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The Distribution with Respect to Smoke Particle Size of Dotriacontane, Hexadecane and Decachlorobiphenyl Added to Cigarettes*

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INTRODUCTION

Study of the biological activity of cigarette smoke requires a method for determining the doses of smoke reaching the target organs of the animal subject under investigation. For inhalation studies, this can be most readily established by using known chemical species to act as tracers for smoke. Several types of tracer can be considered for use as potential markers, the two main types being:

(i) Non-radioactive organic compounds,
(ii) Radioactive organic compounds.

In choosing a tracer for dosimetry experiments it is necessary to take into consideration both the biological and smoke chemical requirements. One of the smoke chemical requirements of an ideal tracer is that it should be distributed in a constant ratio with particulate matter (TPM) throughout the different smoke particle sizes.

This paper describes work to examine the distribution of three potential TPM tracers (1, 2, 3, 4) with respect to the smoke aerosol particle size from a cigarette containing flue-cured tobacco. Two of these tracers, n-[1-^{14}C]hexadecane and [16,17-^{14}C]dotriacontane, are of the radioactive type while the third, decachlorobiphenyl, is non-radioactive. As a check on the radioactive tracer techniques used, the distribution of [N-methyl-^{14}C]nicotine was also determined.

EXPERIMENTAL DETAILS

Radiochemicals

n-[1-^{14}C]Hexadecane s. a. 57 mCi/mmol and [N-methyl-^{14}C]nicotine s. a. 36 mCi/mmol were obtained from The Radiochemical Centre, Amersham, and [16, 17-^{14}C]-dotriacontane s. a. 55 mCi/mmol was obtained from Fluorochem Ltd.

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Preparation of Cigarettes

Plain cigarettes (70 × 25.0 mm) made from flue-cured tobacco were used in all experiments. The cigarettes were selected within the limits: weight 1.03—1.07 g, and draw resistance 11.0—12.0 cm W.G. at 1050 ml/min. The cigarettes were conditioned over saturated sodium bromide solution for at least 24 hours before being injected with the solutions of tracer described below, using the method described in the literature (5, 6). After injection, the solvent was allowed to evaporate (ca. 0.5 h) and the cigarettes were re-conditioned, except in the case of hexadecane where they were placed in a sealed jar (after evaporation of the solvent) in order to minimize losses of the tracer.

(i) *Cigarettes Containing n-[1-^{14}C]Hexadecane:* A solution was prepared containing the n-[1-^{14}C]hexadecane (50 µCi) and radio-inactive n-hexadecane (7.7 mg) in cyclohexane (20 ml). Cigarettes were injected with the solution (120 µl) to give a loading of 0.3 µCi and 0.047 mg (18 × 10^{-5}mmol) of n-hexadecane per cigarette.

(ii) *Cigarettes Containing [16,17-^{14}C]Dotriacontane:* A solution was prepared containing the [16,17-^{14}C]dotriacontane (100 µCi) and radio-inactive dotriacontane (15.8 mg) in cyclohexane (20 ml). Cigarettes were injected with the solution (120 µl) to give a loading of 0.55 µCi and 0.08 mg (18 × 10^{-5} mmol) of dotriacontane per cigarette.

(iii) *Cigarettes Containing [N-methyl-^{14}C]Nicotine:* Cigarettes were injected with 120 µl of a chloroform solution of [N-methyl-^{14}C]nicotine to give a loading of 0.6 µCi per cigarette.

(iv) *Cigarettes Containing Decachlorobiphenyl:* Cigarettes were injected with 120 µl of a solution of decachlorobiphenyl in chloroform (18.2 mg/ml) to give a loading of 2.18 mg per cigarette.

Collection of Smoke Particle Size Fractions

The cigarettes were pressure smoked to a butt length of 23 mm by means of a smoking machine which compresses air through the cigarette to produce a square profile 35 ml puff of 2 seconds duration once every minute. The smoke passed without dilution and with minimal time delay into a centrifugal size classifier. This instrument is similar to the Conifuge described by *Keith* and *Derrick* (7), except that it is fitted with a series of seven fractionation rings. These are designed to collect small quantities of condensate from particles in the particle diameter range from 0.15 to 1.54 micron. At least five batches (each containing twelve cigarettes) were smoked so that the experimental errors could be assessed.

Determination of Radioactivity

The samples of particulate matter were taken in random order and were washed into volumetric flasks (50 ml) with aldehyde-free methanol. The solutions were made homogeneous by agitation in an ultra-sonic bath. They were then assayed for radioactivity using a Packard Tri-Carb Model 3003 Liquid Scintillation Spectrometer with gain and window settings optimized for maximum ^{14}C counting efficiency. Aliquots (1.0 ml) of the methanolic solutions were counted in toluene scintillator (10 ml), prepared by dissolving 5-diphenyloxazole [5.0 g PPO] and dimethyl-2,2-phenylene-bis-(5-phenyloxazole) [0.3 g dimethyl POPOP] in A.R. toluene (1 litre).

Determination of Concentration of Decachlorobiphenyl (DCBP)

The samples of particulate matter were dissolved in methanol. The methanol was removed by evaporation and the condensate extracted with warm cyclohexane (40° C) in an ultra-sonic bath for 15 minutes. The cyclohexane was allowed to cool and was made up to volume (100 ml). An aliquot (1 ml) of this solution was diluted in a volumetric flask (10 ml) and a standard solution of dichlorodiphenyl-dichloroethane [DDD (100 µl)] added as an internal standard.
The solutions were analysed for decachlorobiphenyl on a Perkin-Elmer F17 gas chromatograph equipped with a Ni^{63} electron capture detector at 300° C. An all-glass column (2 m × 3.0 mm i. d.) packed with 3.8% OV-1 on 80—100 mesh Chromosorb W(HP) was used for the analysis. The other operating conditions were:

Injection temperature:	300° C
Carrier gas:	High purity nitrogen
Flow rate:	38 ml/min (cold)
Purge gas:	High purity nitrogen
Flow rate:	27 ml/min
ECD pulse setting:	6
Oven temperature:	245° C

The concentration of DCBP in each solution was calculated from a calibration curve prepared from a series of standard solutions of DCBP and DDD.

RESULTS AND DISCUSSION

To check the experimental smoking and extraction procedures, the percentage transfer of each compound was calculated, averaged over all particle size fractions. The results, which are shown in Table 1, have been calculated on the basis of the whole cigarette loading and are similar to those obtained by normal smoking procedures and collection on a Cambridge filter. This confirms that these components occur in the particulate phase and that this phase was essentially fully recovered in the Conifuge and by the associated extraction procedures.

Table 1. Overall transfer of added compound.

Compound	% Transfer (mean ± standard deviation
[N-methyl-^{14}C]Nicotine	11.9 ± 0.9
[16,17-^{14}C]Dotriacontane	22.3 ± 1.8
n-[1-^{14}C]Hexadecane	24.9 ± 1.0
Decachlorobiphenyl	19.0 ± 1.3

To assess whether each compound is transferred independently of particle size, the fractions of the total amount of tracer and of the total weight of condensate in each particle size range were compared. As shown in Figure 1a, the distribution of nicotine added to the cigarettes appears to be independent of particle size. This result indicates that the radioactive technique is suitable for investigating TPM tracers, and the uniform distribution confirms that any redistribution of mass within the Conifuge is likely to be negligible.
Figure 1b shows that the transfer of dotriacontane is particle size dependent, the largest particles being enriched and the smallest particles depleted. Like any spectrometer, the Conifuge has finite resolution and therefore the true relationship with particle size is underestimated to an unknown extent. This variation, however, may be small in relation to the other variables present in inhalation experiments. It is considered that the dependence of transfer of dotriacontane on particle size should not by itself preclude the use of this compound as a tracer, but if it is used the accuracy of its relationship to the particulate matter deposited in the respiratory system should be assessed by other means.

Figure 2a shows that the transfer of added hexadecane is less particle size dependent. On the basis of particle size considerations, hexadecane can also be regarded as a potential tracer.
Figure 2b shows that the dependence of transfer on particle size of decachlorobiphenyl is similar to dotriacontane, and similar considerations apply in this case.

On the basis of the measurements described in this paper there is no clear reason for rejecting any of the

Figure 1. Relative concentration of added compound as a function of smoke particle diameter.

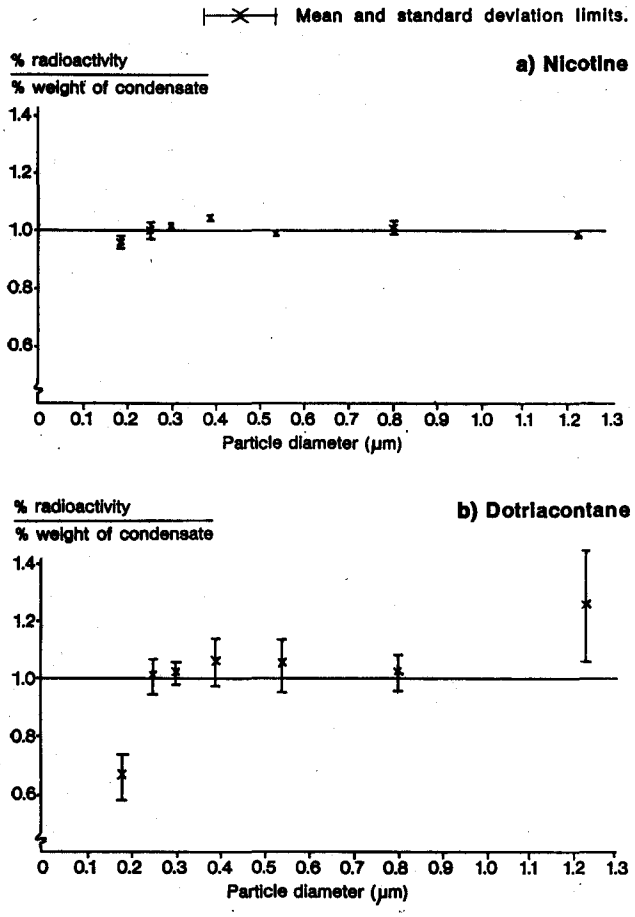
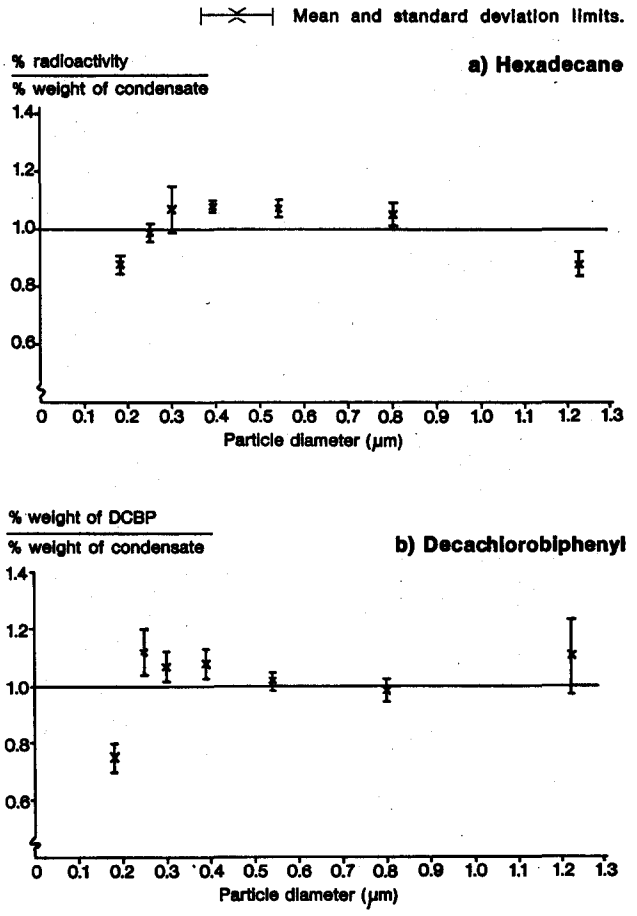


Figure 2. Relative concentration of added compound as a function of smoke particle diameter.



three tracers investigated. It should, however, be appreciated that the lung environment is considerably different from that to which the aerosol has been exposed in this work, and that the results may also depend on other factors such as the type of cigarette.

SUMMARY

When studying the distribution of smoke deposited in the lung, it is necessary to use a tracer which indicates the levels of particulate matter deposited at various sites. Because the site of deposition can be dependent on the particle size of the aerosol, it is important that the tracer should be uniformly distributed with respect to particle size, otherwise the levels of particulate matter may be wrongly estimated. The distributions in the smoke from a cigarette containing flue-cured tobacco of three possible tracers (dotriacontane, hexadecane and decachlorobiphenyl) have been measured; all three show a slight dependence but probably not sufficient to exclude them from possible use in inhalation studies. However, because of the different environment which exists in the lung, and the possible dependence of the results on the type of tobacco, it should not be assumed that there are no selective effects.

ZUSAMMENFASSUNG

Die Untersuchung der Verteilung von Rauchablagerungen in der Lunge setzt die Benutzung eines radioaktiven Indikators voraus, der die Menge an Partikelphase anzeigt, die sich an den verschiedenen Stellen niederschlägt. Da der Ablagerungsort von der Größe der Aerosolpartikel abhängen kann, ist es wichtig, daß sich der Indikator in bezug auf die Partikelgröße gleichmäßig verteilt, da sich sonst falsche Befunde ergeben können. Bei drei anwendbaren Indikatoren (Dotriacontan, Hexadecan und Decachlorbiphenyl) wurde die Verteilung im Rauch einer Cigarette aus „flue-cured"-Tabak mit dem Ergebnis gemessen, daß alle drei Verbindungen eine leichte Abhängigkeit von der Partikelgröße zeigten, die aber wahrscheinlich nicht so groß ist, daß man von einer Verwendung bei Inhalationsexperimenten absehen sollte. In Anbetracht der unterschiedlichen Umgebungsbedingungen in der Lunge und der Möglichkeit, daß die Ergebnisse von der Art des Tabaks abhängen, sollte man jedoch das Vorhandensein von selektiven Wirkungen nicht ausschließen.

RESUME

Pour étudier la distribution des dépôts de fumée dans les poumons, il faut utiliser un traceur qui indique les

quantités de matière particulaire déposée aux différents endroits. L'endroit de dépôt pouvant dépendre de la taille des particules de l'aérosol, il est important que le traceur soit réparti uniformément par rapport à la taille des particules. On pourrait sinon estimer de façon erronnée les quantités de matière particulaire déposée. On a mesuré la distribution de trois traceurs (dotriacontane, hexadécane et décachlorobiphényle) dans la fumée d'une cigarette contenant du tabac «flue-cured»; tous trois montrent une certaine dépendance, cependant probablement insuffisante pour en exclure l'emploi dans des études d'inhalation. On ne peut cependant pas être certain qu'il n'y aura pas d'effets sélectifs dûs aux différents milieux dans le poumon, et à la dépendance possible des résultats de différents types de tabac.

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