Chapter Three

ON THE CARCINOGENICITY OF MARIJUANA SMOKE

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INTRODUCTION

Marijuana *(Cannabis sativa* L.*)* has become the second most widely used smoke product in the Western World[1-2-3-4]. Marijuana smoke, however, is inhaled in significantly lower doses than tobacco smoke and its effect is predominantly psychotomimetic with some acute toxic side effects. Nevertheless, information as to the carcinogenicity of this inhalant is needed. Since most marijuana smokers are also cigarette smokers, it needs furthermore to be determined whether marijuana smoke can potentiate the carcinogenic effect of tobacco smoke.

Until now, no data have been published as to the presence of tumorigenic agents in and the carcinogenic effects of marijuana smoke[4-5-6]. Recently, a short-term test on mouse skin showed that the particulate matter of marijuana smoke ("tar") induced dose-related sebaceous gland

destruction and epidermal hyperplasia with acenthosis[7,8]. Marijuana smoke has been reported to increase abnormal cell proliferation in epithelial cells of lung explants from mice[9].

The present study was designed to determine the carcinogenic potential of marijuana smoke. In the first phase we smoked marijuana cigarettes under conditions established for tobacco cigarettes[11]; we analyzed the mainstream smoke for toxic and tumorigenic agents and bioassayed its particulate matter for carcinogenicity and tumor-promoting activity on mouse skin. For comparison and as a positive control, we analyzed the smoke of a blended tobacco cigarette and tested its particulate matter for tumorigenicity and tumor-promoting activity.

EXPERIMENTAL METHODS

Marijuana Cigarettes

The marijuana cigarettes were made available by the Division of Cancer Cause and Prevention of the National Cancer Institute and were prepared from marijuana bricks which originated from confiscations. The leaves were separated from the stems and twigs and cut to about 30 cuts per inch. No humectants or other additives were used for the marijuana blend. The paper used for the cigarettes had comparable porosity to the paper used for the tobacco control cigarettes.

Tobacco Cigarettes

Both the tobacco and the marijuana cigarettes were 85 mm long and without filter tips. The tobacco cigarettes were the standard blended cigarettes SEB-I of the Tobacco Working Group of the National Cancer Institute.

Smoking of Cigarettes

The cigarettes for the chemical studies were smoked with 20-channel[12] and single-channel (H. Borgwalt, Hamburg) smoking machines. The cigarettes for the bioassays were

smoked with a multiple unit automatic machine.

Analytical Methods

In Table 1 we have summarized the methods used in this study. For each individual determination in which we needed 10 or less cigarettes, we selected the cigarettes by weight and draw resistance. The experimental deviations of the analytical methods averaged between 4 and 6%, and, in the case of polynuclear aromatic hydrocarbons (PAH), within ±8%.

Determination of Cannabinols

For each analysis, 10 cigarettes were smoked on a 20-port Phipps & Bird smoking machine through Cambridge filters. The loaded filters were extracted with 10 ml absolute ethanol, and the extracts were analyzed by glc. The separation was achieved under the following conditions: 2 m x 3 mm stainless steel column filled with 1.4% OV-17 on Gas Chrom Q (100/120 mesh). The temperatures were: injector block, 250°C; column 200°C; and FID, 290°C. The carrier gas was helium, at a flow rate of 40 ml/min. Under these conditions the retention times were: cannabidiol (CBD), 8.5 min.; Δ'-tetrahydrocannabinol (Δ'-THC), 11.5 min.; and cannabinol (CBN), 16.5 min. The detection limit for Δ'-THC was about 10 ng.

Bioassays

For the bioassays we used Swiss albino female mice (Ha/ICR/Mil). The application of the test suspension was started during the second telogen phase of the hair cycle[23].

a. Complete Carcinogenicity

The experimental groups for the complete tumorigenicity assay were started with 100 mice each. Three times weekly we painted a 50% "tar"-suspension on the shaved backs of the mice. On the average 75 mg of "tar" were given per application. All moribund animals were killed as were the remaining mice, 74 weeks after the

Table 1

SMOKE OF MARIJUANA CIGARETTES AND TOBACCO CIGARETTES
METHODS FOR ANALYSIS AND BIOASSAYS

PARAMETER TESTED	NO. OF CIGARETTES	INTERNAL STANDARD	REFERENCE
I. ANALYSIS			
MOISTURE, CIGARETTE FILLER	2 x 10	-	13
PRESSURE DROP	10 x 1	-	14
STATIC BURNING RATE	10 x 1	-	15
CARBON MONOXIDE, CARBON DIOXIDE	4 x 1	-	16
AMMONIA	2 x 10	^{14}C-CH_3NH_2	17
HYDROGEN CYANIDE	3 x 1		
DIMETHYL- AND METHYLETHYL-NITROSAMINE	300	^{14}C-$(CH_3)_2N$-NO	19
ORGANIC GAS PHASE CONSTITUENTS	3 x 1	-	14
ISOPRENE	3 x 1	-	UNPUBLISHED
pH	3 x 1	-	20
TOTAL PARTICULATE MATTER, DRY	4 x 4	-	21
NICOTINE	2 x 4	^{14}C-NICOTINE	14
CANNABINOLS	3 x 10	^{14}C-Δ^9-CANNABINOL	THIS STUDY
VOLATILE PHENOLS	2 x 10	^{14}C-PHENOL	14
POLYNUCLEAR AROMATIC HYDROCARBONS	300	^{14}C-NAPHTHALENE ^{14}C PHENANTHRENE ^{14}C BENZO[a]PYRENE ^{14}C-BENZ[a]ANTHRACENE	22
II. BIOASSAYS			
COMPLETE TUMORIGENICITY (100 MICE)	50,000	-	23
TUMOR PROMOTING ACTIVITY (60 MICE)	25,00	-	23

first application. A skin lesion of a diameter of at least 1 mm was regarded as a papilloma if the tumor did not regress during the first 3 weeks. The lateral invasion of a tumor into adjacent skin was macroscopically considered as transformation into a carcinoma.

Continued growth of such a lesion, however, was required along with a histologic confirmation of epitheliomas. During autopsy all other suspicious lesions were examined.

b. Tumor-Promoting Activity

Initiation was carried out by treatment of the backs of the mice with a single 75 µg dose of 7,12-dimethylbenz α anthracene (DMBA) in 0.05 ml of acetone during the second telogen phase of the hair cycle and between hours 5 and 7 of the 11-hour light cycle. Ten days after initiation we applied, thrice weekly, 0.1 ml of a 50% acetone suspension of marijuana or tobacco "tar", respectively, on the initiated area (average of 75 mg per application) and, in the case of a control group, 0.1 ml of acetone alone. The experiments were terminated after 12 months of promoter application. Tumors were identified as described in the preceding section.

RESULTS AND DISCUSSION

Smoke Analysis

The smoking of marijuana leaves in the form of cigarettes produces a dense aerosol similar to that from tobacco cigarettes. Both aerosols are generated within and in front of a burning cone. The formation of smoke is a chain of oxidations, hydrogenations, crackings, distillations, and sublimations. The group of toxic agents generated as gas phase constituents defined as those components of which 50% or more pass through a glass fiber filter[11] includes carbon monoxide, nitrogen oxides, ammonia, hydrogen cyanide, and several other volatile organic agents.

Figure 1 compares the gas chromatograms of the mainstream smoke of marijuana and tobacco cigarettes. Qualitatively the organic gas phases are similar; quantitatively only one major difference emerges, namely, the higher concentration of isoprene in tobacco smoke. We found 83 and 310 µg, respectively, isoprene in the mainstream of marijuana and tobacco cigarettes (Table 2). We attribute this difference to the high percentage of terpenoids in the

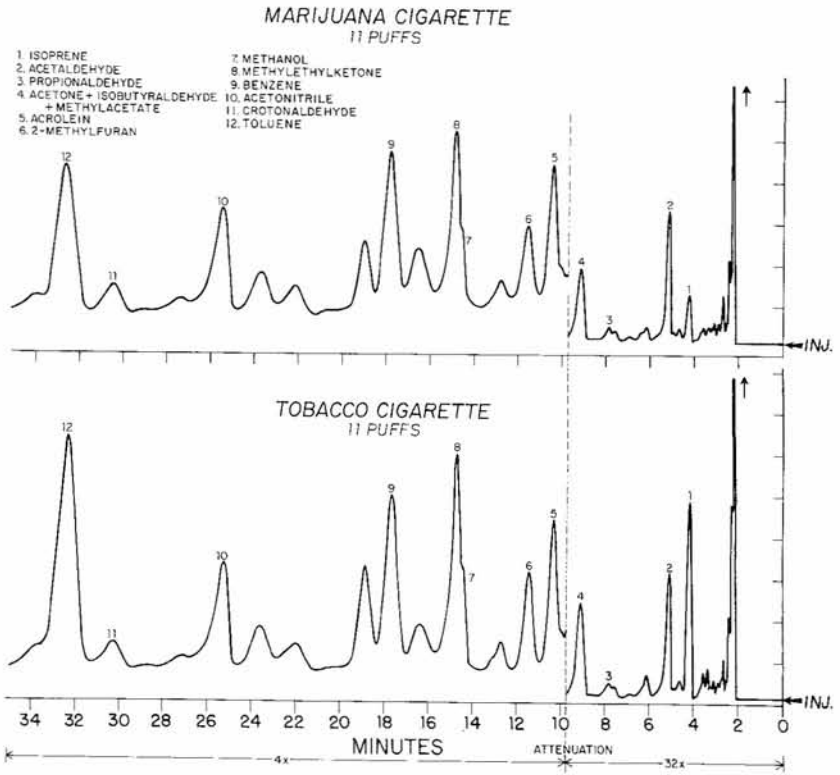

Figure 1. Gas chromatograms of organic compounds in the gas phase of marijuana cigarettes and tobacco cigarettes.

wax-rich layer of tobacco leaves which yield isoprene upon combustion. The burning of a leaf is also influenced by the amount of waxes as indicated by the higher CO concentration in tobacco smoke. In general, the porosity of the cigarette paper is a major factor for the CO concentration in the smoke; however, in this study both cigarettes were wrapped in the same paper.

The amounts of ammonia, hydrogen cyanide, acrolein, acetonitrile, benzene and toluene, and the volatile *N*-nitrosamines are not significantly different between the

Table 2

MARIJUANA AND TOBACCO REFERENCE CIGARETTE
ANALYSIS OF MAINSTREAM SMOKE

	MARIJUANA CIGARETTE 85 mm	TOBACCO CIGARETTE 85 mm
A. ANALYTICAL DATA		
AVERAGE WEIGHT, mg	1115	1100
MOISTURE, %	10.3	11.1
PRESSURE DROP, cm	14.7	7.2
STATIC BURNING RATE, mg/sec.	0.88	
PUFF NUMBER	10.7	11.1
B. MAINSTREAM SMOKE		
I. Gas Phase		
CARBON MONOXIDE, Vol.%	3.99	4.58
mg	17.6	20.2
CARBON DIOXIDE, Vol.%	8.27	9.38
mg	57.3	65.0
AMMONIA, µg	228	198
HCN, µg	532	498
ISOPRENE, µg	83	310
ACETALDEHYDE, µg	1200	980
ACETONE, µg	443	578
ACROLEIN, µg	92	85
ACETONITRILE, µg	132	123
BENZENE, µg	76	67
TOLUENE, µg	112	108
DIMETHYLNITROSAMINE, ng	75	84
METHYLETHYLNITROSAMINE, ng	27	30
PH, 3rd	6.56	6.14
5th	6.57	6.15
7th	6.58	6.14
9th	6.56	6.10
10th	6.58	6.02

two types of smoke. Although the analysis of dimethyl- and methylethylnitrosamine is time-consuming and cumbersome, we determined these agents because they are strong animal carcinogens, though not on mouse skin[24,25]. We detected diethylnitrosamine in both aerosols; however, the amounts were below 5 ng per cigarette. In addition, tobacco smoke contains about 140 ng of nitrosonornicotine as a possible carcinogen[19].

A major toxic factor in tobacco smoke is nicotine, especially if the smoke is alkaline. With increasing pH above 6.0, increasing amounts of nicotine are present in unprotonated form, about 50% at pH 7.8[20]. The unprotonated

form of nicotine is the most toxic form of this alkaloid. Although we expected comparable pH values for marijuana smoke and for the smoke of a blended cigarette, this fact had to be established. For this determination the mainstream smoke of a cigarette was passed over a highly sensitive combination electrode. The recorded pH values for marijuana and tobacco smoke are compared in Figure 2. Since the major toxic agents in marijuana smoke, Δ^1- and Δ^6-tetrahydrocannabinol are phenols (Fig. 3), at pH 6.6 they will be present in unionized form.

Since tobacco alkaloids serve as precursors for volatile pyridines, we found these volatile bases in significantly higher concentrations in tobacco smoke. The values for one marijuana cigarette and one tobacco cigarette were: pyridine, 14.4 and 24.8 μg; α-picoline, 7.4 and 13.1 μg;

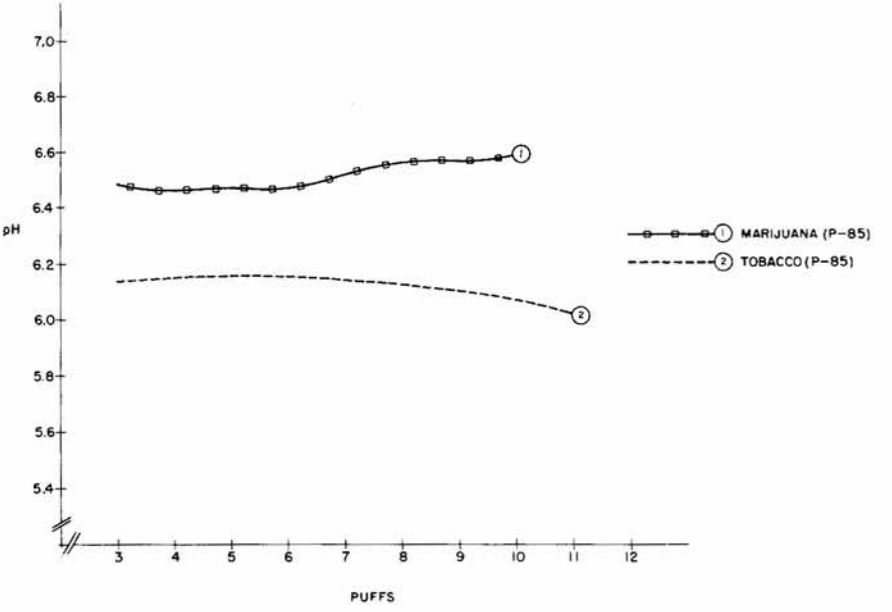

Figure 2. pH of total mainstream smoke of experimental cigarettes: 1) marijuana (P-85), 2) tobacco (P-85).

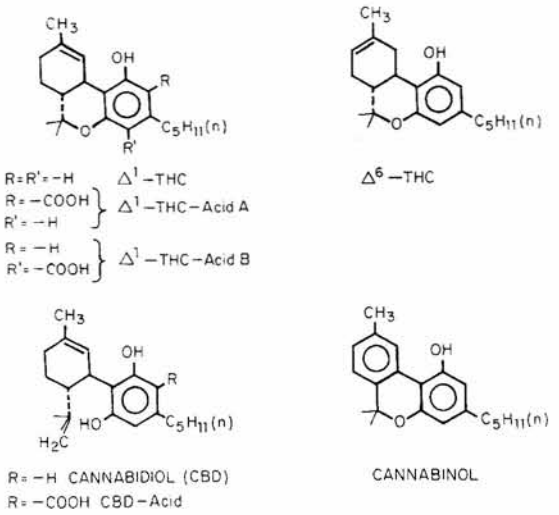

Figure 3. Structures of cannabinoids.

total volatile pyridines measured, 29.0 and 82.8 μg, i.e. about one third the concentration in marijuana smoke compared to tobacco smoke. In contrast to cigar smoke, it is not expected that the pyridines play an important role in toxicity and/or flavor of marijuana smoke.

The particulate matter of tobacco smoke contains about 50% more volatile phenols than marijuana smoke (Table 3). In the experimental animal, volatile phenols have been shown to act as weak tumor promoters[11].

In general, more than half of Δ^1-THC and (CBD) is present in marijuana as the respective acids, Δ^1-THC acids A and B and cannabidiolic acid. During gas chromatographic analysis, these acids are decarboxylated to the free phenolic cannabinols. Average values for the filler of the experimental marijuana cigarettes were found to be 0.61% of Δ^1-THC, 0.32% of CBD, 0.23% of cannabinol (CBN) and about 0.01% of Δ^6-THC (Fig. 3). These values are rather low compared to Mexican marijuana with 3-4% of Δ^1-THC[2] and suggest that the confiscated marijuana used in this study was probably

Table 3

MARIJUANA AND TOBACCO REFERENCE CIGARETTE
ANALYSIS OF MAINSTREAM SMOKE

	MARIJUANA CIGARETTE 85 mm	TOBACCO CIGARETTE 85 mm
B. MAINSTREAM SMOKE		
II. PARTICULATE PHASE		
TOTAL PARTICULATE MATTER, DRY, mg	22.7*	39.0*
PHENOL, µg	76.8	138.5
o-CRESOL, µg	17.9	24
m- and *p*-CRESOL, µg	54.4	65
2,4- and 2,5-DIMETHYLPHENOL, µg	6.8	14.4
CANNABIDIOL, µg	190	
Δ^9-TETRAHYDROCANNABINOL, µg	820	
CANNABINOL, µg	400	
NICOTINE, µg	-	2850
NAPHTHALENE, ng	3000 (136)	1200 (53)
1-METHYLNAPHTHALENE, ng	6100 (270)	3650 (162)
2-METHYLNAPHTHALENE, ng	3600 (160)	1400 (63)
BENZ[a]ANTHRACENE, ng	75 (3.3)	43 (1.9)
BENZO[a]PYRENE, ng	31 (1.38)	22.1 (1.0)
*SIDESTREAM SMOKE, DRY TPM, mg	40.7	57.3

diluted with domestic marijuana (>0.1% THC). With a 62 mm cigarette column (23 mm butt length) we smoked about 660 mg of marijuana (10.3% moisture) and with it about 4.0 mg of Δ^1-THC, 2.01 mg of CBD and 1.5 mg of CBN. Since we found 0.82 mg of Δ^1-THC, 0.19 mg of CBD and 0.4 mg of CBN in the mainstream smoke of these cigarettes, we observed transfer rates of about 20%, 9%, and 26%, respectively. The low transfer rate of cannabidiol indicates its relatively low stability when compared to Δ^1-THC and CBN. The Δ^1-THC concentration of 3.5% in the particulates of the smoke compared to 0.61% in the cigarette filler demonstrates that the smoking of marijuana represents an enrichment process for the most powerful cannabinol in form of a fine, inhalable aerosol and supports the observation that marijuana intake is preferred in the form of smoke.

The nicotine value of 2.85 mg per cigarette is rather high compared with present day commercial nonfilter cigarettes (<2.0 mg; ref. 26); however, the blend for the standard cigarette was formulated about 8 years ago.

Polynuclear aromatic hydrocarbons (PAH) are formed during incomplete combustions of organic matter. Some of the four- to six-ring condensed aromatic hydrocarbons are known carcinogens to animals including mouse skin and serve as tumor initiators when the particulate matter of inhalants is bioassayed[10-27]. We found significantly higher amounts of PAH in marijuana smoke, not only of the naphthalenes, but also of the weak carcinogen, benz[a]anthracene and the strong carcinogen, benzo[a]pyrene. These results do not come as a surprise, since tobacco, especially in a commercial cigarette, burns better and contains less cellulose and cellulose-like constituents than marijuana. Qualitatively, both inhalants contain the same type of PAH, although they differ significantly in their concentration. This fact is best demonstrated by establishing PAH profiles[22]. In order to arrive at reliable quantitative data, we add ^{14}C-labeled naphthalene, phenanthrene, benz[a]anthracene, and benzo[a]pyrene to the "tar" at the beginning of the analysis (Fig. 4). After 2 distribution steps the PAH concentrate is chromatographed on alumina, resulting in 8 PAH fractions. These fractions are analyzed individually by gas chromatography and high speed liquid chromatography. Figure 5 compares the glc profiles for the phenanthrene fractions of the "tars" of four smoking products, and shows the higher concentrations of PAH in the phenanthrene and fluorene fraction of marijuana smoke.

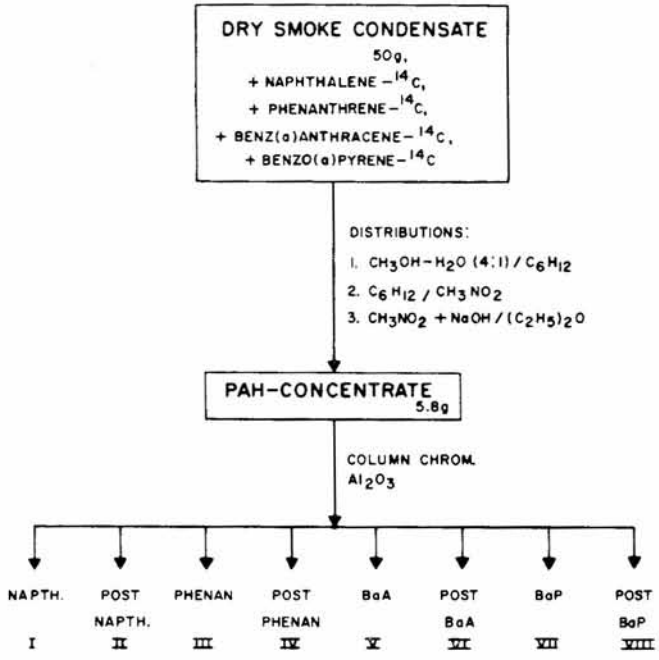

Figure 4. Enrichment method for polynuclear aromatic hydrocarbons from combustion products.

In summary, chemical analysis of the mainstream smoke of marijuana and tobacco has shown that, with the exception of the cannabinoids and tobacco alkaloids, both inhalants are qualitatively similar. Quantitatively, tobacco smoke contains about 3 times the concentration of isoprene and 50% more of the weakly tumor-promoting volatile phenols. Due to its poorer combustibility, marijuana smoke contains about 50% more of the carcinogenic hydrocarbons.

Bioassays

In Figure 6 we have summarized our findings on the mouse skin bioassay for the complete tumorigenicity of marijuana smoke condensate and for tobacco smoke condensate, the positive control. The chronic toxicity of tobacco "tar" was somewhat higher as indicated by the lower survival rate of the mice after about 6 months of treatment. After 74

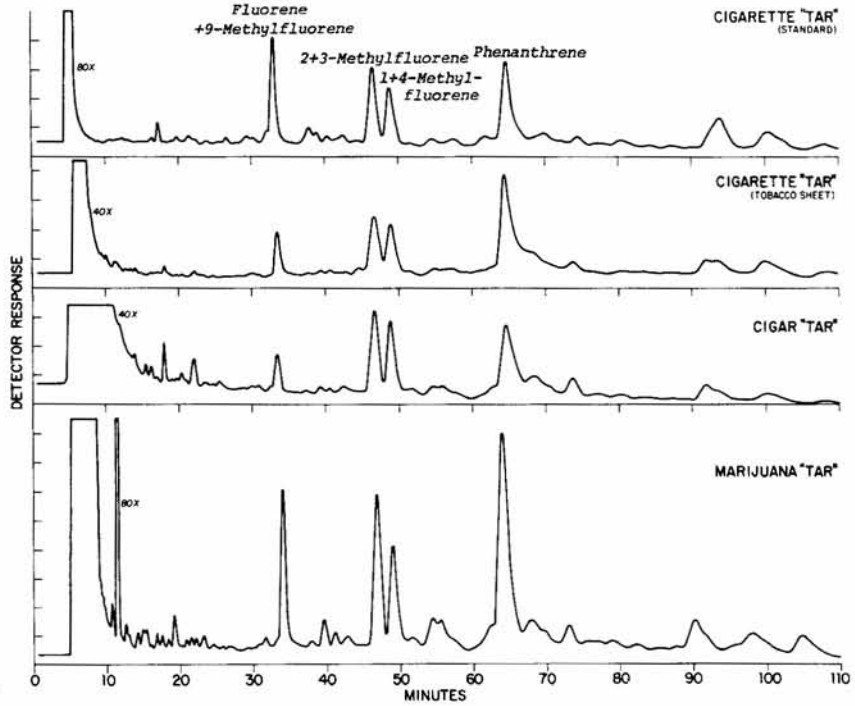

Figure 5. Gas chromatograms of phenanthrene fractions of condensates from various smoke products (each chromatogram was a concentrate from 48 mg condensate).

weeks of "tar" applications, 34% of the "marijuana mice" had survived as compared with only 20% of the "tobacco mice". The first tumor appeared after 17 weeks of "tar" application with 99 mice at risk. At the end of the experiment (74 weeks "tar" application), 6 out of 100 mice in the marijuana groups had developed 7 skin tumors and 14 out of 100 mice of the tobacco group had developed 18 skin tumors (first tumor, 18 weeks; at risk, 97 mice). All of the skin tumors observed after marijuana "tar" application were benign tumors (papillomas) whereas 2 out of the 14 tumor-bearing mice of the tobacco group had malignant tumors (carcinomas), and the

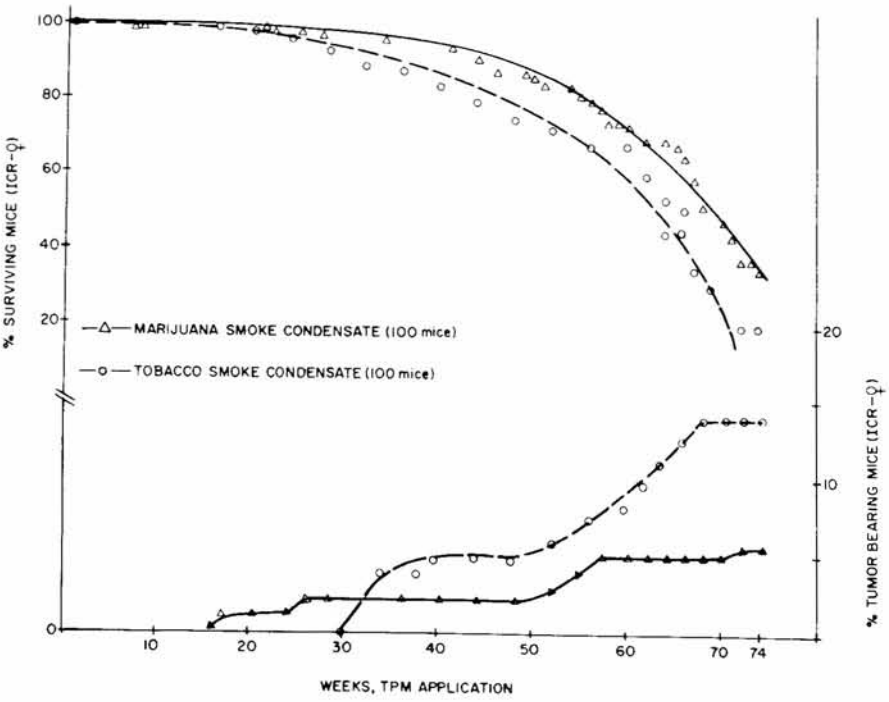

Figure 6. Survival rate and tumor response to cigarette smoke condensate: Complete carcinogenicity.

rest had papillomas. For this strain of Swiss mice we rarely observed skin tumors after treatment with the solvent acetone alone[11]. We can conclude, therefore, that in the mouse skin bioassay marijuana smoke condensate is a weak tumorigenic agent. The activity of marijuana "tar", however, is significantly lower than that of smoke condensate from tobacco cigarettes. In addition to the skin tumors, we observed, in the marijuana group, 25 mice with 34 lung adenomas and 1 mouse with lymphoma compared to 22 mice with 30 lung adenomas and 2 mice with lymphomas for the tobacco group. In the negative control we observed 31 mice with 34 lung adenomas.

The mice which were initiated with 75 µg DMBA were treated for 56 weeks with 50% suspensions of marijuana "tar" or tobacco "tar", respectively. At the end of this time 26 of the 60 mice tested with marijuana "tar" developed 48 skin papillomas and 3 carcinomas, 3 mice had fibrosarcomas, and 8 mice developed 14 lung adenomas (Fig. 7). In the tobacco group we observed that 34 out of 60 mice had 72 skin papillomas, 4 mice had 6 carcinomas and 4 mice had developed 6 lung adenomas. In the negative control group, with 75 µg DMBA as initiator and acetone as "promoter", 5 out of 60 mice developed 8 papillomas, and 1 mouse out of the 5 tumor-bearing mice had 2 carcinomas. In the control

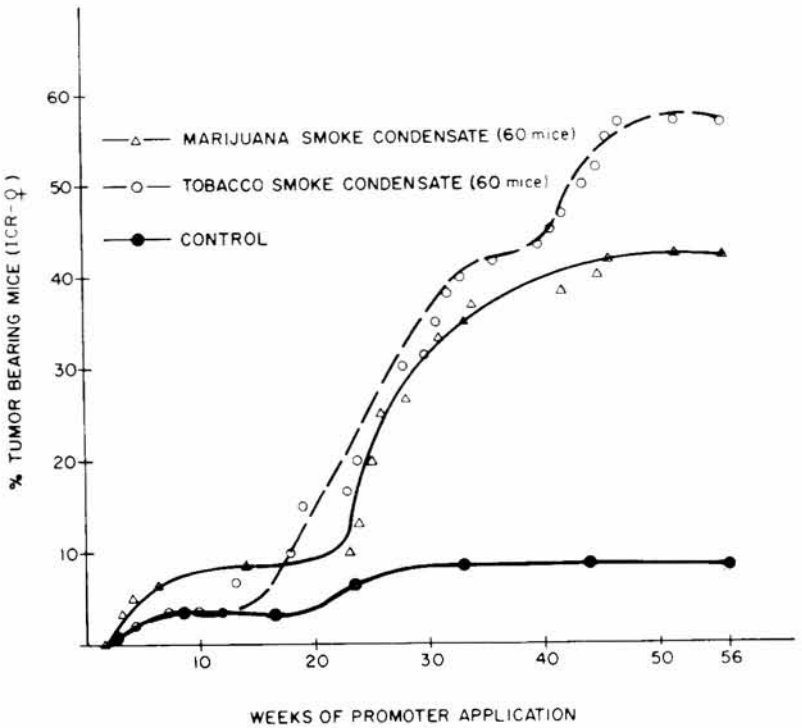

Figure 7. Tumor promoting activity of smoke condensates. Initiator: 75 µg DMBA

group we also found 7 mice with 9 adenomas and 1 mouse with a lymphoma. From these results we conclude that marijuana "tar" has a significant tumor-promoting activity, although lesser in degree than cigarette "tar" ($p<0.05$). Presently, we are engaged in identifying the tumor-promoting agents in marijuana smoke. Based on short-term tests it appears that the majority of the tumor-promoter resides in the weakly acidic fraction within a subfraction which contains non-volatile phenols and long-chain fatty acids.

We conclude from the chemical analytical data and the bioassays that marijuana smoke contains some volatile *N*-nitrosamines as well as some polycyclic hydrocarbon carcinogens. The latter carcinogens may serve in the experimental setting, as used in the present study, as tumor initiators. On mouse skin, marijuana "tar" is a weak tumorigenic agent and has tumor-promoting activity. Both activities, however, are significantly lower than the respective biologic activity of tobacco "tar".

Although we consider these findings of more than academic interest, we should not attempt to relate these bioassays to the human setting without some qualifications. Firstly, no epidemiological data relating marijuana smoke as a possible respiratory carcinogen to man are at hand; secondly, the daily exposure to marijuana smoke, even in extreme cases, is below the dose level of most tobacco cigarette smokers and, furthermore, marijuana has been smoked for fewer years than tobacco cigarettes. It remains as a reasonable question, however, to determine whether, particularly because of its relatively high PAH content, marijuana smoke is capable of potentiating the carcinogenic effects of cigarette smoke.

SUMMARY

The mainstream smoke of experimental marijuana cigarettes was analyzed for toxic and tumorigenic agents and compared with smoke of standard tobacco cigarettes.

Both aerosols are qualitatively comparable, except for differences in cannabinoids and tobacco alkaloids.

Quantitatively we found higher concentrations in the tobacco smoke for carbon monoxide, isoprene, volatile phenols,

and pyridines and in the marijuana smoke higher concentrations for some polynuclear aromatic hydrocarbons. The transfer rate into the mainstream smoke of the major cannabinoid, Δ^1-tetrahydrocannabinol was about 20%, resulting in a significant enrichment of this toxic agent in the particulate matter of this inhalant.

The bioassays on mouse skin for tumorigenicity and tumor-promoting activity. Both activities, however, are significantly below those exchibited by tobacco smoke condensate.

The chemical analytical findings and the results from the bioassays for tumorigenicity are discussed. These data cannot be extrapolated to the human setting without qualification.

Acknowledgements

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REFERENCES

1. Pillard, R.C. 1970. Marijuana in New Engl. J. Med. 283: 294-303.
2. Singer, A.J. 1971. Marijuana: Chemistry, Pharmacology, and Pattern of Social Use. N.Y. Acad. Sci. 191: 1-269.
3. Nahas, G.H. 1973. "Marijuana, Deceptive Weed." p. 325. Raven Press, New York.
4. Mechoulam, R. 1973. "Marijuana: Chemistry, Pharmacology, Metabolism, and Clinical Effects". p. 409. Academic Press, New York.
5. Waller, C.W., J.J. Denny & M.A. Waltz. 1971. "Annotated Bibliography of Marijuana." University of Mississippi, University, Miss., 1964-1970, p. 301.
6. _____, J.J. Denny & M.A. Waltz. 1972 "Annotated Bibliography of Marijuana. University of Mississippi,

University, Miss., 1971 Suppl. p. 155.
7. Magus, R.D. & L.S. Harris. 1971. Carcinogenic potential of marijuana smoke condensate. Federation Proc. 30, Abstr.: 279.
8. Cottrell, J.C., S.S. Sohn, & W.H. Vogel. 1973. Toxic effects of marijuana tar on mouse skin. Arch. Environ. Health, 26: 277-278.
9. Leuchtenberger, C. & R. Leuchtenberger. 1971. Enhancement of abnormal proliferation in lung explants after marijuana cigarette smoke. Experientia, 27: 737-738.
10. _____, R. Leuchtenberger, & A. Schneider. 1973. Effects of marijuana and tobacco smoke on lung physiology. Nature, 241: 137-139.
11. Wynder, E.L. & D. Hoffmann. 1967. "Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis." p. 732. Academic Press, New York.
12. Lipp, G. 1969. Vergleich der Rauchausbeuten von drei verschiedenen Rauchmachinen I. Beitr. Tabakforsch. 5: 39-42.
13. Von Bethmann, M., G. Lipp, & H. Van Nooy. 1961. Feuchtigkeitsbestimmung in Tabak. Beitr. Tabakforsch. 1: 19-29.
14. Rathkamp, G., T.C. Tso, & D. Hoffmann. 1973. Chemical studies on tobacco smoke. XX. Smoke analysis of cigarettes made from Bright tobaccos differing in variety and stalk position. Beitr. Tabakforsch. 7: 179-189.
15. Hoffmann, D. & E.L. Wynder. 1967. The reduction of the tumorigenicity of cigarette smoke condensate by addition of sodium nitrate to tobacco. Cancer Res. 27: 172-174.
16. Brunnemann, K. D. & D. Hoffmann. 1974. Chemical studies on tobacco smoke. XXIV. Quantitative method for carbon monoxide and carbon dioxide in cigarette and cigar smoke. J. Chrom. Sci. 12: 70-75.
17. _____ & D. Hoffmann, unpublished.
18. Artho, A. & P. Koch. 1969. Uber die Bestimmung von Cyanwasserstoff im Cigarettenrauch, Beitr. Tabakforsch. 5: 58-63.
19. Hoffmann, D., G. Rathkamp, & Y.Y. Liu. 1974. Chemical studies on tobacco smoke. XXVI. On the isolation and identification of volatile and nonvolatile *N*-nitrosamines and hydrazines in cigarette smoke. WHO Monograph (in print).
20. Brunnemann, K.D. & D. Hoffmann. 1974. Chemical studies

on tobacco smoke. XXV. The pH of tobacco smoke. Food Cosmet. Toxicol. 12: 115-124.
21. Pillsbury, H., C.C. Bright, K.J. O'Connor, & F.W.J. Irish. 1969. Tar and nicotine in cigarette smoke. J. Assoc. Offic. Anal. Chemists. 52: 458-462.
22. Hoffmann, D., G. Rathkamp, K.D. Brunnemann, & E.L. Wynder. 1973. Chemical studies on tobacco smoke. XXII. On the profile analysis of tobacco smoke. Sci. Total Environ. 2: 157-171.
23. Wynder, E.L. & D. Hoffmann. 1968. Selected laboratory method in tobacco carcinogenesis. Methods Cancer Res. 4: 3-52.
24. Druckrey, H., R. Preussmann, S. Ivankovic, & D. Schmähl. 1967. Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-Nitrosoverbindungen an BD-Ratten. Z. Krebsforsch. 69: 103-201.
25. Magee, P.N. & J.M. Barnes. 1967. Carcinogenic nitroso compounds. Advan. Cancer Res. 10: 163-246.
26. Federal Trade Commission: Tar-Nicotine Report, March 1974, Tobacco Reporter, 101 (4): 26 (1974).
27. Hoffmann, D. & E.L. Wynder. 1968. Chemical analysis and carcinogenic bioassays of organic particulate pollutants. In "Air Pollution" (A.C. Stern, Ed.), Vol. 2. pp. 187-247. Academic Press, New York.