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Insecticide Residues in Cigarette Smoke. Transfer and Fate in Rats

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A quantitative smoking system was devised for mainstream smoke collection or for delivery to the lungs of rats *via* the trachea. Percentage transfer of ^{14}C-labeled insecticide equivalents to the mainstream smoke of cigarettes impregnated with five different ^{14}C-labeled insecticides (carbaryl, carbofuran, leptophos, DDT, and mirex) was approximately the same per milliliter of smoke, 0.2–0.3% of the ^{14}C-labeled insecticide contained in the burned tobacco, with puff volumes of 35 and 5 ml. Thirty-five milliliter puffs represent the average puff volume of man while a 5-ml puff was the volume administered to rats. Total recovery (ash, butt, sidestream smoke, and mainstream smoke) ranged from 88 to 102% of the ring-^{14}C-labeled insecticides added to that tobacco subsequently burned during the smoking process. Rats were administered eight 5-ml puffs at 15-sec intervals from cigarettes impregnated with the radioactive insecticides. Results are presented relative to levels of radiocarbon in the exhaled air, lungs, blood, and heart following the smoking process.

A degree of human exposure to insecticides and other types of pesticidal chemical residues is unavoidable because of their ubiquitous nature in the environment and particularly on food commodities. This source of contamination has long been recognized and stringent use limitations are established for each pesticide in an effort to assure that levels of exposure are kept well below those believed to be hazardous. Tobacco smoke is another possible source of human exposure to insecticides but their actual significance to the health of the smoker is largely unknown (Wynder and Hoffmann, 1967). However, it is known that various insecticide residues occur in commercial tobacco products (Dorough and Gibson, 1972) and that insecticides and their pyrolysis products may be transferred to the mainstream smoke of cigarettes (Chopra *et al.*, 1970; Chopra and Domanski, 1972; Chopra and Sherman, 1972; Guthrie, 1968; Hengy and Thirion, 1970, 1971; Hoffmann and Rathkamp, 1968; Hoffmann *et al.*, 1969; Iso, 1972).

Even so, tobacco smoke has not been generally considered as an important source of human exposure to insecticides and, not being a food crop, tobacco is not included among those crops requiring official pesticide tolerances. Nevertheless, regulatory officials do carefully consider the levels and nature of residues in tobacco and in smoke condensates as part of the evaluation of pesticides whose proposed use includes the control of tobacco pests. If hazardous conditions appear likely, the use may be denied or proper limitations imposed to circumvent the potential problems involved. This type of consideration is relatively new and there is a critical need for information which will assist in determining the significance of pesticide residues in tobacco and tobacco smoke. A most obvious void in this regard is information relating to the fate of insecticide residues, their metabolites, and/or pyrolysis products after being inhaled. Studies of this nature should be conducted with animals and, as is the case with pesticide residues on food crops, the data extrapolated to humans.

In the current study, a simple but quantitative smoking device was constructed whereby smoke containing radioactive insecticide residues was transferred directly from the cigarette to the lungs of rats *via* the trachea. Rats were selected because they are commonly used in metabolic fate studies of pesticides, and the existing information would be useful in interpreting the significance of data obtained in the smoke experiments. While it is desirable to administer the smoke to the animal in a more natural manner, an effective quantitative system that would allow the "smoking" of animals 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) may be sufficient for studying the pathological affects 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 methods. Forced smoking *via* the trachea (Wynder and Hoffmann, 1967; Armitage *et al.*, 1969) allows an excellent means of administering quantitative doses of smoke containing insecticide residues to the lungs of animals, and the subsequent determination of the fate of the inhaled residues.

MATERIALS AND METHODS

Treatment of Cigarettes. Radioactive insecticides were used in all experiments. The identity of the compounds and the position of the ^{14}C label on the molecule are shown in Table I. These insecticides were selected to represent different chemical types and not as examples of compounds used for the control of tobacco pests. In fact, of those insecticides listed in Table I, only carbaryl and carbofuran are currently approved for this use. DDT was registered for use on tobacco until 1970 and residues do exist in commercial cigarettes (Dorough and Gibson, 1972).

Reference research cigarettes 1R1 were provided by the Tobacco and Health Research Institute (University of Kentucky, Lexington, Ky). The desired quantity of insec-

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Table I. Radioactive Compounds Used in the Study of the Transfer of Insecticide Residues to Rats in Cigarette Smoke[a]

Chemical identity and position of carbon-14	Designation	Sp act., mCi/mmol
1-Naphthol-*1*-^{14}C	1-Naphthol-^{14}C	15.2
1-Naphthyl *1*-^{14}C-methylcarbamate	Carbaryl-*naphthyl*-^{14}C	0.6
1-Naphthyl methylcarbamate-*carbonyl*-^{14}C	Carbaryl-*carbonyl*-^{14}C	26.4
1-Naphthyl methyl-^{14}C-carbamate	Carbaryl-*methyl*-^{14}C	1.1
2,3-Dihydro-2,2-dimethyl-7-benzo-^{14}C-furanyl *N*-methylcarbamate	Carbofuran-*ring*-^{14}C	2.85
2,3-Dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate-*carbonyl*-^{14}C	Carbofuran-*carbonyl*-^{14}C	3.83
O-(2,5-Dichloro-4-bromophenyl-^{14}C) *O*-methyl phenylthiophosphonate	Leptophos-*phenoxy*-^{14}C	7.34
O-(2,5-Dichloro-4-bromophenyl) *O*-methyl phenyl-^{14}C-thiophosphonate	Leptophos-*phenyl*-^{14}C	6.22
1,3,4-Methenododecachlorooctahydro-2*H*-cyclobuta[*c*, *d*]pentalene-*U*-^{14}C	Mirex-^{14}C	6.34
1,1,1-Trichloro-2, 2-bis(*p*-chlorophenyl-^{14}C)ethane	DDT-^{14}C	2.7

[a] Of the insecticides listed only carbaryl and carbofuran are currently used on tobacco. Other compounds were included to represent different chemical classes of pesticides.

ticide was dissolved in 0.1 ml of acetone and applied to a 17-mm segment, extending from a point 25 mm from the butt end, of the cigarette using a microsyringe. The needle of the syringe was inserted into the cigarette 10 mm from the treatment zone toward the end to be lighted and passed through the center of the cigarette until the point was at the center of the 17-mm band, and the solution slowly injected into the cigarette. Each insecticide was applied to the 200 mg of tobacco in the treated zone at a concentration of 100 ppm. To evaporate the solvent, the cigarettes were left at room temperature for 2 hr and then refrigerated overnight before use. No degradation of the insecticides occurred during storage of the cigarettes. Impregnation of hundreds of cigarettes in this manner, and subsequent radioassay of subsections of the impregnated zone, showed that the radioactivity was distributed in a bell-shaped fashion with just trace amounts on either side of the marked zone.

Smoking Device. A smoking device was designed to collect, or transfer to the lungs of rats, known volumes of smoke at designated intervals (Figure 1). The mainstream smoke outlet was attached to a valve, B (three-way Teflon valve, Hamilton ML-3000), which allowed a puff to be drawn into syringe C and then transferred to the lungs through a polyethylene tube, D (1.6 mm i.d., 2.1 mm o.d.). Manual operation of the three-way valve and delivery of a 5-ml puff to the animal required 2–3 sec. A 50-ml syringe, E, was attached to the tracheal tube by a 27G needle for the collection of the exhaled smoke. For mainstream smoke analysis, the smoke was collected directly in an air tight syringe at the end of the tracheal tube. For each test, a cigarette was placed in the holder and lit and the cover of the smoking chamber, A, was put into place. Air was passed through the chamber at 500 ml/min to remove the sidestream smoke and to keep the cigarette burning. Sidestream smoke was passed through traps of glass wool (F), cold ethanol (G), and two carbon dioxide trap solutions (H) consisting of a 2:1 mixture of 2-methoxyethanol and 2-aminoethanol. After the appropriate smoking sequence, the air flow through the smoking chamber was stopped to extinguish the cigarette and then operated again momentarily to clear the chamber of any smoke before it was opened to collect the ash and butt.

Inhalation Studies. Female white rats (Sprague-Dawley) weighing 180–200 g and 9–10 weeks old were used in the smoking studies. For each inhalation experiment, an impregnated cigarette was placed in the holder and lit, and the smoking chamber cover put into place. Immediately, a rat was anesthetized with ether, and a midline longitudinal incision made to expose the trachea. A thread was introduced around the trachea and a small hole was clipped between the 4th and 5th tracheal rings caudal to the thyroid cartilage. Just as the cigarette burned to the treatment zone, the tracheal tube of the smoking system was introduced into the trachea and the thread was tied over the trachea and the tube. A 5-ml puff was drawn into syringe C and directed into the lung of the rat. The smoke was held in the lungs for 12 sec, then 5 ml were withdrawn into syringe E. The complete process required 15 sec. After repeating the process for a total of 8 puffs, during which the entire treatment zone was burned, the trachea was immediately clamped and

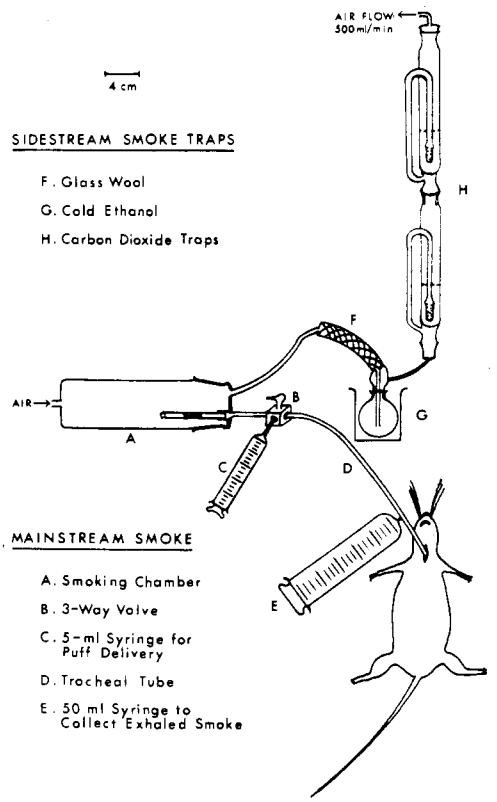

Figure 1. Smoking apparatus used to study the transfer of ^{14}C-labeled insecticides from cigarettes to the lungs of rats.

the air flow through the system was stopped to extinguish the cigarette.

A blood sample was removed from the right ventricle of the heart and the lung and heart were removed and placed in a freezer. Blood was taken about 2.5 min after the last exhalation and the heart and lungs were excised about 1 min later.

Analysis. The blood was immediately radioassayed by combustion in a Beckman Biological Materials Oxidizer and the radioactive carbon dioxide trapped in a 2:1 mixture of 2-methoxyethanol and 2-aminoethanol. Total blood volume of the animals was calculated to be 12.5 ml (Cartland and Koch, 1928). Heart and lung tissues were similarly analyzed after 1 hr.

Radioactive residues in the exhaled air, syringe E, were removed by washing the syringe with ethanol and carbon dioxide trap solution. First, the ethanol was pulled into the syringe, the syringe shaken thoroughly, and the ethanol forced from the syringe until only the needle was filled with solvent. An air space was left between the syringe plunger and solvent to contain any gasses and/or other volatile components not soluble in ethanol. The ethanol wash was repeated, followed by two washings of the syringe with the carbon dioxide trap solution. This same procedure was used in collecting the mainstream smoke radiocarbon for direct analysis. The ethanol wash was concentrated and applied to thin-layer plates (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. Individual components resolved by tlc were quantitated but no attempt was made to identify the pyrolysis products. The radiocarbon suspected of being the original insecticide added to the cigarettes was extracted from the gel and rechromatographed as a mixture with the authentic compound. Cochromatography was achieved with each of the insecticides evaluated.

To determine if the radiocarbon in the carbon dioxide trap solution was actually $^{14}CO_2$, a barium hydroxide trap was used in place of the 2-aminoethanol and 2-methoxyethanol. Approximately the same quantity of material was trapped in the barium hydroxide solution and, upon acidification, about 90% of the radiocarbon dissipated. These data indicated that the radiocarbon was predominately [^{14}C]carbon dioxide and not other volatile components of the insecticides formed during the smoking process.

Radiocarbon in the ash, butt, and smoking chamber was determined by washing or extracting the residues with ethanol. Aliquots of the liquid sidestream smoke traps (G, H) were radioassayed directly while the glass wool, F, was washed with ethanol and then the wash assayed. Ethanol solubles from the sidestream smoke, which included the smoking chamber washes, were analyzed by tlc as described for the mainstream smoke.

All radioassays were performed using liquid scintillation counting (Packard Tri-Carb Model 3380) and a commercial blend of scintillation counting fluid (3a70B, Research Products International Corp., Elk Grove Village, Ill.). All data reported herein were derived from a minimum of five replicates for each experimental parameter investigated.

Inhalation Parameters. During the development of the smoking process described above, various studies were performed to determine the effects of puff volume and lung retention time on the amount of ^{14}C-labeled residues retained in the animal body. The objective was to obtain the maximum level of radiocarbon in the body following the smoking process so that its fate in the rats could be determined.

Cigarettes were impregnated with carbaryl-*naphthyl*-^{14}C as previously described and then the entire treated zone was smoked using 2.5- and 5-ml puff volumes. For each puff volume, the smoke was held in the lungs for 6, 9, and 12 sec. The number of puffs required to smoke the treated portion of the cigarettes decreased as the lung retention time was increased, with the numbers being 22, 18, and 16, respectively, when the 2.5-ml puffs were used. For the 5-ml puffs, the number required was reduced by one-half. Radiocarbon remaining in the body after the last smoke exhalation was determined by radioassay of the lungs, blood, and heart.

In another experiment, the smoking device (Figure 1) was modified by replacing the three-way valve with an in-line flutter valve which opened as the animal inhaled, thus drawing smoke into the lungs, and closed when the animal exhaled, forcing the expired smoke into syringe E. This was a totally automatic process since the plunger of syringe E, lubricated with ethanol, was pushed outward by exhalation but maintained its position during inhalation. Using this system, anesthetized animals smoked the treated zone of the cigarettes in approximately 180 puffs. The average single puff volume was 0.8 ml with the inhalations per minute being between 85 and 95. This situation represented the minimum puff volume and lung retention time attainable with the smoking device used in these studies. Rats were allowed to smoke cigarettes impregnated with carbaryl-*naphthyl*-^{14}C by the self-inhalation method and the level of ^{14}C-labeled residues remaining in the body was compared with those obtained with the standard eight 5-ml puff smoking process, and with a single inhalation of 5 ml of smoke.

The levels of carbaryl-*naphthyl*-^{14}C equivalents in the blood of animals during the self-inhalation and eight 5-ml puff smoking process, and for 4 min after the last puff, were determined. Blood was drawn from the subclavian vein at various intervals after the first inhalation and the total radiocarbon in the samples determined by combustion analyses. In this particular study, ten animals were used for each method of smoke exposure since considerable variation in the blood levels was encountered.

Initially, flutter valves were used in the smoking apparatus (Figure 1) to administer smoke to the lungs of rats rather than using the manually operated three-way valve (B). A flutter valve was inserted on either side of syringe C which allowed the smoke to be drawn into the syringe (valve between syringe C and animal closed) and then transferred to the lungs (valve between cigarette and syringe C closed). Although this arrangement was superior to the three-way valve in regard to ease of operation and rapidity of smoke delivery, it was discarded because 50–75% of the ^{14}C-labeled residues in the mainstream smoke of 5-ml puffs was deposited directly on the valves. With the three-way valve, which operates in a completely open or completely closed manner, the amount of mainstream smoke radiocarbon on the valve was generally less than 1%.

Thirty-five Milliliter Puff Volumes. The use of rats in smoking studies for the purpose of estimating the fate of inhaled insecticide residues in humans would be valid only if the nature of the inhaled residues were the same, and if the amounts were comparable based on lung capacity. These factors were evaluated by determining the quantity and nature of ^{14}C-labeled residues in the mainstream smoke of cigarettes impregnated with each of the insecticides, but not all radioactive preparations listed in Table I, and smoked using 35-ml puffs of 2-sec duration at 1-min intervals. This is the procedure normally used with commercial smoking machines to represent human smoking (Wynder and Hoffmann, 1967). The impregnated zones of the cigarettes were burned completely by three puffs. All other aspects of these studies (treatment of cigarettes, collection of smoke, analyses, etc.) were the same as reported for the 5-ml puffs.

Filters. The effect of filters on the quantity of carbaryl-*naphthyl*-^{14}C equivalents transferred to the mainstream smoke of cigarettes was evaluated using the eight 5-ml

Table II. Effect of Smoke Exposure Method on Retention of Carbaryl-*naphthyl*-^{14}C Equivalents in the Body of Rats Immediately after the Smoking Process

Puffs[a]			% of ^{14}C in smoked portion of cigarette					
				Retained in body[b]				
No.	ml	sec held in lung/puff	Exhaled	Lung	Blood	Heart	Total	Total
1	5.0	12	0.3	0.7	0.2	0.03	0.9	1.2
180[c]	0.8	0.7	4.1	0.2	1.9	0.05	2.2	6.3
22	2.5	6	5.6	1.0	2.4	0.21	3.6	9.2
18	2.5	9	4.1	1.6	2.6	0.33	4.5	8.6
16	2.5	12	2.8	2.1	3.1	0.37	5.6	8.4
11	5.0	6	4.6	2.0	4.5	0.32	6.8	11.4
9	5.0	9	3.7	2.1	4.6	0.44	7.1	10.8
8	5.0	12	3.3	3.5	3.7	0.30	7.5	10.8

[a] With the exception of the single 5-ml puff, the number of puffs represents that necessary for the animal to smoke the entire impregnated zone, 17 mm, of the cigarette. [b] Trachea clamped after exhalation of last puff. Blood collected 2.5 min later; lung and heart excised after 3.5 min. [c] Rats permitted to smoke at normal respiration rate of anesthetized animals.

puff sequence. Four commercial brands of filter cigarettes were impregnated with the insecticide and the radiocarbon in the ash, butt, sidestream smoke, and mainstream smoke was quantitated. Ethanol-soluble ^{14}C-labeled residues in the mainstream smoke were analyzed by tlc. The same procedure was followed using cigarettes from the same package which had the filters removed. Reference cigarettes, 1R1, were included in the study with the butt length adjusted to be equivalent to the tobacco plus filter of the filter cigarettes. When the filters were removed, an equivalent length of the 1R1 cigarettes was also removed. Therefore, it was possible to compare the trapping effect of the filters with a comparable length of plain tobacco.

Commercial cigarettes used in these experiments, designated as brands A–D, each had a filter length of 20 mm except brand B which was 25 mm. The nature of the filters was as follows: brand A, two cellulose acetate filters separated by charcoal granules; brand B, an inner cellulose acetate filter (toward the burning end) containing charcoal granules and an outer cellulose acetate filter; brand C, an inner charcoal-impregnated cellulose acetate filter and an outer cellulose acetate filter; brand D, a single cellulose acetate filter. The treatment zone of the filter cigarettes, 17 mm, was 25 mm from the point where the filter was joined to the cigarettes, making the tobacco-containing butt the same length as for nonfilter cigarettes.

RESULTS AND DISCUSSION

Inhalation Parameters. Retention of carbaryl-*naphthyl*-^{14}C equivalents in the body of rats immediately after exposing the animal to cigarette smoke using different puff volumes, numbers, and lung-contact times is shown in Table II. Of primary importance in this regard was the period of time the smoke was held in the lungs. Maximum retention of the radiocarbon, 76% of that inhaled, was obtained with a single 5-ml puff held in the lungs for 12 sec. Of course, the actual amount of carbaryl-^{14}C equivalents in the body after the single puff was very small, only 0.9% of that radioactivity added to the cigarette. Repeating the 5-ml puff eight times in the same manner, during which the entire impregnated zone of the cigarette was smoked, increased the total carbaryl-^{14}C equivalents inhaled by a factor of 9 and the amount retained by a factor of 8. This smoking sequence resulted in 7.5% of the radioactivity added to the cigarette being retained in the lungs, blood, and heart. Unlike the single 5-ml puff smoking process where 78% of the retained residues were in the lungs, the eight 5-ml puffs resulted in almost equal amounts of residues in the lungs and blood. Because the quantity of radiocarbon remaining in the body using eight 5-ml puffs was greater than with any other smoking procedure, the process was selected as the standard method of exposing rats to insecticide residues in cigarette smoke.

In addition to establishing conditions for maximum body retention of ^{14}C-labeled insecticide residues inhaled in cigarette smoke, the data in Table II indicate some of the factors affecting the degree of transfer and retention of the residues. For example, while the percentage transfer of carbaryl-^{14}C equivalents to the mainstream smoke was obviously greater as the total volume of smoke increased, there was very little difference when considered on a per milliliter basis. This was true not only for carbaryl, but for each of the insecticides evaluated, and for each puff volume used. Under conditions used in these experiments, each milliliter of mainstream smoke contained an equivalent of between 0.2 and 0.3% of the radioactive insecticide in the tobacco burned during the smoking process. Increasing the number of puffs required to burn the same amount of tobacco did tend to reduce the percentage transfer of residues to the mainstream smoke. However, this reduction was grossly evident only when the number of puffs was 180, that required to burn the impregnated zone using the normal respiration rate of anesthetized rats (Table II).

Retention of the inhaled ^{14}C-labeled residues was primarily a function of the period of time the smoke was held in the lungs, and to a lesser extent to the volume of the puff. Increasing either of these parameters resulted in increased retention of the inhaled radiocarbon. Of the retained residues, less was located in the blood as the lung contact time of each puff was increased from 6 to 12 sec; the percentage remaining in the lung tissue showed a corresponding increase. Although there was rapid absorption of the residues by the blood, these data show that, with repeated inhalation, the deposition of insecticide equivalents in the lungs exceeded the rate of their removal by the blood.

Following the smoke-exposure period, carbaryl-^{14}C equivalents continued to increase in the blood (Figure 2). Maximum concentrations were approximately 28% of the total radiocarbon inhaled with both the eight 5-ml puff smoking process and when the animals were exposed to the smoke at normal respiration rates. Blood of rats exposed to smoke by normal respiration rates of anesthetized animals contained maximum levels of radioactivity 1.5 min after the last smoke exhalation, and then the levels began to dissipate gradually. Peak concentrations of carbaryl-^{14}C equivalents in the blood of rats given the eight 5-ml puffs were reached after 3.5 min, and remained unchanged after 4 min, or 6 min after initiating the smoking process. In these experiments, the rats were allowed to continue breathing after smoke exposure, but for all other data reported herein, the trachea was clamped immedi-

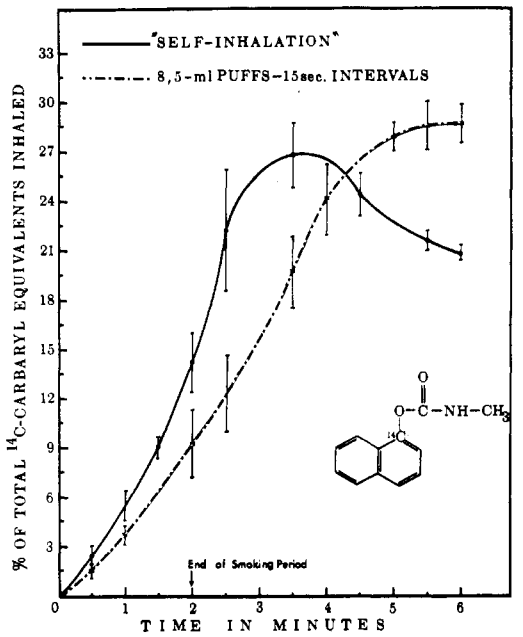

Figure 2. Carbaryl-^{14}C equivalents in the blood of rats resulting from "smoking" cigarettes impregnated with carbaryl-*naphthyl*-^{14}C. The entire impregnated zone of the cigarettes was smoked using the normal respiration rate of anesthetized animals, self-inhalation, or by administering eight 5-ml puffs.

ately after the last smoke exhalation. Blood samples were then collected after about 2.5 min. Data in Figure 2 show that levels in the blood when sampled in this manner would be approximately twice that at the end of the smoking sequence. That the accumulation of carbaryl-^{14}C equivalents in the blood was not appreciably altered by clamping the trachea was demonstrated by the fact that ^{14}C-labeled residue levels in the blood were very similar when the trachea was clamped and when the animals were permitted to continue breathing. This is in agreement with the observation that the heart functioned for about 3 min after clamping the trachea.

Smoking Process and Transfer to Mainstream Smoke. The impregnated zone of the cigarettes weighed 200 ± 29 mg while the amount of tobacco burned per 5-ml puff, including the interval between puffs, was 25 ± 3.8 mg and contained 12.5 ± 6.2% of the compound added to each cigarette. Variation in the amount of ^{14}C-labeled insecticide equivalents per puff was caused largely by the bell-shaped distribution of the insecticide in the spiked zone. Such variations probably did not affect retention of the insecticide equivalents in the rat lung since Egle (1970) reported no difference in acetaldehyde retention in humans with up to 50% variation in concentration. A similar lack of concentration effect was noted in our studies using carbaryl at 25 and 100 ppm.

One of the major objectives of this study was to perfect a simple, reproducible system capable of comparing the potential of different insecticides to be transferred in a toxic form to humans in tobacco smoke. Toxic residues on the leaf at market or in smoke condensates are significant only if they survive the smoking process and are retained in the body at sufficient levels and periods to induce pathological conditions. Based upon the excellent accountability of the radiocarbon added to the cigarettes (Table III), it appears that the procedure described herein could serve as a general method for estimating, at least in part, the significance of pesticides and their metabolites in tobacco products. With the exception of leptophos-*phenyl*-^{14}C, where the recovery was only 67%, total recovery of the compounds ranged from 85% for carbaryl-*carbonyl*-^{14}C to 102% for DDT. The loss of radioactivity with certain of the insecticides indicated that volatile pyrolysis products were formed which were not collected in the traps of the smoking apparatus (Figure 1). Loss of such compounds was reported in a study of the pyrolysis of DDT (Chopra and Osborne, 1971).

There was no consistent difference in the recovery of the insecticides using puff volumes of 5 and 35 ml (Table III). Neither were there any great differences in the percentage transfer of pesticide ^{14}C equivalents to the mainstream smoke when considered on a per milliliter basis. With the 5-ml puffs, 40 ml total, from 7 to 14% transfer occurred, whereas with the 35-ml puffs, 105 ml total, the transfer to the mainstream smoke was 21–32%, resulting in an overall transfer of 0.18–0.34% per milliliter of smoke. Therefore, the quantities of residues inhaled by rats given 5-ml puffs would be comparable in regard to lung capacity to that inhaled in 35-ml puffs by man. With the 35-ml puffs, more of the ^{14}C-labeled residues were deposited in the butt and less given off in the sidestream smoke than with the 5-ml puffs. Only minute residues were in the ash of the cigarettes, regardless of the puff volume or compound involved.

The use of carbaryl, carbofuran, and leptophos radiolabeled at different positions on the molecule showed that the distribution of radiocarbon following smoking of the cigarettes varied little with the position of the radioactive carbon. Carbaryl-*naphthyl*-^{14}C and carbaryl-*methyl*-^{14}C behaved similarly to 1-naphthol-^{14}C, but with carbaryl-*carbonyl*-^{14}C, the levels of residues in the butt were much less. This apparently resulted from the production of $^{14}CO_2$ from the carbonyl carbon which did not condense in the butt as did the other ^{14}C-labeled pyrolysis products. With the exception that radioactive residues in the sidestream smoke were higher with carbofuran-*carbonyl*-^{14}C, the distribution of residues was about the same as for carbofuran-*ring*-^{14}C. In the case of leptophos, the phenyl-labeled material gave lower residues in all fractions than did the phenoxy-^{14}C material. Obviously, some phenyl-^{14}C pyrolysis product(s) was generated which escaped the sidestream and/or mainstream smoke traps.

To compare the distribution and nature of residues in the mainstream smoke using the smoking apparatus in Figure 1 with that of a commercial smoking machine, leptophos-*phenoxy*-^{14}C was added to cigarettes and smoked with both systems using 35-ml puffs. With the syringe method, Figure 1, a 17-mm portion of the cigarette was impregnated with 100 ppm of leptophos in the manner already described. Cigarettes prepared for use on the commercial smoking machine were impregnated throughout their entire length at the same concentration. These latter cigarettes were smoked, 35-ml puffs of 2-sec duration at 1-min intervals, to a butt length of approximately 25 mm and the mainstream smoke, butts, and ash were analyzed. In both cases, the distribution of radiocarbon was calculated on the basis of the leptophos-^{14}C content of the tobacco actually burned during smoking. Results of these analyses showed that the distribution of radiocarbon in the three fractions, and the nature of the ^{14}C-labeled residues in the mainstream smoke, were virtually the same as obtained using the smoking apparatus shown in Figure 1 (Tables III and IV).

As mentioned earlier, 35-ml puffs are used to represent that of man, and have been used in previous studies of the transfer of insecticides to the mainstream smoke of cigarettes. One such study with DDT (Hoffmann and Rathkamp, 1968) showed that 12.4% of the parent compound was transferred to the mainstream smoke. Our 35-ml puff experiment gave almost identical results, showing 12.1% transfer of the applied material. Hengy and Thirion (1971) showed that the amount of the chlorinated hydrocarbon, endosulfan, transferred intact to the mainstream smoke was 15% of that contained in the burned tobacco. This is

Table III. Distribution of Radiocarbon following Smoking of Cigarettes with 5- and 35-ml Puffs

Compd and puff vol, ml	% of radiocarbon added to cigarette[a]								
	Mainstream smoke								
			Sidestream		Traps				Total
	Ash	Butt	smoke	Apparatus	Ethanol	CO_2	Total	Total	Recovery
1-Naphthol-^{14}C, 5	0.3±0.13	19.8±2.0	60.1±2.2	1.3±0.3	7.9±1.4	1.6±0.7	9.5±1.2	10.8±0.4	91.0±4.2
1-Naphthol-^{14}C, 35	0.2±0.09	23.1±3.2	48.3±1.9	6.0±0.8	13.3±1.4	1.5±0.6	14.8±1.8	20.8±2.7	92.4±3.5
Carbaryl-*naphthyl*-^{14}C, 5	0.4±0.11	16.5±2.4	63.8±3.7	1.1±0.2	7.4±1.8	1.8±0.5	9.2±2.1	10.3±2.1	91.0±3.5
Carbaryl-*naphthyl*-^{14}C, 35	0.2±0.03	20.5±0.8	51.1±1.2	5.1±0.3	20.1±0.9	2.0±0.4	22.1±1.1	27.2±0.7	99.0±2.1
Carbaryl-*carbonyl*-^{14}C, 5	0.3±0.07	7.8±1.6	67.4±6.0	0.7±0.1	5.9±1.5	2.5±0.3	8.4±1.4	9.1±0.9	84.6±2.9
Carbaryl-*methyl*-^{14}C, 5	0.5±0.12	14.8±3.2	71.2±4.4	0.8±0.3	7.5±1.1	0.8±0.4	8.3±1.3	9.1±0.8	95.6±3.2
Carbofuran-*ring*-^{14}C, 5	0.5±0.06	13.8±1.3	68.8±2.3	0.8±0.3	7.1±1.6	1.4±0.4	8.5±1.2	9.3±0.6	92.4±2.8
Carbofuran-*ring*-^{14}C, 35	0.4±0.05	18.8±2.5	53.2±2.7	4.0±0.5	22.4±1.7	1.5±0.2	23.9±1.9	27.9±2.0	100.3±3.6
Carbofuran-*carbonyl*-^{14}C, 5	0.2±0.04	10.7±4.0	76.5±4.6	0.8±0.2	6.4±1.4	0.7±0.3	7.1±1.4	7.9±0.7	95.3±3.1
Leptophos-*phenoxy*-^{14}C, 5	0.5±0.16	13.6±1.7	66.3±2.6	1.2±0.1	10.1±0.4	3.2±0.2	13.3±0.8	14.5±0.5	94.9±4.8
Leptophos-*phenoxy*-^{14}C, 35	0.4±0.12	21.6±2.4	38.8±1.8	4.9±0.4	20.8±1.1	1.7±0.5	22.5±2.4	27.4±2.3	88.2±1.1
Leptophos-*phenyl*-^{14}C, 5	0.2±0.04	7.3±2.7	52.6±3.0	0.7±0.1	4.4±0.9	2.0±0.3	6.4±0.4	7.1±0.3	67.2±2.7
Mirex-^{14}C, 5	0.2±0.10	19.6±1.8	61.2±4.1	1.1±0.1	9.0±1.0	2.6±0.4	11.6±0.9	12.7±1.1	93.7±3.7
Mirex-^{14}C, 35	0.4±0.15	25.0±3.6	40.6±3.7	4.2±0.5	23.9±2.7	3.9±0.2	27.8±3.0	32.0±2.9	98.0±5.2
DDT-^{14}C, 5	0.5±0.13	20.2±1.8	65.4±4.2	1.1±0.1	8.7±1.2	2.0±0.3	10.7±0.7	11.8±1.0	97.9±2.1
DDT-^{14}C, 35	0.4±0.09	22.6±1.0	50.0±2.4	4.4±0.6	21.7±2.3	2.4±0.7	24.1±2.1	28.5±2.5	101.5±2.9

[a] The 17-mm portion of cigarette impregnated with 100 ppm of insecticide. Entire impregnated zone smoked using eight 5-ml puffs or three 35-ml puffs.

Table IV. Nature of Radiocarbon in Mainstream Smoke of Cigarettes Impregnated with Ring-^{14}C-Labeled Insecticides

^{14}C-Labeled material	% of total ^{14}C-labeled residues in mainstream smoke/puff vol[a]											
	1-Naphthol		Carbaryl		Carbofuran		Leptophos-*phenoxy*-^{14}C		Mirex		DDT	
	5 ml	35 ml	5 ml	35 ml	5 ml	35 ml	5 ml	35 ml	5 ml	35 ml	5 ml	35 ml
Unaltered compd	53.2	66.4	45.2	41.5	62.5	71.7	20.9	34.6	68.7	71.7	44.1	42.4
Carbon dioxide	14.8	7.2	17.5	7.4	15.0	5.4	22.1	6.2	20.5	12.2	16.9	8.4
Pyrolysis products[b]	32.0	23.0	31.9	44.8	10.8	8.7	50.7	54.2	4.0	7.7	32.1	38.9
Loss[c]	0	3.4	5.4	6.3	11.7	14.2	6.3	5.0	6.8	8.4	6.9	10.3

[a] The 17-mm portion of cigarette impregnated with 100 ppm of insecticide. Entire impregnated zone smoked using eight 5-ml puffs or three 35-ml puffs. [b] Number of pyrolysis products were 6, 7, 2, 4, 3, and 7 for the above compounds, respectively. [c] Loss of ^{14}C-labeled materials during tlc analysis.

in contrast to the 3% transfer reported in another study (Guthrie and Bowery, 1962). Some of the difference in the two values may be explained by the fact that in the latter study, calculations were based on radioactivity added to the total cigarette and not restricted to that in the burned tobacco. Endosulfan was not evaluated in the present study, but with the chlorinated material mirex, 23% was as unaltered compound in the mainstream smoke.

In our studies, 10% of the organophosphorus insecticide leptophos was transferred intact to the mainstream smoke. With the carbamates carbaryl and carbofuran, the transfer was 11 and 20%, respectively. These values are far in excess of the 1% transfer reported for carbaryl and guthion, an organophosphate, in the review article by Guthrie (1968). However, they are similar to the 9% transfer of intact malathion, a rapidly biodegradable organophosphate, obtained in the studies by Hengy and Thirion (1970).

Mainstream Smoke. Smoking the ^{14}C-ring-labeled insecticide-impregnated cigarettes with 35-ml puffs generally did not degrade the compounds as extensively as did the 5-ml puffs (Table IV). Only with carbaryl and DDT were the percentages of parent compound less with the higher puff volume. Mirex was the most stable compound with approximately 70% of the ^{14}C-labeled components in the mainstream smoke as the applied compound. Three pyrolysis products were detected, the major one constituting 52% of their total. This product chromatographed similar to the monodechlorinated derivative of mirex formed by photolysis (Gibson *et al.*, 1972). As with all

Table V. Fate of ^{14}C-Labeled Residues Inhaled by Rats in Cigarette Smoke

Compound	% of total ^{14}C-labeled residues inhaled[a]				
		Retained			
	Exhaled	Lung	Blood	Heart	Recovery[b]
1-Naphthol-^{14}C	29.9	26.7	25.5	2.5	84.6
Carbaryl-*naphthyl*-^{14}C	32.9	31.4	27.2	2.4	93.9
Carbaryl-*carbonyl*-^{14}C	41.9	32.5	15.3	2.0	91.7
Carbaryl-*methyl*-^{14}C	30.9	52.5	6.9	3.2	93.5
Carbofuran-*ring*-^{14}C	52.2	22.8	17.8	3.6	96.4
Carbofuran-*carbonyl*-^{14}C	42.2	30.5	17.3	2.8	92.8
Leptophos-*phenoxy*-^{14}C	30.5	31.8	39.9	3.6	105.8
Leptophos-*phenyl*-^{14}C	53.3	12.0	13.9	1.4	80.6
Mirex-^{14}C	47.3	35.5	11.1	1.3	95.2
DDT-^{14}C	31.3	39.5	27.1	2.1	100.0

[a] Rats given eight 5-ml puffs at 15-sec intervals and the trachea clamped immediately after exhalation of the last puff. Blood then collected after 2.5 min; lung and heart excised after 3.5 min. [b] Based on disintegrations per minute in mainstream smoke collected directly.

other compounds evaluated, the number and relative magnitudes of the mirex pyrolysis products were similar with 5- and 35-ml puff volumes.

Carbofuran was almost as stable as mirex, but only two pyrolysis products of carbofuran were detected by tlc analysis. The products existed in approximately equal concentrations and one chromatographed the same as the phenolic derivative of carbofuran.

The percentage of the ^{14}C-labeled residues in the mainstream smoke as the applied compound, 40–45%, was the same with carbaryl-*naphthyl*-^{14}C and DDT. Both compounds yielded seven pyrolysis products, with 66% of the pyrolysis products of carbaryl consisting of 1-naphthol. DDT has two major degradation products occurring in almost equal quantities which together constituted 64% of the pyrolytic materials resolved by tlc. 1-Naphthol was degraded during the smoking process to form all but one of the pyrolysis products observed with carbaryl-*naphthyl*-^{14}C. However, more 1-naphthol survived the smoking process than did intact carbaryl.

Unaltered leptophos-*phenoxy*-^{14}C accounted for less of the total ^{14}C-labeled residues in the mainstream smoke than with any of the other compounds. Only 21% was as the parent compound with 5-ml puff volumes and 35% with 35-ml puffs. Four pyrolysis products detected by tlc analysis made up about 50% of the mainstream smoke ^{14}C-labeled residues. Of these, 90% was as a compound with the same chromatographic behavior as the phenolic analog of leptophos.

Radioactive carbon dioxide accounted for 15–20% of the ^{14}C-labeled residues in the mainstream smoke when cigarettes were smoked with 5-ml puffs (Table IV). With the 35-ml puffs, the amount of $^{14}CO_2$ ranged from 5 to 12% of the total residues in the smoke. Since more complete degradation, especially to carbon dioxide, of the insecticides took place using eight 5-ml puffs than with three 35-ml puffs, it is apparent that exposure of the compounds to the 850–900° heat at the burning point of the cigarette (Touey and Mumpower, 1957) was greater with the smaller puff volume. Actually, this is probably a function of puff numbers, or frequency, and not the volume of the puff. The amount of parent compound in the mainstream smoke clearly shows that much of the intact insecticides was volatilized away from the intense heat, and suggests that extensive pyrolysis occurred only to that material "trapped," or not volatilized, from the burning zone. This trapping effect apparently takes place to a greater degree during the initial phase of each puff, with increased volatility of the insecticides occurring as the temperature rises in the area just behind the burning zone of the cigarettes. Therefore, less pyrolysis of the insecticide would be expected when the same amount of tobacco was burned with three puffs than with eight puffs.

Fate of Inhaled ^{14}C-Labeled Residues. From 30 to 53% of the inhaled ^{14}C-labeled materials was exhaled, depending upon the individual compound and position of the radioactive carbon in the molecule (Table V). More leptophos-*phenyl*-^{14}C, carbofuran-*ring*-^{14}C, and mirex-^{14}C equivalents were exhaled than with the other materials. With leptophos-*phenyl*-^{14}C and carbofuran-*ring*-^{14}C, this was reflected in the low level of residues in the lung as compared with the other compounds. The highest accumulation of residues in the lungs occurred with carbaryl-*methyl*-^{14}C. The 20% increase in radioactivity between this label and the other two carbaryl labels was probably due to rapid absorption of methylamine-^{14}C by the lung tissues.

Subsequent transfer of the retained residues from the lungs to the blood was quite varied. The levels were low with carbaryl-*methyl*-^{14}C, leptophos-*phenyl*-^{14}C, and mirex-^{14}C. The highest level of ^{14}C-labeled materials in the blood was found with leptophos-*phenoxy*-^{14}C. 1-Naphthol-^{14}C, carbaryl-*naphthyl*-^{14}C, and DDT-^{14}C were similar insofar as the distribution of the retained ^{14}C-labeled residues was concerned. Different levels of ^{14}C-labeled residue in the blood following smoking of the same compound, but radiolabeled at different sites on the molecule, showed that extensive degradation of the compounds had occurred, and that absorption characteristics of the resulting ^{14}C-labeled pyrolysis products were quite different.

There was no apparent difference among insecticides in the distribution of the ^{14}C-labeled residues in the lungs of the rat. Residue levels in different portions appeared to depend more on the surface area of the individual lobe rather than on the nature of the inhaled chemical. Individual analysis of the lobes showed that the distribution of radioactivity in the lungs was directly proportional to the weight of the part being analyzed. In some rats, which were eliminated from the results reported herein, one or more of the lobes were not functional and contained little, if any, radioactivity.

No pattern of retention and/or distribution of inhaled ^{14}C-labeled 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.

Sidestream Smoke. Insecticide residues in the sidestream smoke of cigarettes may serve as a source of contamination to the nonsmoker as well as to the smoker. Analysis of the sidestream smoke of ^{14}C-labeled insecticide-impregnated cigarettes smoked with eight 5-ml puffs showed that the smoke contained considerable quantities of intact pesticide (Table VI). Intact leptophos constituted 21% of the sidestream smoke ^{14}C-labeled residues, the same as it did in the mainstream smoke (Table IV). In fact, the entire composition of leptophos-*phenoxy*-^{14}C equivalents in the sidestream smoke was almost the same as in the mainstream smoke. With all other compounds, the percentage of the residues in the sidestream smoke as the applied material was two- to fourfold less than that in

Table VI. Nature of Radiocarbon in Sidestream Smoke of Cigarettes Impregnated with Ring-^{14}C-Labeled Insecticides and Smoked Using Eight 5-ml Puffs

	% of total ^{14}C-labeled residues in sidestream smoke					
^{14}C-Labeled material	1-Naphthol	Carbaryl	Carbofuran	Leptophos-*phenoxy*-^{14}C	Mirex	DDT
Unaltered compd	17.1	14.9	16.7	20.7	37.1	22.6
Carbon dioxide	62.1	61.4	45.9	28.5	36.4	35.5
Pyrolysis products	15.1	18.2	10.0	45.4	17.0	28.6
Loss	5.7	5.5	27.4	5.4	9.5	13.3

the mainstream smoke; conversion of the insecticides to $^{14}CO_2$ was much greater in the sidestream smoke.

Pyrolysis products of the insecticides, as determined by tlc analysis of the components of the glass wool and ethanol traps, were identical in number and similar in chromatographic behavior in both sidestream and mainstream smoke. No attempt was made to establish that the pyrolysis products were actually the same, but R_f values of corresponding products were similar enough to suggest this possibility. Ratios of the pyrolysis products of each insecticide were different in the sidestream smoke than in the mainstream smoke as evidenced by the autoradiographs; the products in the sidestream smoke were not individually quantitated.

Loss of radioactivity during the analysis of the sidestream smoke was generally of the same magnitude as observed in the mainstream smoke analysis. The major exceptions were with carbofuran and DDT where greater loss occurred during analysis of the sidestream smoke.

Effect of Filters. Different types of commercial filters reduced the carbaryl-*naphthyl*-^{14}C equivalents in the mainstream smoke by 36–49% as compared to the same cigarettes without filters (Table VII). Increasing the butt length of unfiltered 1R1 cigarettes to that equivalent to the filter lengths reduced the mainstream smoke ^{14}C-labeled residues by 28%. Therefore, the reduction of ^{14}C-labeled residues as the result of filters was only 8–21% more effective than an equivalent length of tobacco. Two of the charcoal-containing filters, A and C, were more efficient in trapping the residues than filter D which had no charcoal. However, the third charcoal-containing filter, B, was slightly less efficient than filter D. Very little selectivity was demonstrated by any of the filters. The reduction in the amount of radiocarbon in the mainstream smoke resulted from partial trapping of the parent compound and of the pyrolysis products except $^{14}CO_2$. Consequently, the $^{14}CO_2$ accounted for a greater percentage of the mainstream smoke radiocarbon with the filter cigarettes than with the nonfiltered ones.

Further studies must be conducted to determine the nature and ultimate fate of insecticide residues inhaled in cigarette smoke. Such investigations are now in progress with emphasis on the detailed evaluation of selected insecticides rather than comparing different chemicals. Because the animals, after clamping the incision, can be returned to metabolism cages for further evaluations, it is possible to conduct complete studies of the distribution, metabolism, and excretion of ^{14}C-labeled materials subsequent to smoke inhalation. As is the case for orally consumed residues, an understanding of the nature and fate of insecticide residues inhaled by rats will be useful in estimating their significance in man when similarly exposed.

Table VII. Effect of Filters on the Transfer and Nature of Carbaryl-*naphthyl*-^{14}C Equivalents in the Mainstream Smoke of Cigarettes Smoked with Eight 5-ml Puffs[a]

		^{14}C-Labeled components of mainstream smoke[d]		
Brand	% reduction[b]	Carbaryl	CO_2	Pyrolysis products
A	48.6	0.7	1.4	0.9
B	35.8	0.9	1.2	0.9
C	42.5	0.9	1.4	0.8
D	37.8	0.7	1.6	1.0
1R1[c]	28.4	0.9	1.3	0.9

[a] Cigarettes from same package smoked with and without filters. [b] Reduction in the percentage transfer of radiocarbon from the burned tobacco to the mainstream smoke of filter cigarettes as compared with the same cigarettes with filter removed. [c] Reference cigarettes, unfiltered, with butt length the same as other cigarettes with and without filters. [d] (Per cent of total ^{14}C with filters)/(per cent of total ^{14}C without filters).

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