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# Measurement of $\Delta^9$-Tetrahydrocannabinol (THC) in Whole Blood Samples from Impaired Motorists

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**ABSTRACT:** The major psychoactive cannabinoid in marihuana, $\Delta^9$-tetrahydrocannabinol (THC) was measured in 1792 randomly selected blood specimens from erratic motorists arrested for impairment who submitted to blood alcohol sampling. Of these specimens, 14.4% were positive for THC ($\geq$5.5 ng/mL). In those erratic driver specimens negative for alcohol THC positives rose to 23%. Drivers who used marihuana covered a broad age range. Aliquots of hemolyzed blood (10 $\mu$L) were analyzed by a sensitive radioimmunoassay (RIA) not requiring extraction. RIA accuracy and specificity were validated by gas liquid chromatography/mass spectroscopy (GLC/ MS) split pair analysis (correlation coefficient = 0.93). This initial experience should facilitate and amplify a program designed to set forth the epidemiology of marihuana use in motorists and possible behavioral correlates.

**KEYWORDS:** toxicology, marihuana, tetrahydrocannabinol, driving (motor vehicle operation), radioimmunoassay, impairment

The prevalence of marihuana use in the general driving population and its impact on highway safety are not known. However, recent surveys [*1*] disclose considerable cannabis use in all age groups, while other data [*2*] suggest that driving under the influence of this drug is widespread. Furthermore, laboratory studies [*3,4*] demonstrate marihuana induced impairment of coordinated motor activity such as is needed for motor vehicle operation. Indeed, actual city driving experiments [*5*] document its adverse effect on motor vehicle handling.

These findings clearly indicate the desirability of determining marihuana use in impaired motorists by accurate body fluid analysis for its major psychoactive component, $\Delta^9$-tetrahydrocannabinol (THC). Unfortunately work to date has been hampered by the unavailability of a routine method for THC quantitation in forensic specimens such as hemolyzed blood.

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The present study concerns a large population of erratic motorists arrested for suspected impairment whose blood was analyzed by a recently developed radioimmunoassay (RIA) which specifically quantitates THC directly in small aliquots (10 $\mu$L) of serum or hemolyzed blood. This initial phase was designed to ascertain the prevalence of recent marihuana use by such motorists and to determine the feasibility of large scale testing for THC in hemolyzed blood. If driving impairment is associated with THC in the blood, it may be mandatory to specify concentrations such as those that exist for alcohol.

## Materials and Methods

### *THC RIA*

The 2-(4′-carboxyphenyl)-azo-$\Delta^9$-THC antigen and its resultant antisera have been characterized [*6, 7*] and the detailed assay procedure described [*8*]. Its specific quantitation of $\Delta^9$-THC has been documented [*9*]; there were no significant assay contributions by THC metabolites or other cannabinoids.

Accuracy of the RIA was determined by spiked recovery experiments and by comparison of RIA and gas liquid chromatography/mass spectroscopy (GLC/MS)[6] analyses of coded split specimens. Assay precision was evaluated by calculation of intra- and inter-assay coefficients of variation with laboratory controls.

Additionally, precision was monitored during the course of the study by periodic analysis of specimens with known THC content. Such controls included blood from persons denying marihuana use (negatives), and specimens from impaired motorists whose THC level had previously been determined. All controls were coded (laboratory blind) and interspersed with study samples sent for initial analysis.

### *Impaired Motorist Specimens*

Study specimens were collected as follows.[7] Approximately one of every three erratic motorists detained by California Highway Patrol (CHP) officers for "driving under the influence" submit to blood alcohol determination. The California State Department of Justice (DOJ) district crime labs receive 20 000 such specimens per year of which >90% have blood alcohol concentrations (BAC) above the legal level of impairment (0.1%). Therefore, to assure an adequate sampling of those drivers with BAC <0.1% an equal number of specimens were randomly chosen from each category for THC analysis. These specimens were obtained as hemolyzed blood.[8]

Information (age, sex, blood alcohol level, and so forth) were gathered from the subject's individual arrest sheets [*10*]. Statistical compilation of results were performed after assay data were compiled.

## Results

### *THC RIA*

*Standard Curve*—Mean $\Delta^9$-THC values from eight replicate RIA standard curves were linear from 5 to 50 ng/mL with a correlation coefficient of 0.96. Uniformly low coefficients of variation (CV) for each standard point established precision throughout the response curve

[6]GLC/MS was performed by Jimmie L. Valentine, Ph.D., University of Missouri, Kansas City, MO.
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[8]All specimens were kindly provided, in code, by the DOJ to Receptor Research Institute for RIA.

(Fig. 1). Antibody bound counts per minute (cpm) at 5-ng $\Delta^9$-THC/mL were >3 standard deviations lower than cpm bound in the absence of hapten.

*Accuracy*—Triplicate analysis of blood specimens that were spiked with THC to yield low (5.0 ng/mL), mid (7.5 ng/mL), and high (35 ng/mL) range specimens gave 99, 105 and 102% mean recoveries, respectively. A more stringent test of both accuracy and specificity was performed by measuring THC in 15 split specimen pairs by RIA as well as GLC/MS (Table 1). Regression analyses of the paired data yielded a correlation coefficient of 0.93.

*Precision*—Intra-assay coefficients of variation were calculated in mid (8.0 ng/mL) and high (36 ng/mL) range sampling assayed in quadruplicate in a single assay. They were 5.3 and 7.6%, respectively. A mean inter-assay coefficient of variation of 11.4% (standard deviation ± 4.1) was found when twelve laboratory controls (range 3.1 to 36 ng/mL) were analyzed in seven separate assays over a two-month period.

Eighty-six specimens were obtained from thirteen persons who stated they did not use cannabis. Of these, eighty were negative, five were reported at 5.0 ng/mL (assay sensitivity ≥5.5 ng/mL), and a sixth at 9 ng/mL [*10*]. However, three of the marginal specimens as well as the one positive were obtained from a single individual at different times and represent thirteen assays of which nine were reported as below the cutoff. Thus it is unlikely that spurious THC levels were included in the incidence data because of variations in specimen collection and handling or laboratory procedures.

Three blood samples presumed to be negative for $\Delta^9$-THC obtained from a single individual were submitted for a separate concurrent study[9] as eleven blind aliquots. Nine of the aliquots were reported as 6 to 10 ng/mL, $\Delta^9$-THC, and two were below cutoff. The individual from

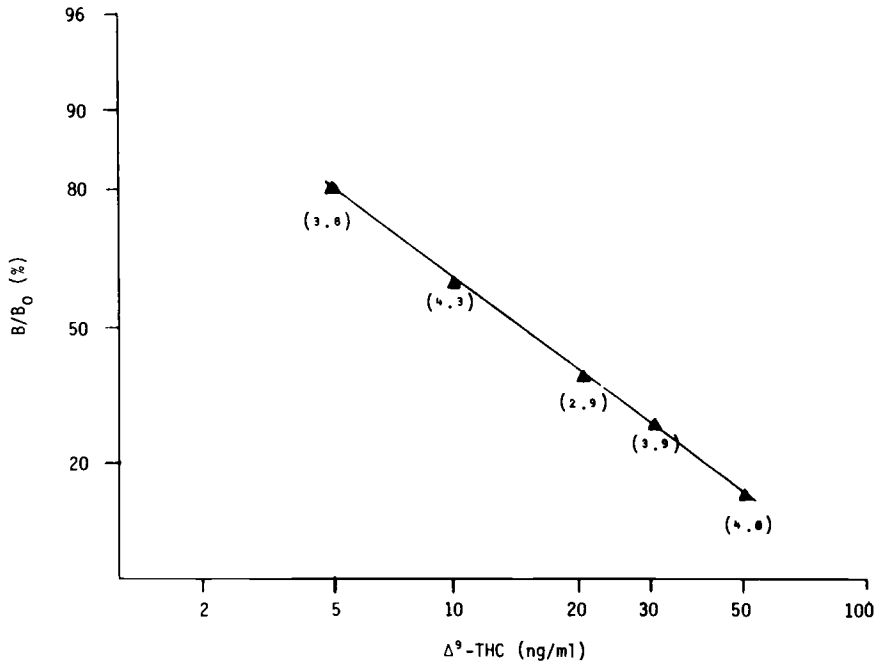


FIG. 1—*Mean values and coefficients of variation for each standard point are depicted for eight replicate blood (▲——▲) standard curves* $B/B_0$ *on logit scale where* $(B/B_0)$ = *binding/maximum binding. Standard curves were constructed by iterative weighted regression of these values.*

[9]Pending publication.

TABLE 1—*Comparison of $\Delta^9$-THC levels measured by RIA and GLC/MS in blood specimens from cannabis smokers.*[a]

| $\Delta^9$-THC, ng/mL | |
|---|---|
| RIA | GLC/MS |
| 36 | 45 |
| 32 | 22 |
| 22 | 32 |
| 21 | 19 |
| 19 | 21 |
| 13 | 13 |
| 13 | 13 |
| 10 | 7 |
| 7 | 10 |
| 7 | 9 |
| 6 | 5 |
| 5 | 6 |
| 0 | 0 |
| 0 | 0 |
| 0 | 0 |

[a]Linear regression analysis yielded a slope of 1.05 and a $y$-intercept of 0.13 (correlation coefficient = 0.93).

whom the specimens were obtained has been exposed repeatedly to THC in the laboratory, hence these must be considered as positives.

Several specimens from apprehended drivers (74) were analyzed twice, the second assay done at varying time periods after the first. Specimens remeasured after a short period of storage showed good correlation with initial results. However, as the time period between the two determinations lengthened, values from the second analysis diverged from those of the first, generally becoming lower. This problem did not surface early because of increasing turn around time of assay and ensuing delays in provision of coded results from DOJ. Only values in the initial analysis of blood specimens were included in incidence calculations. Subsequent work has shown that THC levels do significantly change when stored at 4°C for up to 24 weeks.

### *Impaired Motorist Specimens*

If a positive THC blood level is conservatively defined as $\geq$5.5 ng/mL, 14.4% of all specimens from this impaired motorist group were positive. The figure rose to 23% when only alcohol negative specimens were considered. Distribution by age group is summarized in Fig. 2. It ranges from 13.3% in the 13- to 21-year-old group to 19% in the 40- to 61- and 13.8% in the 62- to 99-year-old categories. While this intriguing pattern in older motorists was unexpected, numbers remained insufficient to assign epidemiologic validity to the age spread. It was not possible to correlate ethnic or occupational factors. Demographic analysis was hindered by the lack of extensive sampling of major metropolitan centers (San Francisco, Los Angeles, and San Diego). The incidence of THC in blood samples in the counties broadly surveyed ranged from 6.7 (Fresno) to 38.1% (Calaveras).

All motorists who had been stopped and who had positive blood THC failed the standard CHP roadside sobriety test. Positive blood THC levels (Fig. 3) ranged up to 23 ng/mL (median = 9 ng/mL). These concentrations are consistent with the time lag [*10*] from arrest to sampling (average, 45 min) during which time a very substantial but incomplete clearance from the blood compartment is known to occur after acute marihuana smoking [*11*].

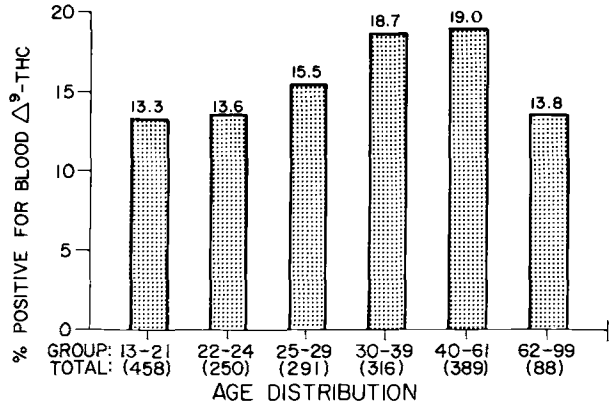


FIG. 2—*The percentage of impaired drivers whose blood contained $\Delta^9$-THC is tabulated by their age (categories are arbitrary).*

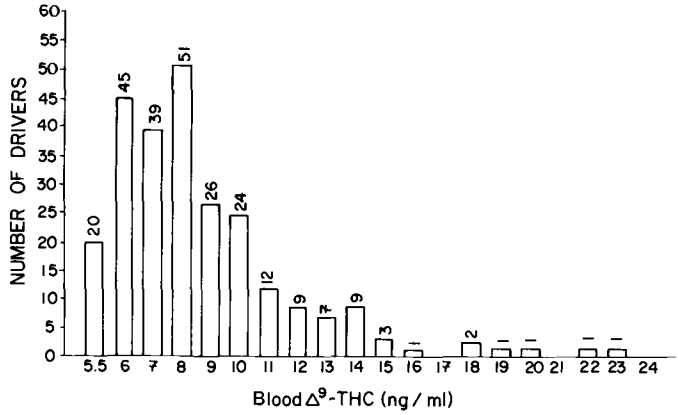


FIG. 3—*The number of drivers having a given blood THC level is depicted. Only specimens with THC concentrations ≥ 5.5 ng/mL were included in the prevalence calculations.*

The percent of positive THC was not increased in driving accidents (fatal and nonfatal) in relation to the general impaired population. However it is important to note that specimens were obtained from single and multiple occupant vehicles. Such statistics may be skewed by blood sampling that included passengers in the involved vehicles. In this connection, CHP reports commonly reflect ambiguities caused by unobserved driver movements such as changed position after accidents. Adequate single occupant accident data should be analyzed to ascertain the relevance of recent marihuana use, if any, to highway safety.

## Discussion

The present study suggests that marihuana is used by a significant number of impaired drivers. An overall incidence of 14.4% THC positive blood specimens found in these motorists is probably a conservative estimate because: (1) the time elapsed between arrest and blood sampling permitted substantial clearance from the blood, (2) major metropolitan centers (San Francisco, Los Angeles, and San Diego) were not heavily sampled, and (3) long-term storage under less than ideal conditions may have contributed to low or negative values.

Whether or not subsequent studies demonstrate that recent marihuana use causes highway accidents, the data in the present manuscript suggest that blood $\Delta^9$-THC will generally be under 20 ng/mL in positive cases.

The narrow range of low THC in blood specimens from a large motoring population may reflect higher blood THC concentrations at the time of arrest. Thus it would seem reasonable to pay far more than casual attention to a positive blood specimen obtained from an erratic motorist. The accuracy of the existing method has been documented [*8*] and a confirmatory test for 9-substituted THC metabolites has now been developed [*12*].

Sophisticated laboratory simulation [*3,4*] and "real life" driving studies [*5*] support the view that marihuana in standard doses detrimentally affects driving performance. These findings are especially disturbing in view of the significant incidence and broad age of marihuana smoking motorists.

In conclusion, psychoactive THC was found in 14.4% of a large population of erratic motorists whose blood was analyzed by RIA. The range of blood THC levels was narrow and use was spread over a broad age range. This preliminary effort represents the first large scale field survey using quantitative analysis of blood levels of $\Delta^9$-THC in forensic specimens. This initial experience should facilitate and amplify a program designed to set forth the epidemiology of marihuana use in motorists, and possible behavioral correlates.

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