# Distribution of Aflatoxin in Pistachios. 6. Seller's and Buyer's Risk

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The seller's risk—the probability of a set of samples exceeding an agreed upon aflatoxin level when the lot mean does not—and the buyer's risk—the probability of a lot exceeding this level when a set of samples do not—have been computed using a parametrized experimental aflatoxin distribution and Monte Carlo simulation. The calculations are exemplified using the proposed EC standards (three 10 kg samples, 4 ng/g of total aflatoxin, basis kernels only) as well as for samples up to 250 kg and for varied lot aflatoxin levels. It is found that within this sample size range the seller's risk is as high as 42% at 10 kg and increases with increasing sample size to 80% at 250 kg. Only by reducing lot levels to 0.2 ng/g of total aflatoxin, basis kernels, can the risk be brought down to 2.5%, independent of sample size. The buyer's risk is as high as 58% at 10 kg but falls to 11% at 250 kg samples. The implications for both seller and buyer strategies are discussed.

**Keywords:** *Sampling; lot means; sample distributions; sample sizes; EC protocol; strategies*

## INTRODUCTION

In the commerce of pistachios the aflatoxin level has become a critical parameter. Government agencies wish to minimize this mycotoxin to protect their populations and thus have commonly set limits on the aflatoxin concentration in lots which may be imported or sold. Because of public awareness of the problem, levels even lower than these are frequently set by the marketplace. As a result, the reliable establishment of the aflatoxin level in a lot becomes critical. This level is obtained by taking one or more samples of a predetermined size, measuring the level of aflatoxin in the sample and assuming the level(s) measured are representative of the lot concentration. However, such measurements are subject to analytical, subsampling, and sampling errors. Of these, the sampling errors are by far the largest, so that the other errors may be ignored to a first approximation (Schatzki and Pan, 1996). In fact, the standard error of the measurements for commonly used sample sizes may be of the order of the measurement itself. Thus questions arise as to the significance of the measurement. Generally the seller and buyer agree on an acceptance level $C_a$, i.e., that a lot should not be sold (or imported) if the concentration $C_s$ of a sample of agreed size from the lot tests at $C_s > C_a$ ng/g. (The test may involve a set of samples taken simultaneously or sequentially, but the criterion in the final analysis involves single samples.) Because of the large standard error both seller and buyer are at risk. The seller's risk can be expressed as: Given my lot has a mean aflatoxin level $m < C_a$, what is the probability that a sample, taken from this lot, will exceed $C_a$ ng/g, purely on statistical grounds? In mathematical notation, what is the conditional probability of *not s*, given *m*, written as $P(\textit{not } s|m)$? (Throughout this manuscript it is assumed that the seller knows the aflatoxin concentration of the lot, either from experience or through extensive testing.) Here we use the short hand *s* for any agreed upon sample outcome, a single sample, or a set of samples for which $C_s$ falls into a range of *C*. The buyer's risk is here defined as: Given a sample is taken and tests at $C_s < C_a$, what is the probability that the lot from which that sample came has a mean aflatoxin concentration $m > C_a$? Mathematically, what is $P(m > C_a|s)$, from which one can compute the average lot concentration, averaged over all lots which passed the test *s*? Note that this definition differs from that used commonly in quality control (QC) (Hodges and Lehman, 1964, section 13.1), which is expressed as the following: What is the probability that a sample will test at $C_s < C_a$, given that the lot from which it came has a mean $m > C_a$? This amounts to $P(s|m > C_a)$ and can be taken directly from the seller's risk results which will be presented. We believe the results in terms of $P(m|s)$ are more useful to a buyer facing a decision whether to accept a lot which has been chosen by the seller not at random, but with a strategy in mind, in essence a game theoretic approach. In any event, the two expressions are directly related through Bayes' law (below).

This problem has been addressed previously in QC terms for peanuts by Whitaker et al. (1970, 1989, 1995), using a postulated negative binomial or an experimental distribution of aflatoxin in the lot. Since results can be expected to depend critically on the distribution, as well as sampling design, Whitaker's results cannot be applied directly to pistachios.

The underlying pistachio statistics and the seller's risk have been addressed previously (Schatzki, 1995a,b, 1998). In these publications the quasi-continuous distribution of aflatoxin among the individual nuts of a lot had been approximated as a discrete distribution,

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**Table 1. Aflatoxin Lot Distribution**

| concn, $c_i$, ng/g[a] | probability, $p_i$ | concn, $c_i$, ng/g[a] | probability, $p_i$ |
|---|---|---|---|
| 25 | $7.3 \times 10^{-5}$ | 7900 | $6.9 \times 10^{-6}$ |
| 79 | $6.3 \times 10^{-5}$ | 25000 | $1.4 \times 10^{-5}$ |
| 250 | $5.3 \times 10^{-5}$ | 79000 | $8.9 \times 10^{-6}$ |
| 790 | $4.3 \times 10^{-5}$ | 250000 | $3.4 \times 10^{-6}$ |
| 2500 | $1.0 \times 10^{-5}$ | | |

[a] Midpoint of bin.

binned into half-decile bins and expressed as the set $\{p_i, c_i\}$, with $i > 0$ as the bin index. Here $c_i$ is the geometric midpoint of bin $i$ and $p_i$ is the probability that a single nut has an aflatoxin content $c_i \times 10^{-0.25} < c < c_i \times 10^{0.25}$. All concentrations falling into bin $i$ are approximated by $c_i$. The basis for the latter approximation and the bin size (the root of 10) are justified in Schatzki, 1995a, on experimental grounds. In addition, a single bin $i = 0$ is defined for $c < c_0$, where $c_0$ is the experimental detection limit for aflatoxin and nuts in this bin are approximated by $c = 0$. These approximations are carried into the present work, although some will be released, as indicated. The previous calculations suffered, however, from three limitations.

1. The nut distribution, $\{p_i, c_i\}$, from which the sample distribution $\{P_i, C_i\}$ was derived (the sample concentration $C_s$ was binned as well), had been approximated by a boxcar distribution: $p_i =$ constant for $i > 0$, $c < c_{max}$, where $c_{max} =$ approximately 250 000−1 000 000 ng/g; $p_i = 0$ for $c > c_{max}$; $p_0 = 1 - \sum_{i>0} p_i$.

2. Only the expected mean and standard error of $C_s$ were computed as a function of $m$ and the sample size. While it is true that the distribution $P(C_s)$ will approach a normal one for large enough sample size, due to the central limit theorem (Feller, 1950), the *p(c)* distribution is highly skewed, and the required sample size may not be reached.

3. The buyer's risk was not addressed.

The present manuscript removes these limitations.

1. The actual $\{p_i, c_i\}$, as determined from five lots, was used. It was noted in previous work (Schatzki, 1995a) that the quite different lots (early split nuts in the orchard, nuts as harvested, processed nuts, and pick-outs) showed similar distributions, differing mainly by a constant multiplier of $p_i$, $i > 0$. Accordingly, the five experimental distributions were shifted vertically on a log $p$ plot to coincide as closely as possible to obtain a merged master distribution which is listed in Table 1. (The values of $p_i$ at low $c_i$, $i \neq 0$, are not of importance here.) The distribution for any hypothetical lot of the mean aflatoxin concentration $m$ was then assumed to be given by $\{kp_i, c_i\}$, $i > 0$, and again $p_0 = 1 - \sum_{i>0} kp_i$, $c_0 = 0$, with $k$ chosen to obtain the desired lot mean, $m = \sum_i kp_ic_i$.

2. The actual distribution of the average aflatoxin concentration of $N$ samples of a given sample size $n$ (in number of nuts) was computed as a function of $m$ by the use of a Monte Carlo calculation (i.e. by simulated sampling). From the resulting $C_s$ distribution the fraction of samples within appropriate ranges of $C$ were calculated. This yields *P(s|m)* for a fixed sample size.

3. The buyer's risk was specifically computed, using Bayes's law, $P(m|s) = P(m)P(s|m)/P(s)$, where *P(m)* is the a priori probability that a lot has a mean $m$, which must be derived from past experience with lots which have been presented by the particular seller (or group) under consideration. $P(s) = \int_0^\infty P(m)P(s|m)\,dm$ represents the fraction of all lots presented by the seller which pass test $s$, again based on past experience. The usual derivation of Bayes' Law (Hodges and Lehman, 1964, section 4.4) is carried out in terms of discrete events, in which case functions of $m$, such as *P(m)* and *P(m|s)* are, in fact, probabilities. However, Bayes' law may be generalized to apply to a continuous distribution in $m$, for which *P(m)* and *P(m|s)* become density functions ($s$, the outcome of a test, remains discrete). For consistency with the usual derivations we have chosen to retain the symbols *P(m)* and *P(m|s)* with the understanding that they represent functions of $m$ (densities) here. To obtain the buyer's risk it becomes necessary to integrate over an appropriate range of $m$. The choice of this range is contained in the definition of risk. We have chosen to express it as $P(m > m'|s) = \int_{m'}^\infty P(m|s)\,dm$, i.e., the probability that a lot mean will exceed $m'$ even though it satisfies $s$. (This is a slight extension in that $m'$ need not be $C_a$). Other integrals could be used as well, of course, and are readily derived from our graphical results. A particularly interesting quantity is the expectation of the average aflatoxin concentration of all lots which pass $s$, $E(m|s) = \int_0^\infty P(m|s)\,m\,dm$, as that allows a regulator to compute how much aflatoxin a population will be subject to, given $s$, by multiplying the average by the total imports. This is derived as well. It is important to note that the a priori density *P(m)* plays a crucial part in our definition. This density represents the buyer's belief regarding the lot before testing, i.e., the probability density that this particular seller (or seller group) will present a lot of mean $m$, and thus the buyer's confidence in the seller.

As an example, we specifically address the proposed EC (European Community) standard for tree nuts and some other commodities (Commission of the European Community, 1998). This standard requires that for acceptance of a lot (≥10 000 kg) three commutated subsamples of 10 kg from a 30 kg aggregate sample shall each test below 4 ng/g of total aflatoxin (aflatoxin $B_1 + G_1 + B_2 + G_2$), basis kernels only. If this test fails but if the average of the three samples falls below 10 ng/g, the lot may be reprocessed (resorted, but not blended) and, presumably, retested. Otherwise, the lot is to be seized. Generally, kernel and shell weights are approximately equal, while the shells carry little or no aflatoxin (Schatzki and Pan, 1996), so the standard can be converted to in-shell kernels by halving the above values to 2 and 5 ng/g, basis kernels plus shells. All values reported here are basis kernels plus shells, total aflatoxin, unless indicated otherwise. Furthermore, EC requires that aflatoxin $B_1$, which is separately tested for, be no more than half of total aflatoxin. Previous work with pistachios (Schatzki and Pan, 1996) indicated that the $G_1/B_1$ ratio averaged 0.26, while some limited, unpublished work in our laboratory suggests $B_2/B_1$ averages 0.37. $G_2$ is almost never seen in pistachios. From these values one concludes that, on average, $B_1$ is 60% of total so that the $B_1$ and total aflatoxin standards are reasonably consistent. Here only the acceptance limit for total aflatoxin is explored, although in a specific case both must be addressed. In what follows then, two sets of $s$ are considered. In terms of single sample events, $s_1$ corresponds to acceptance, three samples below 2 ng/g, and the required probability is computed from $[P(C_s^{10} \leq 2|m)]^3$, where the superscript on $C_s$ indicates the sample weight. The reprocessing outcome is represented by the symbol $s_2$, whose probability is computed from the failure of $s_1$ times the

success of a single 30 kg sample testing below 5 ng/g. The required probability is thus given by $\{1 - [P(C_s^{10} \leq 2|m)]^3\} \times P(C_s^{30} \leq 5|m)$. (For the larger sample weights, considered below, the superscripts will be 50 and 150 or 250 and 750, respectively.)

## MATERIALS AND METHODS

The Monte Carlo sampling simulation was carried out by means of a program in C++ language. In previous work (Schatzki, 1995a) it was pointed out that if the number of nuts/sample $n$ was small enough so that $nkp_i < 0.1$, the probability was high (>95%) that at most a single contaminated nut determined the sample aflatoxin concentration. Under these conditions the distribution $\{P_i, C_i\}$ of the aflatoxin concentration in $n$-sized samples could be directly related to $\{kp_i,c_i\}$ of individual nuts, with $P_i = nkp_i$, $i > 0$, $P_0 = 1 - \sum_{i>0}P_i$, and $C_i = c_i/n$. In this case sampling can be simulated by choosing a single random concentration for each sample according to $P_i$, rather than $n$ values using $kp_i$. The former is, of course, much faster (were $nkp_i \geq 0.1$, such $n$-fold sampling would be required). To make the sampling a bit more realistic, it was assumed here that the contaminated nuts which fell within bin $i$ did not contain $c_i$ ng/g of aflatoxin, as was done previously, but were distributed evenly in ln $c$ throughout bin $i$ and $C_s$ was chosen within bin $i$ accordingly. This choice of sampling within a bin was based on the observation that $p_i$ was reasonably constant on a ln scale (Table 1 here and Figure 1 of Schatzki, 1998). This sampling causes a shift of the arithmetic average concentration within a bin. This shift is most easily evaluated by dividing the bin into $r$ ln equal size subbins, summing their midpoints, and dividing by $r$, i.e., by evaluating

$$[\sum_{j=1}^{r-1} C_i\, 10^{-0.25+j(0.5/r)} + 0.5C_i(10^{-0.25} + 10^{0.25})]/r$$

With increasing $r$ this expression converges very rapidly to $1.056C_i$ (reaching $1.057C_i$ at $r$ =10).

In the present work it was desired to simulate samples of 10, 50, and 250 kg, as well as samples at 30, 150, and 750 kg, converted at 700 nuts/kg. The value of $n$, derived from $nkp_i \leq 0.1$, would be much too large to obtain samples that contain but a single significantly infected nut. However, a large sample can be thought of as a set of $N$ small samples, each adequately small, which are ground and analyzed separately and from which the large sample aflatoxin concentration is derived by arithmetically averaging the results. While this would not be as efficient as blending the ground samples before analysis, the results would be the same. Yet each of the small samples would not contain more than a single significantly contaminated nut, provided $nkp_i \leq 0.1$ for each small sample. Accordingly, the C++ program was run as follows. The desired mean concentrations of the lot and the sample size ($nN$) were input. A value of $k$ was chosen to obtain an approximately correct output mean $m$ (basically from $\sum_i kp_ic_i$). Since $p_1 > p_{i>1}$, a first approximation of $n$ was obtained from $nkp_1 = 0.1$. $N$ was set as $(nN)/n$. To avoid the effect of integer rounding, a second approximation of $n$ was then obtained as $(nN)/N$. Next, $N$ samples of size $n$ were obtained by simulation, as described above, the resulting $N$ $C_s$ values were averaged, and the average was written to a text file, one value/line. This simulation was repeated 8000 times, i.e., for 8000$N$ samples.

Although the remaining calculations could have been completed using C++, it was found convenient to complete them using a spreadsheet (Corel Quattro Pro 8, Corel, Inc., Orem UT 84097), linked to the C++ output file. While spreadsheet calculations are awkward, they do show trends (and errors) rapidly and may be plotted immediately using the graphics capabilities of Quattro Pro. The $P(s|m)$ distribution for a given sample size was computed using the function @array(@freqdist-([link]A:A1..A8000,A:binset)), where [link] is the C++ output file and binset is an array containing the bin limits. This function computes the number of entries in the list of 8000 (i.e. results of the Monte Carlo simulation) which fall into each bin. Division by 8000 yields $P(C_i|m)$, the probability of an outcome falling into a particular bin. Summing over the bins appropriate to $s$ then gives $P(s|m)$. The bin limits chosen were 0, 1, 2, ..., 20, 22, ..., 40, 45, ..., 60 ng/g. This entire calculation was repeated for each desired input value of $m$ and for each desired sample size. Input values of $m$ chosen were $m$ = 0.3, 0.6, 1, 2, ..., 60, 62, ..., 70, 75, and 80 ng/g. The output $m$ was about 6% larger than the input, as noted above. All further computer calculations used the output $m$ and not the input. Integrals were obtained by linear interpolation.

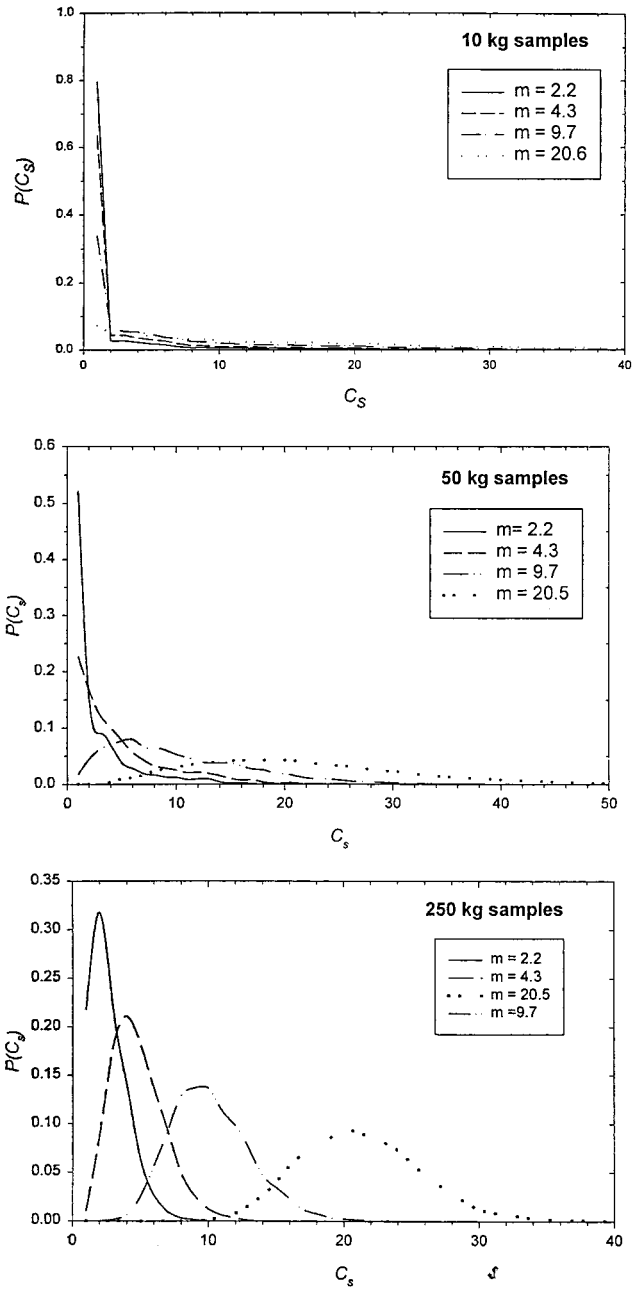


**Figure 1.** Single sample aflatoxin distribution for lots of various means $m$.

The C++ code, the Quattro Pro spreadsheet, and the resulting graphs are available via anonymous ftp @aggie.pw.gov/pub/dropbox/jafc6/monte.cpp,spreads.wk3 and graphs.jnb. The graphics were produced using Sigma Plot 4.0 (Jandel Scientific Software, San Raphael, CA) by cutting and pasting from the spreadsheet results, using splined line plots (except for the sampling frequency at 10 kg which was plotted without splining).

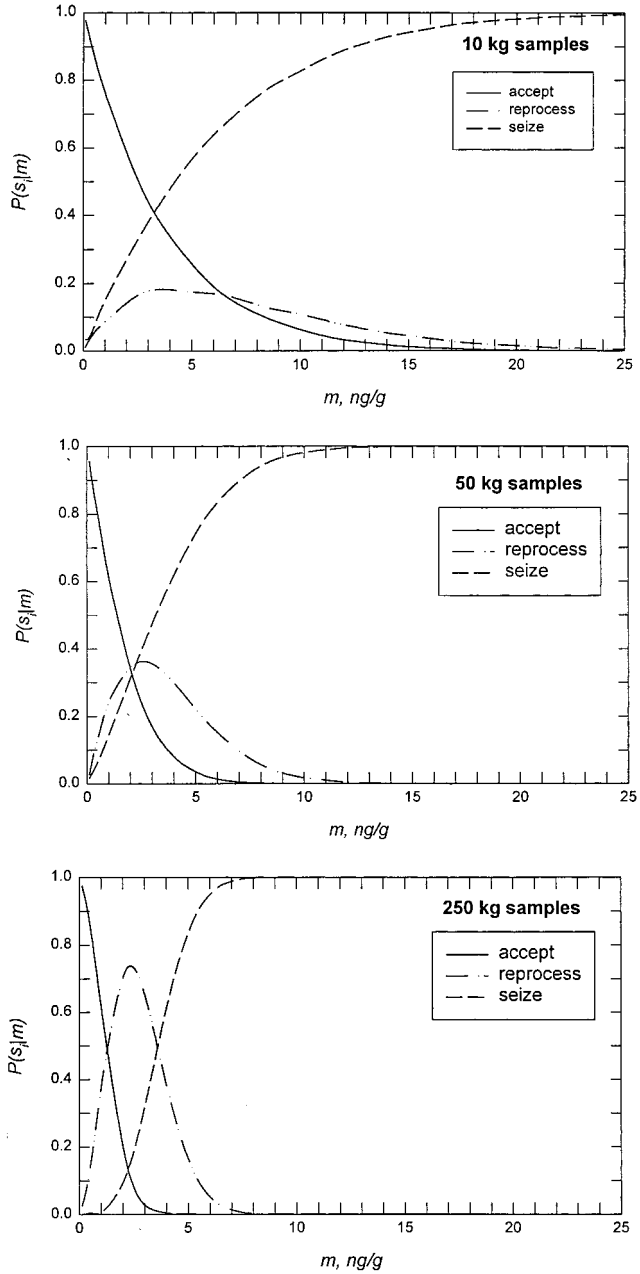


**Figure 2.** The seller's risk. Probability of having a lot accepted, returned for processing, or seized, following current EC import standards as a function of lot mean $m$.

**Table 2. Seller's Risk at Low Lot Mean Aflatoxin, %**

| sample size, kg | lot mean, ng/g | accept | reprocess | seize |
|---|---|---|---|---|
| 10 | 0.113 | 0.975 | 0.025 | 0 |
| 50 | 0.115 | 0.954 | 0.027 | 0.019 |
| 250 | 0.114 | 0.975 | 0.025 | 0 |

## RESULTS AND DISCUSSION

For small $nN$ (total nuts in all $N$ samples) the sample distribution $P(C_s|m)$ was found to be exceedingly broad and skewed. This is shown in Figure 1 where the sample aflatoxin concentration $C_s$ is shown as the $x$-axis, while the $y$-axis shows the probability of obtaining a sample, $P(C|m)$, in the range $C_s - 1 \leq C < C_s$ for four different lot means of about $m = 2$, 4, 10, and 20 ng/g. At 10 kg the distributions show a large contribution for $C_s = 1$ (mostly from noncontaminated samples), even when $m$ is as high as 10 ng/g, and show a wide spread, not centered at $C_s = m$. The situation is quite different for

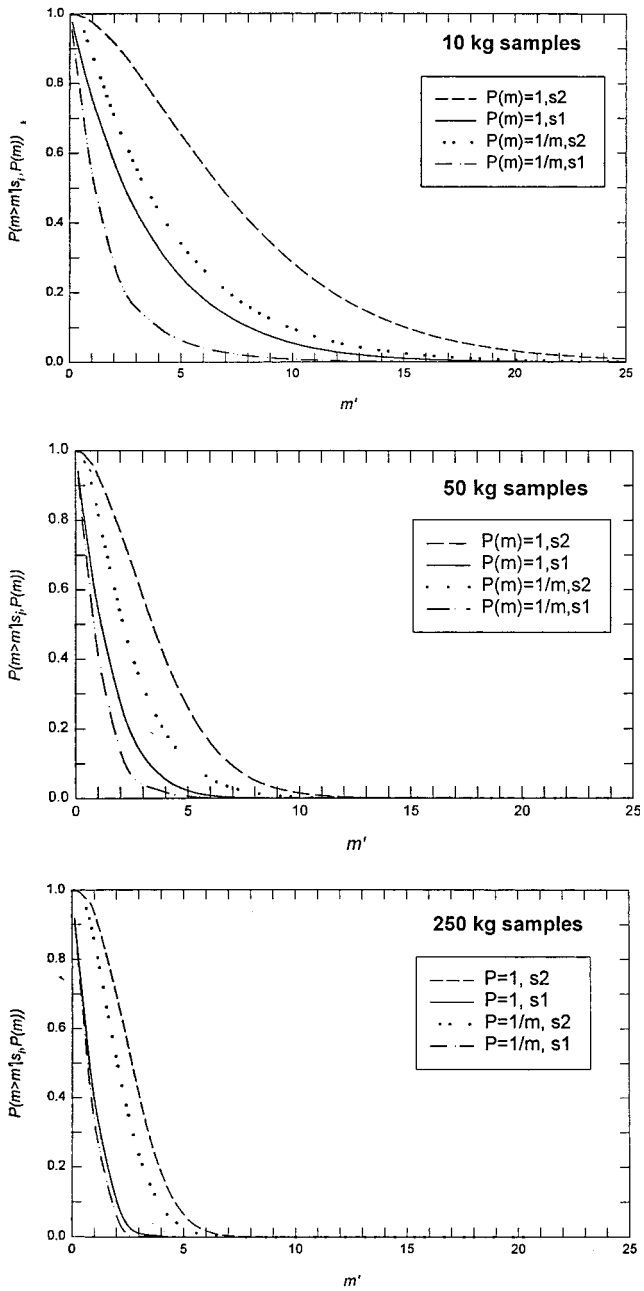


**Figure 3.** The buyer's risk. Probability of having a lot mean $m$ no larger than $m'$, as a function of $m'$ for two sample results and two prior probability distributions $P(m)$.

larger samples. The corresponding plot for 250 kg shows that now the sample size ($nN = 175\,000$) is large enough for the sample distribution to be close to normal (kurtosis at $m = 10$ is 0.64); i.e., the central limit value is approached. At 50 kg only lots of $m > 20$ ng/g show normallike distributions. As the sample size increases, the curves sharpen; the variance and its effect on seller's risk was discussed in Schatzki (1995a, 1998).

The seller's risk or the buyer's risk in QC terms is obtained by simply integrating these curves. For the EC situation the probability of acceptance is given by $P(s_1|m)$, the risk of having to reprocess by $P(s_2|m)$, and the risk of seizure by $1 - P(s_1|m) - P(s_2|m)$. These quantities are plotted vs $m$ in Figure 2. As these curves are difficult to read with precision, additional data at low $m$ are given in Table 2. A seller would like to obtain acceptance rates of 95−98%, not only to reduce costs and loss of product but also to build buyer confidence.

It is seen that to achieve such rates a lot mean of around 0.1 ng/g (0.2 ng/g, basis kernels only) is required, much lower than the 2 ng/g acceptance level. This low lot mean is required at 250 kg as well as at 10 kg, so the seller gains nothing from increasing the sample size in this range. Quite the opposite, the acceptance level drops as sample size increases. Only at much larger, and impractical, sample sizes would the acceptance curve approach the step function: 1.0 if $m < 2$, 0 if $m > 2$. (On the basis of standard error, one expects 97.5% acceptance for a 1 ng/g lot if the sample size exceeds 860 kg.) For lots with $C_a > 2$ ng/g the chance of acceptance decreases monotonically with sample size. Thus, the chance of a 5 ng/g lot being accepted amounts to 26% at 10 kg and 3% at 50 kg and would be negligible at 250 kg or higher. In this range of sample sizes, a seller gains little or nothing but a buyer considering QC risk gains quite a lot as the sample size is increased.

In game theoretic terms, the buyer's risk is expressed here as the probability that the lot mean $m$ exceeds some value $m'$. The expression for $P(m > m'|s)$ was given above. There is no obvious mathematical expression which can be used for $P(m)$. Accordingly, calculations were made for two cases. If there is no prior information, it would seem reasonable to assume that any lot mean is as likely as any other, which would set $P(m) = 1$ or a constant. To illustrate the effect of $P(m)$, calculations were also made for $P(m) = 1/m$, $m > 1$ and $P(m) = 1$, $m < 1$, which is equivalent to having $P(m)$ equally distributed in ln $m$ space ($dm/m = d \ln m$) and which makes high aflatoxin lots less likely. Plots of $P(m > m'|s)$ for $s_1$ and $s_2$ and the two choices of $P(m)$ are shown in Figure 3. Facing an unknown or suspected seller, the buyer's risk is not negligible. E.g., assuming three 10 kg samples each tested at less than 2 ng/g of total aflatoxin [$P(m) = 1$, $s_1$], there is a 25% chance that the lot mean $m$ exceeds 5 ng/g and a 6% chance that it exceeds 10 ng/g. Here the buyer gains a good deal if the sample size is increased. If three 250 kg samples each test at less than 2 ng/g, the chances are vanishingly small that the lot exceeds 4 ng/g. [The technique of testing such large samples without material loss by presorting and testing only the rejects has been discussed previously (Schatzki, 1998; Pearson and Schatzki, 1998).] However, facing a seller known to want a long-term quality relation with the buyer, the situation is quite different. Here the seller would probably not submit lot containing aflatoxin of more than a few tenths ng/g, at least in good years, for the reasons discussed above. The buyer would be aware of this and thus could introduce a prior $P(m)$ decreasing sharply with $m$, reducing his risk substantially. A few 10 kg tests early each year would suffice to test whether this confidence remained warranted. The average aflatoxin level of all lots which passed $s_1$ is given in Table 3.

**Table 3. Average Aflatoxin Level of Accepted Lots ($s_1 = 3$ Samples, Each below 2 ng/g) in ng/g**

| | sample weight | | |
|---|---|---|---|
| prior distribution | 10 kg | 50 kg | 250 kg |
| $P(m) = 1$ | 3.52 | 1.51 | 0.99 |
| $P(m) = 1/m$ | 1.76 | 1.09 | 0.87 |

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