CHLORINATED INSECTICIDE RESIDUES IN THE UNIVERSITY OF KENTUCKY REFERENCE AND ALKALOID SERIES CIGARETTES

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The chlorinated hydrocarbon insecticide content of the Research and Reference cigarettes produced by the Tobacco and Health Research Institute, University of Kentucky, has been examined. Total chlorinated insecticide residues on the cigarettes manufactured in 1969 ranged from 12.5 to 64.3 ppm with the 1R1 reference cigarette exhibiting the highest level. DDT-TDE residues accounted for >97% of the total content in this series. The remainder was composed of dieldrin and endrin residues. No endosulfan insecticide was detected. For the reference and research cigarettes manufactured in 1974, levels of chlorinated insecticide residues ranged from 3.6 to 7.1 ppm. Again, DDT-TDE accounted for the majority (62 to 77%) of the residues, with the balance composed of endosulfan. Smoke condensate derived from the 1R1 cigarette under standard smoking conditions contained an average of 8.3 μg of DDT-TDE residues/cigarette. Approximately 9.2% of the p,p'-DDT was transferred intact to the mainstream smoke.

INTRODUCTION

Until recently one of the major obstacles in determining the relationship of smoking to human health was the lack of a standardized cigarette. In 1969, the Tobacco and Health Research Institute embarked upon a program to produce for scientists in the tobacco and health area reference and research cigarettes for use in their investigations. The first reference cigarettes, coded 1R1, were produced in June 1969 by a commercial tobacco company using tobacco contained in its inventory (2, 14). The tobacco composition of these 85 mm cigarettes was designed to reflect the blend of an "average" domestic unfiltered cigarette. Production of the reference cigarette was repeated in March 1974, using recently produced tobacco and labeled 2R1. In addition, two other types of reference cigarettes were produced, coded 1R2 and 1R3, which consisted basically of the 2R1 blend plus a designated quantity of reconstituted tobacco.

An "alkaloid series" of cigarettes, coded A, also was produced in 1969. These research cigarettes contained blends of low-nicotine burley and flue-cured tobaccos designed to deliver varying levels of nicotine to the mainstream smoke. Cigarettes coded 1A1 and 1A2 consisted entirely of experimental tobacco. The 1A3 and 1A4 cigarettes contained the commercial flue-cured tobacco used in the 1R1 cigarettes, in addition to the low-nicotine burley strain. The 1A1 alkaloid series cigarettes were prepared again in 1974 and designated as 2A1 cigarettes. The reference and research cigarettes have been well characterized with respect to natural leaf components and mainstream smoke composition (4).

The intent of the program was the production, by a fixed manufacturing process, of reference and research cigarettes containing standardized blends from known sources of tobacco. However, no consideration was given specifically to the pesticide content of these tobaccos. Probably, this would be a rather constant variable because of the other commonalities attributed to the experimental cigarettes.

The rationale for using reference cigarettes was to relate specific biological responses to smoking and to attempt to correlate these responses with naturally occurring components of tobacco and or tobacco smoke. Should foreign toxicants such as pesticides exist on tobacco and in the smoke, any adverse response attributed to smoking might actually be caused, at least in part, by the foreign material. Naturally, the identity of the toxic agent is vitally important in the production of a safe cigarette. There is no doubt that the feasibility of removing, or reducing to a safe level, pesticide residues from tobacco is much greater than accomplishing the same with natural components of tobacco. In addition, it is well known that certain xenobiotics added to cigarette tobacco prior to smoking have a significant effect upon the composition of the smoke phase (8). However, the influence of pesticide compounds on the composition of smoke has not been examined.

It has been well documented that cigarettes and other tobacco products contain insecticide residues (10, 11). Most of these residues consist of the chlorinated insecticides, particularly DDT and more recently endosulfan. Moreover, appreciable quantities of these insecticide residues are transferred to the mainstream smoke (1, 5, 15). These studies suggest that residues of the chlorinated insecticides would be present on experimental cigarettes and in mainstream smoke from them. The present study was conducted to determine the nature and levels of chlorinated insecticides in the reference and research cigarettes and in 1R1 smoke condensate. The actual health significance of these residues, if any, must await evaluations conducted with pesticide-free tobacco products.

METHODS

Cigarette Tobacco

The research cigarettes 1A1, 1A2, 1A3, 1A4, 1R1, 1R2, 2A1, and 2R1 were obtained from the cold storage facilities of the Tobacco and Health Research Institute, University of Kentucky, Lexington, Kentucky. The tobacco from a single carton (200 cigarettes) was separated from the paper and thoroughly mixed. Three 10 g subsamples were removed for analysis of the chlorinated hydrocarbons.

Tobacco samples were submitted to Soxhlet extraction with 9:1 chloroform: methanol (7). Cleanup of the extracts for DDT-TDE, endrin, dieldrin, and endosulfan

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Table I. Chlorinated Insecticide Residues in Reference and Alkaloid Series Cigarettes Available from the Tobacco and Health Research Institute

	Ppm in cigarettes[a]/year produced and Institute code								
	1969					1974			
Insecticides	1A1	1A2	1A3	1A4	1R1	2R1	2A1	1R2	1R3
DDT-TDE's									
p,p'-DDT	3.3	4.7	12.7	16.4	18.0	2.4	1.0	1.4	1.5
p,p'-TDE	6.4	10.1	28.0	27.3	34.5	1.9	1.0	1.2	.6
others[b]	2.5	5.3	9.7	13.2	10.7	1.2	.7	.9	.5
Total	12.2	20.1	50.4	56.9	63.2	5.5	2.7	3.5	2.6
Endosulfan									
I	0	0	0	0	0	0	0	0	0
II	0	0	0	0	0	.7	.4	.6	.4
Sulfate	0	0	0	0	0	.7	1.1	.6	.4
Total	00	0	0	0	0	1.4	1.5	1.2	.8
Dieldrin	.2	.3	.3	.3	.6	.2	.1	.1	.1
Endrin	.1	.3	.5	.2	.5	0	0	.1	0
Total	12.5 ±0.1	20.7 ±0.7	51.2 ±1.9	57.4 ±2.7	64.3 ±0.4	7.1 ±0.1	4.3 ±0.1	4.9 ±0.3	3.5 ±0.1

[a] *Zero indicates residues of less than 0.05 ppm. Calculations based on 12% moisture.*
[b] *Includes p,p'-DDE; o,p'-DDT; o,p'-TDE; p,p'-TDEE.*

Table II. Transfer of DDT-TDE Residues from 1R1 Reference Cigarettes into Mainstream Smoke

Residue	Tobacco (A)[a]	Smoke (B)[b]	B/A
p,p'-DDT	13.0	1.2	.092
p,p'-TDE	24.8	2.5	.10
p,p'-DDE	.7	.2	.29
p,p'-TDEE	.6	3.2	5.3
o,p'-DDT	1.9	.2	.10
o,p'-TDE	4.5	1.0	.22
DCS	–	.3	–
Total	45.5 ± .40	8.6 ± .59	.19

[a] *Average weight of the smoked tobacco at 12% moisture was 724 mg.*
[b] *Average weight of dried smoke condensate per cigarette was 32.3 mg.*

I analyses required an activated florisil column using 6% diethyl ether-hexane (200 ml) and 15% diethyl ether-hexane as eluents. A 2% deactivated florisil column eluted with hexane (200 ml) followed by 30% methylene chloride-hexane (300 ml) was employed for sample cleanup prior to endosulfan II and endosulfan sulfate analyses (11, 13).

Resolution and quantitation of the insecticide residues were performed on Varian Aerograph 1700 instruments equipped with tritium electron capture detectors. The analyses of DDT-TDE, endrin, dieldrin, and endosulfan I residues were conducted on a 180 cm × 2 mm glass column containing a 1.5% OV-17/2% SP-2401 mixed liquid phase on 100/120 mesh chromosorb W HP. Column, injector and detector temperatures were maintained at 210°C, 220°, 220° respectively. The nitrogen carrier gas flow was 44 ml/min. A 180 cm × 2 mm glass column packed with 5% OV-101 on 100-120 mesh Gas Chrom Q was used for endosulfan II and endosulfan sulfate analyses. Instrument parameters were maintained as described above.

DDT-TDE residues were confirmed by co-chromatography on an OV-210 volumn and by alkaline dehydrochlorination (21) and oxidation to dichlorobenzophenone isomers (20). Dieldrin, endrin and endosulfan residues were confirmed by co-chromatography on OV-210 and DC-200 columns. In addition, the presence of dieldrin was confirmed by extraction p values in two solvents systems (6). Recoveries of chlorinated hydrocarbons from fortified tobacco at the 0.5 and 1.5 ppm levels generally exceeded 90%.

Smoke Condensate

Two hundred and thirty-five 1R1 cigarettes, previously conditioned at 75°F and 60% relative humidity, were smoked to a 30 mm butt length under standard conditions (35 ml puff of 2-second duration at 60 second intervals) on a modified M. Borgwaldt apparatus (4). Whole mainstream smoke was collected in a cold trap (12) and dried to yield 7.59 g of smoke condensate. The samples were frozen until analyzed.

Approximately 200–400 mg of condensate, corresponding to 6–12 cigarettes, was dissolved in 100 ml of warm chloroform and partitioned once with 100 ml of 2% aqueous sodium chloride. The aqueous phase was separated and washed twice with 50 ml of chloroform. The organic extracts were combined, dried with anhydrous sodium sulfate and concentrated to 4 ml. Sample cleanup and gas chromatographic analysis were conducted as described for cigarette tobacco. Residues identified as DDT-TDE components were confirmed by dehydrohalogenation and oxidation as described above, followed by co-chromatography with dichlorobenzophenone. Because the SP 2401/0V-17 column was unable to separate trans-*p,p'*-dichlorostilbene (DCS) and DDE, the quantitation of DCS was conducted on a 180 cm column containing 3% SE-30/chromosorb W with the first 12 cm packed with 12% SE-30/chromosorb W support. Oven temperature was 187°C and nitrogen flow rate was 25 ml/min. Control experiments designed to establish recoveries of DDT, TDE and DCS in the analytical method were accomplished by fortifying pesticide-free smoke condensate with the chlorinated hydrocarbons at the 30 ppm level. Recoveries exceeded 95%. The smoke condensate was obtained from cigarettes made from burley tobacco shown to be essentially free of the insecticide (<.2 ppm). The stabilities of DDT and TDE in the analytical procedure were confirmed.

The trans-*p,p'*-dichlorostilbene was synthesized by the acid catalyzed dehydration of 1,2-di-*p*-chlorophenyl-1-ethanol (19). Thus, 53 g (0.2 M) of the crude alcohol was dissolved in 600 ml of dry benzene containing 3 g of *p*-toluene sulfonic acid. The reaction was refluxed until about 3.6 ml of water (0.2 M) had been collected by azotropic distillation. The solution was cooled, diluted with 100 ml of ethyl acetate, washed with 20% potassium carbonate and dried. Concentration of the yellow solution to half volume led to the crystallization of 18 g of pure product (m.p. 176–178°C). The reported melting point was 175–176°C (19). Further concentration of the solution gave an additional 26 g of the dichlorostilbene. The yield was 88%.

RESULTS

Results of the insecticide analyses of the research cigarettes are summarized in **Table I**. The 1R1 cigarettes contained 64.3 ppm of total chlorinated insecticide of which greater than 98% were DDT-TDE residues; the remainder (< 2%) was composed of approximately equal amounts of endrin and dieldrin. Total chlorinated insecticide residues on the other 1969 samples ranged from 12.5 to 57.4 ppm, with > 97% being DDT-TDE. No endosulfan was detected in any of the 1969 research cigarettes.

Total chlorinated residues on the 2R1 cigarettes were 7.1 ppm; 5.4 ppm (77%) was DDT-TDE, 1.4 ppm (20%) endosulfan and 0.2 ppm (3%) was dieldrin. No endrin was detected. Residue levels of these compounds on other 1974 research cigarettes ranged from 3.6 to 4.9 ppm and exhibited a similar distribution of individual insecticides.

Whole smoke condensate contained 18% of the same chlorinated insecticide residues contained in the burned portion of the 1R1 cigarettes (**Table II**). Approximately 9.2% and 10.0% of DDT and TDE residues, respectively, were transferred intact to the mainstream smoke.

An average of 3.2 and 0.3 micrograms of *p,p'*-TDEE (TDE-HCL) and DCS respectively were found in the smoke condensate from one 1R1 cigarette. Trace amounts of *p,p'*-dichlorobenzophenone were also detected.

DISCUSSION

The 1R1 and 2R1 cigarettes were produced from the tobacco inventories of the manufacturer. Consequently, the level and distribution of residual insecticides on these samples were expected to reflect those of the commercial cigarettes made during the same periods. The ban on the use of DDT-TDE on tobacco was initiated in late 1969 and early 1970. But, tobacco is normally stored for at least two years before manufacture into smoking materials; and a general decrease in levels of these insecticides on commercial cigarettes was not expected until 1972. The average DDT-TDE contents of cigarettes purchased from 1966 to 1971 was 40 ppm, with an exhibited range of 20 to 56 ppm depending on brand (10, 11). In 1972, the level had decreased to 27 ppm and in 1974, to 7.8 ppm (22). Before 1971, DDT-TDE residues constituted more than 98% of the total chlorinated insecticides found on cigarettes. Since then, this value has gradually declined to 88% with endosulfan constituting most of the remaining residues. Similar trends were found for the 1R1 and 2R1 reference cigarettes.

The relatively low level of residual insecticide found on 1A1 and 1A2 research cigarettes produced in 1969 likely resulted from the carefully controlled conditions under which the special strains of the flue-cured and burley tobaccos were grown (14). On the other hand, the source of the higher residue levels of DDT-TDE found on the 1A3 and 1A4 cigarettes (49.7 and 56.1 ppm, respectively) was probably the flue-cured tobacco which was produced using standard agricultural practices.

Residues of endosulfan were not detected on cigarettes purchased before 1971. However, these residues averaged 0.2 ppm in 1971 (10), 0.4 ppm in 1972 and 0.8 to 1.0 ppm from 1973 to 1975 (11, 22). Depending on brand, the insecticide comprised 6 to 20% of the total chlorinated insecticide residues in 1973 and 5 to 13% of those on cigarettes in 1974. This presumably reflects its position as a DDT substitute. Endosulfan was not detected in 1969 research cigarettes. However, this compound was found on all 1974 research cigarettes and constituted 20 to 38% of the chlorinated residues. Dieldrin levels of 0.1 and 0.2 ppm in 1974 research cigarettes are similar to the amounts detected in 1973 and 1974 commercial cigarettes (22). Thus, the chlorinated insecticide content of the reference and research cigarettes closely reflects the levels and trends of these insecticides on commercial cigarettes.

The transfer of DDT-TDE residues to mainstream smoke has been the subject of several investigations. Generally, 12 to 18% of the *p,p'*-DDT and *p,p'*-TDE residues on cigarettes are transferred intact to the mainstream smoke (1, 5, 15, 16). In addition, *p,p'*-TDEE has been found at levels amounting to 13 to 46% of the identified chlorinated hydrocarbon residues in smoke condensate. While a portion of the *p,p'*-TDEE in the smoke condensate probably comes from the transfer of intact compound, dehydrochlorination of *p,p'*-TDE during the smoking process is likely the major pathway for its formation (9). Previously, it has been observed that 18% of the *p,p'*-TDE present on the burned tobacco was transferred intact to the smoke phase (5, 15). However, it should be noted that the benzene soxhlet (15) and hexane (5) extraction techniques used by these authors were less efficient than the chloroform-methanol method in removing total DDT-TDE residues from tobacco (21). Correcting the cigarette residue data of Hoffmann and Rathkamp (15) for the increased efficiency of the chloroform-methanol extraction, the corrected percentage transfer of the intact *p,p'*-TDE and *p,p'*-DDT to the mainstream smoke is 9.0% and 13.7%, respectively, in close agreement with our results.

Other DDT-TDE pyrolysis products detected in the smoke condensate included trans-*p,p'*-dichlorostilbene and *p,p'*-dichlorobenzophenone. Both compounds have been produced during pyrolyses of DDT (9), while the former had been observed in the pyrolyzate of TDE (16) and in the smoke condensate of commercial cigarettes (15). Dieldrin and endrin residues were not detected in the smoke condensate.

The carcinogenic activity of *p,p'*-DDT, *p,p'*-TDE, *p,p'*-TDEE, *o,p'*-DDT, *o,p'*-TDE, and trans-*p,p'*-dichlostilbene has been summarized (17). With the possible exception of the latter two compounds, these substances are noncarcinogens. *o,p'*-TDE may possess a tumor initiating ability (18) while dichlorostilbene may be a tumor accelerator (17).

From this study, as well as others, it may be assumed that 15 to 30% of the DDT-TDE residues present on the tobacco will be carried over to the mainstream smoke. Accordingly, the chlorinated hydrocarbon content of smoke condensate from cigarettes made in 1974 will be significantly lower than those from the cigarettes manufactured in 1969.

In view of the lack of information concerning the possible effects of pesticide residues upon biological activity of smoke condensate, this variable content of chlorinated hydrocarbons should be kept in mind when evaluating the effects of various smoke condensates.

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LITERATURE CITED

1. Atallah, Y. H. and H. W. Dorough. 1975. Insecticide residues in cigarette smoke: Transfer and fate in rats. **J. Agr. Food Chem.** *23:* 64–71.

2. Atkinson, W. O. 1970. Production of sample cigarettes for tobacco and health research. Proceedings of the University of Kentucky Tobacco and Health Research Institute. *2:* 28–30.

3. Benner, J. F. 1970. Tentative summary of leaf and smoke analysis of the University of Kentucky reference and alkaloid series cigarettes. *Ibid. 2:* 30–34.

4. Benner, J. F. and C. K. Keene. 1972. Condensate preparation and fractionation. *Ibid. 3:* 185–190.

5. Bowery, T. G., W. R. Evans, F. E. Guthrie and R. L. Rabb. 1959. Insecticide residues on tobacco. **J. Agr. Food Chem.** *7:* 693–702.

6. Bowman, M. C. and M. Beroza. 1965. Extraction p-values of pesticides and related compounds in six binary solvent systems. **J. Assoc. Anal. Chem.** *48:* 943–52.

7. Bowman, M. C., M. Beroza and C. R. Gentry. 1969. GLC determination of residues of disulfoton, oxydemetonmethyl and their metabolites in tobacco plants. *Ibid. 52:* 157–62.

8. Burton, H. R. and J. F. Benner. 1972. Investigations on the effect of chemical modifiers on tobacco and tobacco smoke. Proceedings of the University of Kentucky Tobacco and Health Research Institute. *3:* 341–351.

9. Chopra, N. M. and J. T. Thekkekandam. 1973. A mechanistic study on the formation of the non-volatile degradation products of *p,p'*-DDT and *p-p'*-TDE in *p,p'*-DDT- and *p,p'*-TDE-treated tobacco smoke. **Beitr. Tabakforsch.** *7:* 88–92.

10. Domanski, J. T., J. W. Laws, P. L. Haire, and T. J. Sheets. 1973. Insecticide residues on 1971 U. S. tobacco products. **Tob. Sci.** *17:* 80–81.
11. Dorough, H. W. and J. R. Gibson. 1972. Chlorinated insecticide residues in cigarettes purchased 1970–1972. **Environ. Entomol.** *1(6):* 734–43.
12. Elmenhorst, H. 1965. Eine neue Kältefalle zur Gewinnung grosser Mengen von Tabakrauchkondensat. **Beitr. Tabakforsch.** *3:* 101–107.
13. Gibson, J. R., G. A. Jones, W. H. Dorough, C. I. Lusk, and R. Thurston. 1974. Chlorinated insecticide residues in Kentucky burley tobacco: crop years 1963–1972. **Pest. Monitor. J.** *7:* 205–213.
14. Griffith, R. O., J. Calvert, and W. O. Atkinson. 1972. The production of large samples for tobacco and health research. Proceedings of the University of Kentucky Tobacco and Health Research Institute. *3:* 337–383.
15. Hoffmann, D. and Rathkamp. 1968. Chemical studies on tobacco smoke. **Beitr. Tabakforsch.** *4:* 201–214.
16. Hoffmann, D., G. Rathkamp, and E. L. Wynder. 1969. Chemical studies on tobacco smoke. *Ibid. 5:* 140–148.
17. Hoffmann, D. and E. L. Wynder. 1971. A study of tobacco carcinogenesis. **Cancer** *27:* 848.
18. Lacassagne, A. and L. Hurst. 1965. Experimental tumors of the interstitial gland of the rat testical as an oncogenous manifestation of *o,p'*-TDE. **Bull. Assoc. Franc. Etude Cancer** *52:* 89.
19. Lutz, R. E. and R. S. Murphy. 1949. Substituted amino ketones and alcohols related to 4,4'-dichlorobenzoin. **J. Am. Chem. Soc.** *71:* 478.
20. Miles, J. R. W. 1972. Conversion of DDT and its metabolites to dichlorobenzophenones for analysis in the presence of polychlorinated biphenyls. **J. Assoc. Anal. Chem.** *3:* 1039–1041.
21. Skrentry, R. F. and H. W. Dorough. 1971. Efficiencies of several extracting procedures in removing organochlorine insecticides from tobacco. **Tob. Sci.** *15:* 111–113.
22. Thorstenson, J. H. and H. W. Dorough. 1975. Unpublished results.