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MARIHUANA CONTACT TEST, EVALUATION AND DEVELOPMENT

Contract No. DOT-HS-4-01003
February 1976
Final Report

PREPARED FOR:

U.S. DEPARTMENT OF TRANSPORTATION

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16. Abstract

A colorimetric swab test for detecting human contact with marihuana was evaluated. The test was found to be capable of detecting only 83% of marihuana smokers immediately after smoking and was also demonstrated to be subject to a wide range of possible interferences.

An alternative test was developed using thin-layer chromatography as the method for detecting marihuana constituents from a skin swab. This test detected 86% of the smokers immediately after smoking and was not demonstrated to be subject to any interferences.

Recommendations are presented on practical uses for this test.

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PREFACE

This report was prepared under Contract No. DOT-HS-4-01003 for the Department of Transportation, National Highway Traffic Safety Administration. The author gratefully acknowledges the assistance provided by Dr. Fred B. Benjamin, NHTSA, the Contract Technical Manager. The discussions and assistance provided by Dr. Kurt M. Dubowski of the University of Oklahoma, Health Sciences Center, Oklahoma City, and Dr. Bryan S. Finkle of the Center for Human Toxicology, Salt Lake City, Utah, were also much appreciated.

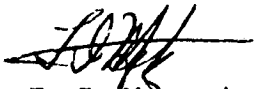
Dr. Edward J. Woodhouse, Principal Chemist, was the project leader and principal investigator. He was responsible for all aspects of the program. He was assisted by Dr. Florence I. Metz, Assistant Director of the Physical Sciences Division, and Dr. Sophia S. Fotopoulos, Director of the Behavioral Sciences Group at MRI.

Many other MRI staff members played a key role in the project. Mr. Jim Windels, Junior Chemist, and Mr. Steve W. Graves, Assistant Chemist, performed the chemical evaluations and analyses. Ms. Diane Dintruff and Mr. Michael Sharp were responsible for the statistical analyses. Dr. Mary Cook, Dr. Charles Graham, Dr. Stan Butts, Mr. Harvey Cohen, Mr. Edward LeCluyse, Ms. Kathryn Haskins, Ms. Pat Emily, Mr. Whitney Sunderland, Mr. William Jellison, Ms. Mary Gerkovitch, Ms. Susan Shockley and Mr. Jack Kohn assisted in the operation of the smoking sessions and testing of subjects.

MRI gratefully acknowledges the assistance of the National Institute on Drug Abuse in providing the marihuana necessary for this project, and for the cooperation provided by Dr. Bryan S. Finkle of the Center for Human Toxicology, Salt Lake City, Utah, and Dr. Joe Cochin of the Boston University School of Medicine in analyzing swab specimens.

Approved for:

MIDWEST RESEARCH INSTITUTE



F. I. Metz, Assistant Director
Physical Sciences Division

October 27, 1975

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SUMMARY

Methods for detecting human contact with marihuana have been investigated in a program designed to both evaluate a previously used method and develop a new method for future use.

The previously used method, and the only method available at that time, was the colorimetric swab (CS) test. This test was used on driver/drug surveys, "The Incidence of Drugs in Fatally Injured Drivers," DOT Contract No. DOT-HS-119-3-627 and "Drug Use Among Drivers," DOT Contract No. DOT-HS-119-2-440. The test relies on the formation of a color reaction with cannabinoids on a skin swab. The CS test was evaluated for sensitivity and specificity. It was found capable of detecting 2 µg of tetrahydrocannabinol (THC) per swab, but was subject to interference by a large number of natural products and cosmetics. The test was found to work best on the lip areas of subjects, the hands producing orange brown color reactions on the swabs which could be confused with the red color formed by a positive reaction with THC. Swabs from corpses showed no false positive reactions which could be attributed to chemical changes in the human skin after death. The CS test was evaluated in a human subject smoking experiment in which 83% of the smokers were identified immediately after smoking by showing a positive swab result from the lips and/or one or both hands. Immediately after smoking 83% of the smokers were also identified by showing a positive swab result from the lips only. This agrees with earlier findings (LWL Report No. LWL-CL-08C72) which indicated a 78% detection rate on the lips of subjects immediately after smoking using the CS test. The results of this evaluation study indicate that the lips are the most reliable area to swab using the CS test, and, using a correction factor obtained from controlled smoker studies, yield an incidence rate of 25.0% marihuana contact for the fatally injured drivers survey and an incidence rate of 7.7% marihuana contact for the survey of drivers on the road. The reliability of these incidence rates is questionable, however, due to the deficiencies found in the CS test in terms of potential interferences and the lack of ability of the test to detect all smokers immediately after smoking.

An alternative method for detecting marihuana contact was developed. This method utilizes a swabbing technique similar to the CS method, but the cannabinoids are extracted from the swab and subjected to thin-layer chromatography (TLC). Specimen collection by the swab and cannabinoid extraction from the swab were evaluated and optimized for use in conjunction with a specially developed TLC system. The TLC system separates the cannabinoids from themselves and from many of the substances producing interferences in the CS test. The optimized test has a sensitivity of 100 ng THC on the swab, and shows a very distinct cannabinoid pattern when positive. Interferences, as documented for the CS test, are eliminated. The

new test was evaluated and validated using human subject smoking programs. Immediately after smoking, 86% of the smokers were detected, 74.4% being detected on the lips. A 60.3% overall detection rate was accomplished for a range of smokers tested up to 3 hr after smoking. The detection rate using the new test was found to be dependent on elapsed time between smoking and testing, but independent of dosages studied. Three separate laboratories were engaged to analyze the swabs; two laboratories agreed very closely with 75.9 and 77.4% overall smoker detection rates, whereas the third laboratory yielded a 52.0% detection rate.

Thus, the TLC test, while not proven suspect of any false positives, still lacks the ability to detect all smokers even immediately after smoking. Because of the uncertainties involved in the false negative problem with the TLC test, it is our opinion that this test should be used for survey data only with great caution, and that the inadequacy of applying correction factors be borne in mind at all times. Since other analytical techniques presently under development, such as radioimmunoassay and mass spectrometry seem likely to offer greater sensitivity for marihuana constituents, it is recommended that these analytical techniques be considered for future use in surveys of the incidence of marihuana contact. Such techniques also offer the promise of detection of marihuana constituents in body fluids, and thus, possible detection of the state of marihuana intoxication of individuals.

A questionnaire survey conducted on this program revealed interest by parties other than the NHTSA in a simple, inexpensive test for evidence of marihuana contact on automobile surfaces, personal clothing and belongings, furniture and other household items. The swab test, utilizing the thin-layer chromatographic analysis, is recommended for such purposes. It must be borne in mind, though, that additional tests would be necessary to provide acceptable legal proof of marihuana contamination of the surfaces tested.

I. INTRODUCTION

This report, the final report in a series of 15 reports, details the accomplishments, results and conclusions of a 16 month project designed to evaluate and develop a marihuana contact test. Specific objectives of the project were to determine the nature and extent of possible error introduced by the colorimetric swab (CS) test used in previous driver studies to detect marihuana contact, and to develop a new test procedure suitable for use in future research on the incidence of marihuana in fatally injured drives and drivers on the road.

Described below are the history of marihuana contact tests; the research approach and methodology of the present project; experimental procedures, experimental results, conclusions and recommendations for each phase of the project.

II. THE HISTORY OF MARIHUANA CONTACT TESTS

The pervasiveness of marihuana use in today's society has raised the question as to whether or not marihuana use constitutes a significant factor in highway safety. This is particularly relevant when considering the well-known statistics on alcohol use and highway safety. In contrast to alcohol, however, there is no known and established method as yet for accurately assaying or even reliably detecting the presence of marihuana constituents or metabolites in the blood or urine of users.

Certain tests, however, have been developed to detect traces of marihuana constituents on the skin of marihuana smokers. These tests involve the removal of the marihuana constituents from appropriate skin areas of the body using a swabbing or washing technique with an organic solvent and then subjecting the swab or washing to a chemical analysis. These techniques are adaptations of original work conducted by Stone and Stevens.[1/] Such techniques have recently been used in NTHSA studies on the incidence of marihuana use in fatally injured and living drivers. A description of the use of these marihuana detection techniques and the problems encountered are detailed below. Included is a description of the original alcohol wash test developed for fatally injured driver testing, the modified alcohol wash test, the modified alcohol swab test, and the colorimetric alcohol swab test.

A. Original Alcohol Wash Test

The National Highway Traffic Safety Administration recently completed a program to determine the incidence of drugs in both fatally injured and living drivers. The inclusion of a test for marihuana use was an important factor in this program, and the only test available to the NHTSA was an alcohol wash test. Upon initiation of the program, conducted by Midwest Research Institute, the NHTSA developed such an alcohol wash test for detecting marihuana constituents on the skin of drivers. This test consisted of washing the oronasal area and fingers of the subject with cotton balls soaked in 70% ethanol. The cotton balls were squeezed dry and the ethanol containing the marihuana residues was combined and subjected to analysis.

After evaporation of the ethanol, the residues were dissolved in 1 ml of a 1:1 mixture of benzene and petroleum ether. The solutions were placed on alumina columns and washed with 10 ml of the same benzene:petroleum ether solution. The cannabinoids (marihuana constituents) were then eluted with 5 ml of a 1:1 mixture of benzene chloroform. The eluate was evaporated to 1/2 ml and spotted on a silica gel G TLC plate. The plate was developed in benzene and sprayed with Fast Blue B (0.25% in 0.1 N HCl), followed by sodium hydroxide solution (0.5 N). A standard solution of tetrahydrocannabinol (THC) was applied to each plate before developing to check on the validity of the results. Smokers and control subjects were used in these tests, and spiked ethanol solutions were also carried through the test procedure as an additional check.

It was found that when this test was applied to human volunteers who had smoked marihuana in the laboratory or when applied to the spiked ethanol solutions, the washings showed either no cannabinoids present or extremely faint indications of their presence. The standard THC spot showed up very well each time and washes on subjects before sampling and washes on control subjects gave no spots on the TLC plate. The conclusion was that the cannabinoids were being trapped on the column along with the fatty fraction of the washings. Further elution of the columns did not alleviate this problem. A modified alcohol wash test was therefore developed which eliminated the use of the alumina column.

B. Modified Alcohol Wash Test

Alcohol washes were obtained in an identical manner to the previous alcohol wash test. Upon evaporation, however, the residues obtained were immediately dissolved in 1 ml methanol and further evaporated to 1/2 ml. This solution was spotted onto TLC plates which were developed as in the previous test. In the case of the control subjects, this test gave no trace

of cannabinoids; likewise, subjects washed before smoking gave no traces of cannabinoids. The standard THC spot gave a red spot of R_f 0.40; the spiked wash gave a positive and so did the subject's washes after smoking. Thus it seems reasonable to conclude that the column technique, while removing fat from the samples, also removed much or most of the very fat soluble cannabinoids. Elimination of the column steps resulted in a solution from which fat could be physically removed if desired, and this solution then yielded positives on a TLC system for subjects after smoking marihuana.

At this point it seemed that a method suitable for testing fatally injured and living drivers for marihuana had been developed. However, another problem emerged. Contact with coroners and medical examiners revealed that a method which employed washes with ethanol and cotton balls was likely to be too cumbersome for practical use. The program to determine the incidence of marihuana (and other drugs) in fatally injured drivers depended on the ability to dispatch kits containing supplies and instructions to coroners and medical examiners so that on-site washes could be obtained in locations all over the United States. These washes would then be mailed back to MRI for laboratory testing. The coroners in this program were cooperating free of charge and indicated that swabs such as Q-Tips® would be much preferred to cotton balls. In order to facilitate the use of the test by coroners and medical examiners, the decision was made to use Q-Tips® type swabs with 70% ethanol as a test method.

Because of the small size of a Q-Tip® swab, a "wash" would not be possible, i.e., it could not be squeezed out on the spot like a cotton ball and discarded, leaving only a solution for analysis. The swab could only be moistened with a small amount of alcohol, used, and then packaged and mailed back to the laboratory for elution of the cannabinoids. This procedure is described in the next section as the modified alcohol swab test.

C. Modified Alcohol Swab Test

The modified alcohol swab test was identical to the modified alcohol wash (cotton ball) test except the Q-Tips® were used instead of cotton balls. The swab test was employed on the fatally injured drivers study and the living drivers study as follows.

Swabs were moistened with 70% ethanol, and the lips (exposed) and areas between the upper lip and the nose, the thumb and first two fingers of each hand, swabbed with a gentle rolling motion. The swabs were then placed in separate protective tubes and mailed back to the laboratory by air. Upon arrival at the laboratory, the swabs were washed with a small

amount of methanol, squeezed dry, and the washing solution evaporated to dryness and subjected to thin-layer chromatography. The results on the first 265 cases revealed 6 cases in which positives were identified. Upon investigation with spiked swabs in the laboratory, it was revealed that the majority of the cannabinoids were adhering to the cotton swab and were not eluted by the methanol wash. This was especially true if the swabs had dried out in the time period between swabbing and laboratory analysis. This problem had, of course, not existed with alcohol wash techniques using cotton balls.

At this point, a decision was made to analyze the swabs by a direct colorimetric test performed on each swab. The logic behind this decision was based on the results of an U.S. Army Land Warfare Laboratory Contract (No. DAAD05-72-C-0187) just completed by Midwest Research Institute which indicated that such a colorimetric swab (CS) test was 78% effective for lip swabs of marihuana smokers. The CS test also revealed no false positives with volunteer marihuana smokers.

D. Colorimetric Alcohol Swab Test

The colorimetric swab test was developed and evaluated by Midwest Research Institute under contract to the U.S. Army Land Warfare Laboratory (Report No. LWL-CL-08C72). The test is performed on swabs obtained by gently rolling an alcohol-moistened Q-Tips® swab on the skin areas under investigation. The swabs are simply dried, if necessary, and then moisturized with 2 drops of 0.25% Fast Blue B in 0.1 N HCl. After drying, 2 drops of 0.2 N NaOH is added to the swab, and a positive is indicated by any red or pink color appearing within 2 sec of the addition of the NaOH. This colorimetric swab test was proven effective for lip swabs from marihuana pipe smokers and is fully documented in the U.S. Army Land Warfare Laboratory Report No. LWL-CL-08C72. Seventy-eight percent of a population of 100 smokers yielded positive results in this study after smoking 600 mg of marihuana containing 1.7% THC. No false positives were recorded. Table 1 summarizes the results of this evaluation. Interfering substances in this test were evaluated using spiked swabs and the only substances found constituting an interference were Areca, Catechu, Mormon Tea and Yohimbe. Table 2 lists the substances examined for interferences in this test and the color readings obtained.

The colorimetric swab test was employed on 345 fatally injured drivers and the number of positive responses was surprisingly high. Thirty-eight percent of the fatally injured drivers showed at least one positive response. These results, as indicated in Table 3, lead one to suspect that an unknown factor associated with fatally injured drivers is contrib-

TABLE 1

SUMMARY OF RESULTS ON SMOKING TEST USING THE COLORIMETRIC SWAB TEST[a/]

Substance[b/] Smoked	Test Results Before				Test Results After				Percent
	Controls		Smokers		Controls		Smokers		
	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(+)
0.6 g marihuana	9	0	50	0	9	0	13	37	74 (37/50)
0.4 g marihuana	5	0	50	1	5	0	15	35	70 (35/50)
0.25 g marihuana	7	0	50	4	7	0	19	31	62 (31/50)
0.6 g marihuana	5	0	50	2	5	0	9	41	82 (41/50)
0.4 g marihuana	8	0	50	1	8	0	13	37	74 (37/50)
0.25 g marihuana	7	0	50	2	7	0	20	30	60 (30/50)
0.6 g hashish	3	0	50	1	3	0	9	41	82 (41/50)

a/ As report in LWL Report No. LWL-CR-08C72, LWL Contract No. DAAD05-72-C-0187. The swabs in this study were lip swabs.

b/ Marihuana (1.7% THC) and hashish (0.1% THC) were smoked in pipes.

TABLE 2

EVALUATION OF POSSIBLE INTERFERENCES IN THE COLORIMETRIC LIP SWAB TEST FOR MARIHUANA SMOKERS [a/]

Material Tested	Color Observed	Material Tested	Color Observed
THC (Active Cannabis Ingredient)	Bright Red	Mescaline	Pale Brown/Orange
		Lobeline	Pale Brown/Orange
		Nalorphine	Pale Brown/Orange
Phenobarbital	Pale Brown/Orange	Phenmetrazine	Pale Brown/Orange
Pentobarbital	Pale Brown/Orange	Tripelennamine	Pale Brown/Orange
Amobarbital	Pale Brown/Orange	Methapyrilene	Pale Brown/Orange
Secobarbital	Pale Brown/Orange	Phenylpropanolamine	Pale Brown/Orange
Butabarbital	Pale Brown/Orange	Oxymorphone	Pale Brown/Orange
Butobarbital	Pale Brown/Orange	Areca	Dark Brown/Pink
Diphenylhydantoin	Pale Brown/Orange	Catechu	Dark Brown/Pink
Meperidine	Pale Brown/Orange	Chamomile	Pale Pink/Orange
Acetyl Salicylic Acid	Pale Brown/Orange	Damiana	Brown
Salicylic Acid	Pale Brown/Orange	Hops	Brown
Chlorpheniramine	Pale Brown/Orange	Horsetail	Brown/Green
Diphenhydramine	Pale Brown/Orange	Kava Kava	Brown
Amitriptyline	Pale Brown/Orange	Kola	Brown
Thioridazine	Pale Brown/Orange	Lobelia	Brown
Propoxyphene	Pale Brown/Orange	Mistletoe	Brown
Quinine	Pale Brown/Orange	Mormon Tea	Orange/Pink
Methylphenidate	Pale Brown/Orange	Tobacco	Brown
Oxazepam	Pale Brown/Orange	Mustard	Brown
Promazine	Pale Brown/Orange	Onion	Brown
Trifluoperazine	Pale Brown/Orange	Paprika	Brown
Chlorpromazine	Pale Brown/Orange	Passion Flower	Brown
Imipramine	Dark Brown/Pink	Skull Cap	Brown
Diazepam	Pale Brown/Pink	Valerian	Brown
Morphine	Pale Brown/Pink	Wormwood	Brown
Codeine	Pale Brown/Pink	Yohimbe	Orange/Pink
Glutethimide	Pale Brown/Pink	Nutmeg	Brown/Pink
Cocaine	Pale Brown/Pink	Cinnamon	Brown
Methadone	Pale Brown/Pink	Cloves	Brown/Pink
Hydromorphone	Dark Brown/Pink	Ginger	Brown/Pink
Quinine Extract	Dark Brown/Pink	Mace	Pink/Orange
Nicotine	Dark Orange/Yellow	Pepper	Brown
MDA	Pale Brown/Pink	Rosemary	Brown
STP	Pale Brown/Orange	Sage	Brown
Amphetamine	Pale Brown/Orange	Thyme	Brown
Methamphetamine	Pale Brown/Orange		
DMT	Pale Brown/Orange		
DET	Pale Brown/Orange		

a/ As reported in LWL Report No. LWL-CR-08C72, LWL Contract No. DAAD05-72-C-0187.

TABLE 3

INCIDENCE OF MARIHUANA IN FATALLY INJURED DRIVERS
AS TESTED BY THE COLORIMETRIC SWAB TEST
(323 Drivers Total)

Test Swab	Swabs Tested	Number of Positives	Percent Positives	Total Incidence[a]
Right hand	303	80	26.4	33.8
Left hand	305	77	25.2	32.3
Mouth	201	44	21.9	28.1
Complete set	323 sets	124 sets[b]	38.4	49.2
Complete set of three swabs clean enough for testing	195 sets	23 sets[c] 67 sets[b]	11.8 34.4	15.1 44.1

a/ Adjusted incidence takes into account that the colorimetric swab test yielded 78% positives on tests with smokers in laboratory controlled experiments (U.S. Army LWL Report No. LWL-CR-08C72).

b/ Incidences in which at least one swab was positive per set.

c/ Incidences in which all three swabs were positive.

uting an interference with the colorimetric swab test. Swab tests conducted at MRI with living volunteers, showed that cigarette smoke and excessive perspiration did not contribute this interference. The same swab test, when used on living drivers, produced a positive response in only 6% of the drivers.*

E. Conclusions from Previous Work

Precisely stated, the conclusions that can be drawn from the previous work conducted by Midwest Research Institute in the area of skin-washing or swabbing tests are as follows:

1. Cannabinoids can be removed from the skin of a marihuana smoker using a suitable organic solvent, e.g., ethanol.

2. Washing with cotton balls which are immediately squeezed dry will produce a liquid wash containing these cannabinoids.

3. Thin-layer chromatography of washing residues will separate the cannabinoids from one another and from any of the other materials seen during any of these experiments. Visualization of the cannabinoids on the TLC plates using Fast Blue B is extremely sensitive (better than one microgram) and reasonably specific.

4. When cotton swabs (Q-Tips®) are used to remove the cannabinoids from the skin, removal of the cannabinoids from the swab necessitates more than a simple elution or wash with methanol. This is particularly true if the swabs are allowed to dry after taking the sample.

5. Colorimetric swab tests, i.e., testing for the presence of cannabinoids directly on the swab by means of a color reation with Fast Blue B, works well with experimental living human subjects. No false positives have been recorded. It is suspected that an interference is present in swabs from fatally injured drivers (or deceased persons in general), since a very high percentage of positives was recorded in the recently conducted NHTSA study.

The colorimetric swab test may, in fact, be yielding accurate information on the incidence of marihuana use in fatally injured drivers. However, the interpretation of the colorimetric swab test results needs more information in terms of the validity (sensitivity and specificity) of the colorimetric technique. Research to provide both adequate information on the validity of the colorimetric swab technique and an improved skin-washing or swabbing procedure for future use are the objectives of the research project described in this report.

* Results are from DOT Contract No. DOT-HS-119-2-440, final report.

III. RESEARCH APPROACH AND METHODOLOGY

The research program described in this report was designed to determine the nature and extent of possible error introduced by the colorimetric swab (CS) test procedures used in previous studies. Furthermore, the program was designed to determine how such information could be used in the interpretation of the results from previous studies. A final objective of the project was to develop a new test procedure suitable for use in future research on the incidence of marihuana on fatally injured drivers, injured drivers, and drivers on the road.

In order to gain useful information to evaluate and develop the marihuana tests, many factors were taken into consideration. The operations involved in the collection of the samples, especially by remote personnel, must be fully understood and taken into consideration. The delay between sample acquisition and analysis is important. The choice of swab materials, washing solvents, packaging of the swabs, and storage must be taken in consideration. The chemistry of the cannabinoids and other materials native to the skin in living and deceased subjects, as well as other materials which may be present, must be emphasized. The techniques of elution or washing of swabs and the subsequent thin-layer chromatography of residues must undergo careful scrutiny. The chemistry of the color reaction on the swab needs very close examination in terms of possible interfering substances. Once initial development and evaluation is completed, vigorous evaluation of new techniques and comparison with prior techniques must be carried out in blind studies on populations of living and deceased subjects.

A research plan was adopted to accomplish the following objectives:

* Evaluation of the colorimetric swab test.

* Development and evaluation of other analytical techniques for detecting marihuana constituents in skin washings.

* Evaluation of specimen collection and extraction techniques used in the colorimetric swab test and other promising techniques.

* Determination of how this information should be used in the interpretation of previous colorimetric swab test results.

* Recommendation of a procedure for future use.

* Validation of the recommended procedure.

The research program was conducted in two phases. The tasks involved in Phase I are depicted in Figure 1. Validation of the recommended new procedures constituted Phase II of the program. The accomplishment of specific tasks in the two phases of the program is described in the next sections.

IV. EXPERIMENTAL PROCEDURES, PHASE I

This section details accomplishments in the successive steps of the experimental program. The following steps are described in order:

A. Task 1. Identification of Test and Evaluation Requirements

B. Task 2. Evaluation of Analytical Methods

C. Task 3. Evaluation of Specimen Collection and Extraction Methods

Task 4, as outlined in Figure 1 is discussed in a succeeding section.

A. Task 1. Identification of Test and Evaluation Requirements

In order to identify uses to which the marihuana contact test will be put by NHTSA and other government agencies and individuals, a letter and questionnaire was compiled and sent to individuals and agencies across the country A copy of this letter and questionnaire is attached to this report along with a listing of the recipients as Appendix A.

The letter and questionnaire were sent to a cross section of those individuals who were judged most likely to be interested in a relatively simple and inexpensive test for contact with marihuana.

Out of 51 individuals contacted, 20 expressed interest by returning the completed questionnaire. A summary of the respondents' replies is shown in Figure 2 (a filled-in copy of the questionnaire). The consensus of opinion was as follows:

- 13 out of the 20 respondents would have a use for the test.
- 12 out of the 20 respondents would use it for swabbing skin.
- 10 respondents showed an interest in using the test for other surfaces, in particular, vehicle interiors and clothing, pipes, teeth and containers in general.

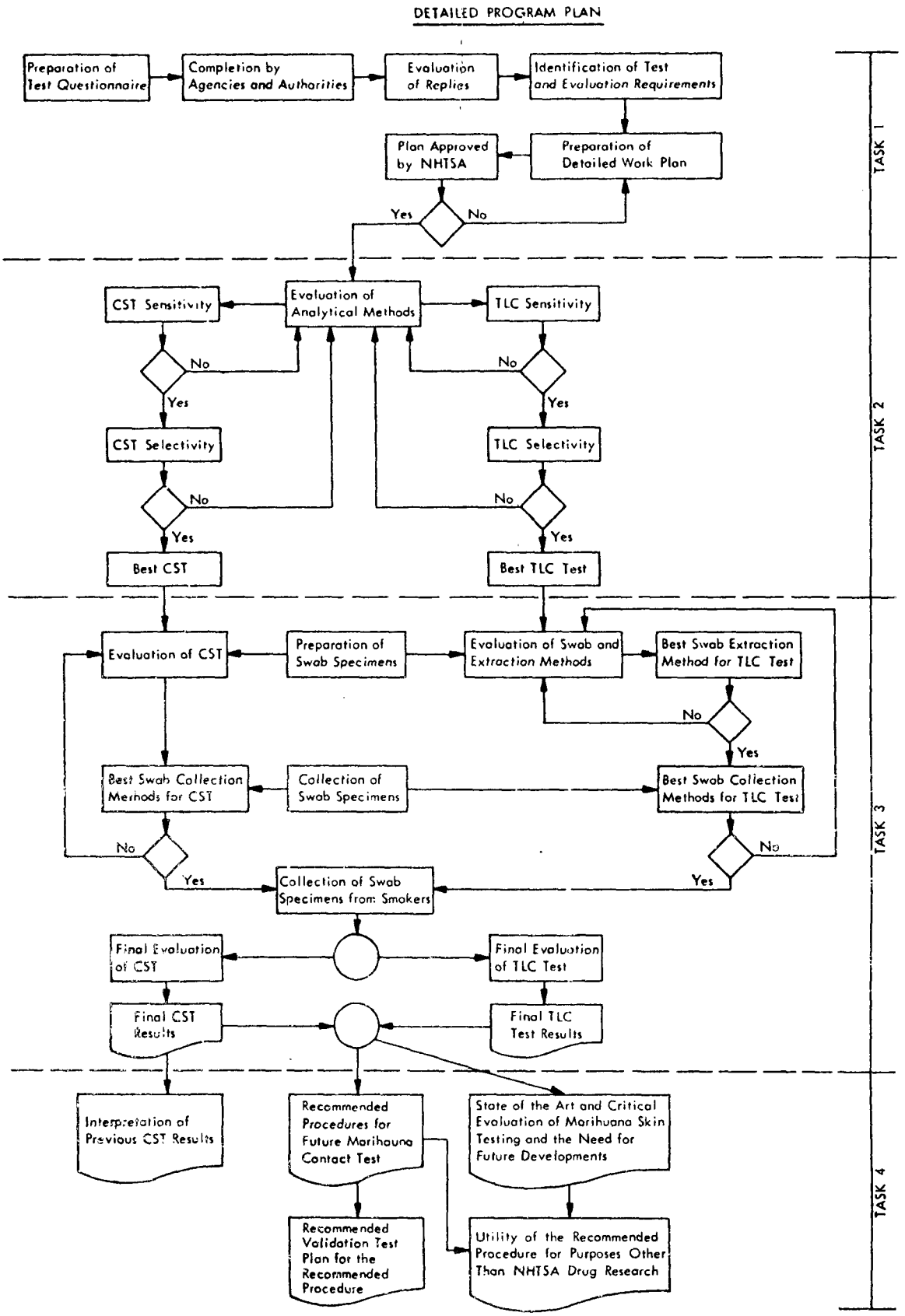

Figure 1 - Detailed Program Plan

Assuming that a simple contact (swab) test for detecting marihuana was available, would you have a use for it?	[13] Yes	[6] No
Would you use it for:		
- Detecting marihuana smokers by swabbing skin areas (lips, etc.)	[12] Yes	[6] No
- Detecting the presence of marihuana residues on surfaces other than skin, and if so, what surfaces:	[9] Yes	[9] No

pipes (1); teeth (1); containers (1); windshields (1); clothes (3); vehicle interiors (3).

Describe briefly any other uses you could conceive or would like to use the contact test for:

Please describe briefly any programs in which you feel the contact test would be of use (surveys, enforcement programs, forensic applications, etc.):

Driving under the influence of marihuana (8); surveys (4); forensic work (5); accident investigations (3); enforcement (2); school surveys (2); personnel on the job (1).

Figure 2 - Marihuana Contact Test Questionnaire--Summary of Results.

Which criteria do you consider most important in such a simple contact test:

	Very Important	Important	Un-important
Specificity (minimum of false positives)	18	1	1
Sensitivity (minimum of false negatives)	7	11	2
Cost of test--state maximum cost ____¢/test	1	7	10
Ease of use	6	13	1
Adaptability for field use	6	9	5

Any other comments: Toxicity of solvents (2); more interested in methods for THC in body fluids (2); training of test administrators (1); time studies (1); effect of temperature (1); quantitation from swabs (1).

(Please use additional space if necessary.)

Name: _____

Title: _____

Organization: _____

_____

Address: _____

_____

City: _____

State: _____ Zip: _____

Figure 2 (Concluded) - Marihuana Contact Test Questionnaire - - Summary of Results.

- Eight respondents expressed interest in using the test for driver surveys or tests to determine if drivers were smoking marihuana. Four respondents indicated interest in using the test for surveys in general, two were interested in school surveys. Enforcement use was specified by two respondents, forensic applications by five respondents, accident investigations by three respondents and employee personnel screens mentioned by one respondent.

- 18 of the 20 respondents stressed specificity as "very important."

- Seven of the 20 respondents felt sensitivity was "very important," and 11 felt that it was "important."

The majority of the respondents did not feel the cost of the test was important. Out of six quoted costs, seven were in the $1.00/test range--the rest were less.

- Ease of use of the test was considered "important" by 13 respondents--and "very important" by six respondents.

- Adaptability for field use was considered "important" by nine respondents and "very important" by six respondents.

- Two respondents were more interested in methods for THC in physiological specimens. Two respondents stressed considerations of the toxicity of swab solvents. One reply stressed the training of test administrators, one the effect of temperature on the test and one respondent questioned the possibility of quantitating from the swab.

Overall, the majority of respondents would have a use for the test, especially for skin swabbing and motor vehicle and clothing swabbing, especially for drivers surveys. Specificity was considered very important, as was ease of use of the test, and adaptability to field testing.

The research program was therefore designed to evaluate the previously used colorimetric swab (CS) test and to develop a new test with the above respondents' criteria in mind as well as the criteria of sensitivity and specificity needed for NHTSA use in the future.

B. Task 2. Evaluation of Analytical Methods

The analytical methods to be considered in this program are the previously used colorimetric swab (CS) test and a new test. The new test to be considered will, in the light of the previous data and history of testing for marihuana, consist of a thin-layer chromatographic (TLC) test. Thin-layer chromatography offers the advantage of separation of the compounds picked up by a swab as well as the high sensitivity offered by well known visualization agents such as Fast Blue B.

The evaluation in this task included determination of the sensitivity and validity for the techniques considered, i.e., the colorimetric swab test and thin-layer chromatography.

1. Colorimetric swab test: The colorimetric swab (CS) test was evaluated for sensitivity and specificity by conducting the test on swabs spiked with alcoholic solutions of THC and possible interferences, as follows:

A spiked swab (Q-Tip®) is allowed to dry for a specific period of time, two drops of Fast Blue B solution (0.25% in 0.1 N HCl) added, the swab allowed to dry for 2 min, then one drop of 0.2 N NaOH added. Any color change is noted; a red or pink color appearing immediately is taken as a positive result.

a. Evaluation of sensitivity: In preliminary experiments it was noted that the solvent used in spiking the THC (ethanol or isopropanol) had an inhibiting effect on the color reaction; thus it was necessary to dry the swabs for 20 min. Also, the Fast Blue B solution must be made up fresh daily to consistently yield negative blanks. These preliminary tests indicated the sensitivity of the CS test was of the order of 2 µg THC on the swab (identical to a swab dipped in a 10.0 ppm THC solution). A series of 100 blind tests were devised to test out the CS test for sensitivity. The results, as shown in Table B-1 (Appendix B), indicate that the sensitivity limit is achieved with a swab dipped in a 10 ppm THC solution, i.e., 2 µg THC per swab. No false positives were recorded.

b. Evaulation of specificity: The colorimetric swab test was also evaluated for interfering substances by applying 200 µg of a selection of substances to the swab and then applying the colorimetric test. Table B-2 lists the substances tested, and the colors achieved on the swabs. Possible interfering substances are:

Areca	Old Spice® After Shave
Catechu	Listerine®
Kava-kava	Jaguar® After Shave
Kola	Breck® Creme Rinse
Yohimbe	Blistik® Lipstick
Nutmeg	Clearasil®
Mace	British Sterling® After Shave
Sage	Viva Patchouly® After Shave
Thyme	Desire® Perfume
Methylphenidate	Cachet® Perfume
Oxymorphone	Jacques® After Shave
Hydromorphone	Ritz® Cologne
Licorice	Extract of Wood Q-tip® Handles
Prop® Electric Preshave	

Solid or semisolid substances were extracted by mixing with methanol in a Waring Blender. Liquids were used unchanged or were extracted with methanol and filtered to obtain a clear extract.

2. Thin-layer chromatographic test: Thin-layer chromatography was evaluated in terms of sensitivity and interferences on solutions which could represent washings from swabs. Preliminary experiments using ethanolic solutions of THC indicated that evaporation of solutions by compressed air or hood draft at room temperature or in a water bath at 60°C all produced equally good residues of THC which, after reconstitution in 1 ml of petroleum ether, evaporation and reconstitution in 0.1 ml of ethanol, could be spotted onto TLC plates.

Several solvent systems were evaluated for the thin-layer chromatography of THC. Glass TLC plates, 5 x 10 cm, coated with 0.25 mm silica gel G were spotted with THC. After development in a solvent, the plates were dried in air and sprayed with 0.25% Fast Blue B in 0.1 N HCl, followed by a spray of 0.2 N NaOH. In all cases, the sensitivity of detection on the plate was approximately 100 ng THC. Table B-3 lists the solvents evaluated, and the mobilities (R_f values) of the THC.

a. Evaluation of sensitivity: To adequately test the sensitivity of the TLC system, a blind test was conducted on 56 5 ml ethanol aliquots, spiked with THC. The ethanol was evaporated to dryness, reconstituted in 1 ml petroleum ether, evaporated to dryness and reconstituted in 0.1 ml ethanol. After spotting on TLC (silica gel G) plates, developed in benzene and visualized with the Fast Blue B, results were obtained as shown in Table B-4. The limit of sensitivity is about 250 ng THC. No false positives were recorded.

To further test the sensitivity and specificity of the TLC systems, ethanol washings from human forearms were used instead of pure ethanol. These washings were spiked with THC, 5 ml aliquots being used in each experiment. Fifty-six forearm washing experiments were tested in a blind study, the results are shown in Table B-5. The results are listed in parts per million THC in the 5-ml washings. The sensitivity limit is seen to be 0.05 ppm THC in the washings, i.e., 250 ng/5-ml washing. All the washings gave identical TLC background patterns due to substances in the skin oil. However, no false positives were encountered.

The above experiments were repeated using the same conditions except that the TLC operations of spotting, developing and visualization were all conducted within 1 hr. The sensitivity limit improved under these conditions to approximately 100 ng THC per washing.

b. Evaluation of specificity: The thin-layer chromatographic system utilizing benzene (unsaturated conditions) as the developing solvent was tested for interferences with the same potential interfering substances used in the CS test. Table B-6 lists the mobilities and colors of materials tested. Twenty micrograms of most substances were used in these experiments. Interferences, in terms of R_f and color, could possibly be caused by:

Emeraude® Perfume
British Sterling® After Shave
Desire® Perfume
Jacques® After Shave
Ritz® Cologne

However, none of the above cosmetics give a pattern similar to the cannabinoids, THC, cannabinol (CBN) and cannabichromene (CBCH) which is likely to be observed from a swab contacted with marihuana. In order to further reduce the possibility of interferences, a second thin-layer chromatographic developing solvent was sought. A suitable solvent was found in a mixture of hexane/ether, 4:1 (unsaturated). Table B-7 shows the R_f values and colors for those materials which posed a possible interference with THC in the benzene solvent. Although the R_f values of some of the spots approach that of THC, the materials containing the substances responsible for these spots also contain many other materials giving a multitude of spots. In no cases are the spots close to the R_f of THC, the same color as THC.

The TLC system of choice, therefore, is:

- Silica gel G plates - 250 μ on glass.
- Developing solvents 1. Benzene
  2. Hexane/ether, 4:1
- Visualization reagent - 0.25% Fast Blue B in 0.1 N HCl followed by 0.2 N NaOH.

The mobilities of the cannabinoids will vary with the degree of saturation of the developing tanks. If an unsaturated condition is used, the mobilities of the cannabinoids are:

	R_f (benzene)	R_f (hexane/ether)
THC	0.57	0.59
CBN	0.63	0.52
CBD	0.67	0.69
CBCH	0.46	0.40

Saturated developing solvent conditions produce lower mobilities as follows:

	R_f (benzene)	R_f (hexane/ether)
THC	0.40	0.35
CBN	0.44	0.31
CBD	0.47	0.41
CBCH	0.32	0.24

The same possible interferences are noted under either conditions with the benzene solvent, and are eliminated by the hexane/ether solvent under both conditions. Saturated conditions seem to render slightly more sensitive a detection and are therefore used in future work in this program. All TLC operations should be conducted within 1 hr to avoid loss of sensitivity.

Sensitivity is 100 ng THC on the plate using benzene as the developing solvent and 500 ng THC on the plate using hexane/ether as the developing solvent.

The observation of the cannabinoid (THC + CBN + CBCH) pattern on the TLC plate with the first developing solvent (benzene) can be taken as definite evidence of the presence of marihuana. Additional confirmation using the less sensitive hexane/ether developing solvent is considered a worthwhile but not a necessary step.

C. Task 3. Evaluation of Specimen Collection and Extraction Methods

In order to maximize the sensitivity of the CS or TLC test methods, the swab collection method should remove as much cannabinoid material as possible from the skin surface. For the TLC method to achieve maximum sensitivity, extraction of cannabinoids from the swab should be efficient. These factors are discussed below.

1. Specimen collection: Experiments were conducted to determine the most appropriate materials for removing THC from the skin.

A laboratory Plexiglas glovebox was fitted with loose flaps for the easy entry of forearms and hands. The box was situated in a hood to remove smoke and fumes from the breathing atmosphere of the laboratory. A small amount of marihuana was slowly burned in an open crucible with a draft of air or a marihuana cigarette was held in the box by the subject to produce an atmosphere of marihuana smoke. Both techniques produced comparable results.

Hands and forearms were exposed to the marihuana smoke for approximately 4 min per experiment. Controls were also run on subjects not exposed to marihuana smoke. The following tests were performed.

a. CST: Areas of approximately 4 sq in. of flesh were swabbed with a Q-Tip® dipped in 100% ethanol and the standard test was performed. In most cases, the test swab showed a positive reaction which quickly turned brown. The control swabs also turned orange/brown making them a poor example for comparison with the test swabs. It appears that a certain material may be present on normal skin which is not present on lips as this problem was not encountered in previous laboratory-controlled lip swabbing experiments. It already appears that the CS test is unsuitable for swabbing areas other than the lips.

b. TLC test: Areas of approximately 4 sq in. of flesh located on forearms and hands were swabbed with a Q-Tip® or a cotton ball dipped in 100% ethanol. The resulting swabs were examined by shredding the Q-Tip® and eluting with hot acetone or, in the case of cotton balls, just collecting the liquid squeezed from the ball. The eluates were evaporated to dryness, reconstituted in 0.1 ml ethanol and subjected to TLC, using benzene as the developing solvent. The results are shown in Table C-1, Appendix C, and indicate that the Q-Tip® and cotton ball with 100% ethanol picked up the cannabinoids. Little, if any, difference was noted between cotton balls and Q-Tips®. Repetition of the experiment using 70% ethanol also produced similar results.

In order to further examine different types of swabbing materials, Q-Tips®, paper, wool, cotton cloth, cotton balls, foam rubber, glass wool and nylon cloth were examined for retention of cannabinoids (i.e., ease of elution). Amounts of the above materials, equal to the weight of one Q-tip® cotton end were spiked with 2 µg THC, eluted with hot acetone, the eluate dried, reconstituted and subjected to TLC.

The elutions were performed immediately after spiking, 2 days, and 1 week later after storage at room temperature in the dark. All materials gave the same result, an easily observable spot matching with THC on the TLC plate, except nylon which gave a much weaker spot. After 2 days and even 1 week, the spots were still of the same intensity, except those from nylon which disappeared after 1 week. The conclusion is that Q-Tip® swabs are as good as any material tested and that the cannabinoids can be eluted from the swab material.

2. Specimen extraction: Extraction of the swabs is necessary in the TLC test in order to elute the cannabinoids from the swab for thin-layer chromatographic examination. Such extraction is not necessary in the CS test which is performed on the swab itself.

Further tests have been conducted to determine the optimum elution conditions to remove THC from Q-Tip® swabs. The parameters studied were: elution solvents; temperature; time elapsed between sample collection and elution; agitation of elution solvent; physical disintegration of the swab; and duration of elution time. These factors are considered below.

Elution solvents and temperature - Q-Tip® swabs were spiked with 0.1 to 0.5 µg THC, the cotton removed from the swabs, shredded, placed in the elution solvent and allowed to soak for 30 min. The elution solvent (2 ml) was then evaporated to dryness and the residue treated by the standard TLC method using benzene as the developing solvent. Experiments were conducted using methanol, ethanol, chloroform, petroleum ether, benzene and acetone at room temperature and at 60°C.

Each solvent was tested 10 times at each temperature.

In each and every case, a positive was recorded for all the elution solvents tested at both temperatures. Attempts to reduce the amount of THC on the swab resulted in positives being recorded in all cases until the limit of sensitivity of the TLC spray (Fast Blue B Salt) was reached (~ 50 ng/swab). Below 50 ng/swab no positives were recorded in any cases.

This indicates that under the conditions of elution, the only factor affecting the results is the sensitivity of detection of the THC on the TLC plate.

Thus all the solvents tested are capable of eluting the cannabinoids as required by the swab test using TLC. We have chosen acetone to continue our experimentation since it is of amenable volatility and low toxicity. Methanol would be the next candidate solvent.

Elapsed time - Q-Tip® swabs treated with 0.1, 0.2, 0.3, 0.4, and 0.5 µg THC were eluted with acetone at room temperature for 30 min, evaporated and subjected to TLC using benzene as the developing solvent. Samples were eluted immediately, after 2 days, and after 1 week. Storage was in the dark at room temperature. The results are shown in Table C-2, and indicate that the sensitivity limit is 100 ng/swab or better and that no deterioration in THC is seen within 1 week of storage.

Agitation and shredding - Q-Tip® swabs were spiked with 0.1 and 0.5 µg THC, left whole or shredded, eluted with cold acetone for 30 min, with and without stirring. The elution solvents were then evaporated to a residue which was subjected to TLC using benzene as the developing solvent. The results are shown in Table C-3 and indicate that no dramatic effect results from either shredding or agitation. Again, the limit of detection is 100 ng THC per swab or less. Judging from TLC spot intensity, shredding and agitation do improve the elution very slightly.

Elution time - Q-Tip® swabs spiked with 0.1 and 0.5 µg THC were subjected to the elution procedure and TLC analysis using benzene as the developing solvent. Swabs were subjected to 5, 10, 20, and 30 min elution times. The results, as shown in Table C-4 indicate that 5 min elution time is as good as 30 min and sufficient for detection of 100 ng THC per swab.

Optimized swab extraction procedure: The above data indicate that the optimized swab extraction procedure is as follows:

The cotton on the sample swab is removed by slicing through it with a razor blade held parallel to the stem and scraping gently to lift the cotton away from the adhesive on the stem. The cotton will come off easily and completely when the fibers are cut cleanly all the way from the exterior of the swab to the plastic of the stem. The cotton is dropped into a 30 ml glass conical evaporation vessel and the stem itself placed in the vessel for use in agitating the sample and removing the cotton after elution. Approximately 3 ml of reagent grade acetone is added to the vessel and the THC allowed to elute for 5 min at room temperature. The swab stem is used to stir the solvent and tamp on the cotton periodically during the elution period. After extraction, the cotton is removed by using the swab stem and excess solvent squeezed out. The vessel walls are washed with 1 ml acetone and the sample evaporated to dryness by placing the vessel in a draft in a warm-water bath.

The residue is reconstituted in 0.1 ml ethanol and subjected to TLC. The optimized TLC system described earlier on p. 20 is used.

To investigate the actual extraction efficiency, swabs were spiked with THC in 70% ethanol at such concentrations as to place 200 ng, 500 ng and 1 µg of THC on the swabs. Three swabs were spiked at each concentration level. The swabs were allowed to dry, and then extracted and analyzed by the standard TLC procedure using benzene as the developing solvent. THC standards of known concentration were placed on the TLC plates for comparison of spot intensity and rough quantitation. In the case of all three concentration levels, it appears that between 60 and 70% of the THC originally on the swab appears on the TLC plate. Since the sensitivity of the TLC visualization reagent is 50 to 100 ng THC, the sensitivity of the total method is approximately in the range of 70 to 140 ng THC per swab which should be entirely adequate for the contact test. A sensitivity of 100 ng/swab was documented in earlier studies in this program.

3. Instruction effectiveness: The removal of cannabinoids from skin using a swabbing technique depends not only on the swabs used, but also on the technique used by the swabbers. It was not known how effective the instructions were and how they were interpreted by naive swabbers.

To further investigate this, an experiment was devised at MRI in which naive swabbers would be requested to follow written instructions. Over a 1-week period, 20 persons were asked to swab an investigator's hand exposed to marihuana smoke. Ten additional persons were asked to swab another investigator's hand not exposed to smoke. The investigator exposed his hand to marihuana smoke by burning a 500 mg reefer of marihuana (2.61% THC, 0.17% CBCH, 0.01% CBD, 0.15% CBN) in a vented glove box. The swabber was then asked to read the following instructions and swab the exposed hand. No aid or further explanation was given to the swabbers:

MARIHUANA SWAB INSTRUCTIONS

> This test uses a swab in a glass tube and a tube containing a 70% alcohol solution.
>
> Remove the swab from the swab tube, dip it in the alcohol and swab the thumb and tips of the fingers of the hand to be tested.
>
> Place the moist swab back in the swab tube, and screw the cap on firmly.

During the swabbing, the investigator noted the method of swabbing used. The investigator then washed his hands and stored the swab for later analysis.

The swabs were analyzed using the standard extraction and TLC system using benzene as the developing solvent. The results on exposed hands are listed in Table C-5. The analyses were run in a blind manner. All 10 swabs of hands not exposed to marihuana smoke yielded negative results. The results on exposedhhands yielded positives in all but one case. Positives always consisted of a THC spot with smaller amounts of cannabinol and cannagichromene also present. No cannabidiol was detected, probably due to its low concentration in the marihuana. TLC positives were rated as weak, medium or strong.

The investigation revealed that many people will just use a single swab stroke on the face (flesh side) of each digit (every swabber confined the swabbing to the first phalange). Ten (one-half) of the swabbers used one stroke on the face of the digits. The negative results appeared when this one stroke was extremely light in pressure. Six weak strength TLC results and three medium strength TLC results were yielded by the other swabbers in this category.

Four of the remaining swabbers used several strokes on the face of each digit and their swabs yielded medium strength positives. Two more swabbers still used only the face of the digits but scrubbed hard, yielding strong TLC results.

Of the remaining four swabbers, one used medium pressure strokes all around the digits (face, sides and nail) producing a swab which yielded medium strength TLC spots. The remaining three swabbers used heavy scrubbing strokes all around the digits, producing swabs which yielded strong TLC spots.

The table below shows the results by sex of the swabber. Twelve males and eight females volunteered for this program. The majority of females used several strokes or a scrubbing action, mostly just on the face of the digits, resulting in medium or strong results. Seven of the males used a single stroke resulting in weak or medium results and five used several strokes or a scrubbing action resulting in medium or strong TLC results.

	Results				
Swabber	Negative	Weak	Medium	Strong	Total
Male	0	5	4	3	12
Female	1	1	4	2	8
Total	1	6	8	5	20

The implications of this study seem to be that more precise instructions should be used. In particular:

- Digits should be scrubbed hard.

- Digits should be scrubbed all around (face, sides and nail).

Thus a new set of swabbing instructions was drawn up as follows:

This test uses a swab in a glass tube and a tube containing a 70% alcohol solution.

Remove the swab from the swab tube. Dip the swab in the alcohol and scrub the thumb and fingers of the hand to be tested. Scrub the thumb and fingers from the tips down to the first joint. Be sure to scrub all the way around the digits, front, sides and back (nail).

Place the moist swab back in the swab tube, and screw the cap on firmly.

An experiment, identical to the previous one--except for the new instructions, was conducted to determine the effectiveness of the new instructions as follows:

Over a 1-week period, 20 persons were asked to swab an investigator's hand exposed to marihuana smoke. Ten additional persons were asked to swab another investigator's hand not exposed to smoke. The investigator exposed his hand to marihuana smoke by burning a 500 mg reefer of marihuana (2.61% THC, 0.17% CBCH, 0.01% CBD, 0.15% CBN) in a vented glove box. The swabber was then asked to read the new instructions and swab the exposed hand. No aid or further explanation was given to the swabbers.

During the swabbing, the investigator noted the method of swabbing used. The investigator then washed his hands and stored the swab for later analysis.

The swabs were analyzed using the standard extraction and TLC system using benzene as the developing solvent. Positives revealed the presence of THC, CBN and CBCH. No cannabidiol (CBD) was seen on the plates due to its low concentration in the marihuana and its low contrast color (orange).

The analyses were run in a blind manner. All 10 swabs of hands not exposed to marihuana smoke yielded negative results. The results on exposed hands yielded positives in all cases. Compared to the previous experimental results, which were rated on a scale of negative/weak/medium/strong, the present experiment yielded results which would be medium, strong, and very strong on the same scale.

The results of the experiment are shown in Table C-6. The sex of the swabber, and the technique used (pressure), are indicated as well as the result of the test. One swabber (male) used an extremely rough scrubbing technique with a resulting very strong positive. Two swabbers (both females) used a mild scrubbing technique resulting in strong and very strong results. The remaining 17 swabbers used a strong scrubbing action producing medium, strong and very strong results. In all cases, the swabbers scrubbed all around the digits and not just on the front (pad).

The table below shows the results by sex of the swabber. Ten males and 10 females volunteered for this program.

	Results					
Swabber	Negative	Weak	Medium	Strong	Very Strong	Total
Male	0	0	5	4	1	10
Female	0	0	0	5	5	10
Total	0	0	5	9	6	20

The results seem to indicate that females swab harder than males, but this may be due to the fact that the exposed hands were those of males.

The implications of this study seem to be that new instructions emphasizing vigorous scrubbing all around the digits definitely reduce the probability of false negative results.

The recommended colorimetric swab (CS) and thin-layer chromatographic (TLC) methods are thus as follows:

SWABBING INSTRUCTIONS (for both the CS and TLC tests)

Swab 1. Dip a Q-Tip® swab (blue plastic stem type) into a 70% ethanol solution. Press the swab gently against a glass surface to remove excess (dripping) alcohol. Swab the subject's lips with a hard rolling motion combined with a scrubbing action. Swab the fleshy part of the upper and lower lip and the facial skin immediately adjacent to the lips.

Swab 2. Dip the swab in the alcohol and remove excess alcohol as before. Swab (scrub) the thumb and fingers of the right hand from the tips down to the first joint. Be sure to scrub all the way around the digits, front, sides, and back (nail).

Swab 3. Same as Swab 2, except on the left hand.

Place the swabs in separate glass tubes and screw caps on firmly. Store in a refrigerator until analysis.

THE COLORIMETRIC SWAB (CS) TEST

Allow the swab to dry for about 5 min. Add two drops of Fast Blue B Salt solution (0.25% in 0.1 N HCl). Allow the swab to dry for 2 min. Add one drop of 0.2 N sodium hydroxide. Note any color change on the swab. A red or pink color forming immediately is a positive test. The sensitivity of the test is approximately 2 µg THC per swab.

THE THIN-LAYER CHROMATOGRAPHIC TEST (TLC test)

1. Extraction of Cannabinoids from the Swabs

A stainless steel rack (Brinkmann Cat. No. 35-00-450-5) is set up with glass conical vessels, 30-ml capacity (Brinkmann Cat. No. 35-00-420-3). Swabs are removed from their glass tubes and placed in the conical vessels, one swab per vessel. The cotton on the swab is removed by slicing through it with a razor blade held parallel to the stem and scraping gently to lift the cotton away from the adhesive on the stem. The cotton will come off the stem easily and completely when the fibers are cut cleanly all the way from the exterior of the swab to the plastic of the stem.

The cotton is dropped into the glass concical and the stem itself is placed in the vessel for use in agitating the cotton and removing it after extraction. Three (3) ml of reagent grade acetone is then added to the vessel and the cannabinoids allowed to extract for 5 min at room temperature. The swab stem is used to stir the solvent and tamp on the cotton periodically during the extraction period. After extraction, the cotton is removed by

using the swab stem, excess solvent being squeezed out. The vessel walls are then washed with 1 ml of reagent acetone and the solvent evaporated to produce a dry residue by placing the vessel in a draft at approximately 50°C (water bath). The vessel walls are then rinsed with petroleum ether (1 ml) which is evaporated off a room temperature. The residue is then subjected to thin-layer chromatography as described below.

2. Thin-Layer Chromatography of the Extract

Extract residues are reconstituted in 0.1 ml of reagent grade ethanol and subjected to TLC using the following conditions.

One-half of the residue is spotted 2 cm from the bottom of a warm TLC plate (20 x 20 cm, 250 μ silica gel G on glass, "Silplate-22," Brinkmann Cat. No. 68-10-200-6). Approximately 15 residues may be spotted per plate. Standards of THC, cannabinol and cannabichromene are also spotted on the plate (20 μl of a solution containing a mixture of these cannabinoids).

The plate is cooled and developed in benzene (saturated) for a distance of 10 cm. The plate is than air-dried and visualized by spraying with a fresh solution of 0.25% Fast Blue B in 0.1 N HCl followed by 0.2 N NaOH. The sprays should dampen the plates in both cases.

Should a positive be noted for cannabinoids in any residue (the cannabinoid pattern is unique and easy to recognize), the other half of that residue can be subjected to TLC using hexane/ether (4:1) (Saturated) as the developing solvent on a TLC plate previously washed by running for 20 cm in pure methanol. This will provide additional confirmation of the presence of cannabinoids, although the test is less sensitive than that using benzene as the developing solvent.

Note: Fast Blue B should be made fresh about every 3 days. To make the Fast Blue B solution, add the Fast Blue B salt powder to previously chilled 0.1 N HCl. Keep the solution in the refrigerator when not in use. Use gloves when spraying.

If using a glass sprayer for the NaOH solution, be sure to rinse it out daily to prevent clogging and seizing.

Some of the cannabinoid spots on the finished plates will be weak and may be observed best if the plate is held up to the light. Some spots show up better after the plate has stood and dried for a few minutes.

The R_f values of the standard cannabinoids will vary slightly, but should approximate the following values:

	Benzene Developing Solvent	Hexane/Ether (4:1) Developing Solvent
Tetrahydrocannabinol (THC)	0.40 (Red)	0.35 (Red)
Cannabinol (CBN)	0.44 (Purple)	0.31 (Purple)
Cannabidiol (CBD)	0.47 (Orange)	0.41 (Orange)
Cannabichromene (CBCH)	0.32 (Purple)	0.24 (Purple)

The sensitivity of the test is approximately 200 ng THC per swab when using the benzene developing solvent and 1 µg THC when using the hexane/ether developing solvent. These sensitivities are one-half of those quoted earlier since only half the extract is spotted for each developing solvent. All TLC operations should be conducted within 1 hr to avoid loss of sensitivity.

4. Evaluation of the CS and TLC analytical methods: With the evaluation of the specimen collection and extraction methods completed, the two test methods, the CS and TLC methods, were evaluated with the following types of swabs:

- Blank swabs, swabs spiked with THC, and swabs spiked with possible interfering compounds.
- Swabs from hands exposed to marihuana smoke.
- Swabs from corpses.
- Swabs from marihuana smokers.

a. Blank swabs, swabs spiked with THC and interferences: Evaluation was conducted on 100 blank swabs, 500 swabs spiked with THC and 560 swabs spiked with possible interferences. The three types of swabs were randomly mixed and the whole operation conducted by a blind operator. Half of the swabs were tested by the CS test, the other half by the TLC test. The results of the 1,160 swabs are summarized in Tables C-7 and C-8.

The results include swabs tested immediately after spiking and after 1 week's storage at room temperature in the dark.

Using the TLC test, no blank swabs yielded positives, and the sensitivity limit with the first solvent (benzene) is seen to be about 100 to 200 ng THC per swab. The confirmation solvent (second TLC plate) was used only with those swabs yielding a positive with the first solvent and it can be seen that the sensitivity limit is 500 to 1,000 ng THC. In other words, we have lost some sensitivity in gaining selectivity. Possible interferences, numbering 1 through 28 and shown in Table C-9, and each tested five times fresh, five times 1-week old, produced spots which could possibly be confused with THC in the first TLC developing solvent in the cases of Kava-Kava, Mace, Methylphenidate, British Sterling® After-Shave, Desire® Perfume, Cachet® Cologne, Jacques® After-Shave and Emeraude® Perfume, None of these interferences yielded interferences when run with the second TLC solvent. Similar results were found on samples stored 1 week as were found on samples tested the day of production.

Using the CS test, no blank samples yielded positives, and the detection limit is about 2 µg THC per swab. The week old samples yielded results similar to the fresh samples. Interferences were much more significant, however, than in the TLC test with every one of the substances listed in Table C-9 except methylphenidate yielding at least one false positive (each interference was tested five times fresh and five times 1 week-old).

b. Swabs from hands exposed to marihuana smoke: One hundred volunteer subjects were employed in an evaluation of the colorimetric swab (CS) test and the thin-layer chromatography (TLC) test for marihuana. Marihuana joints were prepared for this purpose from approximately 300 mg marihuana containing approximately 1.7% THC. Prior to exposure, one hand of the subject was swabbed with 70% ethanol using a blue-stem Q-Tip®. The tips and first phalange of the thumb and fingers were swabbed. The other hand of the subject was then placed in a glove box inside a hood and the subject requested to hold a lighted joint with this hand. The subject could artificially "draw" on the joint by placing the end of the joint onto an orifice, through which air was being drawn. The subjects were requested to conduct this artificial smoking until only the butt of the joint was left. The subject's thumb and fingers were continuously exposed to the smoke from the joint. Finally, the hand exposed to the smoke was swabbed in a manner identical to that of the unexposed hand. The swabs were coded by an experimenter other than the tester so that the tests would be conducted blind. The first 10 smokers' hands were tested by the TLC method, the second 10 by the CS method, etc.

Out of the 50 subjects tested by the TLC test (using both solvents) all the control samples except one yielded a negative with no indication of any TLC spots remotely resembling any cannabinoids (standards of THC, CBN, CBD, and CBCH were used on all TLC plates). Colored spots on the TLC baseline were evident in some cases. One false positive was recorded (both solvents showed a positive THC spot), and there was no indication that this subject had used any extraordinary substances on his hands within his memory before the experiment. It is possible that the swabber's hand contaminated the subject's control hand, although the swabber washed with soap and water after each subject was swabbed.

The same subjects' hands were swabbed again, in an identical manner on four occasions, once in the morning and once in the afternoon on two successive days. Both the right and left hand were swabbed and neither were exposed to marihuana smoke. All eight swabs gave absolutely negative results when extracted and analyzed by the thin-layer chromatographic method. The subject's hand lotion, "Dermassage," was diluted into an acetone/ethanol mixture and subjected to thin-layer chromatography. No spots were formed at the R_fs of cannabinoids, although a strong purple spot was observed at a very low R_f.

It appears that the positive obtained previously on this subject was due to contamination by the swabber or swabbee.

All 50 of the hands exposed to marihuana smoke showed excellent positives, with CBN and either CBD or CBCH showing on the plates in many cases as well as THC.

Out of the 50 subjects tested by the CS test, three false positives were recorded on the control hands, and only 46 of the 50 exposed hands yielded positives. There was again no indication of the use of any unusual substances by the persons exhibiting false positives (e.g., we would consider hand cream an unusual substance).

c. Swabs from corpses: Since one of the primary goals of the program is to evaluate the CS test previously used on traffic fatalities, the evaluation of the swab tests on corpses is considered as a valuable study. In particular, any positive tests recorded from the corpses should be given careful examination in the context of their being false positives.

Fifty corpses were subjected to swabs of the lips, fingers and, when possible, the palate. The corpses were those received by the medical examiner for Jackson County, Missouri, at the morgue. Intervals between death and swabbing were recorded as well as age and sex of the victim and the cause of death. A copy of the information sheet completed for each corpse is shown in Figure 3. The corpses were alternately tested by the TLC method and by the CS method. The residues of all swabs tested by the TLC method have been saved and stored, frozen, for possible further examination. Tables C-10 and G-11 list the data obtained on the corpses when using the TLC and CS method, respectively. The results indicate one positive, Corpse No. 43, which was tested by the TLC method. This subject was actually shot while smoking marihuana and died with a joint in his lips. It is interesting to note that he yielded a positive palate swab. This may indicate that most palates yield negatives because of the washing action of the tongue and saliva on the roof of the mouth. In this particular case there was no chance for such washing to take place. All other corpses indicated no positives by either method although some colored spots were observed on the TLC plates (developing solvent, benzene).

d. Swabs from marihuana smokers: Smoking experiments were conducted with 90 subjects for the collection of specimens for the final evaluation of the recommended analytical methods.

The subjects were all male, 21 years of age or over All subjects were required to complete a psychological test before admittance to the program. Before participating in the program, subjects were informed of the nature and goals of the program and the risks involved, and were then asked to sign an informed consent form if they wished to participate.

Upon entering the experiment, subjects were swabbed on the lips, palate, and the five digits of each hand using the standard technique as described earlier.

Cadaver Code No. (M.E.) _____

Date of death: _____ Time of death: _____
Date of swabs: _____ Time of swabs: _____

Cause of death: _____

_____

_____

Sex: _____ Age: _____

Was subject treated with drugs prior to death? _____ If so, please list: _____

_____

_____

Were any drugs (including marihuana) found on the subject? _____

If so, please list: _____

_____

_____

Is there any indication that the subject may have been a marihuana smoker?

_____

_____

Was the subject a tobacco smoker? _____

Please indicate if a medical examiner's report (anonymous), including examination for evidence of drug presence, can be supplied for this subject at a later date. _____

Figure 3 - Corpse Swab Information Sheet

The subjects were then requested to smoke one cigarette containing 400 mg of marihuana (2.1% THC, 0.4% CBN, 0.02% CBD, 0.2% CBCH) or 400 mg of placebo marihuana prepared from the active material by solvent extraction of the cannabinoids. Immediately after smoking or 2, 6, or 24 hr after smoking, subjects were again swabbed in a manner identical to that before smoking. The swabs were coded and stored in closed glass vials under refrigeration to await analysis.

Prior to the program, subjects were requested not to smoke marihuana for 24 hr prior to the experiment. During the experiment, details of health, unusual food and drink, drugs and cosmetics used during the prior 24 hr were gathered. Facial details and smoking habits were also recorded.

Swabs from one-third of the subjects were tested using the colorimetric swab test (CS) and two-thirds were tested using the thin-layer chromatographic test (TLC using both developing solvents) developed earlier in this program. The number of subjects in each category is shown below:

CS Test: 24 test subjects, six at 0, 2, 6, and 24 hr after smoking.
6 placebo subjects, all tested at 0 hr after smoking.

TLC Test: 48 test subjects, 12 at 0, 2, 6, and 24 hr after smoking.
12 placebo subjects, all tested at 0 hr after smoking.

The above analyses were conducted blind.

The analytical results using the CS Test are shown in Table C-12 those using the TLC Test are shown in Table C-13. These results are discussed in the next section.

V. EXPERIMENTAL RESULTS, PHASE I

The experimental results from the evaluation of the CS and TLC tests on marihuana smokers, as shown in Tables C-12 and C-13, have been analyzed and interpreted as described below. An evaluation of the CS tests results is followed by a similar evaluation of the TLC tests results. The number of subjects involved in these smoking tests does not allow the valid use of statistical analysis. Descriptive statistics are provided for each test.

A. CS Test Results

In the placebo group:

0% right hands, 0% left hands, 50% (3) lips and 33% (2) palates were positive before smoking. One out of the three subjects contributing to these positives admitting smoking marihuana during the 24 hr preceding the experiment.

0% right hands, 0% left hands, 33% (2) lips and 0% palates were positive after smoking. The two lips positive after smoking were also positive prior to smoking.

In the 0 hr test group:

17% (1) right hands, 17% (1) left hands, 17% (1) lips and 17% (1) palates were positive before smoking. In all cases, subjects admitted smoking during the 24 hr preceding the experiment. Positive hands correlated with later observed smoking habits.

50% (3) right hands, 33% (2) left hands, 83% (5) lips and 0% palates were positive after smoking. The right and left hand positives correlated with hands noted as being used in smoking.

In the 2 hr test group:

17% (1) right hands, 33% (2) left hands, 33% (2) lips and 0% palates were positive before smoking. Two out of the three subjects yielding these positives admitted smoking during the 24 hr preceding the experiment. Two of the three hand positives correlated with smoking habits observed later.

17% (1) right hands, 0% left hands, 50% (3) lips and 0% palates were positive after smoking. The one right hand positive correlated with observed smoking habits.

In the 6 hr test group:

17% (1) right hands, 33% (2) left hands, 17% (1) lips and 0% palates were positive before smoking. One of the three subjects yielding these positives admitted smoking during the 24 hr preceding the experiment. All hand positives correlated with smoking habits observed later.

17% (1) right hands, 17% (1) left hands, 0% lips and 0% palates were positive after smoking. The right hand positive correlated with observed smoking habits; the left hand positive did not.

In the 24 hr test group:

67% (4) right hands, 0% left hands, 17% (1) lips and 17% (1) palates were positive before smoking. Only one subject admitted smoking during the 24 hr preceding the experiment. Three of the four hand positives correlated with smoking habits observed later.

17% (1) right hands, 17% (1) left hands, 0% lips and 0% palates were positive after smoking. These hands did not correlate with observed smoking behavior.

In summary:

17% (1) of the placebo smokers showed positives before smoking.
17% (1) showed positives after smoking.

50% (3) of the 0 hr test subjects showed positives before smoking.
83% (5) showed positives after smoking.

50% (3) of the 2 hr test subjects showed positives before smoking.
50% (3) also showed positives after smoking.

50% (3) of the 6 hr test subjects showed positives before smoking.
33% (2) showed positives after smoking.

83% (5) of the 24 hr test subjects showed positives before smoking.
33% (2) showed positives after smoking.

The above figures represent smokers showing one or more positive swabs.

Since it is not possible to control a subject's behavior before the experiments (unless hospitalization is used), positives before smoking may be anticipated. It is also not possible to be certain of 100% admittance of prior marihuana smoking.

Since palate swabs do not seem to yield significant positives, they are omitted from further discussion in this test.

Of the 30 subjects in the test, 12 admitted smoking marihuana 24 hr prior to the experiment. Of these 12, 6 (50%) yielded positive tests prior to smoking marihuana or placebo in the program. Of the 18 subjects not smoking marihuana 24 hr prior to the experiment, 10 (56%) yielded positive tests prior to smoking marihuana or placebo in the program. Of the 16 subjects showing positives before smoking in the program, six (38%), admitted smoking marihuana in the previous 24 hr.

The distribution of positive findings (after smoking marihuana) among the lips, left and right hands are as shown below.

Findings	Right Hand	Left Hand	Lip	Total
+	6	4	8	18
-	18	20	16	54
Total	24	24	24	72

25% of the right hands were positive.
17% of the left hands were positive.
33% of the lips were positive.

There is no statistical difference (Chi-Square test, $X^2 = 1.778$, $df = 2$, $p < 0.05$) between these percentages because of the low number of subjects involved.

Chi-Square tests reveal no significant correlations of positive results with aftershave/cologne, hand cream/lotion, elapsed time between smoking and the test, hands used to smoke, amount of cigarette smoked, use of roach clips, type of inhalations, skin features, facial features or the prior use of alcohol.

B. TLC Test Results

These results were obtained using the standard TLC test as described on p. 28 using both developing solvents. Positive results are those showing a positive identification using the first TLC developing solvent (benzene). The second developing solvent (hexane/ether, 4:1) results in some loss in sensitivity and thus did not confirm all positive results indicated by the first developing solvent. Eighty-eight percent of the positive results obtained using the first developing solvent were confirmed using the second developing solvent. In the 12% of the results not confirmed by the second developing solvent, the first developing solvent showed a weak cannabinoid pattern (as did the 88% confirmed results), which is unique and totally different from any pattern observed from potential interferences previously investigated. The following statistics resulted from this test.

In the placebo group:

17% (2) right hands, 8% (1) left hands, 0% lips and 0% palates were positive before smoking. The left hand and one right hand positive resulted from a subject who admitted smoking during the 24 hr preceding the experiment. He was observed to use his left hand during our experiments. The other subject was observed using both hands during our experiments.

No positives at all were recorded on the 12 subjects after smoking the placebo.

In the 0 hr test group:

17% (2) right hands only were positives before smoking. One of these resulted from a subject who admitted smoking during the 24 hr preceding the experiment, although he used his left hand during our experiments. The other subject used both hands during smoking.

After smoking, all 12 subjects recorded at least one positive result. 67% (8) right hands, 42% (5) left hands, 83% (10) lips and 8% (1) palates were positive. In all cases, positives on hands were consistent with observed smoking habits.

In the 2 hr test group:

8% (1) left hand and 8% (1) lips were positive before smoking. In both cases, the subjects had admitted smoking during the 24 hr preceding the experiment. Both smokers used both hands when smoking.

After smoking, 67% (8) subjects recorded at least one positive. 17% (2) right hands, 25% (3) left hands, 42% (5) lips and 0% palates were positive. In all cases, positives on hands were consistent with observed smoking habits.

In the 6 hr test group:

8% (1) right hand and 8% (1) lips were positive before smoking. The one subject responsible for those positives admitted smoking during the 24 hr preceding the experiment. He was a right-handed smoker.

After smoking, only one subject (8%) showed a positive (lips). This was the same subject who showed positives prior to smoking.

In the 24 hr test group:

8% (1) right hand, 8% (1) left hand and 8% (1) lips were positive before smoking. The one subject responsible for these positives did not admit to smoking during the 24 hr preceding the experiment. It should be noted that when he did smoke, he used both hands.

After smoking, only one subject (8%) showed positives (left hand and lips). He was observed to be a left-handed smoker.

Since palate swabs do not seem to yield significant positives, they are omitted from further discussion in the test.

In summary:

17% (2) of the placebo smokers showed positives before smoking. 0% showed positive after smoking.

17% (2) of the 0 hr test subjects showed positives before smoking. 100% (12) showed positives after smoking.

17% (2) of the 2 hr test subjects showed positive before smoking. 67% (8) showed positives after smoking

8% (1) of the 6 hr test subjects showed a positive before smoking. 8% (1) showed a positive after smoking.

8% (1) of the 24 hr test subjects showed a positive before smoking. 8% (1) showed a positive after smoking.

The above figures represent smokers showing one or more positive swabs.

Of the 60 subjects in the test, 18 admitted smoking marihuana 24 hr prior to the experiment. Of these 18, six (28%) yielded positive tests prior to smoking marihuana or placebo in the program. Of the 42 subjects not smoking marihuana 24 hr prior to the experiment, three (7%) yielded positive tests prior to smoking marihuana or placebo in the program. Of the eight subjects showing positives before smoking in the program, five (63%) admitted marihuana smoking in the previous 24 hr.

The distribution of positive findings, after smoking marihuana, among the lips, left and right hands are as shown below. These figures include results from smokers tested at 0, 2, 6 and 24 hr elapsed time between smoking and testing.

Findings	Right Hand	Left Hand	Lip	Total
+	10	9	17	36
-	38	39	31	108
Total	48	48	48	144

21% of the right hands were positive.
19% of the left hands were positive.
35% of the lips were positive.

There is no statistical difference (Chi-Square test) between these percentages. Chi-Square tests reveal no significant correlations of positive results with aftershave/cologne, hand cream/lotion, hands used to smoke, amount of cigarette smoked, use of roach clips, type of inhalations, skin features, facial features or prior use of alcohol. There is, however, a significant relationship between the number of positive smokers and the elapsed time between smoking and the testing; i.e.,

100% show at least 1 positive at 0 hr elapsed time.
67% show at least 1 positive at 2 hr elapsed time.
8% show at least 1 positive at 6 hr elapsed time.
8% show at least 1 positive at 24 hr elapsed time.

($X^2 = 31.150$, $df = 3$, $p < 0.05$).

VI. TASK 4. CONCLUSIONS AND RECOMMENDATIONS, PHASE I

The conclusions and recommendations based on Phase I of the program are presented in five separate discussions, as follows:

A. Interpretation of previous CS test results.

B. Recommended procedures for future marihuana contact tests.

C. State of the art and critical evaluation of marihuana skin testing and need for future developments.

D. Utility of the recommended procedure for purposes other than NHTSA drug research.

E. Recommended validation procedures for the procedure recommended in B above.

The conclusions and recommendations are derived from the results of the preceding three tasks in Phase I of the program.

A. Interpretation of the Previous Colorimetric Swab (CS) Test Results

The colorimetric swab (CS) test was previously used on driver/drug surveys, "The Incidence of Drugs in Fatally Injured Drivers," DOT Contract No. DOT-HS-119-3-627, and "Drug Use Among Drivers," DOT Contract No. DOT-HS-119-2-440.

In the first survey, "The Incidence of Drugs in Fatally Injured Drivers," 323 sets of swabs (lips, right hand, left hand) were collected from fatally injured drivers. Of these, only 195 sets were judged clean enough for testing by the CS method. Twenty-three (11.8%) of these 195 sets yielded positives on all three swabs by the CS test. The distribution of lip and hand positives in the 195 sets is shown below. It should be noted here that the "+" and "-" findings refer to lips and hands, and not to individual drivers.

Finding	Right Hand	Left Hand	Lip	Total
+	43	43	38	124
-	152	152	157	461
Total	195	195	195	585
% +	22.1%	22.1%	19.5%	21.2%

The ratio of % + right hand/left hand/lip, 22.1/22.1/19.5 is significantly different from that observed in the CS test results obtained in the present program (25.0/16.7/33.3), and in the TLC test data obtained in the present program (20.8/18.8/35.4). The ratios obtained from the CS and TLC tests performed in the present program resulted from tests conducted after smoking, but also after the initial presmoking swab which cleaned the hands and lips. We presume that the results obtained in the present program are thus more reliable than those obtained in the previous program in terms of false positives. It would seem that the results from the previous program are either:

- too low in lip positive results, or
- too high in hand positive results.

The latter alternative is judged the more likely since it would involve false positives, which the CS test has been demonstrated to yield in the present program. False positives (those occurring before smoking in which the subject denied smoking before the experiment) in the present CS test program numbered 10 out of 30 subjects. Of these 10, 4 involved right hands, 4 involved left hands and 2 involved lips. This supports the hypothesis that hands are more likely to yield false positives than are lips. It was also noted earlier (p. 21) that swabs from skin areas other than lips tended to turn orange/brown when tested by the CS method, thus leading to the possibility of more false positives on hand swabs. Also the LWL study on lips only (p. 7) yielded no false positives. (In the TLC test, of course, many or all false positives are eliminated so comparison here cannot be made.)

It would thus seem reasonable to assume that the lips are the most reliable contact area to swab in terms of reducing the false positive count. Lips also yield the least false negatives of the contact areas tested in the present program. Thirty-three percent of the lips of the 24 subjects smoking in the present CS study were positive after smoking. This figure includes those tested at 0, 2, 6, and 24 hr after smoking. At 0 and 2 hr elapsed times after smoking, the lip to hand positive ratio is even higher with the lip positive percentage reaching 83 and 50%, respectively.

If the lip areas are considered the most reliable contact area swabbed, the results in the previous program involving fatally injured drivers can be best interpreted utilizing the lip data from the 195 complete sets obtained. The inclusion of dirty or incomplete sets will confound the data to an unknown extent and is therefore not included. A figure of 19.5% positive drivers is therefore estimated for the fatally injured drivers involved in the previous program.

A correction factor should be applied to these positives. Eighty-three percent is the maximum percentage positives found immediately after smoking in the present CS test described in this report. However, this is based on a small population of six subjects. Since only the lips are involved, a better correction factor could be applied using data obtained from 100 subjects who were tested, immediately after smoking, on the lips by the CS method in a program supported by the U.S. Army (Report No. LWL-CR-08C72), and yielding a 78% positive lip swab detection rate. Thus a corrected detection of 19.5%/78%, i.e., 25.0%, is obtained.

The incidence of positive drivers would be 34.4% if a positive driver is identified as one showing a positive swab on one or more swab locations (see Table 3, p. 9). However, as previously mentioned, we feel that hand swabs are more subject to false positives than are lip swabs, and no reliable correction factor can be applied to results which include hand swabs.

In the second survey, "Drug Use Among Drivers," the lips of drivers on the road were collected and subjected to the CS test. This previous program did not involve the swabbing of hands. In light of the above arguments we feel that the lip results obtained here should be interpreted simply by adjusting them by the 78% factor obtained from the LWL study.

Thus, the 2.92 and 9.20% positive lip incidences obtained from drivers on the road in Lincoln, Nebraska, and Dade County, Florida, in the previous study should be interpreted as 3.74 and 11.79%, respectively (7.7% average). The populations of fatally injured drivers from Lincoln, Nebraska, and Dade County, Florida, in the first survey are too small for comparison with these living driver results. If, however, the nationwide population of fatally injured drivers from the first survey is divided into two groups, those from locations similar to Lincoln and those from populations similar to Dade County (see DOT Contract No. DOT-HS-119-2-440, Final Report, August 1974, p. 32), then the incidences of fatally injured drivers showing positive lip swabs from the 195 complete swab sets are 22.2% for the Lincoln-type locations and 18.6% for the Dade County-type locations. Using the 78% lip positive correction factor (LWL-CR-08C72) these figures translate to 28.5% for Lincoln-type locations and 23.8% for Dade County-type locations.

The CS test does have its limitations. Positives (red color) are often difficult to judge because of orange colors often seen on true negative swabs, especially on hand swabs. The CS test should be regarded as a presumptive test of rather a crude nature, yet very simple and inexpensive to perform. Its reliability will always be in doubt because of the simplicity of the nature of the test, its lack of ability to detect all smokers, even immediately after smoking, and possible interferences which have been discovered in the present program investigations of the CS test.

B. Recommended Procedures for Future Marihuana Contact Tests

The work conducted in this program has indicated that a swabbing procedure followed by extraction of the cannabinoids from the swab and consequent TLC of the extracts yields a reliable test method for marihuana contact. The TLC procedure almost always produces a cannabinoid pattern on the TLC plates which because of its uniqueness provides a high degree of confidence in the results. Subsequent TLC of the extract in a second solvent will also provide additional confirmation of results. There is rarely any question about the presence or absence of the annabinoids. In the smoking experiments conducted in Phase I of this program, 100% of the test subjects yielded positives after zero elapsed time and 67% yielded positives after 2 hr. The recommended procedure is as described earlier in this report (pp. 27-29), and repeated below.

SWABBING INSTRUCTIONS (for both the CS and TLC tests)

Swab 1. Dip a Q-tip® swab (blue plastic stem type) into a 70% ethanol solution. Press the swab gently against a glass surface to remove excess (dripping) alcohol. Swab the subject's lips with a hard rolling motion combined with a scrubbing action. Swab the fleshy part of the upper and lower lip and the facial skin immediately adjacent to the lips.

Swab 2. Dip the swab in the alcohol and remove excess alcohol as before. Swab (scrub) the thumb and fingers of the right hand from the tips down to the first joint. Be sure to scrub all the way around the digits, front, sides, and back (nail).

Swab 3. Same as Swab 2, except on the left hand.

Place the swabs in separate glass tubes and screw caps on firmly. Store in a refrigerator until analysis.

THE COLORIMETRIC SWAB (CS) TEST

Allow the swab to dry for about 5 min. Add two drops of Fast Blue B Salt solution (0.25% in 0.1 N HCl). Allow the swab to dry for 2 min. Add one drop of 0.2 N sodium hydroxide. Note any color change on the swab. A red or pink color forming immediately is a positive test. The sensitivity of the test is approximately 2 µg THC per swab.

THE THIN-LAYER CHROMATOGRAPHIC TEST (TLC test)

1. Extraction of Cannabinoids from the Swabs

A stainless steel rack (Brinkmann Cat. No. 35-00-450-5) is set up with glass conical vessels, 30-ml capacity (Brinkmann Cat. No. 35-00-420-3). Swabs are removed from their glass tubes and placed in the conical vessels, one swab per vessel. The cotton on the swab is removed by slicing through it with a razor blade held parallel to the stem and scraping gently to lift the cotton away from the adhesive on the stem. The cotton will come off the stem easily and completely when the fibers are cut cleanly all the way from the exterior of the swab to the plastic of the stem.

The cotton is dropped into the glass conical and the stem itself is placed in the vessel for use in agitating the cotton and removing it after extraction. Three (3) ml of reagent grade acetone is then added to the vessel and the cannabinoids allowed to extract for 5 min at room temperature. The

swab stem is used to stir the solvent and tamp on the cotton periodically during the extraction period. After extraction, the cotton is removed by using the swab stem, excess solvent being squeezed out. The vessel walls are then washed with 1 ml of reagent acetone and the solvent evaporated to produce a dry residue by placing the vessel in a draft at approximately 50°C (water bath). The vessel walls are then rinsed with petroleum ether (1 ml) which is evaporated off a room temperature. The residue is then subjected to thin-layer chromatography as described below.

2. Thin-Layer Chromatography of the Extract

Extract residues are reconstituted in 0.1 ml of reagent grade ethanol and subjected to TLC using the following conditions.

One-half of the residue is apotted 2 cm from the bottom of a warm TLC plate (20 x 20 cm, 250 µ silica gel G on glass, "Silplate-22," Brinkmann Cat. No. 68-10-200-6). Approximately 15 residues may be spotted per plate. Standards of THC, cannabinol and cannabichromene are also spotted on the plate (20 µl of a solution containing a mixture of these cannabinoids).

The plate is cooled and developed in benzene (saturated) for a distance of 10 cm. The plate is then air-dried and visualized by spraying with a fresh solution of 0.25% Fast Blue B in 0.1 N HCl followed by 0.2 N NaOH. The sprays should dampen the plates in both cases.

Should a positive be noted for cannabinoids in any residue (the cannabinoid pattern is unique and easy to recognize), the other half of that residue can be subjected to TLC using hexane/ether (4:1) (saturated) as the developing solvent on a TLC plate previously washed by running for 20 cm in pure methanol. This will provide additional confirmation of the presence of cannabinoids, although the test is less sensitive than that using benzene as the developing solvent.

Note: Fast Blue B should be made fresh about every 3 days. To make the Fast Blue B solution, add the Fast Blue B salt powder to previously chilled 0.1 N HCl. Keep the solution in the refrigerator when not in use. Use gloves when spraying.

If using a glass sprayer for the NaOH solution, be sure to rinse it out daily to prevent clogging and seizing.

Some of the cannabinoid spots on the finished plates will be weak and may be observed best if the plate is held up to the light. Some spots show up better after the plate has stood and dried for a few minutes.

The R_f values of the standard cannabinoids will vary slightly, but should approximate the following values:

	Benzene Developing Solvent	Hexane/Ether (4:1) Developing Solvent
Tetrahydrocannabinol (THC)	0.40 (Red)	0.35 (Red)
Cannabinol (CBN)	0.44 (Purple)	0.31 (Purple)
Cannabidiol (CBD)	0.47 (Orange)	0.41 (Orange)
Cannabichromene (CBCH)	0.32 (Purple)	0.24 (Purple)

The sensitivity of the test is approximately 200 ng THC per swab when using the benzene developing solvent and 1 µg THC when using the hexane/ether developing solvent. These sensitivities are one-half of those quoted earlier since only half the extract is spotted for each developing solvent. All TLC operations should be conducted within 1 hr to avoid loss of sensitivity

C. State of the Art and Critical Evaluation of Marihuana Skin Testing and Need for Future Developments

Literature reports of marihuana skin testing are essentially limited to hand and oral swabbings and washings.[1-4] Stone and Stevens[1] first described swabbing methods for fingers using chloroform as a solvent and conducting thin-layer chromatography on the washings or swab extracts. The same workers also conducted an initial evaluation of mouth washes using a saline alcohol solution. Their techniques, however, have not been reported used in any large marihuana detection surveys. The evaluation programs described in this report represent the major critical evaluation work conducted on marihuana skin testing methods.

As in Stone and Steven's work, the two major test methods, the CS and TLC tests, utilized the very sensitive reaction of Fast Blue B to produce bright visible colors in the presence of cannabinoids. The use of this visualization agent enables one to detect approximately 2 μg of THC on a swab (Q-Tip®) or 100 ng of THC on a TLC plate which translates to approximately 200 ng of THC per swab if extraction efficiencies are taken into consideration.

The specificity of skin testing using the Fast Blue B color reaction is questionable when using the CS test, i.e., a color reaction performed directly on the swab. A number of substances (listed on p. 18 of this report) yield false positives in the CS test. The TLC test, however, is able to separate possible interfering substances and produce results with a higher degree of specificity. Skin swabs from marihuana smokers will not only yield a positive TLC spot for THC, but will display, in many cases, a spectrum of separated cannabinoids on the TLC plate which provide very strong evidence for marihuana constituents on the skin.

Recent programs using the colorimetric swab (CS) test have indicated that 78%[2] to 83%* of marihuana smokers can be detected by lip swabs immediately after smoking. This percentage drops significantly after 2 hr, and is practically insignificant after 24 hr.

Recent surveys[3,4] using the CS test have indicated that 25% of fatally injured drivers had traces of marihuana on the lips and that 3.7 to 11.8% of drivers randomly stopped on the road had marihuana on their lips.

The colorimetric swab (CS) test is very simple and inexpensive to conduct. Its simplicity, however, dictates its vulnerability to interferences and its decreased sensitivity over the TLC test. The evaluation of the TLC test described in this report indicates both a better specificity and a better sensitivity for the TLC test than for the CS test. Sixty sub-

* Results from this report.

jects were evaluated in a smoking test using the TLC method described earlier in this report (pp. 28-29). Zero percent placebo smokers showed positives after smoking, whereas,

100% showed positives immediately after smoking,
67% showed positives 2 hr after smoking,
8% showed positives 6 hr after smoking, and
8% showed positives 24 hr after smoking.

The lips were detected as positive in 35% of the subjects, whereas the right and left hands were detected as positive in only 21% and 19% of the subjects, respectively.

The present status of marihuana skin testing, based on the TLC test, can be summarized as follows:

- A test of good specificity is available (no known interferences).
- The sensitivity of the test (200 ng/swab) is useful only for detecting recent smokers (0 to 2 hr). If one sample is taken per subject, it is best performed on the lips. The test will not detect 100% of marihuana smokers, even immediately after smoking.
- Swabbing with alcohol-moistened Q-Tips® is effective in removing cannabinoids from the skin.
- Extraction procedures recommended in this report will remove over 50% of the cannabinoids from the swab for examination by TLC.

The present skin testing methods need both further evaluation and development. The present CS test interpretation would benefit from a larger controlled study using an order of magnitude of more subjects than has been used to date. The present TLC test should also be validated with a large number of subjects under controlled conditions. (This has been conducted in Phase II of this program.) Marihuana skin testing would benefit also if more sensitive detection methods were developed. Methods such as radioimmunoassay and mass spectrometry, if developed, could offer vastly increased sensitivity in detection of cannabinoids on skin swabs. If specific enough, these methods could be applied directly to the swab or swab washings. It must be remembered, however, that these new methods would be considerably more expensive than the present CS or TLC methods. The physiological and pshychological effects of marihuana are usually present for up to several hours after smoking, and a reliable test for marihuana traces for several hours after smoking is a definite need.

D. Utility of the Recommended Procedure for Purposes Other Than NHTSA Drug Research

The recommended procedure for the analysis of marihuana contact on the skin is the TLC procedure for swab extracts. This procedure is described in detail on pp. 28-29 of this report.

The TLC test procedure is applicable to the testing of surfaces of all types, including skin. The questionnaire survey (pp. 14-15 of this report) indicated interests in the use of a swab test for testing motor vehicle surfaces and personal clothing as well as skin testing. The use of the swab test, involving swabbing an object with an alcohol-moistened Q-Tip®, is adaptable to almost any surface. Materials, other than marihuana constituents picked up by the swab, will be separated from the marihuana constituents when the swab extract is subjected to TLC.

It is suggested that, for purposes other than NHTSA drug research, the TLC test using a Q-Tip® swab would be ideal for marihuana detection on living and inanimate surfaces involving automobiles, clothing, personal belongings, furniture and other household items, etc. Use of the test could be made by law enforcement officials, educational administrators, and state and local government agencies interested in incidence data. The test is simple and inexpensive to perform. The swabs can be collected and then analyzed at a later data with little or no deterioration in the result.

The recommended procedure should be regarded as a presumptive test only; further and more sophisticated tests would be necessary to provide acceptable proof of marihuana contamination of the skin or other surfaces.

E. Recommended Validation Procedures for the Recommended Procedure

Validation of the recommended procedure (Section B, TLC Test Results, pp. 28-29 of this report) is necessary in light of the small number of subjects evaluated in Phase I of this program.

The purpose of this validation study is to ensure the utility of the recommended marihuana contact test procedure issuing from Phase I of this program. The subjects and test personnel in this study will be others than those used in Phase I. Positive results in the cross-validation will increase the confidence that the recommended procedure will work when used by other researchers and subjects.

Figure D-1 (Appendix D) indicates the scope of the proposed cross-validation study. The study is designed to include nonexposed subjects, subjects exposed to marihuana smoke and subjects actually smoking

marihuana. Subjects will also smoke one of three different doses of marihuana; exposed and smoking subjects will also be tested at one of three different elapsed periods after exposure or smoking; all subjects will be swabbed by one of three different personnel; and each swabber's swab will be analyzed by one of three analysts. All exposed and smoking subjects will be swabbed on the lips and both hands before and after exposure or smoking.

The total scope of the study will consist of 234 different subjects and result in the production and analysis of 1,350 swabs. In addition, 135 artificially spiked and control swabs will be randomly mixed in with the subjects' swabs. Pertinent points in the validation study, which comprises Phase II of this program, are discussed in the next section of this report.

VII. PHASE II. VALIDATION OF THE RECOMMENDED PROCEDURE

A. Experimental Procedures

Described below are the experimental procedures adopted for a program designed to validate the Phase I test results obtained on the recommended procedure for marihuana contact skin testing. The recommended procedure is the TLC test described earlier on pp. 28-30 of this report.

1. Collection of swab specimens from marihuana smokers: Smoking experiments were conducted with human subjects for the collection of specimens for validation of the recommended analytical methods.

The subjects were all male and 21 years of age or over. All subjects were required to complete a psychological test before admittance to the program. Before participating in the program, subjects were informed of the nature and goals of the program and the risks involved, and were then asked to sign an informed consent form if they wished to participate.

Upon entering the experiment, subjects were swabbed on the lips and the five digits of each hand using the standard technique as described on p. 28 of this report.

The subjects were then requested to do one of the following:

- Smoke 400 mg of marihuana in cigarette form.
- Smoke 700 mg of marihuana in cigarette form.

- Smoke 1,000 mg of marihuana in cigarette form.

- Expose themselves to the smoke of 1,000-mg smokers seated adjacent to them.

The marihuana contained 2.1% THC, 0.4% CBN, 0.02% CBD and 0.2% CBCH. Either immediately after smoking or exposure to smoke, or 1 hr after smoking or exposure, or 3 hr after smoking or exposure, subjects were swabbed again in a manner identical to that used before smoking. The swabs were coded and stored in closed glass vials under refrigeration to await analysis.

Subjects were requested not to smoke marihuana for 24 hr prior to the experiment. During the experiment, details of health, unusual food and drink, drugs and cosmetics used during the prior 24 hr were gathered. Facial details and smoking habits were also recorded.

The purpose of this validation study was to ensure the utility of the recommended marihuana contact test procedure issuing from Phase I of this program. The subjects and test personnel in this study were others than those used in Phase I. Positive results in the cross-validation will increase the confidence that the recommended procedure will work when used by other researchers and subjects.

Figure D-1 indicates the scope of the cross-validation smoking study. The study was designed to include nonexposed subjects, subjects exposed to marihuana smoke and subjects actually smoking marihuana. All subjects were swabbed by one of three different personnel, and each swabber's swab was analyzed by one of three analysts.

The total scope of the program consisted of 234 subject runs and resulted in the production of 1,350 swabs. In addition, 36 artificially spiked swabs were randomly mixed in with the subjects' swabs. Pertinent points in the program are discussed below:

a. Subjects: The subjects were drawn from a pool of approximately 200 males, 21 or over. Some subjects participated more than once but never without a 48-hr gap in time between experiments. It was necessary to allow this because of the difficulty in massing a pool large enough to accommodate the program with 234 different subjects. Subjects were paid $2 to $5 for their participation in the study depending on the length of time they were required to stay.

b. Swabbers: Three different swabbers were employed equally in the program. These swabbers did not have any prior experience with the program. They consisted of technician-level persons with no particular chemical or scientific knowledge which would bias the results. They were trained in the technique of swabbing in a preliminary program. Training was conducted by MRI personnel previously experienced in the program and training of all three was identical. The swabbers were allowed to refer to a standard set of swabbing instructions as shown on p. 27 of this report, and as Figure D-2.

2. Analysis of the swab specimens: The analysis of the 1,386 swabs resulting from the above smoking program was conducted using the standard extraction and TLC procedures issuing from Phase I.

The swabs were separated into three groups to be analyzed by Analysts A, B, and C as shown in Figure D-1. Each analyst tested 462 swabs. Analysts were given instructions, the necessary equipment, and chemicals and standards to perform the analyses according to the instructions. None of the analysts chosen for this work had prior experience in the program. All analysts were technical grade personnel, previously acquainted with the average analytical techniques used in common drug analysis. The program was conducted in a blind manner with each separate swab provided with a code number. The results of the analysts' tests were forwarded to both DOT and MRI personnel at the same time, thus avoiding any question in the interpretation of results.

The program was designed so that each analyst tested two sets of swabs from each swabber for each elapsed time period and for each marihuana dosage.

The analysts were chosen from three laboratories: Dr. Cochin's laboratory at the Boston University School of Medicine; Dr. Finkle's laboratory at the Center for Human Toxicology, Salt Lake City, Utah; and Dr. Woodhouse's laboratory at Midwest Research Institute, Kansas City, Missouri. Analysts A, B and C will not be identified laboratory-wise.

B. Experimental Results

The experimental results from the validation study described in the previous section are tabulated in Tables E-1 to E-9 (Appendix E). The nine tables represent results from each of the three analysts on swabs taken by each of the three swabbers. Due to a misunderstanding of the analytical procedures, a proportion of the results from Analyst C are missing.

The data in Tables E-1 through E-9 have been interpreted by examination of all the variables involved. An analysis is presented below on the following experimental aspects of the study.

- Accuracy of detection after smoking versus swabbers.
- Accuracy of detection after smoking versus laboratories.
- Detection after smoking versus dosage level.
- Detection after smoking versus elapsed time.
- Interaction between dosage level and elapsed time.
- Detection after smoking versus hand(s) used for smoking.
- Detection after smoking versus location (lip, hand).
- Detection after smoking versus amount of cigarette smoked.
- Detection after smoking versus use of roach clips.
- Detection after smoking versus type of inhalation.
- Detection (lips) after smoking versus facial features.
- Detection after smoking versus skin features.
- Detection after smoking versus prior use of alcohol.
- Detection before smoking versus use of aftershave/cologne.
- Detection before smoking versus use of hand cream/lotion.
- Detection before smoking versus use of marihuana previous to the experiment.

In the statistics comparing the accuracy of detection, an identification is considered accurate (+) if a smoker is identified as a smoker and a nonsmoker is identified as a nonsmoker; an identification is considered inaccurate (-) if a smoker is not identified as such (168 cases) or a nonsmoker is identified as a smoker. In all other statistics examining detection, a positive detection will be considered a positive result and a negative detection a negative result.

In these analyses a result is considered positive if it is "++" or "+-." A subject will also be considered positive if any one of the swab locations reveal a positive result, except in detailed examinations of detection versus swab location.

In order to accomplish the statistical evaluation, subjects who yielded positive swabs before experimentation and subjects with missing data are not included in analyses involving the swabs examined after smoking.

a. Accuracy of detection after smoking or exposure versus swabbers: The following figures are revealed for accurate/inaccurate detections from swabs by the three swabbers (including swabs from smokers and exposed subjects).

Finding	Swabber A	Swabber B	Swabber C	Total
Accurate	37	41	40	118
Inaccurate	21	10	21	52
Total	58	51	61	170
% acc.	63.8%	80.4%	65.6%	69.4%

There is no statistical difference between the proportion of accurate detections made by the three swabbers ($X^2 = 4.182$, df = 2, $p < 0.05$).

b. Accuracy of detection after smoking or exposure versus analysts: The observed frequency of accurate and inaccurate detections made by the three analysts (including swabs from smokers and exposed subjects) is as shown below:

Finding	Analyst A	Analyst B	Analyst C	Total
Accurate	44	48	26	118
Inaccurate	14	14	24	52
Total	58	62	50	170
% acc.	75.9%	77.4%	52.0%	69.4%

There is a significant difference ($X^2 = 10.153$, df = 2, $p < 0.01$) between the proportion of accurate detections made by the three analysts. Ryans tests indicate that Analyst C made significantly fewer detections than Analysts A or B, the latter not differing significantly from each other.

c. Detection after smoking or exposure versus dosage level and elapsed time, and interaction between dosage level and elapsed time: The frequencies of positive subject results by dosage level and elapsed time are shown below:

Dose	0 Hr Elapsed Time	1 Hr Elapsed Time	3 Hr Elapsed Time
High	80.0%	50.0%	37.5%
Medium	92.9%	71.4%	40.0%
Low	85.7%	55.6%	35.3%
Exposed	11.8%	0.0%	0.0%

Using an arcsine transformation of proportions it is concluded that there were significantly fewer detections in the exposed subjects than in the smoking subjects. Within elapsed time groups, dosage levels did not significantly differentially influence the frequency of detections. Regardless of dosage level, there were significantly more detections in the 0 hr group than in the 3 hr group. The high dosage/1 hr group made significantly less detections than all three 0 hr groups. The low dose/1 hr group made significantly less detections than the low dose/0 hr group and medium dose/0 hr group. The medium dose/1 hr group did not differ significantly from the 0 hr group. All conclusions are based on $p < 0.05$.

The reason why high dosage smokers did not yield as high a positive detection as medium and low dose smokers in the 0 and 1 hr groups may be due to the fact that the high dosage smokers were required to smoke two cigarettes. They rarely smoked the second cigarette completely, thus exposing their hands and lips to the tarry residues of only one cigarette of 500 mg (approximately the same as the low dosage). Since the tarry residues are probably responsible for much of the cannabinoids adhering to the lips and hands, the high dosage smokers may have only been exposed to as much cannabinoid material as a low dosage smoker.

d. Detection after smoking versus hand(s) used for smoking: The frequency of positive detections on lips, hands used for smoking and hands not used for smoking are as follows (subjects at 0, 1, and 3 hr elapsed times):

Finding	Hand Used	Hand Not Used	Lip	Total
+	63	20	60	123
-	110	59	66	235
Total	173	79	126	378
% +	36.4%	25.3%	47.6%	37.8%

Chi-Square tests using Ryan's methods indicate that the percentage positives observed on the hands not used is not significantly different from that on the hands used. The percentage positives observed on the lips (47.6%) is significantly different from the percentage positives observed on the hands not used for smoking (25.3%). ($X^2 = 10.149$, df = 2, $p < 0.017$).

If the same analysis is conducted on the 0 hr elapsed time group only, the following figures are revealed:

Finding	Hand Used	Hand Not Used	Lip	Total
+	36	13	32	81
-	26	11	11	48
Total	62	24	43	129
% +	58.1%	54.2%	74.4%	62.8%

There is no significant difference between these results as determined by a Chi-Square test at $p < 0.05$. If the same analyses are conducted using only subjects who smoked using one hand, no significant differences between detection rates on lips/hands used or hands not used are found at $p < 0.05$.

e. Detection after smoking versus location of swab: The frequency of positive detections on lips, right and left hands after smoking are as follows (subjects at 0, 1, and 3 hr elapsed time):

Finding	Lip	Right Hand	Left Hand	Total
+	60	44	39	143
-	66	82	87	235
Total	126	126	126	378
% +	47.6%	34.9%	31.0%	37.8%

A Chi-Square test indicates a significantly higher percentage of detections on the lips (47.6%) than on the left hand (31.0%) ($X^2 = 7.336$, $df = 1$, $p < 0.05$) or on the right hand (34.9%) ($X^2 = 4.192$, $df = 1$, $p < 0.05$). There is no statistical difference between the percentage positives found on the right and left hands.

f. Detection after smoking versus amount of cigarette smoked: The frequency of positive detections after smoking versus the amount of the cigarette smoked is shown below in terms of whether or not the subjects smoked the cigarettes down to the butt.

Finding	Complete	Not Complete	Total
+	45	31	76
-	29	21	50
Total	74	52	126
% +	60.8%	59.6%	60.3%

There is no statistical difference between the positives found in complete and incomplete cigarette smokers. It was noted earlier though, that the high dose smokers yielded less positive results than the medium dose and low dose smokers, and this was attributed to incomplete smoking, even though it was not statistically significant.

g. Detection after smoking versus use of roach clips: In this program 80.9% of the smokers used roach clips towards the end of the smoke. No smokers used roach clips at the start of a smoke. The frequencies of roach clip use versus detections is shown below for 0, 1 and 3 hr groups:

Finding	Roach Clip Used	No Roach Clip	Total
+	61	15	76
-	41	9	50
Total	102	24	126
% +	59.8%	62.5%	60.3%

There is no statistical difference between the above percentages at the $p < 0.05$ level.

h. Detection after smoking versus type of inhalations: Inhalations were observed as medium, heavy or variable. The frequency of positive detections after smoking (0, 1 and 3 hr elapsed time groups) versus type of inhalations is shown below:

	Type of Inhalation			
Finding	Medium	High	Variable	Total
+	42	16	18	76
-	31	7	12	50
Total	73	23	30	126
% +	57.5%	69.6%	60.0%	60.3%

There is no statistical difference between the above percentages at the $p < 0.05$ level.

i. Detection of lips after smoking versus facial features: Since facial features such as beards and mustaches are likely only to affect the lip detection, the frequency of positive lip detections after smoking (0, 1 and 3 hr elapsed time groups) versus facial features are shown below:

Finding	Mustache and Beard	Mustache only	Beard only	Clean Shaven	Total
+	11	25	3	21	60
-	10	37	1	18	66
Total	21	62	4	39	126
% +	52.4%	40.3%	75.0%	53.8%	47.6%

There is no statistical difference between these percentages at the $p < 0.05$ level. Even though 75.0% of those smokers possessing a beard only were positive, the total number of subjects possessing a beard only totaled just four.

j. Detection after smoking versus skin features: All subjects were asked to identify their skin as normal, oily or dry. The frequency of detection after smoking (0, 1 and 3 hr elapsed time groups) versus skin type is shown below:

Finding	Dry Skin	Normal Skin	Oily Skin	Total
+	5	63	8	76
-	4	41	5	50
Total	9	104	13	126
% +	55.6%	60.6%	61.5%	60.3%

There is no statistical difference between the above percentages at the $p < 0.05$ level.

k. Detection after smoking versus the prior use of alcohol: All subjects were asked if they had consumed alcohol in the 24 hr prior to the experiment. The frequency of detection after smoking (0, 1 and 3 hr elapsed time groups) versus the prior use of alcohol is shown below:

Finding	Used Alcohol	Not Used Alcohol	Total
+	38	38	76
-	23	27	50
Total	61	65	126
% +	62.3%	58.5%	60.3%

There is no statistical difference between the above percentages at the $p < 0.05$ level.

1. Detection before smoking versus use of aftershave/cologne: All subjects were asked if aftershave or cologne had been used within the past 24 hr. Presented below are the frequencies of detection before smoking or exposure versus the prior use of aftershave and/or cologne:

Finding	Used Aftershave/ Cologne	Not Used Aftershave/ Cologne	Total
+	8	18	26
-	59	111	170
Total	67	129	196
% +	11.9%	13.9%	13.3%

There is no statistical difference between the above percentages at the $p < 0.05$ level.

m. Detection before smoking versus use of hand cream/lotion: All subjects were asked if hand cream or skin lotion had been used within the 24 hr preceding the experiment. Presented below are the frequencies of detection before smoking or exposure versus the prior use of hand cream/lotion:

Finding	Used Hand Cream/ Lotion	Not Used Hand Cream/Lotion	Total
+	4	22	26
-	18	152	170
Total	22	174	196
% +	18.2%	12.6%	13.3%

Although hand cream/lotion users showed a higher percentage of positives than those not using hand cream/lotion, the difference in the percentages is not statistically significant at the $p < 0.05$ level.

n. Detection before smoking versus prior marihuana use: All subjects were asked whether or not they had smoked marihuana in the 24 hr prior to the experiment. All subjects were asked not to smoke during this period, but we experienced difficulty in persuading them not to do so. Presented below are the frequencies of detection before smoking marihuana in the experiment versus prior use of marihuana:

Finding	Used Marihuana	Not Used Marihuana	Total
+	20	6	26
-	66	104	170
Total	86	110	196
% +	23.3%	5.5%	13.2%

The difference between the 23.3% who showed positives and had used marihuana and the 5.5% who showed positives and did not admit smoking marihuana is significant at the $p < 0.05$ level ($X^2 = 13.294$, $df = 1$).

C. Conclusions and Recommendations

The conclusions that can be drawn from the recommended procedure, the TLC test, are as follows.

The accuracy of detection in marihuana smokers using this test depends much more on the time elapsed between smoking and testing than on dosage. Eighty-six percent of the smokers were detected immediately after smoking, whereas only 60% were detected after 1 hr, and 37.5% detected after 3 hr. Different swabbers did not have a significant effect on accuracy rates. Of the three laboratories analyzing swabs, two laboratories agreed very closely at 75.9 and 77.4% detection accuracy rates, while the third laboratory had only a 52.0% detection rate.

The total detection rate on lips (47.6%) is significantly higher than that on either of the hands (34.9 and 31.0% for the right and left hands, 41.3% for right and/or left hands, and 60.3% for one or more positive locations). The ratios of lip positive percentages to right and left hand positive percentages in the tests conducted in Phases I and II of this program are:

	Lip	R. Hand	L. Hand
CS test	1.94	1.47	1.00
Phase I TLC test	1.84	1.05	1.00
Phase II TLC test	1.55	1.13	1.00

Persons exposed to marihuana smoke are not readily detected, and only then immediately after exposure at an 11.8% rate at maximum.

The amount of cigarette smoked, the use of roach clips, the type of inhalations, facial features, skin features, and the prior use of alcohol did not significantly affect the results.

The use of aftershave and/or cologne or hand cream and/or skin lotion did not contribute to false positives. The prior smoking of marihuana did result in positive detections before smoking. Out of 26 subjects positive before experimental smoking, 20 (77%) admitted smoking 24 hr prior to the experiment.

Thus, the recommended TLC test has a smoker detection rate of 86% immediately after smoking (74.4% lips, 58.1 and 55.8% right and left hands). This rate of detection falls to 60.0% 1 hr after smoking and to 37.5% after 3 hr. The detection rate over the whole range of smokers (0, 1 and 3 hr elapsed time between smoking and testing) is 60.3% (47.6% lips, 34.9 and 31.0% right and left hands).

Based upon the above findings, the TLC test is not proven suspect of false positive but can detect only 86% of smokers immediately after smoking. It will yield an underestimate of smokers who have smoked marihuana within several hours prior to the test. It is recommended that a more sensitive technique for detecting cannabinoids be sought for use in a marihuana contact test using the swabbing procedures employed in this investigation. It is recommended therefore that the TLC test be used on survey programs involved in the determination of the incidence of marihuana contact only with great caution, and that the inadequacy of the test in terms of false negatives be borne in mind at all times. Since other analytical techniques, presently under development, such as radioimmunoassay and mass spectrometry, seem likely to offer greater sensitivity for marihuana constituents, it is recommended that these analytical techniques be considered for future use in surveys of the incidence of marihuana contact. Such techniques also offer the promise of detection of marihuana constituents in body fluids, and thus, possible detection of the state of marihuana intoxication of individuals.

A questionnaire survey conducted on this program revealed interest by parties other than the NHTSA in a simple, inexpensive test for evidence of marihuana contact on automobile surfaces, personal clothing and belongings, furniture and other household items. The swab test, utilizing the TLC analysis, is recommended for such purposes. It must be borne in mind, though, that additional tests would be necessary to provide acceptable legal proof of marihuana contamination of the surfaces tested.

REFERENCES

1. Stone, H. M., and H. M. Stevens, "Detection of Cannabis Constituents in the Mouth and on the Fingers of Smokers," Forensic Sci. Soc. J., 9(1-2):31-34 (1969).

2. U.S. Army Report No. LWL-CL-08C72.

3. DOT Contract No. DOT-HS-119-3-627.

4. DOT Contract No. DOT-HS-119-2-440.

APPENDIX A

LETTER AND QUESTIONNAIRE RECIPIENTS

July 26, 1974

Name
Address
City, State

Dear :

The National Highway Traffic Safety Administration of the U.S. Department of Transportation has recently funded a program with Midwest Research Institute (MRI) to critically evaluate a marihuana "contact test."

This test is capable of detecting minute traces of THC and other species unique to marihuana. It consists of a swab (e.g., a Q-tip) which is moistened with a solvent and applied to the surface to be tested for traces of marihuana or its residues. A simple color test is then performed on the swab, or the marihuana components can be removed from the swab and examined by chromatography in the laboratory.

This test has already proven to be approximately 80% effective in detecting marihuana traces on the lips of human smokers immediately after smoking, with no false positives apparent. The reliability of the test on deceased persons and inanimate objects is still under investigation.

Since the new evaluation program may produce a simple and fully evaluated test for marihuana and its residues, both the NHTSA and MRI are anxious to learn of your interest and possible uses for such a test.

If you could spare a moment to complete the attached questionnaire and return it to us in the envelope enclosed, it would be most appreciated.

If you have any questions concerning this request, please feel free to call us--we look forward to receiving your completed questionnaire.

Sincerely yours,

MIDWEST RESEARCH INSTITUTE

E. J. Woodhouse, Ph.D.
Principal Chemist

EJW:sps

MARIHUANA CONTACT TEST QUESTIONNAIRE

Assuming that a simple contact (swab) test for detecting marihuana was available, would you have a use for it? ☐ Yes ☐ No

Would you use it for:

- Detecting marihuana smokers by swabbing skin areas (lips, etc.) ☐ Yes ☐ No

- Detecting the presence of marihuana residues on surfaces other than skin, and if so, what surfaces: ☐ Yes ☐ No

_____

_____

_____

_____

Describe briefly any other uses you could conceive or would like to use the contact test for: _____

_____

_____

_____

_____

Please describe briefly any programs in which you feel the contact test would be of use (surveys, enforcement programs, forensic applications, etc.):

_____

_____

_____

_____

_____

(OVER)

Which criteria do you consider most important in such a simple contact test:

	Very Important	Important	Un-important
Specificity (minimum of false positives)	☐	☐	☐
Sensitivity (minimum of false negatives)	☐	☐	☐
Cost of test--state maximum cost _____¢/test	☐	☐	☐
Ease of use	☐	☐	☐
Adaptability for field use	☐	☐	☐

Any other comments: _____

_____

_____

_____

_____

(Please use additional space if necessary.)

Name: _____

Title: _____

Organization: _____

_____

Address: _____

_____

City: _____

State: _____ Zip: _____

Thank you

LIST OF RECEIPIENTS OF THIS LETTER

Dr. James G. Arey
Temple University School of Medicine
Huntingdon and Lawrence Streets
Philadelphia, Pennsylvania 19133

Mr. Arthur J. Bilek
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APPENDIX B

CS AND TLC SENSITIVITY AND SPECIFICITY TESTS

TABLE B-1

COLORIMETRIC SWAB (CS) TEST SENSITIVITY TEST RESULTS

Swab No.	[THC]a/	Result	Swab No.	[THC]a/	Result
1	17.5	+	36	2.5	-
2	0	-	37	0	-
3	20.0	+	38	12.5	+
4	10.0	+	39	5.0	-
5	5.0	-	40	0	-
6	17.5	+	41	15.0	+
7	2.5	-	42	17.5	+
8	7.5	-	43	2.5	-
9	25.0	+	44	1.0	-
10	15.0	+	45	7.5	-
11	5.0	-	46	25.0	+
12	1.0	-	47	12.5	+
13	10.0	+	48	17.5	+
14	17.5	+	49	7.5	-
15	0	-	50	20.0	+
16	25.0	+	51	15.0	+
17	15.0	+	52	10.0	-
18	20.0	+	53	12.5	+
19	0	-	54	5.0	-
20	10.0	+	55	25.0	+
21	5.0	-	56	12.5	+
22	17.5	+	57	0	-
23	15.0	+	58	7.5	-
24	20.0	+	59	15.0	+
25	17.5	+	60	5.0	-
26	1.0	-	61	1.0	-
27	15.0	+	62	20.0	+
28	2.5	-	63	2.5	-
29	17.5	+	64	10.0	+
30	7.5	-	65	0	-
31	1.0	-	66	25.0	+
32	25.0	+	67	7.5	-
33	10.0	+	68	0	-
34	5.0	-	69	12.5	+
35	20.0	+	70	1.0	-

TABLE B-1 (Concluded)

Swab No.	[THC][a]	Result	Swab No.	[THC][a]	Result
71	12.5	+	86	7.5	+
72	1.0	-	87	2.5	-
73	2.5	-	88	10.0	+
74	25.0	+	89	7.5	-
75	20.0	+	90	0	-
76	2.5	-	91	17.5	+
77	25.0	+	92	5.0	-
78	20.0	+	93	1.0	-
79	12.5	+	94	15.0	+
80	5.0	-	95	10.0	+
81	1.0	-	96	2.5	-
82	15.0	+	97	20.0	+
83	0	-	98	12.5	+
84	12.5	+	99	7.5	-
85	25.0	+	100	10.0	+

a/ [THC] is in parts per million and is the concentration of THC solution in which the swabs were dipped. Ten parts per million corresponds to 2-μg THC on a swab.

TABLE B-2

COLORIMETRIC SWAB (CS) TEST INTERFERENCES TEST RESULTS

Substance	Swab Color	Substance	Swab Color
Areca	Dark red	Salicylic acid	--
Catechu	Dark red	Chlorpheniramine	--
Chamomile	Green-brown	Diphenhydramine	--
Danivana	Brown	Amitriptyline	--
Hops	Red-brown	Thioridazine	--
Horsetail	Brown	Propoxyphene	--
Kava-kava	Red	Quinine	--
Kola	Dark red	Methylphenidate	Faint pink
Lobelia	Brown	Oxymorphone	Faint pink
Mistletoe	Brown	Promazine	--
Mormon tea	Dark brown	Trifluoperazine	--
Tobacco	Orange	Chlorpromazine	--
Mustard	Green	Imipramine	--
Onion	Negative or light brown	Diazepam	--
		Morphine	--
Paprika	Light brown	Codeine	--
Passion flower	Brown	Glutethimide	--
Skull cap	Brown	Cocaine	--
Valerian	Light brown	Methadone	--
Wormwood	Brown	Hydromorphone	Pink
Yohimbe	Red	Nicotine	--
Nutmeg	Dark red	MDA	--
Cinnamon	Yellow	STP	--
Cloves	Brown	Amphetamine	--
Ginger	Dark brown	Methamphetamine	--
Mace	Dark red	DMT	--
Pepper	--	DET	--
Rosemary	Light brown	Mescaline	--
Sage	Pink-orange	Lobeline	--
Thyme	Pink-orange	Nalorphine	--
Phenobarbital	--	Phendimetrazine	--
Pentobarbital	--	Tripelenamine	--
Amobarbital	--	Methapyrilene	--
Secobarbital	--	Phenylpropanolamine	--
Butabarbital	--	Vitamin D_3	--
Butobarbital	--	11B-OH-Etidiolanolone	--
Diphenylhydantoin	--	Tryptophan	--
Meperidine	--	Glycine	--
Aspirin	--	Tyrosine	--

TABLE B-2 (Continued)

Substance	Swab Color
Cortisone	--
Hydrocortisone	--
Prednisone	--
Gumdrop extract	--
Licorice extract	Faint pink
Jelly bean extract	--
Hot Tamale® candy extract	--
Juicyfruit® candy extract	--
Emeraude® perfume	Brown
Prop® electric preshave	Red
Old Spice® after shave	Dark red
Colgate 100® mouthwash	--
Listerine® mouthwash	Red/Orange
Jaguar® after shave	Dark red
Right Guard® powder deodorant	--
Helene Curtis® protein hair spray	--
Clear nail polish	--
Red nail polish	--
Amolin® deodorant powder	--
Mexsana® medicated powder	--
Iodent No. 2 tooth powder	--
Mennen® shave talc	--
Breck® creme rinse	Dark red
Vaseline® intensive care lotion	--
Suave® shampoo with egg	--
Old Spice® deodorant soap	--
Rapid Shave®	--
Blistik®	Pink
Red lipstick	--
Compact	--
Wood Q-tips®	Faint pink
Tussy® cream deodorant	--
Vaseline® petroleum jelly	--
Mint Crest® toothpaste	--
Blue plastic Q-tips®	--
Paper Q-tips®	--
Band-Aids®	--
Johnsons® baby shampoo	--
VO5® hairdressing	--
QT® suntan lotion	--
Coppertone® tanning butter	--
Noxema® skin cream	--
Medicated throat discs	--
Lipstick	Pink orange

TABLE B-2 (Concluded)

Substance	Swab Color
Clearasil®	Dark red
Oil control make-up	--
English Leather® deodorant soap	--
British Sterling®	Red
Viva Patchouly® after shave	Brown/red
Desire® perfume	Purple/red
Cachet® perfume	Purple/red
Jacque® after shave	Purple/red
Ritz® cologne	Purple/red

TABLE B-3

TLC SOLVENT SYSTEMS FOR THC[a/]

Solvent		R_f (THC)
Chloroform/methanol	8.5/1.5	0.96
Chloroform/methanol	9/1	0.96
Chloroform/methanol	9.5/0.5	0.90
Chloroform/methanol	9.8/0.2	0.86
Benzene/chloroform	3/7	0.61
Benzene/chloroform	2/8	0.61
Benzene/chloroform	4/6	0.61
Benzene		0.57
Chloroform		0.69
Cyclohexane		0.00

a/ TLC performed on silica gel, 250 μ on glass. Visualization by Fast Blue B (unsaturated developing conditions).

TABLE B-4

TLC SENSITIVITY TEST RESULTS USING PURE ETHANOL SOLUTIONS AS SIMULATED WASHINGS

Test No.	[THC]a/	Result	Test No.	[THC]a/	Result
1	0.05	-	29	0.1	+
2	0.3	+	30	0.05	+
3	0.4	+	31	0.3	+
4	0.1	+	32	0.05	-
5	0.02	-	33	0.2	+
6	0.2	+	34	0.1	+
7	0.05	+	35	Blank	-
8	Blank	-	36	0.4	+
9	0.4	+	37	0.3	+
10	0.2	+	38	0.1	+
11	0.3	+	39	0.02	-
12	0.02	-	40	0.2	+
13	Blank	-	41	0.1	-
14	0.02	-	42	Blank	-
15	0.1	+	43	0.4	+
16	0.4	+	44	0.05	-
17	0.3	+	45	0.3	+
18	0.05	+	46	0.02	-
19	0.2	+	47	0.1	-
20	0.4	+	48	0.05	-
21	0.02	-	49	0.4	+
22	Blank	-	50	0.2	+
23	0.02	-	51	0.02	-
24	0.4	+	52	0.3	+
25	0.05	-	53	Blank	-
26	0.2	+	54	0.2	+
27	Blank	-	55	0.1	+
28	0.3	+	56	Blank	-

a/ [THC] is in parts per million. One part per million represents 5 μg of THC per wash solution.

TABLE B-5

TLC SENSITIVITY TEST RESULTS USING FOREARM WASHINGS

Test No.	[THC][a]	Result	Test No.	[THC][a]	Result
1	0.2	+	29	Blank	-
2	0.05	+	30	0.02	-
3	0.4	+	31	0.2	+
4	Blank	-	32	0.1	+
5	0.1	+	33	0.05	+
6	0.3	+	34	0.4	+
7	0.05	+	35	0.3	+
8	0.4	+	36	0.05	+
9	0.02	-	37	Blank	-
10	0.1	+	38	0.4	+
11	0.2	+	39	0.02	-
12	0.3	+	40	0.1	+
13	Blank	-	41	0.2	+
14	0.1	+	42	0.3	+
15	0.3	+	43	Blank	-
16	0.02	-	44	0.02	-
17	Blank	-	45	0.1	+
18	0.2	+	46	0.05	+
19	0.3	+	47	0.3	+
20	0.05	+	48	0.4	+
21	0.4	+	49	0.1	+
22	0.02	-	50	Blank	-
23	0.1	+	51	0.05	+
24	Blank	-	52	0.3	+
25	0.4	+	53	0.2	+
26	0.2	+	54	0.02	-
27	0.02	-	55	0.4	+
28	0.05	+	56	0.2	+

a/ [THC] is in parts per million. One part per million represents 5 µg of THC per wash solution.

TABLE B-6

TLC INTERFERENCE TEST RESULTS

Substance	TLC Results: Rfs and Colors[a]	Decision
THC	0.57-R	+
Areca	0.0-R	-
Catechu	1.0-Pu	-
Chamomile	1.0-Pu	-
Damiana	0.04-Pu	-
Hops	0.0-R	-
Horsetail	--	-
Kava-kava	0.05-Pu	-
Kola	--	-
Lobelia	--	-
Misteltoe	--	-
Mormon tea	--	-
Tobacco	0.0-Or, 1.0-Pu	-
Mustard	0.0-V, 0.05-V	-
Onion	--	-
Paprika	--	-
Passion flower	--	-
Skull cap	--	-
Valerian	--	-
Wormwood	--	-
Yohimbe	--	-
Nutmeg	0.0-R, 0.04-Or, 0.09-Br	-
Cinnamon	0.11-Br, 0.17-Pu, 0.28-V, 0.40-Br	-
Cloves	0.46-Br, 0.24-BrOr	-
Ginger	--	-
Mace	0.44-Br, 0.0-Or, 0.04-R, 0.19-P, 0.35-Pu	-
Pepper	--	-
Rosemary	0.14-Pu, 0.68-Br	-
Sage	0.05-Pu, 0.46-YBr	-
Thyme	0.51-Br, 0.45-YBr	-
Phenobarbital	--	-
Pentobarbital	--	-
Amobarbital	--	-
Secobarbital	--	-
Butabarbital	--	-
Butobarbital	--	-

TABLE B-6 (Continued)

Substance	TLC Results: Rfs and Colors[a]	Decision
Diphenylhydantoin	--	-
Meperidine	0.19-Br	-
Aspirin	--	-
Salicylic acid	--	-
Chlorpheniramine	--	-
Diphenhydramine	--	-
Amitryptyline	--	-
Thioridazine	--	-
Propoxyphene	--	-
Quinine	--	-
Methylphenidate	0.00-Br	-
Oxymorphone	0.00-Or	-
Promazine	--	-
Trifluoperazine	--	-
Chlorpromazine	0.00-Br	-
Imipramine	--	-
Diazepam	--	-
Morphine	0.00-Br	-
Codeine	--	-
Glutethimide	--	-
Cocaine	--	-
Methadone	--	-
Hydromorphone	0.00-Or	-
Nicotine	--	-
MDA	0.30-Or,Br	-
STP	--	-
Amphetamine	--	-
Methamphetamine	--	-
DMT	--	-
DET	--	-
Mescaline	--	-
Lobeline	0.0-Br	-
Nalorphine	--	-
Phendimetrazine	--	-
Tripelennamine	--	-
Methapyrilene	--	-
Phenylpropanolamine	--	-
Vitamin D_3	--	-
11-β-OH Etiocholanone	--	-
Tryptophan	--	-
Glycine	--	-
Tyrosine	--	-
Cortisone	--	-

TABLE B-6 (Continued)

Substance	TLC Results: R_fs and Colors[a]	Decision
Hydrocortone	--	-
Prednisone	--	-
Gumdrops	0.0-Br	-
Licorice	0.0-Br	-
Jelly beans	--	-
Hot Tamale® candy	--	-
Juicyfruit® candy	--	-
Emeraude® perfume	0.66-Pu	+
Prop® electric preshave	0.0-P, 0.13-P, 0.23-Or,Br	-
Old Spice® after shave	0.0-Br, 0.45-Y	-
Colgate 100® mouthwash	0.39-Br	-
Listerine®	0.43-Y,Br	-
Jaguar® after shave	0.23-Or, 0.34-P, 0.43-Br	-
Right Guard® powder deodorant	--	-
Helene Curtis® protein hair spray	--	-
Clear nail polish	--	-
Red nail polish	--	-
Amolin® deodorant powder	0.43-Y	-
Mexsana® medicated powder	0.59-Y	-
Iodent® tooth powder	--	-
Mennen® shave talc	--	-
Breck® creme rinse	0.00-Pu	-
Vaseline® intensive care	--	-
Suave® shampoo with egg	--	-
Old Spice® deodorant soap	0.42-Br	-
Rapid Shave®	--	-
Blistik®	0.45-Y,Br	-
Red lipstick	0.06-Br	-
Compact	--	-
Wooden Q-tip® stick extract	0.00-P,Br	-
Tussy® cream deodorant	0.42-Br	-
Vaseline® petroleum jelly	--	-
Mint Crest® toothpaste	0.46-Y,Br	-
Blue Plastic Q-tip® stick extract	--	-
Paper Q-tip® stick extract	--	-
Band Aids®	--	-
Johnsons® baby shampoo	0.00-Y	-
VO5® hairdressing	--	-
QT® suntan lotion	0.92-Y	-
Coppertone® tanning butter	--	-
Noxema® skin cream	0.48-Y, 0.43-Br, 0.22-Br, 0.14-Br, 0.05-Br,Or	-

TABLE B-6 (Concluded)

Substance	TLC Results: R_f's and Colors[a]	Decision
Medicated throat discs	--	-
Lipstick	0.49-Y, 0.40-Br	-
Clearasil®	0.00-Pu	-
Oil control make-up	--	-
English Leather® deodorant soap	--	-
British Sterling® after shave	0.00-Pu, 0.08-Br,Pu, 0.15-R, 0.40-Br, 0.23-Or, 0.53-Br, 0.00-Pu, 0.89-Gy, 0.77-Gy	
Viva Patchouly® after shave	0.40-Br, 0.89-V	-
Desire® perfume	0.22-R,Pu, 0.25-P, 0.34-Br, 0.38-V, 0.44-Y, 0.64-Pu	+
Cachet® perfume	0.0-Pu, 0.14-Or, 0.28-Or, 0.60-P, 0.89-Gy	+
Jacques® after shave	0.0-Pu, 0.14-Or, 0.26-Or, 0.41-Br, 0.60-P,Pu, 0.77-Y	+
Ritz® cologne	0.08-Y, 0.36-Or, 0.47-Br, 0.62-Pu, 0.68-Y, 0.71-R, 0.76-P, 0.86-Gy	+
THC, CBN, CBD, CBCH (Cannabinoids)	0.57-R, 0.63-Pu, 0.67-Or, 0.46-Pu	+

a/ Legend: Br - Brown
Gy - Gray
Or - Orange
P - Pink
Pu - Purple
R - Red
V - Violet
Y - Yellow

Solvent = benzene (unsaturated).

TABLE B-7

TLC CHARACTERISTICS OF POTENTIAL INTERFERENCES USING SECOND SOLVENT

Substance	R_f's and Colors[a/]	Decision
THC	0.59-R	+
Emeraude® perfume	0.48-B, 0.95-Gy	-
British Sterling® after shave	0.18-R, 0.23-R, 0.30-Br, 0.42-Br, 0.73-R, 0.90-Y, 0.95-Gy	-
Cachet® cologne	0.20-Pu, 0.28-R, 0.36-Br	-
Jacques® after shave	0.03-R, 0.17-R, 0.24-Br, 0.30-Br, 0.44-Br	-
Ritz® cologne	0.08-Br, 0.33-Br, 0.49-Gy, 0.56-Pu	-
THC	0.59-R	+
CBN	0.52-Pu	
CBD	0.69-Or	
CBCH	0.40-Pu	
Cannabinoids		

a/ Legend: Br - Brown
Gy - Gray
Pu - Purple
R - Red
Y - Yellow
Or - Orange

Solvent = Hexane/ether, 4:1 (unsaturated).

APPENDIX C

EVALUATION OF SPECIMEN COLLECTION AND EXTRACTION

TABLE C-1

TLC RESULTS FROM Q-TIP® AND COTTON BALL SWABS
FROM EXPOSED AND CONTROL FOREARMS

Swab	TLC Result[a/]	Decision
Blank Q-tip®	0.55-Or	-
Blank Q-tip®	0.55-Or	-
Test Q-tip®	0.43-R, 0.55-Or	+
Test Q-tip®	0.43-R, 0.55-Or	+
Blank cotton ball	0.55-R	-
Blank cotton ball	0.55-R	-
Test cotton ball	0.36, 0.42-R, 0.55-Or	+
Test cotton ball	0.36, 0.42-R, 0.55-R	+
THC (spike)	0.41-R	+

a/ Legend: Or - Orange
R - Red

Solvent - benzene (saturated).

TABLE C-2

EFFECT OF ELAPSED TIME ON ELUTION

THC on Swab (μg)	Results		
	Immediately	After 2 Days	After 1 Week
Blank	-	-	-
Blank	-	-	-
0.1	-	+	+
0.1	+	+	+
0.1	+	+	+
0.1	+	+	+
0.2	+	+	+
0.2	+	+	+
0.3	+	+	+
0.3	+	+	+
0.4	+	+	+
0.5	+	+	+

TABLE C-3

EFFECT OF AGITATION AND SHREDDING ON ELUTION

	THC on Swab (µg)	Results												
		W	S	W	S	W	S	W	S	W	S	W	S	B
Agitated	0.1	+	+	+	+	+	+	+	+	+	+	+	+	-
	0.1	-	+	+	+	+	+	+	+	+	+	+	+	-
	0.5	+	+	+	+	+	+	+	+	+	+	+	+	-
	0.5	+	+	+	+	+	+	+	+	+	+	+	+	-
Non-agitated	0.1	+	+	+	+	+	+	+	+	+	+	+	+	-
	0.1	+	+	+	+	+	+	+	+	+	+	+	+	-
	0.5	+	+	+	+	+	+	+	+	+	+	+	+	-
	0.5	+	+	+	+	+	+	+	+	+	+	+	+	-

W = whole
S = shredded
B = blank

TABLE C-4

EFFECT OF ELUTION TIME

THC on Swab (µg)	Result: Elution Time 5 min	10 min	20 min	30 min
0	- - -	- - -	- - -	- - -
0	- - -	- - -	- - -	- - -
0.1	+ + +	+ + +	+ + +	+ + +
0.1	+ + +	+ + +	+ + +	+ + +
0.5	+ + +	+ + +	+ + +	+ + +
0.5	+ + +	+ + +	+ + +	+ + +

TABLE C-5

RESULTS OF SWAB TEST USING NAIVE SWABBERS

Swabber No.	Sex	Analytical Result	Investigator Comments
1	Female	-	Used one light small stroke on face of each digit.
2	Male	Weak +	Used one small stroke on face of each digit.
3	Male	Weak +	Used one small stroke on face of each digit.
4	Male	Medium +	Used several medium pressure strokes all around digits.
5	Female	Weak +	Used one small stroke on face of each digit.
6	Male	Medium +	Used several medium pressure strokes on face of each digit.
7	Male	Strong +	Used several heavy scrubbing type strokes all around each digit.
8	Male	Weak +	Used one small stroke on face of each digit.
9	Female	Medium +	Used several small strokes on face of each digit.
10	Male	Weak +	Used one small stroke on face of each digit
11.	Female	Medium +	Used several small strokes on face of each digit.
12.	Female	Medium +	Used one small stroke on face of each digit.
13.	Male	Weak +	Used one small stroke on face of each digit.
14.	Male	Strong +	Used several heavy scrubbing strokes all around digits.

TABLE C-5 (Concluded)

Swabber No.	Sex	Analytical Result	Investigator Comments
15.	Female	Strong +	Used several heavy scrubbing strokes on face of each digit.
16.	Male	Medium +	Used one heavy stroke on face of each digit.
17.	Male	Strong +	Used several heavy scrubbing strokes on face of each digit.
18.	Male	Medium +	Used one medium stroke on face of each digit.
19.	Female	Medium +	Used several strokes on face of each digit.
20.	Female	Strong +	Used several heavy scrubbing strokes all around digits.

TABLE C-6

RESULTS OF SWAB TEST USING NAIVE SWABBERS AND NEW INSTRUCTIONS

Swabber No.	Sex	Analytical Result	Swabbing Pressure			
			Very High	High	Medium	Low
1	F	Very strong +		X		
2	M	Strong +		X		
3	M	Medium +		X		
4	M	Strong +		X		
5	F	Strong +		X		
6	F	Strong +		X		
7	M	Strong +		X		
8	M	Medium +		X		
9	M	Strong +		X		
10	F	Strong +		X		
11	M	Medium +		X		
12	M	Very strong +	X			
13	F	Very strong +			X	
14	M	Medium +		X		
15	F	Strong +			X	
16	M	Medium +		X		
17	F	Very strong +		X		
18	F	Very strong +		X		
19	F	Very strong +		X		
20	F	Strong +		X		

F = Female
M = Male

TABLE C-7

RESULTS OF TLC EVALUATION OF SWABS (Blanks, THC, and Interferences)

	[THC] on Swab	Results			
		Fresh Swabs		Week Old Swabs	
		Positive	Negative	Positive	Negative
Benzene developing solvent (First Solvent)	Blank	0	25	0	25
	50 ng	10	15	4	21
	100 ng	16	9	17	8
	500 ng	25	0	24	1
	2 µg	25	0	25	0
	5 µg	25	0	25	0
Hexane/Ether 4:1 developing solvent. (Second Solvent)	Blank	0	0	0	0
	50 ng	3	7	1	3
	100 ng	6	10	5	12
	500 ng	18	7	22	2
	2 µg	22	3	25	0
	5 µg	25	0	25	0

TABLE C-8

RESULTS OF CS EVALUATION OF SWABS (Blanks, THC, and Interferences)

[THC] on Swab	Results			
	Fresh Swabs		Week Old Swabs	
	Positive	Negative	Positive	Negative
Blank	0	25	0	25
50 ng	0	25	0	25
100 ng	0	25	0	25
500 ng	0	25	1	24
2 µg	18	7	22	3
5 µg	25	0	25	0

TABLE C-9

INTERFERING SUBSTANCES TESTED IN THE EVALUATION STUDY

1. Areca
2. Catechu
3. Kava Kava
4. Kola
5. Yohimbe
6. Nutmeg
7. Mace
8. Sage
9. Thyme
10. Methylphenidate
11. Oxymorphone
12. Hydromorphone
13. Licorice
14. Prop® Electric Pre-Shave
15. Old Spice® After-Shave
16. Listerine®
17. Jaguar® After-Shave
18. Breck® Creme Rinse
19. Blistick® Lip Balm
20. Clearasil®
21. British Sterling® After-Shave
22. Viva Patchouly® After-Shave
23. Desire® Perfume
24. Chachet® Cologne
25. Jacques® After-Shave
26. Ritz® Cologne
27. Wood Q-Tip® Extract
28. Emeraude® Perfume

TABLE C-10

RESULTS OF TESTS ON SWABS FROM CORPSES USING THE TLC METHOD

Subject No.	Age	Sex	Interval Between Death and Swabbing	Swab	Results	Cause of Death
1	48	F	9.8 hr	RH	Neg.	Suicide (firearm to head)
				LH	Neg.	
				LP	Neg.	
				P	No Sample	
3	29	M	5.3 hr	RH	Neg.	Murder (knife to throat)
				LH	Neg.	
				LP	Neg.	
				P	No Sample	
5	54	M	13.5 hr	RH	Neg.	Head Injuries
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
7	28	M	8.3 hr	RH	Neg. R_f 0.0-Red R_f 0.5-Yellow	Murder (gunshot wounds)
				LH	Neg. R_f 0.0-Red R_f 0.5-Yellow	
				LP	Neg.	
				P	Neg.	
9	41	M	17.8 hr	RH	Neg.	Suicide (overdose of Valium and Darvon)
				LH	Neg.	
				LP	Neg. R_f 0.3-Orange	
				P	Neg.	
11	56	M	13.2 hr	RH	Neg. R_f 0.8-Tan	Traffic Fatality
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
13	54	M	8.2 hr	RH	Neg.	Unknown
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
15	42	M	16.6 hr	RH	Neg. R_f 0.0-Red	Murder (gunshot wounds)
				LH	Neg. R_f 0.0-Red	
				LP	Neg.	
				P	Neg. R_f 0.7-Tan	
17	53	M	14.1 hr	RH	Neg. R_f 0.34-Yellow	Heart Failure
				LH	Neg. R_f 0.34-Yellow	
				LP	Neg.	
				P	Neg.	

TABLE C-10 (Continued)

Subject No.	Age	Sex	Interval Between Death and Swabbing	Swab	Results	Cause of Death
19	57	F	13.8 hr	RH	Neg.	Traffic Fatality
				LH	Neg.	
				LP	Neg.	
				P	No Sample	
21	37	F	9.6 hr	RH	Neg.	Drug Overdose (Placidyl, Mellaiill, Chlortrimeton, Sinequan)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
23	36	M	9.6 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
25	49	M	2.0 hr	RH	Neg.	Heart Failure
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
27	59	M	8.8 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
29	39	M	22.0 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
31	43	F	6.0 hr	RH	Neg.	Natural Causes
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
33	53	M	4.5 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	No Sample	
35	18	M	17.3 hr	RH	Neg.	Head Injury
				LH	Neg.	
				LP	Neg.	
				P	No Sample	

TABLE C-10 (Concluded)

Subject No.	Age	Sex	Interval Between Death and Swabbing	Swab	Results		Cause of Death
37	44	M	18.3 hr	RH	Neg.		Natural Causes
				LH	Neg.		
				LP	Neg.		
				P	Neg.		
41	51	M	1.4 hr	RH	Neg.	R_f 0.61-Black R_f 0.44-Yellow	Alcohol Overdose
				LH	Neg.		
				LP	Neg.		
				P	Neg.		
43	14	M	17.8 hr	RH	Pos.	R_f 0.61-Black R_f 0.37-Red	Murder (gunshot wounds) N.B. victim was smoking marihuana at the time of death
				LH	Neg.	R_f 0.61-Black	
				LP	Pos.	R_f 0.37-Red	
				P	Pos.	R_f 0.37-Red	
45	34	M	2.25 hr	RH	Neg.		Acute Alcoholism
				LH	Neg.		
				LP	Neg.		
				P	Neg.		
47	41	M	18.5 hr	RH	Neg.		Fall from 20th Floor of Building--Multiple Traumatic Injuries
				LH	Neg.		
				LP	Neg.		
				P	Neg.		
49	22	M	16.5 hr	RH	Neg.		Fall from Building--Multiple Traumatic Injuries
				LH	Neg.		
				LP	Neg.		
				P	Neg.		

Legend:

RH - right hand
LH - left hand
LP - lips swab
P - palate swab
M - male
F - Female

R_fs Standards: THC 0.37 Red
CBN 0.42 Purple
CBD 0.46 Orange
CBC 0.39 Purple

TABLE C-11

RESULTS OF TESTS ON SWABS FROM CORPSES USING THE CS METHOD

Subject No.	Age	Sex	Interval Between Death and Swabbing	Swab	Results	Cause of Death
2	15	F	3.5 hr	RH	Neg.	Suicide (carbon monoxide)
				LH	Neg.	
				LP	Neg. (light orange)	
				P	Neg. (light orange)	
4	16	F	5.0 hr	RH	Neg.	Unknown
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
6	22	M	5.4 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
8	39	M	8.3 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
10	35	M	18.2 hr	RH	Neg.	Heart Failure
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
12	24	M	9.6 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
14	21	M	7.3 hr	RH	Neg.	Traffic Fatality (head wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
16	34	M	18.2 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
18	21	M	20.0 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg. (orange)	
				LP	Neg.	
				P	No Sample	

TABLE C-11 (Continued)

Subject No.	Age	Sex	Interval Between Death and Swabbing	Swab	Results	Cause of Death
20	51	F	11.4 hr	RH	Neg.	Traumatic Injury from Fall
				LH	Neg.	
				LP	Neg.	
				P	No Sample	
22	19	M	8.5 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
24	29	M	22.8 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
26	49	M	17.5 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
28	17	F	6.6 hr	RH	Neg.	Epileptic Siezure
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
30	60	M	11.5 hr	RH	Neg.	Traumatic Injuries
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
32	30	M	17.0 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
34	51	M	13.4 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
36	23	M	18.4 hr	RH	Neg.	Drug Overdose?
				LH	Neg.	
				LP	Neg.	
				P	Neg.	

TABLE C-11 (Concluded)

Subject No.	Age	Sex	Interval Between Death and Swabbing	Swab	Results	Cause of Death
38	89	F	20.0 hr	RH	Neg.	Heart Failure
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
40	48	M	11.0 hr	RH	Neg.	Natural Causes
				LH	Neg.	
				LP	Neg.	
				P	Neg. (orange)	
42	33	M	Unknown	RH	Neg.	Murder (stab wounds)
				LH	Neg.	
				LP	Neg.	
				P	Neg. (orange)	
44	21	M	12.6 hr	RH	Neg.	Murder (gunshot wounds)
				LH	Neg.	
				LP	Neg. (orange)	
				P	Neg. (orange)	
46	26	M	15.5 hr	RH	Neg.	Gunshot Wounds
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
48	28	M	11.0 hr	RH	Neg.	Gunshot Wounds
				LH	Neg.	
				LP	Neg.	
				P	Neg.	
50	46	F	1.5 hr	RH	Neg. (orange)	Natural Causes
				LH	Neg. (orange)	
				LP	Neg. (orange)	
				P	No Sample	

Legend:

RH - right hand swab
LH - left hand swab
LP - lips swab
P - palate swab
M - male
F - female

TABLE C-12

ANALYTICAL RESULTS OF THE CS TEST ON 30 SUBJECTS

Subject	Elapsed Time Between Smoking and Swabbing (hr)	Before Smoking				After Smoking				Smoking Habits					Facial Features	Skin Features	Aftershave/ Cologne	Handcream Lotion	During Last 24 Hr	
		R	L	Lp	P	R	L	Lp	P	R	L	C	RC	IN					Alcohol	Marihuana
Placebo	0	-	-	+	+	-	-	+	-	X	-	X	X	M	-	N	-	-	-	X
Placebo	0	-	-	+	+	-	-	-	-	X	-	X	-	H	-	D	X	-	-	-
Placebo	0	-	-	+	-	-	-	+	-	X	-	X	X	H	-	O	X	-	-	-
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	X	M	-	O	-	-	-	X
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	X	V	M,B	N	-	-	X	X
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	-	M	M,B	O	-	-	-	-
Test	0	-	-	-	-	+	+	+	-	X	X	X	X	M	M,B	N	-	-	-	-
Test	0	-	+	-	-	-	+	+	-	-	X	X	X	H	M	D	-	-	X	-
Test	0	-	-	+	+	+	-	+	-	X	-	X	-	M	-	N	-	-	X	X
Test	0	+	-	-	-	-	-	+	-	X	X	X	X	H	M	N	-	-	X	-
Test	0	-	-	-	-	-	-	-	-	X	-	X	-	M	-	O	-	-	-	X
Test	0	-	-	-	-	+	-	+	-	X	X	X	X	H	M,B	N	-	X	-	X
Test	2	+	-	+	-	-	-	+	-	X	X	X	-	V	M	N	X	-	X	X
Test	2	-	+	-	-	-	-	-	-	X	X	X	-	H	M	N	X	X	X	-
Test	2	-	-	-	-	-	-	-	-	-	X	X	X	M	-	N	X	-	X	X
Test	2	-	+	+	-	-	-	+	-	X	-	-	-	M	M,B	O	-	-	X	X
Test	2	-	-	-	-	-	-	+	-	X	X	X	X	M	M,B	O	-	-	X	X
Test	2	-	-	-	-	+	-	-	-	X	-	X	-	M	M	N	-	-	X	-
Test	6	-	+	-	-	-	-	-	-	X	X	X	X	H	M,B	D	-	-	-	-
Test	6	-	-	-	-	-	-	-	-	X	-	X	X	V	M	N	X	-	X	-
Test	6	-	-	-	-	-	+	-	-	X	-	X	-	H	M	N	-	-	-	-
Test	6	+	-	+	-	-	-	-	-	X	-	X	X	M	M	O	-	-	-	X
Test	6	-	+	-	-	-	-	-	-	-	X	X	X	M	-	N	-	-	-	-
Test	6	-	-	-	-	+	-	-	-	X	-	X	X	M	M,B	N	-	-	X	-
Test	24	-	-	-	+	-	-	-	-	-	X	X	X	H	-	O	X	-	-	-
Test	24	-	-	-	-	-	-	-	-	X	-	X	X	M	M,B	N	X	-	-	-
Test	24	+	-	-	-	-	-	-	-	X	-	X	-	M	M,B	N	-	-	-	-
Test	24	+	-	+	-	-	+	-	-	X	-	X	X	M	-	N	-	-	-	X
Test	24	+	-	-	-	+	-	-	-	-	X	-	X	M	-	N	-	X	-	-
Test	24	+	-	-	-	-	-	-	-	X	X	X	X	H	M	N	-	-	X	-

Legend:

Analytical Results:
+ = positive result
- = negative result
R = right hand
L = left hand
Lp = lips
P = palate

Facial Features:
M = mustache
B = beard

Skin Features:
N = normal
D = dry
O = oily

Smoking Habits:
L = light
M = medium
H = heavy
V = variable
R = right hand
L = left hand
C = smoked completely (down to butt)
RC = used roach clip
IN = type of inhalations

TABLE C-13

ANALYTICAL RESULTS OF THE TLC TEST ON 60 SUBJECTS

Subject	Elapsed Time Between Smoking and Swabbing (hr)	Analytical Results: Before Smoking R	L	Lp	P	After Smoking R	L	Lp	P	Smoking Habits: R	L	C	RC	IN	Facial Features	Skin Features	Aftershave/ Cologne	Handcream/ Lotion	During Last 24 Hr: Alcohol	Marihuana
Placebo	0	++	-	-	-	-	-	-	-	X	X	X	X	H	M,B	N	X	-	-	-
Placebo	0	++	++	-	-	-	-	-	-	-	X	X	X	M	M	N	-	-	-	X
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	X	M	-	N	-	-	-	-
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	X	V	-	0	X	-	X	-
Placebo	0	-	-	-	-	-	-	-	-	X	X	-	-	M	-	N	X	X	-	-
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	X	M	M,B	0	-	-	-	-
Placebo	0	-	-	-	-	-	-	-	-	X	X	X	-	M	M,B	N	X	-	X	-
Placebo	0	-	-	-	-	-	-	-	-	X	X	X	-	V	M	N	-	-	X	X
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	X	H	-	D	X	-	-	-
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	-	M	M	N	-	-	X	-
Placebo	0	-	-	-	-	-	-	-	-	X	-	X	-	M	M,B	0	-	-	-	-
Placebo	0	-	-	-	-	-	-	-	-	X	X	X	X	V	-	N	-	-	-	-
Test	0	-	-	-	-	-	-	+-	++	-	X	X	-	H	-	D	-	-	X	X
Test	0	-	-	-	-	++	++	-	-	X	-	X	X	M	-	D	X	-	X	-
Test	0	-	-	-	-	++	++	++	-	X	-	X	-	H	M,B	0	-	-	-	-
Test	0	-	-	-	-	-	-	++	-	-	X	X	X	H	M	N	-	-	-	-
Test	0	-	-	-	-	++	-	++	-	X	-	X	X	M	M	0	-	-	X	-
Test	0	++	-	-	-	++	++	++	-	-	X	X	X	M	M	N	-	-	-	X
Test	0	-	-	-	-	-	-	+-	-	X	X	X	X	H	M	N	-	-	X	-
Test	0	-	-	-	-	+-	-	-	-	X	-	X	X	M	M,B	0	-	-	-	-
Test	0	-	-	-	-	++	-	++	-	X	-	X	X	M	M	0	-	-	X	-
Test	0	++	-	-	-	++	++	+-	-	X	X	X	X	H	M,B	N	X	-	-	-
Test	0	-	-	-	-	++	-	++	-	X	X	X	-	V	M	N	-	-	X	X
Test	0	-	-	-	-	-	++	++	-	X	X	X	-	M	M,B	N	X	-	X	-
Test	2	-	-	-	-	-	++	-	-	X	X	X	X	V	M	0	X	X	X	X
Test	2	-	-	++	-	-	++	++	-	X	X	X	X	M	M,B	N	-	X	-	X
Test	2	-	-	-	-	++	-	-	-	X	-	X	X	M	M	N	-	X	-	X
Test	2	-	-	-	-	-	-	-	-	-	X	X	-	H	-	N	X	-	-	-
Test	2	-	-	-	-	-	-	-	-	X	-	X	-	H	M	D	-	-	X	-
Test	2	-	++	-	-	-	++	-	-	X	X	X	X	H	M,B	D	-	X	-	X
Test	2	-	-	-	-	-	-	-	-	X	X	X	X	L	M	N	X	X	-	-
Test	2	-	-	-	-	++	-	++	-	X	-	X	X	V	M	0	X	-	X	X
Test	2	-	-	-	-	-	-	++	-	X	X	X	X	M	M	0	-	-	-	X
Test	2	-	-	-	-	-	-	+-	-	X	-	X	-	M	M,B	0	-	-	-	-
Test	2	-	-	-	-	-	-	++	-	X	-	X	X	M	M,B	N	-	-	-	-
Test	2	-	-	-	-	-	-	-	-	X	-	X	X	M	M,B	0	-	X	-	X

TABLE C-13 (Concluded)

Subject	Elapsed Time Between Smoking and Swabbing (hr)	Analytical Results: Before Smoking R	L	Lp	P	After Smoking R	L	Lp	P	Smoking Habits R	L	C	RC	IN	Facial Features	Skin Features	Aftershave/ Cologne	Handcream Lotion	During Last 24 Hr: Alcohol	Marihuana
Test	6	-	-	-	-	-	-	-	-	X	X	X	X	M	M,B	N	-	-	X	-
Test	6	-	-	-	-	-	-	-	-	X	-	X	-	V	M	D	X	-	X	-
Test	6	-	-	-	-	-	-	-	-	-	X	X	X	M	M	N	-	-	-	-
Test	6	++	-	++	-	-	-	++	-	X	-	X	X	H	M	D	X	-	X	X
Test	6	-	-	-	-	-	-	-	-	X	-	X	-	V	M	N	-	-	X	-
Test	6	-	-	-	-	-	-	-	-	-	X	X	-	V	-	D	X	-	X	-
Test	6	-	-	-	-	-	-	-	-	X	-	X	X	H	-	N	-	-	X	X
Test	6	-	-	-	-	-	-	-	-	X	-	X	X	M	-	N	-	-	-	-
Test	6	-	-	-	-	-	-	-	-	X	X	X	X	M	M	O	X	-	X	-
Test	6	-	-	-	-	-	-	-	-	X	-	X	X	H	M	N	X	-	X	-
Test	6	-	-	-	-	-	-	-	-	X	-	X	-	M	-	O	-	-	X	X
Test	6	-	-	-	-	-	-	-	-	X	X	X	X	V	-	N	-	-	-	-
Test	24	-	-	-	-	-	-	-	-	X	-	X	X	M	M,B	N	-	-	-	-
Test	24	-	-	-	-	-	-	-	-	X	X	X	X	M	M,B	N	X	-	-	-
Test	24	++	++	++	-	-	-	-	-	X	X	X	X	M	-	N	-	-	X	-
Test	24	-	-	-	-	-	-	-	-	-	X	X	-	H	M	D	X	-	-	X
Test	24	-	-	-	-	-	-	-	-	-	X	X	X	M	M,B	N	-	-	X	-
Test	24	-	-	-	-	-	++	+-	-	-	X	-	-	H	-	N	X	-	X	-
Test	24	-	-	-	-	-	-	-	-	X	-	X	X	M	M	N	X	-	-	X
Test	24	-	-	-	-	-	-	-	-	-	X	X	X	M	-	N	-	-	-	-
Test	24	-	-	-	-	-	-	-	-	X	-	X	X	H	M	N	X	-	-	X
Test	24	-	-	-	-	-	-	-	-	X	-	X	-	M	M,B	O	-	-	-	-
Test	24	-	-	-	-	-	-	-	-	X	-	X	-	H	M	N	-	X	-	-
Test	24	-	-	-	-	-	-	-	-	X	-	-	-	H	M,B	N	-	-	X	-

Legend:

Analytical Results:
- ++ = positive results on both TLC plates
- +- = positive result on first TLC plate
- - = negative result on first TLC plate
- R = right hand
- L = left hand
- Lp = lips
- P = palate

Facial Features:
- M = mustache
- B = beard

Smoking Habits:
- L = light
- M = medium
- H = heavy
- V = variable
- R = right hand
- L = left hand
- C = smoked completely (down to butt)
- RC = roach clip used
- IN = type of inhalations

Skin Features:
- N = normal
- D = dry
- O = oily

APPENDIX D

VALIDATION STUDY PROCEDURES

Figure D-1 - Validation Study Outline
Figure D-2 - Swabbing Instructions

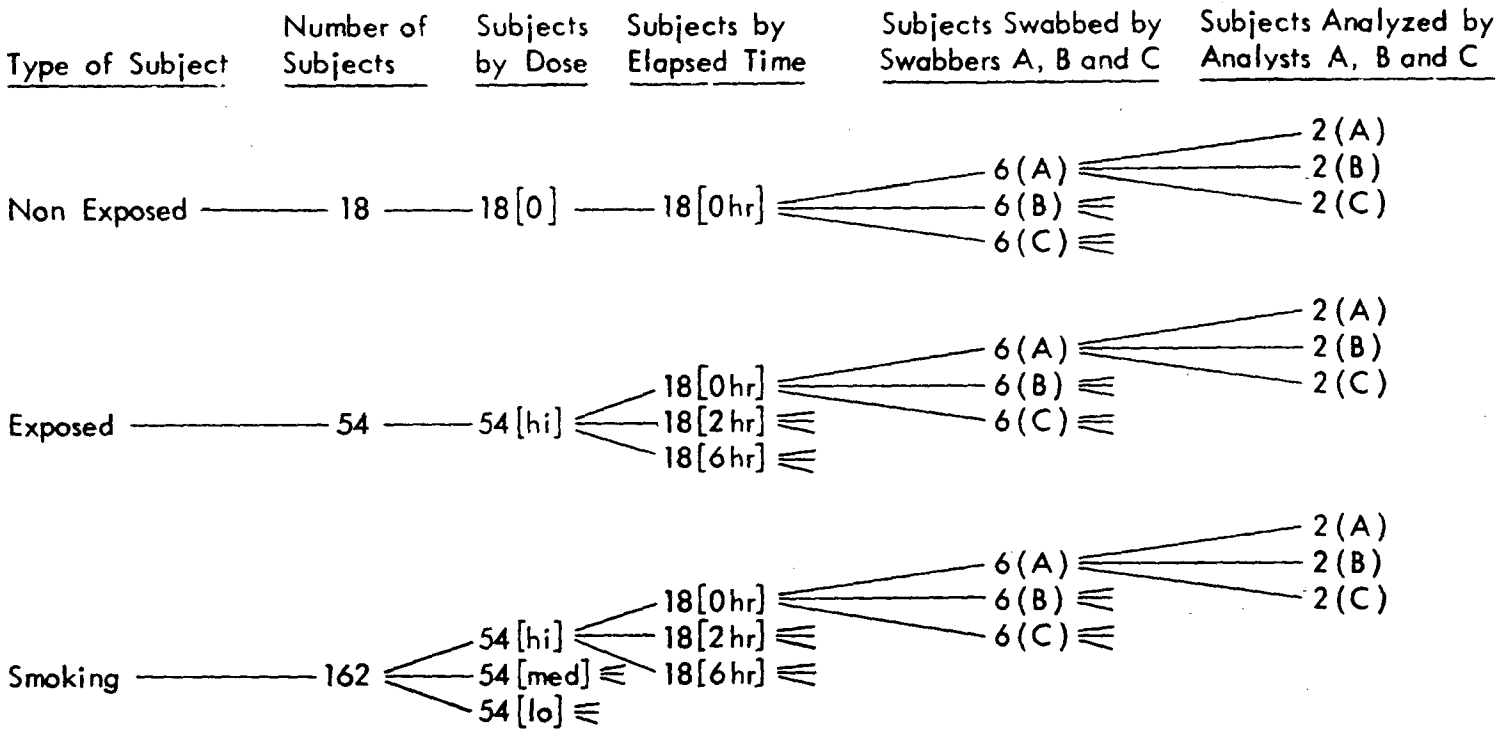

Figure D-1 - Validation Study Outline

SWABBING INSTRUCTIONS

Swab 1. Dip the swab in the 70% alcohol solution. Press the swab gently against the neck of the alcohol vessel to remove excess (dripping) alcohol. Swab the lips with a hard rolling motion combined with a scrubbing action. Swab the fleshy part of the upper and lower lip and the facial skin immediately adjacent to the lips.

Swab 2. Dip the swab in the 70% alcohol solution. Press the swab gently against the neck of the alcohol vessel to remove excess (dripping) alcohol. Swab (scrub) the thumb and fingers of the right hand from the tips down to the first joint. Be sure to scrub all the way around the digits, front, sides, and back (nail).

Swab 3. Same as Swab No. 2, except swab the left hand.

Place the swabs in separate glass tubes (coded and labeled) and screw caps on firmly. Store in refrigerator.

Figure D-2 - Swabbing Instructions

APPENDIX E

VALIDATION STUDY RESULTS

LEGEND FOR TABLES E-1 THROUGH E-9

Analytical Results

++ = positive results on both TLC plates
+- = positive results on first TLC plate
\- = negative results on first TLC plate
* = results unavailable
R = right hand
L = left hand
Lp = lips
P = palate

Smoking Habits

L = light
M = medium
H = heavy
V = variable
R = right hand
L = left hand
C = smoked completely (down to butt)
RC = roach clip used
IN = type of inhalations

Facial Features

M = mustache
B = beard

Skin Features

N = normal
D = dry
O = oily

TABLE E-1

SWABBER A, ANALYST A

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: H	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream/ Lotion	During Previous 24 Hr: Alcohol	During Previous 24 Hr: Marihuana
224	6/9/75	High	0	-	-	+-	++	++	++	-	X	-	X	M	M,B	O	X	-	X	X
245	6/11/75	High	0	-	-	-	++	++	++	X	X	-	-	H	-	N	-	-	-	-
148	6/9/75	High	1	-	-	-	++	++	++	-	X	-	X	M	-	N	-	X	-	-
247	6/10/75	High	1	-	-	-	++	-	-	X	X	-	X	M	M	N	-	-	-	-
309	6/14/75	High	3	-	-	-	-	-	-	X	-	-	X	M	M	N	-	-	-	X
333	6/15/75	High	3	-	-	-	+-	+-	-	X	X	-	X	M	-	N	-	-	X	-
177	6/9/75	Medium	0	+-	-	-	++	++	++	X	X	-	X	V	M	N	-	-	-	X
246	6/12/75	Medium	0	-	-	-	++	++	++	X	-	X	-	M	M	N	-	X	-	-
186	6/9/75	Medium	1	-	-	-	-	-	+-	X	-	X	X	H	-	D	-	-	X	-
133	6/12/75	Medium	1	-	-	-	-	-	-	-	X	X	X	M	M	N	X	-	-	-
246	6/18/75	Medium	3	-	-	-	+-	+-	-	X	-	X	-	V	M	N	-	X	-	-
177	6/18/75	Medium	3	+-	+-	+-	+-	+-	+-	-	X	X	X	M	M	N	X	-	-	X
242	6/9/75	Low	0	++	++	-	+-	+-	-	X	-	X	X	H	-	O	-	-	X	X
318	6/12/75	Low	0	-	-	-	++	++	-	-	X	X	X	H	-	N	-	-	-	-
001	6/9/75	Low	1	++	++	+-	++	+-	++	X	-	X	X	V	-	N	-	-	-	X
217	6/10/75	Low	1	-	-	-	-	+-	-	X	-	X	X	M	M	N	X	-	X	X
308	6/14/75	Low	3	-	-	-	-	-	-	X	X	X	-	M	-	N	X	-	X	X
154	6/15/75	Low	3	-	-	-	-	-	-	X	-	X	X	M	-	N	-	-	-	-
301	6/11/75	Exposed	0	-	-	-	-	-	-						-	N	-	-	-	-
305	6/11/75	Exposed	0	-	-	-	-	-	-						-	N	X	X	-	X
184	6/11/75	Exposed	1	-	-	-	-	-	-						M,B	N	-	-	-	X
242	6/12/75	Exposed	1	+-	+-	-	+-	+-	-						-	N	-	-	X	X
114	6/14/75	Exposed	3	-	-	-	-	-	-						M	N	X	-	-	-
202	6/15/75	Exposed	3	-	-	-	-	-	-						B	N	-	-	-	X
C1	6/19/75	Control	-	-	-	-									M	D	-	-	-	-
C10	6/19/75	Control	-	-	-	-									-	N	X	-	X	-

TABLE E-2

SWABBER A, ANALYST B

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream/ Lotion	During Previous 24 Hr: Alcohol	During Previous 24 Hr: Marihuana
207	6/10/75	High	0	-	-	-	+-	-	++	X	X	-	X	M	M,B	N	X	-	X	X
248	6/12/75	High	0	-	-	-	++	++	++	X	X	-	X	M	-	N	-	-	-	X
106	6/10/75	High	1	-	-	+-	-	-	-	X	X	-	X	M	M,B	N	-	-	X	X
169	6/11/75	High	1	-	-	-	-	-	-	X	-	-	X	M	M	N	-	X	-	-
116	6/14/74	High	3	-	-	-	-	-	-	X	X	-	X	V	-	N	-	-	X	X
151	6/15/75	High	3	-	-	-	-	-	-	X	-	-	X	M	M	N	-	-	-	X
240	6/9/75	Medium	0	-	-	-	++	++	++	X	X	-	X	H	M,B	D	X	-	X	X
143	6/9/75	Medium	0	-	-	-	-	+-	-	-	X	-	X	V	-	N	X	-	X	-
151	6/9/75	Medium	1	-	-	-	-	-	++	X	-	X	X	V	M	O	-	-	X	-
130	6/10/75	Medium	1	-	-	-	-	-	++	-	X	X	X	M	-	N	X	-	-	-
133	6/14/75	Medium	3	-	-	-	-	-	-	-	X	X	X	M	M	N	X	-	X	X
176	6/14/75	Medium	3	-	-	-	+-	-	-	X	-	X	X	M	M	N	-	X	-	-
313	6/12/75	Low	0	-	-	-	-	++	++	X	-	X	X	H	-	N	-	-	-	-
149	6/12/75	Low	0	-	-	-	-	-	++	X	-	X	-	M	M	N	-	-	X	-
115	6/11/75	Low	1	-	-	-	+-	-	+-	-	X	X	X	M	M	N	X	-	-	-
155	6/11/75	Low	1	-	-	-	-	-	-	X	-	X	X	M	M,B	N	-	-	-	-
134	6/15/75	Low	3	-	-	-	-	-	-	X	-	X	X	M	M	N	X	-	X	-
331	6/18/75	Low	3	-	-	-	+-	+-	+-	X	X	X	X	M	M	N	-	-	X	-
145	6/9/75	Exposed	0	-	-	-	-	-	-						M	O	-	-	-	X
254	6/10/75	Exposed	0	-	-	-	-	-	-						M	N	-	-	X	-
308	6/12/75	Exposed	1	-	-	-	-	-	-						-	N	-	-	-	-
213	6/12/75	Exposed	1	-	+-	-	-	-	-						M,B	N	X	-	-	-
115	6/14/75	Exposed	3	-	-	-	-	-	-						M	N	X	-	X	X
174	6/14/75	Exposed	3	-	-	-	-	-	-						M	N	-	-	X	X
C2	6/19/75	Control	-	-	-	-	-								M,B	N	-	-	X	-
C11	6/19/75	Control	-	-	-	-									-	O	X	-	X	-

TABLE E-3

SWABBER A, ANALYST C

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	LN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream/ Lotion	During Previous 24 Hr: Alcohol	Marihuana
127	6/9/75	High	0	-	-	-	-	-	-	X	X	-	X	M	M,B	N	-	-	X	X
260	6/10/75	High	0	+	*	-	-	-	-	-	X	-	X	M	M	N	-	-	-	-
300	6/11/75	High	1	-	-	-	-	-	-	X	-	-	-	M	M	N	X	-	-	-
105	6/12/75	High	1	-	-	-	-	-	-	X	X	-	X	M	-	O	X	-	X	-
123	6/15/75	High	3	-	-	-	-	-	-	X	-	-	X	M	M	D	-	-	X	X
329	6/15/75	High	3	-	-	-	-	-	-	X	X	-	X	M	M	N	-	-	X	-
210	6/11/75	Medium	0	-	-	-	++	++	++	X	-	X	X	M	M,B	N	-	-	X	X
254	6/12/75	Medium	0	-	-	-	+	++	+	X	X	X	X	V	M	N	-	-	X	-
238	6/11/75	Medium	1	-	-	-	-	-	-	X	-	X	X	V	M	N	-	-	-	X
001	6/12/75	Medium	1	-	-	-	-	-	-	X	X	X	X	M	-	N	-	-	X	-
326	6/18/75	Medium	3	*	*	-	*	-	*	-	X	X	-	V	M	N	-	-	-	X
322	6/18/75	Medium	3	-	-	-	-	-	-	X	X	X	X	M	M	O	X	-	-	-
154	6/9/75	Low	0	-	-	-	++	+	++	X	-	-	X	V	-	N	-	-	X	-
209	6/10/75	Low	0	-	-	-	++	-	-	X	-	X	X	M	M	N	X	-	-	-
250	6/10/75	Low	1	*	*	*	*	-	*	X	-	X	X	H	-	N	-	-	X	X
114	6/12/75	Low	1	-	-	-	-	-	-	X	-	X	X	M	M	N	X	-	-	-
303	6/15/75	Low	3	-	-	-	-	-	-	X	-	X	X	M	M	N	-	-	-	-
169	6/18/75	Low	3	-	-	-	-	-	-	X	-	X	X	V	M	N	-	X	X	X
116	6/9/75	Exposed	0	-	-	-	-	-	-						-	N	-	-	X	X
257	6/10/75	Exposed	0	*	-	*	*	*	*						-	N	X	-	-	-
175	6/10/75	Exposed	1	-	-	-	-	-	-						M	O	-	-	X	X
212	6/12/75	Exposed	1	*	-	-	-	-	-						M	N	X	-	X	-
238	6/15/75	Exposed	3	-	-	-	-	-	-						M	N	-	-	X	X
200	6/14/75	Exposed	3	-	-	-	-	-	*						-	D	-	X	X	X
C12	6/19/75	Control	-	-	-	*									-	N	-	-	X	-
C3	6/19/75	Control	-	-	-	-									M	N	X	-	-	-

TABLE E-4

SWABBER B, ANALYST A

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream Lotion	During Previous 24 Hr: Alcohol	Marihuana
258	6/10/75	High	0	-	-	-	++	++	++	-	X	-	X	M	-	N	X	X	X	-
244	6/11/75	High	0	-	-	++	++	++	++	X	-	-	X	M	-	N	-	-	-	X
105	6/10/75	High	1	-	-	-	-	-	-	X	X	-	X	M	M	N	-	-	X	-
177	6/11/75	High	1	-	-	-	++	++	++	X	X	-	X	M	M	N	-	-	X	-
301	6/14/75	High	3	++	++	-	++	+-	-	X	-	-	X	V	-	N	-	-	-	X
305	6/14/75	High	3	-	-	-	-	-	-	-	X	-	X	M	-	O	X	-	X	X
211	6/11/75	Medium	0	-	-	-	++	++	++	X	-	X	X	M	M	O	-	-	X	X
316	6/12/75	Medium	0	+-	-	+-	++	-	++	X	-	X	X	M	B	N	-	-	X	-
126	6/9/75	Medium	1	-	-	-	++	++	++	X	-	X	X	H	-	O	X	-	X	X
133	6/10/75	Medium	1	-	-	-	-	+-	-	X	X	X	X	V	M	N	X	-	-	X
175	6/14/75	Medium	3	-	-	-	-	-	-	X	X	X	X	M	M	O	-	X	X	X
143	6/14/75	Medium	3	+-	+-	-	+-	++	-	X	-	X	X	L	-	N	X	-	X	-
244	6/9/75	Low	0	++	++	++	++	++	++	-	X	X	X	V	-	N	-	-	-	X
152	6/11/75	Low	0	-	-	-	-	-	++	X	X	X	X	M	M,B	N	-	-	X	-
252	6/10/75	Low	1	-	-	-	++	++	++	X	X	X	X	V	-	O	X	-	X	-
195	6/11/75	Low	1	++	-	-	++	++	++	-	X	X	X	M	M	N	-	-	X	-
341	6/18/75	Low	3	-	-	-	-	-	-	X	X	X	X	H	M,B	N	-	-	-	-
353	6/18/75	Low	3	-	-	-	+-	+-	+-	X	X	X	-	V	M	N	-	-	-	-
169	6/9/75	Exposed	0	-	-	-	-	-	-						M	N	-	X	-	-
303	6/11/75	Exposed	0	-	-	-	-	-	-						M	N	-	-	X	-
176	6/10/75	Exposed	1	-	-	-	-	-	-						M	N	-	-	X	-
224	6/11/75	Exposed	1	+-	-	-	+-	-	++						M,B	O	X	X	X	X
211	6/15/75	Exposed	3	-	-	-	-	-	-						M	O	-	X	-	X
327	6/15/75	Exposed	3	-	-	-	-	-	-						-	N	-	-	-	X
C13	6/19/75	Control	-	-	-	-									-	D	X	X	X	-
C4	6/19/75	Control	-	-	-	-									-	N	X	-	-	-

TABLE E-5

SWABBER B, ANALYST B

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave Cologne	Handcream Lotion	During Previous 24 Hr: Alcohol	Marihuana
205	6/10/75	High	0	-	-	-	+-	-	+-	X	X	-	X	H	M	N	X	-	-	-
186	6/11/75	High	0	-	-	-	++	+-	+-	X	-	-	X	M	-	N	X	-	-	-
184	6/10/75	High	1	-	-	+-	++	+-	+-	X	X	-	X	M	M,B	N	-	-	-	X
106	6/12/75	High	1	-	-	++	++	++	+-	X	X	-	X	M	M,B	N	-	-	X	X
330	6/15/75	High	3	-	-	-	-	-	+-	-	X	-	-	M	M,B	N	-	-	X	-
184	6/14/75	High	3		-	-	-	-	+-	X	-	-	X	M	M,B	N	-	X	X	X
241	6/9 75	Medium	0	-	-	-	+-	+-	+-	X	-	-	X	H	M,B	N	X	-	-	X
232	6/9/75	Medium	0	-	-	-	-	+-	+-	X	-	X	X	H	-	N	-	-	-	-
221	6/10/75	Medium	1	-	-	++	-	-	+-	X	X	X	X	V	-	N	-	-	X	X
167	6/11/75	Medium	1	-	-	+-	-	-	+-	X	X	X	X	M	M,B	N	-	X	X	X
329	6/18/75	Medium	3	-	-	-	-	-	+-	X	X	X	-	M	M	N	-	-	-	-
321	6/18/75	Medium	3	-	-	-	-	-	+-	X	X	X	-	M	-	N	-	-	-	-
174	6/10/75	Low	0	-	-	-	++	+-	+-	X	-	X	X	M	M	N	-	-	X	-
312	6/12/75	Low	0	-	-	-	+-	-	+-	X	-	X	X	M	B	N	-	-	X	-
238	6/9/75	Low	1	-	-	-	-	-	+-	X	-	X	X	M	M	N	-	-	-	X
124	6/12/75	Low	1	-	-	++	-	-	+-	-	X	X	X	M	M,B	N	X	-	X	X
340	6/18/75	Low	3	-	-	-	+-	-	-	X	-	X	X	V	-	O	-	-	-	X
327	1/18/75	Low	3	-	-	-	++	-	-	X	-	X	X	V	-	N	-	-	-	X
246	6/10/75	Exposed	0	-	-	-	-		-						M	N	-	X	-	-
344	6/14/75	Exposed	0	-	-	-	-	-	-						M	N	-	-	X	-
134	6/10/75	Exposed	1	-	-	-	-	-	-						M	N	X	-	-	-
113	6/11/75	Exposed	1	-	+-	-	-	-	+-						-	N	X	X	X	-
224	6/15/75	Exposed	3	-	-	-	-	-	-						M,B	O	X	-	X	X
331	6/15/75	Exposed	3	-	-	-	-	-	-						M	N	-	-	-	-
C5	6/19/75	Control	-	-	-	-									-	N	-	-	-	-
C14	6/19/75	Control	-	-	-	-									M	N	X	-	X	-

TABLE E-6

SWABBER B, ANALYST C

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave Cologne	Handcream/ Lotion	During Previous 24 Hr: Alcohol	Marihuana
220	6/9/75	High	0	-	-	-	-	-	-	X	-	-	X	H	M	N	-	-	-	-
239	6/9/75	High	0	-	-	-	-	++	++	X	-	-	X	M	-	N	X	-	-	-
112	6/9/75	High	1	*	-	-	-	*	-	X	X	-	X	M	M	N	X	-	X	-
143	6/12/75	High	1	-	-	-	+-	-	-	X	-	-	X	M	-	N	X	-	X	-
161	6/15/75	High	3	-	-	-	-	-	-	-	X	-	-	M	M	N	-	-	-	X
332	6/15/75	High	3	*	-	*	*	-	-	X	-	-	X	M	M,B	N	-	-	X	-
257	6/12/75	Medium	0	-	-	-	++	+-	++	X	X	X	X	M	M	N	-	-	-	-
209	6/14/75	Medium	0	-	-	*	++	-	++	X	-	X	X	M	M	N	-	-	X	X
131	6/12/75	Medium	1	-	-	-	-	-	++	X	X	X	X	V	M	N	-	-	X	X
221	6/12/75	Medium	1	++	-	++	+-	+-	++	X	X	X	X	M	-	N	X	-	-	X
336	6/15/75	Medium	3	-	-	-	-	-	-	-	X	X	X	M	M,B	N	-	-	-	X
227	6/18/75	Medium	3	-	-	-	-	-	-	-	X	X	-	V	M	D	-	-	-	-
139	6/12/75	Low	0	-	-	-	++	++	++	X	X	X	X	M	M,B	N	-	-	-	X
124	6/14/75	Low	0	-	-	-	-	++	-	-	X	X	X	M	M	N	X	-	X	X
251	6/10/75	Low	1	*	*	*	*	*	-	X	-	X	X	M	-	N	-	-	-	-
175	6/12/75	Low	1	-	-	-	*	*	-	X	X	X	X	V	M	N	-	X	-	X
106	6/15/75	Low	3	-	-	-	-	-	-	X	-	X	X	H	M,B	N	-	-	X	-
337	6/15/75	Low	3	-	-	-	-	-	-	X	-	X	X	H	M,B	O	X	-	-	-
255	6/10/75	Exposed	0	-	-	-	-	-	-						-	N	X	-	X	-
345	6/14/75	Exposed	0	-	-	-	-	-	-						M	N	-	-	X	-
227	6/11/75	Exposed	1	-	-	++	-	-	-						M	N	-	-	-	-
139	6/15/75	Exposed	1	-	-	-	-	-	-						M,B	N	-	-	-	X
107	6/15/75	Exposed	3	-	-	-	-	-	-						M,B	N	-	-	X	-
237	6/15/75	Exposed	3	*	*	*	-	-	*						-	D	-	X	X	-
C15	6/19/75	Control	-	-	-	*									-	D	-	-	-	-
C6	6/19/75	Control	-	-	-	-									M	N	X	-	X	-

TABLE E-7

SWABBER C, ANALYST A

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream Lotion	During Previous 24 Hr: Alcohol	During Previous 24 Hr: Marihuana
304	6/11/75	High	0	-	-	-	++	++	++	X	X	-	X	M	-	D	-	-	-	-
302	6/11/75	High	0	-	-	-	++	++	++	X	X	-	-	H	M	D	X	-	X	-
146	6/10/75	High	1	-	-	-	++	-	++	X	X	-	X	M	M	N	-	-	-	-
134	6/12/75	High	1	-	-	-	++	++	++	X	-	-	X	M	M	N	X	-	-	-
174	6/15/75	High	3	-	-	-	-	-	-	X	-	-	-	M	M	N	-	-	X	X
334	6/15/75	High	3	-	-	-	-	+-	++	X	X	-	X	M	B	N	-	-	X	X
236	6/9/75	Medium	0	-	-	-	-	-	++	X	X	-	X	H	-	N	X	-	X	X
152	6/9/75	Medium	0	-	-	-	-	-	++	-	X	-	X	H	M,B	N	-	-	-	-
193	6/9/75	Medium	1	-	-	-	-	-	-	X	X	X	X	V	-	D	-	-	X	X
109	6/12/75	Medium	1	-	-	-	+-	++	++	X	X	X	X	M	-	N	-	-	-	-
338	6/15/75	Medium	3	-	-	-	-	-	+-	-	X	X	X	M	M	N	-	-	-	-
323	6/18/75	Medium	3	-	-	-	+-	+-	-	X	X	X	X	V	M	N	X	-	X	-
245	6/9/75	Low	0	-	-	-	++	++	++	X	-	-	-	H	-	N	-	-	X	-
310	6/12/75	Low	0	-	-	-	-	-	-	X	X	X	-	V	M	N	X	-	X	X
126	6/12/75	Low	1	-	+-	-	-	-	-	X	-	X	X	V	M,B	N	-	-	X	X
233	6/12/75	Low	1	-	-	-	-	-	-	X	-	X	X	M	-	N	X	-	X	-
152	6/15/75	Low	3	-	-	-	-	-	-	X	-	X	-	M	M,B	N	X	-	-	-
352	6/18/75	Low	3	-	-	-	-	-	+-	X	-	X	-	V	-	N	X	X	X	-
256	6/10/75	Exposed	0	-	-	-	-	-	-						M	N	X	-	X	X
145	6/11/75	Exposed	0	-	-	-	-	++	-						M	O	-	-	X	X
248	6/10/75	Exposed	1	-	-	-	-	-	-						-	N	-	-	X	X
234	6/11/75	Exposed	1	-	-	-	-	-	-						-	N	X	-	-	-
169	6/15/75	Exposed	3	-	-	-	-	-	-						M	N	-	-	-	-
105	6/14/75	Exposed	3	-	-	-	-	-	-						-	O	X	-	X	-
C7	6/19/75	Control	-	-	-	-									M,B	N	-	-	X	-
C16	6/19/75	Control	-	-	-	-									M	O	X	-	X	-

TABLE E-8

SWABBER C, ANALYST B

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream/ Lotion	During Previous 24 Hr: Alcohol	Marihuana
259	6/10/75	High	0	-	-	-	++	++	-	X	X	-	X	H	-	N	X	-	X	X
147	6/12/75	High	0	-	-	-	++	-	+-	X	-	-	-	M	M,B	N	-	-	X	-
220	6/11/75	High	1	-	-	-	-	-	-	X	-	-	X	M	M	N	-	-	-	X
214	6/12/75	High	1	-	-	-	-	-	-	X	-	-	X	H	M	N	-	-	-	X
335	6/15/75	High	3	-	-	-	-	-	++	X	X	-	X	H	B	N	-	-	-	X
200	6/15/75	High	3	-	-	-	+-	+-	-	-	X	-	X	M	-	D	-	X	-	X
154	6/11/75	Medium	0	-	-	-	++	-	++	X	X	X	X	M	-	N	-	-	-	-
315	6/12/75	Medium	0	-	-	++	-	++	++	X	X	X	X	M	M,B	N	-	X	X	X
124	6/10/75	Medium	1	-	-	-	-	-	+-	-	X	X	X	V	M	N	X	-	-	-
233	6/10/75	Medium	1	-	-	-	-	-	++	X	X	X	X	V	-	O	X	-	X	-
155	6/18/75	Medium	3	-	-	-	-	-	-	X	-	X	X	M	M,B	N	-	-	-	-
324	6/18/75	Medium	3	-	-	-	-	-	-	-	X	-	X	V	M	N	-	-	-	-
243	6/9/75	Low	0	-	-	++	+-	-	++	-	X	X	X	H	-	O	-	-	X	X
208	6/10/75	Low	0	-	-	-	-	-	-	-	X	-	X	V	-	N	X	-	X	-
155	6/9/75	Low	1	-	-	-	-	-	-	-	X	X	X	M	M,B	N	-	-	-	-
176	6/12/75	Low	1	-	-	-	-	-	++	X	X	X	X	V	M	N	X	X	X	-
202	6/18/75	Low	3	-	-	-	-	-	-	X	-	X	X	V	M,B	N	-	-	-	X
224	6/18/75	Low	3	-	-	-	-	-	+-	X	X	X	-	V	M,B	O	X	-	-	X
131	6/9/75	Exposed	0	-	-	-	-	-	-						M,B	N	-	-	X	-
120	6/9/75	Exposed	0	-	-	-	-	-	+-						M	N	X	-	X	X
162	6/11/75	Exposed	1	-	-	-	-	-	-						M,B	N	-	-	-	-
344	6/15/75	Exposed	1	-	-	-	-	-	-						M	N	-	-	-	-
319	6/15/75	Exposed	3	-	-	-	-	-	-						M	N	-	-	X	-
161	6/14/75	Exposed	3	-	-	-	-	-	-						M	D	X	-	X	X
C8	6/19/75	Control	-	-	-	-									-	N	X	-	X	-
C17	6/19/75	Control	-	-	-	-									M	D	-	-	-	-

TABLE E-9

SWABBER C, ANALYST C

Subject Code	Experiment Date	Dose	Elapsed Time (hr)	Analytical Results: Before Smoking R	Before Smoking L	Before Smoking Lp	After Smoking R	After Smoking L	After Smoking Lp	Smoking Habits: R	L	C	RC	IN	Facial Feature	Skin Feature	Aftershave/ Cologne	Handcream- Lotion	During Previous 24 Hr: Alcohol	Marihuana
161	6/9/75	High	0	-	-	-	-	-	-	X	X	-	X	M	M	D	-	-	X	-
206	6/10/75	High	0	-	-	-	-	-	++	-	X	-	X	M	M	N	X	-	X	X
002	6/11/75	High	1	*	*	-	-	*	+-	X	-	-	X	M	M	N	X	X	-	-
215	6/12/75	High	1	*	*	-	-	-	*	X	-	-	X	H	M,B	N	-	X	-	-
328	6/15/75	High	3	-	-	-	-	-	-	-	X	-	X	M	M	N	X	-	X	X
222	6/14/75	High	3	-	-	-	-	-	-	X	-	-	X	H	B	N	-	-	X	-
115	6/9/75	Medium	0	-	-	-	-	-	++	X	-	X	-	M	M	N	X	-	-	-
317	6/12/75	Medium	0	-	-	-	-	-	-	X	X	X	X	V	M	N	X	X	X	-
116	6/11/75	Medium	1	-	-	-	*	*	*	X	-	X	X	M	-	N	-	-	X	X
151	6/11/75	Medium	1	-	-	-	-	-	++	X	-	X	X	H	M	N	X	-	X	-
350	6/18/75	Medium	3	-	-	-	-	-	-	X	-	X	-	H	M	N	X	-	-	-
220	6/18/75	Medium	3	-	-	-	-	-	-	X	-	X	-	V	M	N	-	-	-	X
314	6/12/75	Low	0	*	*	*	-	*	-	X	X	X	X	H	M,B	N	-	-	X	-
311	6/12/75	Low	0	-	-	-	-	-	++	X	X	X	-	V	M	O	-	-	X	X
187	6/9/75	Low	1	*	*	*	*	*	*	X	X	X	X	M	M	N	-	-	X	X
249	6/10/75	Low	1	-	*	-	*	*	*	X	X	X	X	M	-	N	-	-	-	-
335	6/18/75	Low	3	-	-	-	-	-	-	X	-	X	X	V	-	N	-	-	-	-
334	6/18/75	Low	3	*	*	*	*	-	*	X	X	X	X	V	M,B	N	-	-	X	-
167	6/10/75	Exposed	0	-	-	-	-	-	-						M,B	N	-	X	X	X
253	6/10/75	Exposed	0	-	-	-	-	-	-						-	N	-	-	X	-
309	6/11/75	Exposed	1	*	*	*	*	*	*						M	N	-	-	X	X
232	6/11/75	Exposed	1	-	-	-	-	-	-						-	N	-	-	-	X
226	6/15/75	Exposed	3	-	-	-	-	-	-						B	N	X	-	X	-
210	6/14/75	Exposed	3	-	-	-	-	-	-						M,B	N	-	-	X	X
C9	6/19/75	Control	-	-	-	-									-	N	X	-	X	-
C18	6/19/75	Control	-	-	-	-									-	N	X	-	X	-