Further Investigations Into the Metabolism of Endosulfan I, Endosulfan II and Endosulfan Sulfate in Tobacco Leaf*

by N. M. Chopra** and Amal M. Mahfouz

Tobacco and Pesticide Research Laboratory, North Carolina Agricultural and Technical State University, Greensboro, North Carolina, U.S.A.

Even though several laboratories carry out pesticide residue analysis in cured tobacco leaf routinely, very little has been published on the fate of pesticides during the tobacco growing period and subsequent curing. Some, though scanty, information is available on the rate of pesticide applied and the residue in green and cured leaf (1, 3, 5, 7, 9). However, the information available is not enough for a proper understanding of what happens to the pesticide from the time it is applied to the tobacco plant to the time when tobacco leaf is finally cured. In our previous paper (2) we described the fate of endosulfan I, endosulfan II and endosulfan sulfate in green tobacco leaf. This work is an extension of those investigations, and consists of a comparison between the endosulfan I and II and endosulfan sulfate residues in tobacco leaf at the time it is harvested, and the time when it is finally cured.

EXPERIMENTAL

Material and Methods

Tobacco, variety Coker 347, was planted on May 10, 1973 in a field at Chinqua Penn, North Carolina. On July 10, 1973 the plants were separately treated with 0.75 lb./acre and 1.50 lbs./acre of endosulfan I, endosulfan II and endosulfan sulfate. For studies on the metabolism of endosulfan I and II and endosulfan sulfate in green leaf, tobacco leaves from the middle of the plants were harvested on August 4, 13 and 23, 1973 (5, 14 and 24 days after the pesticide treatments), and endosulfan* and its metabolites were estimated according to the methods developed by Domanski and Sheets (4) and Nesemann and Seehofer (8). A detailed description of these operations is given in our previous publication (2).

* Based on the paper "Metabolism of endosulfan I, endosulfan II and endosulfan sulfate in tobacco leaf" presented at the 6th International Tobacco Scientific Congress (Coresta) held in Tokyo, Japan, in November 1976.

Contribution 10 of the Tobacco and Pesticide Research Project.

** Author to whom correspondence should be addressed.

* Endosulfan, the commercial pesticide, is a mixture of endosulfan I and II.

For studies into the fate of endosulfan I and II and endosulfan sulfate in flue-cured tobacco leaf, leaves from the same tobacco plants used in the previous study were harvested conventionally on August 13 and 22, and September 6, 1973 (14, 23 and 37 days after the pesticide treatments) and flue-cured according to the established commercial practices. We attempted to make "average commercial" cured tobacco leaf samples by thoroughly mixing 10 lbs. each of cured tobacco leaves harvested on August 13 and 22, and September 6, 1973 for any particular treatment. Samples from these mixtures were powdered and analyzed for endosulfan I and II and endosulfan sulfate according to the method described above. As very little of other metabolites of endosulfan, such as endosulfan diol, endosulfan ether, endosulfan hydroxyether, and endosulfan lactone, was found in the cured tobacco leaf samples, their estimations were not carried out.

Endosulfan and its metabolites in tobacco leaf were identified by their retention time and by co-chromatographing them with authentic reference substances on two different columns (2). In addition, the presence of endosulfan I and endosulfan sulfate in the green leaf of all the three 1.50 lbs./acre pesticide treatments harvested on August 23, and the presence of endosulfan II in the green tobacco leaf from 1.50 lbs./acre endosulfan II treatment tobacco harvested on August 23 was confirmed by comparing their retention times and mass spectra on a Finnigan F3100 GC/MS with that of pure reference compounds.

RESULTS

The results of the quantitative estimations of endosulfan I and II and endosulfan sulfate are given in Tables 1 and 2. In Table 1 the results given are on the residues in green tobacco leaf, and are calculated on the basis of 12% moisture content. In our previous paper (2) we reported the same results, in the form of a graph, calculated on the basis of 80% moisture content.

In Table 2 results are given on the residues in both the green leaves harvested on August 23, 1973, and the cured

leaves. They are both based on 12% moisture content, and a comparison is made between the residue levels in the green leaf and the cured leaf.

DISCUSSION

Comparison between the nature and the amounts of endosulfan I and II and endosulfan sulfate in the green leaf and cured leaf is an interesting study. For quantitative comparison purposes we have made two assumptions:

[1] We have assumed that the combined residue levels in the flue-cured leaf of any particular treatment, at the time it was harvested, average the residue levels in green tobacco leaf harvested on August 24, 1973. We have based our assumption on the fact that the change in the residue level in green leaf between August 13 and August 22 is very similar to that between August 22 and September 6. Since equal amounts of cured tobacco were taken from leaf harvested on August 13 and 22, and September 6, the average residue level in the combined cured tobacco leaf for any particular treatment is equal to that of residue levels on Aug. 13 + Aug. 22 + Sept. 6 divided by 3.

$(14 + 23 + 37)/3$ days after pesticide treatment
= 24.66 days after pesticide treatment (average)
= August 23.66.

[2] Our second assumption is that since the cured tobacco was made up of tobacco leaf from all parts of the plant, the average pesticide level in the tobacco leaf used for flue-cured tobacco was not very different from the middle plant leaf harvested on August 23, 1973 for endosulfan and metabolite residue analyses in green tobacco leaf. This assumption is consistent with the work done by Corbaz et al. (3) and Dorough and co-workers (5). Corbaz et al. found that the residue level of Patoran in tobacco leaf is independent of the leaf position, whereas, the Molipan residues were higher in the lower tobacco leaves, and lower in higher tobacco leaves; while Dorough and co-workers found that endosulfan levels in tobacco leaf, 28 days after the application of pesticides, were the same in top, middle and bottom leaves of the tobacco plant.

A study of the residue levels reported in Tables 1 and 2 shows that in endosulfan sulfate treatments* the principal metabolic product in green leaf is endosulfan I, found in very small quantities. No endosulfan II is formed in detectable quantities; whereas, when this same leaf is flue-cured, the residue pattern is reversed. It may thus be seen that in green leaf endosulfan sulfate is metabolized into endosulfan I, while during curing processes endosulfan sulfate is metabolized into endosulfan II. This conversion of endosulfan sulfate into endosulfan II during curing is also seen in other treatments; for

instance, in 0.75 lb./acre endosulfan I treatment (see Table 2) the total endosulfan level in green leaf and cured leaf is about the same. However, the amounts of endosulfan II and endosulfan sulfate in green leaf are 0.08 ppm and 0.90 ppm, and in cured leaf 0.46 ppm and 0.70 ppm, respectively. A similar conversion of endosulfan sulfate into endosulfan II may also be seen, during the curing processes, in 1.50 lbs./acre endosulfan I treatment.

The reduction of sulfate into sulfite in whole tobacco leaf has been previously reported (6). However, our report is, to the best of our knowledge, the first in which the formation of such different reduction products during growing and curing periods in tobacco leaf appears. It is reasonable to assume that the stereoselective conversion of endosulfan sulfate into endosulfan I in the green tobacco leaf is catalyzed by an enzyme which is destroyed soon after the tobacco leaf is harvested, and that endosulfan sulfate is subsequently converted non-enzymically or by less dominant enzyme in the harvested leaf into endosulfan II.

Table 2 also shows that when the pesticide residues in the green leaf are higher, the loss of pesticide during the curing process tends also to be higher. Thus, when the total endosulfan + metabolites in green leaf is 1.12 ppm (0.75 lb./acre endosulfan I treatment) the loss during the curing process is nearly zero; whereas, when the residue level of endosulfan + metabolites is 5.08 ppm (0.75 lb./acre endosulfan II treatment) the pesticide loss during curing is 53.7%. Corbaz et al. (3) report a loss of 40% Molipan residue during fermentation process, while Dorough and co-workers (5) report a 55% loss of endosulfan during curing process under circumstances similar to ours. (A very close comparison between our work and that of Dorough et al. cannot be made since we worked with pure endosulfan I and II and endosulfan sulfate, while Dorough and co-workers worked with commercial endosulfan.) Thus, we can see that the higher the residue level of pesticide in tobacco leaf the higher the loss of pesticide during the curing process.

Also, since very little of other endosulfan metabolic products was found in cured leaf, we conclude that the loss of endosulfan I and II and endosulfan sulfate residues during the curing process is mainly due to volatilization. This conclusion is consistent with the work done by Cheng and Steffens (1), who found that with the non-volatile maleic hydrazide, the loss of maleic hydrazide in tobacco occurred only during the growing period.

Lastly, Table 2 shows that of the three compounds, endosulfan I gives the least amount of residue in cured tobacco leaf. This finding in itself may be of some commercial value.

SUMMARY

Tobacco plants were separately treated with 0.75 lb./acre and 1.50 lbs./acre of endosulfan I, endosulfan II and endosulfan sulfate. Leaves from the tobacco plants were analyzed for endosulfan and its metabolites at the har-

* Endosulfan sulfate in endosulfan sulfate-treated tobacco leaf is not considered as a metabolite.

Table 1. Endosulfan I, endosulfan II and endosulfan sulfate residues in green leaves on 5th, 14th and 24th day after treatment from tobacco plants treated with 0.75 lb./acre and 1.50 lbs./acre of endosulfan I, endosulfan II and endosulfan sulfate*.

Treatment	Endosulfan I residues (in ppm)			Endosulfan II residues (in ppm)			Endosulfan sulfate residues (in ppm)		
	5th day	14th day	24th day	5th day	14th day	24th day	5th day	14th day	24th day
0.75 lb./acre endosulfan I	1.76	0.28	0.14	0.35	0.13	0.08	2.64	1.76	0.90
1.50 lbs./acre endosulfan I	10.4	0.64	0.57	0.88	0.40	0.35	2.70	1.76	1.70
0.75 lb./acre endosulfan II	0.16	0.06	traces	37.0	7.50	4.20	0.46	0.70	0.88
1.50 lbs./acre endosulfan II	0.40	0.24	0.88	70.2	37.0	13.4	0.53	0.88	0.88
0.75 lb./acre endosulfan sulfate	0.06	0.044	0.05	ND**	ND	ND	61.6	27.3	26.4
1.50 lbs./acre endosulfan sulfate	—	—	0.07	ND	ND	ND	123.2	52.8	52.8

* Calculated on the basis of 12 % moisture content.

** ND = not detected.

Table 2. Endosulfan I, endosulfan II and endosulfan sulfate residues in green leaves on 24th day after treatment and in the cured leaves of tobacco plants treated with 0.75 lb./acre and 1.50 lbs./acre of endosulfan I, endosulfan II and endosulfan sulfate, and their comparison.

Treatment	Endosulfan I residues (in ppm) in		Endosulfan II residues (in ppm) in		Endosulfan sulfate residues (in ppm) in		Total endosulfan residue in green leaf (ppm)	Total endosulfan residue in cured leaf (ppm)	Percentage loss of endosulfan during curing process
	green leaf (A)	cured leaf (R)	green leaf (B)	cured leaf (S)	green leaf (C)	cured leaf (T)	A+B+C=D	R+S+T=W	$\frac{D-W}{D} \times 100$
0.75 lb./acre endosulfan I	0.14	traces	0.08	0.46	0.90	0.70	1.12	1.16	nil
1.50 lbs./acre endosulfan I	0.57	0.12	0.35	0.80	1.70	1.20	2.62	2.12	19.1
0.75 lb./acre endosulfan II	traces	traces	4.20	1.75	0.88	0.50	5.08	2.25	53.7
1.50 lbs./acre endosulfan II	0.88	traces	13.4	4.60	0.88	1.12	15.2	5.72	62.5
0.75 lb./acre endosulfan sulfate	0.05	ND*	ND	0.54	26.4	11.4	26.5	11.9	55.1
1.50 lbs./acre endosulfan sulfate	0.07	ND	ND	0.56	52.8	22.5	52.9	23.1	54.5

* ND = not detected.

vesting stage, and later, when flue-cured. From the residue data obtained we conclude that [1] In green tobacco leaf endosulfan I, found in very small amounts, is the only metabolite of endosulfan sulfate, and that endosulfan II is the main endosulfan sulfate metabolite in flue-cured tobacco leaf; [2] The loss of residues during the curing process depends largely on the residue level of this pesticide in the green tobacco leaf; [3] The loss of pesticide during the curing process is mainly due to volatilization; and [4] Smallest amounts of residues are left in tobacco leaf with endosulfan I treatments.

ZUSAMMENFASSUNG

Tabakpflanzen wurden jeweils gesondert mit 0.75 lb./acre (841 g/ha) und 1.50 lbs./acre (1682 g/ha) Endosulfan I, Endosulfan II bzw. Endosulfansulfat behandelt. Die Blätter der Pflanzen wurden zum Zeitpunkt der Ernte und später nach der Trocknung (flue-curing) auf den Gehalt an Rückständen von Endosulfan und dessen Metaboliten untersucht. Aus den erhaltenen Rückstandswerten schließen wir: [1] Im grünen Blatt ist das in sehr kleinen Mengen gefundene Endosulfan I das einzige Abbauprodukt von Endosulfansulfat; im getrockneten Blatt ist Endosulfan II das hauptsächliche Abbauprodukt von Endosulfansulfat. [2] Die Verminderung der Rückstandsmengen während des Trocknungsvorganges hängt in hohem Maße von der im grünen Tabakblatt befindlichen Menge des jeweiligen Pestizids ab. [3] Die Verringerung des Pestizidgehaltes während der Trocknung ist vor allem auf Verdampfung zurückzuführen. [4] Die geringsten Rückstandsmengen finden sich im Tabakblatt, das mit Endosulfan I behandelt wurde.

RÉSUMÉ

On a traité séparément des plantes de tabac avec 0.75 lb./acre et 1.50 lbs./acre d'endosulfan I, d'endosulfan II et de sulfate d'endosulfan. Les feuilles de ces plantes de tabac ont été analysées en ce qui concerne l'endosulfan et ses métabolites, au moment de la moisson et plus tard lorsqu'elles sont «flue-cured». Des données provenant des résidus, nous pouvons conclure [1] que l'endosulfan I, trouvé en quantité très minime, est le seul métabolite du sulfate d'endosulfan dans la feuille verte, et que l'endosulfan II est le métabolite principal du sulfate d'endosulfan dans la feuille de tabac «flue-cured»,

[2] que la perte de résidus durant le processus de séchage dépend à un haut degré du niveau de résidus de ce pesticide dans la feuille verte du tabac, [3] que la perte de pesticide durant le processus de séchage est due principalement à la volatilisation et [4] qu'il reste les plus petites quantités de résidus dans la feuille de tabac lorsqu'elle a subi des traitements à l'endosulfan I.

REFERENCES

1. Cheng, A. L. S., and G. L. Steffens: *Tob. Sci.* 20 (1976) 90.
2. Chopra, N. M., and A. M. Mahfouz: *J. Agric. Food Chem.* 25 (1977) 32.
3. Corbaz, R., A. Artho, P. Ceschini, M. Häusermann and J. Cl. Plantefève: *Beitr. Tabakforsch.* 5 (1969) 80.
4. Domanski, J. J., and T. J. Sheets: *Tob. Sci.* 17 (1973) 55.
5. Dorrough, H. W., G. A. Jones and C. I. Lusk: *Environ. Entomol.* 2 (1973) 845.
6. Fromageot, P., and H. Perez-Milan: *Biochem. Biophys. Acta* 32 (1959) 457.
7. Guthrie, F. E., and T. G. Bowery: *J. Econ. Entomol.* 55 (1962) 1017.
8. Neesemann, E., and F. Seehofer: *Beitr. Tabakforsch.* 5 (1970) 207.
9. Tappan, W. B., C. H. van Middelem and H. A. Moye: *J. Econ. Entomol.* 60 (1967) 765.

Acknowledgements

The authors wish to thank the personnel of the School of Agriculture and Life Sciences, North Carolina State University, Raleigh, for their assistance in growing tobacco, spraying the plants with pesticide formulations, and flue-curing the harvested leaves; FMC Corporation, Niagara Falls, for kindly supplying us with pure endosulfan I, endosulfan II, endosulfan sulfate, endosulfan diol and endosulfan ether; Farbwerke Hoechst AG, Frankfurt, W. Germany, for the gift of pure endosulfan ether, endosulfan hydroxyether and endosulfan lactone; Finnigan Corporation, Sunnyvale, California, for carrying out GC/MS analyses; and CSRS-USDA for a research grant (No. NCX-PR-0002-309-108).

Authors' address:

Tobacco and Pesticide Research, North Carolina Agricultural and Technical State University, Greensboro, N. C., 27411, U.S.A.