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Weight and activity in male mice after daily inhalation of cannabis smoke in an automated smoke exposure chamber

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An automatic smoking machine capable of delivering marihuana smoke to groups of 10 mice at a time at a constant concentration of Δ^9-THC is described. In all cases the flow rate (50 ml s^{-1} or 100 ml s^{-1}) of a smoke:air mixture, and the dilution factor (1:20 or 1:10 smoke:air) was adjusted to give the same equilibrium concentration of Δ^9-THC in the chamber atmosphere. At a concentration of 0·123 mg Δ^9-THC $litre^{-1}$ of exposure chamber atmosphere (1:20 smoke:air; 100 ml s^{-1} flow rate), a seven day schedule of 20 min daily treatments significantly decreased activity of the marihuana-treated animals by the sixth day over both air and placebo smoke controls. This concentration and exposure schedule failed to affect weight gain during this same period. A significant decrease in activity, although not as great as in the marihuana-treated group, was also seen in the placebo control group compared with the air control animals, indicating the need for a smoke control group in all experiments involving the administration of marihuana via inhalation. An exposure period of 102 min, with marihuana smoke diluted with air 1:10 (50 ml s^{-1} flow rate), at the same equilibrium concentration of 0·123 mg Δ^9-THC $litre^{-1}$, resulted in a mortality of 90% following administration or within 48 h. 102 min of exposure to an atmosphere of placebo smoke at a smoke:air dilution of 1:10 (50 ml s^{-1} flow rate), resulted in no mortality.

Experimental designs involving inhalation of marihuana, the major route in man, have rarely been used in animal studies because of the difficulty of quantifying and standardizing the volume of smoke produced, air dilution factors and correct combustion products (Ho, Fritchie & others, 1970; Ho, An & others, 1972). De Ropp, Kastl & Balbus (1972) administered marihuana smoke to mice by forcing air, for 6 s at 27 litres min^{-1} through a burning cigarette into a plastic holder capable of housing 20 mice. The smoke was held in the chamber for 4 s and then flushed out with air at 13·3 litres min^{-1}. Fried & Nienan (1973) devised a system allowing the administration of marihuana smoke to the nostrils and mouth of rats using a respirator and a cylinder chamber in which the rat's nose and mouth protruded. Rosenkrantz & Braude (1974) studied the acute and subacute inhalation toxicity in Fischer rats exposed to marihuana smoke from cigarettes impregnated with Δ^9-THC under smoking conditions of a 60 ml puff of 2 s duration for 30 s min^{-1}.

We have used an automatic smoking machine capable of delivering marihuana smoke to mice at a constant concentration to determine weight changes and behavioural effects after daily short term exposure for seven days.

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MATERIALS AND METHODS

Meaningful toxicologic data on the effect of marihuana smoke on animals can be obtained by achieving reproducible smoking conditions in an exposure chamber, and by estimating Δ^9-THC concentration by frequent sampling of the chamber atmosphere, and by relating its concentration with time of exposure and effects observed.

Marihuana cigarettes

All studies were conducted with marihuana cigarettes (QCD-72986) assayed by gas chromatography to have 2·1% Δ^9-THC (National Institutes of Mental Health, Rockville, Maryland) and 0·2% each of cannabinol and cannabidiol (NIMH). Placebo cigarettes* (SSC-69417) were of ethanol-extracted marihuana with a Δ^9-THC concentration of approxi-

* Tightness of packing varied between the marihuana and placebo cigarettes due to the extraction process which consisted of percolating the marihuana four times with 96% ethanol over a 10 day period to obtain placebo material. The plant material was then dried air and this was followed by drying at 60° in a forced draft oven. The material was then placed in a continuous extractor using hot 95% ethanol for three days, air dried, oven dried and packed into cigarettes.

mately 0·05%. The mean weights of 84 mm marihuana and placebo cigarettes were 965 ± 10·5 mg (s.e.) and 845 ± 19·7 mg (s.e.), respectively. All cigarettes were kept in an atmosphere of approximately 60% humidity at room temperature (20°) before use. To prevent increased air flow resistance from accumulated tars, cigarettes were burned until 34 mm remained (50 mm smoked). The rate of burn was set at 95 s per 50 mm.

Estimation of Δ⁹-THC concentration in exposure chamber atmosphere

An estimation of the Δ⁹-THC concentration in the chamber atmosphere was by gas chromatographic analysis of samples (20 ml) withdrawn at 5 min intervals, with all-glass syringes, during exposure. The syringes were sealed and the samples allowed to condense on the sides overnight and were then quantitatively transferred with light petroleum (b.p. 40–60°) into vials. Methadone hydrochloride 25 μl of a 2 mg ml^{-1} solution as an internal standard, was added to each sample. The mixture was then evaporated to dryness, and made up in 100 μl of light petroleum for analysis on a Varian Aerograph 2740 with 6 ft × ¼ inch glass columns (2 mm i.d.) packed with 3% OV-17 on 100/120 Chromosorb W HP at a temperature of 235°. The helium carrier gas had a flow rate of 40 ml min^{-1} amp/mV for a 1·0 μg sample injection.

Smoking machine-exposure chamber

An automatic smoking machine, capable of holding 10 mice during each exposure, designed to offer standardization of smoking conditions, was used for inhalation. A vacuum pump (Fig. 1) (L) creates a negative pressure of 5 inches of water (manometer; 0) in a 21 litre exposure chamber (battery jar). The total animal volume is not more than 5% of the total chamber volume, conforming to the loading limits proposed by Silver (1946) as the level above which animal surface effects can cause excessive concentration decreases. A 1 rev min^{-1} electric motor and cam (D) operates a single pole double throw microswitch which alternately opens each valve solenoid for 5 s (A. Skinner, New Brittain, Ct.). Marihuana or placebo cigarettes are fitted to the valves with special adaptors (Philip Morris). Since the vaccuum is maintained by a sensitive vacuum regulator (I; Fairchild, Winston-Salem, N.C.) there is always a constant flow of smoke into the exposure chamber, provided one of the solenoids is always open. This procedure allows correct combustion products to be formed in the cigarette smoke through the generation of puffs

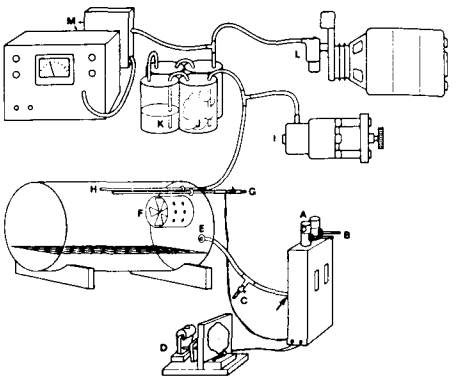

FIG. 1. Diagram of smoking machine and exposure chamber. A Solenoids. B Cigarettes. C Air dilution valve. D Cam motor. E Intake port. F Distributing fan. G Gas sampling port. H Exhaust port. I Vacuum regulator. J Glass wool filter. K Oil filter. L Vacuum pump. M. Capnograph. Arrow points to Y-joint. A manometer was inserted between the exposure chamber and the glass wool filter. Drawing not to scale.

of smoke, and, through continuous influx of smoke, allows an equilibrium concentration to be reached. Immediately before entry into the chamber the smoke from either cigarette was diluted with room air through an adjustable valve (C) set at a ratio of smoke to air of 1:20 or 1:10. This avoids carbon monoxide poisoning in the test animals (Wright, 1972), and more accurately simulates 'real' smoking conditions. Smoke entered through the intake port (B) into the exposure chamber, and was distributed uniformly with a small fan. The exhaust pipe (H) leads to the vacuum regulator and then to a series of 2 glass wool filters (J) and two oil filters (K), allowing filtration of tars and resins before reaching the vacuum pump. A Capnograph (M. Godart Instruments, New York) monitored CO_2 concentrations of filtered smoke during the 20 min exposure time. The Capnograph was calibrated using a 5% CO_2 in oxygen mixture. Smooth-surfaced foam weatherstrip material was used to seal the Plexiglas front and glass exposure chamber. Flow through the cigarette was determined before each run by the use of a calibrator cigarette (mean weight: 965 mg for marihuana; 845 mg for placebo), which was placed in a closely fitting metal tube. Air flow was adjusted to draw 25 ml/5 s interval as determined by a bubble flow meter. Air flow through the dilution valve was determined using a gas flow meter (5–150, American Meter Co., Philadelphia, Pa.) and set to deliver either a 20:1 or a 10:1 dilution (100 or 50 ml s^{-1},

respectively). Following calibration, all tubing between flow meters and the smoke exposure chamber remained attached to prevent change of air resistance factors that would affect cigarette flow. Temperature rise in the chamber was only 2° per 20 min exposure. A ¼ inch mesh animal support was placed on the bottom of the exposure chamber to provide a platform for the animals and allow faeces and urine to drop through to the glass below.

The distance between the solenoid assembly and the intake port was kept at a minimum to avoid excessive condensation of smoke before entry into the chamber. Sharp angles and small diameters in the glass and plastic tubing between the solenoids to the chamber were avoided.

The machine offered the ability to give repeatable concentrations of smoke, Δ^9-THC concentrations, exposure time and to ensure correct combustion products by allowing puffing during 5 s intervals to occur. Animals were free to move around during exposure, although movements decreased rapidly following the introduction of either placebo or marihuana smoke into the chamber. Some periods of hyperactivity during exposure in both groups was observed. All mice had been conditioned to the smoking apparatus in the absence of smoke before exposure periods commenced. Total flow of smoke-air through the chamber was either 105 ml s^{-1} at a 1:20 air dilution (525 ml/5 s interval) or 55 ml s^{-1} at a dilution of 1:10 (275 ml/5 s interval). Preliminary studies revealed an equilibrium concentration of Δ^9-THC was obtained in the exposure chamber within 60 s, and this concentration was maintained during the entire exposure period due to the constant circulation of the chamber atmosphere via the fan and exhaust port. Animals used were random bred male Ha/ICR mice (Flow Labs, Dublin, Virginia), 6–7 weeks old and weighing 20–25 g. They were randomly divided into groups to receive either marihuana smoke, placebo smoke or serve as an air control.

Inhalation protocol

For the determination of the effect of marihuana smoke on activity and weight, groups of 10 animals were simultaneously exposed to either marihuana, placebo smoke or air once daily for 20 min for seven consecutive days. Approximately 12 cigarettes (placebo or marihuana) were burned during each 20 min exposure period. The air dilution factor was calibrated to deliver a 1:20 smoke:air ratio into the chamber at a total flow of 105 ml s^{-1}. Immediately after removal from the exposure chamber, animals were placed individually into activity chambers and monitored for 2 min. The activity testing chamber consisted of a bell jar fitted against a metal platform. The 4500 ml volume bell jar had a 6 inch diameter and was made of clear glass. A light beam source was set up so that a thin beam was directed across the diameter of the chamber, causing a number to register every time a mouse broke the beam (Lafayette Instrument Co., Lafayette, Indiana). Activity counts were taken for 2 min following a 30 s acclimatization period each time the animal was placed in the chamber, and all animals had been acclimatized to the chamber before treatment began. Each animal was observed for incoordination, hyperactivity and ataxia while in the chamber. Between exposure periods, food and water were freely available, all animals were weighed daily. Animals were kept in a room at 25° ± 2, and were exposed at the same time each day for the seven day study. The statistical significance of differences between marihuana, placebo and air groups was evaluated by Student's *t*-test.

RESULTS

The chamber concentration of Δ^9-THC was maintained at 0·123 mg $litre^{-1}$. At this concentration, animals showed some incoordination of movement and a deep but slower rate of respiration during exposure to marihuana smoke. Hyperactivity was

Table 1. *Activity of male mice after administration of marihuana or placebo smoke for seven days.* Daily treatment of 20 min. Values shown as mean ± s.e., n = 10 for all groups.

	Air control	Placebo smoke	Marihuana smoke
Pre-treatment	29·2 ± 3·6	30·6 ± 3·1	35·6 ± 3·4
Day 1	29·8 ± 4·6	11·0 ± 2·1[a]	8·6 ± 2·6[a]
Day 2	19·1 ± 3·5	7·7 ± 1·7[a]	8·1 ± 1·5[a]
Day 3	23·4 ± 3·1	15·8 ± 3·9[a]	15·3 ± 3·5[a]
Day 4	21·4 ± 4·1	13·6 ± 3·1[a]	6·2 ± 2·5[b]
Day 5	18·5 ± 4·2	13·5 ± 2·4	12·0 ± 3·8
Day 6	20·5 ± 4·3	20·3 ± 2·8	10·4 ± 5·1[b]
Day 7	17·7 ± 3·6	14·1 ± 2·6	8·5 ± 2·9[b]
3 days post-treatment	28·2 ± 5·0	27·0 ± 5·1	29·1 ± 4·6

[a] Significantly different from air control $P<0·05$.
[b] Significantly different from both placebo smoke and air control $P<0·05$.

noted for all marihuana-treated animals for approximately 2 min after removal from the chamber, followed by a period of depressed activity lasting over 1 h.

In the seven day study, the three groups of 10 animals, during their pretreatment acclimatization period showed no significant difference in group mean activity (see Table 1). A significant decrease ($P < 0.05$) in activity in the marihuana-treated group relative to the air control and placebo treated group was seen on the fourth, sixth and seventh days of treatment. The marihuana-treated group also showed significant decreased activity ($P < 0.05$) compared to the air control group on days 1–4, 6, 7.

The placebo-treated group showed a significant decrease in activity, although less than the marihuana-treated group, in relation to the air control group on days 1–4. There was no evidence of any degree of tolerance to the placebo smoke before the fourth day. Three days after the last treatment, all activity levels were non-significant. Final body weight/initial weight in the study was + 1·05 for the air control, + 1·06 for the placebo-treated animals, and + 1·02 for marihuana-exposed animals (see Table 2).

Table 2. *Weight change in male mice following administration of marihuana or placebo smoke for seven consecutive days.* Values shown as mean ± s.e., n = 10 for all groups.

	Air control	Placebo smoke	Marihuana smoke
Pretreatment weight	22·7 ± 2·7	20·9 ± 1·7	24·2 ± 2·3
Day 7 Weight	23·9 ± 1·2	22·3 ± 1·2	24·9 ± 0·8
change (g)	+1·2	+1·4	+0·7
% change of pretreatment weight (%)	+5·3	+7·0	+3·0

An exposure of 102 min, with marihuana smoke diluted with air at a ratio of 1:10, at the same concentration of 0·123 mg Δ^9-THC *litre*$^{-1}$, resulted in a mortality of 90% in groups of 10 mice while exposure for 86 min produced death in 1/10 and exposure for 68 and 20 min in 0/10 animals. All deaths occurred within 48 h of exposure to smoke. Representative animals showed pulmonary oedema and some small focal haemorrhages on the surface of the lungs. Exposure to an atmosphere of placebo smoke for 102 min, at a dilution factor of 1:10 (smoke:air), resulted in no mortality.

DISCUSSION

Exposure to atmospheric Δ^9-THC at 0·123 mg litre^{-1} produced effects in mice. The exposure chamber offered repeatable and standardized smoking conditions allowing an analysis of the effects.

The determination of an inhalation dose in this system was not attempted due to the factors affecting absorption from the respiratory tract: respiration rates, tidal volume and blood flow.

At 0·123 mg litre^{-1} of Δ^9-THC, activity was depressed in the initial few treatments with both marihuana and placebo smoke, and not until the fourth exposure did the activity of marihuana-exposed animals drop significantly lower than placebo animals. Thus, it is necessary to have a placebo smoke control. The relatively low dose used caused a biphasic behavioural change of initial hyperactivity in the first few minutes after exposure followed by depressed activity in the marihuana-treated animals, as measured by activity counts and observation. This biphasic pattern has also been reported by Rosenkrantz & Brande (1974). There were no signs of cyanosis in either the placebo or the marihuana groups at the end of any of the 20 min exposure periods. Full recovery of all animals occured within 24 h. There were no significant variations in weight over the seven days, so the marihuana dose was high enough to affect activity counts, but not to affect weight gain. Manning, McDonough & others (1971) have reported a decreased rate of growth in male rats treated chronically with 8 mg kg^{-1} Δ^9-THC orally over many weeks.

After the last exposure, levels of activity returned to almost identical values in all three groups, indicating the depression of activity seen in marihuana-treated animals could possibly be due to an accumulation of active agents. At 0·123 mg of Δ^9-THC *litre*$^{-1}$ of exposure chamber atmosphere, it was not until the fourth day after exposure that there was a significant effect on activity levels in the marihuana-treated animals over both the placebo and air control groups.

In the second series of experiments dealing with mortality, a dilution of smoke:air of 1:10 was employed for different time exposures. The mortality among the 10 mice exposed for 102 min was 90%, with all deaths occurring near the end of the exposure period or within 48 h. All surviving animals were observed for 14 days following exposure. Exposure of placebo-treated animals at the same dilution of 1:10 smoke:air produced no deaths during the same observation period. The mechanism producing death

in the marihuana exposed animals is not known.

Our findings are in accord with those of others for marihuana smoke or Δ^9-THC and our method has been shown to be a vital and controllable experimental procedure for the investigation of this material in animal models.

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