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Residues and Persistence of Endosulfan in Dry Tobacco Leaves and Cigarettes

Euphemia Papadopoulou-Mourkidou and Anastasia Milothridou

Agricultural Chemistry Laboratory, Aristotelian University, P. O. Box 19678, Thessaloniki, Greece

Pesticide residue surveys in agricultural products have never been conducted in Greece and data concerning pesticide residues are circumstantial and fragmentary. These are derived from few research projects going on occasionally in Greece but mostly from national surveys and pesticide residue surveillance programs in countries importing agricultural products from Greece. Recently a research program on pesticide residues and pesticide persistence studies in different agricultural products has beeen approved and initiated with respective research in tobacco leaves and cigarettes. Tobacco is considered to be one of the main agricultural products of Greece. Many tobacco varieties (basmas, koulak, samsous, tsebel, burley, virginia etc) are cultivated in Greece and a high percentage of the annual production is exported. Therefore, data concerning the residues and behaviour of pesticides in tobacco leaves as well different tobacco products is of concern to the Greek as well to other country cigarette and tobacco smokers.

The use of most organochlorine insecticides has been banned in Greece except for endosulfan and lindane which are still in use for the protection of many crops including tobacco. The residues and behaviour of these two pesticides in dry tobacco leaves and cigarettes will be discussed in the present communication.

MATERIALS AND METHODS

Samples of dry tobacco leaves consisting of approximately 500 g each were collected either from individual tobacco growers from different tobacco growing areas of Central and Northern Greece or were supplied by Tobacco Export Companies. These samples were taken mostly from the 1987 tobacco yield. Cigarette samples from different brands either produced in Greece or imported consisting of 200 to 60 cigarettes each were purchased from the local market (1987). Before the analysis, individual leaf samples were further dried at 45 °C for two days and pulverized using a Wiley Mill. The cigarettes from each brand sample were opened and the already cut tobacco leaf material was mixed thoroughly and dried as previously.

Three subsamples of one gram (±0.005 g) each were taken from each of the ground tobacco bulk sample and the tobacco mixture derived from each cigarette brand and processed. For the analysis of the organochlorine insecticides, endosulfan and lindane, the method reported by Waliszewski and Szymczynski (1986) appropriately modified to be used as a micromethod was applied. In brief, each subsample was transferred into a 50 ml glass beaker and immersed into 25 ml of a mixture consisting of acetonitrile:water (65:35, v/v). The beakers were covered with a piece of alumimum foil and kept at ambient temperature for 16 hours. At the end of this wetting and equilibration period each subsample was homogenized for 3 min at high speed by use of a Polytron homogenizer (Kinematica). The mixture was filtered under vacuum through a Whatman No. 1 filter covered by a thin layer of Celite 545. The tissue extraction was repeated for two additional times using each time 15 ml of the extraction solvent mixture. The filtrates were combined into a separatory funnel where 50 ml of a 10% sodium chloride solution was added and the organochlorines were partitioned into 3 x 10 ml of petroleum ether. The petroleum ether phases were collected into a round bottom flask after being dried with anhydrous sodium sulfate by passing through a funnel plugged with a small piece of glass wool topped with two tea spoons of anhydrous sodium sulfate. The organic phase was concentrated to a small volume at 40 °C by use of a rotary evaporator operated under vacuum and the concentrated extract was tranferred into a graduated centrifuge tube by use of Pasteur pippete. The round bottom flask was thoroughly rinsed with small volumes of petroleum ether and all the rinses were collected into the centrifuge tube. The final volume of this concentrated extract was recorded.

Two drops of concentrated sulfuric acid were added in each centrifuge tube containing the concentrated tobacco leaf extracts. Each tube was vortexed for 1 min and centrifuged at 3000 rpm for 10 min. A portion of the upper petroleum ether phase was transferred onto a mini anhydrous sodium sulfate column packed in a disposable Pasteur pipette and the eluate collected was directly analyzed by gas chromatography.

The gas chromatographic analysis was conducted on a Varian Model 3700 gas chromatograph equipped with a ^{63}Ni detector and a 2 m glass column packed with either 3% OV-101 coated on Gas Chrom Q 80/100 mesh or 3% OV-17 coated on Chromosorb HP DMCS 100/120 mesh or 3% OV-210 coated on Gas Chrom Q 80/100 mesh. The gas chromatographic system was operated under the following temperature regime: 280 °C (detector), 240 °C (injector), 220 °C (OV-101 column), 200 °C (OV-17) and 160 °C (OV-210). The carrier (Nitrogen) gas flow was invariably set at 30 ml/min. The gas chromatograph was connected either to a LKB (Sweden) strip chart recorder or a Varian integrator, Model 2400.

RESULTS AND DISCUSSION

The recovery of the micromethod applied for the analysis of organochlorine insecticides in tobacco samples was checked

daily. Therefore, each day in addition to the field treated tobacco leaf samples and respective control and fortified samples with endosulfan and lindane were also processed. The endosulfan and lindane analytical standard solutions made in hexane were dispersed over the surface of the control tobacco tissues and the samples were let at room temperature for about one hour for the hexane to evaporate. Thereafter, the extraction solvent was added and the samples were processed. The recovery data are presented in the Table 1. The recovery of endosulfan ranged from 81.6 to 93.0 % and 96.7 to 100.0 % for the 0.1 and 7.00 ppm level, respectively. Control samples fortified with lindane at 0.1 ppm level gave recoveries ranging from 92.2 to 108.0 %. The detection limits of this method were 0.004 and 0.001 ppm for the endosulfan and lindane, respectively.

Sample chromatograms from the analysis of control, fortified, and field treated samples and pesticide standard materials are presented in the Figure 1. Tobacco extracts analysed by GC on either of the columns reported in the experimental section gave chromatograms with the endosulfan and lindane completely separated from the tobacco coextractives. Sample cleanup was the main problem encountered during the analysis of endosulfan and lindane in tobacco extracts. Cleanup of extracts on silica gel or Florisil columns was not sufficient and there were always coextractives interfering with the GC analysis of the sought analytes. Therefore, the sulfuric acid cleanup method applied by Waliszewski and Szymczynski (1986) for the analysis of some organochlorines (HCB, HCH isomers, DDT, op'-DDT, and DDE) in tobacco was also evaluated in our laboratory and found to be the most efficient as well the quickest and cheapest cleanup procedure of tobacco extracts when endosulfan and lindane were also the sought analytes.

In 1987, tobacco leaf samples were collected from 428 individual growers from a total of 83 villages of tobacco growing areas of Central and Northern Greece. The mean residue level of endosulfan (a- and b-isomer combined) in the samples collected from the growers of the same village was determined first and according to this residue mean level corresponding to each village the different villages considered in this investigation were ranked into 10 groups. For each of these groups a new residue mean level was calculated taking in account all the individual samples collected from all the growers of the villages rated together. The mean residue levels of the aforementioned groups are presented in the Table 2. In the group with the rate number 1 are included 4 villages and its residue mean ($\pm$ standard deviation) value, calculated from the residue levels of the samples collected from a total of 17 growers, is 0.046 $\pm$0.03 ppm. The second group, rate number 2, includes 21 villages and its respective residue mean ($\pm$ standard deviation) value, based on the residue levels in the samples collected from 74 growers is 0.123 $\pm$ 0.04 ppm. In the third group, rate number 3, are included 14 villages and its residue mean ($\pm$ standard deviation) value, based on the residue levels found in the samples collected from 70 growers, is 0.205 $\pm$0.11 ppm. The respective values for the

Table 1. Recovery Data of Endosulfan and Lindane from Dry Tobacco Leaves

Fortification level (ppm)	% Recovery
Endosulfan	
0.10	81.6- 93.0
0.50	88.8-100.0
1.55	94.6-100.0
7.00	96.7-100.0
lindane	
0.10	92.2-108.0

Table 2. Residues (ppm) of Endosulfan in Dry Tobacco Leaves of the 1987 Tobacco Yield.

Group	Number of Villages	Number of growers	Mean ± SD
1	4	17	0.046 ± 0.03
2	21	74	0.123 ± 0.04
3	14	70	0.205 ± 0.11
4	12	70	0.316 ± 0.20
5	14	79	0.557 ± 0.50
6	5	25	1.093 ± 1.32
7	4	36	1.477 ± 1.56
8	4	24	2.498 ± 3.16
9	4	28	3.333 ± 4.36
10	1	5	5.158 ± 10.04

SD denotes standard deviation

Table 3. Residue Levels (ppm) of Endosulfan in Cigarettes (1987)

Group	Mean ± SD	Group	Mean + SD
A	0.098 ± 0.10 (11)	E	0.478 ± 0.18 (9)
B	0.282 ± 0.02 (2)	F	0.555 ± 0.27 (4)
C	0.371 ± 0.27 (4)	G	0.668 ± 0.55 (8)
D	0.450 ± 0.17 (12)		

The number in parenthesis represents the number of cigarette brands consisting the respective group. SD denotes standard deviation.

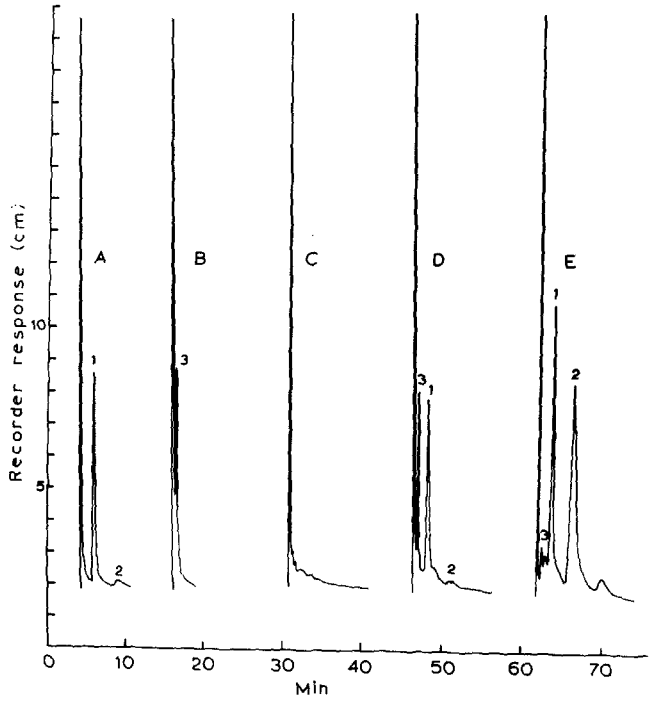

Figure 1. Sample chromatograms from the analysis of 0.2 ng of endosulfan standard (A), 0.03 ng of lindane standard (B), 0.666 mg of control (C), 0.399 mg of fortified at 0.1 ppm with endosulfan and lindane, respectively (D), and 0.192 mg from a field treated tobacco sample. The peaks numbered 1 and 2 are the a- and b-isomers of endosulfan, respectively, and lindane is the peak 3.

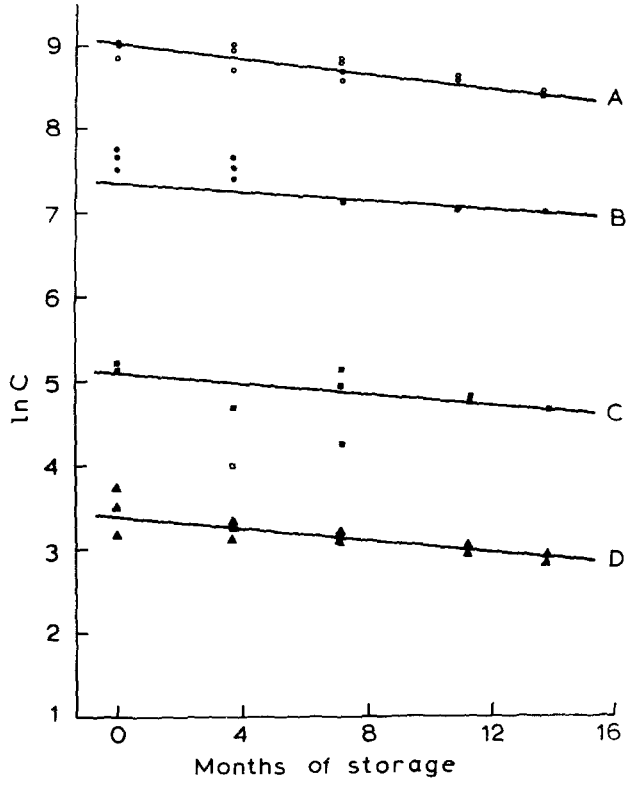

Figure 2. Dissipation plots of endosulfan in dry tobacco leaves during storage at ambient temperature.

forth (rate 4, 12 villages and 70 growers) and the fifth group (rate 5, 14 villages and 79 growers) are 0.316±0.20 and 0.557 ± 0.50 ppm, respectively. The following five groups are less numerous, in terms of villages and respective grower numbers included, rate 6 (5 villages and 25 growers), rate 7 (4 villages and 36 growers), rate 8 (4 villages and 24 growers), rate 9 (4 villages and 28 growers), and rate 10 (1 village and 5 growers) and their respective residue mean (± standard deviation) values are 1.09 ±1.32, 1.48 ± 1.56, 2.50 ± 3.16, 3.33 ± 4.36, and 5.16 ± 10.1 ppm.

There is no relation between the endosulfan residue levels found in the dry tobacco leaves and the geographical location of the areas where tobacco plants are grown. However, high mean residue levels were found in villages of more intense tobacco growing areas. The great variation in endosulfan residue levels found sometimes among tobacco samples derived from growers of neighboring villages or from growers of the same village is merely due to the great differences of agricultural practices exercised among individual tobacco growers. However, in spite of the fact that most of the tobacco samples analysed were collected from villages of the most intense tobacco growing areas of Greece, among the 428 samples collected only 34 samples, corresponding to 7.9% of the samples collected, contained endosulfan residues exceeding the 3.00 ppm level.

The levels of lindane residues in the tobacco samples collected were generally very low. In about 10% of the samples analysed the residues of lindane were in the range of 20 to 50 ppb, in another 50% they were below 20 ppb, and in the rest of the samples lindane was present in quantities below the detection limit of this procedure (1 ppb). In only one sample out of 428 samples analysed lindane was present at the 227 ppb level.

The persistence of endosulfan in dry tobacco leaves during storage was also investigated. For this purpose four bulk dry tobacco leaf samples, derived from the 1986 tobacco yield and each sample consisting from leaves of a different tobacco variety, were stored at room temperature rapped in paper bags and subsamples taken after certain storage periods were analysed. The storage persistence plots of endosulfan in these samples are shown in the Figure 2. These are lnC vs storage time (months) plots, where C is the concentration of endosulfan in ppb at different storage intervals. All four plots are almost parallel to each other indicating thus that endosulfan is dissipated with the same rate in all the samples, irrespective of the tobacco variety, and that this dissipation of endosulfan in dry tobacco leaves is a very slow process. The endosulfan residues being 8.300, 3.500, 0.170, and 0.022 ppm in the samples dessignated as A, B, C, and D, respectively, were decreased to 4.800, 1.150, 0.107, and 0.017 ppm, respectively, after about 13 months of storage.

The endosulfan and lindane residues were also determined in the tobacco of cigarettes purchased from the local market (1987).

Samples from a total of 50 different cigarette brands either manufactured or imported to Greece were analysed. The residue data of endosulfan in the tobacco of cigarettes are presented in the Table 3. The different cigarette brands were separated into seven groups each group representing the brands manufactured by a single tobacco company except for the group labelled A which consists from brands of different manufacturing companies other than the ones included in the groups B to G. The residue levels of lindane in most of the cigarette brand samples examined were close to the detection level of the micromethod applied. The levels of endosulfan in some of the cigarette brand groups (Table 3) are relatively high compared to the levels of endosulfan in the majority of the grower tobacco leaf samples collected from the 1987 tobacco yield (Table 2). Apparently the use of endosulfan in tobacco plant protection has been significantly reduced during 1987 compared to the previous years. High levels of endosulfan residues were also reported by Domanski and Guthrie (1974) in different brands of cigars sold in the USA.

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