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Exposure of consumers to morphine from poppy seeds in Hungary

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Abstract:	<p>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 $78.64 \mu\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$ for adults, and $116.90 \mu\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$ for children. Based on probabilistic estimations, the 97.5 and 99 percentile exposures ranged between $18.3\text{-}25.4$ and $25.6\text{-}47.4 \mu\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$ for adults, and 32.9 and $66.4 \mu\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$ for children, respectively. As no effect level (NOEL) has not been established, the significance of exposure could not be assessed.</p>

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1 Exposure of consumers to morphine from poppy seeds in Hungary

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6 7 Abstract

8 Poppy seed containing foods are popular dishes in Hungary and some other Central European
9 countries. The alkaloids of poppy are used in the production of medicines. Poppy seeds used
10 as food may also contain considerable amounts of alkaloids, which raise the question of food
11 safety. Morphine, codeine, thebaine and noscapine concentrations of poppy seed samples
12 from the time period 2001-2010, and consumption data from two Hungarian surveys, carried
13 out in 2003 and 2009 were evaluated. Exposure calculations were made for morphine intake
14 both by point estimate and probabilistic methods, and the uncertainty of the calculated values
15 was estimated. The point estimate for the acute consumer exposure, calculated using the 97.5
16 percentiles of morphine concentration and of poppy seed consumption and taking into account
17 the reduction of morphine content by processing, was $78.64 \mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ for adults, and
18 $116.90 \mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ for children. Based on probabilistic estimations, the 97.5 and 99
19 percentile exposures ranged between 18.3-25.4 and 25.6-47.4 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ for adults,
20 and 32.9 and 66.4 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ for children, respectively. As no effect level (NOEL) has
21 not been established, the significance of exposure could not be assessed.

22 **Keywords:** consumers' exposure, probabilistic modelling, point estimate of exposure, poppy
23 seed alkaloids

24 Introduction

25 Foods made of poppy seeds are popular in Hungary. However, their natural alkaloid content
26 can be of concern. The poppy plant (*Papaver somniferum* L.) contains several alkaloids, for
27 instance morphine, codeine, noscapine and thebaine. These are important substances in the
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3 28 production of medicines. Poppy plant varieties are grown for industrial and dietary purposes
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5 29 as well. Published literature indicates that poppy seeds contain only very low or non-
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7 30 detectable levels of alkaloids (Lachenmeier et al 2010), but the seeds may be contaminated
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9 31 during harvest. The poppy seeds from the plants cultivated for industrial alkaloid production
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11 32 in Hungary are allowed for human consumption only after proper cleaning. Occasionally, the
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13 33 poppy seed marketed as food ingredient could contain high amounts of the alkaloids. During
14
15 34 the past five years, the poppy seed was subject of increased research, particularly regarding
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17 35 the reduction of its morphine contents. This was the result of a serious intoxication case of an
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19 36 infant in 2005 in Germany (Lachenmeier et al 2010).

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21 37 The consumption of large amounts of highly contaminated poppy seeds may lead to light-
22
23 38 headedness and enteroparesis in sensitive individuals. The symptoms described are in
24
25 39 agreement with the toxicological actions of morphine. In particular, reports indicate that
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27 40 morphine intake from poppy seeds may be on the scale of therapeutic morphine doses
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29 41 (Battilani et al. 2009). In Hungary, official authorities have recorded data of illnesses caused
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31 42 by poppy seed consumption since 1985. The symptoms ranged from paleness to nausea,
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33 43 vomiting, diarrhoea, headache, stomach cramps, trembling, flushing, bitter sensation, stupor,
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35 44 dizziness, drowsiness, shivering, depression and itchy skin. Ground raw poppy seed was
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37 45 consumed in all cases. However, the amount eaten was not recorded (unpublished report of
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39 46 National Institute for Food and Nutrition Science 2006).

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41 47 After ingestion, morphine is relatively quickly absorbed from the gastrointestinal tract, mainly
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43 48 from the upper small intestine and, to a lesser degree, from the stomach. The maximum effect
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45 49 of morphine is achieved about 30 min after ingestion. The effect of a single dose lasts about
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47 50 4-6 hours (Federal Institute for Risk Assessment 2005). Morphine is metabolized mainly in
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49 51 the liver and undergoes conjugation with glucuronic acid principally at the 3-hydroxyl group.
50
51 52 Morphine is excreted in urine mainly as morphine-3-glucuronide. In addition to the
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53 53 3,6diglucuronide, other minor metabolites that have been identified include normorphine and
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55 54 the 3-ethereal sulfate (Battilani et al. 2009). Secondary conjugation also occurs at the 6-
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57 55 hydroxyl group to form the 6-glucuronide, which is pharmacologically active, and to a limited
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59 56 extent the 3,6-diglucuronide. The low bioavailability (20% - 40%) in the case of oral
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61 57 morphine administration can be attributed to elimination through metabolism in the intestinal
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63 58 mucosa and liver (Battilani et al. 2009).

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3 59 There is no internationally accepted reference point for the toxicity of poppy seed alkaloids.
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5 60 On national level, the German Federal Institute for Risk Assessment (BfR) estimated 6.3 μg
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7 61 $(\text{kg bw})^{-1}\text{day}^{-1}$ as a provisional daily upper intake level of morphine from poppy seeds in
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9 62 2005, based on the lowest oral therapeutic dose of 1.9 mg morphine (2.5 mg morphine
10
11 63 sulphate pentahydrate) per person and a safety factor of 5 (Federal Institute for Risk
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13 64 Assessment 2005). The lowest starting oral dose administered to adults with normal kidney
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15 65 function for pain killing is 2x30 mg/day and 2x10 mg/day for patients with restricted renal
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17 66 diuretic capacity (Embey-Isztin 1996). Taking into account the lowest 10 mg single dose, it is
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19 67 about 5 times higher than the oral therapeutic dose used in BfR study. In the European Union,
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21 68 only Hungary has a national legislation (17/1999 EüM rendelet 1999) that specifies the
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23 69 maximum permitted levels at 30 mg kg^{-1} for morphine, 40 mg kg^{-1} for morphine and
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25 70 noscapine, 20 mg kg^{-1} for noscapine, 20 mg kg^{-1} for codeine and 20 mg kg^{-1} for thebaine in
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27 71 poppy seed.

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29 72 According to several studies, proper cleaning, washing, grinding and baking could reduce
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31 73 significantly the alkaloid contents of poppy seeds. Sproll et al. (2006, 2007) studied the effect
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33 74 of processing on the morphine level of poppy seed, and found that the optimal treatment for
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35 75 reduction consists of washing, drying and grinding. All washing treatments led to a significant
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37 76 reduction of the morphine content with a minimal removal of 48% and a maximum removal
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39 77 of the total morphine content. With cold water, only about 60% of morphine was removed. At
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41 78 temperatures above 60°C, significantly higher proportion, around 90%, could be removed.
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43 79 The length of poppy seed rinsing also increased the level of reduction. Grinding reduced the
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45 80 morphine and codeine contents on an average by 24% and about 20%, respectively (Sproll et
46
47 81 al. 2007). In another study (Sproll et al. 2006), the authors reported morphine losses of 34 ± 5
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49 82 % due to grinding. They also found that during baking up to 135°C the reduction of morphine
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51 83 was relatively low (around 30%), but at 220°C a reduction of 80-90% was observed.

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53 84 In this study we present the results of the evaluation of the acute exposure of Hungarian
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55 85 poppy seed consumers to morphine, calculated with both point estimate and probabilistic
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57 86 method, based on the alkaloid content of poppy seed marketed in Hungary during 2001-2010,
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59 87 and on consumption data from the surveys conducted in 2003 (Rodler et al. 2005) and 2009
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61 88 (Szeitzné-Szabó et al. 2011), taking also into account the effect of processing. The uncertainty
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63 89 of the calculated values was also estimated.

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91 **Materials and Methods**

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

96 During the first study period the alkaloid content was measured with thin layer
97 chromatography according to the Hungarian standard MSZ 690:1993. In this method the
98 morphine was extracted with chloroform-isopropyl alcohol, purified with 0.1 mol/l HCl
99 solution, separated and detected on silica GF₂₅₄ TLC plate. Recovery was 80%. The LOQ for
100 morphine was 2 mg kg⁻¹, and it varied between 2, 3, 4, 5, 8 mg kg⁻¹ for noscapine, codeine
101 and thebaine. The results were confirmed with HPLC–UV detection.

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

112 Consumption data obtained with 3-day dietary record method from the years 2003 and 2009
113 were used for the assessment. In 2003, 1360 adult consumers recorded 79 poppy seed
114 consumption days (1.94%), while 4992 consumers (age range between 0-101 years) reported
115 327 poppy seed consumption days (2.18%) in 2009. Regarding consumption data of children
116 and adolescents of 1-18 years of age, information was only available from the consumption
117 survey conducted in 2009, where 1010 young responders reported 85 poppy seed
118 consumption days in total (2.81%). In the 2009 survey there were data available about the
119 recipes of foods, so it was possible to evaluate data of processing. The poppy seed was
120 consumed in ground form on 65% of days and only in 35% of the cases was consumed in
121 baked cakes.

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3 122 In view of the infrequent consumption of poppy seed and the rapid decrease of the potential
4 123 adverse effects of its alkaloid content, only the acute exposure of consumers is a concern. For
5 124 the estimation of acute exposure, both the alkaloid concentration data and the poppy seed
6 125 consumption data (eaters only) obtained during the two periods were considered separately,
7 126 and after combining them for the whole time period (2001-2010 for alkaloid concentrations,
8 127 and 2003+2009 for the poppy seed consumptions).

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13 128 For point estimate, the exposure was calculated using the 97.5th percentile of daily poppy seed
14 129 consumptions and 97.5th percentile of morphine concentrations (EFSA 2007).

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18 130 As consumption data for ground poppy seed and baked poppy seed were available from the
19 131 2009 survey, the effect of processing on morphine intake could also be studied. The
20 132 processing factor (PF) was calculated as the ratio of the concentration of morphine in
21 133 processed (ground or ground and baked) poppy seed and raw poppy seed (FAO 2009). As
22 134 poppy seed is not washed as part of the commercial or bakery operations in Hungary, the
23 135 effect of washing was not considered. The processing factor of 0.71 for grinding of poppy
24 136 seed was calculated from the 34% and 24% average losses of morphine (Sproll 2006, 2007).
25 137 The strudels and cakes are baked in Hungary typically at 180°C. The processing factor of 0.31
26 138 for baking of cakes was estimated using MS Excel exponential trend line fitting function from
27 139 the reduction of morphine content observed at 135 °C and 220 °C temperatures (Sproll et al.
28 140 2006) assuming the reported quadratic relationship. The combined processing factor including
29 141 grinding for morphine content in baked poppy seed is $0.71 \times 0.31 = 0.22$. The morphine
30 142 intakes were reduced by the calculated processing factors according to the form of consumed
31 143 poppy.

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42 144 For probabilistic modelling, parametric distributions were fitted to the morphine concentration
43 145 values and consumption data applying @RISK programme package. The uncertainty of the
44 146 fitted parameters was not estimated. The relative frequency distributions of original data and
45 147 the fitted distributions were visually evaluated too. Parametric distributions giving the best fit
46 148 for both the morphine concentrations and consumption data were used to generate 200 000
47 149 and 500 000 random values. The generated lognormal or gamma distributions were expected
48 150 to cover the high experimental values and beyond those observed. Morphine exposure of
49 151 children and adolescents, as a subset of the total population, was calculated similarly.

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56 152 In summary, the exposure was calculated with each of the following methods, for comparison:

57 153 (a) All consumption data were multiplied by all morphine concentration data;

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3 154 (b) The 200,000 or 500,000 pairs of random values of morphine concentrations and poppy
4 155 seed consumptions, drawn from the selected parametric distributions, were multiplied
5 156 with each other. This is a parametric alternative to method (a).

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8 157 (c) The combined original data populations (737 morphine concentrations and 406 poppy
9 158 seed consumptions) were bootstrapped 10,000 times and the resulting morphine
10 159 concentrations were multiplied with the resulting consumption values (10,000
11 160 iterations). The cumulative relative frequencies of exposure were calculated from each
12 161 iterations, using the NIST definition for percentile calculations (NIST 2011). This
13 162 non-parametric bootstrapping procedure (Efron and Tibshirani 1994) was used to
14 163 quantify uncertainty due to the limited sample sizes.

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20 164 (d1) and (d2) Morphine intake was calculated using the abovementioned processing
21 165 factors for ground and baked poppy seeds applying procedures (b) and (c).

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23 166 The poppy seed consumption in raw ground form and in baked cakes showed distinctly
24 167 different pattern, therefore parametric distributions were separately fitted on the two data sets.
25 168 The exposures of consumers to morphine derived from consuming ground poppy seed and
26 169 cakes containing poppy seed were calculated separately from the re-sampled distributions of
27 170 consumption and morphine data. As the separate consumption data were only available from
28 171 the 2009 survey, the lognormal distribution fitted on 2007-2010 morphine concentration data
29 172 were used for the calculations. In order to obtain representative exposure values for the
30 173 combined poppy seed consumption, the proportion of consumption days of ground poppy
31 174 seed (65%) and ground and baked poppy seed (35%) were taken into account and 325,688
32 175 and 174,312 random samples were drawn from the generated ground and baked poppy seed
33 176 consumption data population, respectively. The same number of concentration data were
34 177 drawn from the generated morphine population. The exposures calculated from the two sets of
35 178 data were evaluated separately and in combination.

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45 179 The results obtained with these different methods were compared and evaluated together.

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49 50 181 **Results and Discussion**

51 52 182 *Alkaloid levels in poppy seeds in Hungary*

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55 183 Altogether 737 poppy seed samples were analysed. Morphine was detected in 736 out of 737
56 184 samples. Codeine and thebaine were detected in 61.3% and 63.0% of the cases, respectively.

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3 185 Noscapine was detected in 6.2% of the samples only. In more than half of the samples 3 or 4
4 186 alkaloids were detected together. Table 1 presents the summary of the detectable alkaloids
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6 187 and their selected percentiles, in the samples.
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9 188 Morphine content of the samples varied to a great extent, with median of 11 mg kg⁻¹ and
10 189 highest values of 222, 238, 267 and 533 mg kg⁻¹. The 97.5th and 95th percentiles and the
11 190 median values of morphine concentrations were calculated with Harrell-Davis (Craig personal
12 191 communication), and the binomial (Hamilton et al. 2004) methods, and with MS Excel (Table
13 192 2). The binomial method enabled estimating the probability that a given ranked value is the
14 193 selected percentile of the population. The Harrell-Davis (HD) and the binomial methods
15 194 resulted in very similar estimates, while the 97.5th percentile values obtained with Excel were
16 195 lower. Consequently, the average values of median, 95th and 97.5th estimates obtained with
17 196 HD and binomial methods are given in Table 3 for the alkaloids detected in poppy seeds. The
18 197 average estimated 97.5th percentile of morphine concentrations and the similarly estimated
19 198 97.5th percentile of poppy seed consumptions were used in calculating the point estimates of
20 199 morphine intake.
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30 200 The relative frequency distributions of morphine concentrations measured during the two
31 201 survey periods were similar (Figure 1). The ratios of the 97.5th percentile and median values
32 202 of the morphine 2007-2010 and 2001-2006 data sets were 1.064 and 1.055, respectively. The
33 203 ratios indicate that the morphine concentrations measured with different methods during
34 204 2001-2006 and 2007-2010 were not substantially different. The Mann-Whitney U-test
35 205 confirmed that the difference was not significant (P=0.35) (McDonald 2009).
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40 206 The medians and 97.5 percentile concentrations of alkaloids calculated for the 10 years of
41 207 data separately (Table 1), did not indicate clear trend in changing of morphine content. The
42 208 calculated 97.5th percentiles are lower in the last years (2009 and 2010), but it may be
43 209 attributed to the smaller number of samples analysed, as the probability of detecting at least
44 210 one value above the 97.5 percentile is only 40-45% in case of 21-25 samples. Based on these
45 211 results, the combination of morphine data of the 10 years is justified.
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50 51 212 *Consumption of poppy seeds in Hungary*

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53 213 In the 2003 food consumption survey, poppy seed consumption occurred on 1.94 % of all
54 214 consumption days. Considering body weight of consumers, the highest, the 97.5th percentile
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215 and the average daily consumption was 1 g (kg bw)⁻¹day⁻¹, 0.80 g (kg bw)⁻¹day⁻¹ and 0.34 g
216 (kg bw)⁻¹day⁻¹, respectively.

217 Poppy seed consumption was recorded on 327 out of 14 976 days (2.18%) in the 2009 food
218 consumption survey. The average and 97.5th percentile consumptions were 0.44 g (kg bw)⁻¹
219 day⁻¹ and 1.20 g (kg bw)⁻¹day⁻¹, respectively. The highest adult consumption was recorded by
220 a 20-year-old, 62 kg man (1.68 g (kg bw)⁻¹day⁻¹). The average and 97.5th percentile
221 consumptions of children and adolescents were 0.67 g (kg bw)⁻¹day⁻¹ and 1.77 g (kg bw)⁻¹day⁻¹
222 respectively. The highest poppy seed consumption was recorded by a 4-year-old, 22 kg girl
223 (2.73 g (kg bw)⁻¹day⁻¹), while the youngest poppy seed consumer was 1 year old. It should be
224 pointed out, that none of the consumption survey periods included Christmas, when the cakes
225 are traditionally prepared with poppy seed.

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

230 There was detailed information about the form of consumed poppy seed from the 2009 survey
231 only. Out of the 327 consumption days, pasta with ground, raw poppy seed was consumed on
232 213 (65%), and baked cakes were consumed on 114 (35%) occasions. Consumption levels of
233 raw, ground poppy seed were generally higher than those of baked poppy (Table 4.)

234 *Morphine intake from the consumption of poppy seeds*

235 *Point estimate*

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

240 In order to assess the potential consequence of morphine concentrations and consumption
241 values obtained during the two periods, the intakes were calculated from the two consumption
242 data sets separately and from the combined consumption data as well as the separate and
243 combined morphine concentration data sets. The results are summarised in Table 5.

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3 244 Based on consumption of raw poppy seed, the point estimate (using average percentiles
4 245 calculated by binomial and Harrell-Davis calculation methods) for the acute consumer
5 246 exposure ranges between 73.1 and 116.7 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$. The lowest exposure (73.1 $\mu\text{g (kg}$
6 247 $\text{bw)}^{-1}\text{day}^{-1}$) was obtained from the combination of the 2003 consumption data and all
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8 248 morphine concentrations, the highest exposure (116.7 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$) resulted from the
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10 249 combination of 2009 consumption and 2007-2010 morphine data.

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13 250 The approximate 95% confidence limits for the estimated intake values were calculated, with
14 251 the binomial method, from the ranked values bracketing the 68% confidence intervals ((1-
15 252 0.683)/2=0.1587; $0.1587 \times 0.1587=0.025$) of the 97.5th percentiles of corresponding data sets
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17 253 estimated based on the binomial method (Figure 3). The standard deviation, SD, of the
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19 254 selected percentile 'p' is calculated as $\text{SD}=\sqrt{(\text{Npq})}$, where N is the number of data points and
20 255 $p+q=1$ (Diem and Seldrup 1982). The 68% confidence intervals around the estimated 97.5
21 256 percentile for $N=737$ correspond to the $17-4=13^{\text{th}}$ and $18+4=22^{\text{nd}}$ ranked values (67 and 112
22 257 mgkg^{-1}) where the largest value has the 1st rank. As the best estimate (i.e. average of two
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24 258 calculation methods) of the 97.5th percentile was close to the value estimated with the
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26 259 binomial method, the confidence intervals calculated with the values obtained from the
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28 260 binomial method provide an acceptably close approximation. Due to low number of data
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30 261 points, the 95% confidence limits for the 2003 consumption data and 2007-2010 morphine
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32 262 concentrations encompassed practically the whole data sets. Therefore confidence limits could
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34 263 not be calculated for those combinations.

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37 264 Morphine short-term intakes of children and adolescents (1-18 years) calculated from the
38 265 2007-2010 and 2001-2010 morphine concentrations and the 85 consumption days data of raw
39 266 poppy seed were 164.7 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ and 150.2 $\mu\text{g(kg bw)}^{-1}\text{day}^{-1}$, respectively. The
40 267 morphine intake was higher than that of the whole population due to the higher average and
41 268 97.5th percentile of the poppy seed consumption of the age group of 1-18 years. The small
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43 269 number of data did not allow the calculation of the confidence intervals for the estimated
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45 270 intakes.

50 271 ***Probabilistic estimation of morphine intake***

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53 272 Probabilistic modelling was carried out with options (a), (b) (c) and (d) described under
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55 273 methods. The results are summarized in the following.

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3 274 • The 200K values generated from the fitted lognormal distributions covered the
4 275 maximum recorded consumption figures in each data set; however the maximum
5 276 experimental morphine concentration could only be covered in two out of three cases
6 277 (columns B, C and D1 in Table 6). When 500K values were generated, the highest
7 278 values obtained were substantially higher than the highest experimental value (column
8 279 D2). The results suggest that the heavy tail of the empirical consumption data can only
9 280 be covered if large number of data points are generated from the fitted distribution;
10 281 • The random morphine values generated from the fitted gamma distribution, however,
11 282 underestimated the upper tail of morphine experimental data (the highest generated
12 283 and experimental values were $180.1 \mu\text{g g}^{-1}$ and $202 \mu\text{g g}^{-1}$, or $191.2 \mu\text{g g}^{-1}$ and $533 \mu\text{g}$
13 284 g^{-1} respectively). Consequently, the gamma distribution was not suitable for estimating
14 285 high quantiles of exposure;
15 286 • When random samples were drawn from the fitted lognormal distributions, the intake
16 287 estimates obtained from the different data sets were in the same range below the 95th
17 288 percentile, but showed larger differences at or above the 95th percentile of the
18 289 cumulative relative frequency distributions (Table 6 columns B, C, D1 and D2). The
19 290 difference between the cumulative relative frequencies corresponding to the intake
20 291 values obtained with the three combinations of data represents the uncertainty of the
21 292 exposure estimation based on the calculations with the limited subsets of data;
22 293 • The results obtained with multiplying all of experimental data and bootstrapping
23 294 (methods (a) and (c)) are much higher than those obtained by method (b) and therefore
24 295 represent worst case situations (see columns A and D2, and columns H and I).
25 296 • The comparison of columns C and E, illustrates the effect of processing based on the
26 297 2009 consumption and 2007-2010 morphine concentrations. The ratio of exposure
27 298 from raw poppy seed and from processed poppy seed in the percentiles above 95th is
28 299 between 0.639 and 0.646, which indicates that the grinding ($P_f=0.71$) was the main
29 300 source of reduction of exposure.
30 301 • The comparison of columns F and G illustrates the effect of processing based on
31 302 exposure results of 10 000 bootstrapped data (the exposure percentiles were calculated
32 303 from each iteration and the medians are shown in the table). The ratios of
33 304 corresponding percentiles are between 0.621 and 0.675 at the higher percentiles.
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3 305 • The exposure of children ($\mu\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$) to poppy seed shown in columns H, I and
4 306 J is about 1-2 times higher than of adults (columns D2, A, and G) at the 97.5th - 99.99th
5 307 percentiles.

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8 308 The cumulative distribution of the exposure calculated by multiplying the morphine
9 309 concentration and consumption data obtained from their fitted lognormal distributions and
10 310 with multiplying all of experimental data (method (a)) are close to each other up to about 90th
11 311 percentile of the exposure (columns A, D1 and D2). At 99.9th and 99.99th percentiles, however
12 312 the differences are more than two times even in the cases where the highest modelled values
13 313 for poppy seed consumption and morphine concentrations are higher than the corresponding
14 314 experimental values. The possible reason for the large differences at high percentiles was
15 315 further studied. The highest morphine concentrations observed in poppy seed samples were
16 316 187, 200, 202, 222, 238, 267 and 533 mgkg^{-1} , and the highest poppy seed consumptions were
17 317 1.30, 1.32, 1.33, 1.54, 1.58, 1.68 and 2.73 $\text{g}(\text{kg bw})^{-1}\text{day}^{-1}$. It was found that the 4 highest
18 318 morphine concentrations in combination with the highest 20 poppy seed consumption values
19 319 resulted in the exposures above the 99.99th percentile cumulative exposure ($489.1 \mu\text{g}(\text{kg bw})^{-1}\text{day}^{-1}$).
20 320 If the highest morphine and poppy seed consumption values (533 mg kg^{-1} and 2.73 g
21 321 $(\text{kg bw})^{-1}\text{day}^{-1}$) were omitted from the calculation, then the 99.99th percentile values would be
22 322 $267 \mu\text{g}(\text{kg bw})^{-1}\text{day}^{-1}$. These results explain why lower exposure values were obtained when
23 323 they were calculated from the results of random sampling of fitted populations (columns D1
24 324 and D2 in Table 6), because in the latter cases the probability of selecting the highest values
25 325 from both populations of 200,000 or 500,000 with random sampling is very low.

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39 326 The range of estimated cumulative relative frequency values were calculated with
40 327 bootstrapping drawing 10,000 random samples with replacement from both the combined 737
41 328 morphine concentration values and the 406 poppy seed consumption data, and multiplying all
42 329 concentration values with all consumption data in each case to calculate the morphine intake
43 330 [$\mu\text{g}(\text{kg bw})^{-1}\text{day}^{-1}$]. The procedure was repeated 10,000 times (bootstrap method (c)). The
44 331 median and the 95% confidence limits of the calculated cumulative relative frequencies are
45 332 shown in Figure 4. The horizontal lines within the confidence limits show the intake range
46 333 belonging to a given cumulative percentile. The vertical line between the confidence intervals
47 334 indicates the range of the percentage of the consumption days of morphine intake around the
48 335 selected one. The results showed that the 95% confidence interval of the estimated cumulative
49 336 frequencies, indicating the uncertainty of the estimation, was the narrowest at 50th percentile
50 337 and increased at higher and lower percentile values. For instance, the relative 95% interval of
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3 338 the estimated 50th, 95th, 97.5th, 99th, 99.9th and 99.99th cumulative percentile values were in the
4 339 order of: 13.2%, 27.7%, 40.1%, 50.3%, 69.7% and 80.5%.

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7 340 Figure 5 shows examples for the frequency distributions of estimated percentile values
8 341 obtained from 10 000 bootstrap samples. At or below the 99th percentile the calculated values
9 342 approximate normal distribution. However, at higher percentile values the distribution is
10 343 getting scattered and their ranges are getting wider, as expected.

14 344 *Effect of processing on morphine intake*

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17 345 The calculations assuming that the morphine concentrations measured in raw poppy seed are
18 346 present in consumed food provide an overestimate of the intake. In order to get a more
19 347 realistic estimate, the effects of washing, grinding and baking were taken into consideration.
20 348 In Hungary, the poppy seed produced at commercial scale is not washed. Taking into account
21 349 that 65% proportion of poppy seed is consumed unbaked, and 35% is consumed baked, the
22 350 acute intake of morphine was recalculated applying the calculated processing factors (0.71
23 351 and 0.22). The estimated exposure decreased by 32.6% for adults and 29.0% for children,
24 352 respectively (from 116.70 to 78.64 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ and from 164.70 to 116.90 $\mu\text{g (kg bw)}^{-1}$
25 353 day^{-1} respectively).

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33 354 Regarding probabilistic exposure estimate of processed poppy seed consumption, it was found
34 355 that the intake was decreased by about 33-38% at the upper percentiles. These findings are in
35 356 accordance with the higher intakes of ground poppy seed (estimated processing factor is 0.71)
36 357 than that consumed with cakes, and consequently the ground poppy seed consumption is the
37 358 cause of the high morphine intake.

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42 359 Counting for a consumer of 60 kg (body weight), the starting oral therapeutic dose reported
43 360 by Embey-Isztin would be about 167 $\mu\text{g (kg bw)}^{-1}$, while the lowest oral therapeutic dose
44 361 reported by BfR would be about 31.7 $\mu\text{g (kg bw)}^{-1}$. The results from probabilistic calculations
45 362 suggest that the 31.7 $\mu\text{g (kg bw)}^{-1}$ dose could be reached only above the 97.5 percentile of the
46 363 adult poppy seed eaters (see Table 6, columns B, C, D1 and D2). Looking at childrens'
47 364 exposure (Table 6, column H), above 90-95 percentiles could be in the range of the estimated
48 365 lowest therapeutic dose referred by BfR. Probability of reaching the starting oral therapeutic
49 366 dose of 167 $\mu\text{g (kg bw)}^{-1}$ is very low, practically negligible.

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56 367 The provisional daily upper intake level (6.3 $\mu\text{g (kg bw)}^{-1}\text{day}^{-1}$) established by BfR has an
57 368 incorporated uncertainty factor of 5. This is not likely to be exceeded by the median adult

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3 369 exposure; however, children have higher intake than adults. Around the 65% of the
4 370 cumulative distribution of morphine intake of adults and 50% of children corresponds to the
5 371 $6.3 \mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ provisional daily upper intake, calculated with the morphine content of
6 372 unprocessed poppy seed.

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10 373 Taking into account that poppy seed is consumed in processed form, it was concluded that
11 374 exposures are about 35% lower than the scenarios calculated without counting for processing
12 375 losses.

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16 376 Applying the point estimation methodology the calculated acute intake exceeds the BfR
17 377 reference value but does not reach the starting oral therapeutic dose. It should be noted that
18 378 this is a very conservative way of intake calculation; the probability that a 'big eater'
19 379 consumes from the highly contaminated food is low.

20 21 22 23 24 380 **Conclusion**

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26 381 The most prominent alkaloid of poppy seed is morphine being present in detectable amounts
27 382 practically in all samples analysed in Hungary during 2001-2010. As the poppy seed was
28 383 consumed on only a small fraction of the dietary survey days, the chronic intake is not of a
29 384 concern. Consequently only the short-term intakes of poppy seed alkaloids were estimated in
30 385 our study, based on the measured alkaloid content of raw poppy seed, taking also into account
31 386 the estimated effect of grinding and baking. The intake calculation performed with the fitted
32 387 lognormal distributions gave the best fit on the original data. The 97.5th percentile exposure
33 388 of adults ranged between $25.6\text{-}34.7 \mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ depending on the time period. The
34 389 calculated 97.5th percentile of childrens' exposure was $59.1 \mu\text{g (kg bw)}^{-1}\text{day}^{-1}$. Taking into
35 390 account the reduction of morphine content by processing, the 97.5 and 99 percentile
36 391 exposures based on probabilistic estimations ranged between $18.3\text{-}25.4$ and $25.6\text{-}47.4 \mu\text{g (kg}$
37 392 $\text{bw)}^{-1}\text{day}^{-1}$ for adults, and 32.9 and $66.4 \mu\text{g (kg bw)}^{-1}\text{day}^{-1}$ for children, respectively.

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47 393 The poppy seed was used on 65% of consumption days as flavouring ingredient of pasta in
48 394 ground form. The average and 97.5th percentile consumption of poppy seed in baked form is
49 395 60% and 77% of that eaten in ground form, respectively. The high morphine intake primarily
50 396 derives from the consumption of ground raw poppy seed. According to the current marketing
51 397 practice, the poppy seed is cleaned only by ventilation and screening, but not washed.
52 398 Consequently, morphine content of the commercialised raw poppy seed is reduced before
53 399 consumption by about 35% due to the effect of grinding. The consequences of pattern and
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3 400 form of the consumption is reflected in the results of estimated short term exposure which
4 401 indicated that the morphine intake from processed poppy seed was about 35% lower than the
5 402 values calculated from the morphine content of raw poppy seed.
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9 403 The 5-fold difference in reported lowest oral therapeutic doses of $167 \mu\text{g (kg bw)}^{-1}$ and 31.7
10 404 $\mu\text{g (kg bw)}^{-1}$ of morphine indicates the large uncertainty in the estimation of the reference
11 405 dose value for assessing the potential adverse effects of exposure of consumers to morphine.
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14 406 Reliable risk assessment was not possible because internationally accepted acute reference
15 407 doses for morphine and the other alkaloids detected in poppy seeds have not been established
16 408 yet. Even less or no information is available on the potential adverse effects of codeine,
17 409 thebaine and noscapine content of poppy seeds individually or in combination, and the
18 410 bioavailability of these alkaloids.
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21 411 Further scientific studies, meeting the relevant standards, are required in order to establish
22 412 acute reference dose to assess the actual risk. The effect of technological steps, the
23 413 degradation of morphine in the digestive system during absorption, and the effect of poppy
24 414 alkaloid "cocktail" compared to purified morphine should also be taken into account. Upon
25 415 that the necessary risk management options could be established to reduce the alkaloid intake.
26 416 Our findings contribute to the scientific risk assessment with providing exact, statistically
27 417 well-founded exposure data of poppy seed alkaloids, with specific focus on morphine.
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37 38 419 **Acknowledgement**

39
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44 424 Harrel-Davis method.
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51 52 426 **References**

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3 491 Figure captions:
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5 492 Figure 1. Relative frequency distributions of morphine concentrations detected in poppy seed
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7 493 Figure 2. (a, upper figure) Distributions of poppy seed consumption data in 2003 and 2009
8 494 and (b, lower figure) Distribution of poppy seed consumption in ground raw and baked form
9 495 in 2009.
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12 497 Figure 3. Probability density function of the 97.5th percentile of all morphine concentration
13 498 values (2001-2010 data). Explanation for the confidence limits: the 97.5th percentile lies
14 499 between the 17th and 18th ranked values from largest to smallest. The 68% of the values
15 500 (median \pm SD) are between the 17-4=13 and 18+4=22 ranked values.
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18 501 Figure 4. The median and 95% confidence intervals of the exposure to morphine through
19 502 consumption of poppy seed.
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21 503
22 504 Figure 5. Relative frequency of estimated quantile values calculated from 10 000 bootstrap.
23 505 Solid line: normal distribution fitted on results. Dashed line: lognormal distribution fitted on
24 506 results.
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Table 1. Number of poppy seed samples analysed and percentage of alkaloids detected in the samples above LOQ.

Years	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Sample number	80	77	173	55	82	99	71	54	25	21
<i>Morphine</i> % detected	100.0	100.0	100.0	100.0	100.0	99.0	100.0	100.0	100.0	100.0
Median	7.92	16.99	10.38	9.48	15.05	10.93	11.14	12.88	12.70	8.08
97.5 percentile	68.12	300.01	82.40	41.01	89.63	128.80	137.87	110.41	27.63	26.69
<i>Codeine</i> % detected	30.0	64.9	48.0	56.4	75.6	69.7	80.3	75.9	96.0	52.4
Median of codeine concentrations	0.30	2.00	0.30	2.00	2.00	2.00	2.00	1.70	2.00	1.10
97.5 percentile of codeine concentrations	10.13	33.95	21.10	8.60	29.98	20.55	25.28	12.40	4.28	3.50
<i>Noscapine</i> % detected	3.8	5.2	4.0	0.0	0.0	3.0	9.9	18.5	32.0	19.0
Median of noscapine concentrations	0.30	0.30	1.30	1.30	1.30	1.30	0.20	0.20	0.20	0.20
97.5 percentile of noscapine concentrations	2.05	5.20	4.00	1.30	1.30	4.98	6.28	6.22	11.58	5.10
<i>Thebaine</i> % detected	27.5	61.0	50.9	61.8	67.1	78.8	90.1	70.4	92.0	71.4
Median of thebaine concentrations	0.30	2.00	2.00	2.00	2.00	2.00	3.40	1.70	2.50	1.40
97.5 percentile of thebaine concentrations	10.00	46.20	22.00	15.55	22.00	16.40	42.33	15.03	7.16	4.00

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 (mgkg⁻¹)

Year	97.5th percentile of concentration					Median concentration				
	Excel	HD	Binomial method			Excel	HD	Binomial method		
			LCL	Calc. Value	UCL			LCL	Calc. Value	UCL
2001-06 566	80.00	85.74	67.00	89.21	160.00	11.00	10.95	10.00	10.95	11.00
2007-10 171	52.70	90.67	2.90	95.40	>202.00 ¹	11.40	11.53	10.80	11.57	12.70
2001-10 737	80.00	83.57	67.00	86.09	141.00	11.00	10.99	11.00	11.00	11.00

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 (mgkg^{-1}) in all (2001-2010) samples.

	Median ¹	Average ²	P0.95 ¹	P0.975 ¹	Maximum
Morphine	11.0	18.7	57.6	84.8	533.0
Codeine	2.0	3.6	17.2	25.7	60.0
Noscapine	1.2	1.2	1.6	4.7	40.0
Thebaine	2.0	4.0	15.8	23.9	120.0

¹ The median, 95th and 97.5th 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 ($\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$) based on the 2003 and 2009 surveys.

	Survey data					
	2009			2003	2003+2009	Ratio ¹
	All	Ground	Baked			
No. of days	327	213	114	79	406	
Min	0.042	0.048	0.042	0.031	0.031	
Average	0.440	0.516	0.300	0.340	0.421	1.294
Max	2.727	2.727	1.677	1.000	2.727	
SD	0.304	0.294	0.271	0.183	0.287	
CV	0.689	0.570	0.902	0.538	0.681	
Median ²	0.395	0.439	0.217	0.337	0.382	1.172
P0.975 ²	1.255	1.235	0.954	0.862	1.190	1.456

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 [$\mu\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$] from raw poppy seed consumption.

Best estimates ¹ (and 68% confidence intervals) ² for P0.975 consumptions in time periods [$\text{g}(\text{kg bw})^{-1}\text{day}^{-1}$]		Best estimates ¹ (and 68% confidence intervals) ² for morphine P0.975 concentrations in time periods [$\mu\text{g g}^{-1}$]		
		2001-2006	2007-2010	2001-2010
		87.48 (67.00-120.00)	93.04	84.83 (67.0-112.0)
Acute exposure				
2003	0.86	75.42	80.21	73.14
2009	1.26 (1.18-1.33)	109.73 (78.79-159.96)	116.70	106.41 (78.79-149.30)
2003+2009	1.19 (1.11-1.32)	104.02 (74.44-158.88)	110.63	100.87 (74.44-148.29)

¹ Best estimate calculated as the average of estimated 97.5th percentile with HD and binomial method.

² 68% confidence interval calculated as $\text{P0.975} \pm \text{SD}$ 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.

ECDF	A	B	C	D1	D2	<u>E</u>	F	<u>G</u>	H	I	<u>J</u>
0.2	1.7	1.4	1.9	1.7	1.6	<u>0.7</u>	1.7	<u>0.6</u>	2.5	2.9	<u>1.4</u>
0.3	2.4	2.0	2.7	2.4	2.3	<u>1.1</u>	2.5	<u>1.1</u>	3.6	4.1	<u>2.3</u>
0.4	3.2	2.7	3.5	3.2	3.0	<u>1.7</u>	3.4	<u>1.7</u>	4.9	5.3	<u>3.2</u>
0.5	4.1	3.5	4.5	4.2	4.0	<u>2.5</u>	4.3	<u>2.4</u>	6.5	6.7	<u>4.2</u>
0.6	5.1	4.6	5.8	5.5	5.3	<u>3.3</u>	5.4	<u>3.1</u>	8.7	8.6	<u>5.4</u>
0.65	5.8	5.2	6.6	6.3	6.1	<u>3.9</u>	6.1	<u>3.6</u>	10.1	9.5	<u>6.1</u>
0.7	6.6	6.1	7.5	7.3	7.2	<u>4.5</u>	6.9	<u>4.2</u>	11.8	10.9	<u>7.0</u>
0.8	9.2	8.4	10.1	10.2	10.2	<u>6.3</u>	9.7	<u>5.9</u>	16.8	15.0	<u>9.4</u>
0.9	15.4	13.1	15.2	16.0	16.6	<u>9.7</u>	16.1	<u>9.9</u>	27.6	24.6	<u>14.4</u>
0.95	25.0	18.7	21.2	23.3	24.7	<u>13.7</u>	26.1	<u>16.2</u>	41.5	39.3	<u>21.7</u>
0.975	38.8	25.6	28.4	32.0	34.7	<u>18.3</u>	40.9	<u>25.4</u>	59.1	63.3	<u>32.9</u>
0.99	72.6	36.8	39.7	46.5	52.4	<u>25.6</u>	76.2	<u>47.4</u>	88.8	112.0	<u>66.4</u>
0.999	222.1	76.7	78.9	101.7	121.8	<u>50.4</u>	224.4	<u>151.4</u>	204.7	320.0	<u>166.0</u>
0.9999	489.1	147.4	131.9	195.9	235.5	<u>84.9</u>	499.7	<u>336.9</u>	398.1	702.7	<u>321.7</u>
* 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

Explanation:

1: Poppy seed consumption [$\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$]: maximum value of 200 000 or 500 000 generated data with the parameters providing the best fit.

2: Poppy seed consumption [$\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$]: maximum experimental value.

3: Morphine concentration [$\mu\text{g g}^{-1}$]: Maximum value of 200 000 or 500 000 generated data with the parameters providing the best fit.

4: Morphine concentration [$\mu\text{g g}^{-1}$]: Maximum experimental value.

Method (a), unprocessed poppy

A: Multiplying all experimental 2003+2009 poppy consumption data [$\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$] with all (2001-10) morphine data [$\mu\text{g g}^{-1}$]. [406*737 data]

Method (b), unprocessed poppy

B: \ln (2003 poppy seed consumption [$\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$]) & \ln (morphine 2001-6 [$\mu\text{g g}^{-1}$]), 200K data populations.

C: \ln (2009 poppy seed consumption [$\text{g}(\text{kgbw})^{-1}\text{day}^{-1}$]) & \ln (morphine 2007-10 [$\mu\text{g g}^{-1}$]), 200K data populations.

1
2
3 **D1:** $\ln(2003+2009 \text{ poppy seed consumption } [\text{g}(\text{kgbw})^{-1}\text{day}^{-1}])$ & $\ln(\text{morphine } 2001-10 [\mu\text{g g}^{-1}])$, 200K data
4 populations.

5 **D2:** $\ln(2003+2009 \text{ poppy seed consumption } [\text{g}(\text{kgbw})^{-1}\text{day}^{-1}])$ & $\ln(\text{morphine } 2001-10 [\mu\text{g g}^{-1}])$, 500K data
6 populations.

7
8 Method (b), processed poppy

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

10
11 Method (c), unprocessed poppy

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

14 Method (c), processed poppy

15 **G:** Median of percentile exposure values obtained bootstrapping of experimental 2009 poppy consumption data
16 $[\text{g}(\text{kgbw})^{-1}\text{day}^{-1}]$ with 2001-10 morphine data $[\mu\text{g g}^{-1}]$.

17
18 Children, method (b), unprocessed poppy

19 **H:** $\ln(2009 \text{ children poppy seed consumption } [\text{g}(\text{kgbw})^{-1}\text{day}^{-1}])$ & $\ln(\text{morphine } 2001-10 [\mu\text{g g}^{-1}])$, **500K** data
20 populations.

21 Children, method (a), unprocessed poppy

22 **I:** Multiplying all experimental 2009 poppy consumption data of **children** with all (2001-10) morphine data $[\mu\text{g}$
23 $\text{g}^{-1}]$. [85*737 data]

24 Children, method (c), processed poppy

25 **J:** Median of percentile exposure values obtained bootstrapping of experimental 2009 children poppy
26 consumption data $[\text{g}(\text{kgbw})^{-1}\text{day}^{-1}]$ with 2007-10 morphine data $[\mu\text{g g}^{-1}]$.

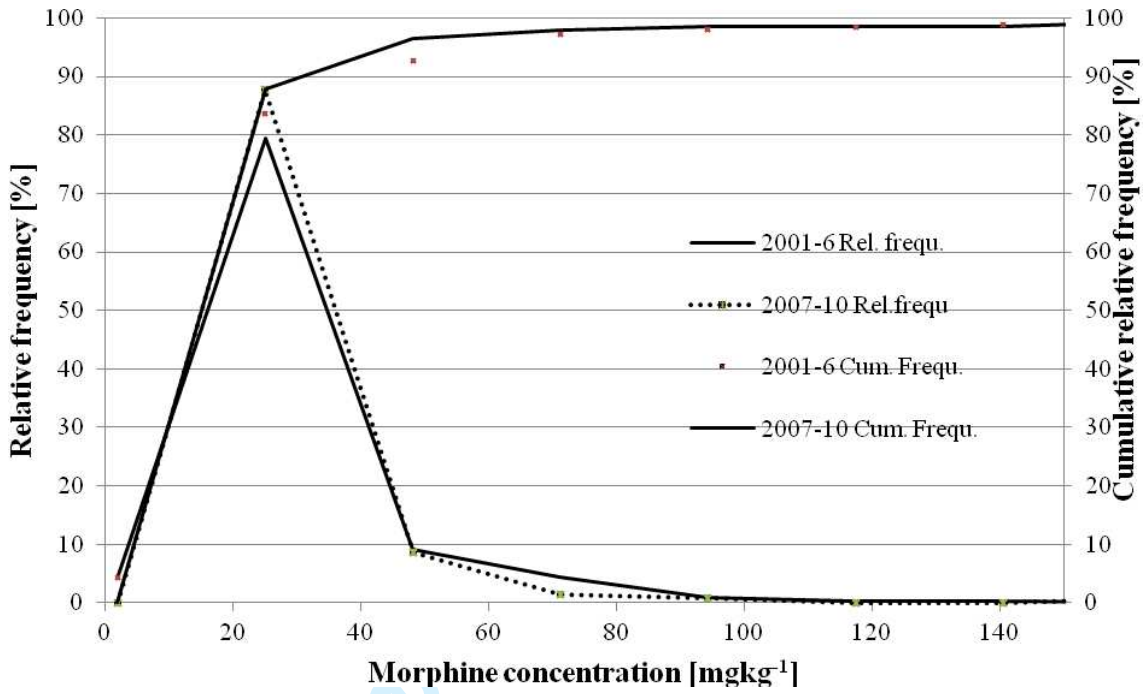


Figure 1. Relative frequency distributions of morphine concentrations detected in poppy seed

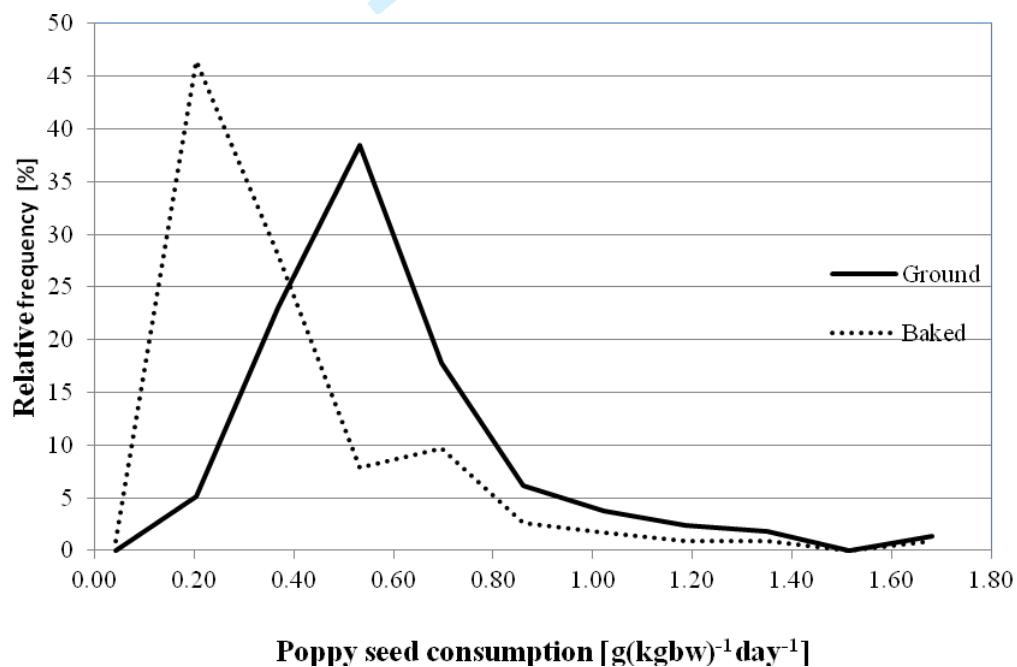
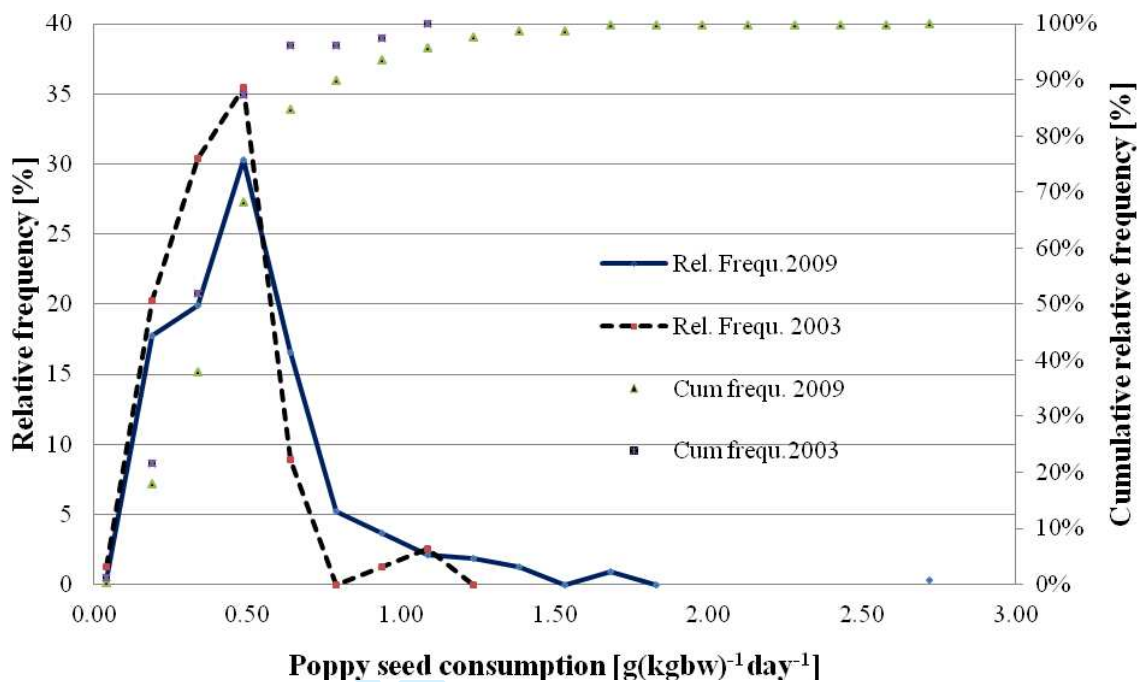


Figure 2. (a, upper figure) Distributions of poppy seed consumption data in 2003 and 2009 and (b, lower figure) Distribution of poppy seed consumption in ground raw and baked form in 2009.

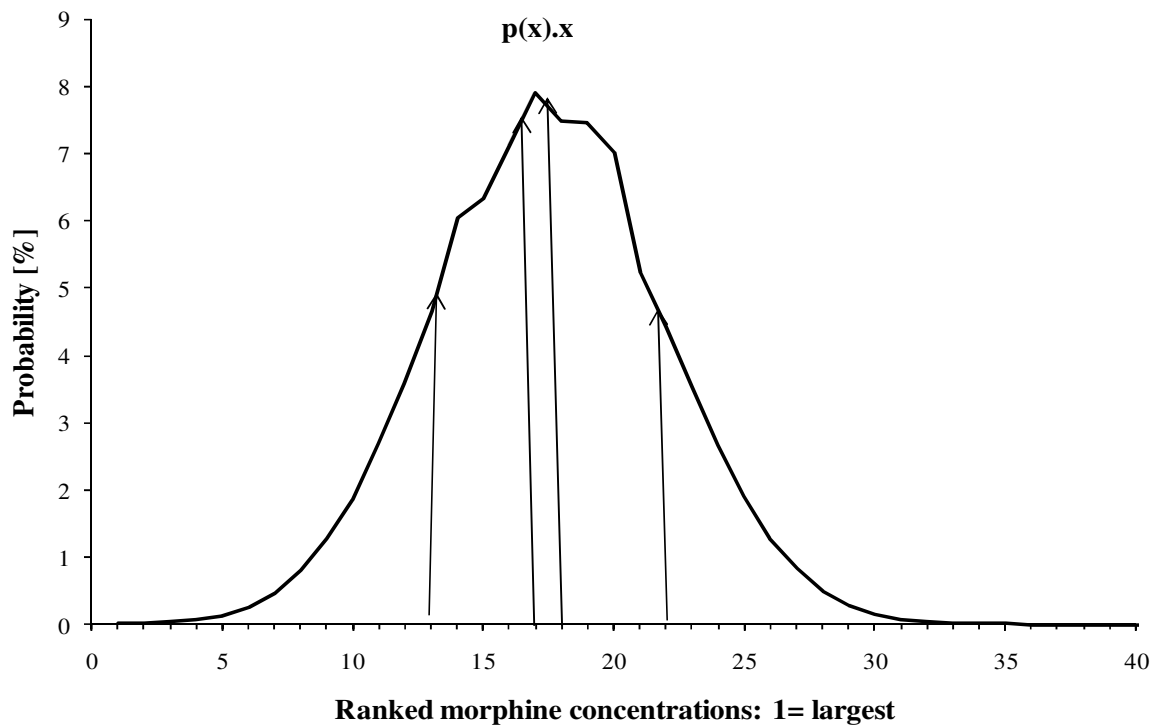


Figure 3. Probability density function of the 97.5th percentile of all morphine concentration values (2001-2010 data). Explanation for the confidence limits: the 97.5th percentile lies between the 17th and 18th ranked values from largest to smallest. The 68% of the values (median \pm SD) are between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

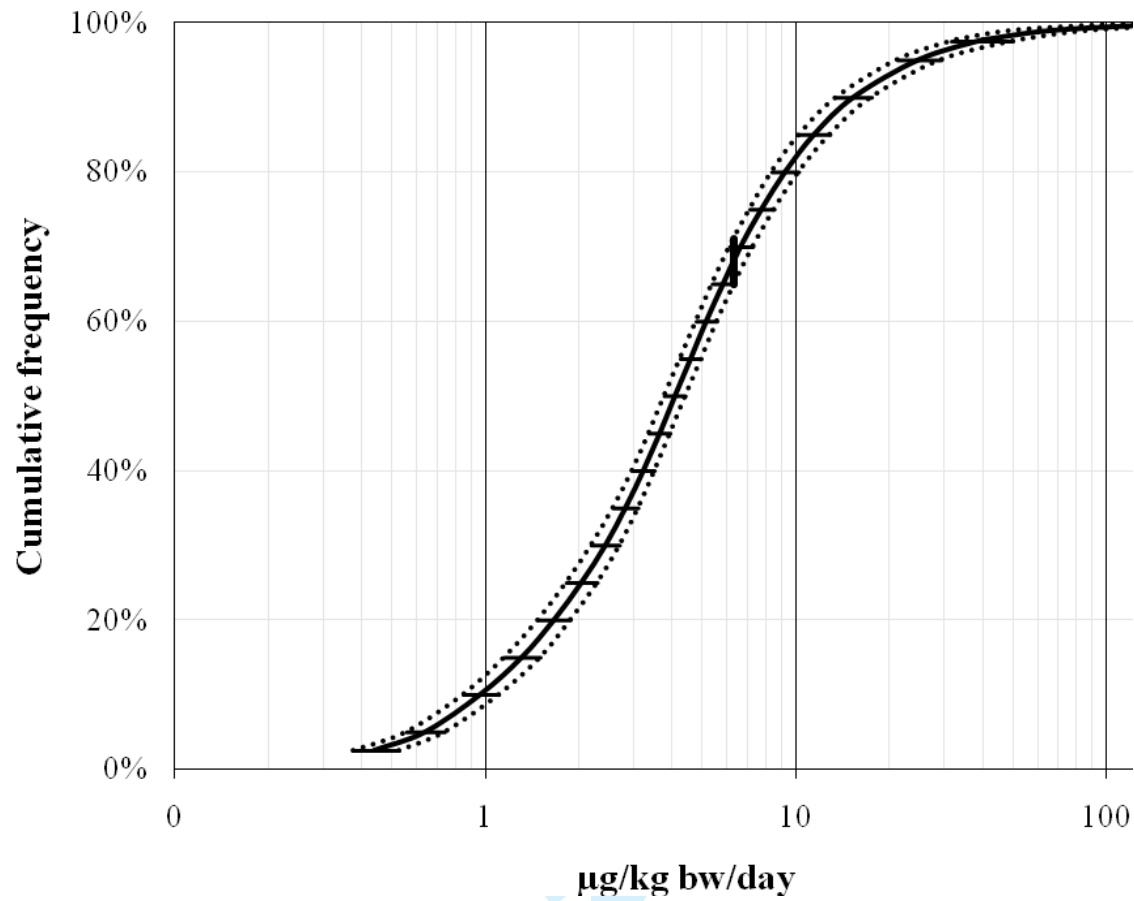


Figure 4. The median and 95% confidence intervals of the exposure to morphine through consumption of poppy seed.

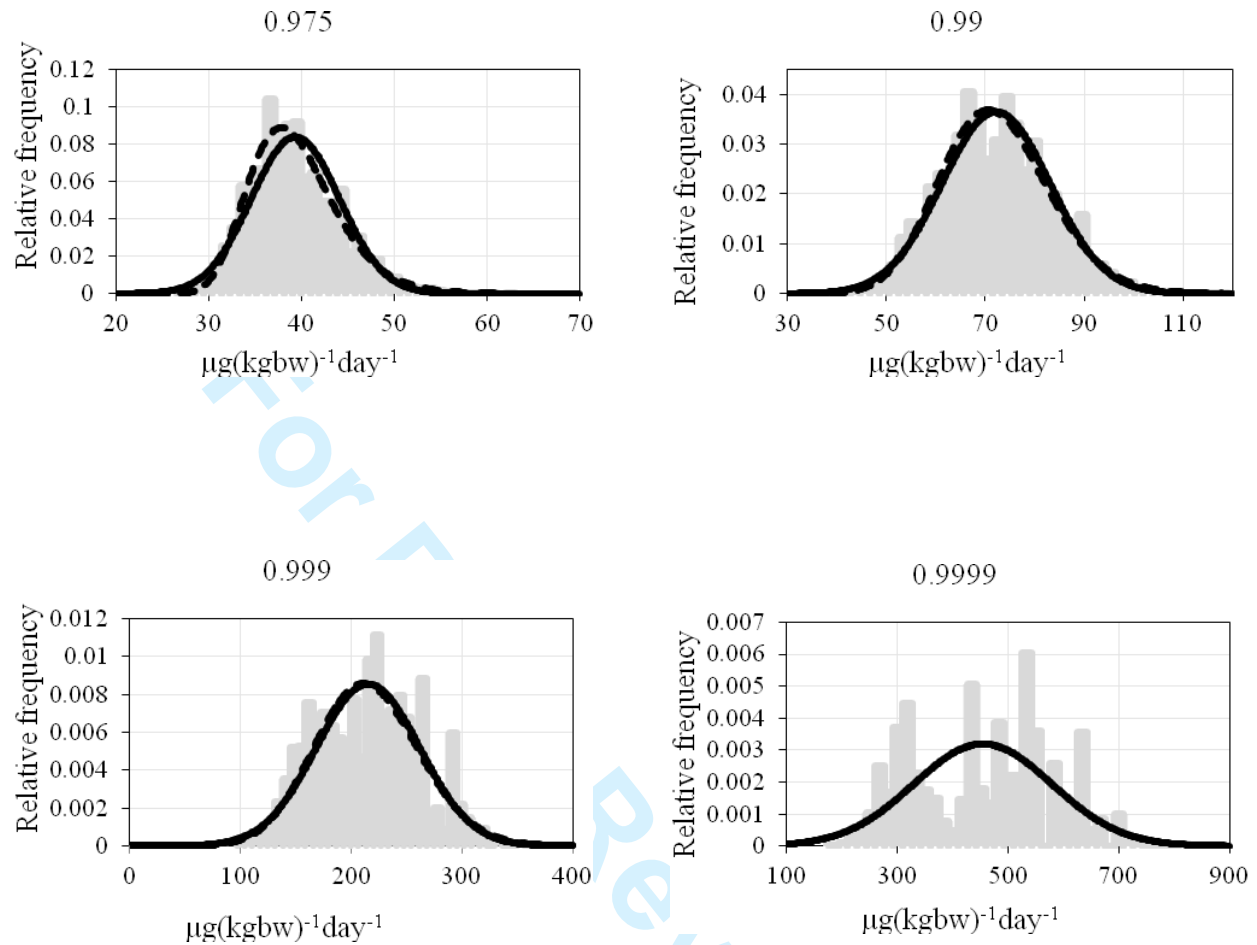


Figure 5. Relative frequency of estimated quantile values calculated from 10 000 bootstrap. Solid line: normal distribution fitted on results. Dashed line: lognormal distribution fitted on results.