Cigarette Smoke Formation Studies

VII. The Fate of (5-¹⁴C)-Methoprene In Cigarette Smoke *

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INTRODUCTION

The economic consequences of damage to tobacco products by the cigarette beetle (*Lasioderma serricorne*) and tobacco moth (*Ephestia elutella*) have long been of concern to the tobacco industry. Considerable effort has been expended to effect control of these pests at all stages of processing and storage. Nevertheless, with all the controls currently in use, the total dollar loss per year from beetle infestation remains large.

In late 1973, Strong and Diekman (1) reported the apparent effective control of the cigarette beetle and several pests of stored products by insect growth regulators (IGR). Additional testing (2) of a material called methoprene⁺ demonstrated excellent control of the tobacco beetle.

Methoprene (Figure 1) or isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate is classified as a juvenile hormone mimic which prevents the larvae of a series of insects from developing into normal adults (3). This material is unique as an insecticide since it exhibits a morphogenetic rather than a terminal toxic effect upon the target insect. Long et al. (4) have reported that methoprene, when applied to laboratory stored tobacco, is completely effective in controlling the cigarette beetle for up to thirty months following the initial application. Most of the IGR is retained on the tobacco. It follows that some of this residual methoprene would find its way into cigarettes. This work describes the total distribution of (5-¹⁴C)-methoprene in cigarette smoke and its combustion and pyrolysis products in mainstream smoke.

Figure 1. Structure of (5-¹⁴C)-methoprene.

EXPERIMENTAL

Radiochemicals

The (5-¹⁴C)-methoprene (5) was provided by Zoecon Corporation of Palo Alto, California. The chemical purity as received was 99.2% (2*E*,4*E*)-methoprene, 0.4% (2*Z*,4*E*)-methoprene. The 500 µCi (58 mCi/mmol) sample was received in a solution of hexane/benzene. The solvents were removed at water aspirator vacuum with a warm water bath. The residual (5-¹⁴C)-methoprene was then transferred to 50 ml absolute ethanol and refrigerated. The ¹⁴C-activity of the ethanolic solution was 10 µCi/ml.

Cigarette Preparation

University of Kentucky (1R1) cigarettes were employed in this study. The filler was removed from the paper tubes by hand and sprayed by means of a technique previously described (6). The level of application for

* Presented, in part, at the 31st Tobacco Chemists' Research Conference at Greensboro, North Carolina, U.S.A., in October 1977.

Received: 27th November 1981 — accepted: 23rd June 1982.

+ Methoprene is the active ingredient in KABAT® and in Altosid®; both are trademarks of Zoecon Corporation, Palo Alto, California.

the ethanolic solution of (5-¹⁴C)-methoprene was 1 ml/g of filler. After evaporation of the solvent and equilibration at 60% relative humidity and 24 °C, the labeled filler was reinserted in the 1R1 paper tubes using a Laredo® (Brown & Williamson Tobacco Corp.) cigarette maker. Two sets of (5-¹⁴C)-methoprene cigarettes were prepared in this fashion. One set had a 30 mm butt section which contained unlabeled 1R1 filler and a forward 55 mm section prepared from (5-¹⁴C)-methoprene sprayed filler; the second set contained the ¹⁴C-labeled filler uniformly distributed throughout the entire length of the 1R1 tobacco rod. All the cigarettes containing sprayed filler were refrigerated until required for smoking. The amount of (5-¹⁴C)-methoprene present in the equilibrated cigarettes was determined by extracting the filler in 25 ml of 95% ethanol and counting an aliquot of the extract.

Total Smoke Distribution Studies

The distribution of the (5-¹⁴C)-methoprene and its products in cigarette smoke were determined using a total recovery smoking machine (7) as illustrated in Figure 2. The total particulate matter (TPM) was determined by weighing the Cambridge filter pad before and after smoking. After the final weighing, the pad was immediately extracted in 25 ml of 95% ethanol. The radioactivity in the 23 mm unconsumed tobacco butt was also determined by extraction into 25 ml of 95% ethanol for 24 hours. All extracts were subsequently radioassayed by liquid scintillation counting.

The sidestream and mainstream gas phases were converted to carbon dioxide (CO₂) and absorbed directly into Hyamine 10-X® (Rohm and Haas) previously cooled to 0 °C. Aliquots of the Hyamine 10-X® absorbent solution and of the extracts from the Cambridge pads were taken for liquid scintillation counting.

Mainstream ¹⁴C-Smoke Analyses

a) *Gas Phase:* Evaluation of the mainstream gas phase was conducted using a smoking machine similar to that described by Newman et al. (8) and a trapping arrangement as shown in Figure 3. This trapping system was similar in design and operation to that reported by Lawrence et al. (9). The trap consisted of 0.635 cm × 1.22 m coiled copper tube immersed in liquid nitrogen. The collection of gas phase was accomplished by smoking one cigarette to a 23 mm butt length through a 4.5 cm diameter Cambridge pad. After the cigarette butt was removed from the Cambridge pad holder, one clearing puff was taken through the pad. The shunt valve (Figure 3) was closed and both valves 1 and 2 were simultaneously rotated 180° to direct the helium flow through the cold trap. The trap was purged with helium for 10 minutes in order to remove air. At this time the liquid nitrogen was removed and the trap heated for 5 minutes with a heat gun. At the end of the heating period the gas chromatographic program was initiated. All gas phase analyses were obtained using a Fisher Victoreen 4800 gas chromatograph with a 0.32 cm outside diameter × 7.2 m stainless steel Porapak® Q 80/100 mesh column. Helium flow was 30 cm³/min, and the program rate was 20 °C/min from -150 °C to 0 °C and 5 °C/min from 0 °C to 230 °C. The column effluent was split 9:1, with nine parts to the flow proportional counter for ¹⁴C detection, and one part to the flame ionization detector. The balance of the analytical procedure and description of the equipment have been previously reported (10).

b) *Semi-Volatiles:* The analysis of the semi-volatile portion (11) of mainstream smoke was accomplished using an injection port machined from stainless steel and capable of accepting a 12 mm outside diameter × 5 cm quartz sleeve (Figure 4). For any given analysis the injection port was pre-heated to 200 °C, and the gas radiochromatograph was always pre-cooled to -50 °C. A portion of the Cambridge pad containing particulate

Figure 2. Total recovery smoking machine.

Figure 3. Gas phase collection system.

Figure 4. Injection port assembly for semi-volatiles analysis.

matter from one cigarette was placed into a quartz sleeve, the injection port opened, the sleeve with pad in place inserted and the port resealed immediately. The injection port was swept with helium for 20 minutes. At the end of this period the oven temperature of the chromatograph was advanced to 0°C. The oven equilibrated in about 2 minutes at which time programming was started at 5°C per min to a maximum temperature of 240°C (OV-101 column). The distribution of activity from the Cambridge pad was determined using two different columns:

- 1) Carbowax® 20M-TPA, 5% on Chromosorb G-HP 80/100 mesh, 3.2 mm outside diameter × 4 m with 30 cm³/min helium flow (maximum temperature of column: 220°C).
- 2) OV-101, 3% on Chromosorb G-HP 80/100 mesh, 3.2 mm outside diameter × 3 m with 30 cm³/min helium flow.

Counting efficiency for the flow proportional counting system was determined using ¹⁴C-toluene (New England Nuclear) after each gas radiochromatographic run. Mainstream gas phase and semi-volatile compound identifications were obtained with a Finnigan GC/MS (Model 3300) in an electron impact mode using previously indicated gas chromatographic parameters.

Liquid Scintillation Counting

A Packard Tricarb liquid scintillation spectrometer (Model 3390) with optimized settings was used. Aliquots of all ethanol extracts were counted in a scintillator solution containing 4 g PPO [2,5-diphenyloxazole] and 50 mg POPOP [*p*-bis-2-(5-phenyloxazolyl)-benzene] per liter of toluene. All Hyamine 10-X® aliquots were placed in Insta-Gel® (Packard) scintillator cocktail prior to counting. Counting times were varied to establish good counting statistics.

RESULTS AND DISCUSSION

The cigarettes labeled with (5-¹⁴C)-methoprene were first extracted in ethanol to determine the activity level per cigarette. The uniform distribution of the labeled methoprene throughout the cigarette was also checked by the extraction techniques described earlier and found to fall within acceptable limits (6).

The cigarettes were smoked on a total smoke recovery machine, and the average distribution of activity in the various portions of smoke for six replicate runs is presented in Table 1. The percent distributions are calculated from the amount of tobacco consumed during smoking. The mainstream contributions from methoprene are similar to those of added menthol which has been reported by Jenkins et al. (12). The amount of ¹⁴C-activity for uniformly labeled (U) menthol in the mainstream gas phase was 1.4% compared to 1.3% for methoprene, while the activity in the mainstream particulate phase for menthol was 39.0% compared to 38.2% for methoprene. It should also be pointed out that the menthol delivery to the particulate phase was due in part to a 2.3% depletion of menthol in the butt. The converse has been found with methoprene, where 8.1% of the activity consumed is filtered out by the unburned tobacco column (23 mm butt). This value was attained by measuring the total activity in the butt after smoking and subtracting the amount of radioactivity present before smoking. In order to confirm this 8.1% value, cigarettes were prepared which contained unlabeled or "cold" filler in the last 30 mm of the tobacco rod. Five additional smoking

Table 1. Distribution of radioactivity (μCi) in smoke from cigarettes labeled with (5-¹⁴C)-methoprene (based on tobacco consumed).

Smoke phase	Cigarette No.						Average	Percent distribution
	1	2	3	4	5	6		
Mainstream gas	0.04	0.04	0.04	0.04	0.04	0.04	0.04	1.3
Mainstream total particulate matter	1.11	1.26	1.12	1.17	1.24	1.18	1.18	38.2
Sidestream gas	0.51	0.57	0.50	0.51	0.65	0.55	0.55	17.8
Sidestream total particulate matter	1.07	1.06	1.08	1.06	1.10	1.07	1.07	34.6
Butt*	0.45	0.26	0.18	0.25	0.10	0.25	0.25	8.1
Total recovery**	3.18	3.19	2.92	3.03	3.13	3.09	3.09	100

* Value obtained by subtracting calculated activity present on 23 mm butt length prior to smoking.

** Calculated activity recovery based on tobacco consumed during smoking = 3.09 μCi/cigarette.

Table 2. Identification of the radioactive components in mainstream smoke gas phase from (5-¹⁴C)-methoprene treated cigarettes.

Peak Identification	% of total activity in mainstream gas phase*
1 Carbon monoxide	23.5 ± 2.0
2 Methane	1.3 ± 0.4
3 Carbon dioxide	62.3 ± 1.5
4 Ethylene	0.9 ± 0.3
5 Ethane	0.7 ± 0.2
6 Propene	0.2 ± 0.1
7 1,3-Butadiene (plus some C ≡ C—C = C and C ≡ C—C ≡ C)	0.9 ± 0.3
8 <i>n</i> -Butane (plus some 1- or 2-butene)	0.9 ± 0.3
9 Isoprene (mainly)	2.0 ± 0.4
10 1,3-Pentadiene (possibly some C ₆ H ₁₀ and C ₆ H ₁₂)	0.8 ± 0.2
11 Benzene	3.9 ± 0.5
12 Toluene	2.6 ± 0.4

* These figures represent 1.3% of total activity consumed during smoking.

runs using these cigarettes produced excellent agreement with the data in Table 1. The butt filtration in this instance was 8.6%. Even with the careful attention that was given to insure the proper intertwining of the tobacco shreds between the labeled and unlabeled filler, the possibility always exists that an abnormal interface or junction resulted. Therefore, the value of 8.1% is thought to better reflect the true value for butt filtration. This relatively high value of 8.1% is comparable to the 8.3% reported for anthracene by *Thornton* (13), and 8.5% reported by *Jenkins* (12) for l-proline. Both of these materials were shown to undergo sublimation and subsequent vapor phase condensation when exposed to the lower temperatures in the butt. This supports the premise that methoprene exists to a large degree in the volatile portion of mainstream particulate smoke.

The chemical composition of the radioactivity in the mainstream smoke was studied by gas radiochromatography. The mainstream smoke gas phase components arising from the added labeled methoprene (1.3% of activity consumed during smoking) are detailed in Table 2. It is apparent that almost 86% of the labeled gas phase is composed of CO and CO₂. The balance of gas phase constituents containing ¹⁴C-activity indicate no single component greater than 4% of the total gas phase.

Listed in Table 3 is a comparison of the mainstream gas phases from (5-¹⁴C)-methoprene and ¹⁴C-bright tobacco (U). Although much less total mass is contributed by the methoprene (1.3% of the 12.4 μg methoprene consumed *vs.* 13.7% of the 730 mg of tobacco consumed), the CO₂/CO ratios are identical, indicating no selective generation of either CO₂ or CO by the added methoprene.

Table 3. Mainstream smoke gas phase comparison.

Component	Labeled additive	
	(5- ¹⁴ C)-methoprene	¹⁴ C-tobacco
Carbon monoxide	23.5 %	23.0 %
Carbon dioxide	62.3 %	62.1 %
Other gases	14.2 %	14.9 %
Fraction of total activity consumed during smoking	1.3 %	13.7 %

Table 4. Percent distribution of radioactive components in the semi-volatile portion of smoke from (5-¹⁴C)-methoprene treated cigarettes.

Compound	% of mainstream particulate	% of sidestream particulate
Unknown "A"	1.1 ± 0.4	14.2 ± 2.0
(2Z,4E)*-methoprene	1.8 ± 0.5	4.0 ± 1.0
(2E,4E)-methoprene	96.8 ± 2.0	81.5 ± 2.0
Residual unknown "B"	0.3 ± 0.1	0.3 ± 0.1

* 0.4% of the added (5-¹⁴C)-methoprene was initially present as (2Z,4E)-methoprene.

The particulate contribution to mainstream and sidestream smoke was also evaluated by gas radiochromatography. These data are shown in Table 4. A 3% OV-101 column was used to generate the data. A comparative run was conducted using a 5% Carbowax® 20M-TPA column which gave a similar value (96.2%) for the unchanged methoprene. These data demonstrate that methoprene transfers essentially intact to the mainstream particulate phase. In the sidestream particulate phase, the degree of pyrolytic breakdown is enhanced, but the methoprene still to a large degree undergoes distillation. Unknown "A", a compound eluting ahead of methoprene, was not identified. Unknown "B" is the residual activity remaining on the TPM pad after sweeping with helium. No chemical analyses were performed on the residuals.

A small percentage of the (2E,4E)-methoprene underwent isomerization to the (2Z,4E) form during the distillation process. A small amount of the (2Z,4E) isomer (0.4%) was initially present in the (5-¹⁴C)-methoprene.

SUMMARY

An insect growth regulator, (5-¹⁴C)-methoprene [5-¹⁴C-isopropyl(2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-decadienoate], has been applied to tobacco filler from which cigarettes were made. The Kentucky 1R1 reference cigarette was employed in this study to determine the distribution of the (5-¹⁴C)-methoprene and its combustion and pyrolytic products in cigarette smoke.

The distribution of smoke activity as determined using a total smoke recovery device showed 38.2 percent of the ^{14}C -activity to be in mainstream smoke with 52.4 percent in sidestream and 8.1 percent filtered by the tobacco butt (23 mm).

Analyses of mainstream particulate matter and gas phase by gas radiochromatography indicated the major portion of the activity found in mainstream smoke to be unchanged (5- ^{14}C)-methoprene (96.8 percent). Limited sidestream particulate matter analyses were also conducted. Analysis of the mainstream gas phase (1.3 percent of total activity) for carbon-14 distribution showed that carbon monoxide and carbon dioxide accounted for 86 percent of the gas phase activity. The remaining 14 percent was distributed among ten additional gas phase components.

ZUSAMMENFASSUNG

Der Wirkstoff (5- ^{14}C)-Methopren [5- ^{14}C -Isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoat] zur Regulierung des Wachstums von Insekten wurde auf den Cigarettenfülltabak der Versuchscigarette 1R1 der Universität von Kentucky gesprüht, in deren Rauch die Verteilung von (5- ^{14}C)-Methopren und dessen Verbrennungs- und Pyrolyseprodukten untersucht wurde.

Unter Benutzung einer Wiederauffindungsvorrichtung für den Gesamtrauch wurden 38,2 % der ^{14}C -Aktivität im Hauptstromrauch, 52,4 % im Nebenstromrauch und 8,1 % im Tabakstummel (23 mm) gefunden.

Die Untersuchung der Partikelphase und der Gasphase des Hauptstromrauches durch Gasradiochromatographie ergab, daß der größte Teil der im Hauptstromrauch aufgefundenen ^{14}C -Aktivität unverändertes (5- ^{14}C)-Methopren ist (96,8 %). In begrenztem Maße wurde auch die Partikelphase des Nebenstromrauches untersucht. Die ^{14}C -Aktivität der Gasphase des Hauptstromrauches (1,3 % der Gesamtaktivität) rührt zu 86 % von Kohlenmonoxid und Kohlendioxid her. Die restlichen 14 % verteilen sich auf zehn andere Gasphasenbestandteile.

RÉSUMÉ

Un régulateur de croissance des insectes, le (5- ^{14}C)-méthoprene [5- ^{14}C -isopropyl (2*E*,4*E*)-11-méthoxy-3,7,11-triméthyl-2,4-dodécadiénoate] a été appliqué au tabac à partir duquel on a fabriqué des cigarettes. La cigarette de référence 1R1 (Université de Kentucky) a été utilisée dans cette étude pour déterminer la répartition du (5- ^{14}C)-méthoprene et de ses produits de combustion et de pyrolyse dans la fumée de cigarette.

La distribution de l'activité de la fumée, définie au moyen d'un appareillage collectant la totalité de la fumée, montre que 38,2 % de l'activité due à ^{14}C est dans le courant principal, 52,4 % dans le courant secondaire

et que 8,1 % est retenue par filtrage dans le mégot (23 mm).

L'analyse de la phase particulaire et de la phase gazeuse du courant principal par radiochromatographie en phase gazeuse montre que la plus grande partie de l'activité trouvée dans le courant principal est du (5- ^{14}C)-méthoprene inchangé (96,8 %). Des analyses plus limitées de la phase particulaire du courant secondaire ont été aussi conduites. Une analyse de la phase gazeuse du courant principal (1,3 % de l'activité totale), montre que CO et CO₂ interviennent pour 86 % de l'activité de cette phase. Les 14 % restants sont répartis sur dix autres constituants de la phase gazeuse.

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Acknowledgements

The application of (5-¹⁴C)-methoprene to tobacco filler and cigarette fabrication by Mr. R. Bass and Mr. G. Newell and GC/MS assistance of Dr. R. Kornfeld are gratefully acknowledged.

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