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Retention and Fate of Inhaled Hexachlorocyclopentadiene in the Rat

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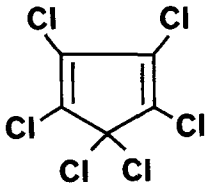
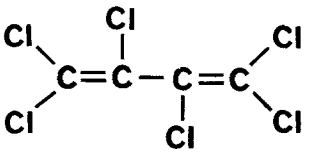
Hexachlorocyclopentadiene (C56) is a volatile oil used in the manufacture of a variety of flame retardants, fungicides, and chlorinated insecticides. It has been shown to be extremely toxic when inhaled by rats, but only moderately toxic when given orally (TREON 1955). The fate of orally administered C56 in rats has been examined (MEHENDALE 1977), but the retention and fate of inhaled C56 vapors have not previously been investigated. The lack of knowledge about the retention, distribution, and ultimate fate of inhaled C56 is the basis for the present study. The distribution and fate of orally administered C56 were also determined to provide a basis for comparison between the two routes of exposure.

MATERIALS AND METHODS

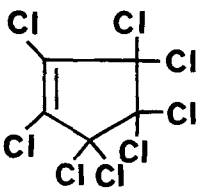
Chemicals. Uniformly-labeled ^{14}C-C56 was obtained from Velsicol Chemical Corporation, Chicago, Illinois, and purified to greater than 95% radiopurity by HPLC (Lichrosorb 10µm; RP-18; 100% methanol at 1.0 mL/min). Non-radioactive analytical standards of C56 and two common impurities (C46 and C58) were also supplied by Velsicol Chemical Corporation. Structures of these compounds are shown below.



Hexachlorocyclopentadiene (C56)



Hexachloro-1,3-butadiene (C46)



Octachlorocyclopentene (C58)

0007-4861/81/0026-0663 $01.20

Dose Administration. Female Sprague-Dawley rats, weighing between 175 and 225 g, were used throughout this study. They were given Purina Laboratory Rodent Chow and water ad libitum. Rats were exposed to ^{14}C-C56 vapors for single 1-h periods with the apparatus shown in Fig. 1. The exposure apparatus and methods of dose quantitation were initially described by STUBBLEFIELD & DOROUGH (1979). The apparatus consists of a generating flask (the inner surface of which is coated with the test material), a one-

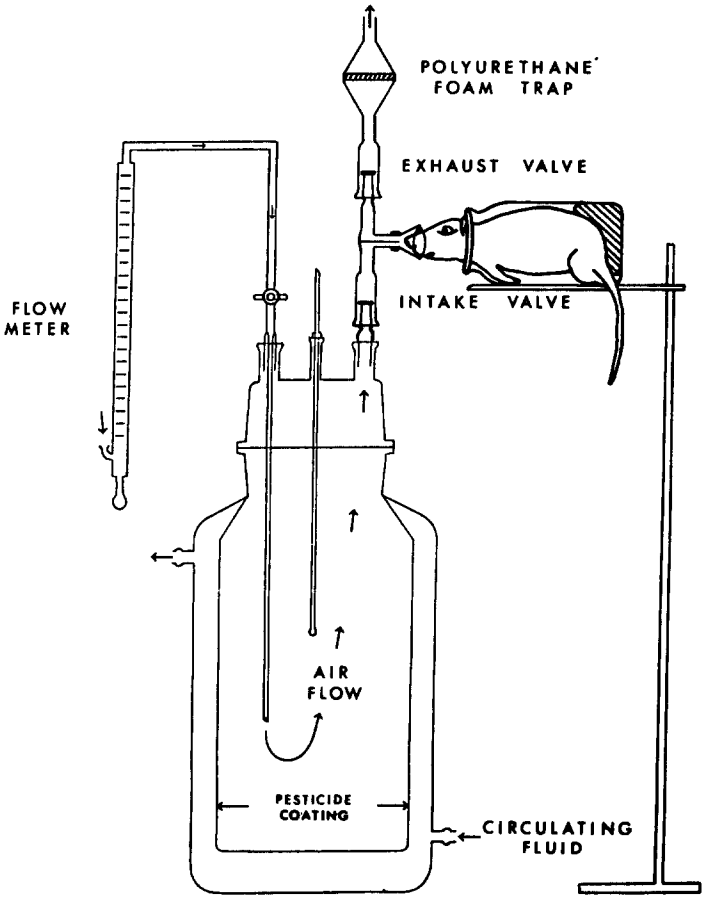


Fig. 1. Apparatus used to expose rats to ^{14}C-C56 vapors.

way valve system which permits separate monitoring of inhaled and exhaled radiocarbon, and a polyurethane foam trap which allows quantitative collection of the exhaled compound. Immediately prior to and following exposure, a standard curve is established which relates air flow to the amount of toxicant contained in the inhaled air, thereby allowing calculation of the inhaled exposure from the rats' mean minute volume (monitored with the bubble flow meter, Fig. 1).

Oral doses of ^{14}C-C56 were administered in 0.5 mL of corn oil with feeding needles (18ga., 12 cm). The reliability of the dosing procedures and methods of dose calculation were confirmed by radioassay of whole rat bodies immediately following treatment.

Radioassay. All radioassays were performed with a Beckman LS 9000 liquid scintillation counter. Ten mL of scintillation cocktail (3a70B, Research Products International, Elk Grove, IL) were used in standard size plastic vials. Aliquots of urine (0.5 mL) were counted directly. All feces, tissues, and tissue homogenates were combusted with a Packard 306 Sample Oxidizer and the trapped $^{14}CO_2$ was assayed.

Analysis of Expired Air. Immediately following treatment with ^{14}C-C56, rats were placed in glass metabolism cages for separate collection of urine, feces, and expired gasses. Air was drawn from the cages (400 mL/min) and passed through traps designed to collect ^{14}C-C56 and $^{14}CO_2$. Polyurethane foam and hexane traps were used to collect ^{14}C-C56. A 2:1 mixture of 2-methoxyethanol and 2-aminoethanol in gas dispersion traps was used to collect $^{14}CO_2$.

RESULTS AND DISCUSSION

When rats were exposed to ^{14}C-C56 vapors for 1-h periods, they retained 83.9% of the inhaled compound (Table 1). Within the range of treatment included in this study (1.4 to 37.4 µg/kg body weight), retention did not appear to be related to the quantity of C56 received. Less than 1% of the administered radiocarbon was eliminated as ^{14}C-C56 in the 0-24 h expired air. No $^{14}CO_2$ was detected. Similar results were obtained when rats were treated orally.

The 72-h fate of radiocarbon was determined following inhalation and oral exposure to ^{14}C-C56. These data are summarized in Table 2.

TABLE 1

Retention of ^{14}C-C56 vapors by rats during a single one-hour exposure period.

Dose (μg/kg body wt)		Ng of ^{14}C-C56 Inhaled	Ng of ^{14}C-C56 Exhaled	Percent of Inhaled C56 Retained
Rat 1	1.4	326	49	85.0
Rat 2	17.3	5023	705	86.0
Rat 3	37.4	9962	1925	80.7
Mean ± S.D.				83.9 ±2.8

TABLE 2

Fate of radiocarbon following inhalation and oral exposure to ^{14}C-C56 in rats.[a]

Substrate	Cumulative Percent of Dose Inhalation	Oral
	24 Hours	
Urine	29.7 ±4.5	22.8 ±1.8
Feces	17.0 ±7.5	62.2 ±8.0
	48 Hours	
Urine	32.5 ±5.1	24.0 ±1.9
Feces	21.0 ±7.5	67.7 ±5.1
	72 Hours	
Urine	33.1 ±4.5	24.4 ±1.9
Feces	23.1 ±5.7	68.2 ±5.1
Body	12.9 ±4.7	0.2 ±0.2
Total	69.7 ±9.6	92.8 ±4.7

[a]All values are the mean ± S.D. of three replicate animals treated with 5 μg ^{14}C-C56/kg body weight.

Inhaled ^{14}C-C56 was primarily excreted in the urine (33.1% of the dose) and 23.1% of the dose was eliminated in the feces. After 72 h, 12.9% of the administered radioactivity was still present in the body.

When ^{14}C-C56 was administered orally, the feces were found to be the primary route of elimination (68.2% of the dose). Radiocarbon excreted in the urine was equivalent to 24.4% of the dose and only trace amounts of radioactivity remained in the tissues after the 72-h period (0.2% of the dose).

The distribution of the 72-h residues among selected tissues was determined for rats exposed to ^{14}C-C56 via inhalation and orally. These data appear in Table 3 and are expressed as the percentages of dose present in the entire tissue and as the concentration of C56 equivalents in each tissue. This presentation of the data allows comparison between the two routes of exposure, even though the quantity of C56 administered was different for the two groups of animals. The minimal retention of radiocarbon by the tissues of orally treated rats (Table 2) made it necessary to increase the dose and the total amount of administered radioactivity in order to obtain residue levels high enough to permit radioassay.

TABLE 3

Tissue distribution of radiocarbon 72 hours after inhalation and oral exposure to ^{14}C-C56 in rats.

	Inhaled Dose[a] (24µg/kg)		Oral Dose[a] (6mg/kg)	
Tissue[b]	% of Dose	PPB	% of Dose	PPM
Trachea	0.3 ±0.1	107.0 ±65.0	0.01 ±0.00	0.29 ±0.17
Lungs	2.0 ±0.4	71.5 ±55.2	0.07 ±0.04	0.42 ±0.25
Liver	0.4 ±0.2	3.6 ± 1.9	0.39 ±0.06	0.54 ±0.07
Kidneys	0.8 ±0.2	29.5 ±20.2	0.47 ±0.06	3,27 ±0.08
Carcass	7.8 ±2,0	1.3 ± 0.6	1.87 ±1,16	0.06 ±0.04
Total	11.4 ±2.5		2.82 ±1.10	

[a] Large difference in dose was necessary because of low residues following oral dosing. All values are the mean ± S.D. of three replicate animals.

[b] Fat contained only trace quantities following both exposure routes. Carcass includes total homogenate of body excluding tissues shown.

For rats exposed to ^{14}C-C56 vapors, the trachea and lungs were the tissues of highest residue concentration (107.0 and 72.5 ppb, respectively). The concentration of C56 equivalents was 7 times higher in the kidneys than the liver (29.5 vs. 3.6 ppb); however, the liver contained about half as much total radioactivity as the kidneys because of its greater size.

When ^{14}C-C56 was administered to rats as a single oral dose, the kidneys and liver were also major sites of residue deposition (3.27 and 0.54 ppm, respectively). The concentration of radiocarbon in the kidneys was about 6 times as great as in the liver, but the total amount of radioactivity was only slightly higher in the kidneys, as was the case for inhalation exposure. The lungs were also a site of residue deposition following oral treatment, with the concentration of C56 equivalents being only slightly lower in the lungs than in the liver (0.42 ppm compared to 0.54 ppm). The fat was not a site of residue accumulation for either route of exposure.

Results obtained in this study indicate that the route of C56 exposure has a significant effect on the elimination and retention patterns. Inhaled C56 is primarily excreted in the urine, whereas orally administered C56 is primarily eliminated in the feces. These results are in conflict with those reported by MEHENDALE (1977), which indicated that only 10% of a single oral dose was eliminated in the feces and more than 50% of the administered radioactivity was eliminated in the expired air. Reasons for these differences are not clear at present.

Differences in the elimination and retention patterns of C56 as a function of exposure route study may be attributable to poor absorption of the orally administered compound. The basis for these differences and the effect they may have on the toxicity of C56 as a function of the route of exposure will be explored further.

ACKNOWLEDGMENTS

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