Chemical Studies on Tobacco Smoke

IX: Quantitative Analysis of Chlorinated Hydrocarbon Insecticides*

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The carcinogenic, neutral subfraction BI of cigarette smoke condensate amounts to about 0.6% of the dry particulate matter and contains most of the polynuclear aromatic hydrocarbons as well as several other weakly polaric smoke constituents (8, 9, 15; Figs. 1 and 2). In

order to substantiate results from bioassays with this smoke condensate fraction we attempt to identify and quantitatively determine its chemical constituents. So far, we have quantitatively analyzed fraction BI for PAH (7), indoles (6), carbazoles (5), and chlorinated

Figure 1

Fractionation of cigarette smoke condensate

C = Relative carcinogenic activity
P = Relative tumor promoting activity

* Presented at the CORESTA Symposium on Products for Tobacco Treatment, Stockholm, September 1968. This study was supported by American Cancer Society Grant E-231 and in part by NCI Grant CA-08748.

Figure 2 Fractionation of cigarette smoke condensate fraction B

Figure 3 Chemical constituents of tobacco smoke condensate fraction BI

Group of constituents	Number of compounds		Percent of fraction BI
	Non-carcinogen	Carcinogen	
I Polynuclear aromatic hydrocarbons			
Naphthalenes	5		2.07
Anthracenes, phenanthrenes, fluorene	7		0.46
Four-ring hydrocarbons	7	3	0.21
Five-ring hydrocarbons	4	5	0.036
Six-ring hydrocarbons	2	3	0.007
II N-Heterocyclics			2.8
Indole + derivatives	6		10.8
Carbazole + derivatives	5		0.50
9,9-Dimethylacridane			0.12
III Insecticides			
2,2-Bis (p-chlorophenyl) 1,1-dichloroethane (DDD)			0.54
2,2-Bis (p-chlorophenyl) 1-chloroethylene (DDM)			0.03
4,4'-Dichlorostilbene (DCS)			0.51
2,2-Bis (p-chlorophenyl) 1,1,1-trichloroethane (DDT)			0.13
2, (o-chlorophenyl), 2 (p'-chlorophenyl) 1,1-dichloroethane (o, p'-DDD)			Trace
Groups I-III 51 components			18.4
IV Esters			
Di-isooctyl and n-alkylphthalates			
Saturated and unsaturated C ₁₂ -C ₂₈			
Fatty acid esters of solanesol, phytol, and long-chain alcohols			
V Paraffins C ₁₈ -C ₃₃			Below 1.0
VI Olefins			
VII Terpenes			
VIII Cyclic and acyclic carbonyl components			
IX Quinones			

insecticides with some of their combustion products (11). Other constituents remain to be individually identified (Fig. 3)*. The presence of chlorinated hydrocarbon insecticides in fraction BI stimulated our interest in tobacco pesticides and in their fate during the burning of tobacco.

Extraction of Insecticides from Tobacco

One of the most widely applied methods of extraction of chlorinated insecticides from tobacco is the one by Bowery et al. (1), which uses cold n-hexane as solvent. However, we found that with this technique only 70-85% of the chlorinated insecticides are extracted from tobacco, compared with complete extraction in 24 hours with benzene (5) or with acetonitrile-water (15; Fig. 4).

The n-hexane extraction method appears to be based on the assumption that the sprayed insecticides remain on or in the waxy layer of the cuticle and do not diffuse into the deeper layers of the leaf, or the midribs. In this case, cold n-hexane extraction would suffice to dissolve the chlorinated insecticides. It needed to be determined to what extent chlorinated insecticides are resorbed by the tobacco plant. In cooperation with Dr. G. L. Steffens from the U. S. Department of Agriculture in Beltsville, Maryland, we investigated this question. Single middle leaves of a flue-cured type of a tobacco plant (N. C. -95) were sprayed with 2 concentrations of C¹⁴-labelled DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)-C¹⁴[U])ethane; spec. activity 8.3 mc/mM) while the rest of the plant was shielded. Two weeks after the single application the plant was cut and each leaf and two stem sections were individually extracted with benzene. The relative concentrations of DDT-C¹⁴ on the leaves are shown on a slide (Fig. 5). Cold n-hexane extraction of a sprayed leaf yielded only 90.9%. Based on these data we may assume that some of the DDT diffuses throughout the plant. Therefore, the use of cold n-hexane does not suffice for the quantitative extraction of chlorinated tobacco insecticides. This observation is of importance not only for the analyses

Figure 4 Extraction of chlorinated hydrocarbon insecticides from tobacco

* Since the presentation of this paper we identified in BI six N-alkylcarbazoles (0.08 %), eight N-alkylindoles (0.45 %) and five dibenzofuranes (0.2 %).

Figure 5 Relative β -activity on tobacco leaves
(Two weeks after application of DDT- C^{14} on the middle leaf; rel. act. 100.0)

Figure 6 A: Separation scheme for chlorinated insecticides from cigarette smoke

Figure 7 B: Column chromatography separation

Column Chromatography
on Alumina (rel. act. II)
(4,000 g)

1.000 ml N-Hexane	I	788.4 mg	No Cl-insecticide
200 ml N-Hexane	II	56.3 mg	DCS
200 ml N-Hexane	III	60.1 mg	DCS + o, p-DDT + DDM
500 ml N-Hexane	IV	59.6 mg	DDM + o, p-DDT
630 ml Hex. + Benzene (4:1)	V	56.1 mg	DDT + DDM
500 ml Hex. + Benzene (3:1)	VI	46.1 mg	DDD + o, p-DDD
50 ml Hex. + Benzene (2:1)	VII	8.4 mg	DDD
50 ml Hex. + Benzene (2:1)	VIII	6.9 mg	DDD + m, p-DDD
200 ml Hex. + Benzene (2:1)	IX	15.5 mg	Trace DDD
400 ml Acetone	X	3,210 mg	No Cl-insecticide

but may have a bearing on the balance of the thermic decomposition products of insecticides in tobacco smoke.

Enrichment of Chlorinated Insecticides

Cigarettes are smoked under standard conditions to 23 mm butt length (6, 20). The smoke is drawn through a trap immersed in dry ice-acetone and through a gas wash bottle with distilled water. The trapped particulate matter, or the benzene extract of the tobacco is consecutively distributed between three pairs of solvents, (a) cyclohexane and methanol-water (4:1); (b) n-hexane and acetonitrile, and (c) n-hexane with acetonitrile-water (4:1). These three distributions concentrate the chlorinated hydrocarbon insecticides 12–15 fold. In the next step the insecticides are further enriched by column chromatography on alumina.

Identification of Chlorinated Hydrocarbons

For the identification we enriched the chlorinated hydrocarbons from large samples of tobacco extracts or from cigarette smoke condensate (Figs. 6 and 7). The column endfractions were separated at 220° C on 2 m all-glass columns filled with 7.5% Silicon QF-1 and 5% DC 200 on Gas Chrom Q (Mesh 80–100). The gas chromatograph had a flame ionization detector and a glass splitter in the ratio of 4:1. The 80% portions of those components which gave significant maxima were collected in glass capillary tubes at the end of the splitter.

Figure 8 Mass spectra of DDM

In this manner we obtained material to be identified by additional physicochemical parameters. This is essential for our studies since we do not accept retention time and volume as the sole means of identification.

Mass spectrometry is most useful for the characterization of chlorinated insecticides since Cl occurs in nature in an isotope distribution from Cl^{35} to Cl^{37} of about 3:1. This is reflected in the mass spectra by the occurrence of $n+1$ parent ions for components with n -chloratoms as well as by the occurrence of $n+1$ fragment ions for fragments with n -chloratoms. Furthermore, the isotopic ions have a specific ratio to each other (Fig. 8). In cigarette smoke we were able to identify the following components by gas chromatography in two systems (flame ionization detector and electron capture detector) and by mass spectrometry (Fig. 9): 1-chloro-2,2-bis(p-chlorophenyl)ethylene [DDM,

Figure 10 Mass spectra of DCS and DDE

Figure 9 Gas chromatogram of concentrate of insecticides from cigarette smoke

Column: 2 m; 6 mm (glass)
Phase: 7.5 % Silicon QF-1 + 5 %
Temperature: 220°C

Carrier gas: Helium
Detector: Flame ionization

also DDD-HCl]; trans-4,4'-dichlorostilbene [DCS]; 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene [DDE, also DDT-HCl]; 1,1-dichloro-2-(o-chlorophenyl)-2-(p'-chlorophenyl)ethane [o,p'-DDD]; 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p'-chlorophenyl)ethane [o,p'-DDT]; 1,1-dichloro-2-(m-chlorophenyl)-2-(p'-chlorophenyl)ethane [m,p'-DDD]; 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane [DDD, also TDE] and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane [DDT]. Despite repeated, considerable efforts we failed to detect endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene) in cigarette smoke as well as in the tobacco of a blended U.S. cigarette purchased in the winter months 1967/1968.

In 1964 the U.S. Department of Agriculture recommended the withdrawal of endrin as tobacco insecticide (18), thus our finding appears plausible although it is not in accord with two recent studies (15, 17), in which this insecticide was found. In these investigations traces of endrin were determined with tests that lack the specificity of mass spectrometry. Endrin has 6 chloratoms and, therefore, 7 parent ions, with m/e 378, 380, 382, 384, 386, 388 and 390, and has 6 fragmentation-ions

Figure 11 Ultraviolet-absorption spectra
 I Trans-4,4'-dichlorostilbene [DCS]
 II 1,1-Dichloro-2,2-bis (p-chlorophenyl)ethylene [DDE]
 Solvent: Cyclohexane

Figure 12 Gas chromatogram of concentrate of insecticides from cigarette smoke

Column: 6.0 m, 3 mm (glass)
 Phase: 7.5 % silicon QF-1 + 5 %
 Temperature: 230° C
 Carrier Gas: N₂
 Detector: Flame ionization

Figure 14 Gas chromatogram of a concentrate of chlorinated hydrocarbon insecticides from cigarette smoke

Column: 1.7 m, 3 mm (glass)
 Phase: 7.5 % silicon QF-1 + 5 % DC-200 on Gas Chrom Q 80/100
 Temperature: 220° C
 Carrier gas: N₂
 Detector: Electron capture

Figure 15 Gas chromatogram of concentrate of „aged“ cigarette smoke condensate

(Experimental conditions – see legend to Figure 14)

Figure 13 Possible pathway for the pyrosynthesis of trans-4,4'-dichlorostilbene [DCS] from DDT and DDD

Table 1 Chlorinated insecticides in cigarette mainstream smoke*

	I μg/Cig.	II μg/Cig.	III μg/Cig.	IV μg/Cig.	V μg/Cig.	Average m	Standard deviation	Deviation coefficient
DDM	0.76	0.84	0.82	0.82	0.80	0.81	0.034	4.2 %
DCS**	1.76	1.73	1.78	1.67	1.71	1.73	0.043	2.5 %
o,p'-DDD	0.40	0.42	0.47	0.46	0.50	0.45	0.040	8.8 %
o,p'-DDT	0.68	0.72	0.69	0.67	0.73	0.70	0.026	3.7 %
DDD	1.70	1.72	1.78	1.73	1.85	1.75	0.060	3.4 %
DDT	0.77	0.84	0.71	0.76	0.79	0.77	0.048	6.2 %

* For each determination - 50 cigarettes were smoked under standard conditions (6).

** 21 % of the trans-4,4'-dichlorostilbene peak is made up by DDE.

with m/e 343 and higher. Mass spectrometry is highly specific for endrin and thus offers more accurate identification of the insecticide.

Trans-4,4'-dichlorostilbene and DDE were determined together by mass-spectra (Fig. 10). Ultraviolet absorption spectra allow the determination of trans-4,4'-dichlorostilbene in the gas chromatographic effluent without interference from DDE (Fig. 11). The ratio of DCS to DDE was found to be 4:1 for the smoke of the tested cigarettes. Recently we were able to improve our gas chromatographic system so that we can partially separate these two chlorinated aromatic hydrocarbons (Fig. 12).

Theoretically, it appears that the thermodynamically preferred trans-4,4'-dichlorostilbene may be formed in the reducing atmosphere of a burning cigarette (21). This could occur via several intermediates, as suggested by *in vitro* tests (2, 14, 16; Fig. 13).

Quantitative Analysis

As internal standard for the quantitative analysis we employed C¹⁴-labelled DDT. Each analysis was carried

out with 2.0 g cigarette tobacco or with the mainstream smoke from 50 cigarettes. After the enrichment or "clean-up" process, we used a gas chromatograph with an electron capture detector for which tritiated titanium foil is the source (Fig. 14). The sensitivity of the instrument was better than 1 nanogram. The results for the smoke analysis are presented in Table 1. Depending on the concentration of the insecticides in the smoke, the experimental deviation coefficients for five smoke analyses ranged from 2.5 to 8.8 %. The recovery rate for DDT-C¹⁴ varied from 80-90 %.

Table 2 presents the data for the analysis of the type of tobacco smoked in the test cigarettes. The transfer rate of DDT, o,p'-DDD and DDD from cigarette tobacco into mainstream smoke varied from 12-18 % (Table 3), which is in agreement with that reported by Guthrie and Bowery (4). However, the rate is about twice as high as found by Neseemann et al. (15).

Table 2 Chlorinated insecticides in tobacco

	Per 1 g of tobacco* μg
DDM	1.1
o,p'-DDD	4.8
DDD	11.7
o,p'-DDT	3.55
DDT	7.8

* 11.2 % moisture content

Table 3 Transfer rates of insecticides into mainstream smoke

DDD	18.0 %
o,p'-DDD	11.6 %
DDT	12.4 %
DDM (DDD - HCl)	(88.3 %)*

* DDM derives mainly from the dehydro-chlorination of DDD and, only to a minor extent, from unchanged DDM already present in tobacco.

"Aging" of Tobacco Smoke Condensate

During our investigations we also analyzed "aged tar"* and found that the gas chromatogram of its insecticide concentrate shows a new maximum (Fig. 15) unless the "tar" had been stored away from light and in the cold. The mass spectrum of the new component was identical with that of trans-4,4'-dichlorostilbene. Since it is known that trans-stilbene can photoisomerize to cis-stilbene (2), we assumed that a similar photoisomerization may occur with trans-4,4'-dichlorostilbene during "aging". The cis-form of 4,4'-dichlorostilbene was indeed synthesized by exposure of the trans-form to sunlight and under our experimental conditions at 20° C, the maximal conversion rate was 48 % (5). The cis-trans isomers were separated at 32° C on a Sephadex L.H.-20 column. Cis-4,4'-dichlorostilbene was identified by elementary analysis, mass spectra and infrared analysis. When a 0.05 molar solution of trans-4,4'-dichlorostilbene was exposed to sunlight in a gas wash bottle under a constant flow of air, we obtained up to 0.5 % yield of a component identical with 3,6-dichlorophenanthrene recently synthesized by C. I. Lewis (13; Fig. 16). However, so far we did not isolate 3,6-dichlorophenanthrene from "aged" smoke condensate.

* The term "tar" is used throughout for convenience, although it is not chemically accurate in the strictest sense.

Figure 16 Photoisomerization and oxydation of *trans*-4,4'-dichlorostilbene

Pyrolysis Experiments

Currently we are analyzing the 880° C pyrolysis products of DDT and DDD. The final figure shows a summary of components which have been identified as pyrolysis products of DDD (Fig. 17). We hope that these investigations may be helpful in identifying addi-

Figure 17 Some pyrolysis products of DDD*

* Pyrolysis conditions: N₂-atmosphere; 880° C

+ Present; N. D. so far not detected

? Identified by mass spectra without reference component

		μg/cigarette	μ/g DDD pyrolyzed
DDD		1.75	31,400
DDM (DDD-HCl)		0.81	103,600
1-chloro-2,2-bis (p-chlorophenyl) ethane		N. D.	?
3,6-Dichloro-9- chloromethyl- fluorene		N. D.	?
9-Methylene- fluorene		N. D.	+
DCS (<i>Trans</i> -4,4'- dichlorostilbene)		1.52	64,500
Chlorobenzene		+	+

tional decomposition products of chlorinated tobacco insecticides in the mainstream smoke. A case in point may be the highly reactive pyrolysis product of DDD, the monomer 9-methylenefluorene (Fig. 17). This alkylating agent may react with other smoke components or may polymerize in the smoke or smoke condensate.

Discussion

Our interest in chlorinated hydrocarbon insecticides has been motivated by their presence in the tumorigenic subfraction BI of cigarette smoke condensate. At present we are testing *trans*-4,4'-dichlorostilbene for its tumorigenicity on the experimental animal (19).

Biological experiments with other major pyrolysis products of DDT and DDD are planned. It has been suggested that the liver detoxification of aromatic hydrocarbons (by hydroxylation) is inhibited by insecticides and thus the exposure of the target organs to carcinogenic hydrocarbons is increased (21). Presently, we test this concept for its applicability to the mouse skin bioassay system.

Recently Lacassagne and his co-workers reported that *o,p'*-DDD, a byproduct of the technical synthesis of DDD, is highly toxic to and can also induce tumors in the testicles of rats (11, 12). Lacassagne et al. concluded that "these results should be of interest to all investigators concerned with the degradation of the life conditions in the normal environment and the possible destruction of the animal species by the abusive use of insecticides".

SUMMARY

An analytical method was developed for the qualitative and quantitative determination of chlorinated hydrocarbon insecticides in tobacco products. It is based on three consecutive liquid-liquid distributions, followed by column chromatography on deactivated alumina. This procedure leads to a degree of enrichment which permits the direct assessment of the insecticides by gas chromatography. For the isolation and identification of the individual components the column chromatography endfractions are separated by gas chromatography and collected from the effluent of the column. These materials are used for mass spectrometric analysis. For the quantitative analysis C¹⁴-labelled DDT is employed as internal standard and the amount of insecticides is determined with the aid of a gas chromatograph with an electron capture detector with a sensitivity for chlorinated insecticides of 1 nanogram (10⁻⁹ g).

In 1.0 g cigarette tobacco were found 11.7 μg 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane [DDD]; 4.8 μg 1,1-dichloro-2-(o-chlorophenyl)-2-(p'-chlorophenyl)ethane [o,p'-DDD]; 7.8 μg 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane [DDT] and 3.6 μg of an admixture of 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p'-chlorophenyl)ethane [o,p'-DDT] and 1,1-dichloro-2-(m-chlorophenyl)-2-(p'-chlorophenyl)ethane [m,p'-DDD]. The mainstream smoke of an 85 mm U.S. blended cigarette without filter tip contained 1.75 μg DDD, 0.45 μg o,p'-DDD, 0.81 μg

DDM, 0.77 µg DDT, 0.70 µg o,p'-DDT plus m,p'-DDD, 0.21 µg 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and 0.21 µg 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene [DDE] and 1.52 µg trans-4,4'-dichlorostilbene [trans-DCS]. Endrin was neither detected in cigarette smoke nor in commercial U.S. tobacco samples purchased during the winter 1967-68. The transfer rates for unchanged chlorinated insecticides from cigarette tobacco into mainstream smoke were 18% for DDD, 11.6% for o,p'-DDD and 12.4% for DDT.

The extraction of tobacco with n-hexane does not yield a complete extraction of chlorinated insecticides. A study in which a single leaf of a tobacco plant was sprayed with C¹⁴-labelled DDT indicated that the insecticide to some degree permeates the tobacco plant by diffusion into the lower layers of the leaf.

In tobacco smoke condensate, which is exposed to sunlight or white laboratory light, one pyrolysis product of DDD and DDT, trans-4,4'-dichlorostilbene, is photoisomerized to its cis-isomer. In an *in vitro* test cis-4,4'-dichlorostilbene was found to be dehydrogenated in air up to 0.5% to 3,6-dichlorophenanthrene.

Pyrolysis experiments at 880° C with DDD and DDT have been discussed briefly. In addition to components already found in cigarette smoke, chlorobenzene, the highly reactive 9-methylenefluorene and tentatively 1-chloro-2,2-(p-chlorophenyl)ethane as well as 3,6-dichloro-9-chloromethylfluorene were identified in the pyrolyzate.

The findings of this study are compared with earlier investigations and discussed in respect to the formation of some of the chlorinated aromatic hydrocarbons. Animal studies are needed to evaluate the possible tumorigenicity of the major pyrolysis products of DDT and DDD.

ZUSAMMENFASSUNG

Eine analytische Methode zur qualitativen und quantitativen Bestimmung chlorhaltiger Insektizide in Tabakprodukten wird beschrieben. Die Methode basiert auf Verteilung des Untersuchungsmaterials zwischen drei Lösungspaaen und wird durch Säulenchromatographie an inaktiviertem Aluminiumoxid fortgeführt. Die Säulenchromatographie bewirkt genügende Anreicherung der Insektizide zur direkten gaschromatographischen Bestimmung. Die Isolierung und Identifizierung der Einzelkomponenten erfolgt durch gaschromatographische Auftrennung der Säulenfraktionen mit massenspektrographischer Auswertung der Gaschromatographiefraktionen. Zur quantitativen Bestimmung dient C¹⁴-markiertes DDT. Ein Elektronendetektor ermöglicht die gaschromatographische Bestimmung chlorhaltiger Insektizide mit einer Empfindlichkeit bis zu 10⁻⁹ g.

In einem Gramm eines Zigarettentabaks wurden die folgenden Insektizide gefunden: 11,7 µg 1,1-Dichlor-2,2-di(p-chlorophenyl)äthan [DDD]; 4,8 µg 1,1-Dichlor-2-(o-chlorophenyl)-2-(p-chlorophenyl)äthan [o,p'-DDD]; 7,8 µg 1,1,1-Trichlor-2,2-di(p-chlorophenyl)äthan [DDT] und 3,6 µg eines Gemisches von 1,1,1-Trichlor-2-(o-

chlorphenyl)-2-(p'-chlorophenyl)äthan [o,p'-DDT] mit 1,1-Dichlor-2-(m-chlorophenyl)-2-(p'-chlorophenyl)äthan [m,p'-DDD]. Der Hauptstromrauch einer 85 mm langen filterlosen Zigarette enthielt 1,75 µg DDD; 0,45 µg o,p'-DDD; 0,81 µg DDM; 0,77 µg DDT; 0,70 µg o,p'-DDT mit m,p'-DDD sowie 0,21 µg 1,1-Dichlor-2,2-di(p-chlorophenyl)äthylen [DDE] und 1,52 µg Trans-4,4'-dichlorstilben [Trans-DCS]. Endrin konnte weder im Hauptstromrauch der Zigarette noch im Tabak des Handels vom Winter 1967/68 nachgewiesen werden. Die Überführung unersetzer chlorierter Insektizide vom Tabak in den Zigarettenhauptstrom betrug 18% für DDD, 11,6% für o,p'-DDD und 12,4% für DDT.

Behandlung des Tabaks mit n-Hexan ist nicht ausreichend, um chlorierte Insektizide quantitativ zu extrahieren. Befunde nach Sprühen einzelner Tabakblätter mit C¹⁴-markiertem DDT zeigten an, daß Diffusion des Insektizides in die unteren Blattschichten zur Verteilung in andere Pflanzenteile gelangt.

Im Tabakrauchkondensat führt Belichtung durch Sonnen- oder weißes Laboratoriumslicht zur Photoisomerisation von Trans-4,4'-dichlorstilben, einem Pyrolyseprodukt des DDD und DDT, ins cis-Isomer.

In vitro Versuche zeigten, daß Cis-4,4'-dichlorstilben durch Dehydration an der Luft bis zu 0,5% in 3,6-Dichlorphenanthren übergeht.

Pyrolyse-Versuche mit DDD und DDT bei 880° C sind kurz erörtert. Außer den im Zigarettenrauch identifizierten Verbindungen enthält das Pyrolysat nachweislich das hoch reaktionsfähige 9-Methylenfluoren sowie möglicherweise 1-Chlor-2,2-(p-chlorophenyl)äthan und auch 3,6-Dichlor-9-chlormethylfluoren.

Die Ergebnisse der vorliegenden Arbeit werden mit denen vorangegangener Veröffentlichungen verglichen und in bezug auf die Bildung einiger der chlorierten aromatischen Kohlenwasserstoffe diskutiert.

Zukünftige Tierversuche müssen die Frage der karzinogenen Eigenschaften der Insektizide und der Pyrolyseprodukte aufklären.

RÉSUMÉ

Une méthode analytique a été mise au point pour la détermination qualitative et quantitative d'insecticides organo-chlorés dans des produits du tabac. L'analyse est basée sur trois distributions consécutives liquide-liquide suivies d'une chromatographie sur colonne d'alumine inactivée. Le procédé conduit à un enrichissement suffisant pour permettre la détermination directe des insecticides par chromatographie en phase gazeuse. L'isolement des composants individuels se fait par séparation des fractions finales de la chromatographie sur colonne grâce à une chromatographie en phase gazeuse. Les effluents correspondant aux pics chromatographiques sont ensuite identifiés par spectroscopie de masse. Pour les analyses quantitatives, le DDT marqué au carbone 14 a été utilisé comme standard interne et le dosage a été fait en chromatographie gazeuse avec détection par capture d'électrons (limite de sensibilité 10⁻⁹ g).

Dans 1,0 g de tabac on a trouvé 11,7 µg de 1,1-dichloro-2,2-bis(p-chlorophényl)éthane [DDD]; 4,8 µg de 1,1-dichloro-2-(o-chlorophényl)-2-(p'-chlorophényl)éthane [o,p'-DDD]; 7,8 µg de 1,1,1-trichloro-2,2-bis(p-chlorophényl)éthane [DDT] et 3,6 µg d'un mélange additionnel de 1,1,1-trichloro-2-(o-chlorophényl)-2-(p'-chlorophényl)éthane [o,p'-DDT] et 1,1-dichloro-2-(m-chlorophényl)-2-(p'-chlorophényl)éthane [m,p'-DDD]. Le courant principal de la fumée d'une cigarette en mélange américain de 85 mm, sans filtre, contenait 1,75 µg de DDD, 0,45 µg de o,p'-DDD, 0,81 µg de DDM, 0,77 µg de DDT, 0,70 µg de o,p'-DDT plus m,p'-DDD, 0,21 µg de 1,1-dichloro-2,2-bis(p-chlorophényl)éthylène [DDE] et 1,52 µg de trans-4,4'-dichlorostilbène [trans-DCS]. La présence de l'endrine n'a pu être détectée ni dans la fumée de cigarette ni dans des tabacs américains de l'hiver 1967-1968. Le taux de transfert des insecticides organo-chlorés non modifiés du tabac de cigarette dans le courant principal de la fumée était 18% pour le DDD, 11,6% pour le o,p'-DDD et 12,4% pour le DDT.

L'extraction de tabac avec le n-hexane ne permet pas l'extraction complète des insecticides organo-chlorés. Une étude d'une seule feuille de tabac arrosée de DDT marqué au carbone 14 a montré que l'insecticide pénètre dans une certaine mesure dans la plante de tabac par diffusion dans les couches inférieures de la feuille.

Si l'on expose le condensat de fumée de cigarette à la lumière du soleil ou à la lumière blanche du laboratoire, un produit de pyrolyse du DDD et du DDT, le trans-4,4'-dichlorostilbène, est photo-isomérisé à son cis-isomère. Par un test *in vitro* on a trouvé que le cis-4,4'-dichlorostilbène se déshydrogénait à l'air dans une proportion s'élevant jusqu'à 0,5% pour donner du 3,6-dichlorophénanthrène.

On a discuté succinctement des essais de pyrolyse à 880° C du DDD et du DDT. En plus des composants déjà trouvés dans la fumée de cigarette, on a identifié dans les produits de pyrolyse, le chlorobenzène, le très réactif 9-méthylène-fluorène, et expérimentalement du 1-chloro-2,2-(p-chlorophényl)éthane aussi bien que du 3,6-dichloro-9-chlorométhylfluorène.

Les résultats obtenus dans cette étude sont comparés avec ceux provenant de recherches antérieures et sont discutés sous le rapport de la formation de quelques dérivés chlorés des hydrocarbures aromatiques. Des études biologiques sont nécessaires pour évaluer le pouvoir tumorigène éventuel des principaux produits provenant de la pyrolyse du DDT et du DDD.

REFERENCES

1. Bowery, T. G., Evans, W. R., Guthrie, F. E., and Rabb, R. L.: J. Agr. Food Chem. 7 (1959) 693.

2. Brizer, E., Dallnigk, E., and Rucca, M.: Helv. Chim. Acta 41 (1958) 1390.
3. Forrest, J., Stephenson, O., and Waters, W. A.: J. Chem. Soc. 1946, 333.
4. Guthrie, F. E., and Bowery, T. G.: Residue Rev. 19 (1967) 31.
5. Hoffmann, D., and Rathkamp, G.: Beitr. Tabakforsch. 4 (1968) 201.
6. Hoffmann, D., and Rubin, J.: Beitr. Tabakforsch. 3 (1966) 409.
7. Hoffmann, D., and Wynder, E. L.: Cancer 13 (1960) 1962.
8. Hoffmann, D., and Wynder, E. L.: Proc. Am. Assoc. Cancer Res. 7 (1966) 32.
9. Hoffmann, D., and Wynder, E. L.: Monogr. Natl. Cancer Inst. 28 (1968) 151.
10. Hoffmann, D., Rathkamp, G., and Woziwodzki, H.: Beitr. Tabakforsch. 4 (1968) 253.
11. Lacassagne, A.: U. I. C. C. Monogr. 7 (1967) 241.
12. Lacassagne, A., Hurst, L., and BaGiav, N.: Compt. Rend. 266 (1968) 1532.
13. Lewis, C. I.: personal communication.
14. Li, H. Y.: J. Chem. Soc. Japan 62 (1941) 562.
15. Neesemann, E., Schröder, R., and Seehofer, F.: Beitr. Tabakforsch. 4 (1968) 182.
16. Ramart-Lucas, Mme., and Amagat, P.: Bull. Soc. Chem. 51 (1932) 108.
17. Sheets, T. J., Smith, J. W., and Jackson, M. D.: Tobacco Sci. 12 (1968) 66.
18. U. S. Department of Agriculture: Chem. Eng. News 42 (1964) 21.
19. U. S. Department of Health "Smoking and Health" Public Service Publ. No. 1103 (1964) 62 and 145.
20. Wissenschaftliche Forschungsstelle im Verband der Cigarettenindustrie: Beitr. Tabakforsch. 1 (1962) 307.
21. Wynder, E. L., and Hoffmann, D.: Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis (1967), Academic Press, New York.

Acknowledgement

We express our appreciation to Dr. G. L. Steffens for his collaboration on the study of DDT-C¹⁴ in the tobacco plant and to Dr. C. I. Lewis for supplying us with a sample of 3,6-dichlorophenanthrene.

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