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Optimized LC/MS/MS Analysis of Morphine and Codeine in Poppy Seed and Evaluation of Their Fate during Food Processing as a Basis for Risk Analysis

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The opiate alkaloids present in poppy seed intended for use in food recently have raised major concerns. An efficient method for routine analysis of morphine and codeine using liquid chromatography in combination with tandem mass spectrometry on a triple quadrupole instrument (LC/MS/MS) was therefore developed. The optimal sample preparation was found to be cold extraction of 10 g of unground poppy seed with 30 mL of methanol containing 0.1% acetic acid for 60 min shaken at 250 rpm. The fate of morphine during food processing was also studied. All experiments led to a significant reduction of morphine and codeine. For poppy cake only 16−50% of the morphine was recovered, and in poppy buns at the highest temperature (220 °C) only 3% of the original morphine content was found. Ground poppy seed showed significantly lower recoveries than untreated seed. Morphine elimination during food processing has to be taken into account in the current discussion about its maximum limits in poppy seed.

KEYWORDS: Morphine; codeine; opiates; poppy seed; *Papaver somniferum* L.; liquid chromatography/tandem mass spectrometry (LC/MS/MS)

INTRODUCTION

The seeds of the opium poppy (*Papaver somniferum* L.) are commonly used in dishes and pastries in central Europe. The alkaloid content in the latex of the plant, with morphine as its major constituent, has raised major concerns. For this reason only the low-morphine variety Mieszko is certified for cultivation in Germany (*1*). However, Germany's annual poppy seed requirement for baking and food use of up to 10000 t is so far almost exclusively covered by imported goods. Important producer countries are Turkey, the Czech Republic, Hungary, and Austria (*2*).

The poppy seed itself does not contain latex and older (and even newer) botanical literature describes the seeds as alkaloid-free (*3*−*6*). In 1965 morphine and codeine were detected in the seeds by Preininger et al. (*7*) using thin-layer chromatography. The first quantitative study applying gas chromatography/mass spectrometry (GC/MS) was conducted by Grove et al. in 1976 (*8*) and trace amounts of morphine in the range from 0.6 to 2.3 mg/kg were detected in commercial samples. Starting in the 1980s a large number of studies were conducted due to concerns that poppy seed consumption might lead to positive drug tests (*9*−*29*). Internationally published morphine contents of poppy seed were recently reviewed in the works of Moeller et al. (*25*), Rochholz et al. (*2*), and Westphal et al. (*29*). The alkaloid content of poppy seed varies over very large ranges, and morphine (0.1−620 mg/kg) and codeine (0.08−57.1 mg/kg) are usually detected in the seed (*2*, *25*, *28*, *29*). Besides the poppy variety, geographical origin, and time of harvest (*26*, *28*), external contaminations were given as the most likely cause (*2*, *8*, *25*, *26*). A change in harvesting technology was speculated as an explanation for high morphine contents in the last 20 years (*25*, *26*).

Because poppy seed is usually consumed in small quantities, e.g., poppy buns contain around 3 g of poppy seed, and regarding the past opinion that they are nearly morphine-free, it is not surprising that the European Union has so far established no maximum limits for morphine in poppy seed. Today, especially for babies, infants, and old or ill people, the consumption of large quantities of poppy seed can represent a health risk (*2*). Imported seed must be controlled by manufacturers and official food authorities regarding its content of alkaloids using efficient analytical methods.

Most methods for the determination of alkaloids in poppy seed apply gas chromatography-mass spectrometry (GC/MS) or liquid chromatography-diode array detection (HPLC/DAD) (*8*−*11*, *15*, *17*, *19*, *21*, *26*, *27*, *30*). All methods require an extensive extraction and cleanup step. The GC methods require an additional derivatization. HPLC-DAD is restricted by the similar chromatographic behavior of the minor alkaloids such as codeine and noscapine, as well as matrix interferences that

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Table 1. Variables and Ranges Used in the Experimental Designs for Method Optimization and To Evaluate Influences of Food Processing

	design type	no. of experiments	variables	name	levels
extraction expt 1	D-optimal	23	A	extraction time [min]	5, 30, 60
			B	sample pretreatment	not ground, ground
			C	agitation	ultrasonication, magnetic stirring
			D	solvent	aqueous acetic acid, methanolic acetic acid
extraction expt 2	D-optimal	16	A	extraction time [min]	30, 68, 105, 143, 180
			B	agitation	magnetic stirring, automatic agitation
extraction expt 3	one-factor	10	A	sample weight [g]	1, 5, 10, 15, 25, 30
poppy cake expt	full factorial	8	A	pretreatment	not ground, ground
			B	cooking time	short, long
			C	swelling time	short, long
poppy bun expt	D-optimal	11	A	baking temperature [°C]	50, 90, 135, 220
			B	pretreatment	not ground, ground

necessitate an SPE ion exchange step. Liquid chromatography in combination with tandem mass spectrometry on a triple quadrupole instrument (LC/MS/MS) is an efficient method for routine analysis because it commonly does not require extensive sample cleanup, and the high specificity of mass selective detection avoids matrix interferences and compensates for separation problems. There are a number of methods to determine morphine in forensic samples (*31*−*36*). The first LC/MS/MS method for the determination of morphine in poppy seed used as food was proposed by Trafkowski et al. (*28*). Our work presents a simplified LC/MS/MS procedure consisting of optimized cold extraction of the unground poppy seed with acidified methanol and improved chromatography with alkaline gradient system on pH-stable HPLC column.

The observation that a case of intoxication after consuming poppy cake with even quite high poppy contents has never been reported to our knowledge leads to the question of whether the alkaloid content changes during food processing, e.g., grinding, swelling, heating, cooking, or baking. This work studies the fate of morphine in poppy cake and poppy buns, the two best-liked poppy foods, to be able to evaluate the actual exposure.

MATERIALS AND METHODS

Samples. A total of 83 poppy seed samples and 12 baking mixes containing poppy seed submitted to the CVUAs Karlsruhe and Stuttgart were analyzed for morphine, codeine, papaverine, and noscapine. The samplings were conducted by local authorities, either directly from the manufacturers and importers or from the retail trade.

Reagents and Materials. Methanol (>99.8%), ammonia solution (25%), morphine hydrochloride, and codeine phosphate hemihydrate were purchased from Merck (Darmstadt, Germany). Morphine-d_3 (deuterated at $=N-CD_3$; 100 mg/mL in methanol), ammonium hydrogen carbonate, acetic acid (99−100%), noscapine hydrochloride, and papaverine hydrochloride were obtained from Sigma-Aldrich (Taufkirchen, Germany). Disposable syringe filters with a pore width of 0.2 μm (Chromafil PET-20/25) were from Macherey-Nagel (Düren, Germany).

Liquid Chromatography and Tandem Mass Spectrometry. The LC/MS/MS system consisted of an Agilent (Waldbronn, Germany) 1100 HPLC system (binary pump, degasser, and autosampler) coupled with a Thermo Finnigan (Dreieich, Germany) TSQ 7000 mass spectrometer. LC separation was performed on a reversed phase Phenomenex (Aschaffenburg, Germany) 150 × 2 mm i.d., 3 μm, RP18 Gemini column thermostated at 40 °C using mobile phase A (water, 20 mM ammonium hydrogen carbonate, adjusted with ammonia to pH 9) and mobile phase B (water/methanol 5:95 (v/v), 20 mM ammonium hydrogen carbonate, adjusted with ammonia to pH 9) in a gradient program with a flow of 0.2 mL/min: 0−1 min, 60% A; 1−3 min, 60% A to 5% A; 3−9 min, 5% A; 9−10 min, 5% A to 60% A; 10−15 min, 60% A. Electrospray ionization (ESI) in positive ion mode used a capillary temperature of 280 °C and a spray voltage of 2.8 kV. The sheath gas was nitrogen at 70 psi. Argon was used as collision gas; the collision cell of the triple quadrupole was operated with an offset voltage of −45 eV for all transitions. For quantitative analysis the following fragmentations with the highest intensity were monitored in the selected reaction monitoring (SRM) mode: *m*/*z* 286 → 153 and *m*/*z* 286 → 165 for morphine, *m*/*z* 289 → 165 for morphine-d_3 as the internal standard, *m*/*z* 300 → 165 for codeine, *m*/*z* 414 → 220 for noscapine, and *m*/*z* 340 → 202 for papaverine. For qualitative confirmation the following fragmentations were recorded as qualifiers: *m*/*z* 286 → 181, *m*/*z* 286 → 185, *m*/*z* 286 → 152, and *m*/*z* 286 → 201 for morphine, as well as *m*/*z* 300 → 153, *m*/*z* 300 → 171, *m*/*z* 300 → 199, and *m*/*z* 300 → 209 for codeine. For detailed information about the electrospray fragmentation the studies of Raith et al. (*37*) and Poeaknapo et al. (*38*) are recommended.

Sample Preparation. A portion of 10 ± 0.001 g of poppy seed was placed into a 100 mL glass flask with screw cap, mixed with 30 mL of extraction solvent (methanol with 0.1% acetic acid), and the flask was immediately sealed. The flask was agitated for 60 min in an automatic shaker at 250 rpm. After that, the supernatant liquid was filtered through the disposable syringe filters. Two hundred microliters of the extract were diluted with 700 μL of methanol and 100 μL of morphine-d_3 solution as an internal standard (10 mg/L). In the case of very high morphine contents, the extracts were diluted with extraction solvent before adding the internal standard. One microliter of the extract with added morphin-d_3 solution was injected into the LC/MS/MS system.

Method Optimization. To find the optimal working settings with a minimum amount of experiments, the information gleaned from each experiment and the relationships between the experiments have to be fully exploited. This was done with D-optimal designs (*39*, *40*). The D-optimal algorithm was used because it chooses an ideal subset of all possible combinations and significantly reduces the number of required experiments compared to standard design types. The experiments, parameters, and chosen ranges of variables are shown in **Table 1**. The ground samples were prepared with a Knife Mill Grindomix GM 200 (Retsch, Haan, Germany) using 100 g of sample for 30 s at 8500 rpm. To additionally study the effect of grinding, 37 samples were analyzed before and after grinding.

Validation Studies. To validate the method, commercial poppy samples were extracted and analyzed several times intraday ($n = 7$) and interday ($n = 5$) using the optimized procedure given above. To determine accuracy, samples were spiked with different morphine concentrations. The calibration curve linearity was evaluated between 0.1 and 5 μg/mL (morphine and codeine) or between 0.02 and 1.0 μg/mL (papaverine and noscapine). The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the regression line residual standard deviation using a real matrix of ground and homogenized poppy seed (*41*, *42*).

Food Processing Experiments. The fate of morphine and codeine in poppy seed foodstuff was evaluated using experimental designs with

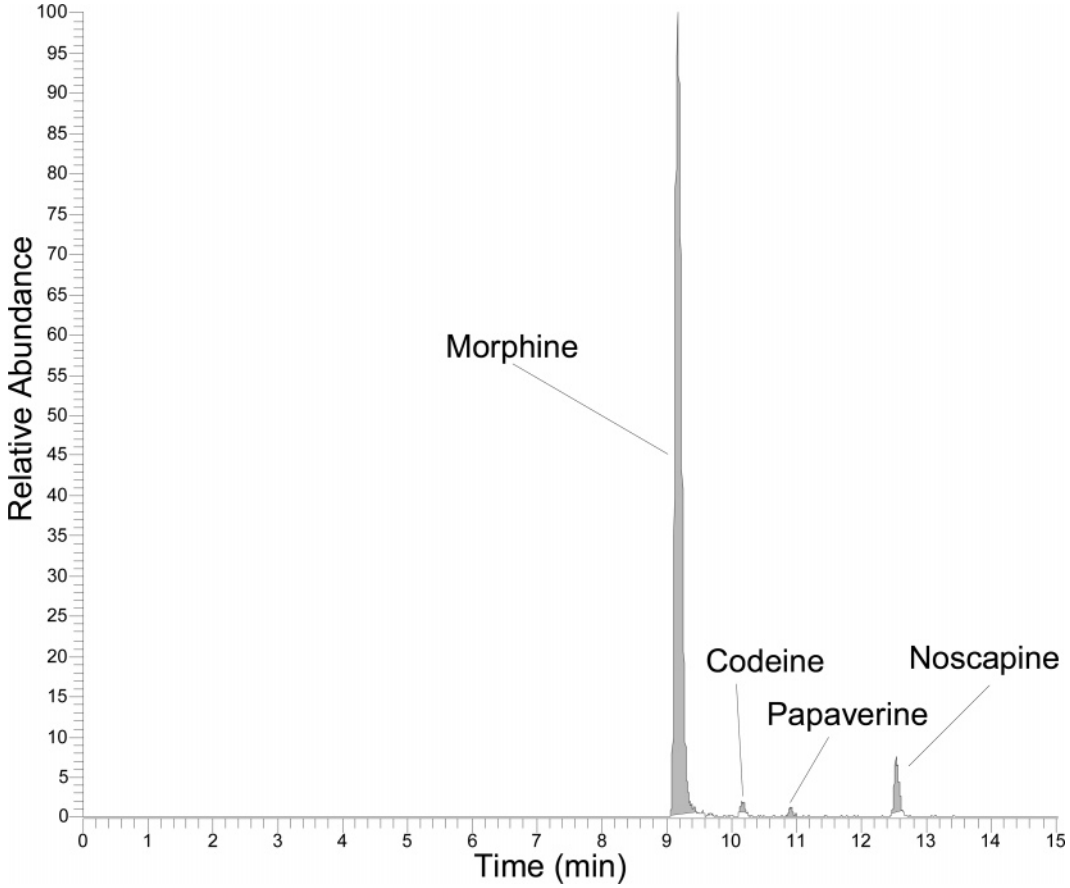

Figure 1. Typical LC/MS/MS (SRM) chromatogram of a poppy seed sample with 11 mg/kg of morphine. Codeine, papaverine, and noscapine were below the limit of quantitation.

different processing conditions. Poppy cakes were prepared by a standard German recipe that required the usually ground seed to be cooked in sugared milk, using different cooking times. The poppy filling was then left to sit for some time (swelling time) and then filled into a cake dough. The influences of cooking time, swelling time, and seed grinding were studied by a full factorial model (**Table 1**). To achieve the highest comparability, little cakes were prepared in muffin baking pans. Ten grams of poppy seed (unground or ground) were cooked (1 or 15 min) with 5 g of sugar and 10 mL of milk, and at least 5 g of full egg was added. After that the filling was cooled in a refrigerator (short swelling time) or left to sit overnight (long swelling time). Then the filling was put into a baking pan lined with shortcrust, and the cakes were baked at 180 °C for 20 min. After cooling, the complete homogenized cakes were extracted as described above.

The second experiment was designed to study poppy seed as a baking topping, e.g., for poppy buns. Unground or ground seed was roasted in an oven for 20 min at temperatures between 50 and 220 °C according to a D-optimal design (**Table 1**).

Statistics. The experimental designs and calculations were done using the Software Package Design Expert V6 (Stat-Ease Inc., Minneapolis, MN). The experiments were evaluated using Analysis of Variance (ANOVA) to find the variables' significance and their interactions in the models. The models were checked for consistency by looking at the lack of fit and possible outliers.

RESULTS AND DISCUSSION

Analysis and Validation Results. The morphine content of 83 poppy seed samples ranged from concentrations below the limit of quantitation (<1 mg/kg) to 270 mg/kg. Baking mixes contained morphine between undetectable concentrations (<0.3 mg/kg) and 4 mg/kg. Seventy poppy seed samples (84%), but none of the 12 ready-for-use baking mixes, exceeded the provisional guidance value of 4 mg/kg proposed by the German Federal Institute for Risk Assessment (*43*). In all cases, codeine was detected in lower concentrations than morphine up to 56 mg/kg in poppy seed and was not detectable in baking mixes. Noscapine and papaverine were detected in isolated cases but were mostly below the limit of quantitation. Only five samples showed noscapine contents up to 2.1 mg/kg. A typical chromatogram of a poppy seed sample is shown in **Figure 1**. All alkaloids exhibited good linearity with regression coefficients greater than 0.99. The detection limits obtained ranged between 0.07 mg/kg for papaverine as well as for noscapine and 0.3 mg/kg for morphine as well as for codeine. The precision resulted in ranges between 7.4 and 9.0%, and the accuracy was between 9.8 and 17.6%.

Method Optimization Results. The first extraction experiment gave a statistically significant model. The regression coefficients were determined and the statistical ANOVA approach calculated the individual significance of each coefficient. The largest influence on the extraction was the sample pretreatment. The ground samples had significantly lower morphine contents than the untreated ones. This effect was confirmed by further analysis of 37 samples. The morphine loss due to grinding was $34 \pm 5\%$ ($p < 0.001$).

The extraction time, as well as the interaction between pretreatment and solvent, significantly influences the models for morphine and codeine. This interaction can be explained by the fact that the use of aqueous solvent with ground poppy leads to emulsions, which are not observed using methanolic solvent. By using Myers' and Montgomery's desirability function (*44*), a numerical optimization was calculated. The desirability is calculated by simultaneously optimizing multiple responses and ranges from 0 to 1 (least to most desirable,

respectively). For extraction the goal was defined to be the maximum extraction yield for morphine. The best setting, with a desirability of 0.91, used 60 min as the extraction time, an untreated sample, magnetic stirring, and a methanolic solvent. Because this optimal extraction time was at the upper limit of the range tested, the second extraction experiment was conducted to evaluate higher extraction times. In this case a significant model could not be fitted to the data. The overall average is the best estimate of the morphine and codeine concentrations. Neither a variation of extraction time between 30 and 180 min, nor the mode of agitation, had a significant influence. Agitation by the automatic shaker was preferred for reasons of handling, as it can simultaneously agitate a number of samples.

The third and last extraction experiment evaluated the influence of sample weight. The sample weight, too, had no significant influence on the determined alkaloid concentrations. The replications at 1 g, however, showed a higher standard deviation than those above 5 g of sample weight. This might be mainly attributable to sample inhomogeneities and a higher weighing error at lower sample weights.

Food Processing Results. The results of the food processing experiments were calculated as percentages of the original morphine content before food processing. All experiments lead to a significant reduction of morphine and codeine. For poppy cake, only 16−50% of morphine and 10−50% of codeine were recovered, in the poppy bun experiment at the highest temperature only 3% of morphine and 7% of codeine were found. Pretreatment had a significant influence in all cases: ground poppy seed showed lower content than untreated seed. The poppy bun experiment proved significant quadratic influence of baking temperature, which means that at first the reduction is relatively low up to 135 °C (around 30%), but at 220 °C a reduction of 80−90% was determined.

Method Optimization and Influence of Grinding. The only systematic study of the extraction of alkaloids from *Papaver* was conducted by Yoshimatsu et al. (*30*). Various aqueous solutions (water, acetic acid, hydrochloric acid, and sodium citrate buffer) or mixtures of these solutions with alcohol were compared. The highest amount of morphine was obtained with 5% acetic acid, and the amount increased after adding ethanol to the solution. In a number of studies methanol was used for the extraction of morphine and codeine (*2, 12, 29*). Based on these preliminary findings, our experiments showed methanolic acetic acid as being the best extraction medium. Hot extraction with citric acid, proposed by some authors (*10*), did not show any advantages over cold extraction with methanolic acetic acid. Both methods were comparable, but extraction with citric acid led to emulsions, requiring further cleanup (e.g., SPE).

For the first time, influence of grinding on the morphine and codeine content was systematically studied. In consideration of the studies of Grove et al. (*8*) and Lo and Chua (*17*) postulating so-called "bound morphine" inside the seed, we did not expect to find significantly lower concentrations in ground seed than in unground seed. Lo and Chua determined the morphine content in two steps: an initial methanolic extraction of the untreated seed was followed by grinding and acidic extraction. It is possible that the first methanolic extraction did not completely extract the morphine and that the remaining morphine content was misleadingly interpreted as "bound morphine". An explanation for the reduction of morphine after grinding can be found in the studies of Schenk et al. (*45*−*47*). Decreases up to 15% were reported due to the oxidation of morphine under the influence of phenol oxidase in combination with transfer agents such as caffeic acid. The degradation of pharmaceutical morphine was recently reviewed by Vermeire and Remon (*48*). They concluded that degradation of morphine is accelerated in the presence of oxygen and at higher pH, whereas temperature and light have only a minor influence on the degradation rate. The influence of oxygen, leading to the formation of pseudomorphine and morphine-*N*-oxide, can especially be assumed in the case of grinding that leads to large active surfaces.

The present study showed that the total morphine content can be determined easily by direct extraction with methanol/acetic acid with no sample pretreatment. The original alkaloid content and the consumer's actual exposure are detected in this way. The optimized extraction method provides extracts with low matrix interferences and does not form emulsions. Measurement with LC/MS/MS requires no sample cleanup at all. In comparison to the method of Trafkowski et al. (*28*) that consisted of grinding in a mortar, preparation of a slurry with buffer solution, and subsequent ultrasonication, the developed procedure is simpler to perform. The chosen conditions are very robust, and the experimental designs show that slight deviations (e.g., of extraction time) have no influence on the accuracy of the results.

Origin of Alkaloids in Poppy Seed. Besides the poppy variety, the method of harvest has the highest influence on the morphine concentrations and leads to the great variability of the alkaloid concentration (*25*). Poppy seed harvest can take place in two ways. Traditionally, the ripe seed is manually shaken (especially where manual labor is cheap) so that the seed falls out of the holes below the many-rayed stigma. In contrast, the high-yield closed-capsule plants have to be crushed open using modified combine harvesters sealed against loss of fine seed (*2, 49*). The latter procedure contaminates the seed with the chyle of unripe capsules, which has to be removed afterward. Otherwise, a higher alkaloid content will result (*26*). This is also proven by the fact that washing the poppy seed can drastically reduce its morphine content (*8, 9, 17, 21, 26*).

This study confirms the observations that poppy seed morphine originates predominantly from external contamination. The nonuniformity of this contamination leads to inhomogeneities in the collective sample and explains the precisions of 7.4−9.0% as well as the requirement of relatively large sample weights.

We did not find any proof of "bound morphine" but in this case our methodology was restricted by the oxidative effects described above. At the very least we can conclude that if "bound morphine" does, in fact, exist, its concentration will be so low that it is directly decomposed if the seed is crushed. Its relevance for the consumer can therefore be neglected, compared to the total morphine content due to contamination. This can be confirmed by a number of seed-washing experiments that significantly reduced the morphine content (*9, 17, 21, 26*). Bjerver et al. (*9*) showed that 40% of the total morphine can be removed by a single washing with slightly acidified water. Soaking poppy seeds in water for 5 min was found to remove about 45.6% of their free morphine and 48.4% of their free codeine (*17*).

High morphine concentrations in the seed can be attributed to insufficient harvest technology, leading to seed contamination with morphine rich latex or to inadequate cleaning and handling. Considering lot-to-lot differences and inhomogeneities in the sampling process, manufacturers were advised of their duty to exercise diligence and to use state-of-the-art measures to limit the seed morphine content. Washing, in combination with blending different batches, would be an adequate way for manufacturers to reduce the poppy seed morphine content.

Adequate control of every batch is required, and batches with very high morphine content should be rejected.

Fate of Opiates during Food Processing. The influence of food processing on the alkaloid content of foodstuff prepared from poppy seed has not yet been systematically studied. Meadway et al. (*21*) reported the first observation of differences in opiate concentration between cooked, sieved, and untreated seed specimens. They concluded that the method of seed preparation influenced the alkaloid concentrations. Brenneisen and Borner (*50*) reported the results of a single baking experiment. In black poppy seed from the food trade they measured 0.002% morphine before processing and only 0.0002% morphine after baking the seed used as topping on a poppy bun. During the baking process, 90% of the morphine was therefore destroyed. Our statistically designed experiments confirmed the significant reduction of morphine during food processing. Mechanical pretreatments such as grinding, as well as heat treatment, were found to have the greatest influence on morphine reduction. These findings may explain the fact that no intoxications were reported from the consumption of cake or buns with poppy seed. The cake recipes bakeries use demand grinding to improve the aroma of the product. Cakes and buns are then baked at high temperatures around 200 °C. These processes make a decrease of the morphine content by at least 80% possible. Our results were recently confirmed by investigations of the food industry. Kniel (*51*) reported results of analyses made on different stages during the manufacture of baking mixes. A median of 6.8 mg/kg was determined in the original poppy seeds. A significant reduction was found in the convenience baking mixes that are manufactured including grinding and heating steps (median 3.9 mg/kg). In the finished products found in bakeries and supermarkets, morphine could not be detected anymore (<1 mg/kg).

Consumers and bakeries should be advised to wash poppy seeds with water before direct use or before grinding the seeds prior to baking at preferably high temperatures.

Intoxications Due to Poppy Seed. Consumption of strongly contaminated poppy seed can lead to detectable contents of free morphine in blood as well as measurable concentrations in urine, sometimes for many days (*2, 24, 29*). Until now, the idea that poppy seed could serve as the source of appreciable amounts of morphine was not given much credence despite the old European custom that recommended quieting a noisy baby with a poppy seed filled pacifier (*10*). In fact, older literature from the 19th century reported isolated cases of accidental morphine poisonings of infants (*52, 53*).

A recent case reported by the Federal Institute for Risk Assessment confirms that old home remedies to encourage infants to sleep through the night are still used today (*54*). A mother had given her six-month-old infant the strained milk of baking poppy seed with the very best intentions of helping it sleep better. She had taken the recipe from a cookbook. Just a few hours later the infant had to be taken by ambulance to a hospital. The child was suffering from breathing disturbances; it was not fully conscious and scarcely reacted at all to pain stimulus. Because of the threat of respiratory arrest, the infant had to be ventilated with an oxygen mask. Because of the suspicion of opiate poisoning, the child was given an antidote. A urine test revealed high levels of the alkaloids morphine and codeine, confirming the suspicion. The mother had given her child 75 mL of strained milk made from a mixture of 200 g of poppy seed containing 1000 mg/kg of morphine in 500 mL of milk. This home remedy had even recommended the 2-fold amount of 400 g of poppy seed. This case prompted the Federal Institute for Risk Assessment to warn against using home remedies with poppy seed. Because of their qualitative fluctuations, baking poppy seed may contain differing amounts of the alkaloids morphine and codeine. These alkaloids may lead to serious health damage in infants, ranging from breathlessness to respiratory arrest.

Symptoms such as "dim feelings in the head", vomiting, and hangover-like feelings on the next day were reported in a recent case submitted to the CVUA Stuttgart. The consumer had eaten a pasta dish (spaghetti) strewn with a mixture of poppy seed and sugar. Approximately 75 g of poppy seed containing 210 mg/kg of morphine and 39 mg/kg of codeine were consumed, corresponding to dosages of 16 mg of morphine and 3 mg of codeine.

Risk Analysis and Proposal of Maximum Limits. The intoxication cases and the fact that poppy seed consumption might cause positive urine and blood tests for drugs-of-abuse have led to a discussion about maximum limits for morphine in poppy seed. In a toxicological study by the Bavarian State Office for Health and Food Safety, poppy seed with a morphine content of less than 10 mg/kg was regarded as safe if consumed in usual quantities (*55*). The Federal Institute for Risk Assessment derived, from the lowest pharmaceutically active dosage of 31.7 μg of morphine/kg of body weight under inclusion of a safety factor of 5, a provisional tolerable daily upper intake level of 6.3 μg/kg of body weight. Taking into account the estimated maximum consumed amount of 100 g/day, a provisional guidance value for poppy seed of 4 mg/kg was derived (*43*).

Both studies did not consider the influences of seed preparation or food processing in their establishment of guidance values. About 85% of all analyzed samples exceed the provisional guidance value. Seed cleaning or blending would also not be adequate in most cases to comply with the guidance value. With regard to our results, the relatively low guidance values are inadequate in reference to the real potential risk of processed poppy seed. The intended purpose must therefore be regarded in evaluation of poppy seed. For raw, untreated poppy without any warning notice labelings, the guidance value of 4 mg/kg has to be used because direct consumption, e.g., as milk extract, cannot be ruled out. The morphine ingestion via poppy buns, the most frequent application of poppy seed in Germany, makes only a small contribution to the total morphine exposure due to food, as only 1−4 g of poppy seed is used per bun. Based on this very small consumed amount and the reduction during baking, the poppy seed could contain up to 100 mg/kg of morphine without even nearing the tolerable daily intake. Appropriate labeling for the intended use (e.g., "only for decoration" or "not for direct consumption") would be required in this case. Relevant morphine quantities can be consumed only in products with poppy fillings. For cake making, ground poppy seed is cooked in milk and this mixture is baked with additional ingredients. The usual recipes prescribe between 10 and 30% of poppy seed in the cake. One piece of cake in German bakeries and pastry shops weighs 150−200 g. Considering the morphine elimination during baking and a maximum consumed amount of 2−4 pieces of cake, a guidance value of 20 mg/kg can be estimated for poppy seed intended for baking.

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Supporting Information Available: Method validation results; regression coefficients for extraction and food processing experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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