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Poppy Seed Ingestion as a Contributing Factor to Opiate-Positive Urinalysis Results: The Pacific Perspective

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ABSTRACT: The possible contribution of poppy seed foods to positive opiate urinalysis results, especially from foods available in the Pacific Rim area, has recently become an issue for the U.S. Army Forensic Toxicology Drug Testing Laboratory in Hawaii. To assess the likelihood of this possible contribution, seven different poppy seed food products were consumed by male and female volunteers, and urine specimens were collected at time increments up to either 24 or 72 h. Specimens were evaluated for opiates using Roche Abuscreen radioimmunoassay (RIA), and all RIA positive specimens were analyzed for morphine and codeine using gas chromatography/mass spectrometry (GC-MS). Poppy seed cake, bagels, muffins, and rolls did not contain sufficient quantities of poppy seeds to give rise to opiate positive specimens by U.S. Department of Defense (DOD) GC-MS cutoff levels (morphine = 4000 ng/mL, codeine = 2000 ng/mL), although a number of specimens were positive by National Institute on Drug Abuse (NIDA) cutoff levels (morphine and codeine = 300 ng/mL). However, ingestion of poppy seed streusel or Danish pastry led to confirmed morphine and codeine positive specimens, irrespective of the use of DOD or NIDA confirmation cutoff values. In addition, significant amounts of codeine were observed in a number of these specimens. These findings argue against the unqualified application of previously published quantitative guidelines for eliminating poppy seed ingestion as a possible cause for a positive opiate urinalysis result.

KEYWORDS: toxicology, opiates, poppy seeds, morphine, codeine, unknowing ingestion, drug testing

For many years, the U.S. Department of Defense (DOD) has used routine, random drug urinalysis to assist commanders in deterring drug use in the military. Today, the DOD drug testing effort represents perhaps the largest drug testing system in the world. On a routine basis, each specimen received by a U.S. Army or Air Force drug testing laboratory is tested for only three drugs—tetrahydrocannabinol (THC), cocaine, and a "third drug." The identity of the third drug or drug class for which specimens are tested changes periodically (daily, weekly, or monthly, depending on the laboratory). This

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"rotational" testing scheme maximizes the deterrent effect of the testing, while maintaining cost-effectiveness.

In the recent past, the U.S. Army and Air Force laboratories added opiates as a third drug class in the rotational testing scheme. The problem of interpretation of positive opiate results was recognized as a potentially significant difficulty from the outset. One of the main causes for concern was the possibility that a service member could unknowingly ingest enough of a poppy seed food to cause a positive opiate urinalysis result. Civilian testing guidelines, as promulgated under the National Institute on Drug Abuse (NIDA) requirements, stipulate that a 300-ng/mL cutoff level is used for both morphine and codeine.

It was the purpose of the DOD Biochemical Testing Commitee to ease the opiate interpretative burden to some degree, by adjusting gas chromatography/mass spectrometry (GC-MS) confirmation cutoff values for morphine and codeine to such levels that only use of controlled substances (morphine, codeine, heroin) could give rise to positive urine opiate levels. The 4000-ng/mL morphine and 2000-ng/mL codeine cutoff levels chosen by DOD were based on literature reports [*1–8*] which suggested that realistic ingestion of poppy seed products would be very unlikely to give rise to such elevated levels of morphine or codeine.

One of the most important pieces of information to be gleaned from previous research with poppy seed foods would seem to be the absence of codeine-positive findings following poppy seed ingestion. In the recent review of the subject by ElSohly and Jones [*9*], a great deal of weight was placed on the lack of codeine findings in urine from poppy seed eaters. The authors created a list of general guidelines to assist in the differentiation of sources of opiate positive urinalysis results, the first two guidelines of which state:

1. High levels of total morphine in urine (more than 5000-ng/mL) are indicative of abuse of an opiate product, such as heroin, morphine, or codeine.
2. High levels of codeine (more than 300-ng/mL), with a morphine-to-codeine ratio of <2, are indicative of codeine abuse *and rule out poppy seed ingestion* [emphasis added].

The cited articles have all dealt with poppy seeds and poppy seed food products available in the continental United States, or in Europe. In addition, although the morphine and codeine contents of poppy seeds from a number of worldwide sources have been ascertained, it was not clear that these were the only possible sources of poppy seeds, especially in the Pacific Rim and Far East. The Forensic Toxicology Drug Testing Laboratory at Tripler Army Medical Center, in Honolulu, Hawaii, tests soldiers and airmen serving in the Pacific Rim, Alaska, and several regions of the continental United States. One of the purposes of the present research was to determine the usefulness of the elevated DOD GC-MS cutoff values for simplifying interpretation of opiate urine positive findings. In addition, this study allowed evaluation of the applicability of the interpretive guidelines set forth by ElSohly and Jones, in the context of a vastly different population than that previously examined.

The intent of the present experiment was to mimic the conditions of ingestion that would probably be followed (or claimed) by soldiers and airmen in the region. Therefore, the poppy seeds and poppy seed foods were purchased locally, at the commissary (military grocery store) when available. The study included six different types of poppy seed foods, as well as seeds sprinkled on buttered bread ("German style"). The seeds (raw or in foods) were not extracted to determine their content of opiates. The ingestion pattern involved eating realistic amounts (although the subjects were always very full after eating). Finally, the urine testing was performed the way it happens in the field—by spot collection and testing of urines.

Based on this research design, the study may not answer some of the more academic questions (such as the total recovery of opiates, the relative extent of metabolism, and

so forth). However, the empirical nature of the study allows the laboratory to better answer interpretive questions for the military population in the Pacific. In addition, the information is useful to DOD in evaluating the efficacy of elevated opiate cutoff levels in assisting interpretation, and it allows evaluation of interpretive guidelines in the literature.

Materials and Methods

Poppy Seeds and Poppy Seed Foods

Poppy seeds used in the preparation of homemade poppy seed cake, and eaten as raw seeds in the "German style" ingestion study, were the Schilling "fancy" brand, purchased from the Schofield Barracks Commissary and Hickam Air Force Base Commissary, respectively. In a previously reported study, Schilling poppy seeds contained 28 μg/g of morphine and no detectable codeine [*6*]. Two poppy seed cakes were prepared from a recipe found in *The Chocolatier*, each containing approximately 77 g of seeds. In eating this cake, each of the seven subjects (three females, four males) consumed the equivalent of between 14 and 19 g of seeds. Specimens were collected before eating; after eating at approximately 2, 4, and 8 h postingestion; at the first void the next morning, and 4 h after wake-up.

Poppy seed Danish pastry and streusel were purchased from Fritz's European Bakery in Kalihi, Hawaii. The poppy seeds used by Fritz Vincken were obtained from Mexico and, based on the proprietary recipe, each piece of streusel contained approximately 12 g of seeds. Mr. Vincken uses an authentic German poppy seed press, which results in a fine, crushed poppy seed paste in the streusel and Danish pastry. Each of the five streusel-eating subjects (two females, three males) consumed two pieces of the pastry, containing a total of approximately 24 g of seeds. When poppy seed streusel ingestion was first studied, the specimen collection protocol was the same as that used for poppy seed cake. In a subsequent repeat study of poppy seed streusel ingestion, the collection period was extended to include sampling at 48 and 72 h postingestion.

There is only one bagel manufacturer on the island of Oahu—the Hawaiian Bagel Factory in Honolulu. The poppy seeds were coated on the outside of the bagels, applied at the bakery just after the final cooking step. Four of the bagels were dissected, and their content of poppy seeds was weighed. The average weight of seeds on each bagel was 1.5 g. The origin of the seeds could not be ascertained. During cutting of the bagels (to make the ham and cheese bagel sandwiches for the study) some of the seeds were dislodged. Each of the nine subjects (four females, five males) ate two bagels, and the specimen collection protocol was the same as that used for poppy seed cake.

Poppy seed muffins were purchased from Costco, Inc., in Salt Lake, Oahu, Hawaii. The four female subjects each ate one of the 180-g muffins, while two of the male subjects ate two muffins, and the third male subject ate three. The recipe for the muffins, and the origin of the seeds, could not be obtained from the distributor. However, by dissecting and weighing the seeds from two muffins, the researcher estimated that each contained about 3 g of seeds. Specimens were collected before eating, and then at intervals of 2, 4, 6, 8, 12, 24, 48, and 72 h postingestion.

Delicatessen (deli) rolls were purchased locally from Safeway, Inc. Again by using dissection and weighing, each 60-g roll was estimated to be coated with approximately 1 g of seeds. The origin of the seeds, and the recipe, were not available. Each of the eight subjects (four females and four males) ate two rolls in the form of ham and cheese sandwiches.

Finally, the four "German style" poppy seed eaters (two females, two males) each consumed approximately 22.5 g of the Schilling "fancy" seeds. The seeds were stuck to

buttered or honeyed bread, to help the subjects choke down the large mass of seeds. The specimen collection protocol for the deli roll and "German style" poppy seed eaters was the same as that followed for the poppy seed muffin eaters.

Analytical Methods

The Abuscreen radioimmunoassay (RIA, Roche Diagnostic Systems, Nutley, New Jersey) for morphine was used in accordance with the manufacturer's protocol. The RIA presumptive positive cutoff level was established using the mean counts per minute (CPM) value from up to 22 cutoff controls included in an RIA batch. All presumptive RIA-positive specimens, as well as a large number of other specimens demonstrating RIA responsiveness, were scheduled for gas chromatography/mass spectrometry (GC-MS) confirmation.

The specimens were prepared for GC-MS using an acidic autoclave hydrolysis, and nonpolar solid-phase extraction of codeine and morphine from basic solution using the DuPont Prep-I automated extraction station and Type-W cartridge (now supported by Creative Technology Systems, Inc., Newark, Delaware). The extraction was followed by formation of the trimethylsilyl (TMS) derivatives.

GC-MS was performed using a Hewlett-Packard (Fullerton, California) 5890/5970A GC/mass selective detector (MSD), equipped with a 12-m (0.2-mm internal diameter, 0.3-μm 5% phenyl methylsilicone film thickness) Hewlett-Packard Ultra-2 column and utilizing helium as the carrier gas at 37 cm/s. A 10:1 split injection was employed, and the temperature program involved an initial temperature at 200°C (with a 2-min hold) and then a ramp at 10°C/min to 280°C (with a 2-min hold).

GC-MS identification and quantitation were performed using Thru-Put Systems' (Orlando, Florida) Target software on the Pascal v.3.2 Hewlett-Packard ChemStation. Selected ion monitoring utilized fragments at 429, 236, and 401 atomic mass units (amu) for morphine, 432, 239, and 404 amu for D_3-morphine, 371, 234, and 343 amu for codeine and 374, 237, and 346 amu for D_3-codeine. The DOD cutoff values of 4000 ng/mL for morphine and 2000 ng/mL for codeine were employed, with deuterated internal standards present at the same levels as the cutoff values. A minimum of 37% of each GC-MS batch was composed of standards and controls.

Data reduction, regression, and graphic display were achieved using MicroSoft Chart and FullPaint software packages on a 512K RAM Macintosh computer.

Results and Discussion

The quantities of each type of poppy seed food consumed were considered by the subjects to be rather large. Most subjects felt very full after consuming the food. For most of the foods ingested, the subjects did not feel any physical effects that could be directly attributed to the opiate content of the foods. However, several subjects became ill after consuming the poppy seed streusel or raw seeds ("German style"), with the primary complaint being the offensive consistency of the seeds. For those subjects who enjoyed the taste and consistency of streusel and raw seeds, the major physical effect described was an overall feeling of lethargy, similar to the low one feels after a large meal. One subject who ate 23 g of poppy seeds "German style" was accused by her coworkers of "giggling and acting silly" in the hours after poppy seed ingestion. The subject did not agree with this assessment of her behavior at the time.

Opiate RIA presumptive positive specimens were obtained as a result of ingestion of every poppy seed foodstuff except poppy seed deli rolls. The time courses of excretion of opiate cross-reacting substances following ingestion of poppy seed foods are provided in Figs. 1 through 9. In all portrayals of the data, the ordinate label "Danish" refers to

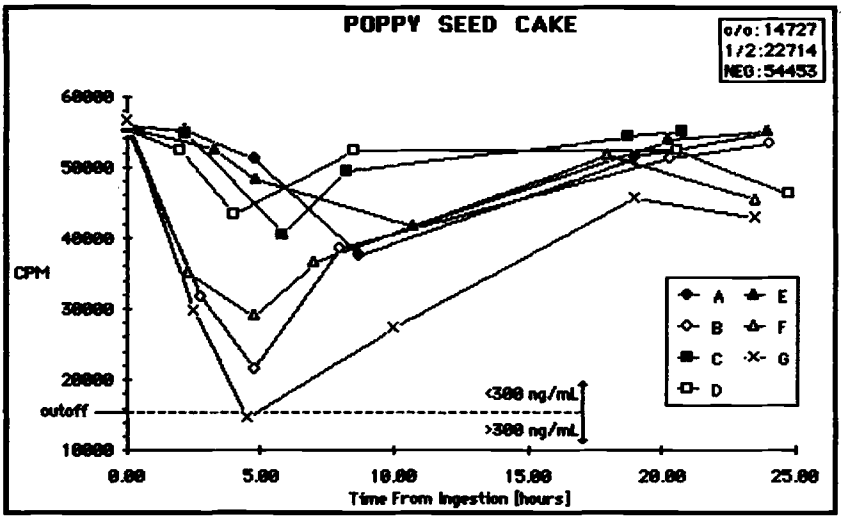

FIG. 1—*RIA results for specimens collected following ingestion of about 300 g of poppy seed cake. The mean CPM values for the cutoff level ("c/o," 300 ng/mL), half-cutoff level ("½," 150 ng/mL), and negative ("NEG," 0 ng/mL) standards and controls are shown in the upper right corner box.*

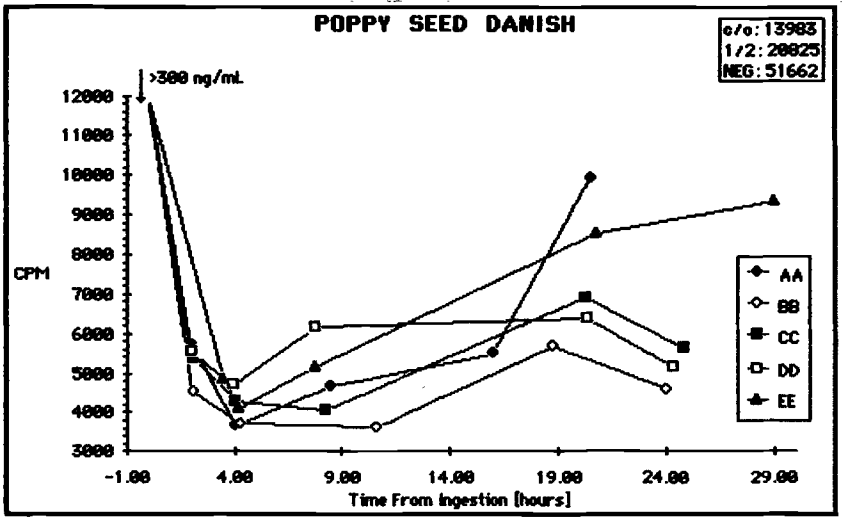

FIG. 2—*RIA results for specimens collected following ingestion of two pieces of poppy seed streusel or Danish pastry.*

the first ingestion trial involving both poppy seed streusel and poppy seed Danish pastry, and includes collection up to 24 h postingestion. The label "streusel" refers to the second trial with only streusel, including collection up to 72 h postingestion.

Poppy seed cake ingestion (Fig. 1) gave rise to only a single presumptive positive specimen (for Subject G at 4.5 h postingestion), which was later confirmed by GC-MS to contain 260 ng/mL of morphine and trace codeine. It is interesting to note that the female subject from whom this specimen was obtained had received only 75% of the largest "dose" of poppy seeds (in grams of seeds per kilogram of body weight) in the study (for Subject B).

Figure 2 demonstrates the surprising results obtained for two females and three males after ingestion of poppy seed streusel or danish, and specimen collection up to 24 h postingestion. Every specimen was presumptively positive, with confirmation values ranging from 820 to 11 571 ng/mL for morphine, and with codeine from trace amounts up to 4861 ng/mL. Of the 25 postingestion specimens, all 25 were morphine positive by NIDA standards (≥300 ng/mL), with 16 NIDA positive for codeine (≥300 ng/mL) as well. Using DOD cutoff levels (4000 ng/mL for morphine and 2000 ng/mL for codeine), 5 specimens were found to be GC-MS positive for morphine, while only 1 of these 5 was also DOD positive for codeine. Four out of five of the subjects gave a specimen which was positive for morphine using the DOD cutoff value.

Ingestion of two poppy seed bagel ham and cheese sandwiches led to the results for the nine subjects shown in Figs. 3 and 4. The data for these subjects fell logically into two groups—Group 1 subjects displayed lower overall peak morphine urine concentrations (ranging from 129 to 452 ng/mL) than those subjects in Group 2 (peak morphine concentrations from 420 to 1456 ng/mL). The sheer number of subjects willing to participate in this phase of the experiment underscored their common motivation to dispel the adage that "there is no free lunch."

Poppy seed muffin ingestion by the seven subjects did not give rise to significant levels of morphine or codeine in postingestion specimens. Data for the three male subjects (two muffins each, except Subject T, who ate three muffins) are displayed in Fig. 5. Figure 6 displays the female data (one muffin each). Peak morphine concentrations for the males ranged from 299 to 730 ng/mL, while those for the female subjects ranged from 152 to 629 ng/mL. Two of the subjects (T and V) did not reach peak urine concentrations of cross-reacting opiates until approximately 12 h postingestion. These extended excretion profiles were not observed in any other subjects in any other phase of the experiment.

The urinalysis results from deli roll ingestion were not surprising, given the relatively small amount of poppy seeds coated on the deli rolls and the likelihood that they would be dislodged during slicing prior to making the sandwiches. Figure 7 illustrates that there was no significant deviation from Negative responsiveness for any specimen from seven of the eight well-fed subjects. Even for Subject L, the "peak" opiate response represents

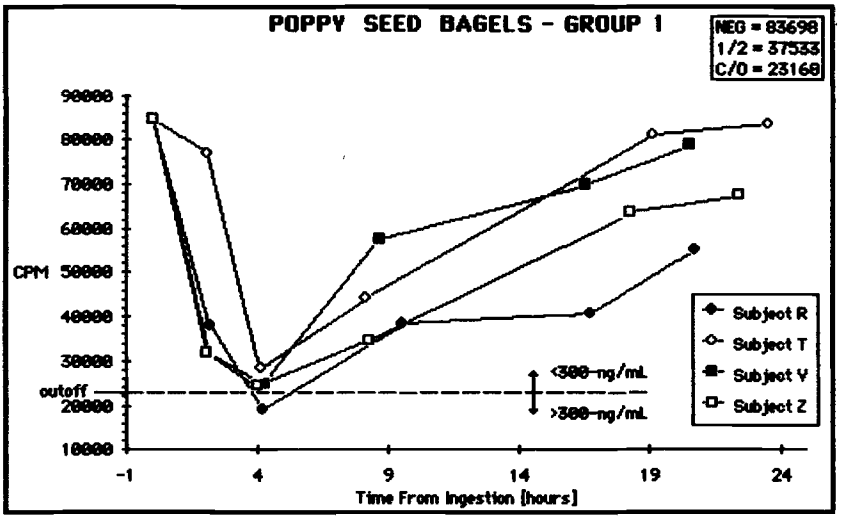

FIG. 3—*RIA results for specimens collected from four subjects following ingestion of two poppy seed bagel sandwiches.*

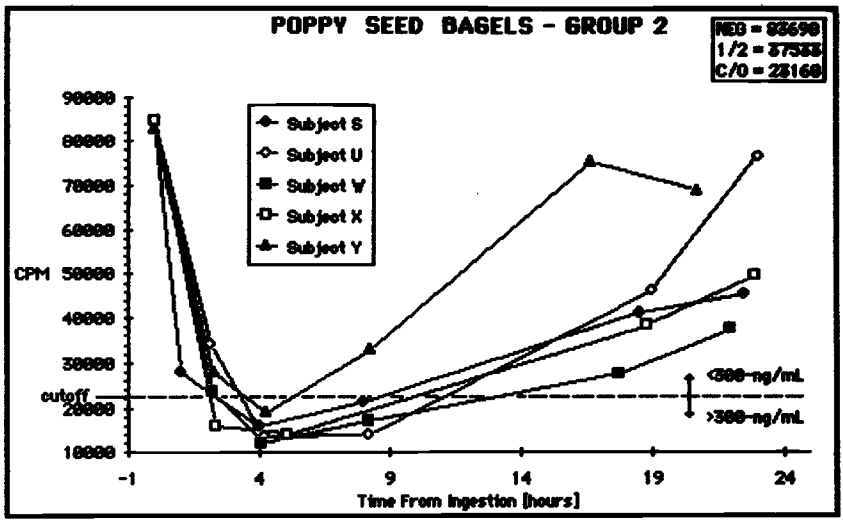

FIG. 4—*RIA results for specimens collected from five subjects following ingestion of two poppy seed bagel sandwiches.*

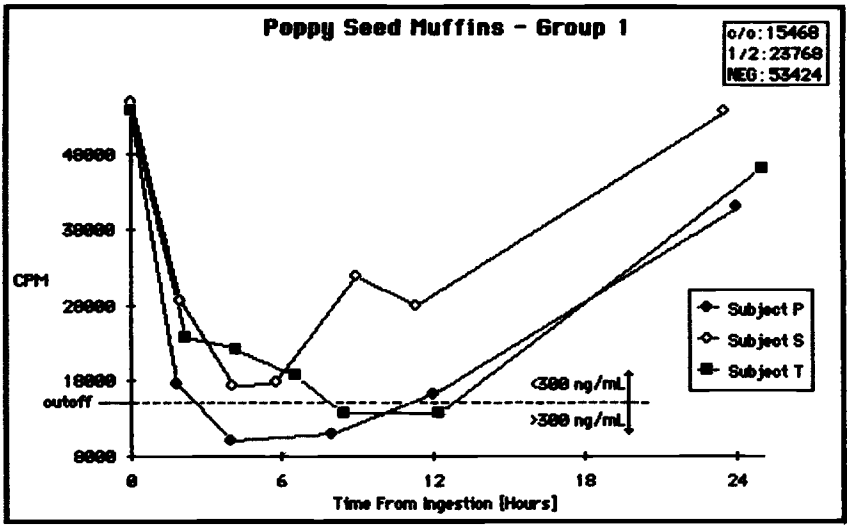

FIG. 5—*RIA results for specimens collected from male subjects following ingestion of two (Subjects S and T) or three (Subject P) poppy seed muffins.*

only a 12% deviation from the mean of the CPM values obtained for the 0-ng/mL morphine standards in the RIA batch.

"German style" ingestion of about 22 g of Schilling poppy seeds led to at least one NIDA confirmed morphine positive for only the two male subjects (see Fig. 8). The two female subjects had peak morphine concentrations of 282 and 207 ng/mL (for Subjects HH and MM, respectively), while the peak morphine levels measured for the two males were 565 and 571 ng/mL (for Subjects FF and JJ, respectively). There were no DOD positives recorded following "German style" ingestion, and all the subjects complained of the strange texture and taste of the food.

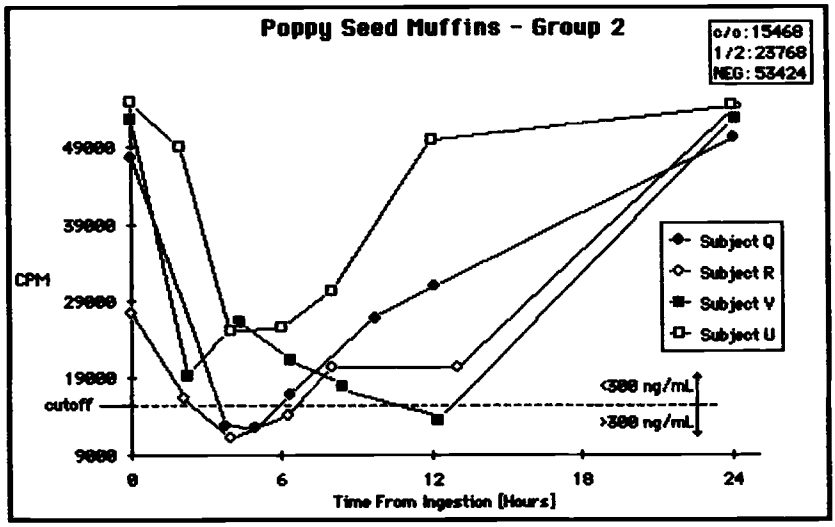

FIG. 6—*RIA results for specimens collected from four female subjects following ingestion of one poppy seed muffin.*

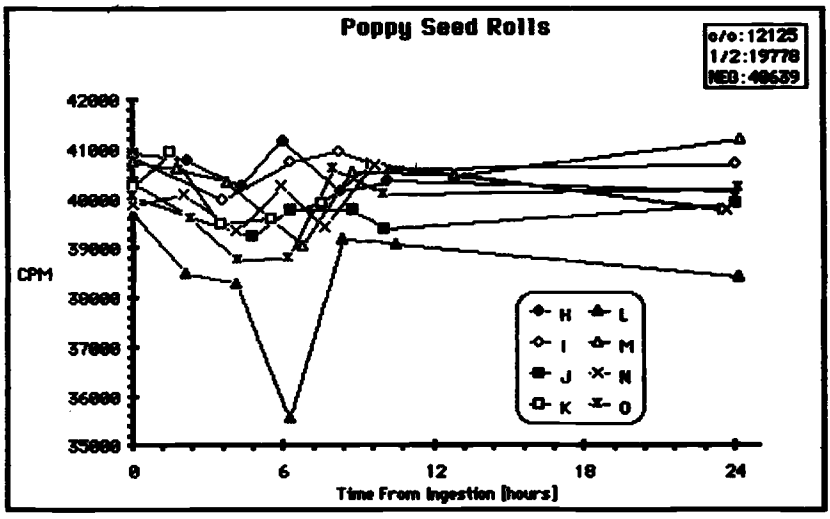

FIG. 7—*RIA results for specimens collected from eight subjects following ingestion of two poppy seed deli roll sandwiches.*

The original results with poppy seed streusel were so surprising that the study was repeated with four new subjects. The results, as shown in Fig. 9, mirror those observed in the first trial with this foodstuff (see Fig. 2). However, the extended specimen collection schedule of the second trial allowed continued evaluation of the urinary excretion of opiates from 24 to 72 hrs postingestion. By 72 h postingestion, three of the four subjects had returned to RIA negative status. The fourth subject (Subject II) was RIA positive and confirmed positive by NIDA standards at 471 ng/mL morphine.

In contrast to the first trial with poppy seed streusel, only a single specimen in the second trial (for Subject II) was confirmed positive for morphine by DOD cutoff levels (at 5159 ng/mL). The other three subjects had peak morphine concentrations of 1934,

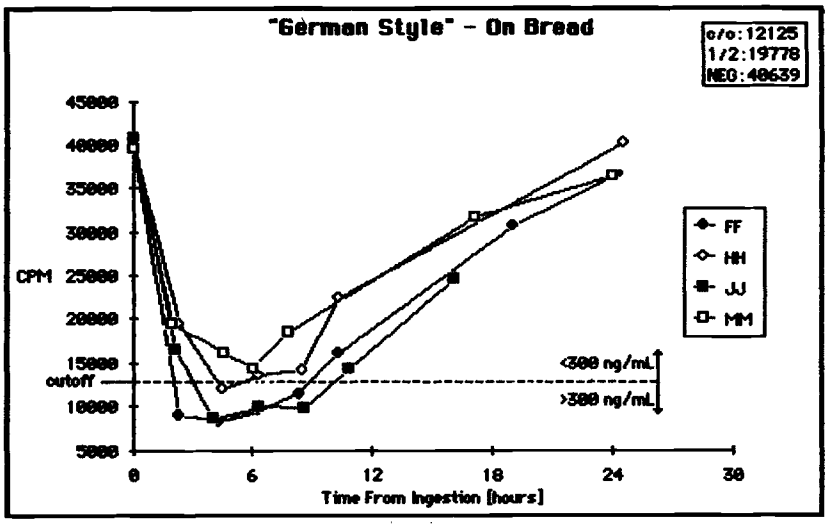

FIG. 8—*RIA results for specimens collected from four subjects following ingestion of approximately 2.5 g of poppy seeds "German style" (with butter or honey) on bread.*

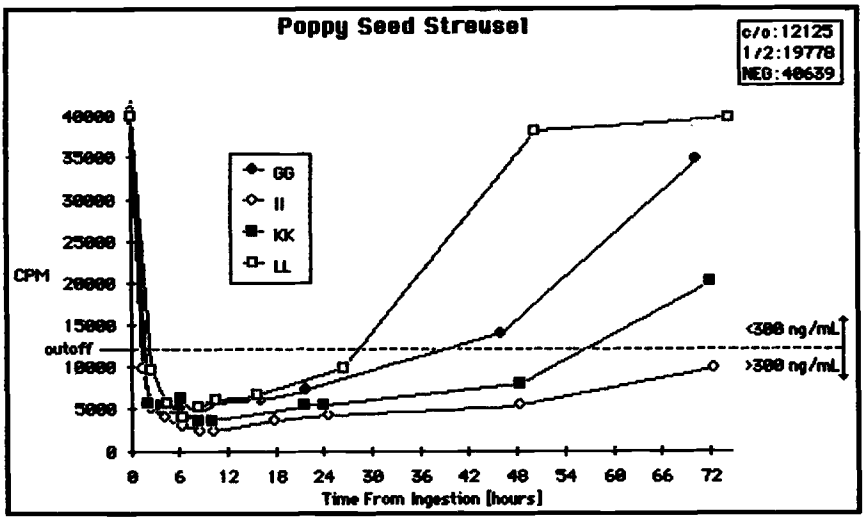

FIG. 9—*RIA results for specimens collected from four subjects following ingestion of two pieces of poppy seed streusel.*

2822, and 2578 ng/mL (for Subjects GG, KK, and LL, respectively). The highest codeine concentrations reached for these four subjects was 852 ng/mL, for Subject II.

The RIA data for postingestion samples for all poppy seed foods are summarized in Fig. 10. It is clear that even ingestion of products containing relatively small quantities of poppy seeds—for example, bagels (3 g of seeds per person) and muffins (3 to 9 g of seeds per person)—can lead to presumptive positive opiate urinalysis results. However, for all foodstuffs except poppy seed Danish pastry and streusel, the *likelihood* that such ingestion will lead to a confirmed morphine positive result by NIDA cutoff values (300 ng/mL) is not great, and it is nonexistent by DOD cutoff values (4000 ng/mL). The GC-MS results for specimens collected within 24 h of ingestion of poppy seed cake, bagels,

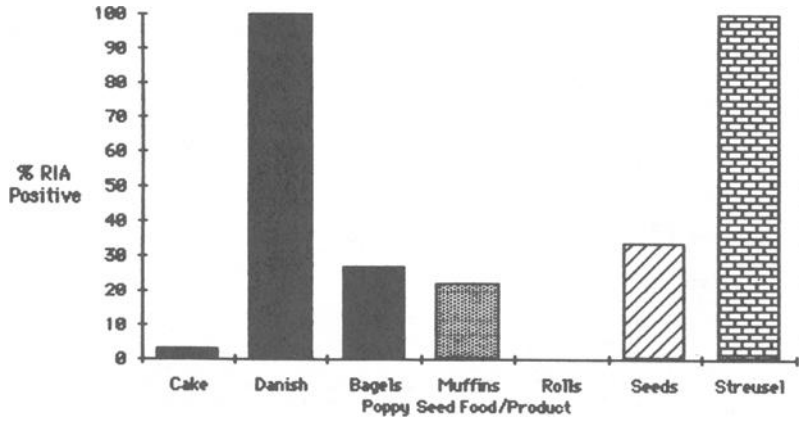

FIG. 10—*Summary chart of the percentage of specimens collected in the first 24 h postingestion which gave presumptive positive (≥ 300 ng/mL of cross-reacting opiates) RIA results, by food product.*

muffins, and raw seeds ("German style") are summarized in Fig. 11. The unshaded "NIDA positive" morphine quantitative zones for each foodstuff in Fig. 11 range from 0% (cake) to 82% (bagels) of the 24-h postingestion specimens.

GC-MS morphine concentrations for specimens collected in the first 24-h after ingestion of poppy seed streusel are summarized in Fig. 12. Every specimen was presumptively positive for opiates, and 100% were confirmed as NIDA morphine positive. Fifteen percent of the specimens collected within this first 24-h period were also confirmed positive using the DOD cutoff value (≥4000 ng/mL).

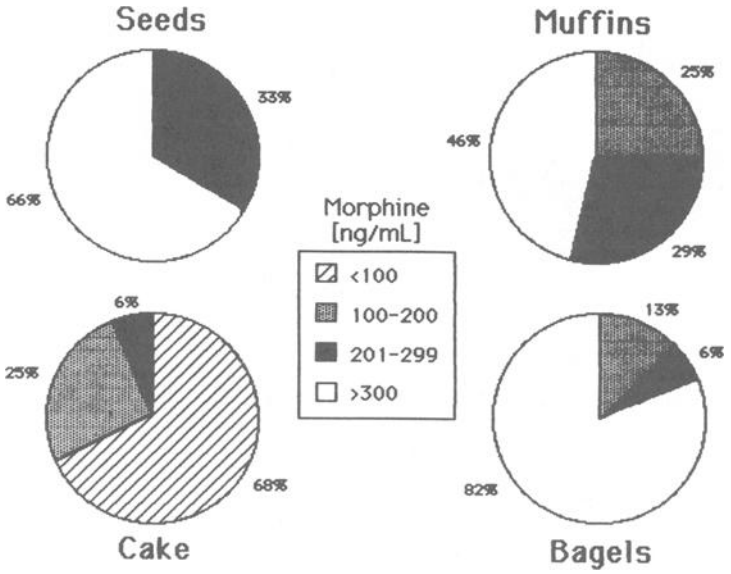

FIG. 11—*Summary pie charts displaying the morphine concentrations of specimens collected within the first 24 h postingestion of raw poppy seeds ("German style"), muffins, cake, and bagels. Percentages of the total number of specimens falling within each concentration range are provided in the margins of each pie chart.*

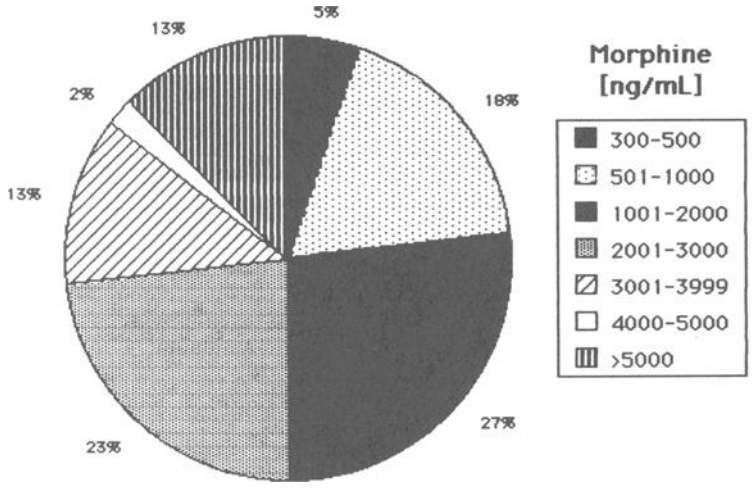

FIG. 12—*Summary of morphine concentrations determined in specimens collected within the first 24 h postingestion of poppy seed streusel. The percentages of the total number of specimens falling within each concentration range are provided in the margins of the chart.*

Table 1 summarizes the maximum peak concentrations of morphine and codeine determined in the subjects' postingestion specimens, as well as the postingestion "time window" within which NIDA positive levels for morphine may be expected for each of the poppy seed foods. Most of the findings of this study support the quantitative "source differentiation" guideline (of ≥5000 ng/mL for morphine) previously espoused by ElSohly and Jones [*9*]. However, 13% of the specimens collected in the first 24 h after ingestion of poppy seed streusel had concentrations above this guideline value. In addition, a significant number of these specimens contained codeine in concentrations greater than the 300-ng/mL "source differentiation" quantitative guideline [*9*]. There was complete agreement on the morphine-to-codeine ratio guideline, in that no specimen had a morphine-to-codeine ratio <2.

In the months of rotational testing of active duty U.S. Army and Air Force members for opiates at the Hawaii laboratory, all but one of the reported opiate positive specimens contained either codeine alone or codeine in a greater concentration than morphine. These testing results are consistent with the use or abuse of codeine and would not be confused with the results obtained for a poppy seed-ingesting service member. In the

TABLE 1—*Summary of the maximum concentration (C_{max}) of morphine and codeine determined after ingestion of each poppy seed food; the collection time at which the last RIA positive was detected for each food is provided as well.*

Food/Product	C_{max} Morphine, ng/mL	C_{max} Codeine, ng/mL	Last RIA +, h
Cake	260	not detected	5
Danish	11 571	4861	24+
Bagels	1 456	159	8
Muffins	730	21	12
Rolls	N/A	N/A	N/A
Seeds	571	not detected	8
Streusel	5 159	852	72+

[a]N/A = not analyzed by GC-MS.

single specimen containing >4000 ng/mL morphine and trace codeine, subsequent testing did not reveal the presence of 6-monoacetyl morphine. Therefore, it is possible that the laboratory findings for this specimen were caused by ingestion of a concentrated poppy seed food within 24 h of the collection.

The present research results argue against the unqualified application of the previously published quantitative guidelines [9] for eliminating poppy seed ingestion as a possible cause for a positive opiate urinalysis result. All poppy seed foods can lead to NIDA opiate positive specimens, and realistic ingestion of some foods can lead to DOD opiate positives, despite the administratively elevated confirmation cutoff values. This fact underscores the importance of the medical review officer in fashioning the correct administrative or judicial response to a positive opiate laboratory result.

Acknowledgments

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