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Quantification of Phencyclidine in Mainstream Smoke and Identification of Phenylcyclohex-1-ene as Pyrolysis Product

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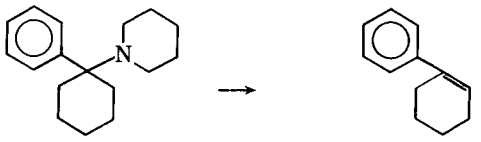
Abstract □ Parsley cigarettes containing [^{3}H]phencyclidine were machine smoked, and the mainstream smoke was trapped in glass wool filters. Radioactivity was extracted from these filters with chloroform. The average recoveries of radioactivity were 76, 85, 70, and 69% for cigarettes containing 3, 10, 30, and 50 mg of [^{3}H]phencyclidine hydrochloride, respectively. TLC and GLC–mass spectrometry were employed to identify and quantify compounds in the filter extracts. Approximately one-half of the recovered radioactivity represented a pyrolysis product, phenylcyclohex-1-ene. Formation of this product involved loss of piperidine from phencyclidine. Piperidine, which was not radiolabeled, also may appear in smoke intact. The remainder of the radiolabeled material represented unchanged phencyclidine. Therefore, the percentage of [^{3}H]phencyclidine delivered was ~40% of the amount smoked. This result was independent of puff frequency and quantity of phencyclidine hydrochloride smoked over the range tested. The [^{3}H]phencyclidine delivery was compared to the quantities of [^{3}H]-Δ^9-tetrahydrocannabinol and [^{3}H]nicotine delivered in mainstream smoke. The recovery of unchanged [^{3}H]-Δ^9-tetrahydrocannabinol from placebo marijuana cigarettes injected with a solution containing 3 mg of Δ^9-tetrahydrocannabinol was 60%. Tobacco cigarettes injected with [^{3}H]nicotine yielded 70% unchanged nicotine in mainstream smoke.

Keyphrases □ Pyrolysis—formation of phenylcyclohex-1-ene from phencyclidine in smoke □ Phencyclidine—identification of pyrolysis product phenylcyclohex-1-ene in smoke □ Phenylcyclohex-1-ene—pyrolysis product of phencyclidine identified in smoke □ Smoke—identification of phenylcyclohex-1-ene, a pyrolysis product of phencyclidine

Phencyclidine [1-(1-phenylcyclohexyl)piperidine] has gained popularity as a drug of abuse, probably because it produces euphoria, dissociation, and hallucinations (1). Smoking phencyclidine-impregnated spices is currently a major method of abuse. The high temperature attained in a burning cigarette raises the possibility that phencyclidine may evolve pyrolysis products when smoked. Phencyclidine is converted to phenylcyclohex-1-ene in the gas chromatograph at low temperatures (2) (Scheme I), which may have led to the conclusion that phenylcyclohexene is a metabolite of phencyclidine (3). Preliminary results indicate that phenylcyclohexene also is formed during smoking (4). In this study, the amount of phencyclidine delivered in mainstream smoke was determined and the pyrolysis products were identified and quantified.



phencyclidine → phenylcyclohex-1-ene

Scheme I

EXPERIMENTAL

Drugs—Phencyclidine hydrochloride[1], piperidinocyclohexane carbonitrile[1], [*phenyl*-1-^{3}H(*n*)]phencyclidine[1] (17 Ci/mmole), Δ^9-tetrahydrocannabinol[1], and [1′,2′-^{3}H]-Δ^9-tetrahydrocannabinol[1] (43 mCi/mmole) were used. [*pyrrolidinyl*-4′,4-^{3}H]Nicotine (4.7 Ci/mmole) was synthesized according to the procedure of Vincek *et al.* (5).

Preparation of Cigarettes—Cigarettes containing phencyclidine were prepared using commercially available parsley flakes[2], 55-mm cigarette tubes[3], and a cigarette machine[4]. Each cigarette (550–600 mg) was prepared immediately prior to its use by injection with 0.5 μCi of [^{3}H]-phencyclidine and 3, 10, 30, or 50 mg of phencyclidine hydrochloride in volumes of 50, 50, 150, or 200 μl of ethanol, respectively. Filterless cigarettes[5] (84 mm) were injected with 0.5 μCi of [^{3}H]nicotine in 50 μl of ethanol, and placebo marijuana cigarettes[1] were injected with 0.5 μCi of [^{3}H]-Δ^9-tetrahydrocannabinol and 3 mg of Δ^9-tetrahydrocannabinol in 50 μl of ethanol. All drug solutions were injected axially and evenly throughout the middle 35 mm of the cigarettes and allowed to dry before smoking.

Procedure—Cigarettes were inserted in a holder and attached to the smoking apparatus (Fig. 1). Smoking was effected by negative pressure provided by water aspiration, and smoke was collected on two contiguous filters (each consisting of 1–1.25 g of glass wool stuffed in 16 cm of 0.635-cm i.d. tubing[6]). Interfaced between the filters and vacuum source were an empty trap (for back-siphoning) and a trap containing 10 ml of chloroform.

Puffing was accomplished with an electric switching valve[7] that opened and closed the system to the vacuum every 5.8 sec. Puff frequency was altered by replacement of a capacitor in the circuit board controlling the valve. A vacuum regulator[8] was set to permit delivery of 45 ml of smoke/5.8-sec puff duration. After an entire cigarette was smoked, each

[1] National Institute on Drug Abuse.
[2] McCormick and Co., Baltimore, Md.
[3] Dominion Cigarette Tube Co., Montreal, Canada.
[4] Premier Supermatic, Central Tobacco Manufacturing Co. Ltd., Montreal, Canada.
[5] Pall Mall, American Tobacco Co., Richmond, Va.
[6] Tygon Tubing and Molded Products, Akron, Ohio.
[7] Skinner, New Britain, Conn.
[8] Fairchild, Winston-Salem, N.C.

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Table I—Recovery of [^{3}H]Phencyclidine from Mainstream Smoke

[^{3}H]Phencyclidine[a], mg	Radioactivity Recovered[b], %	Quantity Recovered[c], mg (micromoles)	
		Phencyclidine	Phenylcyclohexene
2.62	76 ± 4	1.43 (5.88)	0.56 (3.54)
8.73	84 ± 4	4.62 (19.01)	2.71 (17.15)
26.20	70 ± 4	11.19 (48.01)	7.15 (45.20)
43.63	69 ± 5	16.26 (66.92)	13.84 (87.57)

[a] Quantity of [^{3}H]phencyclidine injected into cigarettes that corresponds to 3, 10, 30, or 50 mg of the hydrochloride salt. [b] Means ± *SE* of $n = 3$. [c] Determined by TLC analysis.

filter tubing unit was washed with chloroform (40 ml), and 50-μl aliquots were placed in scintillation vials with 10 ml of aqueous counting scintillant[9]. A rinse of the cigarette holder was included in the wash of the first filter. Each sample was assayed for radioactivity in a scintillation counter[10]. Quench was corrected by external standardization.

Temperature of Burning Cigarette—A thermocouple[11] measured the temperature within a burning parsley cigarette. The temperature-sensitive electrode was inserted perpendicular to the long axis of the cigarette at its midpoint, and the temperature was recorded every 5 sec.

TLC—To quantify [^{3}H]phencyclidine and identify pyrolytic products, 150 μl of each chloroform wash of the glass wool was applied as a band to each of two 5 × 10-cm silica gel TLC plates[12] along with 5 μg of phencyclidine and phenylcyclohexene. One plate was developed in chloroform–methanol–concentrated ammonia (90:10:0.15), in which phencyclidine had an R_f value of 0.42 and phenylcyclohexene moved with the solvent front. The other plate was developed in hexane–concentrated ammonia (5:0.04), in which phencyclidine remained at the origin and phenylcyclohexene had an R_f value of 0.42. The bands were visualized by exposure to iodine vapor, and the plates then were divided into bands and scraped into scintillation vials containing 0.5 ml of methanol and 0.5 ml of water. The samples were sonicated for 30 min, and radioactivity was counted in 10 ml of counting fluid[13]. Counting efficiency was corrected by internal standardization using [^{3}H]toluene[14].

The [^{3}H]nicotine and [^{3}H]-Δ^9-tetrahydrocannabinol smoke condensates were analyzed by TLC as already described. The solvent system for [^{3}H]nicotine was ether–acetone–concentrated ammonium hydroxide (4:1:0.15). The samples were cochromatographed with 3 μg of nicotine (R_f 0.67), and the standard was visualized by spraying with Dragendorff solution. The [^{3}H]-Δ^9-tetrahydrocannabinol samples were cospotted with 3 μg of Δ^9-tetrahydrocannabinol. After development in 40–60% petroleum ether–ether (4:1), Δ^9-tetrahydrocannabinol (R_f 0.56) was visualized by spraying with 0.1% Fast Blue B in 1 *N* NaOH.

GLC–Mass Spectrometry—The instrument[15] was operated at an accelerating voltage of 1.55 kv, an electron energy of 70 ev, a separator temperature of 250°, an ion source temperature of 250°, and an injector temperature of 200°. The glass column (1 m × 2 mm) was packed with 3% (w/w) SP-2250 on 80–100-mesh Gas Chrom Q[16], and the carrier gas (helium) flow rate was 30 ml/min. The initial column temperature of 160° was increased to 180° immediately after phenylcyclohexene was eluted (0.95 min).

RESULTS

The smoking interval for the average cigarette (smoked in its entirety) was ~6 min when a puff interval of 5.8 sec was used. The temperature at the midpoint of the parsley cigarettes ($n = 8$) reached a maximum of 671 ± 13°. The midpoint temperature was >400° for ~0.5 min (5 mm of cigarette burned), indicating that the cigarette components were subjected to high temperatures for a considerable time.

Recovery of Radioactivity from Cigarettes—After the smoking of a cigarette containing 3 mg of [^{3}H]phencyclidine hydrochloride, 76% of the radioactivity was found in the first filter and none was found in the second (Table I). Increasing the quantity of [^{3}H]phencyclidine hydrochloride in the cigarettes did not affect recovery. The possible effect of puff frequency on recovery also was investigated by increasing the interpuff interval from 5.8 to 30 sec. At the lower puff frequency, 67 ± 6% ($n = 3$) of the radioactivity in a cigarette containing 10 mg of [^{3}H]phencyclidine hydrochloride was recovered, which was slightly less than that recovered with the 5.8-sec interpuff interval.

TLC Analysis—In the solvent systems used, either phenylcyclohexene ran with the solvent front or phencyclidine remained at the origin. To ensure complete separation, the samples were chromatographed using two systems. The percentages of recovered radioactivity corresponding to phencyclidine and phenylcyclohexene were the same regardless of the TLC system employed.

All of the condensates contained appreciable quantities of phenylcyclohexene, which ranged from 37 to 54% of the recovered material (Table I). The percentage of phencyclidine converted to phenylcyclohexene increased somewhat as the quantity of phencyclidine was increased. Alteration of puff frequency produced no change in the relative amounts of phencyclidine and phenycyclohexene recovered. Experiments also were carried out to determine whether there was a constant delivery of radioactivity throughout the smoking process. Collection of smoke from the burning of the first third of the cigarette resulted in 16.3 ± 1% of the total recovered radioactivity; the second third accounted for 31 ± 5.6%, and the last third was 52.3 ± 7.0% of the total recovered ($n = 3$/group). The total recovery of the added [^{3}H]phencyclidine was 68 ± 5%.

Identification and Quantification by GLC–Mass Spectrometry—To identify phencyclidine and its pyrolytic products, mass spectra of standard compounds were recorded. Piperidinocyclohexane carbonitrile was injected into the GLC–mass spectrometer where it was readily converted to piperidinocyclohex-1-ene (6), which had a retention time of 0.8 min. Piperidinocyclohex-1-ene had a base peak at m/z 164 and abundant ions at m/z 150 (80% relative intensity), 136 (65%), and 122 (39%). Phenylcyclohexene (retention time 0.9 min) had a molecular ion at m/z 158 (75%) and a base peak at m/z 129. The mass spectrum of phencyclidine (retention time 3.7 min) was similar to that reported previously (2) in that m/z 200 was the base peak and abundant ions were at m/z 158 (57%), 129 (54%), and 91 (93%).

Three smoke condensates were selected randomly for continuous GLC–mass spectrometric scanning. Phencyclidine and phenylcyclohexene were present in all three samples, and no other pyrolysis products were identified. These same three samples also were analyzed by single-ion monitoring. Ions m/z 129 and 200 were detected at the appropriate retention times for phenylcyclohexene and phencyclidine (Fig. 2), but there was no detectable m/z 164 for piperidinocyclohex-1-ene. For quantitative purposes, separate calibration curves were constructed for phencyclidine and phenylcyclohexene in the 50–500-ng/μl concentration range by monitoring ions 200 and 129. The average ratio of phencyclidine to phenylclohexene in the three samples was 1.04 ± 0.02 on a molecular basis, which was in good agreement with the TLC analysis. Since phencyclidine is labile in the gas chromatograph (2), phencyclidine standards were analyzed for degradation products. Less than 0.5% of the phencyclidine was converted to phenylcyclohexene under the GLC–mass spectrometric conditions employed (Fig. 2).

Recovery of Other Drugs of Abuse in Smoke—Placebo marijuana cigarettes ($n = 3$) spiked with [^{3}H]-Δ^9-tetrahydrocannabinol were smoked, and 94 ± 3% of the radioactivity was recovered in the first filter. Likewise, 73 ± 1% ($n = 3$) of the radioactivity in tobacco cigarettes in-

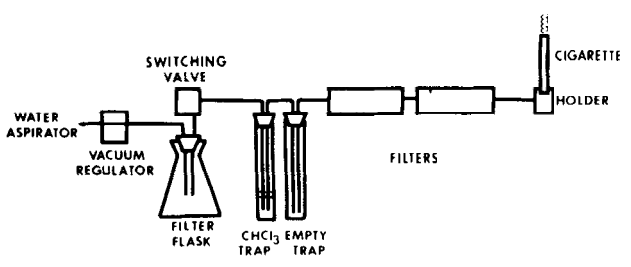

Figure 1—*Smoking apparatus.*

[9] ACS, Amersham Corp., Arlington Heights, Ill.
[10] Beckman Instruments, Palo Alto, Calif.
[11] Wahl Instruments, Culver City, Calif.
[12] Analtech, Newark, Del.
[13] Spectrafluor, Amersham Corp., Arlington Heights, Ill.
[14] New England Nuclear, Boston, Mass.
[15] Finnigan 4000 GLC–mass spectrometer, Finnigan, Sunnyvale, Calif.
[16] Applied Science Laboratories, State College, Pa.

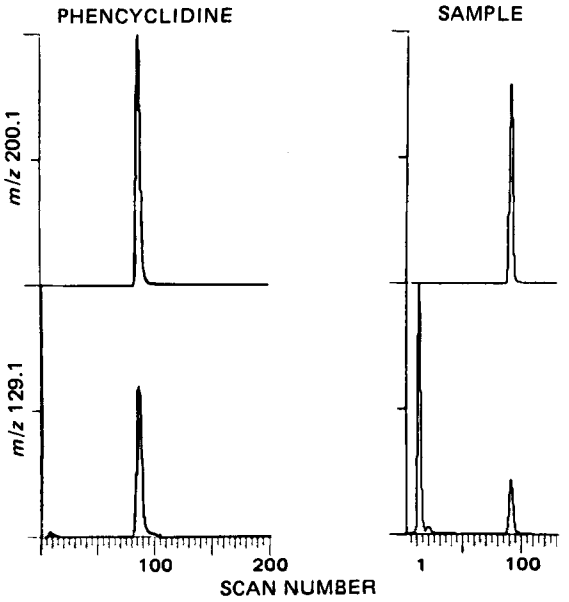

Figure 2—*Single-ion monitoring of phencyclidine and a sample.*

jected with [^{3}H]nicotine was recovered in the first filter. TLC analysis of the [^{3}H]-Δ^9-tetrahydrocannabinol smoke condensate showed that 63% of the radioactivity was due to unchanged material so that 60% of the starting material was delivered in mainstream smoke. Ninety-five percent of the radioactivity recovered from the [^{3}H]nicotine cigarettes remained unchanged. Therefore, 70% of the original [^{3}H]nicotine was recovered from mainstream smoke.

DISCUSSION

In cigarettes containing 3–50 mg of phencyclidine hydrochloride, an amount equivalent to that found in some street samples (7), ~40% of the starting material was recovered from mainstream smoke. The pyrolysis product, phenylcyclohexene, was present in concentrations comparable to those of phencyclidine. Therefore, the percentage of both compounds delivered in mainstream smoke was independent of the quantity of phencyclidine present. Since formation of phenylcyclohexene involves loss of the piperidine moiety from phencyclidine, it would be reasonable to assume that equimolar concentrations of piperidine also are delivered in smoke. Alteration of the puff frequency had little effect on the quantities of phencyclidine and phenylcyclohexene evolved, indicating minor importance of this variable on smoke composition.

This independence of smoke composition from the amount of drug and frequency of puffing suggests that illicit use of phencyclidine-containing cigarettes can be expected to yield amounts of phencyclidine and its pyrolysis product, phenylcyclohexene, similar to those found in this study. Also, the concentration of phencyclidine in mainstream smoke increased as the smoking period progressed, indicating that the drug condensed in the butt as smoking proceeded. This observation suggests that smokers are subjected to varying phencyclidine concentrations during the exposure period.

The recovery of phencyclidine in mainstream smoke was somewhat lower than that found for two other drugs of abuse. However, the recoveries of tetrahydrocannabinol and nicotine were higher than previously found (8, 9). The higher recoveries reported may be due, at least in part, to the high capacity of the glass wool filter to trap smoke components. The short cigarette–filter trap distance used, which approximated the actual smoking situation, minimized drug adsorption on the smoking apparatus. Moreover, cigarettes were burned in their entirety since no data are available concerning butt size of phencyclidine cigarettes. Undoubtedly, the length of cigarette that remains unsmoked varies considerably. The delivery of phencyclidine in smoke as reported in this study would be somewhat higher than the exposure to a smoker.

The relevance of phenylcyclohexene and piperidine intoxication and toxicity due to phencyclidine smoking has yet to be determined. Little is known of the pharmacological effects or toxicity of these products to which phencyclidine abusers are exposed. Preliminary results show the acute lethality of phenylcyclohexene to be an order of magnitude less than that of phencyclidine in rats and mice (4). Likewise, the acute toxicity of piperidine in rats appears to be 10-fold less than that of phencyclidine (10). The pyrolysis and delivery in smoke will be important considerations for experimentation involving inhalation of phencyclidine by smoking.

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