

# Marijuana Use and Increased Risk of Squamous Cell Carcinoma of the Head and Neck<sup>1</sup>

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## Abstract

Marijuana is the most commonly used illegal drug in the United States. In some subcultures, it is widely perceived to be harmless. Although the carcinogenic properties of marijuana smoke are similar to those of tobacco, no epidemiological studies of the relationship between marijuana use and head and neck cancer have been published. The relationship between marijuana use and head and neck cancer was investigated by a case-control study of 173 previously untreated cases with pathologically confirmed diagnoses of squamous cell carcinoma of the head and neck and 176 cancer-free controls at Memorial Sloan-Kettering Cancer Center between 1992 and 1994. Epidemiological data were collected by using a structured questionnaire, which included history of tobacco smoking, alcohol use, and marijuana use. The associations between marijuana use and head and neck cancer were analyzed by Mantel-Haenszel methods and logistic regression models. Controlling for age, sex, race, education, alcohol consumption, pack-years of cigarette smoking, and passive smoking, the risk of squamous cell carcinoma of the head and neck was increased with marijuana use [odds ratio (OR) comparing ever with never users, 2.6; 95% confidence interval (CI), 1.1-6.6]. Dose-response relationships were observed for frequency of marijuana use/day ( $P$  for trend < 0.05) and years of marijuana use

( $P$  for trend < 0.05). These associations were stronger for subjects who were 55 years of age and younger (OR, 3.1; 95% CI, 1.0-9.7). Possible interaction effects of marijuana use were observed with cigarette smoking, mutagen sensitivity, and to a lesser extent, alcohol use. Our results suggest that marijuana use may increase the risk of head and neck cancer with a strong dose-response pattern. Our analysis indicated that marijuana use may interact with mutagen sensitivity and other risk factors to increase the risk of head and neck cancer. The results need to be interpreted with some caution in drawing causal inferences because of certain methodological limitations, especially with regard to interactions.

## Introduction

Marijuana is the second most commonly smoked substance in the United States after tobacco (1, 2). It is estimated that 31% of the United States population 12 years or older in 1992 had ever used marijuana (3). Studies conducted within the past two decades in experimental animals and humans indicate that marijuana smoke can injure the lung and respiratory tract (4). In humans, habitual smoking of marijuana has been shown to be associated with symptoms of chronic bronchitis, an increased frequency of acute bronchitic episodes, extensive tracheobronchial epithelial histopathology including alterations correlated with the subsequent development of malignancy in tobacco smokers (5), DNA injury (6), and abnormalities in the structure and function of alveolar macrophages, key cells in the immune defense system of the lung (7, 8). Further evidence also suggests that marijuana may predispose to the development of cancer of the respiratory tract (9). For example, the tar phase of marijuana smoke contains some of the same carcinogenic compounds found in tobacco smoke, such as phenols and polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene, which is present in ~50% higher concentration in marijuana tar than in the tar from a comparable amount of unfiltered tobacco (10). In addition, a single marijuana cigarette deposits four times as much tar in the respiratory tract as that deposited from a single filtered tobacco cigarette of approximately the same weight (11). The higher content of carcinogenic polycyclic aromatic hydrocarbons in marijuana smoke and the greater deposition of marijuana tar in the lung act together to amplify exposure of the marijuana smoker to carcinogens in the particulate phase. Finally, preliminary *in vitro* studies involving mixed reactions of antigen-presenting dendritic cells and T lymphocytes (12) and *in vivo* studies using a murine model of an immunogenic carcinoma of the lung (12, 13) suggest that  $\Delta^9$ -tetrahydrocannabinol, the major psychoactive ingredient in marijuana smoke, impairs immune responses to tumor antigens. A recent paper reported that habitual marijuana (and/or cocaine) smokers exhibited more molecular genetic abnormalities than nonsmokers (14). The study suggested that smoking marijuana and or cocaine, like tobacco smoking, exerts field cancerization effects

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on bronchial epithelium, which may place marijuana/cocaine smokers at increased risk for the subsequent development of lung cancer.

The above-cited biological evidence pointing to a carcinogenic role for marijuana is supported by several case-series reports, indicating an unexpectedly large proportion of marijuana users among selected cases of upper aerodigestive tract cancer. Since 1986, a total of 74 anecdotal cases of head and neck cancer with a history of marijuana use have been reported in medical literature (15–24). The characteristics of these marijuana-exposed malignancies of the upper aerodigestive tract include young age at diagnosis (<55 years old), extensive field cancerization, and aggressive biological behavior. Although causal inference cannot be made directly from uncontrolled case-series studies, these case reports suggest a need for in-depth epidemiological investigations of the relationship between marijuana use and the risk of cancers of the upper aerodigestive tract.

In the only published epidemiological study of marijuana use and cancer incidence, the authors reported positive associations between lifetime marijuana use (six or more occasions) and both prostate and perhaps cervical cancer among nonsmokers of tobacco cigarettes. No association was observed between marijuana use and all tobacco-related cancers (25). Unfortunately, the specific relationship between marijuana use and cancers of the head and neck, those sites most likely to be affected by marijuana use along with lung, was not explored independently. Moreover, subjects in the latter study (25) may not have been followed long enough for adequate assessment of an effect of marijuana on cancer risk. In addition, there may not have been enough exposure to marijuana to observe an effect in this population.

The aim of the present case-control study was to examine the association between marijuana use as derived from questionnaire data and head and neck cancers, controlling for other known risk factors for the disease, including cigarette smoking and alcohol drinking. We also examined the possible gene-environment interaction between marijuana use and mutagen sensitivity, as well as interactions with other known risk factors for head and neck cancer. Mutagen sensitivity is considered a predisposition marker of cancer risk (26–29). Defects in one or more steps of the DNA repair process may play a significant role in environmental carcinogenesis, and the extent of such defects may be partially responsible for susceptibility or resistance to environmental mutagens (30). Mutagen sensitivity tests are indirect indicators of DNA repair competence. Bleomycin, a radiomimetic agent, was used as the test mutagen to evaluate the rates of induced chromosome breakage as a crude indicator of the response to a genotoxic agent (31, 32).

## Patients and Methods

**Cases and Controls.** Untreated new patients with a histologically confirmed diagnosis of first primary squamous cell carcinoma of the head and neck, seen at Memorial Sloan-Kettering Cancer Center from 1992 to 1994, were considered as cases in this study. We approached 192 patients, and 173 agreed to participate. Sites of disease were classified by the American Joint Committee on Cancer criteria and coded by the International Classification of Diseases Version 9 (ICD-9). The tumor sites included lip (ICD-9, 140;  $n = 2$ ), tongue (ICD-9, 141;  $n = 52$ ), salivary glands (ICD-9, 142;  $n = 1$ , metastatic lesion, squamous cell carcinoma), gum (ICD-9, 143;  $n = 13$ ), floor of mouth (ICD-9, 144;  $n = 15$ ), other parts of the mouth (ICD-9, 145;  $n = 11$ ), oropharynx (ICD-9, 146;  $n = 12$ ), nasopharynx

(ICD-9, 147;  $n = 2$ , squamous cell carcinomas), hypopharynx (ICD-9, 148;  $n = 13$ ), other oral cavity (ICD-9, 149;  $n = 2$ ), esophagus (ICD-9, 150;  $n = 1$ ), nasal cavities (ICD-9, 160;  $n = 1$ ), and larynx (ICD-9, 161;  $n = 48$ ). Age- and sex-frequency matched controls were identified for this study. Controls were without a history of cancer and were identified from the Blood Bank Center of Memorial Sloan-Kettering Cancer Center during the same period. We approached 196 blood donors, and 176 agreed to participate in the study.

**Data Collection.** The study was approved by the Institutional Research Board on Human Subjects of Memorial Sloan-Kettering Cancer Center. All cases and controls were asked to sign an informed consent form if they agreed to participate in the study, to complete a structured questionnaire, and to donate a sample of blood. The questionnaire requested information on the following variables: age, gender, race, year and place of birth, religion, family income, and education; average number of tobacco cigarettes smoked/day, years of smoking, age at initiation of smoking; exposure to environmental tobacco smoking (at home and at work); alcohol consumption, types and frequency of alcohol consumption; occupational and environmental exposures; family history of cancer; sexual history; medical history; and oral hygienic history. In addition, all subjects were asked if they had ever used marijuana. If they responded yes, subjects were asked the average number of times they smoked/day and the number of years of marijuana use.

**Mutagen-Sensitivity Assay.** A total of 91 patients and 131 controls provided a blood specimen for the assessment of mutagen sensitivity. The mutagen-sensitivity assay used in this study has been described in detail previously (33). A peripheral blood sample (10 ml or less) was collected from cases and controls in a heparinized tube prior to initiation of lymphocyte culture. The standard lymphocyte culture procedure used RPMI 1640, supplemented with 15% FCS and phytohemagglutinin, in a ratio of blood:medium of 1:9. At 67 h of incubation, one set of cultures was treated with bleomycin (0.03 unit/ml) for 5 h. Colcemid (0.04 mg/ml) was added in the last hour to induce mitotic arrest prior to harvesting. A conventional cell-harvesting procedure followed. The cells were treated with hypotonic KCl (0.975 M KCl) solution for 15–20 min, fixed, washed with a freshly prepared mixture of methanol:acetic acid (3:1), and air-dried on wet slides. The slides were stained with Giemsa solution without banding. Fifty well-spread metaphases were examined from coded slides. Chromatid aberrations recorded were frank chromatid breaks or exchanges. Bleomycin tends to induce few chromatid exchanges (which, if present, are considered as two breaks). Chromatid gaps or attenuated regions were disregarded. The frequency of breakage was expressed as breaks/cell. The reliability of cytogenetic scoring has been evaluated previously by comparing four separate blood samples from a respective donor with a minimum interval between samples of 1 week. Mutagen sensitivity appeared to be stable and representative in a random-effect, one-way ANOVA model (30).

**Statistical Analysis.** The effects of marijuana use on the risk of head and neck cancer were estimated with ORs<sup>3</sup> and their 95% CIs, derived from logistic regression analysis (34). Continuous variables, such as years of marijuana use and frequency of use, were first analyzed as continuous variables and then divided into three groups according to their marginal distribu-

<sup>3</sup> The abbreviations used are: OR, odds ratio; CI, confidence interval.

tions: frequency of use (marijuana use/day) was categorized as never, less or equal to once per day, and more than once per day; and years of use was categorized as never use, 1–5 years, and >5 years. For eight cases and nine controls who reported previous marijuana use but failed to report frequency of use, the median value of once per day was used to replace the missing values for the continuous variable and for the categorical variable. For five cases and five controls who reported previous use but provided no information on years of use, the median value of 5 years was used for the continuous variable and 1–5 years category for the categorical variable. Results of both replacing missing data with median values and excluding missing data are presented in the results. Dummy variables were used in logistic regression analysis to estimate ORs for each category of exposure. Trend tests for ordered variables were performed by assigning the score  $j$  to the  $j$ th exposure level of a categorical variable (where  $j = 1, 2, \dots$ ) and treating the categorical variable as an interval predictor in unconditional logistic regression. Three models were used to assess marijuana effects: (a) no covariates (crude analysis); (b) statistical adjustment for pack-years of cigarette smoking (continuous variable); (c) statistical adjustment for pack-years of cigarette smoking plus age (continuous variable), sex (male, female), race (white, non-white), education ( $\leq$ high school, college, >college), passive smoking (no, yes), and heavy alcohol drinking (<100 drinks/month;  $\geq$ 100 drinks/month). Stratified analysis was used to assess departures from additive effects between marijuana use and other known risk factors for head and neck cancer, including cigarette smoking, alcohol drinking, and mutagen sensitivity.

## Results

The overall prevalence of lifetime marijuana use was 9.7% in controls and 13.9% in cases. The highest prevalence of marijuana use was found in cases with squamous cell carcinoma of the larynx ( $n = 48$ ; 22.9%) and tongue ( $n = 52$ ; 19.2%). The distributions of marijuana use among cases and controls, stratified by demographic characteristics, cigarette smoking, alcohol drinking, and mutagen sensitivity, are shown in Table 1. Age was strongly associated with marijuana use; large proportions of marijuana smokers were found in younger age groups for both cases and controls. No obvious differences in marijuana use were found between categories of gender, race, or education. Tobacco cigarette smoking was generally independent of marijuana use in both cases and controls, except for those variables related to age such as pack-years of tobacco cigarette smoking, years of smoking, and age at smoking initiation in cases. Heavy alcohol drinking and mutagen hypersensitivity were not related to marijuana use in cases or controls. Passive smoking, not associated with marijuana use in controls, was related to marijuana use in cases.

The estimated crude OR for the effect of lifetime marijuana use (ever *versus* never) on the risk of head and neck cancer was 1.5 (95% CI, 0.8–2.9). Adjusting for age, gender, race, education, heavy alcohol drinking, pack-years of tobacco cigarette smoking, and passive smoking increased the OR to 2.6 (95% CI, 1.1–6.6; Table 2). Strong dose-response relationships were observed for the effects of frequency of marijuana use and years of use. The adjusted ORs were 2.1 for those who smoked marijuana once per day and 4.9 for those who smoked marijuana more than once per day ( $P$  for trend = 0.0358) when missing values were replaced by median values. After excluding those with missing information on frequency of marijuana use, the adjusted ORs were 4.0 (0.9–2.4) and 5.4 (0.9–33) for

those who smoked once per day and more than once per day, respectively ( $P$  for trend = 0.0214). Of those who smoked marijuana for 1–5 years, the adjusted OR was 1.9 (0.6–5.9); for individuals who smoked marijuana >5 years, the adjusted OR was 4.3 (0.99–19) when missing values were replaced by median values ( $P$  for trend = 0.0325; Table 2). After excluding those with missing information on years of marijuana use, the adjusted ORs were 3.9 (0.99–15) and 4.9 (0.8–29) for those who smoked 1–5 years and >5 years, respectively ( $P$  for trend = 0.0134).

The observed association between marijuana use and head and neck cancer was stronger among younger subjects. When the analyses were restricted to 55 cases and 65 controls <55 years, the adjusted OR increased to 3.1 (95% CI, 1.0–9.7; Table 3). Dose-response relationships were also stronger for the effects of frequency of marijuana use and years of use, controlling for the same covariates. When the analysis was further restricted to those between the ages of 40 and 55, the magnitudes of the estimated effects were still persistent. No association was observed between marijuana use and head and neck cancer for those 55 years or older.

Table 4 shows the combined effects of lifetime marijuana use (ever *versus* never) and each of three potential effect modifiers: tobacco cigarette smoking, alcohol use, and mutagen sensitivity. For these analyses, we used >1.0 breaks/cell as the cutoff value to define hypersensitivity and having 100 or more drinks per month as a cutoff point for heavy drinking, and we categorized tobacco cigarette smoking into never smoking, former smoking, and current smoking. These variables were further stratified by marijuana use. The effects of marijuana use and cigarette smoking were more than multiplicative; the adjusted OR for the joint category of current tobacco cigarette smokers and marijuana users was greater than the product of the two component effects for those two exposures, *i.e.*,  $36.1 > 3.6 \times 2.6 = 9.4$ . Similar interaction effects (greater than multiplicative) were found for marijuana use and mutagen sensitivity. The adjusted OR for joint category of marijuana use and mutagen sensitivity was greater than the product of two component effects, *i.e.*,  $77.1 > 6.1 \times 1.1 = 6.7$ . The effects of marijuana use and alcohol consumption appeared more than additive but less than multiplicative, *i.e.*,  $4.3$  (alcohol only) +  $2.7$  (marijuana only) –  $1 = 6.0 < 9.6$  (both exposures)  $< 4.3 \times 2.7 = 11.6$ . In each case, however, power for testing each null hypothesis (effects are additive or multiplicative) and for comparing the fits of additive *versus* multiplicative models was low.

## Discussion

This study has several possible limitations. One limitation is potential selection bias, which might have resulted in an overestimate of the marijuana effect (bias away from null). The controls for this study were blood donors and possibly less likely to be substance abusers. If use of marijuana were inversely associated with blood donation, the selection bias would lead to an overestimate of the marijuana effect. The blood donors at Memorial Sloan-Kettering Cancer Center had to be between the ages of 17 and 75, weigh 110 pounds or more, and be in good health. The prospective donors were asked to give a health history and take a physical examination to ensure the greatest possible safety for both donors and recipients. Nevertheless, the only question directly related to drug abuse was: “Have you used illegal drugs with a needle?” Although marijuana is not generally injected, marijuana use and injected drug use could be positively associated, which might lead to an overestimate of the marijuana effect. Nevertheless, because the

Table 1 The distribution of marijuana use (number and percentage of users and nonusers) in cases and controls, by category of selected demographic factors, smoking, alcohol, and mutagen sensitivity

	Controls				Cases			
	Users	Nonusers	Total	%	Users	Nonusers	Total	%
Total	17	159	176	9.7	24	149	173	13.9
Age								
<40	4	15	19	21.1	5	2	7	71.4
40–54	8	36	44	18.2	15	33	48	31.3
≥55	5	108	113	4.4	4	114	118	3.4
Gender								
Male	11	100	111	9.9	17	92	109	15.6
Female	6	59	65	9.2	7	57	64	10.9
Race								
White	16	143	159	10.1	22	131	153	14.4
Non-White	1	16	17	5.9	2	18	20	10.0
Education								
≤High school	4	42	46	8.7	11	94	105	10.5
College	10	80	90	11.1	8	39	47	17.2
>College	3	36	39	7.7	4	14	18	22.2
Cigarette smoking								
Never	5	56	61	8.2	2	26	28	7.1
Quit	9	62	71	12.7	8	34	42	19.1
Current <sup>a</sup>	2	37	39	5.1	14	84	98	14.3
Pack-Years								
0	5	56	61	8.2	2	26	28	7.1
1–22.4	6	45	51	11.8	9	13	22	40.9
22.5–44.9	3	25	28	10.7	4	37	41	9.8
≥45	1	18	19	5.3	6	62	68	8.8
Cigarettes/day								
0	5	56	61	8.2	2	26	28	7.1
1–20	7	65	72	9.7	11	58	69	15.9
21+	4	29	33	12.1	11	58	69	15.9
Years of smoking								
0	5	56	61	8.2	2	26	28	7.1
1–20	7	39	46	15.2	9	11	20	45.0
21–40	2	37	39	5.1	9	60	69	13.0
>40	1	13	14	7.1	1	42	43	2.3
Age at start smoking (ever smokers only)								
>19	1	26	27	3.7	1	35	36	2.8
18–19	2	24	26	7.7	5	24	29	17.2
16–17	4	21	25	16.0	8	29	37	21.6
<16	4	28	32	12.5	8	30	38	21.1
Years since quitting smoking (ex-smokers only)								
>19	5	27	32	15.6	4	12	16	25.0
5–19	4	35	39	10.3	4	22	26	15.4
<5	0	8	8	0.0	12	56	68	17.7
Passive smoking								
Never	4	23	27	14.8	5	5	10	50.0
Occasionally	5	38	43	11.6	7	35	42	16.7
Regularly	8	88	96	8.3	12	91	103	11.7
Alcohol use (drinks/months)								
<100	15	132	147	10.2	14	90	104	13.5
≥100	2	14	16	12.5	10	51	61	16.4
Mutagen sensitivity (breaks/cell)								
<1	9	91	100	9.0	6	30	36	16.7
≥1	2	28	30	6.7	10	46	56	17.9
Tumor sites (ICD-9)								
Lip (140)					0	2	2	0.0
Tongue (141)					10	42	52	19.2
Salivary glands (142)					0	1	1	0.0
Gum (143)					0	13	13	0.0
Floor of mouth (144)					1	14	15	6.7
Other parts of the mouth (145)					0	11	11	0.0
Oropharynx (146)					1	11	12	8.3
Nasopharynx (147)					1	1	2	50.0
Hypopharynx (148)					0	13	13	0.0
Other oral cavity (149)					0	2	2	0.0
Esophagus (150)					0	1	1	0.0
Nasal cavities (160)					0	1	1	0.0
Larynx (161)					11	37	48	22.9

<sup>a</sup> Includes those who were still smoking and people who had quit smoking for <5 years.



Table 2 Estimated effects of marijuana use (OR and 95% CI) on the risk of head and neck cancer, by covariates selected for adjustment<sup>a</sup>

	No. of cases	No. of controls	Covariates		
			No covariates (crude)	Pack-years of smoking	Pack-years of smoking plus <sup>b</sup>
Marijuana use					
Never	149	159	1.0	1.0	1.0
Ever	24	17	1.5 (0.8–2.9)	1.7 (0.8–3.6)	2.6 (1.1–6.6)
Times/day					
Continuous			1.3 (0.9–1.9)	1.4 (0.9–2.1)	1.5 (0.9–2.4)
0	149	159	1.0	1.0	1.0
1	8	6	1.4 (0.5–4.2)	1.7 (0.5–5.7)	4.0 (0.9–17.2)
>1	8	2	4.3 (0.9–20.4)	5.1 (0.99–26)	5.4 (0.9–33)
<i>P</i> for trend			0.0549	0.0340	0.0214
Times/day <sup>b</sup>					
Continuous			1.3 (0.9–1.8)	1.3 (0.9–1.9)	1.4 (0.9–2.2)
0	149	159	1.0	1.0	1.0
1	16	15	1.1 (0.5–2.4)	1.3 (0.5–2.9)	2.1 (0.8–6.0)
>1	8	2	4.3 (0.9–20.4)	5.0 (0.97–26)	4.9 (0.8–29)
<i>P</i> for trend			0.0972	0.0636	0.0358
Years of use					
Continuous			1.1 (0.98–1.1)	1.1 (0.99–1.21)	1.1 (1.01–1.27)
0	149	159	1.0	1.0	1.0
1–5	8	6	1.4 (0.5–4.2)	1.8 (0.6–5.8)	3.9 (0.99–15.0)
>5	11	6	2.0 (0.7–5.4)	3.3 (0.95–11.4)	4.9 (1.07–22.3)
<i>P</i> for trend			0.1574	0.0360	0.0134
Years of use <sup>b</sup>					
Continuous			1.1 (0.98–1.1)	1.1 (0.99–1.18)	1.1 (1.002–1.2)
0	149	159	1.0	1.0	1.0
1–5	13	11	1.3 (0.5–2.9)	1.2 (0.5–3.0)	1.9 (0.6–5.9)
>5	11	6	2.0 (0.7–5.4)	3.2 (0.94–11)	4.3 (0.99–19)
<i>P</i> for trend			0.1726	0.0751	0.0325

<sup>a</sup> Also adjusted for age (continuous variable), gender (male = 0, female = 1); race (white = 0, non-white = 1); education ( $\leq$ high school = 0; college = 1, >college = 2); heavy alcohol use ( $<100$ /month = 0,  $\geq 100$ /month = 1); and passive smoking (no = 0, yes = 1).

<sup>b</sup> Missing data were replaced by median.

observed prevalence in our controls was similar to the expected prevalence based on national data, the selection of blood donors as controls probably did not affect the association under study (Table 5).

When we evaluated the interaction between marijuana smoking and mutagen sensitivity (Table 4), the possible selection bias might exist because those with blood samples for mutagen assay may be different from individuals without blood samples. A total of 26.1% of controls and 46.8% of cases refused to provide a blood sample for the bleomycin in this study. We have compared the differences between those with and without blood samples on selected variables. This attempt is crucial to show whether there is selection bias attributable to missing samples that may threaten the validity of the interaction between marijuana smoking and mutagen sensitivity. No obvious difference was found between those with and without blood samples in terms of age, gender, race, education, and marijuana smoking. Only a board-line difference for cigarette smoking between those with and without blood samples in head and neck cases was detected ( $P = 0.05$ ), which indicates that the subjects with blood samples might not be a selected group for smoking habits from the original study population. The association between tobacco smoking and risk of head and neck squamous cell cancer was stronger in people with mutagen data than those without mutagen data, which might lead to a stronger confounder effects on the association between marijuana smoking and head and neck cancer. However, when the interaction between mutagen sensitivity and marijuana use was evaluated, the point estimates of the crude ORs were pretty similar to those after controlling for pack-years (Table 4).

A second limitation is differential misclassification of marijuana use, which may also bias the estimated marijuana effect. Because marijuana smoking is illegal, cases and controls might tend to underreport their history of marijuana use, but the degree of underreporting might have been greater for controls than cases who might want to rationalize their disease. Thus, the estimates of marijuana effects could be positively biased. On the other hand, cancer patients, under some duress because of their illness, could underreport their history of marijuana use more than controls, which would negatively bias the estimated marijuana effects. To address this potential source of bias, we compared the reported lifetime prevalence of marijuana use in controls with the corresponding prevalence in the United States population during the same period, stratified by gender and year of birth (Table 5; Ref. 3). We found that the overall (crude) lifetime prevalence of marijuana use in each gender of the controls was approximately equal to the corresponding prevalence in the United States population standardized to the birth-cohort distribution of the controls. For the majority of controls born before 1951 ( $n = 152$ ; 86%), the lifetime prevalence of marijuana use was similar to estimates for the United States population. For a small fraction of those controls born since 1951 ( $n = 24$ ; 14%), however, there is some indirect evidence for systematically underreporting of marijuana use. When we reanalyzed the data by excluding those cases and controls born since 1951, we found little change in the estimated marijuana effects. Because we cannot address issues of either over- or underreporting by cases, it is difficult to evaluate the direction of bias by differential misclassification of past marijuana use on the association under study. The possible limitation of using

Table 3 Estimated effects of marijuana use (OR and 95% CI) on the risk of head and neck cancer for individuals <55 years by covariates selected for adjustment<sup>a</sup>

	No. of cases	No. of controls	Covariates		
			No. of covariates (crude)	Pack-years of smoking	Pack-years of smoking plus <sup>b</sup>
Marijuana use					
Never	35	51	1.0	1.0	1.0
Ever	20	12	2.4 (1.1–5.6)	2.7 (1.02–6.9)	3.1 (0.99–9.7)
Times/day					
Continuous			1.4 (0.95–2.1)	1.4 (0.9–2.2)	1.5 (0.9–2.5)
0	35	51	1.0	1.0	1.0
1	7	4	2.6 (0.7–9.4)	1.9 (0.5–7.8)	3.7 (0.7–20.9)
>1	7	2	5.1 (1.0–26)	5.9 (1.03–34)	6.9 (0.9–50.3)
<i>P</i> for trend			0.0219	0.0344	0.0285
Times/day <sup>b</sup>					
Continuous			1.4 (0.95–2.1)	1.4 (0.9–2.3)	1.4 (0.9–2.3)
0	35	51	1.0	1.0	1.0
1	13	10	1.9 (0.7–4.8)	2.0 (0.7–5.8)	2.4 (0.7–8.6)
>1	7	2	5.1 (1.0–26)	5.9 (1.04–34)	5.9 (0.9–39.9)
<i>P</i> for trend			0.0224	0.0254	0.0376
Years of use					
Continuous			1.1 (1.0–1.2)	1.1 (0.99–1.2)	1.1 (0.98–1.2)
0	35	51	1.0	1.0	1.0
1–5	7	4	2.6 (0.7–9.4)	3.0 (0.7–12.3)	7.0 (1.2–39.2)
>5	10	5	2.9 (0.92–9.3)	3.6 (0.9–14.1)	3.3 (0.7–16.5)
<i>P</i> for trend			0.0380	0.0350	0.0548
Years of use <sup>b</sup>					
Continuous			1.1 (1.0–1.2)	1.1 (0.99–1.2)	1.1 (0.98–1.2)
0	35	51	1.0	1.0	1.0
1–5	10	7	2.1 (0.7–6.0)	2.2 (0.7–6.9)	3.2 (0.8–12.9)
>5	10	5	2.9 (0.92–9.3)	3.6 (0.9–14.1)	3.0 (0.6–13.7)
<i>P</i> for trend			0.0380	0.0409	0.0791

<sup>a</sup> Also adjusted for age (continuous variable), gender (male = 0, female = 1); race (white = 0, non-white = 1); education ( $\leq$ high school = 0; college = 1, >college = 2); heavy alcohol use (<100/month = 0,  $\geq$ 100/month = 1); and passive smoking (no = 0, yes = 1).

<sup>b</sup> Missing data were replaced by median.

mutagen sensitivity assays in case-control study was discussed by Caporaso (29). Cultured cells obtained from patients with cancer or control subjects in a hospital setting can differ for abnormal nutrition, secondary metabolic alterations of neoplastic disease, and effect of treatment, hospitalization, inactivity, or stress, which will allow bias attributable to differential misclassification. However, a recent paper by Cloos *et al.* (28) reported a high heritability estimate of the susceptibility to bleomycin-induced chromatid breaks, which indicates that a clear genetic basis for mutagen sensitivity-related cancer susceptibility may exist in the general population. If the mutagen sensitivity is highly inherited, the differential misclassification bias for this assay might be minimal.

The third limitation is low power and precision. The relatively small sample size and low frequency of marijuana use limits our ability to estimate the effects precisely, especially when analyzing specific sites or when assessing interaction effects with other risk factors.

A fourth possible source of bias is no differential error in measuring confounders of the association under study. It has been shown, for example, that no differential misclassification of a strong confounder will cause the investigator to underestimate both the impact of the confounder on effect estimate and the association of the confounder with the factor under study (35, 36). However, even if the association of major confounders, such as alcohol and tobacco with marijuana, are stronger than they appear, they appear so weak as to represent an unlikely source of bias.

Possible confounding effects also need to be addressed. We have evaluated the possible confounding effects to identify

the potential confounders that induced the large changes in point estimates of ORs and *P*s. Our results showed that age was a major confounder, which causes the largest changes in point estimates of OR and *P* for marijuana smoking after controlling for it. In addition, passive smoking and pack-years of smoking are positive confounders, and alcohol drinking is a negative confounder on the association between marijuana and head and neck cancer.

This is the first epidemiological study to report an effect of marijuana use on the risk of head and neck cancer. Not only did we find an elevated cancer risk among marijuana users, but we also observed dose-response associations for frequency and years of marijuana use, adjusting for several potential confounders.

Marijuana use in the United States increased dramatically among teenagers and young adults in the mid-to-late 1960s, *i.e.*, among persons born between 1941 and 1955. Assuming marijuana use is associated with cancer risk with an induction/latency period of 20–30 years, this cohort will be the earliest possible group to experience and clinically manifest elevated risks of head and neck cancer. This suggests that observed risks should be greater among subjects younger than 55 years. Our analyses, restricted to the younger population (<55 years old) with only 32% of our cases ( $n = 55$ ) and 36% of controls ( $n = 63$ ) suggested a stronger marijuana effect in the subpopulation of younger subjects than in the population as a whole. The dose-response relationships were also stronger in younger subjects. No association was observed for subjects 55 years or older.

Others have speculated that the uniquely characteristic

Table 4 Estimated combined effects (OR and 95% CI) of lifetime marijuana use (ever versus never) and each of three potential modifiers (cigarette smoking, heavy alcohol use, and mutagen hypersensitivity) on the risk of head and neck cancers by covariates selected for adjustment

Potential modifier	Marijuana use	No. of cases	No. of controls	Covariates		
				No. of covariates (crude)	Pack-years of smoking	Pack-years smoking plus <sup>a</sup>
<b>Smoking history</b>						
Never	Never	26	56	1.0	1.0	1.0
Quit	Never	34	62	1.2 (0.6–2.2)	0.4 (0.2–0.99)	0.4 (0.2–0.99)
Current	Never	84	37	4.9 (2.7–9.0)	1.7 (0.7–3.7)	1.8 (0.6–4.8)
Never	Yes	2	5	0.9 (0.2–4.7)	0.9 (0.2–4.7)	0.7 (0.1–5.5)
Quit	Yes	8	9	1.9 (0.7–5.5)	0.8 (0.3–2.7)	2.1 (0.5–8.3)
Current	Yes	14	2	15.1 (3.2–71)	9.7 (1.1–83.5)	18.8 (1.7–204)
<b>Current smoking</b>						
No	Never	60	118	1.0	1.0	1.0
Yes	Never	84	37	4.5 (2.7–7.3)	3.0 (1.7–5.3)	3.6 (1.7–7.4)
No	Yes	10	14	1.4 (0.6–3.4)	1.3 (0.5–3.2)	2.6 (0.8–8.0)
Yes	Yes	14	2	13.8 (3.0–63)	16.3 (2.0–131)	36.1 (3.6–358)
<b>Alcohol (drinks/month)</b>						
<100	Never	90	132	1.0	1.0	1.0
≥100	Never	51	14	5.3 (2.8–10.2)	4.1 (2.0–8.3)	4.3 (2.0–9.3)
<100	Yes	14	15	1.4 (0.6–3.0)	1.6 (0.7–3.8)	2.7 (0.96–7.5)
≥100	Yes	10	2	7.3 (1.6–34.3)	5.9 (1.2–30.4)	9.6 (1.6–56.8)
<b>Mutagen sensitivity (breaks/cell)</b>						
<1	Never	30	91	1.0	1.0	1.0
≥1	Never	46	28	5.0 (2.7–9.3)	6.3 (2.9–13.4)	6.1 (2.4–15.3)
<1	Yes	6	9	2.0 (0.7–6.2)	2.2 (0.5–9.4)	1.1 (0.2–7.4)
≥1	Yes	10	2	15.2 (3.1–73)	15.6 (2.9–84)	77.1 (7.2–826)

<sup>a</sup> Adjusted for age (continuous variable), gender (male = 0, female = 1); race (white = 0, non-white = 1); education (≤high school = 0; college = 1, >college = 2); heavy alcohol use (<100/month = 0, ≥100/month = 1); and passive smoking (no = 0, yes = 1).

Table 5 Lifetime prevalence (%) of marijuana use in the study controls (no. of users/total) and the United States population, 12 years and older, from 1991 to 1993, by birth cohort<sup>a</sup>

Birth cohort	Males			Females		
	Controls Users/Total	Controls (%)	US Pop. <sup>a</sup> (%)	Controls Users/Total	Controls (%)	US Pop. <sup>a</sup> (%)
<1930	2/41	4.9	1.0	0/29	0.0	0.0
1930–40	2/40	5.0	4.0	1/12	8.3	1.0
1941–45	3/9	33.3	11.0	0/4	0.0	2.0
1946–50	3/9	33.3	28.0	2/8	25.0	14.0
1951–55	0/5	0.0	50.0	0/2	0.0	30.0
1956–60	1/4	25.0	59.0	0/2	0.0	47.0
1961–70	0/3	0.0	57.6 <sup>b</sup>	3/8	37.5	48.6 <sup>b</sup>
Total	11/111	9.9	10.9 <sup>c</sup>	6/65	9.2	10.4 <sup>c</sup>

<sup>a</sup> Source: Johnson R. A. and D. R. Gerstein. Initiation of use of alcohol, cigarettes, marijuana, cocaine, and other substances in US birth cohorts since 1919. *Am. J. Pub. Health*, 88: 27–33, 1998. US Pop., United States population.

<sup>b</sup> Weighted average of the prevalence for 1961–1964 and 1965–1970.

<sup>c</sup> Percentage of users in the United States population standardized to the birth-cohort distribution of the controls in each gender.

technique of smoking marijuana might influence the tumor site of development (19, 20). The more rapid and deeper inhalation technique of marijuana smoking may lead to earlier and more pronounced deposition of carcinogens in the particulate phase of the smoke at relatively narrow sites in the upper airway, such as the larynx, as well as in the central portions of the tracheo-bronchial tree, because of turbulence and inertial impaction (11, 37). At the same time, the prolonged inhalation time might permit larger particles in the tar phase to deposit in the oral cavity, especially on the tongue. Because of the limited sample size, we would not be able to analyze marijuana use and head and neck cancer stratified by tumor site. Future studies with larger sample size are warranted to explore this aspect.

Possible interaction effects were suggested between marijuana use and other risk factors for head and neck cancer. The

interplay between carcinogens and intrinsic host susceptibility is an important factor in environmental carcinogenesis. Mutagen hypersensitivity, an indirect marker for DNA repair, interacts with tobacco smoking in head and neck cancer risk (38–41). Synergy between mutagen hypersensitivity and marijuana use was suggested in this study because the effects were more than additive, which suggests that the development of the upper aerodigestive cancers may be affected by gene-environment interaction. Synergy (greater than additive effects) was also suggested between marijuana use and tobacco smoking. These results suggest that the carcinogenic properties of marijuana may include not only the carcinogens present in tobacco but also other potential carcinogens and/or other factors that might particularly predispose marijuana smokers to cancer development, such as the  $\Delta^9$ -tetrahydrocannabinol-related impairment

of antitumor immunity (12). Because of the low power for testing these interactions, however, the present findings will need to be replicated in future studies.

In summary, this is the first epidemiological report that marijuana smoking is associated with a dose-dependent increased risk of head and neck cancer. This association is supported by a series of case reports and by experimental studies that provide a biologically plausible basis for the hypothesis that marijuana is a risk factor for human head and neck cancer. Given the long induction/latency period of head and neck cancer and the first wave of marijuana use in the 1960s in the United States, it is now time to examine the association between marijuana use and cancer risk. Large epidemiological studies are needed to replicate our results, to examine the relationships between marijuana use and increased risk of cancer, and to assess potential interactions between marijuana use and other risk factors.

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