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Estimation of the sampling constants for grains contaminated by mycotoxins and the impact on sampling precision

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Grains are subject to contamination by mycotoxins which are metabolic by-products of various fungi. The fungal infestations produce local concentrations of the mycotoxins thousands of times higher than the average. These facts make the sampling of the grain for assessment of the mean mycotoxin levels potentially very difficult. In the context of sampling theory, this morphology of the infestations creates a very high distributional and intrinsic heterogeneity of the grain shipment.

The distributions of mycotoxin concentrations in seaborne shipments that exist at loading and the number and intensity of infestations that develop under shipping conditions are unknown. All grain shipments carry spores that may give rise to mycotoxin-producing colonies; moisture and temperature determine the final extent of the problem.

This paper develops the value of the sampling constant with respect to intrinsic heterogeneity based on data on observed Ochratoxin A (OTA) concentrations in individual wheat kernels. A useful relationship between the distribution of mycotoxin concentrations on a kernel to kernel basis and the distribution of concentration over subsamples is developed.

Introduction

Grains are subject to contamination by mycotoxins which are metabolites of various fungi. For OTA, the acceptable level in grains is nominally 5 ppb. OTA will grow on wheat at 25°C when the grain contains sufficient moisture to generate an equilibrium water vapour pressure corresponding to 95% relative humidity[1].

There is evidence that the fungal infestations produce very high local concentrations of the mycotoxins. The fact that the local concentrations are so high and highly localized makes the sampling of the grain for assessment of the mean mycotoxin levels potentially very difficult. The calculations presented herein based on data from the Grain Research Laboratory of the Canadian Grain Commission suggest that the sampling problem is difficult and potentially one of the more difficult sampling problems known to sampling practice.

A particulate material can demonstrate two kinds of heterogeneity, namely so-called distributional heterogeneity (DH) and so-called intrinsic heterogeneity (IH). DH relates to a lack of mixing of the material to be sampled while IH relates to differences in concentration of the analyte from one particle to the next.

In the context of a grain sampling problem, a fungal infestation will arise due to a local increase in the water content of the grain, and in this location, the kernels of grain will carry high mycotoxin levels. This situation creates a lack of mixing problem in that all contaminated kernels are close together rather than spread uniformly throughout the shipment of grain. A sample taken at a random point in the shipment has a very low probability of hitting the small zone of contamination and, if it were to hit the contamination partially or fully, it would have a high probability of returning a very high mycotoxin assay rather than the mycotoxin level averaged over the entire shipment. The only means of sampling in a manner that overcomes this extreme DH of the material is either to find some means of effectively mixing the material before sampling or to extract a very large number of small increments from the shipment to make up the sample representing the shipment. Ideally both mixing and frequent increment extraction will be undertaken. Such a policy can be shown to be effective in reducing the variance of the sampling result from commercially unacceptable levels to commercially acceptable levels[2].

Assuming that a shipment of grain has been mixed or blended in such a way that contaminated kernels are distributed in as uniform as possible a manner throughout the mass of grain, it is still true that kernels will either have mycotoxin levels that are relatively high compared to the average level for the entire shipment or be very low in mycotoxin. When taking a small increment of well-mixed grain, the number of contaminated kernels in that increment may be very small or even zero. The assays of these small increments will be very erratic. It is this situation that is indicative of high IH of a material.

If the nature of the contamination of the grain is known, both of these types of heterogeneity can be quantified statistically. Quantification with respect to IH is undertaken in what follows, based on data for the analysis of single kernels of wheat contaminated by OTA.

Development

The heterogeneity of a material is quantified by considering the variance or standard deviation between nominally identical subsamples of a material. Consider some number

of 100 g samples of grain that were drawn from some large mass of grain in a mechanically correct manner (this simply means that the grain was sampled according to the known rules for sampling of particulate materials). If each of the samples were to be assayed in a manner that reported the exact concentration of the analyte (no preparation or analysis variance) in the sample, sampling theory combined with knowledge of the manner in which the analyte was distributed through the sample would correctly predict the assay standard deviation calculated over assay results for the 100 g samples.

To quantify IH, it can be shown that the relative standard deviation (RSD) over nominally identical subsamples of mass M_S is given by

$$RSD_{IH} = \frac{\sigma_{IH}}{\overline{a}} = \sqrt{\frac{K_S}{M_S}} \qquad [1]$$

where K_S is the sampling constant for the material with respect to the analyte of interest and a is the average analyte concentration in the material as a whole. The sampling constant is given by

$$K_S = \sum_{i=1}^{n} x_i v_i \rho_i \left(\frac{a_i - \overline{a}}{\overline{a}} \right)^2 \qquad [2]$$

where x_i is the mass fraction that the i^{th} particle in the sample represents, v_i is the volume of the i^{th} particle, ρ_i is the density of the i^{th} particle and a_i is the assay of the i^{th} particle with respect to the analyte of interest and a is the nominal mean assay for the sample as a whole. The number of particles, n, involved in the calculation is assumed to be large so that the sample is representative of the material and therefore the value of K_S is well-defined.

Note that the particle masses are

$$m_i = v_i \rho_i \qquad [3]$$

and the mass fractions within the sample are

$$x_i = \frac{m_i}{M_S} \qquad [4]$$

To quantify DH, the RSD between nominally identical samples due to DH can be shown to be[3]

$$RSD_{DH} = \frac{\sigma_{DH}}{\overline{a}} = \sqrt{\frac{\xi D_S}{N_I}} \qquad [5]$$

where D_S, the sampling constant due to distributional heterogeneity is given by

$$D_S = \sum_{i=1}^{n} x_i \left(\frac{a_i - \overline{a}}{\overline{a}} \right)^2 \qquad [6]$$

The quantity ξ is a mixing constant which varies between 0 and 1 depending on the extent to which the material has been mixed or blended prior to sampling and N_I is the number of increments extracted during the sampling process. Note that the RSD_{DH} does not depend on particle masses, but only on the proportions of particles having different assays and those assays. In the case of grain in a railcar which has local infestations of fungi, the mixing constant is dependent on the number of local infestations which exist and will decrease as this number increases[2]. As this number is unknown in typical situations, no further consideration of DH will be made herein.

To calculate the RSD due to IH, the distribution of mycotoxin from one kernel to the next is needed. Let this distribution be $g(a)$, where a is the concentration of OTA within a kernel. When the mass fraction (or number fraction if all kernels have the same mass) of contaminated grain in the mixture is f, the average OTA concentration in the grain is

$$\overline{a} = f \, \mathrm{E}\{a\} \qquad [7]$$

where $\mathrm{E}\{a\}$ is the expected value of the kernel contamination level

$$\mathrm{E}\{a\} = \int_0^\infty g(a) a \, da \qquad [8]$$

To find the value of K_S, the expression above for K_S can be split into a contribution for uncontaminated kernels and for contaminated kernels and the contamination level a can be conventionally treated as continuous random variable. The sampling constant is then, for kernels of equal mass, m

$$\begin{aligned} K_S &= m(1-f) + f m \mathrm{E}\left\{ \left(\frac{a - \overline{a}}{\overline{a}} \right)^2 \right\} \\ &= m(1-f) + \frac{f m}{\overline{a}^2} \int_0^\infty (a^2 - 2\overline{a}a + \overline{a}^2) g(a) \, da \\ &= m(1-f) + \frac{m}{f} \left[\frac{\mathrm{var}\{a\}}{\mathrm{E}^2\{a\}} \right] \end{aligned} \qquad [9]$$

This is a completely general result and any form for $g(a)$ can be used. The term

$$\frac{\mathrm{var}\{a\}}{\mathrm{E}^2\{a\}}$$

is simply the variance of the contamination distribution relative to the mean concentration of the contaminant in the contaminated grain, which is a dimensionless ratio. Note that the sampling constant has units of mass (grams when the kernel mass m is in grams).

Application to wheat sampling

The Grain Research Laboratory has undertaken assays of individual kernels of wheat to determine their OTA content; many kernels were found to have low OTA concentrations. The assays for contaminated kernels in Table I show a very wide spread, suggesting that the OTA distribution in contaminated kernels may follow a log-normal distribution. Figure 1 is a plot of the data with a fitted log-normal distribution showing that the log-normal is a plausible model for the OTA concentrations over the contaminated particles, $g(a)$.

The log-normal distribution is characterized by a mean, μ, of the logs of the concentration values and a standard deviation, σ, of those log concentrations. The mean or expected value of the OTA concentration in the kernels is

$$\mathrm{E}\{a\} = \exp\left[\mu + \frac{\sigma^2}{2} \right] \qquad [10]$$

and the variance of the concentrations is

$$\mathrm{var}\{a\} = \mathrm{E}^2\{a\} \left(e^{\sigma^2} - 1 \right) \qquad [11]$$

so

$$\frac{\mathrm{var}\{a\}}{\mathrm{E}^2\{a\}} = e^{\sigma^2} - 1 \qquad [12]$$

for the log-normal, taking all kernels to be of the same mass.

The sampling constant is given by

$$K_S = \frac{m}{f}\left(e^{\sigma^2} - 1\right) + m(1 - f) \qquad [13]$$

For the data of Table I, μ = 5.77 and σ = 1.80 which gives E{a} = 1 632 ppb permitting determination the fractional concentration f for various values of a.

The results applied relate to a full log-normal distribution, but it is possible to truncate the log-normal at some upper concentration, dictated perhaps by knowledge of maximum possible mycotoxin levels that can be achieved by the fungi. Such refinements of the distribution applied demand more detailed data.

In the sampling of wheat in Canada, it is common to retain a subsample mass of 10 kg to represent a given sampling unit (1 500 tonnes for example). This 10 kg subsample may then be further subsampled and ground to provide an analytical aliquot for the determination of some characteristic of the wheat. In the case of OTA determination, it is convenient to grind the 10 kg subsample and then divide out 100 g aliquots for the OTA determination.

Consider first the 10 kg subsamples of unground wheat. On the assumption of a wheat kernel mass[4] of 40 mg, and a full log-normal distribution of OTA over contaminated kernels as derived from the data of Table I, the results in Table II show the impact of IH on OTA content variations between nominally identical 10 kg samples. At low OTA levels, the variance component associated with a 10 kg sample of unground wheat is very significant. This result is sensitive to the maximal OTA concentrations in the kernels which, using a full log-normal distribution, reach very high levels. Truncation of the log-normal at the end of the experimental data-set reduces the RSD at the 2 ppb level to 7.7%. The real result is probably somewhere in the estimated interval (7.7 to 29%).

Table I
OTA results for 15 kernels with OTA levels greater than 20 ppb

Kernel number	OTA (ppb)	Kernel number	OTA (ppb)
1	20.7	9	1100
2	41.6	10	1130
3	57.0	11	1340
4	59.4	12	1400
5	61.7	13	1680
6	121	14	3540
7	142	15	4990
8	190		

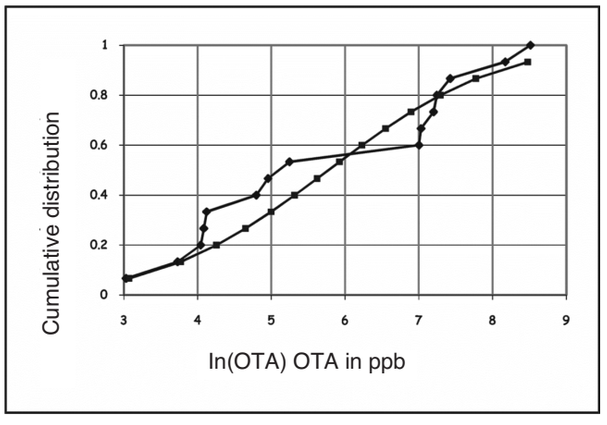

Figure 1. Empirical cumulative distribution of OTA concentrations in contaminated kernels versus ln(OTA) (diamonds) and the fitted log-normal distribution (squares)

Next assume that the wheat is ground to 95% passing 0.5 mm (32 Tyler mesh) from 95% passing 2.5 mm. This upper size limit is estimated from the dimensions of wheat kernel and the lower limit estimated from the properties of wheat ground in the usual manner for OTA determination at the Grain Research Laboratories. Making no allowance for the possible increase in OTA content in ground particles (fragments of the kernel from its surface may be much higher in OTA concentration than fragments from the interior of the kernel), 100 g aliquots of the ground wheat are estimated to show an RSD between nominally identical subsamples as given in Table III. Given that there will be some increase in the maximum OTA concentration in a kernel fragment[5] beyond the levels recorded in Table I, these estimates of uncertainty due to IH are probably realistic.

The analytical uncertainty for a single determination of OTA concentration at levels around 10 ppb is approximately 10% relative. Even at 40 to 50 ppb OTA in the wheat sample as a whole, there is an impact of the IH of the grain on the precision of estimation at the 10 kg sample level. Again, however, these estimates may be a bit high due to the lack of information on the maximal OTA levels that can be encountered in a kernel or kernel fragment.

However, in support of these estimates, an internal study was recently carried out at the Grain Research Laboratories in which a naturally contaminated reference material was mixed with clean grain. The material was subsampled and analyses were made on the subsamples using 100 g aliquots of ground grain. The structure of the test was such that it was possible to estimate the variance due to IH for the ground material at the 100 g sample mass through an appropriate analysis of variance. Estimation was made by maximum likelihood and the methods of moments with close agreement between the outcomes for the two methods. The RSD due to IH was found to be nominally 10% at a mean OTA level of 7.5 ppb. Using the same method as used to generate the results of Table III, the estimated RSD is 13.4%. This estimate is of the same magnitude as the 10% RSD result found experimentally which suggests that naturally contaminated materials have OTA distributions on a kernel to kernel level similar to those provided in Table I and the use of a full log-normal distribution of contamination is appropriate.

Note that in a sampling protocol that involves taking a 10 kg subsample and grinding it and then extracting a 100 g aliquot for analysis, the IH variance at the 100 g level adds to that at the 10 kg level. At 10 ppb, the total RSD due to IH from these two steps is found by combining a 12.7% RSD at the 10 kg level (see Table II) and an 11.4% RSD at the 100g level (see Table III) so the result is

$$\sqrt{12.7^2 + 11.4^2} = 17.1\%.$$

The only way to reduce this loss of precision is to grind the wheat finer before extracting 100 g aliquots of OTA assay. Mixing will not mitigate this problem. However, the IH variance from the 10 kg subsample cannot be reduced by any means other than using larger subsamples.

Due the significant impact of IH on the sampling results, there is considerable incentive to establish the maximal levels of OTA that can occur in wheat kernels.

Table II
IH sampling constant and RSD (%) for 10 kg sample masses as a function of mean OTA level in the grain when kernel contamination levels of OTA are based on data of Table I. A full log-normal distribution is assumed. The grain is unground

Mean OTA (ppb)	f	K_S (g)	RSD (%)
2	0.00123	807.7	28.4
5	0.00306	323.1	18.0
10	0.00613	161.6	12.7
20	0.0123	80.8	9.0
30	0.0184	53.9	7.3
40	0.0245	40.4	6.4
50	0.0306	32.3	5.7
60	0.0368	27.0	5.2
70	0.0429	23.1	4.8
80	0.0490	20.2	4.5
90	0.0552	18.0	4.2
100	0.0613	16.2	4.0
110	0.0674	14.7	3.8
120	0.0735	13.5	3.7
130	0.0797	12.5	3.5
140	0.0858	11.6	3.4
150	0.0919	10.8	3.3

Table III
IH sampling constant and RSD (%) for 100 g sample masses as a function of mean OTA level in the grain when kernel contamination levels of OTA are based on data of Table I. Grain is ground to 95% passing 0.5 mm. A full log-normal distribution is assumed

Mean OTA (ppb)	f	K_S (g)	RSD (%)
2	0.00123	6.461	25.4
5	0.00306	2.585	16.1
10	0.00613	1.293	11.4
20	0.0123	0.646	8.0
30	0.0184	0.431	6.6
40	0.0245	0.323	5.7
50	0.0306	0.259	5.1
60	0.0368	0.216	4.6
70	0.0429	0.185	4.3
80	0.0490	0.162	4.0
90	0.0552	0.144	3.8
100	0.0613	0.130	3.6
110	0.0674	0.118	3.4
120	0.0735	0.108	3.3
130	0.0797	0.100	3.2
140	0.0858	0.093	3.0
150	0.0919	0.086	2.9

The distribution of the sample OTA concentrations

The foregoing development suggested that the OTA concentrations in individual kernels of wheat could be modelled with a log-normal distribution and then sampling theory was used to find the mean and standard deviation of samples as a function of sample mass.

It is of interest to determine whether it is possible to predict the nature of the distribution of the sample OTA levels as this will provide a guide to the possible skewness of the sampling results. Since the kernel distribution is log-normal, it is logical to suspect that the log-normal may also provide a reasonable fit to the sample distribution. This factor is of great interest because it relates directly to the estimation of confidence intervals for shipping results.

To determine the distribution of the sample OTA levels using formal mathematics is in principle possible but is a rather difficult problem. It is far easier to simply simulate the samples kernel by kernel, sampling the kernels from clean or contaminated wheat. For any one realization of a sample, there are two sources of variance: the number of contaminated kernels in the sample and the levels of contamination in those contaminated kernels. The number of contaminated kernels follows a Poisson distribution and the level of OTA in those kernels follows a log-normal distribution. The problem is therefore simple to set up.

Consider unground 100 g subsamples (kernel weight 45 mg) at 6 ppb OTA.

Figure 2 shows the distribution of OTA levels in simulated samples for a mean OTA of 6 ppb. The skewness of the distribution is clearly evident.

Figure 3 shows the corresponding match between the distribution of the log of the sample OTA levels and the corresponding normal distribution: the fit is excellent.

Consider now unground 10 kg samples (kernel weight 45 mg) at 6 ppb OTA.

Figure 4 shows the distribution of OTA levels in simulated samples for a mean OTA of 6 ppb. The skewness of the distribution is still evident.

Figure 5 shows the corresponding match between the distribution of the log of the sample OTA levels and the corresponding normal distribution: the fit is still quite good.

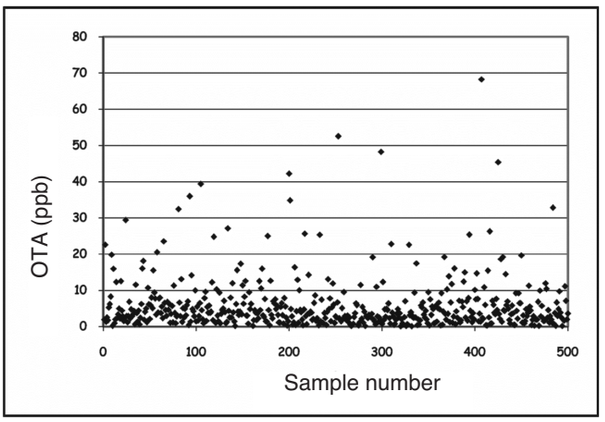

Figure 2. OTA levels in simulated 100 g unground samples. Mean OTA is 5.88 with an SD of 7.55 ppb over a set of 500 samples

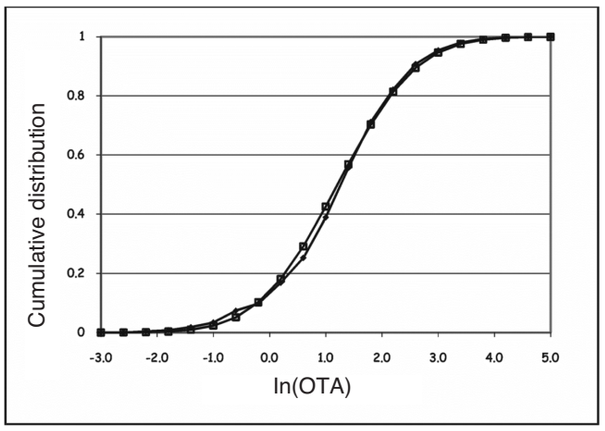

Figure 3. Fit of a normal distribution (open squares) to the distribution of the logs of the OTA levels in 100 g samples (diamonds)

These examples suggest that the distribution of concentration in samples drawn from a mixture of clean grain and contaminated grain, where the kernels have a log-normal distribution of contaminant concentration, can be well approximated by a log-normal distribution.

In such a case, because it is possible to find the variance of the sample distribution using sampling theory, it is possible to predict the distribution of the sample concentrations.

Two parameters characterise the log-normal which will be denoted as μ_S and σ_S^2 for the samples and as μ_k and σ_k^2 for the kernels.

From the results above, one has, for samples of mass M_S,

$$\text{sample variance} = \bar{a}^2 \frac{K_S}{M_S} = \bar{a}^2 \frac{m}{M_S}\left(\frac{e^{\sigma_k^2}-1}{f}+1-f\right) \quad [14]$$

and for a log-normal distribution this must be given by

$$\text{sample variance} = \bar{a}^2\left(e^{\sigma_S^2}-1\right) \quad [15]$$

and note that the distribution considered is that of the samples which include uncontaminated kernels. Equating these two relationships

$$\bar{a}^2 \frac{m}{M_S}\left(\frac{e^{\sigma_k^2}-1}{f}+1-f\right) = \bar{a}^2\left(e^{\sigma_S^2}-1\right) \quad [16]$$

the equation can be solved for σ_S^2 giving

$$\sigma_S^2 = \ln\left[\frac{m}{M_S}\left(\frac{e^{\sigma_k^2}-1}{f}+1-f\right)+1\right] \quad [17]$$

The second parameter can be determined from the expected sample concentration a as

$$\mu_S = \ln \bar{a} - \frac{\sigma_S^2}{2} \quad [18]$$

The last equation can be related to the parameters for the kernel contamination distribution and the fractional contamination as

$$\mu_S = \mu_k + \frac{\sigma_k^2}{2} + \ln f - \frac{\sigma_S^2}{2} \quad [19]$$

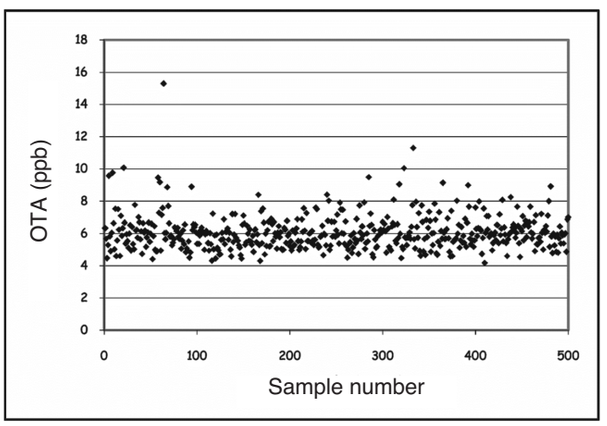

Figure 4. OTA levels in simulated 10 kg samples. Mean OTA is 6.0 with an SD of 1.1 ppb

The equations result from matching the mean and variance for the samples to the mean and variance predicting by sampling theory.

With values of 5.77 and 1.8 for μ_k and σ_k and a value of f of 0.003704 to result in an expected grade of 6 ppb, the estimates of σ_S and μ_S are 0.175 of 1.77 which can be compared to the values determined from the simulation of 0.162 and 1.79 for the 10 kg samples.

Figure 6 compares the distribution found in the simulation with that predicted from sampling theory. The prediction is very good.

This predictive capability is a useful tool to assess the impact of mycotoxin kernel concentration distributions on sample mycotoxin distributions. It can also provide some guidance in relation to setting confidence limits on results in that it provides a means of estimating the possible skewness of the distribution of sample results.

However, in the practical situation of assessing a shipment of grain and putting confidence limits on the results of sampling, the distribution of the true assays of the samples will be masked by the preparation and analytical uncertainties. Further, the distribution of the kernel mycotoxin concentrations in a given shipment is unknown and can be expected to consist of a number of distributions, each of which may be log-normal, arising from individual local fungal infestations.

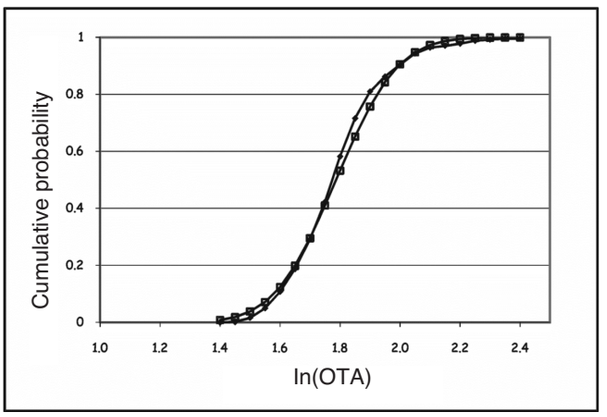

Figure 5. Fit of a normal distribution to the distribution of the logs of the OTA levels in 10 kg samples

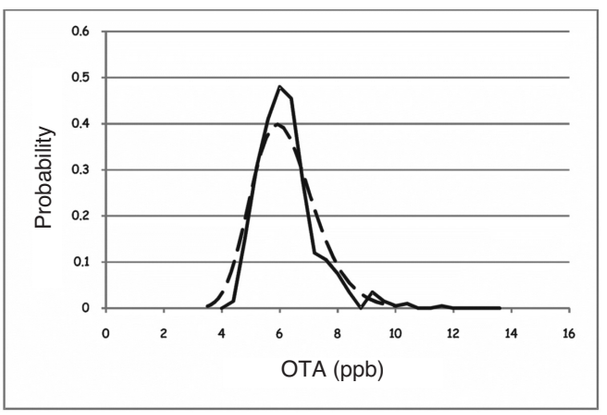

Figure 6. Comparison of the distribution of simulated sample concentrations (solid line) with that predicted from sampling theory (dashed line) for 10 kg samples

Conclusions

Data collected by the Grain Research Laboratory of the Canadian Grain Commission permit the estimation that the levels of OTA contamination in wheat follow a log-normal distribution over individual wheat kernels.

Given knowledge of the distribution of the level of OTA contamination on a kernel by kernel basis, be it log-normal or not, the application of sampling theory permits the calculation of the sampling constants for the unground wheat with respect to intrinsic heterogeneity for mixture of clean and contaminated wheat. It is therefore possible to calculate the sampling variance for any mixture of the contaminated and clean wheat, based on the knowledge of the distribution of contamination levels on a kernel by kernel level. This fact has been borne out by such tests at the Grain Research Laboratories[6]. This conclusion is general and can be applied to the sampling with respect to any contaminant, e.g. deoxynivalenol (DON) arising from fusarium infestation or GMOs.

In the case of a log-normal distribution, simulation of the sampling of a mixture of clean and contaminated wheat kernels points to the fact that the distribution of average concentration in samples of such mixtures can also be described by a log-normal distribution. Since knowledge of the sampling constant for the mixtures permits the calculation of the sampling variance for any mass of sample, it is possible to estimate the distribution of the contamination levels in samples of any mass of unground grain. This is a vital tool in the design of sampling protocols for grain samples.

Finally, under the assumption that the distribution of the contaminant from one kernel fragment to the next is the same as the kernel to kernel contaminant distribution, sampling theory permits the estimation of the sampling variance associated with ground samples of wheat, if the 95% passing size of the ground material is quantified. In the case of OTA, there is evidence that the OTA is concentrated in the outer layers of the wheat kernel, so this sampling variance estimate may be used as a lower bound on the sampling variance with respect to OTA. For other contaminants appropriate knowledge of the morphology of the mycotoxin infestation of the grain can be applied.

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