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Analysis of Fish Tissue for Kepone, Mirex, Atrazine, Linuron, and Alachlor

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Final Report

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Section I - Kepone

Procedures used in analysis of fish for Kepone

The desiccated fish samples were extracted with Ethyl Acetate/Toluene (3+1) and brought to a volume of 25ml except for samples 2, 4, 8, 11, 15, 21, 24, 25, 30, and 36. These 10 samples had aliquots removed equal to 10g of tissue before the Na_2SO_4 tissue mixture was extracted.

0.5ml aliquots of the extracts were placed on 6mm X 165mm florisil columns topped with Na_2SO_4. PCB's, DDT's and other pesticides which might interfere with the Kepone analysis were eluted with 4ml of 50% dischloromethane, 49.65% of Petroleum Ether and 0.35% of Acetonitrile, this fraction was discarded. Kepone was eluted with 15ml of Ethyl Acetate/Toluene (3+1) and concentrated to a volume of 1ml of Ethyl Acetate/Toluene (3+1). Four microliter aliquots of these solutions were injected into a Searle GLC equipped with a ^{63}Ni electron capture detector with the following parameters:

Column - 6' X 4mm I.D., 5% SE-30 on 80/100 GC-Q
Column Temperature - 190°C
Injector Temperature - 215°C
Detector Temperature - 290°C

Kepone was quantitated by comparing peak heights of Kepone in samples to peak heights of Kepone standards. Four fortified samples were analyzed with this group, the average recovery was 72%. All data is uncorrected for recovery.

Samples 37, 35, and 28 have higher than normal detection limits because of interfering lipid materials.

Samples 6, 28, 29, 32, 34, 35, and 37 because of interferences or positive results were reinjected on the column described below:

1.5% OV-17, 2.0% OV-210 on 80/100 GC-Q. 4mm I.D. X 6ft. long
Injector Temperature - 235°C
Column Temperature - 205°C
Detector Temperature - 300°C

Percent Recovery of Kepone Fortified Samples

Lab No.	Fortified Level(ppm)	ppm Control	ppm Fortified Sample	Recovery ppm	Recovery Percent	Applicable to Samples
18385-Sp1	0.5	0.04	0.36	0.32	64	1 → 10
18385-Sp2	0.5	<0.02	0.37	0.37	74	11 → 20
18385-Sp3	0.5	<0.02	0.27	0.27	57	21 → 30
18385-Sp4	0.5	<0.02	0.35	0.35	70	31 → 37

<indicates less than, when present.

Table I - Kepone Results

Lab No.	Customer I.D.	Location	ppm Kepone
18385-1	# 1	Nanticoke	0.04
18385-2	# 2	Nanticoke	0.02
18385-3	# 3	Nanticoke	0.02
18385-4	# 4	Nanticoke	<0.02
18385-5	# 5	Nanticoke	0.02
18385-6	# 6	Potomac	<0.05
18385-7	# 7	Potomac	0.02
18385-8	# 8	Potomac	<0.02
18385-9	# 9	Potomac	0.03
18385-10	#10	Potomac	0.03
18385-11	#11	Sassafras	<0.02
18385-12	#12	Sassafras	<0.02
18385-13	#13	Bohemia	<0.02
18385-14	#14	Bohemia	<0.02
18385-15	#15	Bohemia	<0.02
18385-16	#16	Bohemia	<0.02
18385-17	#17	Bohemia	<0.02
18385-18	#18	Little Elk	<0.02
18385-19	#19	Little Elk	<0.02
18385-20	#20	Elk	<0.02
18385-21	#21	Elk	0.03
18385-22	#22	Elk	<0.02
18385-23	#23	Choptank	<0.02
18385-24	#24	Choptank	<0.02
18385-25	#25	Choptank	0.04
18385-26	#26	Choptank	<0.02
18385-27	#27	Choptank	<0.02
18385-28	#28	James	<0.07
18385-29	#29	James	<0.05
18385-30	#30	James	<0.02
18385-31	#31	James	<0.02
18385-32	#32	James	0.09
18385-34	#34	Rappahannock	0.08
18385-35	#35	Rappahannock	<0.07
18385-36	#36	Rappahannock	0.03
18385-37	#37	Rappahannock	<0.07

<indicates less than, when present.
#33 was not received.

Section II - Mirex

Procedures used in analysis of fish for Mirex

Five milliliter aliquots of the Ethyl Acetate/Toluene (3+1) extracts were cleaned up by gel permeation chromatography with the following parameters: Column 35 X 2.5cm SX-3, Dump 100ml, Collect 100ml, Wash 10ml, flow rate 5ml/minute. The cleaned up extracts were concentrated to 5ml and 1ml aliquots were placed on 165 X 6mm I.D. Silica Gel Columns. Mirex was eluted with 8ml 0.5% Benzene in Petroleum Ether. The eluate was concentrated to a 1ml volume and two to four microliter aliquots were injected into a Searle GLC equipped with a ^{63}Ni electron capture detector at the following conditions:

Column - 6' X 4mm I.D. 1.5% OV-17, 2.0% OV-210 on 100/120 GC-Q
Column Temperature - 200°C
Inlet Temperature - 220°C
Detector Temperature - 300°C

Percent Recovery of Mirex

Lab No.	Fortified Level µg	Blank	Recovery µg	Recovery Percent	Applicable to Samples
385 Mirex GPCSP	0.58	<0.01	0.56	97	all ten

Table II - Mirex Results

Lab No.	Customer I.D.	Location	ppm Kepone
18385-4	# 4	Nanticoke	<0.01
18385-8	# 8	Potomac	<0.01
18385-11	#11	Sassafras	<0.01
18385-15	#15	Bohemia	<0.01
18385-21	#21	Elk	<0.01
18385-24	#24	Choptank	<0.01
18385-25	#25	Choptank	<0.01
18385-30	#30	James	<0.01
18385-36	#36	Rappahannock	<0.01

<indicates less than.

Section III - Atrazine and Simazine

Two ml aliquots of the Ethyl Acetate/Toluene extracts were concentrated to dryness under an air stream. The residue was transferred to a separatory funnel with 10ml of pet ether, and 10ml acetonitrile. The separatory funnel was shaken and the lower (acetonitrile) layer was collected. The hexane layer was extracted with two additional portions of acetonitrile. The combined lower layers were concentrated to just dryness on a rotary evaporator and transferred with benzene to a tube marked at 2ml and brought to volume. A 1ml aliquot was placed on a 15cm X 6mm i.d. florisil column and eluted with 10ml benzene and 15ml 10% acetonitrile in benzene. The eluate was concentrated to just dryness and brought to 1ml with benzene. Two to four microliter aliquots of the sample solutions were injected into a GLC equipped with a ^{63}Ni electron capture detector at the following parameters:

Column - 6' X 4mm i.d., 1.5% OV-17, 2% OV-210 on 100/120 mesh GC-Q
Column Temperature - 170°C
Inlet Temperature - 190°C
Detector Temperature - 300°C

Quantitation was performed by comparing peak heights of standard injections of Simazine and Atrazine to the peak heights of Simazine and Atrazine in the sample.

Percent Recovery of Atrazine and Simazine

Lab No.: 18385-11

	Atrazine	Simazine
Fortified Level (ppm)	5.7	4.9
Control (ppm)	<0.6	<0.4
Recovery (ppm)	4.9	2.9
Recovery (Percent)	85 %	60 %

<indicates less than, when present.

Table III - Atrazine and Simazine Results

Lab No.	Customer I.D.	Location	ppm Atrazine	ppm Simazine
18385-2	# 2	Nanticoke	<0.2	<0.4
18385-4	# 4	Nanticoke	<0.2	<0.4
18385-8	# 8	Potomic	<0.2	<0.4
18385-11	#11	Sassafras	<0.6*	<0.4
18385-15	#15	Bohemia	<0.2	<0.4
18385-21	#21	Elk	<0.2	<0.4
18385-24	#24	Choptank	<0.2	<0.4
18385-25	#25	Choptank	<0.2	<0.4
18385-30	#30	James	<0.2	<0.4
18385-36	#36	Rappahannock	<1.0*	<1.0*

*Detection limits raised because of interference.
<indicates less than, when present.

Section IV - Alachlor and Linuron

Aliquots containing 10g of tissue were taken from the Na_2SO_4, tissue mixture of the designated samples. Alachlor and Linuron were hydrolyzed to diethyl and dichloro analine respectively. The analine hydrolysis products were steam distilled into an acidic trap. This acidic solution was then extracted with petroleum ether to remove codistilled lipophylic substances. The solution was then adjusted to a pH of Ca 8 and the analines extracted with dichloromethane. After concentration to a small volume, the extracts were transferred to a 6 X 0.5 cm florisil column topped with 1cm of Na_2SO_4. Analines were eluted with 11ml of dichloromethane. The eluate was left uncovered at ambient conditions to concentrate, the volume of the solutions adjusted to 1ml. Four microliter aliquots were injected into a Gas Liquid Chromatograph equipped with a flame ionization detector with the following conditions:

Column - 3.8% UCW 98, 4' X 4mm i.d.
Column Temperature - 150°C
Inlet Temperature - 170°C
Detector Temperature - 230°C

Quantitation was performed by plotting peak heights of four standards against amount injected for each analine. From the resultant curve and sample peak height, the amount of analine in the sample injection was obtained. PPM were calculated by multiplying this number by factors accounting for volumes, dilutions, sample weight, and gravimetric factors to express data in terms of the parent compound.

Recovery was very poor for the fish tissue spikes. Factors which may have contributed to this low recovery were the presence of Na_2SO_4 which altered the ionic strength of the hydrolysis solutions. It was difficult to dissolve the Na_2SO_4 and may have irreversibly bound some of the compounds of interest. A great deal of foaming occured with most samples which may have prevented distillation of the analine.

As shown in the recovery table, plant material samples yield quite good recovery.

Table IV - Alachlor and Linuron Results

Lab No.	Customer I.D.	Location	ppm Alachlor	ppm Linuron
18385-2	# 2	Nanticoke	<0.1	<0.2
18385-4	# 4	Nanticoke	<0.1	<0.2
18385-8	# 8	Potomic	<0.1	<0.2
18385-11	#11	Sassafras	<0.1	<0.2
18385-15	#15	Bohemia	<0.1	<0.2
18385-21	#21	Elk	<0.1	<0.2
18385-24	#24	Choptank	<0.1	<0.2
18385-25	#24	Choptank	<0.1	<0.2
18385-30	#30	James	<0.1	<0.2
18385-36	#36	Rappahannock	<0.1	<0.2

<indicates less than, when present.

Percent Recovery of Alachlor and Linuron from Fortified Samples

Lab I.D.:	Spike A		Spike B		
	Alachlor	Linuron	Alachlor	Linuron	
Fortified Level(ppm)	1	1	1	1	All Samples Run In One Set.
Control (ppm)	<0.1	<0.2	<0.1	<0.2	
Fortified Samples(ppm)	0.07	0.47	<0.1	<0.2	
Recovery (ppm)	0.07	0.47	<0.1	<0.2	
Recovery (%)	7%	47%	---	---	

Lab I.D.:	Plant material spikes preceding and following fish analysis.					
	Alachlor	Linuron	Alachlor	Linuron	Alachlor	Linuron
Fortified Level(ppm)	4.0	---	0.05	---	1.0	---
Control (ppm)	<0.1	---	<0.02	---	<0.06	---
Fortified Samples(ppm)	3.9	---	0.034	---	0.87	---
Recovery (ppm)	3.9	---	0.034	---	0.87	---
Recovery (%)	99 %	---	67 %	---	87 %	---
	Peanut Forage after fish analysis.		Peanuts before fish.		Peanut Shells before fish.	

<indicates less than, when present.