0	0.254	0.264	0.259	0.281	0.265	0.273	0.235	0.231	0.233
Spike (ppb)									
0.5	1.33	1.21	1.270	1.325	1.378	1.352	1.212	1.123	1.168
1.0	0.7	0.702	0.701	0.783	0.776	0.780	0.696	0.683	0.690
2.5	0.5	0.461	0.481	0.467	0.437	0.452	0.32	0.376	0.348
5.0	0.225	0.251	0.238	0.264	0.256	0.260	0.187	0.212	0.200

B. Ohmicron test kit assay; con't

INTER-ASSAY

Standard (ppb)	Assay #1			Assay #2			Assay #3		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.323	1.213	1.268	1.420	1.421	1.421	1.210	1.320	1.265
0.1	1.200	1.176	1.188	1.120	0.987	1.054	1.132	1.156	1.144
1.0	0.700	0.687	0.694	0.698	0.721	0.710	0.721	0.776	0.749
5.0	0.220	0.216	0.218	0.243	0.302	0.273	0.277	0.254	0.266
Spike (ppb)									
0.5	1.11	1.105	1.108	1.2	1.12	1.160	0.9	0.918	0.909
1.0	0.667	0.721	0.694	0.62	0.663	0.642	0.784	0.743	0.764
2.5	0.421	0.441	0.431	0.427	0.420	0.424	0.433	0.412	0.423
5.0	0.212	0.221	0.217	0.276	0.296	0.286	0.187	0.178	0.183

Standard (ppb)	Assay #4			Assay #5			Assay #6		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.223	1.256	1.240	1.410	1.306	1.358	1.245	1.250	1.248
0.1	1.212	1.208	1.210	1.214	1.321	1.268	1.156	1.170	1.163
1.0	0.732	0.714	0.723	0.781	0.821	0.801	0.755	0.703	0.729
5.0	0.215	0.235	0.225	0.231	0.247	0.239	0.222	0.217	0.220
Spike (ppb)									
0.5	1.23	1.17	1.200	1.342	1.428	1.385	1.202	1.17	1.186
1.0	0.697	0.677	0.687	0.751	0.789	0.770	0.649	0.621	0.635
2.5	0.452	0.413	0.433	0.437	0.441	0.439	0.31	0.342	0.326
5.0	0.221	0.215	0.218	0.243	0.251	0.247	0.167	0.223	0.195

B. Millipore test kit assay

Standard (ppb)	Assay #1			Assay #2			Assay #3		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.445	1.462	1.454	1.433	1.376	1.405	1.356	1.350	1.353
0.5	1.300	1.298	1.299	1.234	1.312	1.273	1.245	1.249	1.247
2.0	0.867	0.885	0.876	0.858	0.867	0.863	0.765	0.781	0.773
10.0	0.421	0.411	0.416	0.435	0.425	0.430	0.412	0.387	0.400
Spike (ppb)									
0.5	1.312	1.300	1.306	1.333	1.300	1.317	1.315	1.278	1.297
1.0	0.932	0.921	0.927	0.921	0.907	0.914	0.876	0.845	0.861
2.5	0.800	0.821	0.811	0.821	0.810	0.816	0.785	0.8	0.783
5.0	0.735	0.722	0.729	0.727	0.721	0.724	0.678	0.689	0.684
10	0.400	0.431	0.416	0.412	0.421	0.417	0.395	0.390	0.393

Standard (ppb)	Assay #4			Assay #5			Assay #6		
	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean	Abs 1	Abs 2	Mean
0.0	1.316	1.305	1.311	1.336	1.330	1.333	1.455	1.400	1.428
0.5	1.178	1.196	1.187	1.145	1.151	1.148	1.340	1.340	1.340
2.0	0.812	0.822	0.817	0.745	0.750	0.748	0.921	0.925	0.923
10.0	0.400	0.398	0.399	0.368	0.360	0.364	0.475	0.468	0.472
Spike (ppb)									
0.5	1.145	1.189	1.167	1.182	1.157	1.170	1.325	1.327	1.326
1.0	0.936	0.93	0.933	0.886	0.875	0.881	1.105	1.1	1.103
2.5	0.798	0.795	0.797	0.683	0.672	0.678	0.9	0.895	0.896
5.0	0.735	0.722	0.729	0.612	0.6	0.606	0.75	0.765	0.758
10	0.378	0.372	0.375	0.342	0.331	0.337	0.457	0.46	0.459

Millipore test kit assay; con't

Intra-assay

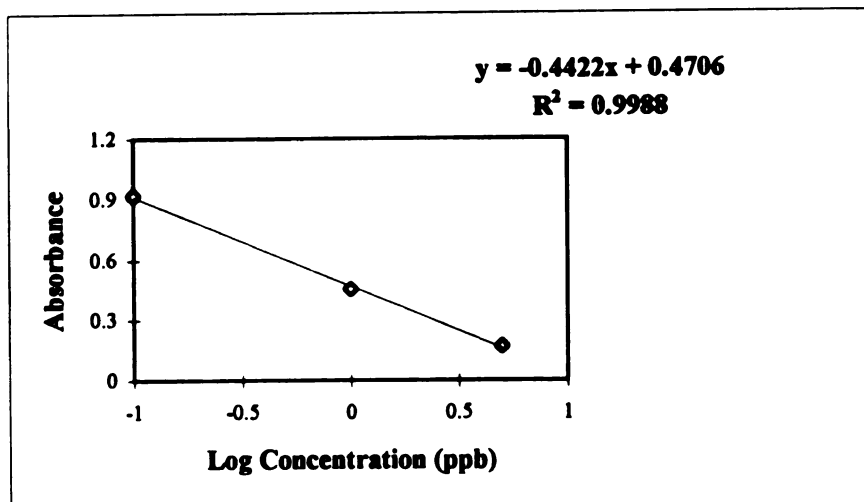
Assay #1	Assay #2		Assay #3	
Abs 1	Abs 2	Mean	Abs 1	Mean
1.326	1.345	1.336	1.423	1.433
1.270	1.247	1.259	1.312	1.223
0.789	0.799	0.794	0.857	0.876
0.320	0.387	0.354	0.423	0.453
1.305	1.312	1.309	1.335	1.312
0.843	0.837	0.840	0.945	0.936
0.77	0.8	0.777	0.811	0.831
0.666	0.66	0.663	0.754	0.721
0.4	0.407	0.404	0.421	0.42

Assay #4	Assay #5		Assay #6	
Abs 1	Abs 2	Mean	Abs 1	Mean
1.510	1.502	1.506	1.216	1.200
1.327	1.316	1.322	1.110	1.120
0.980	0.965	0.973	0.870	0.880
0.412	0.400	0.406	0.376	0.367
1.456	1.455	1.456	0.965	0.978
1.3	1.325	1.313	0.756	0.765
0.941	0.932	0.937	0.635	0.645
0.854	0.832	0.843	0.567	0.565
0.532	0.53	0.473	0.321	0.328

Table B5. Sensitivity study.

A. corn leaf with the Ohmicron test kit

Standard				
Conc (ppb)	Abs1	Abs2	Mean	%Bo
0.0	1.212	1.243	1.228	
0.1	1.121	1.135	1.128	92
1.0	0.555	0.564	0.560	46
5.0	0.211	0.207	0.209	17

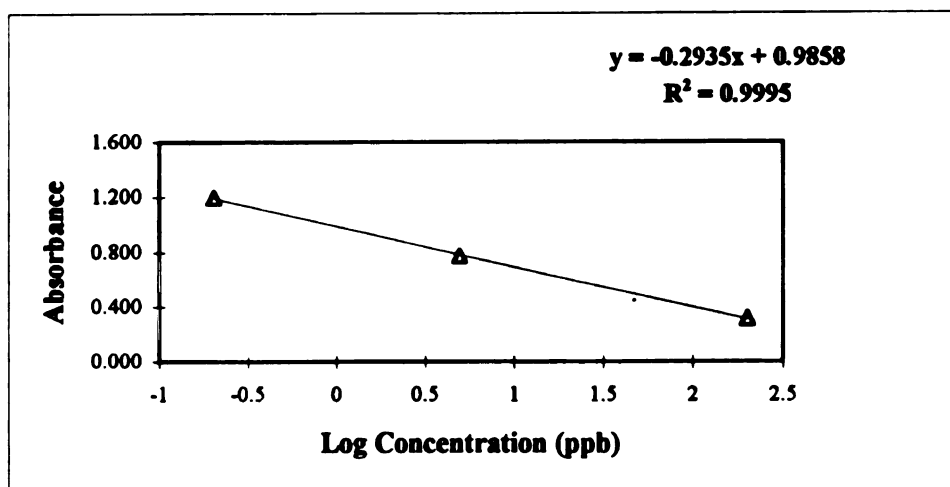


Assay #	Abs	%Bo	Conf
control	1.228		
1	0.279	0.23	0.407
2	0.222	0.18	0.512
3	0.105	0.09	0.727
4	0.200	0.16	0.552
5	0.211	0.17	0.532
6	0.217	0.18	0.521
7	0.225	0.18	0.506
8	0.234	0.19	0.489
Mean	0.212	0.172	0.531
Stdv	0.049	0.040	0.090
3@	0.147	0.120	0.271

B. Corn leaf with Millipore tests kit

Standard

Concentration (ppb)	Ln Con	Abs1	Abs2	Mean
0.0		1.317	1.325	1.321
0.5	-0.69	1.201	1.189	1.195
2.0	0.69	0.767	0.776	0.772
10.0	2.30	0.319	0.311	0.315

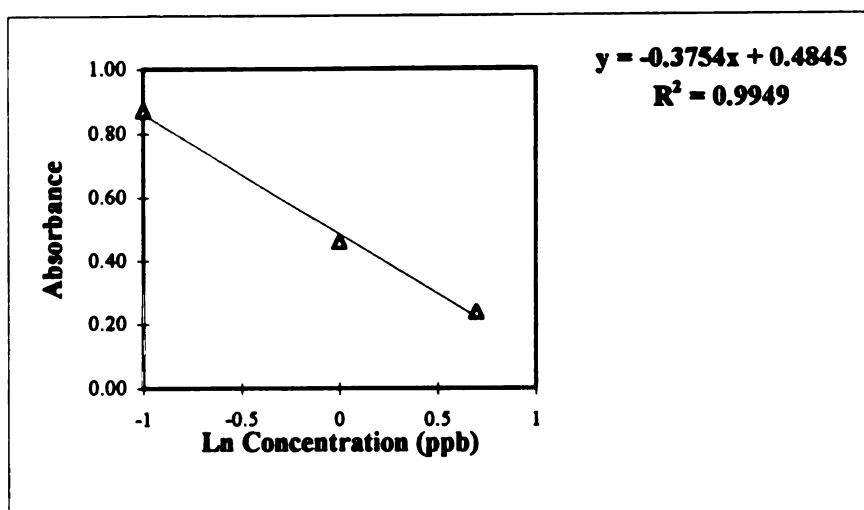


Assay #	Abs	Con	Conf
Blk	1.321		
1	0.535	1.53	4.637
2	0.501	1.65	5.205
3	0.521	1.58	4.863
4	0.498	1.66	5.259
5	0.426	1.90	6.718
6	0.512	1.61	5.014
7	0.504	1.64	5.152
8	0.531	1.55	4.700
Mean	0.504	1.641	5.193
STDv	0.034	0.116	0.657
3@	0.102	0.349	1.972

C. Fish fillet with Ohmicron tests kit

Standard

Conc (ppb)	Abs1	Abs2	Mean	% Bo
0.0	1.130	1.121	1.126	
0.1	0.975	0.985	0.980	87
1.0	0.510	0.521	0.516	46
5.0	0.275	0.260	0.268	24

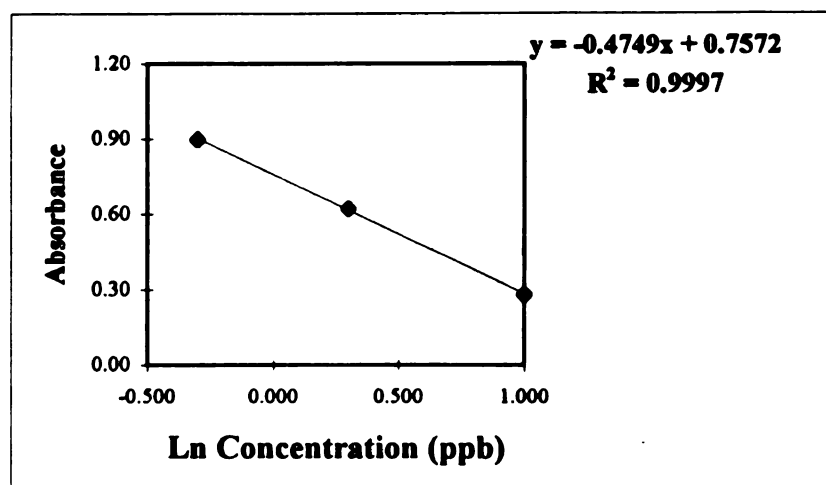


Assay #	Abs	%Bo	Conf
Blk	1.228		
1	0.259	21%	0.729
2	0.212	17%	0.831
3	0.221	18%	0.811
4	0.207	17%	0.842
5	0.201	16%	0.855
6	0.147	12%	0.972
7	0.225	18%	0.803
8	0.217	18%	0.820
Mean	0.211	17%	0.833
STDv	0.031	3%	0.068
3@	0.094	8%	0.204

D. Fish fillet with Millipore test kit

Standard

Conc (ppb)	Abs1	Abs2	Mean
0.0	1.403	1.376	1.390
0.5	1.223	1.270	1.247
2.0	0.865	0.858	0.862
10.0	0.386	0.391	0.389



Assay #	Abs	Ln Con	Con
blank	1.390		
1	0.543	0.451	1.72
2	0.600	0.331	1.82
3	0.621	0.287	1.86
4	0.498	0.546	1.65
5	0.342	0.874	1.41
6	0.410	0.731	1.51
7	0.375	0.805	1.45
8	0.612	0.306	1.84
Mean	0.500	0.541	1.658
Stdv	0.112	0.236	0.183
3@	0.336	0.707	0.548

Table B6. Cross-Reactivity Study Data.

A. Ohmicron test kit assay

A. Atrazine

Assay #1

Concentration (ppb)	Abs1	Abs2	Mean	% Bo
0.0	0.925	0.915	0.920	
0.1	0.786	0.802	0.794	86
1.0	0.457	0.427	0.442	48
5.0	0.132	0.140	0.136	15

Assay #2

Concentration (ppb)	Abs1	Abs2	Mean	% Bo
0.0	0.922	0.935	0.929	
0.1	0.712	0.727	0.720	77
1.0	0.421	0.387	0.404	44
5.0	0.131	0.124	0.128	14

Assay #3

Concentration (ppb)	abs1	abs2	Mean	%Bo
0.0	0.916	0.907	0.9115	
0.1	0.771	0.754	0.7625	84
1.0	0.407	0.412	0.4095	45
5.0	0.133	0.127	0.13	14

Assay #4

Concentration (ppb)	abs1	abs2	Mean	%Bo
0.0	0.912	0.925	0.919	
0.1	0.717	0.731	0.724	79
1.0	0.417	0.391	0.404	44
5.0	0.133	0.131	0.132	14

Ohmicron test kit assay; con't

B. Hydroxy-atrazine

Assay #1

Concentration (ppb)	Abs1	Abs2	Mean	% Bo
0.0	0.925	0.907	0.916	
0.1	0.903	0.895	0.899	98
1.0	0.597	0.607	0.602	66
5.0	0.344	0.321	0.333	36

Assay #2

Concentration (ppb)	abs1	abs2	Mean	%Bo
0.0	0.916	0.907	0.912	
0.1	0.901	0.897	0.899	99
1.0	0.612	0.600	0.606	66
5.0	0.311	0.318	0.315	35

Assay #3

Concentration (ppb)	Abs1	Abs2	Mean	% Bo
0.0	0.922	0.935	0.929	
0.1	0.867	0.897	0.882	95
1.0	0.587	0.596	0.592	64
5.0	0.312	0.319	0.316	34

Assay #4

Concentration (ppb)	abs1	abs2	Mean	%Bo
0.0	0.912	0.925	0.919	
0.1	0.882	0.887	0.885	96
1.0	0.622	0.611	0.617	67
5.0	0.318	0.331	0.325	35

Ohmicron test kit assay; con't

c. Simazine

Assay #1

Concentration (ppb)	Abs1	Abs2	Mean	% Bo
0.0	0.925	0.915	0.920	
0.1	0.912	0.902	0.907	99
1.0	0.800	0.821	0.811	88
5.0	0.645	0.655	0.650	71

Assay #2

Concentration (ppb)	Abs1	Abs2	Mean	% Bo
0.0	0.922	0.935	0.929	
0.1	0.922	0.931	0.927	100
1.0	0.845	0.856	0.851	92
5.0	0.677	0.687	0.682	73

Assay #3

Concentration (ppb)	abs1	abs2	Mean	%Bo
0.0	0.916	0.907	0.912	
0.1	0.847	0.839	0.843	92
1.0	0.775	0.768	0.772	85
5.0	0.663	0.611	0.637	70

Assay #4

Concentration (ppb)	abs1	abs2	Mean	%Bo
0.0	0.912	0.925	0.919	
0.1	0.835	0.830	0.833	91
1.0	0.811	0.789	0.800	87
5.0	0.664	0.678	0.671	73

B. Millipore test kit assay**A. Atrazine**

Concentration (ppb)	Abs1	Abs2	Abs3	Abs4	Mean
0.0	1.510	1.477	1.510	1.477	1.494
0.5	1.100	1.230	1.179	1.227	1.203
2.0	0.927	0.901	0.956	0.946	0.951
10.0	0.470	0.450	0.558	0.555	0.558

B. Hydroxy-atrazine

Concentration (ppb)	Abs 1	Abs 3	Abs 3	Abs 4	Mean
0.0	1.510	1.477	1.51	1.477	1.494
0.5	1.345	1.387	1.365	1.371	1.368
2.0	1.131	1.145	1.170	1.005	1.088
10.0	0.726	0.710	0.719	0.723	0.721

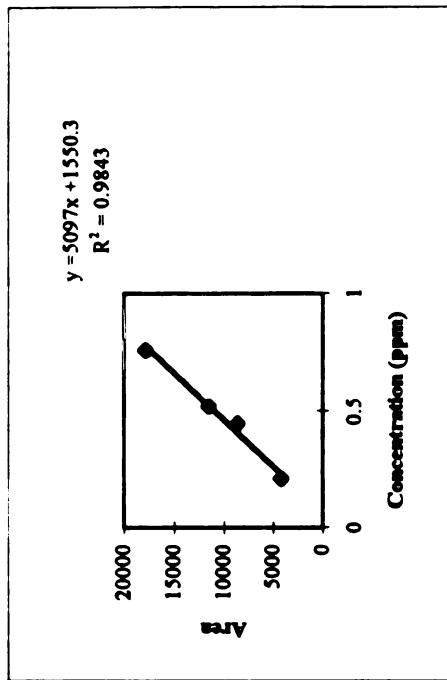
C. Simazine

Concentration (ppb)	Abs1	Abs 2	Abs 3	Abs 4	Mean
0.0	1.510	1.545	1.510	1.561	1.536
0.5	1.512	1.524	1.487	1.494	1.491
2.0	1.237	1.224	1.217	1.203	1.210
10.0	0.767	0.800	0.826	0.819	0.823

Table B8. Incurred Fish Study Data . A. Gas chromatographic determination

Standard curve

Conc	Area
0.208	4231
0.446	8623
0.520	11537
0.756	17845



Control 1

ID	Control 1				TRT #1			
	Area 1	Area 2	Mean	conc	Area 1	Area 2	Mean	conc
1DBT	np	np	np	nd	np	np	np	nd
DOT	np	np	np	nd	7850	7356	7603	0.24
5DAT	np	np	np	nd	np	np	np	nd
7DAT	np	np	np	nd	np	np	np	nd
11DAT	np	np	np	nd	np	np	np	nd

TRT #2

ID	TRT #2				TRT #3			
	Area 1	Area 2	Mean	conc	Area 1	Area 2	Mean	conc
1DBT	*np	np	np	**nd	np	np	nd	nd
DOT	13216	12647	12932	0.45	17320	16345	16833	0.61
5DAT	10010	10017	10014	0.34	13010	13117	13064	0.46
7DAT	8101	7905	8003	0.26	8601	8405	8503	0.28
11DAT	np	np	np	nd	np	np	nd	nd

*No peak of atrazine

**Non-detected

B. Ohmicron test kit data

Standard

Concentration (ppb)	Abs1	Abs2	Mean	B/Bo
0.0	1.223	1.275	1.249	
0.1	1.13	1.125	1.1275	90
1.0	0.567	0.56	0.564	45
5.0	0.201	0.205	0.203	16

Control = non treated

ID	ABS1	ABS2	Mean	%Bo	log Conc	conc	Conf
control	1.467	1.455	1.461				
1DBT	1.450	1.420	1.435	98.22	-1.187	nd	nd
DOT	1.330	1.310	1.320	90.35	-1.008	nd	nd
5DAT	1.367	1.303	1.335	91.38	-1.031	nd	nd
7DAT	1.312	1.333	1.323	90.52	-1.012	nd	nd
11DAT	1.412	1.403	1.408	96.34	-1.144	nd	nd

Treatment #1

ID	ABS1	ABS2	Mean	%Bo	log Conc	conc	conf
control	1.323	1.275	1.299				
1DBT	1.127	1.357	1.242	96	-1.128	nd	nd
DOT	0.452	0.433	0.443	34	0.271	1.87	37.35
5DAT	1.235	1.236	1.236	95	-1.116	nd	nd
7DAT	1.312	1.225	1.269	98	-1.174	nd	nd
11DAT	1.212	1.307	1.260	97	-1.158	nd	nd

Treatment #2

ID	ABS1	ABS2	Mean	%Bo	log Conc	conc	conf
control	1.467	1.455	1.461				
1DBT	1.356	1.362	1.359	93.02	-1.069	nd	nd
DOT	0.281	0.290	0.286	19.54	0.601	3.99	79.87
5DAT	0.407	0.384	0.396	27.07	0.430	2.69	53.86
7DAT	0.421	0.432	0.427	29.19	0.382	2.41	48.20
11DAT	1.232	1.370	1.301	89.05	-0.978	nd	nd

Treatment #3

ID	*ABS1	ABS2	Mean	%Bo	log Conc	conc	**Conf
control	1.323	1.275	1.299				
1DBT	1.312	1.310	1.311	101	-1.248	nd	nd
DOT	0.242	0.235	0.239	18	0.628	4.25	84.96
5DAT	0.304	0.311	0.308	24	0.507	3.22	64.34
7DAT	0.400	0.429	0.415	32	0.320	2.09	41.81
11DAT	1.212	1.250	1.231	95	-1.108	0.08	1.56

*Absorbance at 450 nm

**Final concentration

C. Millipore test kit assay

Standard			
*Conc (ppb)	Abs1	Abs2	Mean % Bo
0.0	1.322	1.356	1.339
0.5	1.107	1.000	1.054 79
2.0	0.532	0.537	0.535 40
10.0	0.210	0.205	0.208 15

*Concentration

Control = non treated

TRT #1									
ID	ABS1	ABS2	Mean	%Bo	Con	**Conf	ABS1	ABS2	Mean %Bo Con Conf
control	1.322	1.356	1.339				1.322	1.356	1.339
1DBT	1.317	1.221	1.269	95	nd	nd	1.305	1.327	1.316 98 nd
DOT	1.320	1.300	1.310	98	nd	nd	0.540	0.510	0.525 39 3,400 34.00
5DAT	1.301	1.312	1.307	98	nd	nd	1.311	1.312	1.312 98 nd
7DAT	1.311	1.306	1.309	98	nd	nd	1.350	1.316	1.333 100 nd
11DAT	1.307	1.355	1.331	99	nd	nd	1.320	1.307	1.314 98 nd

**Final concentration

Standard			
Conc(ppb)	Abs1	Abs2	Mean % Bo
0.0	1.311	1.318	1.315
0.5	1.100	1.070	1.085 83
2.0	0.700	0.690	0.695 53
10.0	0.215	0.225	0.220 17

TRT #2									
ID	ABS1	ABS2	Mean	%Bo	Conc	conf	ABS1	ABS2	Mean %Bo conc conf
control	1.311	1.318	1.315				1.311	1.318	1.315
1DBT	1.302	1.313	1.308	99	nd	nd	1.312	1.377	1.345 102 nd
DOT	0.312	0.320	0.316	24	7.00	70.0	0.275	0.267	0.271 21 8.5 85.0
5DAT	0.424	0.430	0.427	32	4.60	46.0	0.340	0.344	0.342 26 6.5 65.0
7DAT	0.477	0.481	0.479	36	4.00	40.0	0.456	0.435	0.446 34 3.4 34.0
11DAT	1.351	1.333	1.342	102	nd	nd	1.121	1.127	1.124 86 nd

TRT #3

Table B8. Incurred Fish Sample

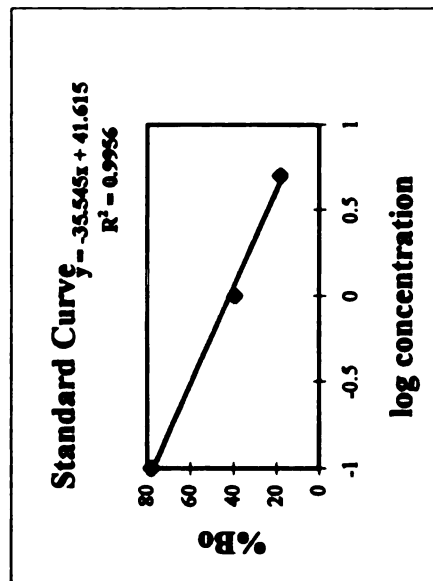
A. Gas chromatographic determination

Treatment #1-1												
ID	Control 1			Control 2			Treatment #1-1					
	Area 1	Area 2	Mean	Conc (ppm)	Area 1	Area 2	Mean	Conc (ppm)	Area 1	Area 2	Mean	Conc (ppm)
Fish1	NP	NP	NP	nd	NP	NP	NP	nd	NP	NP	NP	nd
Fish2	NP	NP	NP	nd	NP	NP	NP	nd	NP	NP	NP	nd
Fish3	NP	NP	NP	nd	NP	NP	NP	nd	NP	NP	NP	nd
Fish4	NP	NP	NP	nd	NP	NP	NP	nd	NP	NP	NP	nd
Fish5	NP	NP	NP	nd	NP	NP	NP	nd	NP	NP	NP	nd
Mean	NP	NP	NP	nd	NP	NP	NP	nd	NP	NP	NP	nd

Treatment #2-2												
ID	Treatment #1-2			Treatment #2-1			Treatment #2-2					
	Area 1	Area 2	Mean	Conc (ppm)	Area 1	Area 2	Mean	Conc (ppm)	Area 1	Area 2	Mean	Conc (ppm)
Fish1	NP	NP	NP	nd	np	np	np	nd	NP	NP	NP	nd
Fish2	NP	NP	NP	nd	np	np	np	nd	NP	NP	NP	nd
Fish3	NP	NP	NP	nd	np	np	np	nd	NP	NP	NP	nd
Fish4	NP	NP	NP	nd	np	np	np	nd	NP	NP	NP	nd
Fish5	NP	NP	NP	nd	np	np	np	nd	NP	NP	NP	nd
Mean	NP	NP	NP	nd	np	np	np	nd	NP	NP	NP	nd

B. Ohmicron test kit assay

Standard				
Conc (ppb)	Abs1	Abs2	Mean	B/Bo
0.0	0.887	0.877	0.882	
0.1	0.679	0.699	0.689	78
1.0	0.343	0.35	0.347	39
5.0	0.153	0.167	0.160	18



Sample	Control #1							Control #2						
	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf
blank	0.887	0.877	0.882					0.887	0.877	0.882				
Fish1	0.884	0.875	0.880	100	-1.603	0.02	nd	0.895	0.866	0.881	100	-1.606	0.02	nd
Fish2	0.823	0.866	0.845	96	-1.493	0.03	nd	0.775	0.895	0.835	95	-1.463	0.03	nd
Fish3	0.821	0.820	0.856	97	-1.529	0.03	nd	0.891	0.776	0.834	95	-1.458	0.03	nd
Fish4	0.856	0.870	0.756	86	-1.214	0.06	nd	0.885	0.865	0.875	99	-1.589	0.03	nd
Fish5	0.788	0.917	0.853	97	-1.518	0.03	nd	0.980	0.981	0.981	111	-1.921	0.01	nd
Mean	0.834	0.870	0.838	95	-1.472	0.03	nd	0.885	0.877	0.881	100	-1.608	0.02	nd

Ohmicron test kit assay; con't

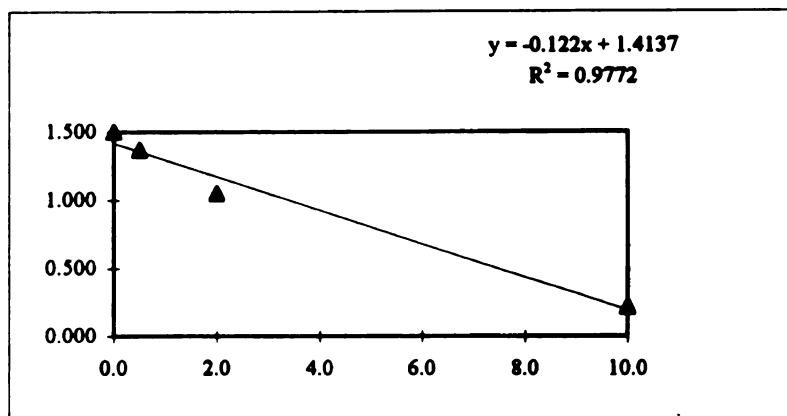
ID	Treatment #1-1							Treatment #1-2						
	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf
BLK	0.887	0.877	0.882					0.887	0.877	0.882				
Fish1	0.845	0.811	0.828	94	-1.441	0.04	nd	0.980	0.815	0.898	102	-1.660	0.02	nd
Fish2	0.807	0.807	0.807	91	-1.375	0.04	nd	0.871	0.837	0.854	97	-1.523	0.03	nd
Fish3	0.801	0.817	0.809	92	-1.381	0.04	nd	0.895	0.891	0.893	101	-1.646	0.02	nd
Fish4	0.825	0.817	0.821	93	-1.419	0.04	nd	0.811	0.845	0.828	94	-1.441	0.04	nd
Fish5	0.822	0.817	0.820	93	-1.414	0.04	nd	0.807	0.870	0.839	95	-1.474	0.03	nd
Mean	0.820	0.814	0.817	93	-1.406	0.04	nd	0.873	0.852	0.862	98	-1.549	0.03	nd

ID	Treatment #2-1							Treatment #2-2						
	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf	Abs1	Abs2	Mean	%Bo	LogConc	Conc	Concf
BLK	1.120	1.143	1.132					0.887	0.766	0.827				
Fish1	0.456	0.435	0.446	39	0.073	1.18	118.3	0.788	0.771	0.780	94	-1.453	0.035	nd
Fish2	0.865	0.755	0.810	72	-0.822	0.15	nd	0.812	0.805	0.809	98	-1.551	0.028	nd
Fish3	0.877	0.852	0.865	76	-0.956	0.11	nd	0.756	0.775	0.766	93	-1.406	0.039	nd
Fish4	0.642	0.616	0.629	56	-0.377	0.42	41.9	0.805	0.813	0.809	98	-1.552	0.028	nd
Fish5	0.746	0.770	0.758	67	-0.694	0.20	nd	0.772	0.815	0.794	96	-1.500	0.032	nd
Mean	0.717	0.686	0.701	62	-0.555	0.28	nd	0.787	0.796	0.791	96	-1.492	0.032	nd

C. Millipore test kit data

Standard #1

Concentration (ppb)	Abs1	Abs2	Mean
0.0	1.510	1.487	1.499
0.5	1.354	1.377	1.366
2.0	1.110	0.987	1.049
10.0	0.213	0.222	0.218

**Control 1**

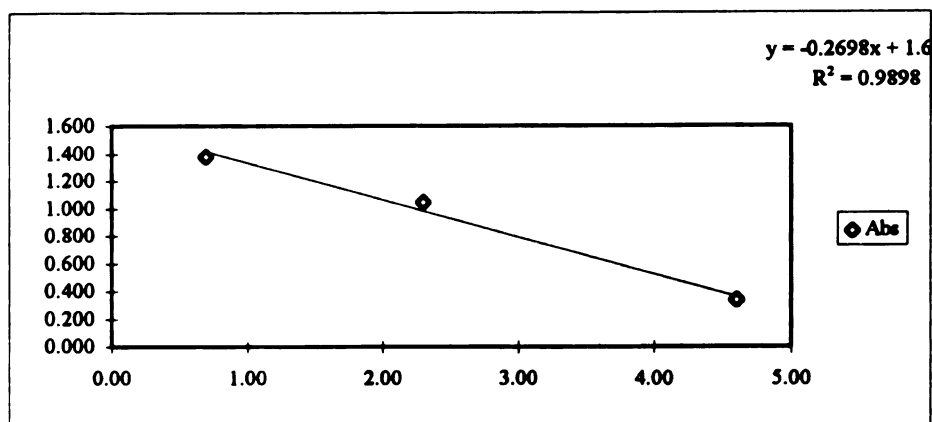
ID	Abs1	Ln Conc	Conc	Concf (ppb)
BLK	1.645			
Fish1	1.507	nd	nd	nd
Fish2	1.316	nd	nd	nd
Fish3	1.400	nd	nd	nd
Fish4	1.585	nd	nd	nd
Fish5	1.431	nd	nd	nd
Mean	1.448	nd	nd	nd

Control 2

ID	Abs1	Ln Conc	Conc	Concf (ppb)
BLK	1.645			
Fish1	1.481	nd	nd	nd
Fish2	1.411	nd	nd	nd
Fish3	1.302	nd	nd	nd
Fish4	1.382	nd	nd	nd
Fish5	1.520	nd	nd	nd
Mean	1.419	nd	nd	nd

Millipore test kit data; con't

Standard #2			
Concentration	Abs1	Abs2	Mean
0.0	1.450	1.477	1.464
2.0	1.385	1.375	1.380
10.0	0.987	1.101	1.044
100.0	0.340	0.331	0.336

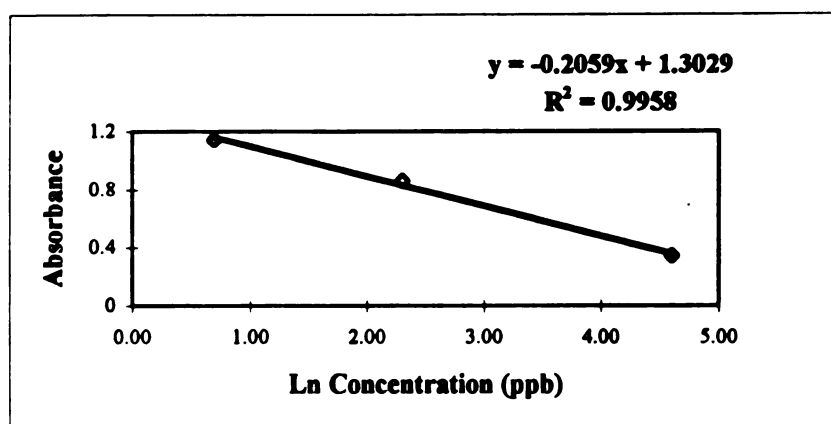


TRT #1-1				
ID	Abs1	Ln Conc	Conc	Concf (ppb)
BLK	1.567			
Fish1	1.423	nd	nd	nd
Fish2	1.400	nd	nd	nd
Fish3	1.326	nd	nd	nd
Fish4	1.415	nd	nd	nd
Fish5	2.512	nd	nd	nd
Mean	1.615	nd	nd	nd

TRT #1-2				
ID	Abs1	Ln Conc	Conc	Concf (ppb)
BLK	1.567			
Fish1	1.325	nd	nd	nd
Fish2	1.317	nd	nd	nd
Fish3	1.341	nd	nd	nd
Fish4	1.312	nd	nd	nd
Fish5	1.292	nd	nd	nd
Mean	1.317	nd	nd	nd

Millipore test kit data; con't

Standard #3				
Concentration (ppb)	Abs1	Abs2	Mean	
0.0	1.567	1.582	1.575	
2.0	1.145	1.140	1.143	
10.0	0.856	0.862	0.859	
100.0	0.345	0.340	0.343	



TRT #2-1				
ID	Abs1	LnConc	Conc	Concf (ppb)
blank	1.760			
Fish1	1.245	nd	nd	nd
Fish2	1.340	nd	nd	nd
Fish3	1.300	nd	nd	nd
Fish4	1.410	nd	nd	nd
Fish5	1.430	nd	nd	nd
Mean				

TRT #2-2				
ID	Abs1	Ln Conc	Conc	Concf (ppb)
BLK	1.760			
Fish1	1.400	nd	nd	nd
Fish2	1.275	nd	nd	nd
Fish3	1.327	nd	nd	nd
Fish4	1.315	nd	nd	nd
Fish5	1.265	nd	nd	nd
Mean	1.316	nd	nd	nd

APPENDIX C

RESULTS OF CARBOFURAN DETERMINATION

Table C1. Standard Curve Data.

A. Ohmicron test kit assay

ASSAY #1						
Concentration (ppb)	Abs 1	Abs 2	Mean	sdv	%CV	%Bo
0.000	1.364	1.397	1.381	0.023	1.7	
0.078	1.315	1.322	1.319	0.005	0.4	96
0.156	1.312	1.307	1.310	0.004	0.3	95
0.312	1.187	1.201	1.194	0.010	0.8	86
0.624	0.877	0.881	0.879	0.003	0.3	64
1.248	0.545	0.540	0.543	0.004	0.7	39
2.496	0.400	0.378	0.389	0.016	4.0	28
4.368	0.302	0.285	0.294	0.012	4.1	21
6.240	0.277	0.295	0.286	0.013	4.5	21

ASSAY #2						
Concentration (ppb)	Abs 1	Abs 2	Mean	sdv	%CV	%Bo
0.000	1.346	1.361	1.354	0.011	0.8	
0.078	1.315	1.310	1.313	0.004	0.3	97
0.156	1.302	1.287	1.295	0.011	0.8	96
0.312	1.221	1.211	1.216	0.007	0.6	90
0.624	0.882	0.867	0.875	0.011	1.2	65
1.248	0.535	0.547	0.541	0.008	1.6	40
2.496	0.386	0.377	0.382	0.006	1.7	28
4.368	0.311	0.306	0.309	0.004	1.1	23
6.240	0.287	0.288	0.288	0.001	0.2	21

ASSAY #3						
Concentration (ppb)	Abs 1	Abs 2	Mean	sdv	%CV	%Bo
0.000	1.337	1.340	1.339	0.002	0.2	
0.078	1.322	1.312	1.317	0.007	0.5	98
0.156	1.297	1.296	1.297	0.001	0.1	97
0.312	1.233	1.213	1.223	0.014	1.2	91
0.624	0.866	0.856	0.861	0.007	0.8	64
1.248	0.530	0.532	0.531	0.001	0.3	40
2.496	0.367	0.371	0.369	0.003	0.8	28
4.368	0.301	0.321	0.311	0.014	4.5	23
6.240	0.300	0.311	0.306	0.008	2.5	23

B. Millipore test kit assay**ASSAY #1**

Concentration (ppb)	Abs 1	Abs 2	Mean	Stdv	%CV
0.000	1.377	1.330	1.354	0.033	2.5
0.156	1.277	1.300	1.289	0.016	1.3
0.312	1.230	1.223	1.227	0.005	0.4
0.624	0.850	0.846	0.848	0.003	0.3
1.248	0.523	0.542	0.533	0.013	2.5
4.368	0.278	0.267	0.273	0.008	2.9
6.240	0.256	0.267	0.262	0.008	3.0

ASSAY #2

Concentration (ppb)	Abs 1	Abs 2	Mean	Stdv	%CV
0.000	1.400	1.356	1.378	0.031	2.3
0.156	1.323	1.367	1.345	0.031	2.3
0.312	1.220	1.267	1.244	0.033	2.7
0.624	0.853	0.861	0.857	0.006	0.7
1.248	0.523	0.542	0.533	0.013	2.5
4.368	0.288	0.255	0.272	0.023	8.6
6.240	0.266	0.247	0.257	0.013	5.2

Table C2. Recovery Study Data for Fish Samples.**A. Water extraction with ohmicron test kit assay**

Standard				
Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.320	1.335	1.328	
0.1	1.235	1.257	1.246	94
1.0	0.727	0.730	0.729	55
5.0	0.262	0.270	0.266	20

ASSAY #1: Dilution 1:10 Standard provided with the kit

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	* %Rec
0.00	1.402	1.377	1.390				
3.28	1.105	1.010	1.0575	76	0.365	3.65	111
5.65	0.955	0.946	0.951	68	0.7	7	124
16.40	0.555	0.560	0.558	40	1.90	19	116
32.80	0.330	0.327	0.329	24	4.00	40	122

* Percent recovery

Assay #2; Dilution 1:50; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	* %Rec
0.00	1.402	1.377	1.390				
3.28	1.345	1.330	1.3375	96	nd	nd	nd
5.65	1.3	1.32	1.31	94	0.1	5	88.50
16.4	1.141	1.157	1.149	83	0.28	14	85.37
32.8	0.977	0.983	0.980	71	0.56	28	85.37

* percent recovery

B. Acetonitrile extraction with Ohmicron test kit**ACETONITRILE EXTRACTION****Standard**

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.345	1.34	1.343	
0.1	1.287	1.265	1.276	95
1.0	0.847	0.865	0.856	64
5.0	0.35	0.325	0.338	25

ASSAY #1; Dilution 1:10; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	%Rec
0.00	1.440	1.435	1.438				
3.28	1.120	1.115	1.1175	78	0.50	5.00	152.44
5.65	0.967	0.976	0.972	68	0.85	8.50	150.44
16.40	0.647	0.610	0.6285	44	2.20	22.00	134.15
32.80	0.395	0.371	0.383	27	3.40	34.00	103.66

Assay #2; Dilution 1:50; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	Rec
0.00	1.440	1.435	1.438				
3.28	1.400	1.397	1.399	97	nd	nd	nd
5.65	1.347	1.354	1.351	94	0.12	6	106.19
16.4	1.129	1.300	1.215	84	0.340	17.00	103.66
32.8	1.000	1.102	1.051	73	0.700	35	106.71

C. Water extraction with Millipore test kit

ASSAY #1: Dilution 1:10 Standard provided with the kit

Standard

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.444	1.422	1.433
0.2	1.243	1.255	1.249
5.0	0.307	0.310	0.309

Sp Levels (ppb)	Abs 1	Abs 2	Mean
0.00	1.444	1.422	1.433
3.28	1.005	1.105	1.055
5.65	0.522	0.946	0.734
16.40	0.343	0.341	0.342
32.80	0.122	0.1267	0.124

Assay #2; Dilution 1:50; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean
0.00	1.444	1.422	1.433
3.28	0.967	0.956	0.9615
5.65	0.723	0.712	0.7175
16.4	0.565	0.545	0.555
32.8	0.235	0.244	0.240

D. Acetonitrile extraction with Millipore test kit**Standard**

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.41	1.425	1.418
0.2	1.215	1.244	1.230
5.0	0.288	0.285	0.287

ASSAY #1; Dilution 1:10; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean
0.00	1.41	1.425	1.418
3.28	0.967	0.955	0.961
5.65	0.775	0.77	0.773
16.40	0.435	0.427	0.431
32.80	0.118	0.105	0.1115

Assay #2; Dilution 1:50; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean
0.00	1.41	1.425	1.418
3.28	0.934	0.924	0.929
5.65	0.755	0.724	0.740
16.4	0.347	0.362	0.355
32.8	0.210	0.221	0.216

Table 3. Recovery Study for Corn Leaf

A. Ohmicron test kit assay

Standard 1

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.311	1.318	1.3145	
0.1	1.213	1.211	1.212	92
1.0	0.731	0.72	0.7255	55
5.0	0.264	0.261	0.2625	20

ASSAY #1: Dilution 1:50 Standard provided with the kit

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	%Rec
Blank	1.455	1.477	1.466				
15.62	0.98	0.975	0.978	67	0.43	21.5	138
39.00	0.735	0.732	0.734	50	0.95	47.5	122
117.00	0.405	0.41	0.408	28	2.8	140	120
195.00	0.297	0.285	0.291	20	4.5	225	115
234.20	0.21	0.201	0.206	14	5.6	280	120

Standard 2

Conc	Abs 1	Abs 2	Mean	%Bo
0.0	1.311	1.318	1.3145	
0.1	1.213	1.211	1.212	92
1.0	0.731	0.72	0.7255	55
5.0	0.264	0.261	0.2625	20

ASSAY #2: Discoloration; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	%Rec
0.00	1.612	1.586	1.599				
15.62	1.212	1.225	1.219	76	0.38	19	122
39.00	0.915	0.923	0.919	57	0.9	45	115
117.00	0.522	0.521	0.522	33	2.45	122.5	105
195.00	0.35	0.344	0.347	22	4.4	220	113
234.20	0.276	0.301	0.289	18	5.8	290	124

Ohmicron test kit assay; con't

Standard 3

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.311	1.318	1.3145	
0.1	1.213	1.211	1.212	92
1.0	0.731	0.72	0.7255	55
5.0	0.264	0.261	0.2625	20

ASSAY #3: Dilution 1:100; Discoloration; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	%Bo	Conc	Conf	%Rec
Blank	1.522	1.517	1.520				
15.62	1.355	1.357	1.356	89	0.14	14	90
39.00	1.145	1.138	1.142	75	0.4	40	103
117.00	0.797	0.799	0.798	53	1.05	105	90
195.00	0.565	0.545	0.555	37	2.1	210	108
234.20	0.53	0.525	0.528	35	2.2	220	94

B. Millipore test kit assay**Standard**

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.412	1.455	1.434
0.2	1.245	1.236	1.241
5.0	0.277	0.286	0.282

ASSAY #1: Dilution 1:50 Standard provided with the kit

Sp Levels (ppb)	Abs 1	Abs 2	Mean	Conc
0.00	1.412	1.455	1.434	*nd
15.62	0.756	0.743	0.750	nd
234.20	0.132	0.141	0.137	nd

* Non-determined

ASSAY #2: Dilution 1:50; Discoloration; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	Conc
0.00	1.412	1.455	1.434	nd
15.62	0.867	0.843	0.855	nd
234.20	0.104	0.107	0.106	nd

ASSAY #3: Dilution 1:100; Discoloration; Standard provided

Sp Levels (ppb)	Abs 1	Abs 2	Mean	Conc
0	1.412	1.455	1.434	nd
15.62	1.115	1.102	1.109	nd
234.20	0.204	0.21	0.207	nd

Table C4. Repeatability Study for carbofuran

A. Intra-assay data

A. Ohmicron test kit

Standard (ppb)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Mean	Stdv	%CV
0.0	1.333	1.327	1.337	1.351	1.311	1.359	1.336	0.017	1.3
0.1	1.219	1.257	1.2	1.3	1.261	1.211	1.241	0.038	3.1
1.0	0.712	0.722	0.687	0.716	0.733	0.775	0.724	0.029	4.0
5.0	0.257	0.234	0.231	0.222	0.212	0.255	0.235	0.018	7.6
Spike (ppb)									
6.56	1.13	1.105	0.996	1.144	0.995	0.956	1.054	0.081	7.7
32.80	0.835	0.775	0.757	0.823	0.83	0.831	0.809	0.034	4.2
65.60	0.612	0.634	0.61	0.655	0.65	0.633	0.632	0.019	3.0
31.20	0.421	0.453	0.456	0.412	0.387	0.425	0.426	0.026	6.1

INTER-ASSAY

Standard (ppb)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Mean	Stdv	%CV
0.0	1.423	1.335	1.321	1.215	1.325	1.312	1.322	0.066	5.0
0.1	1.315	1.2	1.25	1.312	1.205	1.256	1.256	0.050	4.0
1.0	0.701	0.656	0.71	0.767	0.765	0.745	0.724	0.043	6.0
5.0	0.245	0.267	0.223	0.235	0.227	0.244	0.240	0.016	6.6
Spike (ppb)									
6.56	0.978	0.985	1.134	1.138	0.965	0.95	1.025	0.087	8.5
32.80	0.835	0.815	0.845	0.785	0.712	0.865	0.810	0.055	6.8
65.60	0.621	0.627	0.65	0.682	0.650	0.534	0.627	0.051	8.1
31.20	0.356	0.432	0.476	0.435	0.390	0.425	0.419	0.041	9.9

B. Millipore test kit

INTRA-ASSAY

Standard (ppb)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Mean	Stdv	%CV
0.0	1.440	1.411	1.322	1.465	1.312	1.467	1.403	0.070	5.0
0.2	1.125	1.120	1.132	1.210	1.175	1.237	1.167	0.049	4.2
5.0	0.301	0.256	0.265	0.245	0.300	0.256	0.271	0.024	8.9
Spike (ng/g)									
32.80	0.888	0.932	0.912	0.976	0.905	0.877	0.915	0.035	3.9
65.60	0.567	0.532	0.477	0.564	0.574	0.589	0.551	0.041	7.4
312.00	0.215	0.203	0.237	0.273	0.243	0.255	0.238	0.026	10.8

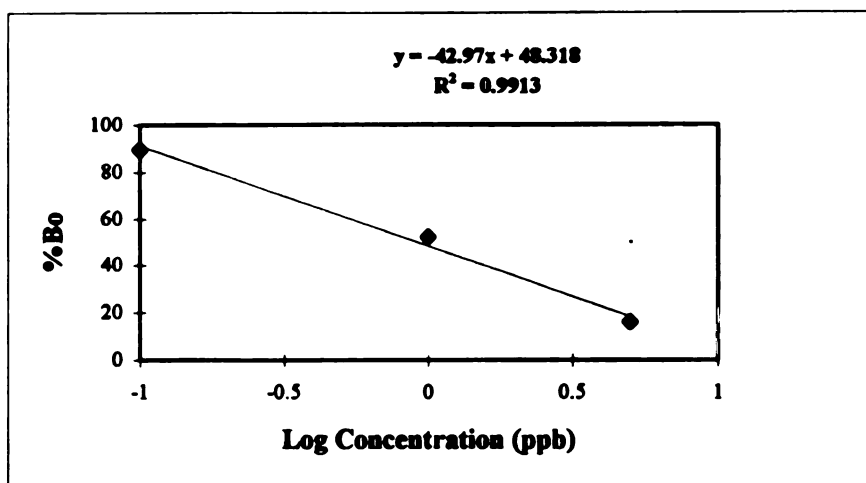
INTER-ASSAY

Standard (ppb)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Mean	Stdv	%CV
0.0	1.534	1.421	1.345	1.440	1.345	1.325	1.402	0.080	5.7
0.2	1.132	1.230	1.255	1.177	1.31	1.367	1.245	0.086	6.9
5.0	0.325	0.255	0.312	0.305	0.265	0.307	0.295	0.028	9.5
Spike (ng/g)									
32.80	0.955	0.853	0.832	0.867	0.845	0.755	0.851	0.064	7.6
65.60	0.701	0.722	0.745	0.867	0.767	0.804	0.768	0.060	7.9
31.20	0.477	0.412	0.532	0.512	0.455	0.407	0.466	0.051	11.0

Table C5. Sensitivity Study Data

A. Corn Leaf with Ohmicron test kit

Standard (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.400	1.338	1.369	
0.1	1.210	1.245	1.228	90
1.0	0.710	0.721	0.716	52
5.0	0.227	0.210	0.219	16

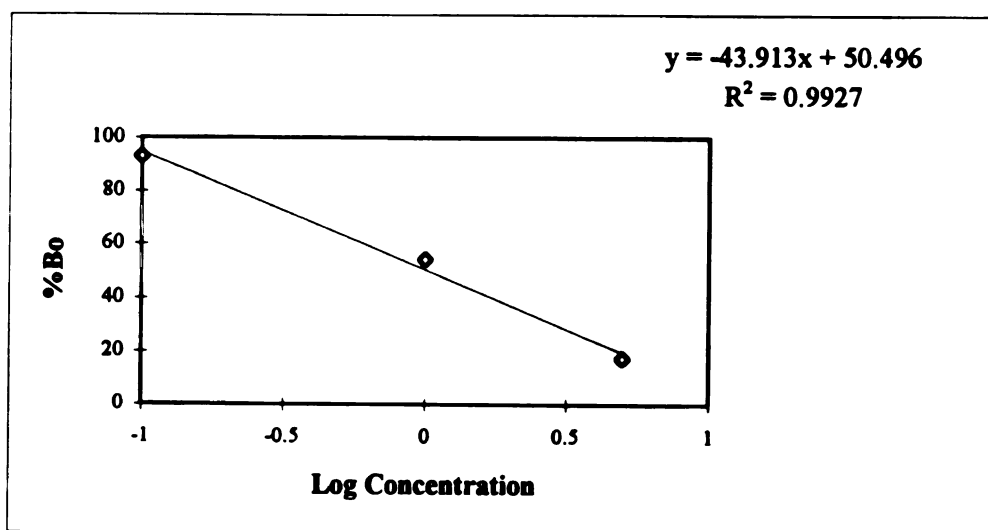


Assay #	Abs	%Bo	Conc
control	1.336		
1	1.321	98.877	0.067
2	1.242	92.964	0.108
3	1.117	83.608	0.176
4	0.967	72.380	0.317
5	1.105	82.710	0.185
6	0.845	63.249	0.512
Mean	1.100	82.298	0.228
Stdv	0.174	13.052	0.164
3@	0.523	39.156	0.491

B. Fish fillet with Ohmicron test kit

Standard

Levels (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.332	1.340	1.336	
0.1	1.225	1.257	1.241	93
1.0	0.707	0.741	0.724	54
5.0	0.241	0.230	0.236	18



Assay #	Abs	%Bo	Concentration
control	1.336		
1	1.212	90.7	0.121
2	1.180	88.3	0.138
3	0.997	74.6	0.282
4	1.210	90.6	0.122
5	0.955	71.5	0.333
6	1.275	95.4	0.095
Mean	1.138	85.2	0.182
Stdv	0.130	9.7	0.100
3@	0.390	29.20	0.299

Table C6. Cross-Reactivity Study Data

A. Ohmicron test kit assay

Sample	Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
Non spiked	0.00	1.355	1.459	1.407	
Carbofuran	6.56	1.13	1.137	1.134	80.6
Carbofuran	32.80	0.835	0.824	0.830	59.0
Carbofuran	65.60	0.612	0.6	0.606	43.1
Carbofuran	131.20	0.421	0.412	0.417	29.6
Carbofuran	196.80	0.321	0.315	0.318	22.6
*3-Keto-Carb	60.0	1.315	1.321	1.318	93.7
3-Keto-Carb	120.0	1.05	1.1	1.075	76.4
3-Keto-Carb	300.0	0.917	0.915	0.916	65.1
3-Keto-Carb	600.0	0.535	0.546	0.541	38.4
**3-Hydroxy-Carb	800.0	1.325	1.343	1.334	94.8
3-Hydroxy-Carb	1280.0	1.156	1.164	1.160	82.4
3-Hydroxy-Carb	1600.0	0.854	0.875	0.870	61.8
carb + 3-Keto-Carb	6.56 + 120	0.976	0.975	0.976	69.3
carb + 3-Keto-Carb	32.80 + 120	0.667	0.672	0.670	47.6
carb + 3-Keto-Carb	65.6 0 + 120	0.505	0.513	0.509	36.2
carb + 3-Keto-Carb	131.2 0 + 120	0.250	0.261	0.256	18.2
carb + 3-Keto-Carb	196.80 + 120	0.135	0.145	0.140	10.0
Carb + 3-Hydro-Carb	6.56 + 1280	1.100	1.050	1.075	76.4
Carb + 3-Hydro-Carb	32.80 + 1280	0.806	0.787	0.797	56.6
Carb + 3-Hydro-Carb	65.6 0 + 1280	0.587	0.603	0.595	42.3
Carb + 3-Hydro-Carb	131.20 + 1280	0.365	0.376	0.371	26.3
Carb + 3-Hydro-Carb	196.8 0+ 1280	0.210	0.235	0.223	15.8

*3-keto-carbofuran

**3-hydroxy-carbofuran

B. Millipore test kit assay

Sample	Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
Ns	0.00	1.421	1.455	1.438	
Carb	6.56	1.310	1.300	1.305	90.8
Carb	32.80	0.932	0.921	0.927	64.4
Carb	65.60	0.733	0.725	0.729	50.7
Carb	131.20	0.482	0.477	0.480	33.3
Carb	196.80	0.300	0.307	0.304	21.1
3-Keto-Carb	60.0	1.376	1.345	1.361	94.6
3-Keto-Carb	120.0	1.157	1.205	1.181	82.1
3-Keto-Carb	300.0	0.923	0.932	0.928	64.5
3-Keto-Carb	600.0	0.712	0.734	0.723	50.3
3-Hydroxy-Carb	800.0	1.410	1.387	1.399	97.3
3-Hydroxy-Carb	1280.0	1.211	1.165	1.188	82.6
3-Hydroxy-Carb	1600.0	0.977	1.105	1.041	72.4
carb + 3-Keto-Carb	32.80 + 120	0.976	0.985	0.981	68.2
carb + 3-Keto-Carb	65.6 0 + 120	0.810	0.787	0.799	55.5
carb + 3-Keto-Carb	131.2 0 + 120	0.396	0.403	0.400	27.8
carb + 3-Keto-Carb	196.80 + 120	0.231	0.241	0.236	16.4
Carb + 3-Hydro-Carb	32.80 + 1280	1.110	1.215	1.163	80.8
Carb + 3-Hydro-Carb	65.6 0 + 1280	0.800	0.800	0.800	55.6
Carb + 3-Hydro-Carb	131.20 + 1280	0.500	0.500	0.500	34.8
Carb + 3-hydro-carb	196.8 0+ 1280	0.300	0.300	0.300	20.9

Carb = carbofuran

3-keto-carb = 3-keto-carbofuran

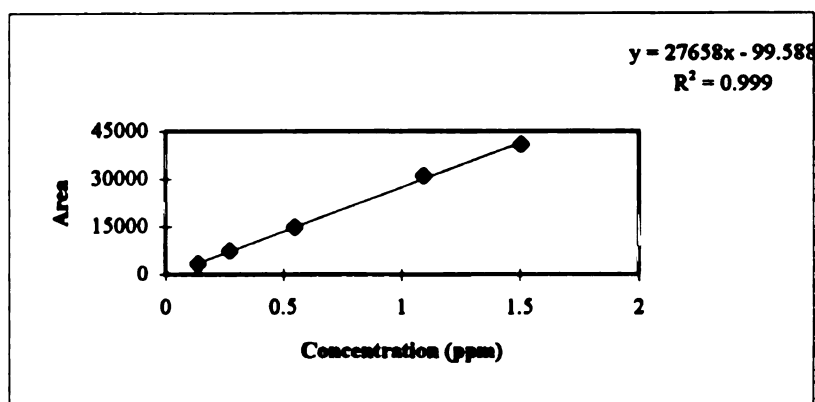
3-hydro-carb = 3-hydroxy-carbofuran

Table C7. Incurred Corn Leaf Study Data

A. Gas Chromatographic determination

Standard

Conc (ppm)	Area
0.137	3415
0.274	7550
0.548	15018
1.096	31005
1.507	41031



*Sp Levels (ppm)	Assay1	Assay 2	Mean	Conc	**Concf	%Rec
0.656	3575	3673	3624	0.135	0.673	103%
1.312	7120	6890	7005	0.257	1.284	98%
3.28	16322	16212	16267	0.592	2.959	90%
4.592	24130	24055	24093	0.875	4.373	95%
6.56	37024	37009	37017	1.342	6.710	102%
Mean						98%

*Spiking Levels

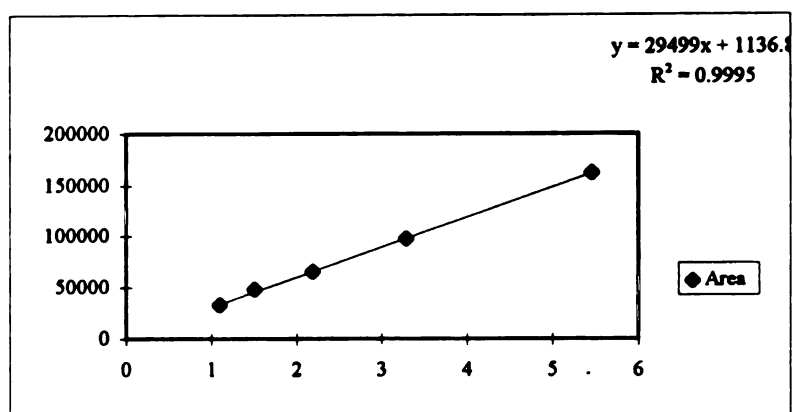
**Final concentration

% Rec = % recovery

Gas chromatographic determination; con't

Standard

Conc (ppm)	Area
1.096	32500
1.507	47562
2.192	65125
3.288	97500
5.460	162500



Non Treated

Sample	Area 1	Area 2	Mean	Conc	Conf
Cut 1	np	np	np	nd	nd
Cut 2	np	np	np	nd	nd
Cut 3	np	np	np	nd	nd
Cut 4	np	np	np	nd	nd
Cut 5	np	np	np	nd	nd

Treatment #1

Sample	Area 1	Area 2	Mean	Conc	Conf (ppm)
1DBT	np	np	nd	nd	nd
DOT	210545	271235	240890	8.205	1.64
5DAT	40546	40215	40380.5	1.407	0.28
7DAT	np	np	nd	nd	nd
11DAT	np	np	nd	nd	nd

Gas Chromatographic determination; con't

Treatment #2

Sample	Area 1	Area 2	Mean	Conc	Conf (ppm)
Cut 1	np	np	nd	nd	nd
Cut 2	275435	276745	276090	9.398	1.88
Cut 3	103250	103112	103181	3.536	0.71
Cut 4	45745	45655	45700	1.588	0.32
Cut 5	np	np	nd	nd	nd

Treatment #3

Sample	Area 1	Area 2	Mean	Conc	Conf (ppm)
Cut 1	np	np	nd	nd	nd
Cut 2	313750	312565	313157.5	10.654	2.13
Cut 3	125455	124765	125110	4.280	0.86
Cut 4	48221	48210	48215.5	1.673	0.33
Cut 5	51212	52342	51777	1.794	0.36

B. Ohmicron test kit determination**Standard**

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.355	1.534	1.445	
0.1	1.335	1.345	1.340	93
1.0	0.756	0.761	0.759	53
5.0	0.286	0.290	0.288	20

Non Treated

Sample	Assay1	Assay2	Mean	%Bo	Conc	Conf (ppm)
control	1.678	1.682	1.680			
1DBT	1.600	1.587	1.594	95	nd	nd
DOT	1.700	1.700	1.700	101	nd	nd
5DAT	1.587	1.605	1.596	95	nd	nd
7DAT	1.631	1.542	1.587	94	nd	nd
11DAT	1.523	1.578	1.551	92	nd	nd

Treatment #1

Sample	Assay1	Assay2	Mean	%Bo	Conc	Concf (ppb)
control	1.712	1.734	1.723			
1DBT	1.700	1.600	1.650	96	**nd	nd
DOT	0.712	0.756	0.734	43	1.70	1700
5DAT	1.456	1.400	1.428	83	0.28	280
7DAT	1.552	1.534	1.543	90	nd	nd
11DAT	1.623	1.635	1.629	95	nd	nd

*Final concentration

Non-detected

Ohmicron test kit determination; con't

Treatment #2

Sample	Assay 1	Assay 2	Mean	%Bo	Con	Conf (ppb)
control	1.512	1.534	1.523			
1DBT	1.412	1.410	1.411	93	nd	nd
DOT	0.580	0.565	0.573	38	2	2000
5DAT	0.947	0.962	0.955	63	0.750	750
7DAT	1.210	1.188	1.199	79	0.36	360
11DAT	1.451	1.431	1.441	95	nd	nd

Treatment #3

Sample	Area 1	Area 2	Mean	%Bo	Con	Conf (ppb)
control	1.512	1.534	1.523			
1DBT	1.478	1.511	1.495	98	nd	nd
DOT	0.531	0.522	0.527	35	2.2	2200
5DAT	0.922	0.915	0.919	60	0.820	820
7DAT	1.210	1.173	1.192	78	0.38	380
11DAT	1.233	1.246	1.240	81	0.285	285

C. Millipore test kit determination

Standard 1

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.650	1.531	1.591
0.2	1.434	1.411	1.423
5.0	0.310	0.341	0.326

Non Treated

Sample	Assay1	Assay2	Mean	Conc	Conf (ppb)
control	1.775	1.734	1.755		
1DBT	1.621	1.612	1.617	nd	nd
DOT	1.735	1.756	1.746	nd	nd
5DAT	1.610	1.602	1.606	nd	nd
7DAT	1.567	1.612	1.590	nd	nd
11DAT	1.665	1.728	1.697	nd	nd

Treatment #1

Sample	Assay1	Assay2	Mean	Conc	Conf
control	1.775	1.734	1.755		
1DBT	1.675	1.710	1.693	nd	nd
DOT	1.612	1.735	1.674	nd	nd
5DAT	1.121	1.105	1.113	>2ppb	>2000 ppb
7DAT	1.465	1.421	1.443	nd	nd
11DAT	1.524	1.561	1.543	nd	nd

Millipore test kit determination; con't

Standard 2

Concentration (ppb)	Abs 1	Abs 2	Mean
0.0	1.464	1.366	1.415
0.2	1.277	1.231	1.254
5.0	0.302	0.312	0.307

Treatment #2

Sample	Area 1	Area 2	Mean	Con	Conf (ppb)
control	1.677	1.655	1.666		
1DBT	1.566	1.545	1.556	nd	nd
DOT	1.267	1.288	1.278	>2	>2000
5DAT	0.922	0.931	0.927	>2	>2000
7DAT	1.121	1.200	1.161	>2	>2000
11DAT	1.662	1.575	1.619	nd	nd

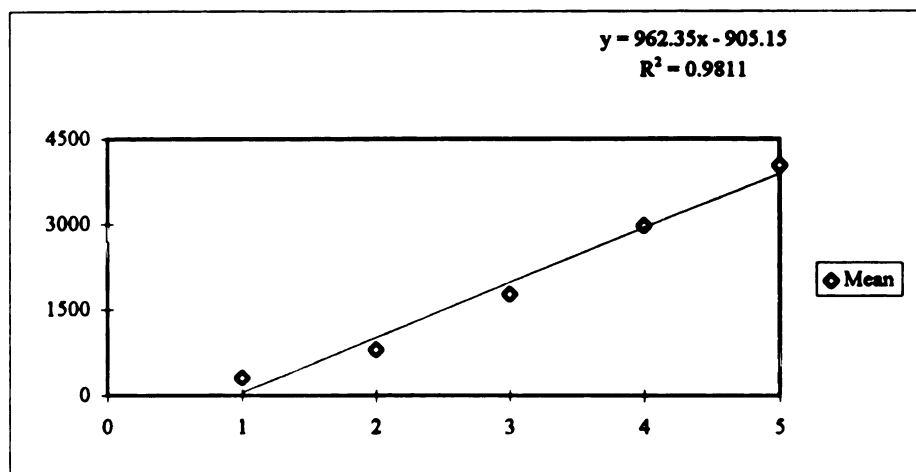
Treatment #3

Sample	Area 1	Area 2	Mean	Con	Conf (ppb)
control	1.677	1.655	1.666		
1DBT	1.632	1.621	1.627	nd	nd
DOT	0.887	0.910	0.899	>2	>2000
5DAT	0.775	0.795	0.785	>2	>2000
7DAT	1.277	1.255	1.266	>2	>2000
11DAT	1.342	1.321	1.332	>2	>2000

Table C8. Incurred Fish Study Data

A. Gas chromatographic determination

Concentration	Standard		Mean
	Area 1	Area 2	
0.137	322	312	317
0.274	812	795	803.5
0.548	1758	1784	1771
1.096	2981	2969	2975
1.507	4048	4038	4043



Control 1

ID	Area 1	Area 2	Mean	Conc (ppm)	Conc
Fish1	*np	np	np	nd	**nd
Fish2	np	np	np	nd	nd
Fish3	np	np	np	nd	nd
Fish4	np	np	np	nd	nd
Fish5	np	np	np	nd	nd
Mean	np	np	np	nd	nd

Control 2

ID	Area 1	Area 2	Mean	Conc (ppm)	Conc
Fish1	np	np	np	nd	nd
Fish2	np	np	np	nd	nd
Fish3	np	np	np	nd	nd
Fish4	np	np	np	nd	nd
Fish5	np	np	np	nd	nd
Mean	np	np	np	nd	nd

*No peak

*Not detected

Gas chromatographic determination; con't

Treatment #1-1

ID	Area 1	Area 2	Mean	Conc (ppm)	Conc
Fish1	np	np	np	nd	nd
Fish2	np	np	np	nd	nd
Fish3	np	np	np	nd	nd
Fish4	np	np	np	nd	nd
Fish5	np	np	np	nd	nd
Mean	np	np	np	nd	nd

Treatment #1-2

ID	Area 1	Area 2	Mean	Conc (ppm)	Conc
BLK	np	np	np	nd	nd
Fish1	np	np	np	nd	nd
Fish2	np	np	np	nd	nd
Fish3	np	np	np	nd	nd
Fish4	np	np	np	nd	nd
Fish5	np	np	np	nd	nd
Mean	np	np	np	nd	nd

Treatment #2-1

ID	Area 1	Area 2	Mean	Conc (ppm)	Conc
Fish1	2092.0	2107.0	2099.5	0.003	0.54
Fish2	np	np	np	nd	nd
Fish3	np	np	np	nd	nd
Fish4	np	np	np	nd	nd
Fish5	np	np	np	nd	nd
Mean	2092	2107	2100	0.003	0.5382

Treatment #2-2

ID	Area 1	Area 2	Mean	Conc (ppm)	Conc
Fish1	nd	nd	nd	nd	nd
Fish2	nd	nd	nd	nd	nd
Fish3	nd	nd	nd	nd	nd
Fish4	nd	nd	nd	nd	nd
Fish5	nd	nd	nd	np	np
Mean	nd	nd	nd	nd	nd

B. Ohmicron test kit determination**Standard 1**

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.337	1.342	1.340	
0.1	1.213	1.207	1.210	90.3
1.0	0.725	0.722	0.724	54.0
5.0	0.254	0.274	0.264	19.7

Non Treated

Sample	Assay 1	Assay 2	Mean	%Bo	Log con	Con	Conf
Blank	1.337	1.342	1.340				
Fish 1	1.327	1.334	1.331	99.3	nd	nd	nd
Fish 2	1.289	1.275	1.282	95.7	nd	nd	nd
Fish 3	1.266	1.254	1.260	94.1	nd	nd	nd
Fish 4	1.312	1.317	1.315	98.1	nd	nd	nd
Fish 5	1.227	1.266	1.247	94.8	nd	nd	nd

Treatment #1-1

Sample	Assay 1	Assay 2	Mean	%Bo	Log con	Con	Conf
Blank	1.337	1.342	1.340				
Fish 1	1.236	1.234	1.235	92.2	nd	nd	nd
Fish 2	1.227	1.228	1.228	91.6	nd	nd	nd
Fish 3	1.264	1.235	1.250	93.3	nd	nd	nd
Fish 4	1.312	1.322	1.317	98.3	nd	nd	nd
Fish 5	1.302	1.278	1.290	97.9	nd	nd	nd

Treatment #1-2

Sample	Assay 1	Assay 2	Mean	%Bo	Log con	Con	Conf
Blank	1.337	1.342	1.340				
Fish 1	1.275	1.270	1.273	95.0	nd	nd	nd
Fish 2	1.244	1.235	1.240	92.5	nd	nd	nd
Fish 3	1.255	1.251	1.253	93.5	nd	nd	nd
Fish 4	1.325	1.312	1.319	98.4	nd	nd	nd
Fish 5	1.321	1.332	1.327	100.6	nd	nd	nd

Ohmicron test kit determination; con't

Standard 2

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.364	1.357	1.361	
0.1	1.225	1.229	1.227	90.2
1.0	0.734	0.741	0.738	54.2
5.0	0.270	0.282	0.276	20.3

Treatment #2-1

Sample	Assay 1	Assay 2	Mean	%Bo	logCon	con	conf
Blank	1.364	1.357	1.361				
Fish 1	0.127	0.136	0.132	9.7	1.01	10.20	509.9
Fish 2	0.235	0.242	0.239	17.5	0.82	6.54	327.1
Fish 3	1.343	1.355	1.349	99.2	nd	nd	nd
Fish 4	1.267	1.270	1.269	93.2	nd	nd	nd
Fish 5	0.756	0.750	0.753	59.4	-0.21	0.62	30.8

Treatment #2-2

Sample	Assay 1	Assay 2	Mean	%Bo	logCon	con	conf (ppb)
Blank	1.364	1.357	1.361				
Fish 1	1.257	1.265	1.261	92.7	nd	nd	nd
Fish 2	1.332	1.338	1.335	98.1	nd	nd	nd
Fish 3	1.244	1.251	1.248	91.7	nd	nd	nd
Fish 4	1.312	1.310	1.311	96.4	nd	nd	nd
Fish 5	1.255	1.250	1.253	95.5	nd	nd	nd

C. Millipore test kit determination

Standard 1

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.423	1.435	1.429	
0.2	1.277	1.265	1.271	88.9
5.0	0.287	0.280	0.284	19.8

Non Treated

Sample	Assay 1	Assay 2	Mean	%Bo	Conc
Blank	1.423	1.435	1.429		
Fish 1	1.423	1.420	1.422	99.5	nd
Fish 2	1.356	1.347	1.352	94.6	nd
Fish 3	1.322	1.231	1.277	89.3	nd
Fish 4	1.412	1.421	1.417	99.1	nd
Fish 5	1.425	1.425	1.425	100.6	nd

Standard 2

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.376	1.365	1.371	
0.2	1.217	1.205	1.211	88.4
5.0	0.301	0.275	0.288	21.0

Treatment #1-1

Sample	Assay 1	Assay 2	Mean	%Bo	Conc
Blank	1.376	1.365	1.371		
Fish 1	1.235	1.255	1.245	90.8	nd
Fish 2	1.312	1.310	1.311	95.7	nd
Fish 3	1.300	1.287	1.294	94.4	nd
Fish 4	1.255	1.256	1.256	91.6	nd
Fish 5	1.311	1.325	1.318	105.0	nd

Standard 2

Concentration (ppb)	Abs 1	Abs 2	Mean	%Bo
0.0	1.376	1.365	1.371	
0.2	1.217	1.205	1.211	88.4
5.0	0.301	0.275	0.288	21.0

APPENDIX D

TANK WATER CHARACTERISTICS IN FISH REARING EXPERIMENT

APPENDIX D

TANK WATER CHARACTERISTICS IN FISH REARING EXPERIMENT

Table D1. Tank Water Characteristics for Alachlor Study

ALACHLOR

Treatment date 03/05/93

		Date								
Treatment		5	6	7	8	9	10	11	12	13
Control #1	ToC	16.00	16.00	16.04	16.23	16.77	16.67	16.70	16.87	16.87
	pH	7.05	7.56	6.77	7.12	7.00	7.25	7.32	7.05	7.45
Control #2	ToC	15.76	16.12	16.10	16.00	16.00	15.78	16.20	16.20	16.10
	pH	7.00	7.00	7.12	7.23	7.45	8.00	8.00	7.77	7.45
Treatment #1	ToC	15.30	16.00	15.67	15.67	16.12	16.10	15.87	15.45	16.15
	pH	8.00	8.12	7.87	7.87	7.56	7.55	7.45	7.45	7.23
Treatment #1	ToC	15.00	15.00	15.30	15.00	15.00	15.21	15.21	15.22	16.00
	pH	7.12	8.00	7.78	8.00	7.56	7.67	8.12	8.12	7.77
Treatment #2	ToC	15.32	15.10	15.20	15.23	15.45	15.10	15.33	16.00	16.10
	pH	7.12	7.20	7.20	7.20	8.00	7.77	7.77	7.65	8.00
Treatment #2	ToC	16.10	15.57	16.00	15.30	15.20	15.22	15.23	15.25	15.50
	pH	8.00	7.40	7.20	7.23	7.30	7.25	7.15	8.00	8.10

		14	15	16	17	18	19	20	21
Control #1	ToC	17.10	17.22	17.23	17.00	17.00	17.12	17.12	17.10
	pH	8.04	8.04	8.12	8.21	7.78	8.35	8.25	8.12
Control #2	ToC	16.77	16.05	16.03	16.12	17.01	17.00	17.21	17.20
	pH	7.55	8.00	8.10	8.12	8.00	7.45	7.77	7.23
Treatment #1	ToC	17.00	16.77	17.20	16.75	16.40	16.4	17	15
	pH	7.45	8.00	8.00	8.10	8.30	8.35	8.15	8.13
Treatment #1	ToC	16.00	16.10	15.77	16.23	17.0	17	16.45	17.12
	pH	8.23	8.23	8.23	8.12	7.76	7.45	7.56	8.12
Treatment #2	ToC	16.20	16.30	16.23	15.77	15.67	15.98	16.12	16.22
	pH	8.20	8.23	8.20	8.30	8.30	8.43	8.32	8.21
Treatment #2	ToC	15.64	16.70	17.01	16.45	17.00	17.00	17.10	17.20
	pH	8.04	8.10	8.30	8.25	8.15	8.3	8.33	8.35

Table D2. Tank Water Characteristics for Atrazine Study

Treatment date 02/2/93

		Date								
Treatment		2	3	4	5	6	7	8	9	10
Control #1	ToC	15.00	15.00	15.00	16.00	15.00	16.00	15.80	15.50	15.50
	pH	7.55	7.61	7.07	7.00	7.35	7.12	7.55	8.01	8.02
Control #2	ToC	15.00	15.00	15.10	15.50	15.20	15.10	16.10	16.50	15.00
	pH	7.23	8.00	7.12	8.01	7.88	7.45	7.67	8.12	8.32
Treatment #1	ToC	15.00	16.00	16.00	16.00	16.02	15.30	16.00	15.23	15.65
	pH	8.22	7.67	7.87	7.03	8.00	7.45	8.05	8.04	
Treatment #1	ToC	15.50	15.00	15.00	15.07	15.26	16.00	16.24	16.25	16.71
	pH	7.56	7.53	8.01	7.67	7.69	8.02	8.02	7.35	8.45
Treatment #2	ToC	15.03	15.10	15.00	15.00	15.45	15.35	14.20	15.45	16.00
	pH	8.00	7.45	7.44	7.32	7.02	7.65	8.00	8.00	8.12
Treatment #2	ToC	16.00	16.00	15.70	15.17	15.02	16.00	16.00	15.77	16.00
	pH	7.10	7.10	7.54	7.45	8.00	8.00	7.35	7.44	7.67

		Date									
Treatment		11	12	13	14	15	16	17	18	19	20
Control #1	ToC	15.00	14.00	16.00	16.00	16.30	16.00	15.60	15.50	16.05	16.20
	pH	7.87	7.45	7.56	8.20	8.23	8.12	7.53	7.01	8.15	8.25
Control #2	ToC	15.00	16.05	16.03	15.00	15.00	16.00	16.30	16.50	17.00	15.30
	pH	7.65	7.02	7.05	8.03	7.45	7.55	7.55	7.35	7.44	7.67
Treatment #1	ToC	15.45	16.00	16.00	16.00	16.02	16.05	16.11	15.87	15.81	16.05
	pH	8.20	7.65	8.56	8.54	8.67	8.11	7.87	8.87	8.71	7.45
Treatment #1	ToC	16.02	16.00	16.45	16.44	16.2	15.77	16.87	16.05	16.12	16.33
	pH	8.01	7.68	7.65	8.21	8.20	7.68	7.45	8.02	8.25	8.77
Treatment #2	ToC	16.00	16.00	15.01	15.23	16.00	16.11	15.64	16.22	16.05	16.12
	pH	8.07	8.12	8.12	8.23	8.51	8.31	7.05	7.75	7.77	7.81
Treatment #2	ToC	16.05	16.23	15.77	15.43	16.02	16.32	16.01	15.78	16.00	16.24
	pH	8.12	8.12	8.25	8.32	7.77	8.15	8.15	8.24	8.56	8.50

Table D3. Tank Water Characteristics for Carbofuran Study

Treatment date 01/07/93

Treatment		7	8	9	10	11	12	13
Control #1	ToC	15.0	15.5	15.5	15.0	15.0	15.5	15.8
	pH	7.55	7.61	7.07	7.11	7.58	7.02	8.06
Control #2	ToC	15.0	15.0	15.5	15.0	15.0	15.5	15.8
	pH	7.12	7.65	7.02	7.56	7.77	7.02	8.08
Treatment #1	ToC	15.0	15.0	15.5	15.0	15.0	15.5	15.5
	pH	7.93	7.87	7.77	7.91	7.93	7.02	7.91
Treatment #1	ToC	15.5	15.5	15.5	15.0	15.0	15.5	15.5
	pH	8.00	7.55	7.76	8.01	8.00	7.01	8.09
Treatment #2	ToC	15.0	7.0	15.0	7.3	15.0	15.5	15.5
	pH	8.06	14.70	7.15	15.00	8.06	7.01	7.85
Treatment #2	ToC	15.0	8.1	15.0	15.0	15.0	15.5	15.5
	pH	8.20	15.00	8.20	8.16	8.20	7.02	8.31

Treatment		Date						
		14	15	16	17	18	19	20
Control #1	ToC	15.5	15.5	15.0	14.0	13.8	13.2	13.5
	pH	7.96	7.50	7.73	8.50	8.19	8.12	8.16
Control #2	ToC	15.5	15.5	15.1	14.0	13.8	13.2	13.5
	pH	8.08	8.02	7.83	8.51	8.38	8.36	8.22
Treatment #1	ToC	15.5	15.5	15.1	14.0	13.4	13.6	13.3
	pH	7.88	7.75	7.88	8.30	8.17	8.17	8.27
Treatment #1	ToC	15.3	15.5	15.2	14.0	13.4	13.1	14.0
	pH	8.23	7.89	7.98	8.25	8.12	8.14	8.05
Treatment #2	ToC	15.5	15.5	15.0	15.0	14.0	13.0	13.0
	pH	7.80	7.82	8.36	8.11	8.06	8.11	8.09
Treatment #2	ToC	15.5	15.0	15.5	14.1	14.0	13.4	13.1
	pH	8.25	7.92	7.93	8.41	8.25	8.12	8.10

LITERATURE CITED

REFERENCES CITED

Anonymous, 1986. "Test Methods for Evaluating Solid Waste". Laboratory Manual Physical/ Chemical Methods. U.S.E.P.A, Office of Solid Waste and Emergency.

Anonymous 1987. "National Pesticide use analysis". Alternative agricultural News 5:1-4.

Anonymous 1990. "REACHING 2020:" Michigan's Food and Agriculture Industry in the 21st Century". Futures Team 2020, Michigan Department of Agriculture.

Anonymous, 1991. "Harnessing the antibody: the fundamentals of enzyme immunoassay as used in environmental diagnostics". Millipore corp, Bedford, MA.

Anonymous, 1991. "Detection of environmental contaminants by immunoassay: Technical reference guide". Millipore Corp., Bedford, MA.

Anonymous. 1994. Herbicide handbook. Weed Science Society of America, 7th Ed.. Eds. William H. Ahrens.

Beetstman, G.B. and Deming, J.M. 1972. "Dissipation modes of acetanilide herbicides from soils". Agron. Abst. p. 94.

Braselton Emmet, 1992. Chemical Toxicology. MPH 450, Spring 92.

Bushway, R.J., Perkins, B., Savage, S.A., Lekousi, S.J., and ferguson, B. S., 1988. "Determination of atrazine residues in water and soil by enzyme immunoassay." Bull. Environ. Contam. Toxicol., 40: 647-654.

Bushway,R.J., Perkins,B., Savage,S.A., Lekousi,S.J., and Ferguson, B.S., 1989. Bull. Environ. Contam. Toxicol., 42: 899-904.

Bushway, R.J., Hurst, H.L., Perkins, L.B., Tian, L., Cabanillas, Guiberteau C., Young, B.E.S., Ferguson, B.S., and Jennings, H.S., 1992. "Atrazine, alachlor and carbofuran contamination of well water in central main". Bull. Environ. Contam. Toxicol., 49: 1-9.

Busser, Hans-Rudolf, 1990. "Atrazine and other s-triazine herbicides in lakes and rain in

Switzerland". Environ. Sci. Technol., 24: 1049-1048.

Cessna, Allan J., 1990. Determination of residues of 2,4-D in post emergence-treated triticale". Pestic. Sci., 30: 141-147.

Cheung, P.Y.K., Gee, S.J., and Hammock, B.D., 1986. "Pesticide immunoassay as a Biotechnology." in *The Impact of Chemistry on Biotechnology: Multidisciplinary Discussions*. ACS symposium series 362: 217-229. American Chemical Society. Washington, DC.

Dunbar, B., Riegle B., and Niswender G., 1990. "Development of enzyme immunoassay for the detection of triazine herbicides". J. Agric. Food Chem. 38: 433-437.

Ebert, E. and Dunford, S.W., 1976. "Effects of triazine herbicides on the physiology of plants". Residue Rev. 65:2-103.

Eisler, R., 1989. "Atrazine hazard to fish, wildlife, and invertebrates: a synoptic review". Contaminant Hazard Reviews Report 18. Biological Report 85. U.S. Fish and Wildlife Service, Washington DC

Ercegovich, Charles D., Vallejo, Remo P., Gettig, Russel R., Woods, L., Bogus Edward R., and Mumma, Ralph O., 1981. "Development of a radioimmunoassay for parathion". J. Agric. Food Chem., 29: 559-563.

Feng, Paul C.C., Wratten, Stephen J., Horton, R., Sharp, Ray C., and Logusch Eugene W., 1990. "Development of enzyme-linked immunosorbent assay for alachlor and its application to the analysis of environmental water samples." J. Agric. Food Chem., 38, 159-163.

Fleeker, James, 1987. "Two enzyme immunoassays to screen for 2,4-Dichlorophenoxyacetic acid in water". J. Assoc. Off. Anal. Chem., 70: 874-878.

Frank, R. and Sirons, G.J., 1979. "Atrazine: Its use in corn production and its loss to stream waters in southern Ontario, 1975-1977". Sci. Total Environ. 12:223-239.

Gee, Shirley J., Miyamoto, T. Goodrow, Marvin H., Buster, D., and Hammock, Bruce D., 1988. "Development of an enzyme-linked immunosorbent assay for the analysis of the thiocarbamate herbicide molinate." J. Agric. Food Chem., 36: 863-870.

Graves, R.L., 1989. "Determination of chlorinated acids in water by gas chromatography with an electron capture detector". Method 515.1. Environmental Monitoring Systems Laboratory Office of Research and Development US.EPA, Cincinnati., pp 221-251.

Graves, R.L., 1989. "Determination of nitrogen and phosphorus-containing pesticides in

water by gas chromatography with a nitrogen-phosphorus detector". Environmental monitoring systems laboratory, Office of Research and development. US.EPA. Cincinnati, Ohio., 143-169.

Gunkel, G., 1981." Bioaccumulation of a herbicide (atrazine, s-triazine) in the white fish (Coregonus). Uptake and distribution of the residue in fish ". J. Hydrobiol. 59:252-287.

Hall,J.C., Deschamps,R.J.A., and Kreig,K.K., 1989. "Immunoassays for the detection of 2,4-D and picloram in river water an urine". J. Agric. Food Chem. 37: 981-984.

Hall, Christopher J., 1990. "Immunoassays to detect and quantitate herbicides in the environment". Weed Technology, 4: 226-234

Hammock, Bruce D., 1988. "Application of Immunochemistry in Crop Protection and biotechnology." in Biotechnology for Crop protection. Ed Paul A. Hedin, Julius J. Menn, and Robert M. Hollingworth. ACS Symposium Series 379: 298-305.

Harrison, Robert O., Gee, Shirley J., and Hammock, Bruce D., 1988. "Immunochemical Methods of Pesticide Residue Analysis". in Biotechnology for Crop protection. Ed Paul A. Hedin, Julius J. Menn, and Robert M. Hollingworth. ACS Symposium Series 379:316-330.

Hargrove, R.S. and Merkle, M.G. 1971. "The loss of alachlor from soils". J. Agr. Food Chem. 18:854-858.

Hoermann, W.D., Tournayre, J.C., and Egli, H. 1979." Triazine herbicide residues in central European streams". Pest. Monit. J. 13:128-135.

Huang, Lee Q. and Pignatello, Joseph J., 1990. "Improved extraction of atrazine and metolachlor in field soil samples." J. Assoc. Off. Anal. Chem., 70(3): 443-446.

Huber, S.J., 1985. "Improved solid-phase immunoassay systems in the ppt range for atrazine in fresh water." Chemosphere, 14(11/12):1795-1803.

Immunochemical Methods for Environmental Analysis. Ed. Jeanette M. Van Emon and Ralph O. Mumma. ACS Symposium Series 442: pp 229. American Chemical Society. Washington, D.C 1990.

Jones, T.W., Kemp, W.M, Stevenson, JC. and Means, J.C., 1982. "Degradation of atrazine in estuarine water/sediment systems and soils". J. Environ. Qual. 11:632-638.

Jung F., Gee, Shirley J., Harrison, Robert O., Goodwrow, Marvin H., Karu, Alexander E., Braun, Adolf L., Li, Quing X., and Hammock, Bruce D., 1989. "Use of immunochemical techniques for the analysis of pesticides". Pesticide Sci., 26: 303-317.

Kelley, Marian M., Zahnow, Edward W., Petersen Christian W. and Toy, Stephen, Toy T., 1985. "Chlorsulfuron determination in soil extracts by enzyme immunoassay." J. Agric. Food Chem., 33: 962-965.

Knopp D., Nuhn P., and Dobberkau, Hans-Joachim, 1985. "Radioimmunoassay for 2,4-dichlorophenoxyacetic acid." Arch. Toxicol., 58: 27-32.

Knuesli, E., Berrer, Depuis, D. and Esser, H. 1969. s-Triazines. In P.C. Kearney and Kaufman, eds., Degradation of herbicides. Marcel Dekker, New York, N.Y., pp 51-70.

Kuhr, R.J. 1970. "Metabolism of carbamate insecticide chemicals in plants and insects". J. Agr. Food Chem., 18:1023-1030.

Leonard, R.A. 1988. "Environmental Chemistry of Herbicides"; Grover, R., Ed., CRC Press: Boca Raton, FL; pp 45-87.

Lankow Richard K., Grothaus, David G., Miller Sally A., 1987. "Immunoassay for crop management systems and agricultural chemistry" in Biotechnology in agricultural chemistry. ACS Symposium Series 334., pp 228-267.

Leavitt, Richard A., Kells, James J., Bunkelmann, Jeffrey R., and Hollingworth, Robert M., 1991. "Assessing atrazine persistence in soil following a severe drought." Bull. Environ. Contam. Toxicol., 46: 22-29.

Lukens, Herbert R. and Williams Collin B., 1977. "Fluorescence immunoassay technique for detecting organic environmental contaminants." Environmental Science & Technology, 11(3): 292-297.

Meagher William R., 1966. "Determination of 2,4-dichloro-phenoxyacetic acid and 2-(2,4,5-trichloro-phenoxy)propionic acid in citrus by electron capture gas chromatography." J. Agric. Food Chem., 14(4): 374-377.

National Research Council, 1987. Regulating Pesticides in Food: The Deleany Paradox. National Academic Press, Washington, DC, pp.52-53.

Newsome, Harvey W. and Collins, Peter G., 197. "Enzyme-linked immunosorbent assay of benomyl and thiabendazole in some foods". J. Assoc. Off. Anal. Chem., 70(5): 1025-1027

Newsome williams, 1985. "An enzyme-linked immunosorbent assay for metalaxyl in foods." J. Agric. Food Chem., 33: 528-530.

Oylypiw, Jr, Harry. M., and Hankin,lester, 1991. "Herbicides in pooled raw milk in

Connecticut". Journal of Food Protection, 54(2):136-137.

Premazzi, G and Steechi, R., 1990. "Evaluation of the impact of atrazine on the aquatic environment". EU 12569. Review. Commission of the European Community. Brussels, Belgium.

Rinder, D.F. and Fleeker J.R., 1981. "A radioimmunoassay to screen for 2,4-dichlorophenoxyacetic acid and 2,4,5-trichloro-phenoxyacetic acid in surface water." Bull. environm. Contam. Toxicol., 26: 375-380.

Rittenburg, J.H., Grothaus, G.D., Fitzpatrick, D.A., Lankow, R.K., 1991. Rapid on-site immunoassay systems: Agricultural and environmental applications in immunoassays for Trace Chemical Analysis, ACS Symposium Series, Vol. 451 (vanderlaan, M., Stanker, R.R., Watkins, B.E., Roberts, D.W., Eds.) American Chemical Society, Washington, DC, pp.28-39.

Schiavon, M., 1988. "Studies of the leaching of atrazine, of its chlorinated derivatives, and of hydroxyatrazine from soil using ¹⁴C ring-labeled compounds under outdoor conditions". Ecotoxocol. Environ. Saf. 15:46-54.

Schlaeppli J-M., Föry W., and Ramstainer K., 1989. "Hydroxyatrazine and atrazine determination in soil and water by enzyme-linked immunosorbent assay using specific monoclonal antibodies." J. Agric. Food Chem., 37: 1532-1538.

Schwalbe M., Dorn, E., and Beyermann K. 1984. "Enzyme immunoassay and fluoroimmunoassay for the herbicide diclofop-methyl." J. Agric. Food Chem., 32: 734-741.

Stratton, G.W., 1984. "Effects of the herbicide atrazine and its degradation products , alone and in combination, on phototrophic microorganisms". Arch. Environ. Contamm. Toxicol. 13:35-42.

Thurman, E.M., Goolsby, D.A., Meyer, M.T., Pomes, M.L., Mills, M.S., and Kolpin, D.W. 1992. "Mapping the regional occurrence of herbicides in surface water of the midwestern United States by immunoassay and GC/MS." Div. of Environ. Chem. , ACS. 867-869.

Thurman, E.M., Meyer, M., Pomes, M., Perry, Charles A., and Schwab, Paul A., 1990. "Enzyme-linked immunosorbent assay compared with gas chromatography/ Mass spectrometry for the determination of triazine herbicides in water." Analytical Chem, 76: 2043-2048.

U.S.EnvironmentalProtection Agency. 1990. "A national survey of pesticides in drinking

water wells". EPA 579/9-90-015. Phase I report. Washington, DC.

Vandelaan, M., Stanker, Larry H., Watkins, Bruce E., and Roberts, Dean W., 1990." Immunoassays for trace chemical analysis: monitoring toxic chemicals in humans, food, and the environment." ACS Series 451., p 374.

Winkelman, D.A. and S.J. Klaine, 1991. "Degradation and bound residue formation of atrazine in western Tennessee soil". Environ. Toxicol. Chem. 10:347-354.

Wolf, D.C. and Marin, J.P., 1975." Microbial decomposition of the ring-14C atrazine, cyanuric acid, and 2-chloro-4,6-diamino-s-triazine". J. Environ. Qual. 4:134-139.

Yoo, J.Y. and Solomon, K.R., 1981. "Persistence of permethrin, atrazine and methoxychlor in a natural lake system". Can. Tech.Rep. Fish. Aquat. Sci. 1151:164-167.

MICHIGAN STATE UNIV. LIBRARIES



31293015649811