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Abstract: Poppy seed containing foods are popular dishes in Hungary and some other Central-European countries. The alkaloids of poppy are used in the production of medicines. Poppy seeds used as food may also contain considerable amounts of alkaloids, which raise the question of food safety. Morphine, codeine, thebaine and noscapine concentrations of poppy seed samples from the time period 2001-2010, and consumption data from two Hungarian surveys, carried out in 2003 and 2009 were evaluated. Exposure calculations were made for morphine intake both by point estimate and probabilistic methods, and the uncertainty of the calculated values was estimated. The point estimate for the acute consumer exposure, calculated using the 97.5 percentiles of morphine concentration and of poppy seed consumption and taking into account the reduction of morphine content by processing, was 78.64 µg(kgbw)⁻¹day⁻¹ for adults, and 116.90 µg(kgbw)⁻¹day⁻¹ for children. Based on probabilistic estimations, the 97.5 and 99 percentile exposures ranged between 18.3-25.4 and 25.6-47.4 µg(kgbw)⁻¹day⁻¹ for adults, and 32.9 and 66.4 µg(kgbw)⁻¹day⁻¹ for children, respectively. As no effect level (NOEL) has not been established, the significance of exposure could not be assessed.
Exposure of consumers to morphine from poppy seeds in Hungary

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Abstract

Poppy seed containing foods are popular dishes in Hungary and some other Central European countries. The alkaloids of poppy are used in the production of medicines. Poppy seeds used as food may also contain considerable amounts of alkaloids, which raise the question of food safety. Morphine, codeine, thebaine and noscapine concentrations of poppy seed samples from the time period 2001-2010, and consumption data from two Hungarian surveys, carried out in 2003 and 2009 were evaluated. Exposure calculations were made for morphine intake both by point estimate and probabilistic methods, and the uncertainty of the calculated values was estimated. The point estimate for the acute consumer exposure, calculated using the 97.5 percentiles of morphine concentration and of poppy seed consumption and taking into account the reduction of morphine content by processing, was 78.64 µg (kg bw)^{-1} day^{-1} for adults, and 116.90 µg (kg bw)^{-1} day^{-1} for children. Based on probabilistic estimations, the 97.5 and 99 percentile exposures ranged between 18.3-25.4 and 25.6-47.4 µg (kg bw)^{-1} day^{-1} for adults, and 32.9 and 66.4 µg (kg bw)^{-1} day^{-1} for children, respectively. As no effect level (NOEL) has not been established, the significance of exposure could not be assessed.

Keywords: consumers’ exposure, probabilistic modelling, point estimate of exposure, poppy seed alkaloids

Introduction

Foods made of poppy seeds are popular in Hungary. However, their natural alkaloid content can be of concern. The poppy plant (*Papaver somniferum* L.) contains several alkaloids, for instance morphine, codeine, noscapine and thebaine. These are important substances in the

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production of medicines. Poppy plant varieties are grown for industrial and dietary purposes as well. Published literature indicates that poppy seeds contain only very low or non-detectable levels of alkaloids (Lachenmeier et al 2010), but the seeds may be contaminated during harvest. The poppy seeds from the plants cultivated for industrial alkaloid production in Hungary are allowed for human consumption only after proper cleaning. Occasionally, the poppy seed marketed as food ingredient could contain high amounts of the alkaloids. During the past five years, the poppy seed was subject of increased research, particularly regarding the reduction of its morphine contents. This was the result of a serious intoxication case of an infant in 2005 in Germany (Lachenmeier et al 2010).

The consumption of large amounts of highly contaminated poppy seeds may lead to light-headedness and enteroparesis in sensitive individuals. The symptoms described are in agreement with the toxicological actions of morphine. In particular, reports indicate that morphine intake from poppy seeds may be on the scale of therapeutic morphine doses (Battilani et al. 2009). In Hungary, official authorities have recorded data of illnesses caused by poppy seed consumption since 1985. The symptoms ranged from paleness to nausea, vomiting, diarrhoea, headache, stomach cramps, trembling, flushing, bitter sensation, stupor, dizziness, drowsiness, shivering, depression and itchy skin. Ground raw poppy seed was consumed in all cases. However, the amount eaten was not recorded (unpublished report of National Institute for Food and Nutrition Science 2006).

After ingestion, morphine is relatively quickly absorbed from the gastrointestinal tract, mainly from the upper small intestine and, to a lesser degree, from the stomach. The maximum effect of morphine is achieved about 30 min after ingestion. The effect of a single dose lasts about 4-6 hours (Federal Institute for Risk Assessment 2005). Morphine is metabolized mainly in the liver and undergoes conjugation with glucuronic acid principally at the 3-hydroxyl group.

Morphine is excreted in urine mainly as morphine-3-glucuronide. In addition to the 3,6diglucuronide, other minor metabolites that have been identified include normorphine and the 3-ethereal sulfate (Battilani et al. 2009). Secondary conjugation also occurs at the 6-hydroxyl group to form the 6-glucuronide, which is pharmacologically active, and to a limited extent the 3,6-diglucuronide. The low bioavailability (20% - 40%) in the case of oral morphine administration can be attributed to elimination through metabolism in the intestinal mucosa and liver (Battilani et al. 2009).
There is no internationally accepted reference point for the toxicity of poppy seed alkaloids. On national level, the German Federal Institute for Risk Assessment (BfR) estimated 6.3 µg (kg bw)^{-1}day^{-1} as a provisional daily upper intake level of morphine from poppy seeds in 2005, based on the lowest oral therapeutic dose of 1.9 mg morphine (2.5 mg morphine sulphate pentahydrate) per person and a safety factor of 5 (Federal Institute for Risk Assessment 2005). The lowest starting oral dose administered to adults with normal kidney function for pain killing is 2x30 mg/day and 2x10 mg/day for patients with restricted renal diuretic capacity (Embey-Isztin 1996). Taking into account the lowest 10 mg single dose, it is about 5 times higher than the oral therapeutic dose used in BfR study. In the European Union, only Hungary has a national legislation (17/1999 EüM rendelet 1999) that specifies the maximum permitted levels at 30 mg kg^{-1} for morphine, 40 mg kg^{-1} for morphine and noscapine, 20 mg kg^{-1} for noscapine, 20 mg kg^{-1} for codeine and 20 mg kg^{-1} for thebaine in poppy seed.

According to several studies, proper cleaning, washing, grinding and baking could reduce significantly the alkaloid contents of poppy seeds. Sproll et al. (2006, 2007) studied the effect of processing on the morphine level of poppy seed, and found that the optimal treatment for reduction consists of washing, drying and grinding. All washing treatments led to a significant reduction of the morphine content with a minimal removal of 48% and a maximum removal of the total morphine content. With cold water, only about 60% of morphine was removed. At temperatures above 60°C, significantly higher proportion, around 90%, could be removed. The length of poppy seed rinsing also increased the level of reduction. Grinding reduced the morphine and codeine contents on an average by 24% and about 20%, respectively (Sproll et al. 2007). In another study (Sproll et al. 2006), the authors reported morphine losses of 34 ± 5 % due to grinding. They also found that during baking up to 135°C the reduction of morphine was relatively low (around 30%), but at 220°C a reduction of 80-90% was observed.

In this study we present the results of the evaluation of the acute exposure of Hungarian poppy seed consumers to morphine, calculated with both point estimate and probabilistic method, based on the alkaloid content of poppy seed marketed in Hungary during 2001-2010, and on consumption data from the surveys conducted in 2003 (Rodler et al. 2005) and 2009 (Szeitné-Szabó et al. 2011), taking also into account the effect of processing. The uncertainty of the calculated values was also estimated.
Materials and Methods

The results of morphine, thebaine, noscapine and codeine analyses in 737 poppy seed samples were obtained from the market surveys carried out by the Hungarian authorities. National Institute for Food Hygiene and Nutrition provided 566 data from 2001-2006, and 171 data were supplied by the Central Agricultural Office for 2007-2010.

During the first study period the alkaloid content was measured with thin layer chromatography according to the Hungarian standard MSZ 690:1993. In this method the morphine was extracted with chloroform-isopropyl alcohol, purified with 0.1 mol/l HCl solution, separated and detected on silica GF$_{254}$ TLC plate. Recovery was 80%. The LOQ for morphine was 2 mg kg$^{-1}$, and it varied between 2, 3, 4, 5, 8 mg kg$^{-1}$ for noscapine, codeine and thebaine. The results were confirmed with HPLC–UV detection.

Morphine was determined with HPLC-MS/MS during 2007-2010. The poppy seed test portions were extracted with methanol containing 1% HCl by shaking for 2 hrs. The filtered extracts were directly injected into the HPLC column (C18 5µm) and detected with MS/MS. The typical method performance parameters reported were: LOQ = 1 mg kg$^{-1}$, LOD = 0.2 mg kg$^{-1}$ recovery: 78-93%; 70-94%; 51-96%; 84-101%, and the reproducibility RSD (relative standard deviation) at around 30 mg/kg: 6.3%, 5.9%, 7.6% and 15.2% for morphine, codeine, thebaine and noscapine, respectively. The accuracy and selectivity of the method were tested by spiking poppy seed with known alkaloid contents and hemp which does not contain alkaloids. The alkaloid standards of known purity were purchased from Sigma-Aldrich Co. LLC. (Dömsödi J, personal communication).

Consumption data obtained with 3-day dietary record method from the years 2003 and 2009 were used for the assessment. In 2003, 1360 adult consumers recorded 79 poppy seed consumption days (1.94%), while 4992 consumers (age range between 0-101 years) reported 327 poppy seed consumption days (2.18%) in 2009. Regarding consumption data of children and adolescents of 1-18 years of age, information was only available from the consumption survey conducted in 2009, where 1010 young responders reported 85 poppy seed consumption days in total (2.81%). In the 2009 survey there were data available about the recipes of foods, so it was possible to evaluate data of processing. The poppy seed was consumed in ground form on 65% of days and only in 35% of the cases was consumed in baked cakes.
In view of the infrequent consumption of poppy seed and the rapid decrease of the potential adverse effects of its alkaloid content, only the acute exposure of consumers is a concern. For the estimation of acute exposure, both the alkaloid concentration data and the poppy seed consumption data (eaters only) obtained during the two periods were considered separately, and after combining them for the whole time period (2001-2010 for alkaloid concentrations, and 2003+2009 for the poppy seed consumptions).

For point estimate, the exposure was calculated using the 97.5\textsuperscript{th} percentile of daily poppy seed consumptions and 97.5\textsuperscript{th} percentile of morphine concentrations (EFSA 2007).

As consumption data for ground poppy seed and baked poppy seed were available from the 2009 survey, the effect of processing on morphine intake could also be studied. The processing factor (Pf) was calculated as the ratio of the concentration of morphine in processed (ground or ground and baked) poppy seed and raw poppy seed (FAO 2009). As poppy seed is not washed as part of the commercial or bakery operations in Hungary, the effect of washing was not considered. The processing factor of 0.71 for grinding of poppy seed was calculated from the 34\% and 24\% average losses of morphine (Sproll 2006, 2007). The strudels and cakes are baked in Hungary typically at 180\°C. The processing factor of 0.31 for baking of cakes was estimated using MS Excel exponential trend line fitting function from the reduction of morphine content observed at 135 °C and 220 °C temperatures (Sproll et al. 2006) assuming the reported quadratic relationship. The combined processing factor including grinding for morphine content in baked poppy seed is 0.71 \times 0.31 = 0.22. The morphine intakes were reduced by the calculated processing factors according to the form of consumed poppy.

For probabilistic modelling, parametric distributions were fitted to the morphine concentration values and consumption data applying @RISK programme package. The uncertainty of the fitted parameters was not estimated. The relative frequency distributions of original data and the fitted distributions were visually evaluated too. Parametric distributions giving the best fit for both the morphine concentrations and consumption data were used to generate 200 000 and 500 000 random values. The generated lognormal or gamma distributions were expected to cover the high experimental values and beyond those observed. Morphine exposure of children and adolescents, as a subset of the total population, was calculated similarly.

In summary, the exposure was calculated with each of the following methods, for comparison:

(a) All consumption data were multiplied by all morphine concentration data;
(b) The 200,000 or 500,000 pairs of random values of morphine concentrations and poppy
seed consumptions, drawn from the selected parametric distributions, were multiplied
with each other. This is a parametric alternative to method (a).

(c) The combined original data populations (737 morphine concentrations and 406 poppy
seed consumptions) were bootstrapped 10,000 times and the resulting morphine
concentrations were multiplied with the resulting consumption values (10,000
iterations). The cumulative relative frequencies of exposure were calculated from each
iterations, using the NIST definition for percentile calculations (NIST 2011). This
non-parametric bootstrapping procedure (Efron and Tibshirani 1994) was used to
quantify uncertainty due to the limited sample sizes.

(d1) and (d2) Morphine intake was calculated using the abovementioned processing
factors for ground and baked poppy seeds applying procedures (b) and (c).

The poppy seed consumption in raw ground form and in baked cakes showed distinctly
different pattern, therefore parametric distributions were separately fitted on the two data sets.
The exposures of consumers to morphine derived from consuming ground poppy seed and
cakes containing poppy seed were calculated separately from the re-sampled distributions of
consumption and morphine data. As the separate consumption data were only available from
the 2009 survey, the lognormal distribution fitted on 2007-2010 morphine concentration data
were used for the calculations. In order to obtain representative exposure values for the
combined poppy seed consumption, the proportion of consumption days of ground poppy
seed (65%) and ground and baked poppy seed (35%) were taken into account and 325,688
and 174,312 random samples were drawn from the generated ground and baked poppy seed
consumption data population, respectively. The same number of concentration data were
drawn from the generated morphine population. The exposures calculated from the two sets of
data were evaluated separately and in combination.

The results obtained with these different methods were compared and evaluated together.

Results and Discussion

Alkaloid levels in poppy seeds in Hungary

Altogether 737 poppy seed samples were analysed. Morphine was detected in 736 out of 737
samples. Codeine and thebaine were detected in 61.3% and 63.0% of the cases, respectively.
Noscapine was detected in 6.2% of the samples only. In more than half of the samples 3 or 4 alkaloids were detected together. Table 1 presents the summary of the detectable alkaloids and their selected percentiles, in the samples.

Morphine content of the samples varied to a great extent, with median of 11 mg kg\(^{-1}\) and highest values of 222, 238, 267 and 533 mg kg\(^{-1}\). The 97.5\(^{th}\) and 95\(^{th}\) percentiles and the median values of morphine concentrations were calculated with Harrell-Davis (Craig personal communication), and the binomial (Hamilton et al. 2004) methods, and with MS Excel (Table 2). The binomial method enabled estimating the probability that a given ranked value is the selected percentile of the population. The Harrell-Davis (HD) and the binomial methods resulted in very similar estimates, while the 97.5\(^{th}\) percentile values obtained with Excel were lower. Consequently, the average values of median, 95\(^{th}\) and 97.5\(^{th}\) estimates obtained with HD and binomial methods are given in Table 3 for the alkaloids detected in poppy seeds. The average estimated 97.5\(^{th}\) percentile of morphine concentrations and the similarly estimated 97.5\(^{th}\) percentile of poppy seed consumptions were used in calculating the point estimates of morphine intake.

The relative frequency distributions of morphine concentrations measured during the two survey periods were similar (Figure 1). The ratios of the 97.5\(^{th}\) percentile and median values of the morphine 2007-2010 and 2001-2006 data sets were 1.064 and 1.055, respectively. The ratios indicate that the morphine concentrations measured with different methods during 2001-2006 and 2007-2010 were not substantially different. The Mann-Whitney U-test confirmed that the difference was not significant (P=0.35) (McDonald 2009).

The medians and 97.5 percentile concentrations of alkaloids calculated for the 10 years of data separately (Table 1), did not indicate clear trend in changing of morphine content. The calculated 97.5\(^{th}\) percentiles are lower in the last years (2009 and 2010), but it may be attributed to the smaller number of samples analysed, as the probability of detecting at least one value above the 97.5 percentile is only 40-45% in case of 21-25 samples. Based on these results, the combination of morphine data of the 10 years is justified.

**Consumption of poppy seeds in Hungary**

In the 2003 food consumption survey, poppy seed consumption occurred on 1.94 % of all consumption days. Considering body weight of consumers, the highest, the 97.5\(^{th}\) percentile...
and the average daily consumption was 1 g (kg bw)\(^{-1}\)day\(^{-1}\), 0.80 g (kg bw)\(^{-1}\)day\(^{-1}\) and 0.34 g (kg bw)\(^{-1}\)day\(^{-1}\), respectively.

Poppy seed consumption was recorded on 327 out of 14,976 days (2.18%) in the 2009 food consumption survey. The average and 97.5\(^{th}\) percentile consumptions were 0.44 g (kg bw)\(^{-1}\)day\(^{-1}\) and 1.20 g (kg bw)\(^{-1}\)day\(^{-1}\), respectively. The highest adult consumption was recorded by a 20-year-old, 62 kg man (1.68 g (kg bw)\(^{-1}\)day\(^{-1}\)). The average and 97.5\(^{th}\) percentile consumptions of children and adolescents were 0.67 g (kg bw)\(^{-1}\)day\(^{-1}\) and 1.77 g (kg bw)\(^{-1}\)day\(^{-1}\), respectively. The highest poppy seed consumption was recorded by a 4-year-old, 22 kg girl (2.73 g (kg bw)\(^{-1}\)day\(^{-1}\)), while the youngest poppy seed consumer was 1 year old. It should be pointed out, that none of the consumption survey periods included Christmas, when the cakes are traditionally prepared with poppy seed.

The relative and cumulative frequency distributions of 2003 and 2009 consumption data are shown in Figure 2. The characteristics of the distributions are given in Table 4. The difference in the consumption figures might be attributed to the relatively small number of consumption days in 2003.

There was detailed information about the form of consumed poppy seed from the 2009 survey only. Out of the 327 consumption days, pasta with ground, raw poppy seed was consumed on 213 (65%), and baked cakes were consumed on 114 (35%) occasions. Consumption levels of raw, ground poppy seed were generally higher than those of baked poppy (Table 4.)

**Morphine intake from the consumption of poppy seeds**

**Point estimate**

Following the FAO/WHO recommended method (WHO 1997) and the current practice of the European Food Safety Authority (EFSA 2011), the 97.5\(^{th}\) percentile of poppy seed consumptions of eaters and the 97.5\(^{th}\) percentile of morphine concentration data were used for calculating point estimates of acute intake.

In order to assess the potential consequence of morphine concentrations and consumption values obtained during the two periods, the intakes were calculated from the two consumption data sets separately and from the combined consumption data as well as the separate and combined morphine concentration data sets. The results are summarised in Table 5.
Based on consumption of raw poppy seed, the point estimate (using average percentiles calculated by binomial and Harrell-Davis calculation methods) for the acute consumer exposure ranges between 73.1 and 116.7 µg (kg bw)^{-1} day^{-1}. The lowest exposure (73.1 µg (kg bw)^{-1} day^{-1}) was obtained from the combination of the 2003 consumption data and all morphine concentrations, the highest exposure (116.7 µg (kg bw)^{-1} day^{-1}) resulted from the combination of 2009 consumption and 2007-2010 morphine data.

The approximate 95% confidence limits for the estimated intake values were calculated, with the binomial method, from the ranked values bracketing the 68% confidence intervals ((1-0.683)/2=0.1587; 0.1587 × 0.1587=0.025) of the 97.5th percentiles of corresponding data sets estimated based on the binomial method (Figure 3). The standard deviation, SD, of the selected percentile ‘p’ is calculated as SD=√(Npq), where N is the number of data points and p+q=1 (Diem and Seldrup 1982). The 68% confidence intervals around the estimated 97.5 percentile for N=737 correspond to the 17-4=13th and 18+4=22nd ranked values (67 and 112 mg/kg^{-1}) where the largest value has the 1st rank. As the best estimate (i.e. average of two calculation methods) of the 97.5th percentile was close to the value estimated with the binomial method, the confidence intervals calculated with the values obtained from the binomial method provide an acceptably close approximation. Due to low number of data points, the 95% confidence limits for the 2003 consumption data and 2007-2010 morphine concentrations encompassed practically the whole data sets. Therefore confidence limits could not be calculated for those combinations.

Morphine short-term intakes of children and adolescents (1-18 years) calculated from the 2007-2010 and 2001-2010 morphine concentrations and the 85 consumption days data of raw poppy seed were 164.7 µg (kg bw)^{-1} day^{-1} and 150.2 µg(kg bw)^{-1} day^{-1}, respectively. The morphine intake was higher than that of the whole population due to the higher average and 97.5th percentile of the poppy seed consumption of the age group of 1-18 years. The small number of data did not allow the calculation of the confidence intervals for the estimated intakes.

**Probabilistic estimation of morphine intake**

Probabilistic modelling was carried out with options (a), (b) (c) and (d) described under methods. The results are summarized in the following.
The 200K values generated from the fitted lognormal distributions covered the maximum recorded consumption figures in each data set; however the maximum experimental morphine concentration could only be covered in two out of three cases (columns B, C and D1 in Table 6). When 500K values were generated, the highest values obtained were substantially higher than the highest experimental value (column D2). The results suggest that the heavy tail of the empirical consumption data can only be covered if large number of data points are generated from the fitted distribution;

- The random morphine values generated from the fitted gamma distribution, however, underestimated the upper tail of morphine experimental data (the highest generated and experimental values were 180.1 µg g\(^{-1}\) and 202 µg g\(^{-1}\), or 191.2 µg g\(^{-1}\) and 533 µg g\(^{-1}\) respectively). Consequently, the gamma distribution was not suitable for estimating high quantiles of exposure;

- When random samples were drawn from the fitted lognormal distributions, the intake estimates obtained from the different data sets were in the same range below the 95\(^{th}\) percentile, but showed larger differences at or above the 95\(^{th}\) percentile of the cumulative relative frequency distributions (Table 6 columns B, C, D1 and D2). The difference between the cumulative relative frequencies corresponding to the intake values obtained with the three combinations of data represents the uncertainty of the exposure estimation based on the calculations with the limited subsets of data;

- The results obtained with multiplying all of experimental data and bootstrapping (methods (a) and (c)) are much higher than those obtained by method (b) and therefore represent worst case situations (see columns A and D2, and columns H and I).

- The comparison of columns C and E, illustrates the effect of processing based on the 2009 consumption and 2007-2010 morphine concentrations. The ratio of exposure from raw poppy seed and from processed poppy seed in the percentiles above 95\(^{th}\) is between 0.639 and 0.646, which indicates that the grinding (Pf=0.71) was the main source of reduction of exposure.

- The comparison of columns F and G illustrates the effect of processing based on exposure results of 10 000 bootstrapped data (the exposure percentiles were calculated from each iteration and the medians are shown in the table). The ratios of corresponding percentiles are between 0.621 and 0.675 at the higher percentiles.
The exposure of children (μg(kg bw)^{-1}day^{-1}) to poppy seed shown in columns H, I and J is about 1-2 times higher than of adults (columns D2, A, and G) at the 97.5th - 99.99th percentiles.

The cumulative distribution of the exposure calculated by multiplying the morphine concentration and consumption data obtained from their fitted lognormal distributions and with multiplying all of experimental data (method (a)) are close to each other up to about 90th percentile of the exposure (columns A, D1 and D2). At 99.9th and 99.99th percentiles, however the differences are more than two times even in the cases where the highest modelled values for poppy seed consumption and morphine concentrations are higher than the corresponding experimental values. The possible reason for the large differences at high percentiles was further studied. The highest morphine concentrations observed in poppy seed samples were 187, 200, 202, 222, 238, 267 and 533 mg kg\(^{-1}\), and the highest poppy seed consumptions were 1.30, 1.32, 1.33, 1.54, 1.58, 1.68 and 2.73 g (kg bw)^{-1}day^{-1}. It was found that the 4 highest morphine concentrations in combination with the highest 20 poppy seed consumption values resulted in the exposures above the 99.99th percentile cumulative exposure (489.1 μg (kg bw)^{-1}day^{-1}). If the highest morphine and poppy seed consumption values (533 mg kg\(^{-1}\) and 2.73 g (kg bw)^{-1}day^{-1}) were omitted from the calculation, then the 99.99th percentile values would be 267 μg (kg bw)^{-1}day^{-1}. These results explain why lower exposure values were obtained when they were calculated from the results of random sampling of fitted populations (columns D1 and D2 in Table 6), because in the latter cases the probability of selecting the highest values from both populations of 200,000 or 500,000 with random sampling is very low.

The range of estimated cumulative relative frequency values were calculated with bootstrapping drawing 10,000 random samples with replacement from both the combined 737 morphine concentration values and the 406 poppy seed consumption data, and multiplying all concentration values with all consumption data in each case to calculate the morphine intake [μg (kg bw)^{-1}day^{-1}]. The procedure was repeated 10,000 times (bootstrap method (c)). The median and the 95% confidence limits of the calculated cumulative relative frequencies are shown in Figure 4. The horizontal lines within the confidence limits show the intake range belonging to a given cumulative percentile. The vertical line between the confidence intervals indicates the range of the percentage of the consumption days of morphine intake around the selected one. The results showed that the 95% confidence interval of the estimated cumulative frequencies, indicating the uncertainty of the estimation, was the narrowest at 50th percentile and increased at higher and lower percentile values. For instance, the relative 95% interval of
the estimated 50th, 95th, 97.5th, 99th, 99.9th and 99.99th cumulative percentile values were in the
order of: 13.2%, 27.7%, 40.1%, 50.3%, 69.7% and 80.5%.

Figure 5 shows examples for the frequency distributions of estimated percentile values
obtained from 10 000 bootstrap samples. At or below the 99th percentile the calculated values
approximate normal distribution. However, at higher percentile values the distribution is
going scattered and their ranges are getting wider, as expected.

**Effect of processing on morphine intake**

The calculations assuming that the morphine concentrations measured in raw poppy seed are
present in consumed food provide an overestimate of the intake. In order to get a more
realistic estimate, the effects of washing, grinding and baking were taken into consideration.

In Hungary, the poppy seed produced at commercial scale is not washed. Taking into account
that 65% proportion of poppy seed is consumed unbaked, and 35% is consumed baked, the
acute intake of morphine was recalculated applying the calculated processing factors (0.71
and 0.22). The estimated exposure decreased by 32.6% for adults and 29.0% for children,
respectively (from 116.70 to 78.64 µg (kg bw)-1 day-1 and from 164.70 to 116.90 µg (kg bw)-1
day-1 respectively).

Regarding probabilistic exposure estimate of processed poppy seed consumption, it was found
that the intake was decreased by about 33-38% at the upper percentiles. These findings are in
accordance with the higher intakes of ground poppy seed (estimated processing factor is 0.71)
than that consumed with cakes, and consequently the ground poppy seed consumption is the
cause of the high morphine intake.

Counting for a consumer of 60 kg (body weight), the starting oral therapeutic dose reported
by Embey-Isztin would be about 167 µg (kg bw)-1, while the lowest oral therapeutic dose
reported by BfR would be about 31.7 µg (kg bw)-1. The results from probabilistic calculations
suggest that the 31.7 µg (kg bw)-1 dose could be reached only above the 97.5 percentile of the
adult poppy seed eaters (see Table 6, columns B, C, D1 and D2). Looking at childrens’
exposure (Table 6, column H), above 90-95 percentiles could be in the range of the estimated
lowest therapeutic dose referred by BfR. Probability of reaching the starting oral therapeutic
dose of 167 µg (kg bw)-1 is very low, practically negligible.

The provisional daily upper intake level (6.3 µg (kg bw)-1 day-1) established by BfR has an
incorporated uncertainty factor of 5. This is not likely to be exceeded by the median adult
exposure; however, children have higher intake than adults. Around the 65% of the cumulative distribution of morphine intake of adults and 50% of children corresponds to the 6.3 µg (kg bw)\(^{-1}\) day\(^{-1}\) provisional daily upper intake, calculated with the morphine content of unprocessed poppy seed.

Taking into account that poppy seed is consumed in processed form, it was concluded that exposures are about 35% lower than the scenarios calculated without counting for processing losses.

Applying the point estimation methodology the calculated acute intake exceeds the BfR reference value but does not reach the starting oral therapeutic dose. It should be noted that this is a very conservative way of intake calculation; the probability that a ‘big eater’ consumes from the highly contaminated food is low.

**Conclusion**

The most prominent alkaloid of poppy seed is morphine being present in detectable amounts practically in all samples analysed in Hungary during 2001-2010. As the poppy seed was consumed on only a small fraction of the dietary survey days, the chronic intake is not of a concern. Consequently only the short-term intakes of poppy seed alkaloids were estimated in our study, based on the measured alkaloid content of raw poppy seed, taking also into account the estimated effect of grinding and baking. The intake calculation performed with the fitted lognormal distributions gave the best fit on the original data. The 97.5th percentile exposure of adults ranged between 25.6-34.7 µg (kg bw)\(^{-1}\) day\(^{-1}\) depending on the time period. The calculated 97.5th percentile of childrens’ exposure was 59.1 µg (kg bw)\(^{-1}\) day\(^{-1}\). Taking into account the reduction of morphine content by processing, the 97.5 and 99 percentile exposures based on probabilistic estimations ranged between 18.3- 25.4 and 25.6- 47.4 µg (kg bw)\(^{-1}\) day\(^{-1}\) for adults, and 32.9 and 66.4 µg (kg bw)\(^{-1}\) day\(^{-1}\) for children, respectively.

The poppy seed was used on 65% of consumption days as flavouring ingredient of pasta in ground form. The average and 97.5\(^{th}\) percentile consumption of poppy seed in baked form is 60% and 77% of that eaten in ground form, respectively. The high morphine intake primarily derives from the consumption of ground raw poppy seed. According to the current marketing practice, the poppy seed is cleaned only by ventilation and screening, but not washed. Consequently, morphine content of the commercialised raw poppy seed is reduced before consumption by about 35% due to the effect of grinding. The consequences of pattern and
form of the consumption is reflected in the results of estimated short term exposure which indicated that the morphine intake from processed poppy seed was about 35% lower than the values calculated from the morphine content of raw poppy seed.

The 5-fold difference in reported lowest oral therapeutic doses of 167 µg (kg bw)^{-1} and 31.7 µg (kg bw)^{-1} of morphine indicates the large uncertainty in the estimation of the reference dose value for assessing the potential adverse affects of exposure of consumers to morphine.

Reliable risk assessment was not possible because internationally accepted acute reference doses for morphine and the other alkaloids detected in poppy seeds have not been established yet. Even less or no information is available on the potential adverse effects of codeine, thebaine and noscapine content of poppy seeds individually or in combination, and the bioavailability of these alkaloids.

Further scientific studies, meeting the relevant standards, are required in order to establish acute reference dose to assess the actual risk. The effect of technological steps, the degradation of morphine in the digestive system during absorption, and the effect of poppy alkaloid "cocktail" compared to purified morphine should also be taken into account. Upon that the necessary risk management options could be established to reduce the alkaloid intake. Our findings contribute to the scientific risk assessment with providing exact, statistically well-founded exposure data of poppy seed alkaloids, with specific focus on morphine.

Acknowledgement

The authors are grateful to the former National Institute for Food Hygiene and Nutrition (recent name National Institute for Food and Nutrition Science) and Central Agricultural Office for providing the results of alkaloid analysis in poppy seed, and to Peter Craig, Durham University, for providing the Excel template for the calculation of percentile values with Harrel-Davis method.

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For Peer Review Only


Sproll C., Perz R.C., Lachenmeier D.W. 2006. 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. J. Agric. Food Chem. 54: 5292-5298


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Figure captions:

Figure 1. Relative frequency distributions of morphine concentrations detected in poppy seed

Figure 2. (a, upper figure) Distributions of poppy seed consumption data in 2003 and 2009
and (b, lower figure) Distribution of poppy seed consumption in ground raw and baked form
in 2009.

Figure 3. Probability density function of the 97.5th percentile of all morphine concentration
values (2001-2010 data). Explanation for the confidence limits: the 97.5th percentile lies
between the 17th and 18th ranked values from largest to smallest. The 68% of the values
(median ± SD) are between the 17-4=13 and 18+4=22 ranked values.

Figure 4. The median and 95% confidence intervals of the exposure to morphine through
consumption of poppy seed.

Figure 5. Relative frequency of estimated quantile values calculated from 10 000 bootstrap.
Solid line: normal distribution fitted on results. Dashed line: lognormal distribution fitted on
results.
Table 1. Number of poppy seed samples analysed and percentage of alkaloids detected in the samples above LOQ.

<table>
<thead>
<tr>
<th>Years</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample number</td>
<td>80</td>
<td>77</td>
<td>173</td>
<td>55</td>
<td>82</td>
<td>99</td>
<td>71</td>
<td>54</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td><strong>Morphine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% detected</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>99.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>7.92</td>
<td>16.99</td>
<td>10.38</td>
<td>9.48</td>
<td>15.05</td>
<td>10.93</td>
<td>11.14</td>
<td>12.88</td>
<td>12.70</td>
<td>8.08</td>
</tr>
<tr>
<td>97.5 percentile</td>
<td>68.12</td>
<td>300.01</td>
<td>82.40</td>
<td>41.01</td>
<td>241.80</td>
<td>137.87</td>
<td>110.41</td>
<td>27.63</td>
<td>26.69</td>
<td></td>
</tr>
<tr>
<td><strong>Codeine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% detected</td>
<td>30.0</td>
<td>64.9</td>
<td>48.0</td>
<td>56.4</td>
<td>75.6</td>
<td>69.7</td>
<td>80.3</td>
<td>75.9</td>
<td>96.0</td>
<td>52.4</td>
</tr>
<tr>
<td>Median of codeine concentrations</td>
<td>0.30</td>
<td>2.00</td>
<td>0.30</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>1.70</td>
<td>2.00</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>97.5 percentile of codeine concentrations</td>
<td>10.13</td>
<td>33.95</td>
<td>21.10</td>
<td>8.60</td>
<td>29.98</td>
<td>20.55</td>
<td>12.40</td>
<td>4.28</td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td><strong>Noscapine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% detected</td>
<td>3.8</td>
<td>5.2</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.0</td>
<td>9.9</td>
<td>18.5</td>
<td>32.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Median of noscapine concentrations</td>
<td>0.30</td>
<td>0.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>97.5 percentile of noscapine concentrations</td>
<td>2.05</td>
<td>5.20</td>
<td>4.00</td>
<td>1.30</td>
<td>1.30</td>
<td>4.98</td>
<td>6.28</td>
<td>11.58</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td><strong>Thebaine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% detected</td>
<td>27.5</td>
<td>61.0</td>
<td>50.9</td>
<td>61.8</td>
<td>67.1</td>
<td>78.8</td>
<td>90.1</td>
<td>70.4</td>
<td>92.0</td>
<td>71.4</td>
</tr>
<tr>
<td>Median of thebaine concentrations</td>
<td>0.30</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>3.40</td>
<td>1.70</td>
<td>2.50</td>
<td>1.40</td>
</tr>
<tr>
<td>97.5 percentile of thebaine concentrations</td>
<td>10.00</td>
<td>46.20</td>
<td>22.00</td>
<td>15.55</td>
<td>22.00</td>
<td>16.40</td>
<td>42.33</td>
<td>15.03</td>
<td>7.16</td>
<td>4.00</td>
</tr>
</tbody>
</table>

1. The calculated 97.5 percentiles are only indicative values, and should be interpreted with caution, as the limited sample number does not make their precise calculation possible. The percentiles are calculated with MS Excel.
Table 2. Calculated 97.5 percentiles and medians of morphine data (mg kg\(^{-1}\))

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of samples</th>
<th>97.5th percentile of concentration</th>
<th>Median concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Excel HD</td>
<td>Binomial method</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LCL Calc. Value UCL</td>
<td>LCL Calc. Value UCL</td>
</tr>
<tr>
<td>2001-06</td>
<td>566</td>
<td>80.00 85.74</td>
<td>67.00 89.21 160.00</td>
</tr>
<tr>
<td>2007-10</td>
<td>171</td>
<td>52.70 90.67</td>
<td>2.90 95.40 &gt;202.00(^1)</td>
</tr>
<tr>
<td>2001-10</td>
<td>737</td>
<td>80.00 83.57</td>
<td>67.00 86.09 141.00</td>
</tr>
</tbody>
</table>

HD: values calculated with Harrel-Davis method
LCL: lower limit of 95% confidence interval; UCL: upper limit of 95% confidence interval
\(^1\)Number of data points was not sufficient for estimation of confidence limits accurately
Table 3. Distribution characteristics of the alkaloid levels (mg kg\(^{-1}\)) in all (2001-2010) samples.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Median(^1)</th>
<th>Average(^2)</th>
<th>P0.95(^1)</th>
<th>P0.975(^1)</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>11.0</td>
<td>18.7</td>
<td>57.6</td>
<td>84.8</td>
<td>533.0</td>
</tr>
<tr>
<td>Codeine</td>
<td>2.0</td>
<td>3.6</td>
<td>17.2</td>
<td>25.7</td>
<td>60.0</td>
</tr>
<tr>
<td>Noscapine</td>
<td>1.2</td>
<td>1.2</td>
<td>1.6</td>
<td>4.7</td>
<td>40.0</td>
</tr>
<tr>
<td>Thebaine</td>
<td>2.0</td>
<td>4.0</td>
<td>15.8</td>
<td>23.9</td>
<td>120.0</td>
</tr>
</tbody>
</table>

\(^1\) The median, 95\(^{th}\) and 97.5\(^{th}\) percentile values are the average of the estimates obtained with Harrell-Davis and binomial methods.

\(^2\) The average concentrations were calculated with the reported LOQ/6 values for non detected results (European Commission 2003).
Table 4. Poppy seed consumption (g(kgbw)^{-1}day^{-1}) based on the 2003 and 2009 surveys.

<table>
<thead>
<tr>
<th>Survey data</th>
<th>2009</th>
<th>2003</th>
<th>2003+2009</th>
<th>Ratio(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>327</td>
<td>213</td>
<td>114</td>
<td>79</td>
</tr>
<tr>
<td>Ground</td>
<td>0.042</td>
<td>0.048</td>
<td>0.042</td>
<td>0.031</td>
</tr>
<tr>
<td>Baked</td>
<td>0.440</td>
<td>0.516</td>
<td>0.300</td>
<td>0.340</td>
</tr>
<tr>
<td>Max</td>
<td>2.727</td>
<td>2.727</td>
<td>1.677</td>
<td>1.000</td>
</tr>
<tr>
<td>SD</td>
<td>0.304</td>
<td>0.294</td>
<td>0.271</td>
<td>0.183</td>
</tr>
<tr>
<td>CV</td>
<td>0.689</td>
<td>0.570</td>
<td>0.902</td>
<td>0.538</td>
</tr>
<tr>
<td>Median(^2)</td>
<td>0.395</td>
<td>0.439</td>
<td>0.217</td>
<td>0.337</td>
</tr>
<tr>
<td>P0.975(^2)</td>
<td>1.255</td>
<td>1.235</td>
<td>0.954</td>
<td>0.862</td>
</tr>
</tbody>
</table>

\(^1\) Ratio of 2009/2003 consumption based on food survey data.
\(^2\) The median and P0.975 estimates were obtained with HD and binomial methods.
Table 5. Point estimate with 95% confidence intervals of acute exposure to morphine [µg(kgbw)^{-1} day^{-1}] from raw poppy seed consumption.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>2001-2006</th>
<th>2007-2010</th>
<th>2001-2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>0.86</td>
<td>75.42</td>
<td>80.21</td>
</tr>
<tr>
<td>2009</td>
<td>1.26</td>
<td>109.73</td>
<td>116.70</td>
</tr>
<tr>
<td>2003+2009</td>
<td>1.19</td>
<td>104.02</td>
<td>110.63</td>
</tr>
</tbody>
</table>

Best estimates¹ (and 68% confidence intervals)² for P0.975 consumptions in time periods [gr(kg bw)^{-1}day^{-1}]

Acute exposure

1. Best estimate calculated as the average of estimated 97.5th percentile with HD and binomial method.
2. 68% confidence interval calculated as P0.975 ± SD as shown in Figure 3. The number of 2003 consumption figures and 2007-2010 morphine results were not sufficient for calculation of the confidence intervals.
Table 6. Estimated cumulative distribution function (ECDF) of morphine intake from consumption of poppy seed calculated with probabilistic modelling.

<table>
<thead>
<tr>
<th>ECDF</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D1</th>
<th>D2</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>1.7</td>
<td>1.4</td>
<td>1.9</td>
<td>1.7</td>
<td>1.6</td>
<td>0.7</td>
<td>1.7</td>
<td>0.6</td>
<td>2.5</td>
<td>2.9</td>
<td>1.4</td>
</tr>
<tr>
<td>0.3</td>
<td>2.4</td>
<td>2.0</td>
<td>2.7</td>
<td>2.4</td>
<td>2.3</td>
<td>1.1</td>
<td>2.5</td>
<td>1.1</td>
<td>3.6</td>
<td>4.1</td>
<td>2.3</td>
</tr>
<tr>
<td>0.4</td>
<td>3.2</td>
<td>2.7</td>
<td>3.5</td>
<td>3.2</td>
<td>3.0</td>
<td>1.7</td>
<td>3.4</td>
<td>1.7</td>
<td>4.9</td>
<td>5.3</td>
<td>3.2</td>
</tr>
<tr>
<td>0.5</td>
<td>4.1</td>
<td>3.5</td>
<td>4.5</td>
<td>4.2</td>
<td>4.0</td>
<td>2.5</td>
<td>4.3</td>
<td>2.4</td>
<td>6.5</td>
<td>6.7</td>
<td>4.2</td>
</tr>
<tr>
<td>0.6</td>
<td>5.1</td>
<td>4.6</td>
<td>5.8</td>
<td>5.5</td>
<td>5.3</td>
<td>3.3</td>
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<td>3.1</td>
<td>8.7</td>
<td>8.6</td>
<td>5.4</td>
</tr>
<tr>
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<td>6.3</td>
<td>6.1</td>
<td>3.9</td>
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<td>10.1</td>
<td>9.5</td>
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<tr>
<td>0.7</td>
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<td>7.5</td>
<td>7.3</td>
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<td>11.8</td>
<td>10.9</td>
<td>7.0</td>
</tr>
<tr>
<td>0.8</td>
<td>9.2</td>
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<td>10.1</td>
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<td>10.2</td>
<td>6.3</td>
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<td>5.9</td>
<td>16.8</td>
<td>15.0</td>
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<tr>
<td>0.9</td>
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<td>13.1</td>
<td>15.2</td>
<td>16.0</td>
<td>16.6</td>
<td>9.7</td>
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<td>9.9</td>
<td>27.6</td>
<td>24.6</td>
<td>14.4</td>
</tr>
<tr>
<td>0.95</td>
<td>25.0</td>
<td>18.7</td>
<td>21.2</td>
<td>23.3</td>
<td>24.7</td>
<td>13.7</td>
<td>26.1</td>
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<td>41.5</td>
<td>39.3</td>
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<td>28.4</td>
<td>32.0</td>
<td>34.7</td>
<td>18.3</td>
<td>40.9</td>
<td>25.4</td>
<td>59.1</td>
<td>63.3</td>
<td>32.9</td>
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<td>72.6</td>
<td>36.8</td>
<td>39.7</td>
<td>46.5</td>
<td>52.4</td>
<td>25.6</td>
<td>76.2</td>
<td>47.4</td>
<td>88.8</td>
<td>112.0</td>
<td>66.4</td>
</tr>
<tr>
<td>0.999</td>
<td>222.1</td>
<td>76.7</td>
<td>78.9</td>
<td>101.7</td>
<td>121.8</td>
<td>50.4</td>
<td>224.4</td>
<td>151.4</td>
<td>204.7</td>
<td>320.0</td>
<td>166.0</td>
</tr>
<tr>
<td>0.9999</td>
<td>489.1</td>
<td>147.4</td>
<td>131.9</td>
<td>195.9</td>
<td>235.5</td>
<td>84.9</td>
<td>499.7</td>
<td>336.9</td>
<td>398.1</td>
<td>702.7</td>
<td>321.7</td>
</tr>
</tbody>
</table>

*1
1.74 | 4.37 | 4.67 | 10.61 | 4.67
*2
1 | 2.73 | 2.73 | 2.73 | 2.73
*3
498.4 | 250.6 | 569.4 | 719.8 | 719.8
*4
533 | 202 | 533 | 533 | 533

Exposure results from processed poppy seed are underlined

Exposure:
1: Poppy seed consumption [g/(kg bw)^1/day^-1]: maximum value of 200 000 or 500 000 generated data with the parameters providing the best fit.
2: Poppy seed consumption [g/(kg bw)^1/day^-1]: maximum experimental value.
3: Morphine concentration [µg g^-1]: Maximum value of 200 000 or 500 000 generated data with the parameters providing the best fit.
4: Morphine concentration [µg g^-1]: Maximum experimental value.

Method (a), **unprocessed poppy**
A: Multiplying all experimental 2003+2009 poppy consumption data [g/(kg bw)^1/day^-1] with all (2001-10) morphine data [µg g^-1]. [406*737 data]

Method (b), **unprocessed poppy**
B: ln (2003 poppy seed consumption [g/(kg bw)^1/day^-1]) & ln(morphine 2001-6 [µg g^-1]), 200K data populations.
C: ln(2009 poppy seed consumption [g/(kg bw)^1/day^-1]) & ln(morphine 2007-10 [µg g^-1]), 200K data populations.
D1: ln(2003+2009 poppy seed consumption [g(kgbw)^{-1} day^{-1}]) & ln(morphine 2001-10 [µg g^{-1}]), 200K data populations.

D2: ln(2003+2009 poppy seed consumption [g(kgbw)^{-1} day^{-1}]) & ln(morphine 2001-10 [µg g^{-1}]), 500K data populations.

Method (b), processed poppy

E: ln(2009 poppy seed consumption [g(kgbw)^{-1} day^{-1}]) & ln(morphine 2007-10 [µg g^{-1}]), 500K data populations.

Method (c), unprocessed poppy

F: Median of percentile exposure values obtained with bootstrapping of all experimental 2009 poppy consumption data [g(kgbw)^{-1} day^{-1}] and 2001-10 morphine data [µg g^{-1}].

Method (c), processed poppy

G: Median of percentile exposure values obtained bootstrapping of experimental 2009 poppy consumption data [g(kgbw)^{-1} day^{-1}] with 2001-10 morphine data [µg g^{-1}].

Children, method (b), unprocessed poppy

H: ln(2009 children poppy seed consumption [g(kgbw)^{-1} day^{-1}]) & ln(morphine 2001-10 [µg g^{-1}]), 500K data populations.

Children, method (a), unprocessed poppy

I: Multiplying all experimental 2009 poppy consumption data of children with all (2001-10) morphine data [µg g^{-1}]. [85*737 data]

Children, method (c), processed poppy

J: Median of percentile exposure values obtained bootstrapping of experimental 2009 children poppy consumption data [g(kgbw)^{-1} day^{-1}] with 2007-10 morphine data [µg g^{-1}].
Figure 1. Relative frequency distributions of morphine concentrations detected in poppy seed
Figure 2. (a, upper figure) Distributions of poppy seed consumption data in 2003 and 2009 and (b, lower figure) Distribution of poppy seed consumption in ground raw and baked form in 2009.
Figure 3. Probability density function of the 97.5th percentile of all morphine concentration values (2001-2010 data). Explanation for the confidence limits: the 97.5th percentile lies between the 17th and 18th ranked values from largest to smallest. The 68% of the values (median ± SD) are between the 17-4=13 and 18+4=22 ranked values.
Figure 4. The median and 95% confidence intervals of the exposure to morphine through consumption of poppy seed.
Figure 5. Relative frequency of estimated quantile values calculated from 10,000 bootstrap. Solid line: normal distribution fitted on results. Dashed line: lognormal distribution fitted on results.