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Study of Pyrolysis Pattern and Transfer Rate of Organochlorine Pesticide in Tobacco

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ABSTRACT : GRLs(Guidance Residue Levels) of agricultural chemicals for tobacco are recommended by the CORESTA Agro-Chemical Advisory Committee guide. In the GRLs list, organochlorine group is one of pesticides commonly used on tobacco cultivation. In this model study, the quantitative correlation in the transfer rate of pesticide residue into tobacco smoke by spiking of organochlorine pesticides to cigarette and pyrolysates were investigated. The spiking concentration referred to the range of GRLs list and the organochlorine pesticides in mainstream smoke were analyzed by GC-MS. For the understanding of the composition variation versus temperature, the behavior of pesticides was investigated by pyrolysis-gas chromatography-mass spectrometry(Py-GC-MS). In this study, the transfer rate of pesticide residue into tobacco smoke at four different spiking concentration and the composition of pyrolysates were analyzed differently. At 10 μg/cig spiking concentrations, the organochlorine pesticides were transferred into tobacco smoke in 0.02 ~ 10.19 % each of component and the most of pesticides were pyrolyzed during smoking. It was found that the decomposition compounds from organochlorine pesticides were mainly composed of oxygenated and nitrogenous compounds. This study could estimate that the transfer rate of pesticides into tobacco smoke is very small amount.

Key words **: Pesticide, transfer rate, pyrolysis, GC/MS**

Public concern over residues of pesticides in food and related products has been increased during the past 20-25 years. The situation has led to regulation setting maximum residue limits (Mueller et al., 1999) of pesticide in agricultural commodities including tobacco or tobacco products. Guidance residue level for ninety nine pesticide residues on tobacco are recommended by CORESTA ACAC, to provide guidance to tobacco growers and those in the tobacco industry (CORESTA GUIDE, 2003). According to the interest in pesticide residues, a few pesticides are studied on the transfer of pesticide residues in tobacco into its mainstream and sidestream smoke (Bowery and Guthrie, 1961; Cai et al., 2002; Ceschini and Chauchaix, 1980). The majority of them is no longer used in tobacco growing due to their environmental toxicity today. Nowadays, several compounds including organochlorine group are most applied pesticides in agriculture. Chlorothalonil is used to fungicide, α-endosulfan and β-endosulfan are used as insecticide. Chlorthal dimethyl, benfluralin and pendimethalin are used as herbicide, butralin and flumetralin are used as herbicide and growth regulator. This study

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determined the transfer rate of eight pesticide on tobacco leaf into its mainstream smoke with different smoking conditions and spiking concentrations. The pesticides are analyzed by gas chromatography-mass spectrometry. And, for the understanding of the composition variation versus temperature of the those pyrolysis pattern, the behavior of pesticides was simulated by investigation by pyrolysis-gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Material and reagent

We used Ky2R4F that is a reference cigarette. The solvents with ultra resi-analyzed grade (J. T. Baker, USA) are used. The standard and spiking reagents, chlorothalonil, α-endosulfan, β-endosulfan, chlorthal dimethyl, benfluralin, pendimethalin, butralin and flumetralin were obtained from Sigma-aldrich Co. (USA)

Method of transfer rate determination Analytical procedure for the eight pesticides in mainstream smoke.

The eight pesticides were spiked in Ky2R4F by additive loading system for which a machine was used to add some materials equally to cigarette. The spiking concentration referred to GRLs and each pesticides was added to 0.1, 1.0, 5.0 and 10 μg/cig. The pesticide of spiked was dissolved in 20 % acetone in hexane. The Ky2R4F reference cigarettes were conditioned at 22 ± 1 ℃ and 60 ± 3 % relative humidity for a minimum of 48 h (ISO condition). The three cigarettes were smoked under ISO and Health Canada conditions (Table 1) by the SM450 (Cerulean, UK). Mainstream smoke was collected in 50 mL acetonitrile in cryogenic bath (dry ice/isopropyl alcohol) and in 44 mm Cambridge filter pad. And then, filter pad was placed in impinger and add an internal standard. The sample was shaken for 30 min and the extract had done with SPE (solid phase extraction) for clean up. A florisil cartridge previously was conditioned with 10 mL of ether. The extract was loaded with 2 mL to wet cartridge and collected into test tube. The SPE column was then eluted with 5 mL of 20 % acetone in hexane. After it was concentrated by nitrogen, it was analyzed by GC/MS in NCI (Negative chemical ionization) mode.

Table 1. Smoking conditions

Conditions	Puff volume (mL)	Puff duration (sec)	Puff interval (sec)	Blocking (%)
ISO	35	2	60	0
HC	55	2	30	100

GC/MS analysis.

It consists of 6890 GC and 5973N mass selective detector (Agilent technology, USA). The separation was carried out on a DB-5MS capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) with helium as carrier gas. One microliter of sample was injected using the split mode (split ratio = 25 : 1). The temperatures of GC inlet and the MSD were both controlled at 250 °C. The initial oven temperature was held at 100 °C for 5 min, it was increased first at 10 °C/min to 150 °C, then 2 °C/min to 250 °C and maintained at that temperature for 10 min. The mass condition was NCI single ion monitoring mode.

Method of pyrolysis pattern study

Pyrolysis was performed in JPS-350 Curie point pyrolyzer (JAI, Japan). Pesticide sample was loaded onto pyrofoil wire, curie temperature of 445 °C and 590 °C was used. A 0.16 sec pyrolysis time was applied and the pyrolysis products were directly injected into GC/MS

system (Agilent 6890 GC with 5973N MSD, USA). The separation was carried out on a DB-XLB capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) with helium as carrier gas. The temperatures of GC inlet and the MSD were controlled at 250 °C and 200 °C, respectively. The initial oven temperature was held at 40 °C for 5 min, it was increased first at 15 °C/min to 150 °C, then 10 °C/min to 280 °C and maintained at that temperature for 20 min. The mass condition was operated in full scan mode with electron ionization. The pyrolysates were identified to match the detected mass spectra against standard mass spectra library. Generally, there are several tentative components in the matching list at a specific peak, and only one of them should be selected as the identified component. The identified components were quantified by their integration peak area.

RESULTS AND DISCUSSION

For the transfer rate experiment, there are some procedures to add pesticides which may be added during the growing of the tobacco, radioactive label pesticides are added to tobacco and the specific amount of pesticides are added in the course of experiment. In this study, the eight pesticides of four different concentrations were spiked in Ky2R4F. Table 2 shows the recoveries of four concentrations, LOD (Limit of detection) and LOQ (Limit of quantitation). The recoveries ranged from 79 to 117 % at each spiking level. The LOD and LOQ were determined to signal/noise ratios of 3 and 10, respectively. The LOD of eight pesticides was in range of 1.33 to 4.13 ng/cig, the LOQ was in 4.43 to 13.76 ng/cig.

Fig. 1 shows the chromatogram of the standard of eight pesticides with introducing experimental methods. The chromatogram of the Ky2R4F cigarette smoke added eight pesticides were well separated without matrix interference.

The transfer rate of pesticide residues of tobacco leaf into tobacco mainstream smoke was determined on different smoking conditions and spiking levels. Table 3 shows the transfer rate of pesticide residues of tobacco leaf into tobacco mainstream smoke under two types of smoking conditions. The transfer rate was shown as different pattern in each pesticide. β-Endosulfan showed the highest value, chlorothalonil showed the lowest value among the eight pesticides. Especially, the transfer rate of chlorothalonil was

Table 2. Recovery of the different spiking level, LOD and LOQ analyzed by GC/MS

Pesticides	Spiking level (μg/cig)	Recovery (%)	Spiking level (μg/cig)	Recovery (%)	Spiking level (μg/cig)	Recovery (%)	Spiking level (μg/cig)	Recovery (%)	LOD (ng/cig)	LOQ (ng/cig)
Chlorothalonil	0.1	115	1.0	88	5.0	103	10	83	1.33	4.43
Chlorthal dimethyl	0.1	113	1.0	90	5.0	90	10	95	2.42	8.06
α-Endosulfan	0.1	79	1.0	90	5.0	117	10	85	3.08	10.26
β-Endosulfan	0.1	79	1.0	89	5.0	102	10	97	4.13	13.76
Benfluralin	0.1	101	1.0	92	5.0	91	10	95	2.12	7.06
Butralin	0.1	87	1.0	97	5.0	88	10	99	3.02	10.06
Flumetralin	0.1	92	1.0	90	5.0	88	10	95	3.32	11.06
Pendimethalin	0.1	85	1.0	96	5.0	86	10	101	3.57	11.90

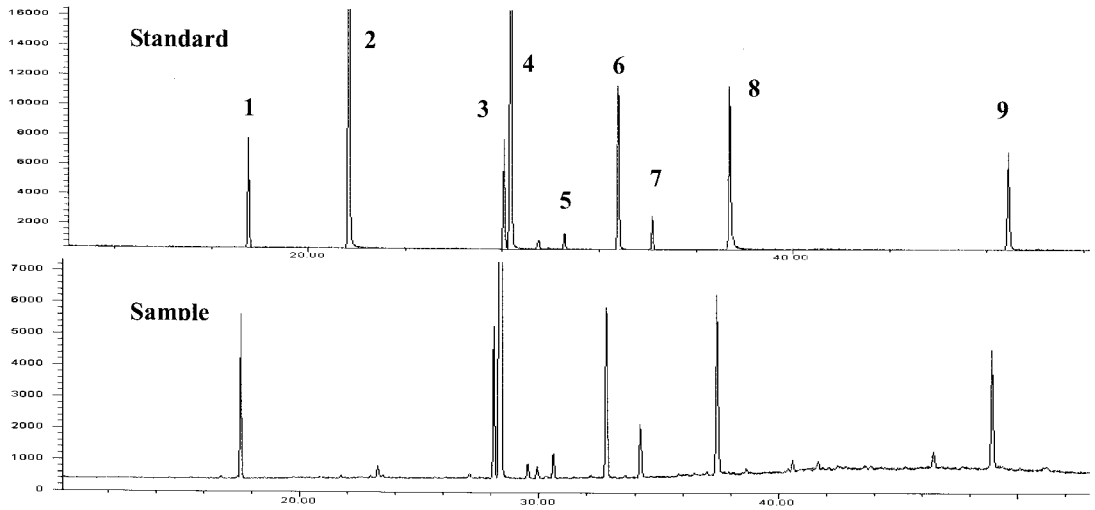

Fig. 1. TICs of the standard and sample of eight pesticides.
1. benfluralin, 2. chlorothalonil, 3. chlorthal dimethyl, 4. butralin, 5. pendimethalin, 6. α-endosulfan, 7. flumetralin, 8. β-endosulfan, 9. mirex

not detected at 0.1 and 1.0 μg/cig spiking level and was shown very low percent at 5.0 and 10 μg/cig spiking level. This result could define that chlorothalonil seems to be disintegrate because it is unstable in solution including acetone. In the result of comparison of the transfer rate under two types of smoking conditions, HC was shown twice or three times higher value than ISO. The transfer rate of ISO and HC was in range of 0.02 to 10.14 % and 0.03 to 18.90 % at 5.0 μg/cig spiking level, respectively. Also, the transfer rate was shown similar or was increased

Table 3. The transfer rate of pesticide residues of tobacco leaf into tobacco mainstream smoke

Pesticides	ISO				HC			
	Spiking level (μg/cig)				Spiking level (μg/cig)			
	0.1	1.0	5.0	10	0.1	1.0	5.0	10
Chlorothalonil	nd[1)]	nd	0.02	0.02	nd	nd	0.03	0.02
Chlorthal dimethyl	5.20	7.69	7.42	6.89	7.75	18.96	16.95	14.50
α-Endosulfan	8.43	10.75	10.10	9.69	11.20	21.42	18.64	17.14
β-Endosulfan	12.72	10.99	10.14	10.19	11.07	23.22	18.90	17.22
Benfluralin	4.04	8.12	8.63	9.57	5.68	15.20	15.65	16.59
Butralin	nd	5.03	5.78	6.62	nd	12.35	13.49	16.03
Flumetralin	5.32	4.53	4.58	5.08	9.70	11.81	10.57	11.57
Pendimethalin	nd	5.07	5.81	6.67	nd	12.73	14.37	16.88

[1)] %, not detect

a little as increasing spiking level.

In this study, the eight pesticides were pyrolyzed at 445 and 590 °C, respectively. The objective is to simulate its pyrolysis behavior and determine the pyrolysis products produced during the pyrolysis process. Fig. 2 shows the total ion chromatography of the pyrolysis products at different temperature. The pyrolysis experiment had a good repeatability in each condition. The results on pyrolysis of eight

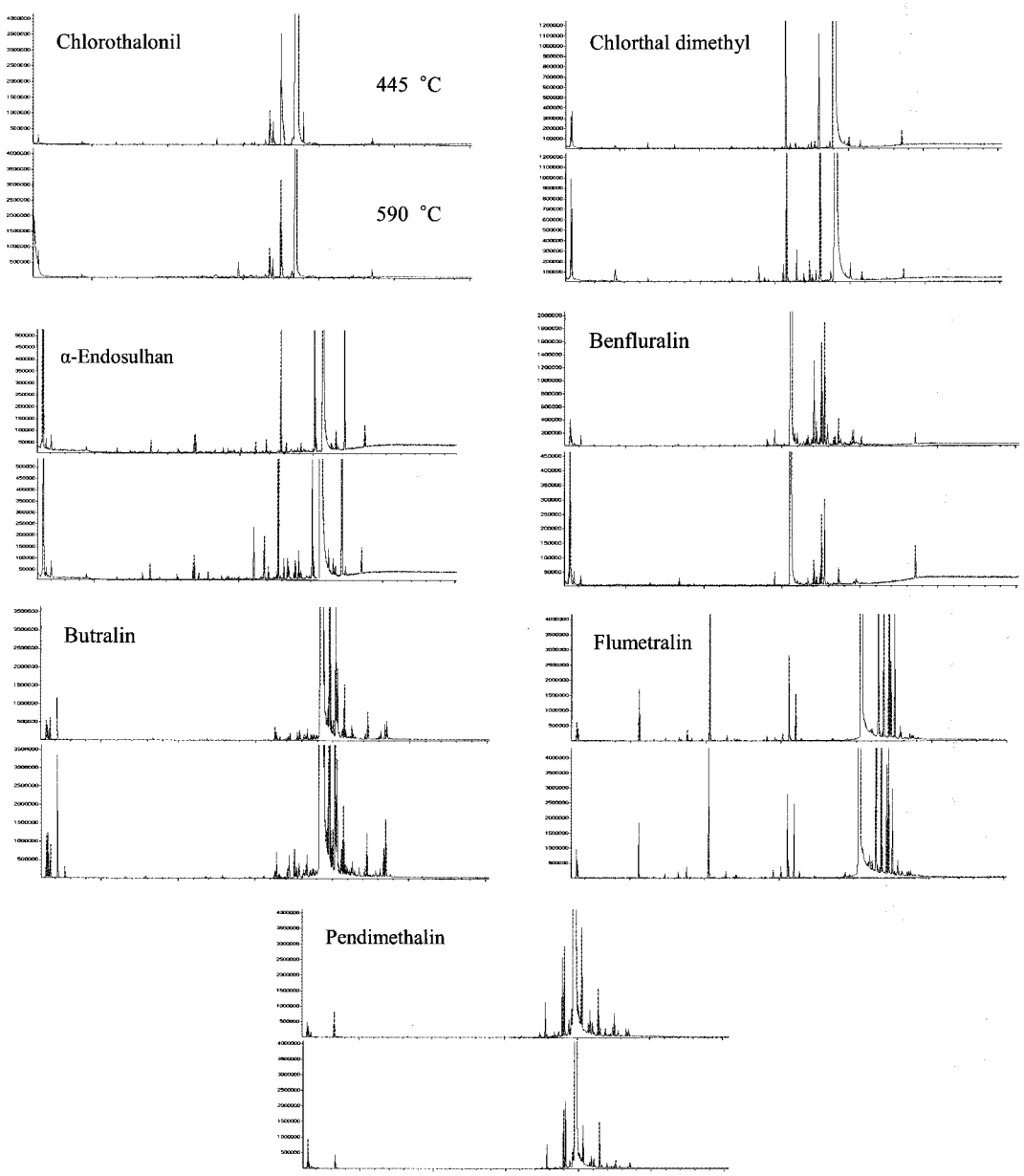

Fig. 2. TICs of the pyrolysis products at different temperatures.

Table 4. Identification and quantitation of the pyrolysis products at 590 °C

NO.	Compounds	Classes	CAS NO.	Area (%)
1	**Chlorothalonil**		000189-45-6	**86.0**
2	Pentachloro benzonitrile	3	020925-85-3	2.27
3	1,3,7-Trichloro naphthalene	1	055720-37-1	0.70
4	2,3,6-Trichloro naphthalene	1	055720-40-6	0.44
5	Hexachloro benzene	1	000118-74-1	0.10
1	**Chlorthal dimethyl**		001861-32-1	**95.2**
2	4,5,6,7-Tetrachloro phthalide	1	027355-22-2	0.60
3	Carbon dioxide	2	000124-38-9	0.34
4	Chloromethane	1	000074-87-3	0.19
5	1,4-Benzenedicarboxylic acid	1	000120-61-6	0.06
1	**α-Endosulfan**		000959-98-8	**90.1**
2	β-Endosulfan		000115-29-7	3.38
3	4,7-Methanoisobenzofuran	2	003369-52-6	1.29
4	sulfur dioxide	2	007446-09-5	0.45
5	Cyclopropanecarboxaldehyde	2	056701-21-4	0.12
1	**Benfluralin**		001861-40-1	**90.7**
2	5-Phenyl-1,2,4-trimethyl imidazole	3	062576-15-2	0.74
3	Acetaldehyde	2	000075-07-0	0.27
4	Pyridine	3	073669-46-2	0.20
5	Butanal	1	000123-72-8	0.12
1	**Butralin**		033629-47-9	**74.6**
2	3-Methoxy-2-methyl-8-nitroquinoline	2	1000242-11-7	6.34
3	2,5-Dimethoxyterephthalic acid	2	007310-97-6	2.19
4	Methyl-α-cyano-4 nitrocinnamate	3	062985-31-3	1.15
5	Ethylene oxide	2	000075-21-8	0.26
6	N,N-Diethyl-3-nitroaniline	3	002216-16-2	0.18
1	**Flumetralin**		062924-70-3	**68.7**
2	2-chloro-6-fluoro- benzaldehyde	2	000387-45-1	1.92
3	N-[4-Chloro-3-nitrophenyl]-3,4-dichlorobenzylamine	3	1000213-60-8	1.90
4	(2-hydroxy-benzylidene)-hydrazide	3	-	1.37
5	1-chloro-3-fluoro- benzene	1	000625-98-9	0.65
6	Acetaldehyde	2	000075-07-0	0.17
7	4-Chloro-2-fluorophenol	2	-	0.15
1	**Pendimethalin**		040487-42-1	**80.9**
2	6-p-nitrophenylimidazol	3	007120-14-1	1.76
3	4-amino-4'-(N,N-dimethylamino)benzene	3	022525-43-5	1.08
4	N-(6-Quinolinyl)acetamide N'-oxide	2	013675-91-7	1.07
5	3-Pentanone	2	000096-22-0	0.32

1. hydtocarbons or aromatic hydrocarbon, 2. oxygenous compounds, 3. nitrogenous compounds

pesticide showed a similar tendency at two different temperature. Most of pesticide residues were identified on original compounds without passing through a lot of pyrolysis process. This result could estimate that the pesticide residues in tobacco volatilized or pyrolyzed some department extremely when the tobacco burn. The decomposition compounds were identified to match the detected mass spectra against standard mass spectra library. The identification and quantitation results at 590 °C were listed in Table 4. The flumetralin and chlorthal dimethyl remained 68.7 and 95.2 % without passing through, respectively. For comparison conveniently, the identified components were divided into three subclasses : (1) hydrocarbons or aromatic hydrocarbon, (2) oxygenated compounds, (3) nitrogenous compounds. The decomposition compounds of chlorothanonil was nitrogenous compounds such as pentachloro benzonitrile. In case of chlorthal dimethyl and benfluralin, there were some decomposition compounds.

The main decomposition compounds of α-endosulfan and butralin were benzofuran and nitroquinoline, respectively. And the main decomposition compounds of flumetralin and pendimethalin were benzaldehyde and 6-p-nitrophenylimidazol, respectively.

CONCLUSIONS

This study determined the transfer rate of pesticide residues under different smoking conditions and the identification and quantitation of pyrolysis product. The transfer rate was shown as different patterns in each compounds and it was generally increased in order of ISO and HC smoking conditions. This study could estimate that the transfer rate of the range of GRLs concentration recommended by the CORESTA ACAC is very small amount that is about 0 to 12.72 % at ISO smoking condition. Also the pyrolysis of eight pesticides at two pyrolysis temperatures showed a similar tendency. The decomposition compounds of the pesticides were composed of hydrocarbon, oxygenated and nitrogenous compounds. This results can provide the useful information for understanding of the thermal behavior of organochlorine pesticide residues.

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