Marijuana-Positive Urine Test Results from Consumption of Hemp Seeds in Food Products

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Abstract

Commercially available snack bars and other foodstuffs prepared from pressed hemp seeds were ingested by volunteers. Urine specimens were collected for 24 h after ingestion of the foodstuffs containing hemp seeds and tested for marijuana using an EMIT immunoassay and gas chromatography–mass spectrometry (GC–MS). Specimens from individuals who ate one hemp seed bar demonstrated little marijuana immunoreactivity, and only one specimen screened positive at a 20-ng/mL cutoff. Specimens from individuals who ate two hemp seed bars showed increased immunoreactivity, and five specimens screened positive at a 20-ng/mL cutoff. A single specimen yielded a quantitative GC–MS value (0.6 ng/mL), but it failed to meet reporting criteria. Several specimens from individuals who ate three cookies made from hemp seed flour and butter screened positive at both 50- and 20-ng/mL cutoffs. Two specimens produced quantitative GC–MS values (0.7 and 3.1 ng/mL), but they failed to meet reporting criteria. Several specimens also tested positive with an FDA-approved on-site marijuana-screening device. Hemp seeds similar to those used in the foodstuffs did not demonstrate the presence of marijuana when tested by GC–MS. In this study, ingestion of hemp seed food products resulted in urine specimens that screened positive for marijuana. No specimens gave a GC–MS quantitative value above the limit of detection for marijuana.

Introduction

Cannabis sativa, commonly called marijuana, is the scientific name for a species of plant that comes in several varieties. One form of cannabis produces hemp (bast) fiber, an age-old source of rope. Fiber hemp is supposed to contain negligible amounts of the psychoactive drug tetrahydrocannabinol (THC). Other varieties of cannabis are the source of THC. In the past, hemp has been raised as a cash crop. For example, during World War II, hemp was intensively cultivated as a part of the war effort to produce rope for the Navy. Fiber hemp is now being touted as a practical source of cloth from which shirts, pants, hats, handbags, knapsacks, and other items can be made. In addition, various food products containing hemp seed are now commercially available. Hemp seed foodstuffs may be eaten simply as a novelty, as a health food, or in the belief that they contain THC and possess psychoactive properties.

The interpretation of urine tests would be complicated if the ingestion of foodstuffs containing hemp seeds could produce marijuana-positive drug-test results. Some individuals testing positive for marijuana because of knowing ingestion of THC might argue that the test result is due to eating hemp seed foods instead of illicit drug use. Urine specimens from volunteers who ate hemp seeds were tested for marijuana using an immunoassay and gas chromatography–mass spectrometry (GC–MS) to determine if specimens could screen positive and if the marijuana presumptive-positive specimens could be confirmed.

Experimental

Hemp seed food

Hemp seeds and hemp seed food products were purchased from Hungry Bear Hemp Foods (Eugene, OR). The seeds are imported, mainly from the Far East, and sterilized as required by the U.S. government. These hemp seeds are legal and approved for culinary use by the Food and Drug Administration. Literature from the company states that hemp seeds will not produce psychoactive effects.

Seedy Sweeties snack bars are made from pressed hemp seeds. The bars were 1.5 oz. (42.5 g) in weight and contained molasses, nonsterilized pressed hemp seed, brown rice, syrup, organic oats, organic sesame seed, unrefined safflower oil, and either sunflower seeds or Brazil nuts. Each bar was individually wrapped in a cellulose bag.

Hemp seed flour and hemp seed butter were used to prepare cookies. The flour was coarse and greenish-brown in color. The butter was black and had a tarlike consistency. The cookie recipe used is as follows: 2 cups hemp seed flour; 1 cup sugar; 1 cup brown sugar; 1 teaspoon each of salt, baking soda, and vanilla; 3 eggs; 1 cup cooking oil; and one-half package of

chocolate chips. After mixing, the batter was baked at 325°F for 12 min.

Test subjects

In the first phase of the study (single dose), ten volunteers, all of whom were employees of PharmChem, each ate one Seedy Sweeties bar. The group included six males and four females ranging in age from 26 to 61. Five of the subjects ate bars with sunflower seeds; five ate bars with Brazil nuts. Two days later, in the second phase (double dose), nine subjects ate two Seedy Sweeties bars. Five volunteers ate bars with sunflower seeds; four ate bars with Brazil nuts. Two days later, in phase three, four subjects each ate three cookies.

Specimens

Urine specimens were collected from each subject before ingestion of the hemp seed foods to establish baseline negative values for each individual. After ingestion, urines voided over the next 24 h were collected. Each specimen was screened by immunoassay for cannabinoids at both 20- and 50-ng/mL cutoffs. Specimens that showed immunoreactivity above the baseline (negative-control value) were tested by GC–MS. The successive phases of the study were separated by approximately 48-h intervals in order to allow return to baseline (negative for marijuana).

Assays

The laboratory's routine marijuana immunoassay, EMIT d.a.u. (Behring, San José, CA), was used to screen specimens. This assay was modified from the manufacturer's protocol to allow for the use of an NAD substrate "extender". Separate 20- and 50-ng/mL calibrators and controls were used to establish cutoff values at each concentration. The negative control absorbance value was used as a baseline against which immunoassay response in specimens was judged.

The laboratory's routine marijuana GC–MS assay was used to analyze specimens that tested above the baseline of the screening immunoassay. Marijuana was extracted from 3 mL of urine via a liquid–liquid manual procedure, and a derivative was formed using tetramethylammonium hydroxide (TMAH) and dimethyl sulfoxide (DMSO). The internal standard was trideuterated 11-nor-Δ^9-tetrahydrocannibinol-carboxylic acid (THCCOOH). Analysis was performed using a single-point calibration with a 15-ng/mL THCCOOH standard. The procedure used a Hewlett-Packard (Atlanta, GA) 5890 GC and 5970 mass selective detector. Selected ion monitoring (SIM) was used with 313, 357, 360, 372, and 375 as the target ions. The limit of detection (LOD) was 4 ng/mL as established by an empirical method requiring 100% of replicates of dilute standards to meet all standard GC–MS acceptance criteria (retention time, ion ratios, chromatography); however, quantitative values did not have to be within 20% of the target value.

On-site marijuana-testing device

Urine specimens that showed the greatest immunoreactivity by the EMIT assay were tested using the PharmScreen™ one-step marijuana test (PharmChem Laboratories, Menlo Park, CA). PharmScreen is a hand-held, on-site screening device that uses a colored anti-THC monoclonal antibody-colloidal gold conjugate methodology to visualize the presence of cannabinoids in a urine specimen. Only a 0.2-mL urine specimen is required, and the result is read in 3–8 min. The device is designed to yield a positive result at a concentration of 50 ng/mL THCCOOH.

Results

The sterilized and pressed hemp seeds were extracted and analyzed by GC–MS for both THC and THCCOOH. No evidence of either the psychoactive drug or of its major metabolite were found in the hemp seed extracts.

None of the participants described the Seedy Sweeties bars as tasty, but some individuals found them to be less palatable than others did. One volunteer withdrew from the study after Phase I (single bar) rather than eat two bars in Phase II because the perceived objectionable taste. Only four of the ten original participants continued into Phase III, at least in part because of the flavor of the hemp seed food. The cookies made from hemp seed flour and butter were considered to taste worse than the bars. One participant indicated that the inclusion of chocolate chips in the cookies was critical to his ability to consume them.

Phase I—single dose of Seedy Sweetie bar

Immunoassay data are summarized in Figure 1 for all of the urine specimens collected from the 10 volunteers. Only 2 specimens of 60 (3%) exhibited screening results appreciably higher than the negative baseline value using the 50-ng/mL cutoff assay. Neither specimen screened positive or even yielded an absorbance close to 75% of cutoff negative control. With the 20-ng/mL cutoff assay, 8 of the same 60 specimens (13%) tested appreciably higher than the baseline. One specimen tested positive, and two specimens were at or greater than the 75% of cutoff negative control. All eight of the specimens quantitated at 0 ng/mL THCCOOH by GC–MS.

Phase II—double dose of Seedy Sweetie bar

Immunoassay data are summarized in Figure 2 for all urine specimens. One individual withdrew from the study after Phase I. Sixteen specimens of 48 (33%) yielded values above the baseline with the 50-ng/mL cutoff assay. Two of these specimens tested at or above 75% of cutoff negative control. Using the 20 ng/mL cutoff assay, 22 of the same 48 specimens (46%) produced values higher than baseline. Four specimens tested positive and three specimens were at or greater than 75% of cutoff negative control. Of the seven specimens tested by GC–MS, six quantitated at 0 ng/mL THCCOOH; the seventh gave a result of 0.6 ng/mL, but it failed to meet the qualifying ion ratio ranges.

Phase III—hemp seed cookies

Figure 3 summarizes the immunoassay data for all urine specimens. Another five individuals withdrew from the study after Phase II. Using the 50-ng/mL cutoff assay, 5 of 13 specimens (38%) were above baseline, three testing positive and two

at or greater than 75% of cutoff negative control. The same five specimens all screened positive with the 20-ng/mL cutoff assay. None of these specimens quantitated above the LOD by GC–MS. Two specimens gave quantitative values of 0.7 and 3.1 ng/mL. The ion ratios were acceptable for both of these specimens, but the chromatography was judged to be unacceptable.

Of the six specimens tested by PharmScreen, all gave positive or borderline-positive results. As noted previously, none of the specimens could be confirmed positive by GC–MS.

Discussion

Marijuana is typically ingested by smoking *Cannabis sativa* plant leaves in cigarettes or through pipes. However, marijuana can be ingested orally as well. The literature reports oral marijuana ingestion in a variety of forms including THC dissolved in ethanol or sesame oil, emulsified in sodium glycocholate or Tween-80, baked in cookies or brownies, and mixed with the filling of a meat sandwich (1–5). These studies indicate that the speed and degree of absorption of the drug and its

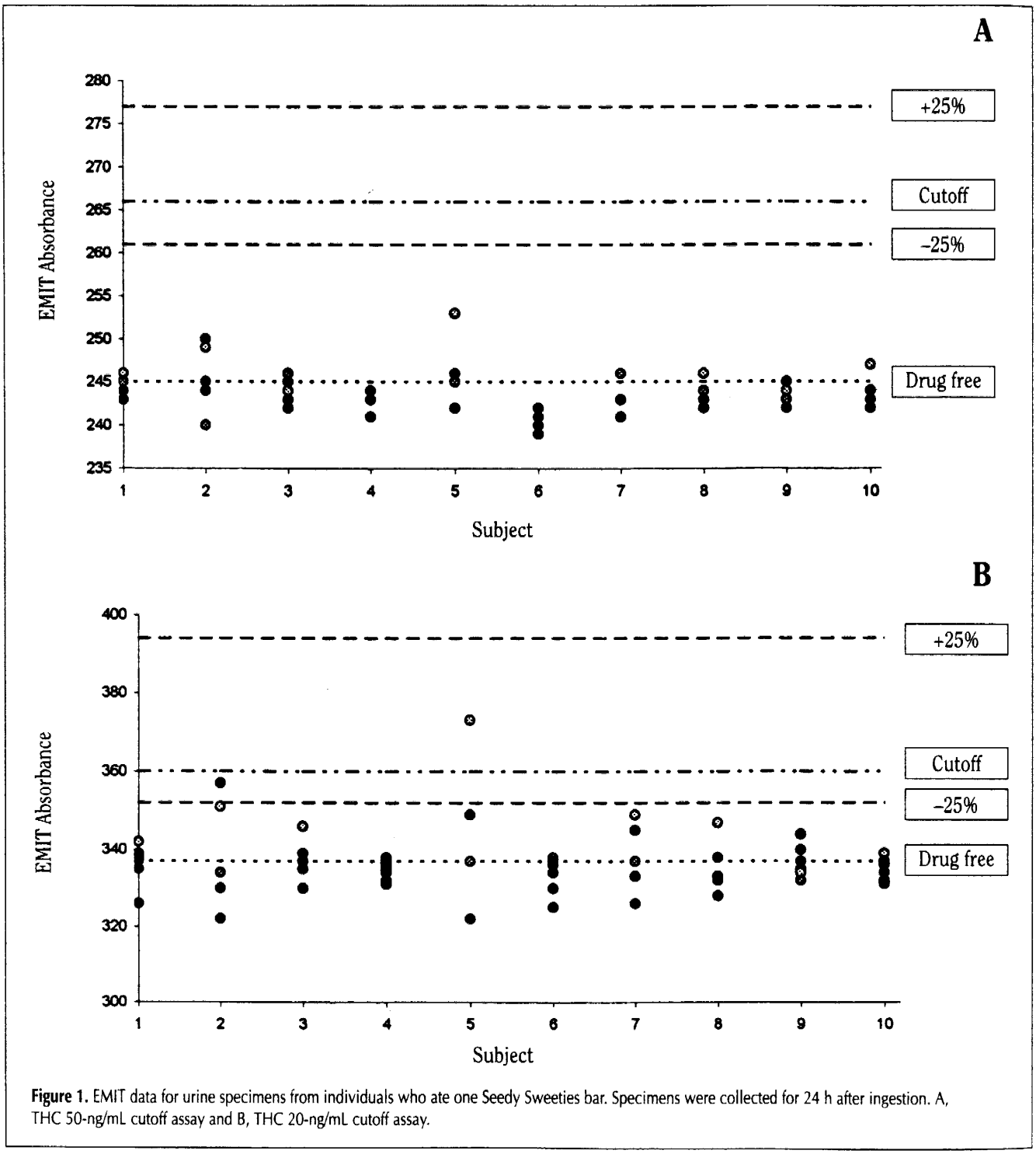

Figure 1. EMIT data for urine specimens from individuals who ate one Seedy Sweeties bar. Specimens were collected for 24 h after ingestion. A, THC 50-ng/mL cutoff assay and B, THC 20-ng/mL cutoff assay.

bioavailability are affected by the vehicle of ingestion (6). The oral ingestion of marijuana results in the same physiological and behavioral effects typical of the drug when it is smoked (1,4). In the marijuana brownie study by Cone et al. (4), urine specimens tested positive by the EMIT d.a.u. assay using a 20-ng/mL cutoff. In fact, urines generally exceeded the 75-ng/mL calibrator value for 1–2 days after ingestion. Thus, oral ingestion of marijuana can result in a positive urinalysis test using the 50-ng/mL cutoff mandated by the Department of Health and Human Services guidelines for drug testing.

Anecdotal accounts from drug users indicate that it is not unusual for marijuana to be consumed in foodstuffs. In the 1960s, brownies, as well as cookies and cakes, became popular vehicles for the ingestion of marijuana. Marijuana-laced baked goods are not reported to taste good because of the amount of plant material typically used. The attraction is not the taste, but rather the amount of THC effectively delivered via oral ingestion. Oral THC ingestion is reputed to produce a "high" that is more pronounced and lasts longer than that from smoking.

The products examined in this study are described by the

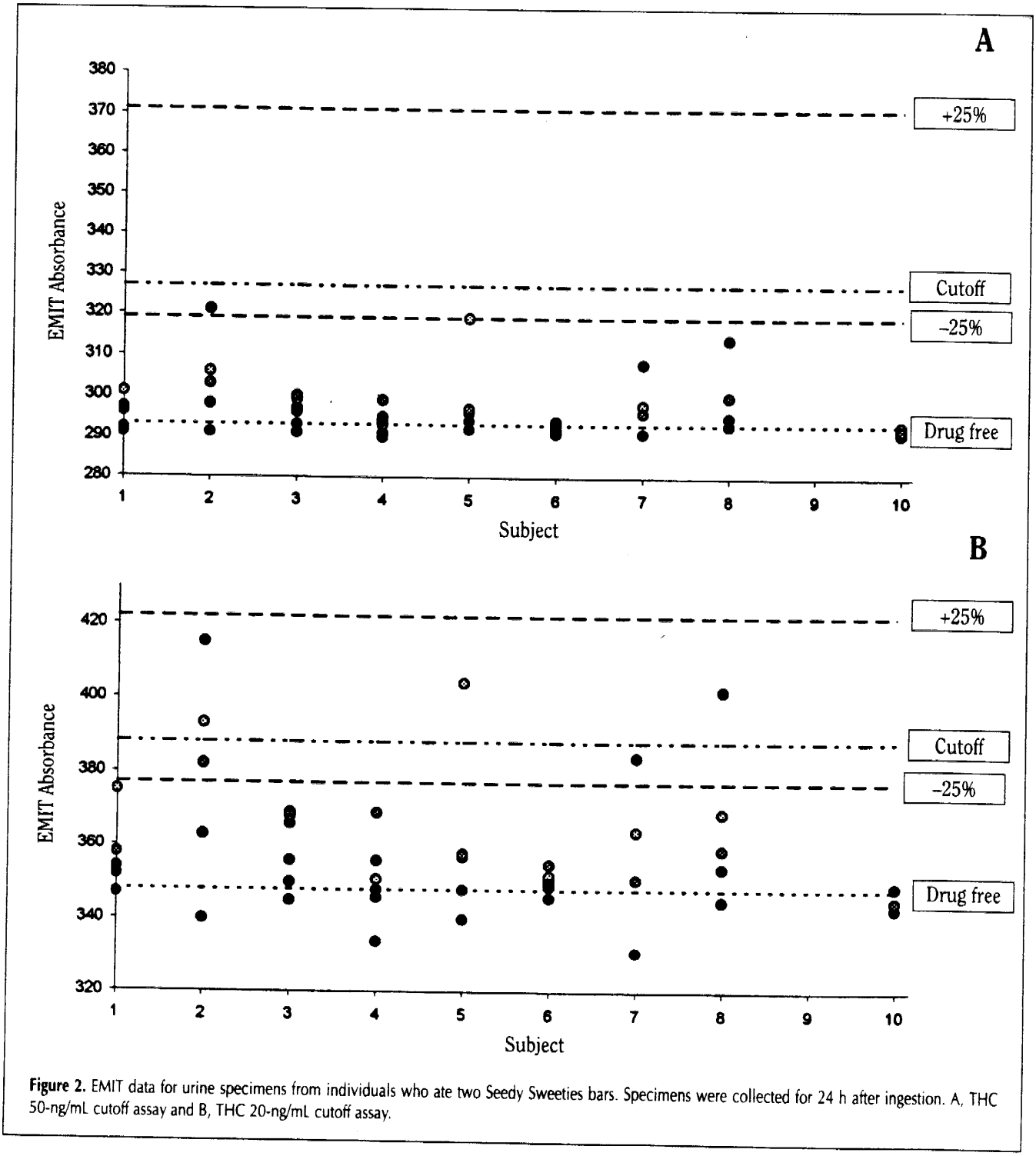

Figure 2. EMIT data for urine specimens from individuals who ate two Seedy Sweeties bars. Specimens were collected for 24 h after ingestion. A, THC 50-ng/mL cutoff assay and B, THC 20-ng/mL cutoff assay.

manufacturer as legal and incapable of producing psychoactive effects. The manufacturer notes that the importation of non-sterilized hemp seeds is not allowed for fear that hemp plants may be grown from them. Sterilized, pressed hemp seeds will not germinate. The purveyors do, however, also state that they are activists as well as food crafters.

Their goal is to bring about a renaissance of the domestic hemp agriculture business. Popularizing hemp foods serves to stimulate interest in the various other uses of hemp (lumber, textiles, paper, medicine, building material, and fuel).

In the present study, it has been shown that positive urine cannabinoid screening tests can result from the ingestion of snack bars or cookies prepared from hemp seed flour and butter. The likelihood of obtaining a positive test result depends on the amount of hemp seeds consumed, the form in which they are ingested, and the testing cutoff value applied. Naturally, the metabolism of the individual and the time of collection of the specimen after ingestion also affect the probability of testing positive. The EMIT d.a.u. assay used for screening is an immunoassay commonly used by drug-testing laboratories.

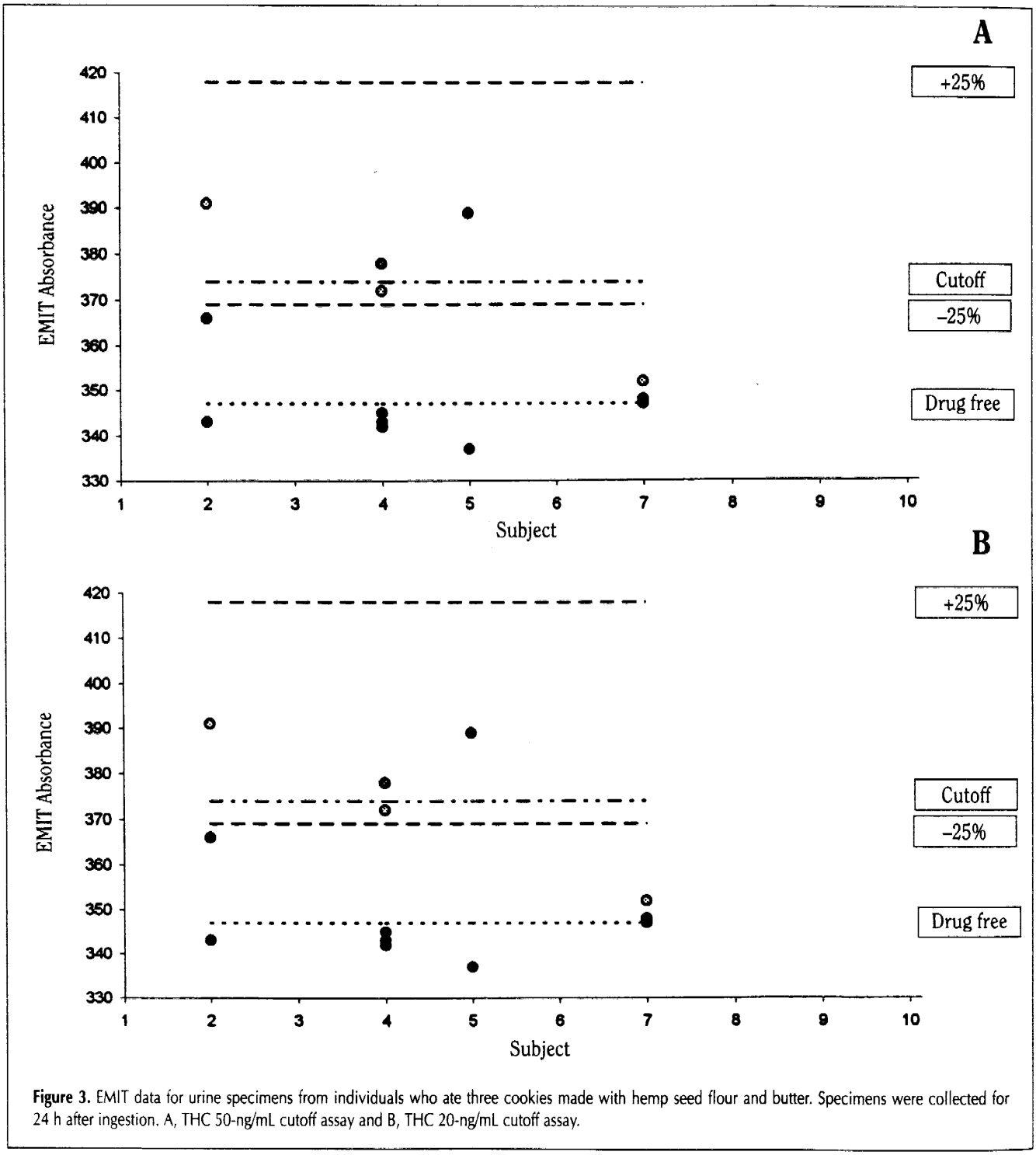

Figure 3. EMIT data for urine specimens from individuals who ate three cookies made with hemp seed flour and butter. Specimens were collected for 24 h after ingestion. A, THC 50-ng/mL cutoff assay and B, THC 20-ng/mL cutoff assay.

The 50-ng/mL cutoff required for federally mandated drug testing is also used by many private companies, and 20 ng/mL is used by many private companies and nonfederal government entities. Based on the findings of this study, a screen-positive marijuana test result is compatible with and can be explained by the oral consumption of hemp seed food products.

None of the urine specimens that screened positive or that showed any substantial immunoreactivity could be confirmed positive for THCCOOH by GC–MS. With few exceptions, the specimens did not even yield a quantitative value above 0 ng/mL. The highest quantitative value obtained was only 3.1 ng/mL, but it failed to meet the routine GC–MS acceptance criteria. Thus, it appears that a confirmed-positive result is unlikely to result from these hemp seed foods. Approximately 60 different molecules make up the family of cannabinoids (7). Although immunoassays are typically calibrated using the major metabolite of THC, THCCOOH, it is not surprising that crossreactivity with other cannabinoids found in hemp seeds results in positive screening tests. The inability to detect and quantitate a significant amount of THCCOOH in these urine specimens suggests that the hemp seeds used in the foodstuffs either do not contain or contain only negligible amounts of the psychoactive substance, THC. The pressed hemp seeds analyzed as part of this study showed no evidence of THC or of THCCOOH, a finding consistent with the urine testing data. Some cannabinoid molecules are present in the foodstuffs, as demonstrated by the positive screening results and GC–MS data.

Consumption of larger amounts of hemp seeds and their chronic ingestion will presumably result in increasingly more positive screening results, and, possibly, GC–MS values approaching the typical cutoff of 15 ng/mL. Still, the data presented here suggest that the hemp seeds consumed contained very little or no THC, making the prospect of a confirmed positive test very unlikely. Hemp seeds from other sources may contain appreciable amounts of THC (8). The volunteers in this study did not find the hemp seed foods appealing or very palatable and were not eager to eat any more than prescribed by the study protocol. Nor did any of the participants report any physiological or behavioral effects consistent with the pharmacological actions of marijuana. It is conceivable that individuals might be willing to eat large quantities of hemp seed foodstuffs in the hopes of legally obtaining a marijuana "high". Our experience suggests that the taste alone of these foodstuffs may serve as an effective deterrent to such an attempt.

Conclusion

It appears that a marijuana-positive urine test due to ingestion of hemp seeds is not as likely as an opiate postive caused by poppy seed ingestion. Hemp seeds from a different lot or from a different variety of hemp plants than those tested in this study may contain only non-negligible amounts of THC and may be capable of producing both screen- and confirm-positive urine drug-test results. Marijuana-positive test results that are due to hemp seed ingestion is a potential problem, especially for on-site tests. Specimens from hemp seed eaters can test positive for marijuana by immunoassay. Some individuals may consume, or claim to consume, hemp seed foodstuffs in order to mask the use of the marijuana and provide a plausible explanation for a positive screening test. It is important not to allow illicit drug users who are subject to urine testing to explain away positive results by attributing them to the innocent ingestion of hemp seeds.

This kind of situation once again underscores the sagacity of following the traditional two-tier approach to drug testing. First, a relatively broad spectrum screening assay for a drug or drug class is used to eliminate negative specimens from consideration (usually, the bulk of specimens submitted) and to identify the presumptive positive specimens. The presumptive positives were then tested by a confirmation assay, based on a distinctly different methodology, that specifically identifies and accurately quantitates one or more drugs or drug metabolites. Taking administrative or legal action against an individual solely on the basis of a positive marijuana-screening test is inappropriate.

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