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Bahavior of Some Herbicides Applied to Oil-bearing Crops

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油脂作物에 施用한 몇가지 除草劑의 行方

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SUMMARY

Three herbicides including nitrofen, alachlor and butachlor were applied to farm soils of oil-bearing crops rape, soybean and paddy rice and their residual levels in the soil and seeds were determined by ECD-attached gas chromatograph without hydrolysis.

Applied herbicides were decreased abruptly 2 weeks after application and slowly thereafter, reaching below 10% level while the extent of disappearance varied depending on the herbicides, crops and soil conditions. The herbicides were not detected at all in the seeds of tested crops within the detection limit of the analytical methods employed.

INTRODUCTION

The use of herbicides in Korea was begun in 1955, however their consumption level increased remarkably since 1970, amounting to about 2,000 tons in 1975 as shown in Table 1. Among the herbicides, nitrofen, butachlor and pentachlorophenol constitute about 84% of total consumption in recent years as shown in Table 2. But the use of pentachlorophenol is decreasing in recent years because of its toxicity toward fishes and shellfishes[1]. Alachlor, an acetanilide herbicide, is expected to be used in large quantities in near future, due to its domestic production. In spite of the wide use of herbicides in Korea, studies in connection with their residue problem are quite limited except a few attempts by Yang et al.[2] and Lim et al.[3].

Nitrofen was studied by several workers; for instance, its photolysis by Crosby et al.[4], its degradation under flooded and uplant conditions by Kuwatsuka[5] and its metabolism in animals by use of ^{14}C-labeled nitrofen by Hunt et al.[6] Recently it was shown by Byeon et al.[7] that nitrofen manifested mutagenic activity in Salmonella/microsome system. However, its absorption, translocation and metabolism in plants and chronic toxicity in animals are not elucidated clearly[8]. On the otherhand, Hargrove et al.[9] reported significant losses of alachlor in soil by acid-catalysed decomposition and volatility, and Beestman et al.[10] showed that the dissipation of propachlor, alachlor and butachlor in soil is mainly caused by microbial decomposition. Yu et al.[11] applied a model ecosystem to assess the effects of alachlor and propachlor in the environment and showed that their metabolites or degradation products were not magnified in food chain.

It is still difficult to predict the actual beha-

Table 1. Consumption trends of herbicides by group in Korea*
(Unit : active ingredient in metric tons/year)

Period \ Group	Phenoxy	Acidamide	Carbamate	Triazin	Others	Total
1955	0.7	—	—	—	—	0.7
1956-60	9.9	—	—	—	—	9.9
1961-65	11.7	0.3	—	—	—	12.0
1966	103.1	0.6	—	0.4	—	104.1
1967	187.1	7.1	—	0.4	—	194.6
1968	253.6	16.6	2.5	3.5	0.2	276.4
1969	346.5	17.3	19.0	1.3	—	384.1
1970	928.7	32.9	25.7	4.1	1.2	992.6
1971	924.8	157.5	26.8	42.2	11.7	1,163.0
1972	553.7	287.1	64.9	38.9	8.5	953.1
1973	455.6	319.3	46.9	15.9	11.3	849.0
1974	824.4	602.9	72.8	16.8	18.3	1,535.2
1975	837.4	1,008.2	65.0	11.9	39.0	1,961.5

*Calculated from Yearbook of Agriculture and Forestry Statistics (1960-1976), Korea

Table 2. Annual consumption pattern of herbicides in Korea
(average for 1974-75 as active ingredients)

Group	Commodity	Total usage (ton)	Distribution (%)
Phenoxy	NIP (nitrofen, TOK)	592.4	33.9
	PCP (pentachlorophenol)	123.6	7.1
	CNP (MO)	82.9	4.7
	2,4-D	21.1	1.2
	MCP	11.1	0.6
Acid amide	Machete (butachlor)	750.8	42.9
	Lasso (alachlor)	32.0	1.8
	DCPA (propanil, stam F-34)	22.8	1.3
Carbamate	Saturn (benthiocarb)	56.7	3.2
	MCC (swep)	12.3	0.7
Triazin	Triazin	12.9	0.7
	CAT (simazine)	1.5	0.1
Others	Paraquat (gramoxone)	27.0	1.5
	Linuron	0.9	0.1
	Devrinol	0.8	0.1
Total		1,748.8	100.0

vior of parent herbicides from soil to crops, especially under practical agronomical conditions in Korea. The reported analytical methods of alachlor and butachlor are not suitable for residue analysis since they employ a hydrolysis step and detect the decomposition product by gas chromatography attached with a flame ionization detector.[11,12] Yang et al.[2] and Lim et al.[3] attempted to use an electron capture detector by eliminating the hydrolysis step in the detection of butachlor and alachlor. Since their objective was confined to the adsorption of the parent material

to the soil, additional clean-up procedure was not investigated. This study was, therefore, attempted to develop a quantitative analytical method for the three herbicides by ECD gas chromatography without hydrolysis and to pursue the behavior of the parent material in the soil-crop system under agricultural pratices in Korea.

MATERIALS AND METHODS

1. Pesticides and other chemicals

Nitrofen (TOK,NIP) was the wettable formulation containing 50% of 2,4-dichlorophenyl-4'-nitrophenyl ether as manufactured by Kyongbook Agricultural Chemicals Company. Alachlor (Lasso) was the emulsifiable concentrate containing 43.7% of 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide and butachlor (Machete) was the emulsifiable concentrate containing 58.8% of 2-chloro-2',6'-diethyl-N- (butoxymethyl) acetanilide as manufactured by Hankuk Agricultural Chemicals Company. Other chemicals were of reagent grade and solvents were of pesticide analysis grade or so purified in the laboratory.

2. Field experiments

Three important oil-bearing crops including rape, soybean and paddy rice were grown in the field and recommended amounts of three major herbicides including nitrofen, alachlor and butachlor were applied to the soil under conventional agricultral practices. Details of soil and culture conditions are given in Table 3. Soil samples were taken from upper 10 cm surface to represent each plot, air-dried and subjected to chemical analysis of pesticide residues. Seed samples of the crops were taken after harvest from each plot.

3. Analytical procedures

1) Nitrofen

Nitrofen in soil and seed samples was extracted and determined according to the FDA method[13] as decribed below.

A representative 50g sample of soil was weighed into a one-liter Waring blendor jar, 50ml isopropanol added and blended 2 min. at high speed. Hundred ml benzene were added, blended 3 min. at high speed and filtered through a Toyo No. 5C filter paper with suction, washing the jar and cake with solvent mixture of isopropanol-benzene(1:2) to effect complete transfer and make the final volume to 200 ml. A hun-

Table 3. Details of field experiments

	Rape	Soybean	Paddy rice
Variety	Yudal	Choongbook-Baek	Jinheung
Culture condition			
Date of beginning	March6(transplanted)	May 17 (seeded)	June 4(transplanted)
Date of harvest	June 16	October 4	October 11
Replication	60plants/plot in duplicate	18m²/plot in duplicate	18m²/plot in duplicate
Watering	When necessary	Natural	Flooded
Total rainfall during growth	148mm	737mm	729mm
Daily highest temp. range	0-29°C	17-30°C	17-30°C
Soil condition			
Texture	Sandy loam	Sandy loam	Silty loam
Organic matter	4.9%	4.5%	8.2%
pH	5.0	5.2	5.6
C.E.C. (meq/100g)	10.6	9.5	11.0
Pesticide application at beginning			
Nitrofen	200g/10a	20g/10a	200g/10a
Alachlor	53g/10a	109g10a	—
Butachlor	180g/10a	180g/10a	180g/10a

dred ml aliquot of the extract equivalent to 25g soil was transferred into a 250ml round bottom flask and evaporated to dryness on a rotary evaporator at 60-65°C under reduced pressure. When all the liquid was removed, the flask was disconnected and traces of solvent removed with the aid of a gentle stream of clean N_2.

A florisil column 1 cm (i.d.)×7cm deep was packed with 60/100 mesh activated florisil and topped with 2 cm Na_2SO_4 granules. The residue was dissolved and transferred quantitatively from the round bottom flask onto the column with a total of 20ml of petroleum ether. The liquid was allowed to go through the column by gravity. As soon as all the liquid has gone into the column, 25 ml of petroleum ether was added and allowed it to go through. The effluent and washings from the column, in the same manner, with 25 ml of 1% ethyl ether in pelroleum ether were discarded.

The nitrofen was eluted with 25ml of 4% ethyl ether in petroleum ether, collecting the eluate in a 1-oz. screw cap vial. The vial was placed in a water bath at 40°C and evaporated to dryness with the aid of a gentle stream of N_2. The residue was dissolved in 1 ml hexane, 0.5g anhydrous Na_2SO_4 added to remove traces of moisture and capped tightly until ready for the next step.

For oilseeds, a 20 g representative sample was weighed into Waring blendor jar and dry-blended at low speed for 30 sec. followed by blending at high speed for 30 sec. One g $MgSO_4$ plus 10 ml methylene dichloride were added for each gram of sample and blended at low speed for 3 min. Half of the extract (equivalent to 10 g sample) was transferred to a 250 ml round bottom flask and evaporated to dryness as in the method of soil. The oily residue was transferred quantitatively from the round bottom flask into a 125ml separatory funnel with a total of 25ml petroleum ether (10+10+5 ml portions). Twenty five ml of acetonitrile were added and shaken vigorously for 2 min. After complete phase separation the lower phase was withdrawn into a clean 300 ml round bottom flask. The petroleum ether was extracted with 25 ml acetonitrile and added to the round bottom flask. The round bottom flask was connected to a rotary flash evaporator and evaporated to dryness at 60-65°C under reduced pressure. The next Florisil clean-up procedure was the same as in the method of soil.

Gas chromatographic conditions were as follows. A standard curve was prepared by injecting 3μl aliquots of hexane solutions containing 0.15, 0.3, 1.5, 3 and 15 ng nitrofen into the GLC column and measuring the peak area by triangle method for the peak having a retention time of 14min.

Column: length and type-2mm×185 cm glass, U-shaped packing-10% SE-30 on 80/100 Gas Chrom Q

Carrier gas flow rate: N_2, 77ml/min.

Temperature: injector, 225°C; column, 200°C; detector, 245°C

Attenuation: 8X

Chart speed: 0.5 inches/min.

Detector: electron capture detector (^{63}Ni)

Confirmatory column: 3% QF-1 Chromosorb G (AW-DMCS) 2mm×92cm glass column

The sensitivity of the analytical mothod was 0.001 ppm for soil or oilseed samples and the recoveries ranged from 75% to 85%.

2) Alachlor and butachlor

These two herbicides were extracted and determined according to the FDA method for nitrofen after modification as tried in this study.

A 50 g soil sample was extracted with isopropanol-benzene(1 : 2) and an aliquot was taken to dryness. The residue was then dissolved in petroleum ether and cleaned up with a florisol column packed with 60/100 mesh activated adsorbant by eluting with petroleum ether, 16% ether in petroleum ether and 48% ether and 48% ether in petroleum ether, in succession. The eluate with the last solvent system was collected and evaporated to dryness for gas chromatographic analysis.

For oilseeds, a 20 g sample was extracted and partitioned as in the case of nitrofen and the

column clean-up was done as specified above.

Gas chromatographic conditions were the same with nitrofen analysis. Retention time was 5 min. for alachlor (column temperature 200°C) and 6.6 min. for butachlor (column temperature 215°C).

The sensitivity of alachlor analysis was 0.001 ppm for soils and 0.013 ppm for oilseeds and that of butachlor was 0.01 ppm for soils and 0.06 ppm for oilseeds. The recoveries ranged 80-90% in alachlor and 75-95% in butachlor.

RESULTS AND DISCUSSION

1. Establishing analytical methods of alachlor residue

The published methods for alachlor analysis is based on hydrolysis followed by detection of the hydrolytic product 2,6-diethylaniline by gas chromatography utilizing a flame ionization detector. However, analytical procedure utilizing an electron capture detector without hydrolysis was tried to substitute the known method. Trial for any variables was tested by adding 10ppm of authentic alachlor to soil or soybean samples and injecting the test solution into gas chromatograph attached with ECD under the conditions employed in the detection of nitrofen.

As the extraction solvent from soil samples, isopropanol-benzene(1:2) and acetone-water(4:1) were tested. Both solvents extracted alachlor to the same extent, but the former solvent system was chosen since it extracted less interfering substances like pigment and easier to concentrate. As a dissolving solvent, n-hexane was preferable to methylene chloride or petroleum ether in order to avoid chlorine molecules and contaminating substances from the sample.

For column clean-up of alachlor extracts, some variables were examined in reference to nitrofen analysis in order to pursue conditions requiring less adsorbant, less solvent and less time. The results are shown in Table 4. It was observed that the particle size of adsorbant was indifferent in the elution pattern while its deactivation caused a slight decrease in the recovery. When

Table 4. Clean-up data of alachlor extracts from soil samples by florisil column

Eluting solvent	60/100 mesh florisil	100/200mesh florisil	
% Ether in petroleum ether	Activated	Activated	Deactivated
0	ND	ND	ND
4	ND	ND	ND
16	ND	ND	4.7
48	75	75	66
60	ND	ND	ND

Data are % recovery of alachlor from 1 ml of 10 ppm test solution.

Table 5. Comparison of two methods for analysis of alachlor (average of duplicate runs)
(Unit: g material)

Procedure	Nitrofen method	Organochlorine method
Afterextraction	1.292	1.278
After partition	0.064	0.275
After column clean-up		
16% eluate	0.036	0.231
48% eluate	0.011	0.027
Recovery	85%	75%

the composition of eluting solvent was further examined with 60/100 mesh activated florisil column by subdividing the ether level in petroleum ether as 4, 16 (first), 16 (second), 32 and 48%, the percent recovery of alachlor was 0, 3.6, 30.7, 51.7 and 1.9%, respectively. It was, therefore, concluded that the column clean-up procedure of utilizing 60/100 mesh activated florisil, washing with 16% ether in petroleum ether followed by elution with 48% ether in petroleum ether would give rise to 80% recovery of alachlor from soil samples.

In case of oilseed samples, two methods known for nitrofen and organochlorine insecticide were compared with respect to the extraction, partition, column clean-up and recovery of alachlor. The results shown in Table5 were obtained by adding 1 ml of 1 ppm alachlor solution to 10g soybean. The elimination of contaminants was measured by weighing the amount of evaporated residue after each step. In terms of recovery and clean-up efficiency, the nitrofen method appeared

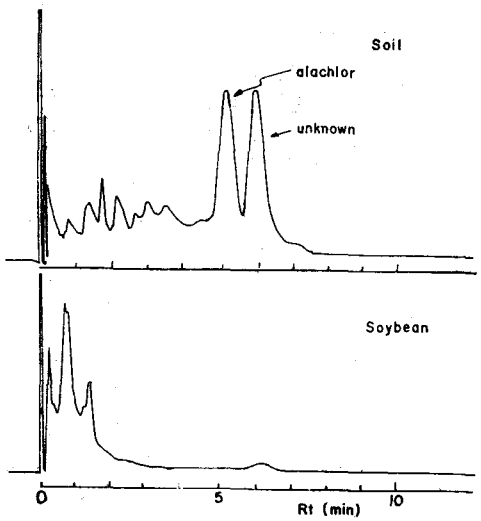

Fig. 1. Typical gas chromatograms of alachlor residues in soil and soybean samples

to be preferrable. Typical gas chromatograms of alachlor residues in soil and soybean samples are given in Fig. 1.

2. Persistence of nitrofen in soil

The results of nitrofen residue in soils of upper 10 cm surface where the oil-bearing crops were grown are shown in Table 6 and Fig. 2. The residual level of the herbicide decreased remarkably 2 weeks after application and continuously did so during the whole growth period of the tested crops to reach 10% level of the applied quantity. The decrease of residual level should be attributed to the decomposition of the herbicide by soil microorganisms and physico-chemical reactions though the possibility of absorption by the crop can not be eliminated. Since this experiment was concerned with the detection of parent compound, any explanation for decomposition products and their significance was not possible. It was, however, apparent that the decomposition rate in flooded rice paddy was faster than that in upland condition, particularly in soybean farm which was drier than rape farm. This finding is in accord with Kuwatsuka's report.[5)]

3. Persistence of alachlor and butachlor in soil

The results of alachlor residue in soils of upper 10 cm surface where the oil-bearing crops were grown are given in Table 7 and Fig. 3. Its residual level decreased abruply after application and the extent of its decrease was more noticeable in rape farm than in soybean farm as is the case in nitrofen.

The results of butachlor residue are given in Table 8. The tendency of disappearance appears to be similar to that of alachlor. However the results were ambiguous likely because the lowest detection limit for butachlor was 10 times higher than alachlor and the disappearance of butachlor

Table 6. Persistence of nitrofen in soils of rape, soybean and rice farm (10 cm surface)

Crop	Days after application	Nitrofen residue (ppm on an air-dry basis) Lot 1	Lot 2	Average	% Residue in soil
Rape	1/6	1.468	2.612	2.040	100
	15	0.840	1.548	1.194	58.5
	30	1.292	0.812	1.052	51.6
	60	0.096	0.264	0.180	8.8
Soybean	1/6	0.605	0.467	0.536	100
	15	0.219	0.376	0.298	55.6
	60	0.328	0.353	0.341	63.6
	90	0.179	0.144	0.162	30.2
	120	0.176	0.147	0.159	29.7
	150	0.037	0.068	0.053	9.9
Paddy rice	1/6	0.055	0.062	0.059	100
	15	0.011	0.012	0.012	20.3
	40	0.009	0.006	0.008	13.6
	70	0.001	0.004	0.003	5.1
	100	0.002	0.002	0.002	3.4
	130	0.002	0.003	0.003	5.1

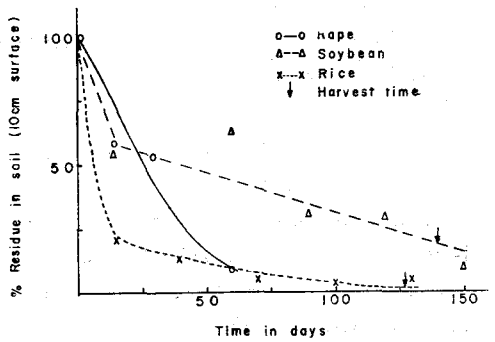

Fig. 2. Residual level of nitrofen applied to soils of rape, soybean and paddy rice farm

Table 7. Persistence of alachlor in soils of rape and soybean farm (10 cm surface)

Crop	Days after application	Alachlor residue (ppm on an air-dry basis) Lot 1	Lot 2	Average	% Residue in soil
Rape	1/6	0.010	0.015	0.0125	100
	15	0.002	0.002	0.0020	16.0
	30	0.002	<0.001	<0.0015	<12.0
	60	<0.001	<0.001	<0.0010	<8.0
Soybean	1/6	0.006	0.015	0.0105	100
	15	0.002	0.008	0.0050	47.6
	60	<0.001	0.002	<0.0015	<14.3
	120	<0.001	<0.001	<0.0010	<9.5
	150	<0.001	<0.001	<0.0010	<9.5

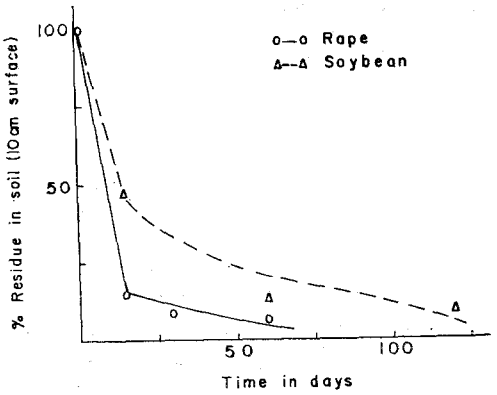

Fig. 3. Residual level of alachlor applied to soils of rape and soybean farm

could not be well demonstrated by this anaytical procedure.

The structural aspect of alachlor and butachlor is so similar that their decomposition in soil is not thought much different.

4. Residue of nitrofen, alachlor and butachlor in seeds of oil-bearing crops

The three herbicides tested here were not detected in the seeds of rape, soybean and rice within the lowest detection limits of 0.0001, 0.013 and 0.06 ppm for respective herbicides. It appears that the absorption of tested herbicides by the oil-bearing crops is negligible, if any, and conventional analytical methods would not give satisfactory results.

Nitrofen as a diphenyl ether herbicide seems to be easily decomposed or metabolized and hardly absorbed by higher plants, thus unlikely to pose any environmental pollution by residue problem. Alachlor and butachlor as acetanilide compounds also should be easily bio-degradable, but the absorption by higher plants might be possible if an analytical method of higher sensitivity like radiotracer technique is used. The possibility of residue problem by decomposition products was not eliminated since they were not examined in this study. Since the report on the residue problem of herbicides is quite limited, more detailed studies into this direction are needed in future.

Table 8. Persitence of butachlor in soils of rape, soybean and paddy rice farm (10 cm surface)

Crop	Days after application	Butachlor residue (ppm on an air-dry basis) Lot 1	Lot 2	Average	% Residue in soil
Rape	1/6	0.193	0.387	0.290	100
	15	0.010	0.034	0.022	7.6
	30	0.015	0.048	0.032	11.0
	45	<0.01	<0.01	<0.01	<3.4
Soybean	1/6	0.015	0.013	0.014	100
	15	0.013	0.029	0.021	150
	30				
	60				
	90				
	120				
	150				
Rice	1/6				
	15				
	30				
	60				
	90				
	110				

non-detectable within the lowest detection limit of 0.01 ppm

요 약

除草劑중 국내에서 많이 사용되는 nitrofen(NIP, TOK), butachlor (Machete) 및 alachlor(Lasso)의 殘留性을 조사하기 위하여 가수분해를 거치지 않고 電子포획검출기가 부설된 깨스크로마토그래피로 분석하는 방법을 확립시킨 후 이들 제초제를 油脂작물인 油菜, 大豆 및 水稻의 재배포장에 施用하였을 때의 殘留量을 분석하였다.

그 결과 세가지 除草劑는 사용후 2주일만에 급격히 감소하였으며 그 이후에는 점차적으로 감소하여 10%수준에 도달하였다. 그러나 油脂원료인 세 작물의 種子에는 分析限界値안에서 전혀 檢出되지 아니하였다.

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