Cigarette Smoke as a Source of Pesticide Exposure

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A simple, but quantitative, smoking system was devised whereby smoke containing radioactive insecticide residues was transferred directly from the cigarette to the lungs of rats via the trachea. While forced-smoking via the trachea is not new (ARMITAGE et al., 1969) and is less desirable than natural inhalation, a quantitative system to allow the "smoking" of animals in a manner similar to the process in humans has yet to be developed. Enclosing animals in cylinders or chambers (MOORE and BOCK, 1956; HOLLAND et al., 1958; BOWERY et al., 1965) may be sufficient for styding the pathological effects of chronic smoke exposure, but the quantitative aspects necessary for pesticide fate studies and for determining exposure potential of pesticides to the smoker are not provided by such means.

MATERIALS AND METHODS

The 5 radioactive insecticides used in this study were (a) 1-naphthyl-1-^{14}C N-methylcarbamate or carbaryl (b) 2,3-dihydro-2,2-dimethylbenzo-^{14}C-furan-7-yl N-methylcarbamate or carbofuran (c) O-(2,5-dichloro-4-bromophenyl-^{14}C)O-methyl phenylthiophosphonate or leptophos (d) 1,3,4-methenododecachloro-octahydro-2H-cyclobuta[c,d]pentalene (^{14}C UL) or mirex and (e) 2,2-bis(p-chlorophenyl-^{14}C) 1,1,1-trichloroethane or DDT.

Unless stated otherwise, all cigarettes were 1R1 reference research cigarettes, 85 mm, supplied by the Tobacco and Health Research Institute (University of Kentucky, Lexignton, Ky.). Each insecticide, in 0.1 ml acetone, was applied to a 17 mm segment, 200 mg tobacco, of the cigarettes with the treatment zone extending from a point 25 mm from the butt end. The needle of a microsyringe was inserted at an angle into the cigarette at a point 10 mm from the zone towards the end to be lighted and passed forward until the point reached the center of the treatment zone. The acetone was then slowly forced from the syringe and the acetone allowed to evaporate. Sectioning and radioassay of the impregnated zones showed that the radioactivity was distributed in a bell-shape fashion with just trace amounts on either side of the marked zone.

The smoking apparatus is shown in Fig. 1. For each experiment, a cigarette was placed in the holder, lit, and the smoking-chamber cover put into place. Air was passed through the system at 500 ml/min. which kept the cigarette burning. Immediately, a female rat (Sprague-Dawley) weighing 180-200 g was anesthetized with ether and a polyethylene tube inserted into the trachea and tied. The animal was lightly anesthetized throughout

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the smoking process. Just as the cigarette burned to the impregnated zone, the distal end of the tracheal tube was connected to the mainstream smoke source.

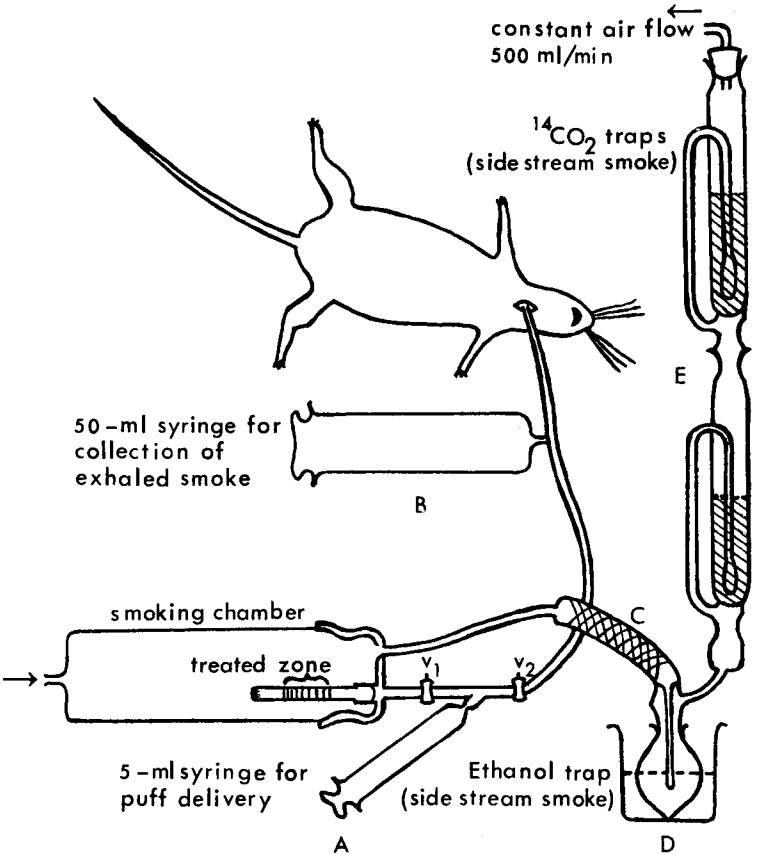

Fig. 1. Apparatus used to transfer mainstream smoke of cigarette to lungs of rats via the trachea.

A 5-ml puff was drawn into syringe A (valve V_1 open, V_2 closed) and directed into the lung of the rat (valve V_1 closed, V_2 open). Since the ultimate objective of this research is to evaluate the fate of retained insecticide residues, the smoke was held in the lungs for 12 sec. to allow maximum retention of radiocarbon in the lungs. Then, 5 ml were withdrawn into syringe B. After repeating the process for a total of 8 puffs and exhalations, during which the entire impregnated zone was burned, the trachea was immediately clamped and the air-flow through the system stopped to extinguish the cigarette.

Radioactive residues in the exhaled air, syringe B, were removed by washing the syringe first with ethanol and then with carbon dioxide-trap

TABLE 1.

Distribution of radiocarbon following smoking of cigarettes impregnated with with-^{14}C-insecticides.

	Percent of ^{14}C-insecticide added to burned zone[a]				
Fraction	Carbaryl	Carbofuran	Leptophos	Mirex	DDT
Ash	0.3 ± .1	0.5 ± .1	0.5 ± .2	0.2 ± .1	0.5 ± .1
Butt	14.3 ± 3.1	13.8 ± 1.3	13.6 ± 1.7	19.6 ± 1.8	20.2 ± 1.8
Sidestream smoke	66.0 ± 5.5	68.8 ± 2.3	66.3 ± 2.6	61.2 ± 4.1	65.4 ± 4.2
Mainstream smoke	10.3 ± .5	9.5 ± .6	15.2 ± .5	13.0 ± 1.1	11.9 ± 1.0
Total Recovery	90.9 ± 2.9	92.6 ± 2.8	95.6 ± 4.8	94.0 ± 3.7	97.9 ± 2.1

[a] 17 mm portion of cigarette impregnated with 100 ppm insecticide. Entire impregnated zone smoked using eight 5-ml puffs at 15-sec. intervals.

solution (2:1 2-aminoethanol: 2-methoxyethanol). This same procedure was used for collecting mainstream smoke ^{14}C-components for analysis. In this case the smoke was collected directly in a syringe and the ethanol was concentrated and applied to thin layer plates, tlc, (Merck, silica gel F-254). Solvent systems for the different compounds were: carbaryl - 7:3 benzene:ether; carbofuran - 5:1 ether:hexane; leptophos - 6:4 chloroform:benzene; mirex - n-heptane; DDT - n-hexane. That the radiocarbon in the carbon dioxide trap solution was actually $^{14}CO_2$ was supported by the fact that approximately the same quantity of ^{14}C-material was collected using a barium hydroxide solution and, upon acidification, the radiocarbon dissipated.

RESULTS AND DISCUSSION

Based upon the excellent accountability of the radiocarbon added to the cigarettes, it appears that the procedure described herein may serve as a general method for estimating the significance of pesticides and their metabolites in tobacco products. Total recovery of the compounds ranged from 91% for carbaryl to 98% for DDT (Table 1). From the smoke-exposure standpoint, the most important residues are those which appear in the mainstream smoke. With all the compounds evaluated, from 10 to 15% of the radiocarbon added to the burned zone was detected in the mainstream smoke.

Evaluation of the nature of the residues in the mainstream smoke showed that the insecticides were degraded to different degrees by the smoking process (Table 2). Mirex, a completely chlorinated material, was the most stable with 69% of the ^{14}C-components in the mainstream smoke as the applied compound. The least stable compound was leptophos where only 21% of the ^{14}C-residues in the mainstream smoke was as the unaltered insecticide. ^{14}C-Carbon dioxide was a major component of the mainstream smoke with all of the ring-^{14}C-labeled insecticides, but the amounts, 15-21%, did not vary greatly.

The mainstream smoke data clearly show that pyrolytic stability is not the only, or always the most important factor involved in the transfer of insecticide residues to the mainstream smoke. It is very unlikely that the compounds, especially the carbamates and phosphates, would withstand the 850-900°C heat (TOUEY and MUMPOWER, 1957) at the burning point of a cigarette. Yet, much of these materials survived the smoking process and were present at significant levels in the mainstream smoke. It is apparent that much of the intact pesticide is vaporized away from the intense heat and transferred to the smoke.

Of those ^{14}C-materials inhaled by the rats, a major portion was exhaled (Table 3). More carbofuran-^{14}C- and mirex-^{14}C-equivalents were exhaled than with the other insecticides. The exhaled air of the carbaryl-exposed animals contained 25% of the unaltered carbaryl which was inhaled. Analysis of exhaled ^{14}C-materials from the other insecticides are incomplete at this time.

The transfer of ^{14}C-insecticide-equivalents from the lungs to the blood, and subsequently to the heart, was less with mirex, and greatest

TABLE 2.

Nature of radiocarbon in mainstream smoke of cigarettes impregnated with ring-^{14}C-insecticides

	Percent of total ^{14}C-residues in mainstream smoke[a]				
^{14}C-Material	Carbaryl	Carbofuran	Leptophos	Mirex	DDT
Unaltered insecticide	44.7	62.7	21.2	69.1	44.2
Carbon dioxide	18.4	14.7	21.0	20.0	16.8
Pyrolysis products	31.5 (7)	11.0 (1)	51.4 (4)	4.0 (3)	32.1 (7)
Loss[b]	5.4	11.6	6.4	6.9	6.9

[a] Number of pyrolysis products shown in parenthesis.

[b] ^{14}C-Material lost during concentration of solvents and tlc analysis.

TABLE 3.

Fate of ^{14}C-residues in cigarette smoke inhaled by rats.

	Percent of total ^{14}C-residues inhaled[a]				
^{14}C-Residues	Carbaryl	Carbofuran	Leptophos	Mirex	DDT
Exhaled	32.9	52.2	30.5	47.3	31.3
Lung	31.4	22.8	31.8	35.5	39.5
Blood	27.2	17.8	39.9	11.1	27.1
Heart	2.4	3.6	3.6	1.3	2.1
Recovery[b]	93.9	96.4	105.8	95.2	100.0

[a] Rats given 8, 5-ml puffs at 15-sec. intervals and the trachea clamped immediately after the exhalation of the last puff. Blood then collected after 2.5 min; lung and heart excised after 3.5 min.

[b] Based on dpm in mainstream smoke collected directly.

with the organophosphorus insecticide, leptophos. DDT and carbaryl were similar insofar as the distribution of the retained ^{14}C-residues was concerned. No pattern of retention and/or distribution of inhaled ^{14}C-insecticide equivalents was observed which could be considered characteristic for a particular chemical type of insecticide. Previous reports that the carbamates and organophosphates are degraded almost completely during the smoking process (GUTHRIE, 1968) were not supported by these findings.

As a part of the current study, carbaryl and leptophos were added to cigarettes and smoked with 5- and 35-ml puffs, the latter representing the inhalation volume in humans. With carbaryl, the amount of ^{14}C-residues in the mainstream smoke was directly proportional to the volume of the puff; on a per ml basis, there was no difference in the 5- and 35-ml puffs. With leptophos, 27.9% of the radiocarbon was transferred to the mainstream smoke using the 35-ml puffs, an amount 30% less per ml than the 5-ml puffs. The ^{14}C-components of the mainstream smoke, and their relative concentrations, were the same with puff volumes of 5 and 35 ml. It was also found that the transfer of ^{14}C-residues from leptophos-impregnated cigarettes was the same using the smoking apparatus, 35-ml, shown in Fig. 1 and a commercial smoking machine. These were important findings in that they demonstrate that the smoke administered to rats or humans would contain the same ^{14}C-residues and at concentrations generally commensurate with lung capacity. Preliminary studies with carbaryl showed that results were similar with concentrations of 30 and 100 ppm in cigarette tobacco. A lack of concentration effect, up to 50% variation, was noted with acetaldehyde in inhalation studies using humans (EGLE, 1970).

Different types of commercial filter cigarettes reduced the ^{14}C-carbaryl equivalents in the mainstream smoke by 36 to 49% as compared to the same cigarettes without filters. Increasing the butt length of unfiltered 1R1 cigarettes to that equivalent to the filter lengths reduced the mainstream smoke ^{14}C-residues by 29%. Therefore, the reduction of ^{14}C-residues as the result of filters was only 7 to 20% more effective than an equivalent length of tobacco.

Mirex, used in this study because of its unique stability, is not used on tobacco and is of no practical importance. Leptophos is an experimental insecticide and is of no immediate concern. DDT was used extensively on tobacco until 1970 and studies in our laboratory showed that average total DDT residues in commercial cigarettes purchased in 1970, 71 and 72 were 36, 44 and 27 ppm, respectively (DOROUGH and GIBSON, 1972). Unpublished data from a continuation of these studies show that the residues declined to an average of 7.7 ppm in cigarettes purchased in 1973. Carbaryl is used on tobacco but residues in cigarettes are reportedly less than 1 ppm (GUTHRIE, 1968). Carbofuran was approved for use on tobacco in 1973 and, therefore, no information is available on its concentration in commercial cigarettes. There are many other pesticides used on tobacco and the results of this study demonstrate that there is a definite need to more critically evaluate their transfer to the mainstream smoke of cigarettes and to determine the nature and fate of the inhaled products in the mammalian system. This can now be accomplished using the techniques described herein since the animals,

after closing the incision with a wound clip, can be returned to metabolism cages and maintained indefinitely.

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