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Dependence of Aflatoxin in Almonds on the Type and Amount of Insect Damage

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The aflatoxin distribution of single insect damaged Nonpareil almonds (1999 crop) has been measured. Separate distributions were obtained for pinhole, insect (feeding), and gross damage. Only a low level of aflatoxin contamination ($\langle c \rangle = 0.0003$ ng/g) was found for pinhole-only damaged nuts. The distributions for insect and gross damage did not differ, but did differ significantly from the distribution previously obtained for gross damaged Ne Plus almonds from a different producer (Schatzki, T. F.; Ong, M. S. *J. Agric. Food Chem*. **2000**, *48*, 489−492; also 1999 crop). The Nonpareil almond distribution could be explained on the basis of a preharvest hull splitting, similar to previous results in pistachios (0−4 weeks versus 2−6 weeks preharvest). The Ne Plus distribution differs in detail from pistachio results and from the Nonpareil results found here. This may indicate additional cultural damage of Ne Plus almonds around harvest time and/or use of different sorting parameters. Aflatoxin lot averages of 31.7 and 3.47 ng/g were obtained for 100% insect damaged Ne Plus and Nonpareil almonds, respectively. (The previous Ne Plus work contained a calculation error, which is corrected here.) The distribution functions were used to compute the seller's risk of nonacceptance of lots in the European Union. To obtain a 95% acceptance rate, aflatoxin B_1 levels of 0.12 and 0.22 ng/g would be required, which would correspond to 3.8 and 1.2% (feeding and gross) insect damage in Nonpareil and Ne Plus almond lots, respectively.

Keywords: *Aflatoxin distribution; sampling; lot means; risk of acceptance; insect damage; source of insect damage*

INTRODUCTION

Among tree nuts, almonds, in particular, are subject to insect damage because of postharvest storage on the ground and/or preharvest insect attack while the crop is still on the trees. The nuts are shaken down at harvest onto ground cover, where they are left for various times before being moved to large outdoor covered piles for storage. Fumigation to control insects, as needed, is common. Nevertheless, damage by navel orange worm larvae, peach twig borer, and other insects may occur in 2−10% of the product, depending on year, cultivar, and growing and handling conditions. Insect damaged nuts are removed from the lots during processing by sorting, visually and by means of electronic color sorters. Processors may segregate damaged nuts into as many as three types. "Minor" damage is defined as small-diameter tunnels ("pinholes") in the nut caused by burrowing navel orange worm larvae. "Insect" damaged nuts show evidence of insect feeding, that is, large gauges removed from the surface of the nut, which are particularly easy to see in natural almonds (nuts with the brown skin in place). Pinholes may also be present. The type of insect that fed can be discerned from details of the gauged hole by a skilled sorter; in most cases the damaging insect is the navel orange worm. "Gross damage" refers to nuts that show evidence of the presence of actual insects, typically body parts or frass (*2*). Contract prices for almond lots commonly include an insect damage level of the lot, as determined by batch testing. Limited levels of insect damage may be acceptable in cases when the final product is used for manufacturing processes, such as ground or chopped almonds, especially in overseas sales.

Aflatoxin content is related to insect damage of the nuts. Several publications have suggested that almonds free of such damage are not contaminated by the toxin (*3*−*5*). The first two publications considered the average aflatoxin level among chopped nuts, each obtained from a representative sample of California processors. No direct study was made of insect damage, but aflatoxin content was found to be related to the type of manufactured product; roughly speaking, the finer the nuts were chopped, the higher the aflatoxin content. Schatzki (*5*) found similar results for samples representing an entire crop year. This at least suggested a relationship between insect damage and aflatoxin, because damage and chopping tend to be related. Schatzki and Ong (*1*) measured aflatoxin in damaged nuts from a particular processor for the 1999 crop year. Substantially all of the nuts showed feeding or gross damage, although pinholes were seen in some nuts as well. Aflatoxin distribution yields the most detail about the aflatoxin contamination. This study did not directly yield information regarding the various processing parameters that may affect this distribution and, thus, overall aflatoxin level, such as storage parameters, general handling practice, weather conditions, cultivars, and the like, although indirect conclusions could be drawn. Knowledge about the effect of such parameters could guide processors to minimize insect damage. On the other hand, it must be realized

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Table 1. Number of Samples Falling into Each Range of Concentration (Bin) for Various Types of Insect Damage in Almonds, Total Aflatoxin

type	no. of samples	sample concn range, C_i, ng/g[a]												
		≤0.02	≤0.03	≤0.10	≤0.31	≤1.0	≤3.16	≤10	≤32	≤100	≤316	≤1000	≤3160	≤10000
pinhole 1999	54	46	0	5	3	0	0	0	0	0	0	0	0	0
pinhole 2000	146	124	0	13	5	3	0	0	0	0	1	0	0	0
feeding	329	205	8	75	18	8	0	5	4	2	2	1	0	0
gross	100	55	3	30	3	2	2	0	1	0	4	0	0	0
Schatzki + Ong	299	88	8	66	66	20	16	8	11	6	4	3	2	1

[a] Column headings indicate the upper limit of each bin. The lower limit is the heading of the next lower bin.

Table 2. Probability, $p_i \times 10^4$, of Single-Nut Total Aflatoxin Contamination in Almonds with Gross and Feeding Damage

source	nut concn, c_i, ng/g										
	11	36	113	360	1130	3600	11300	36000	113000	360000	1130000
pinhole	4.50	2.00	0.75	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00
feeding + gross		2.45	1.17	0.23	0.58	0.58	0.23	0.70	0.12	0.00	0.00
Schatzki + Ong			3.34	2.68	1.34	1.84	1.00	0.67	0.50	0.33	0.17

that such information is difficult to come by directly. The relatively small fraction that comprises the insect damaged part of the lot and the large quantities of material required for a study of aflatoxin distribution (200 samples of 200 nuts each amount to ∼44 kg of damaged product) would require the sorting of a great deal of product, indeed. Accordingly, the present study was undertaken to augment the previous results, to learn whether the aflatoxin contamination was confined to a particular type of insect damage and to determine if processing practices can reduce aflatoxin by removing insect damaged nuts. The present work follows the method of ref *1*, and only significant differences will be pointed out.

MATERIALS AND METHODS

One lot was obtained from a processor in California, referred to below as processor B. The farm product from which this lot originated came from orchards owned by processor B, who exercised tight cultural control. This lot consisted of 1999 crop year Nonpareil nuts mainly of size 23/25 (23−25 nuts/oz). Some 2000 crop year nuts were added late in the study. As is common in the industry, this lot had been presorted by passage through a number of mechanical sorters, which here included gravity tables, a Shellex visible/IR sorter, a SATAKI fluorescence sorter, and an Elbascan red/green two-color laser sorter. The cleaned up product was then hand sorted at the processor to remove all remaining insect damage. Pick-outs were separated into sublots exhibiting pinhole (here referred to as “pinhole 1999"), “feeding” (sometimes referred to simply as “insect”), and “gross” damage and shipped directly to the authors’ laboratory. The sublots were kept separate and were not re-sorted at our laboratory before use in order to obtain a sublot representative of processor practice. An additional, identical sort of early 2000 product was carried out during the year 2000, resulting in an additional batch of pinhole damaged almonds (“pinhole 2000"), which were shipped to us and again used without re-sorting. The latter were analyzed separately.

Another pick-out product, here referred to as from processor A, was described previously (*1*). It differed from that of processor B in the following respects. The cultivar was Ne Plus. The source were orchards owned by a number of farming entities spread over the state of California with presumably differing cultural procedures, particularly with respect to insect spraying. The Ne Plus cultivar is harvested some 15−20 days later than Nonpareil, possibly allowing additional insect damage while the crop is still on the trees. The Ne Plus material had been produced during a commercial sorting operation in essentially the same way as that from processor A, although the depth of the sort may have differed. However, the reject material, consisting almost solely of gross and feeding damaged nuts, had been stored in a bin marked “oil stock”, which might have held some extraneous nuts. As such, its cleanliness was not guaranteed. This material was analyzed here in the same way as that of processor B, and the results were compared with the latter.

A total of 54 samples of pinhole 1999 damage, 329 of feeding damage, and 100 of gross damage were analyzed. An additional 146 samples of pinhole 2000 nuts were analyzed as well. The usual sequence lot (here sublot, as defined by type of insect damage) > sample > homogenizing by grinding > subsampling > analysis was followed. Each sample consisted of 200 nuts. Grinding was carried out in a Waring blender to a particle size of ∼1 mm. Subsample size was 10 g of ground material aliquoted from the sample (∼220 g). For calibration purposes, from about every 13th sample another 10 g subsample was aliquoted and spiked using 100 ng each of aflatoxins B_1 and G_1 and 10 ng of aflatoxin B_2 (the B_1 calibration was used for G_2). Spiked samples were run exactly the same as analytical samples. When the HPLC sample peaks exceeded the linearity of the fluorescence detector, another 10 g sample was extracted and a liquid 9% aliquot prepared by dilution with the extraction fluid. Additional 0.9 and 0.09% aliquots were prepared, if needed. The resulting aflatoxin content was “binned”, defined as a set of contiguous concentration ranges (more of this below). For each sublot all that is needed or reported here is the number of samples which fell into each bin. Results are reported in terms of total aflatoxin ($B_1 + B_2 + G_1 + G_2$) and as B_1 only. Details of the breakdown between individual aflatoxins is noted below. As is customary, results are reported on the basis of an aliquot of the total extraction fluid used. [In ref *1* an error had been made in that the aliquot calculation had been omitted, leading to values ∼20−25% too low. That error in the ref *1* data has been corrected here. In terms of the large scale of the aflatoxin distribution curves, the error is not large, simply moving the distribution curve ∼0.1 unit (in $\log_{10}$) to the left. The recalculated ref *1* values are referred to here as “Schatzki + Ong” results.]

RESULTS

Aflatoxin Values. The repeatability of the analysis between samples can be obtained in two ways. Taking all 18 of the spike results (corrected for the analytical level of the corresponding unspiked sample, if needed), one obtains a mean of 210.3 ng of total aflatoxin. The coefficient of variance (CV) amounted to 13.2, 14.1, and 27.8%, respectively, for aflatoxin B_1, G_1, and B_2 spikes. The large CV for B_2 arose from the difficulty of reading small HPLC areas. Similarly, one can compute the CV for diluted subsamples arising from the same sample, each analyzed separately, as long as two such subsamples fall into the range of the HPLC. The average of 12 such cases amounted to an averaged CV of 12% total aflatoxin. One thus concludes that total aflatoxin

Table 3. Number of Samples Falling into Each Range of Concentration (Bin) for Various Types of Insect Damage in Almonds, Aflatoxin B_1

		sample concn range, C_i, ng/g[a]											
type	no. of samples	≤0.02	≤0.03	≤0.10	≤0.31	≤1.0	≤3.16	≤10	≤31.6	≤100	≤316	≤1000	≤3160
pinhole 1999	54	49	0	4	1	0	0	0	0	0	0	0	0
pinhole 2000	146	124	0	13	5	3	0	0	0	0	1	0	0
feeding	329	207	10	78	14	6	1	4	4	3	1	1	0
gross	100	55	3	30	4	1	2	0	1	1	3	0	0
Schatzki + Ong	299	97	14	80	51	14	13	9	6	6	4	3	2

[a] Column headings indicate the upper limit of each bin. The lower limit is less than the heading of next lower bin.

Table 4. Probability, $p_i \times 10^4$, of Single-Nut B_1 Aflatoxin Contamination in Almonds with Gross and Feeding Damage

	nut concn, c_i, ng/g									
source	11	36	113	360	1130	3600	11300	36000	113000	360000
pinhole	4.25	1.5	0.75	0	0	0	0	0.25	0	0
feeding + gross		2.10	0.82	0.35	0.47	0.58	0.47	0.47	0.12	0
Schatzki + Ong			2.34	2.17	1.51	1.00	1.00	0.67	0.50	0.33

or aflatoxin B_1 measured on different subsamples, each derived from the same sample, agreed to ~13%.

Aflatoxin B_1 comprised virtually all of the aflatoxin found during analysis. Aflatoxin B_2 amounted to but 4.5% of the total, and large values of B_2 were virtually always associated with large values of B_1. Aflatoxin G_1 and especially G_2 values were much lower. The average B_1 value was 3.14 ng/g, and total aflatoxin averaged 3.47 ng/g. The recalculated values for the Schatzki + Ong results amounted to 19.1 and 31.7 ng/g, respectively.

Calculation of Distribution Functions. The number of samples with total aflatoxin concentration, C, falling into each half-decide bin (i.e., bins of size $\sqrt{10}$ on a $\log_{10}$ concentration scale) are shown in Table 1. The Schatzki + Ong results are given as well. In ref *6* it was argued that for a precision of 25% in analysis (and subsampling) a half-decide would be the appropriate bin size. For the present case, where the precision is but 13%, a quarter-decile might be more appropriate. However, the total number of samples, and hence the samples per bin, is limited here, and little would be gained by going to a smaller bin size; that is, one would simply get more scatter between bins. The number of samples per bin can be converted to estimated sample probabilities, P_i, by division by the total number of samples, N, given in the first numeric column. The sample concentration at the (logarithmic) midpoint of the corresponding bin is designated C_i.

It has been pointed out that the estimated probabilities could, under certain assumptions, be converted to a probability distribution, p_i, of aflatoxin among single nuts in the lot (*6*). Before doing so one must ask whether the five estimated distributions, listed in Table 1, are indeed derived from five independent lots (populations) or whether two or more simply represent multiple sampling from the same population and hence an underlying distribution. This can be accomplished by use of the Kolmogorov−Smirnov two-sample test (*7*), which compares cumulative estimated sample distributions bin by bin. On the basis of this test one concludes that the probability is >99% that the pinhole 1999 samples and the pinhole 2000 samples came from the same (or an identical) population. The same is true of the gross and feeding samples. However, it is <10% likely that the Schatzki + Ong samples came from either B population. Accordingly, the pinhole 1999 and pinhole 2000 sample values may be added to yield a combined probability estimate of a population (referred to as the pinhole population), as may the gross and feeding samples (referred to as gross + feeding), yielding three independent populations in all.

In the next step the sample distributions are converted to single-nut distributions, that is, the probability, p_i, that a given nut, chosen at random from the lot, has an aflatoxin concentration, c, within a bin for which the midpoint is designated c_i and for which the size is given by $\sqrt{10}$ on a $\log_{10}$ ng/g scale. This is done following the methods described in ref *6*, which require solely that the sample distributions do not exceed 10% in any one bin. This is the so-called "sparse approximation" and is accomplished by choosing n, the sample size, appropriately. From the results presented in the next section, and a choice of $n = 200$, one finds that the sparse approximation for the gross + feeding data applies (i.e., the fraction of samples, P_i, in a bin is <10%) only to samples with $C > 0.1$ ng/g, or $c > 20$ ng/g, much as the Schatzki + Ong data applied to $C > 0.31$ ng/g. The pinhole data can be applied down to 0.02 ng/g. As before, the high P_i values at low C_i indicate that a large fraction of all nuts carry a low level of aflatoxin, here <0.1 ng/g. This would apply to all gross + feeding nuts, even those in samples with $C > 0.1$ ng/g, simply as an additive background. This low value will have no significant effect on sample aflatoxin values and can be viewed as a separate distribution which needs to be added to the single-nut distribution. Details of this low c distribution cannot be derived from sample data. For the sparse approximation, the conversion from sample probability $P_i(C_i)$ to single-nut probability $p_i(c_i)$ amounts to $p_i = P_i/n$, $c_i = C_i n$, $i > 0$, where $n = 200$ is the sample size (number of nuts/sample). A bin C_0 is chosen to represent those nuts that fall below the detection limit of the contaminant, $C < 0.02$ ng/g; for calculational purposes we set $C_0 = c_0 = 0$. Normalization is achieved by setting $p_0 = 1 - \sum_{i>0} p_i$. Values of $p_i(c_i)$ are given in Table 2 and are plotted in Figure 1. For risk calculations we shall have need of B_1-based data (see below), rather than total aflatoxin. These are shown in Tables 3 and 4 and in Figure 2.

It is seen from the tables that pinhole damaged almonds showed almost no aflatoxin, except for the low-level contamination ($C < 1$ ng/g), common to most tree nut populations. Only a single sample, and hence a single nut, was observed at high concentration ($C = 237$ ng/g, $c = 47500$ ng/g) among the 40000 nuts (roughly 44 kg) that were tested. All samples had been ground without further sorting of the producer-shipped material, so visual inspection of the single nut in question

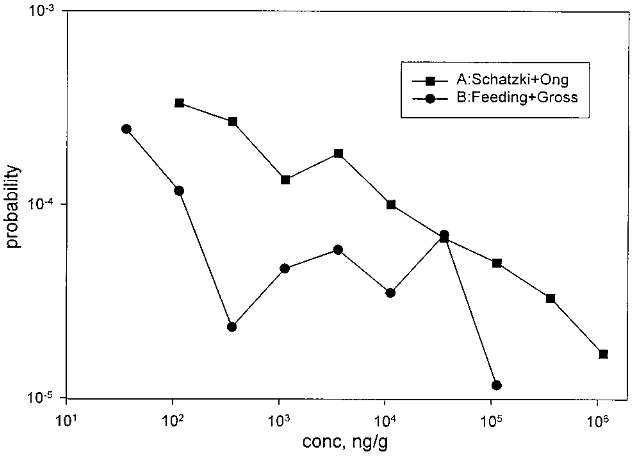

Figure 1. Total aflatoxin distribution among single nuts: lot A, gross insect damaged Ne Plus almonds (*1*); lot B, feeding and gross damaged Nonpareil almonds (this work).

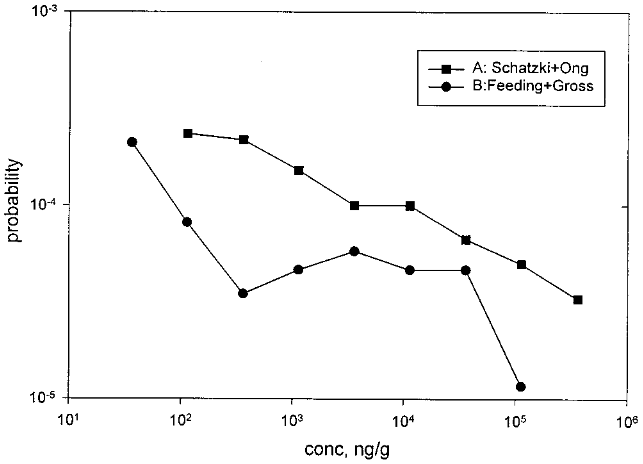

Figure 2. Aflatoxin B_1 distribution among single nuts: lot A, gross insect damaged Ne Plus almonds (*1*); lot B, feeding and gross damaged Nonpareil almonds (this work).

was not possible. However, an interview of the technician who had done the counting and grinding revealed that she had observed “one or two” darkened nuts among the 35 kg of the pinhole 2000 sublot. Accordingly, the remaining 11 kg of unground pinhole 2000 material was inspected, and eight nuts were removed which appeared darkened or were otherwise questionable, although none approached the darkening of the remembered nut. These eight nuts were analyzed individually for aflatoxin. Seven nuts showed no detectable aflatoxin, whereas one, slightly darkened nut, exhibited 0.4 ng/g. The pinhole distribution was not used in any further calculations.

With respect to the feeding + gross results, it can be observed from Tables 1 and 3 that the binned results involve but a few samples at high C_i, where P_i becomes small. As such, the results of Tables 2 and 4 and Figures 1 and 2 are subject to considerable scatter; scatter that arises when samples which fall near a bin boundary may exhibit a small error in concentration which causes them to fall into the adjacent bin. One example occurs in curve B of Figure 1 at c_i = 36000 ng/g. Tracing this peak back to the measured samples, it is seen to arise from six samples falling between 100 and 316 ng/g (Table 1), two of which fall just above the lower bin boundary at 109 and 113 ng/g. Had the bin boundaries been chosen but one standard deviation, that is, 14%, higher, at 114 and 360 ng/g, curve B would have exhibited a smooth maximum. The same is true to a lesser extent of curve B of Figure 2. Such scatter is inherent when the number of samples, *N*, is limited and the results are binned. It can be avoided only by choosing a much larger *N*. It took several weeks of manual labor to sort out the damaged kernels for both processors A and B, with an additional similar effort to measure aflatoxin. Major total sample increases are not realistic. The same considerations apply to the Schatzki + Ong lot.

In addition to bin scatter, limited data cause another problem in selected cases, lack of sensitivity at low p_i. Inspection of Figures 1 and 2 shows curve B terminating at c_i = 113000 ng/g, resulting from a single sample and thus a single nut. The resulting probability $p_i = 1.2 \times 10^{-5}$ corresponds to one sample per total sample size of 429 × 200. To get data at lower p_i requires a larger total sample ($n \times N$), just as in reduction of bin scatter.

Calculation of Risk. As noted above, the feeding + gross almond sample distribution, for samples of any reasonable size (not too large), is extremely broad, that is, shows large variability. This exposes the seller to the risk of having an acceptable lot rejected, simply on statistical grounds. Given the sampling protocol, agreed upon in advance between seller and buyer, and the single-nut distribution, this risk can be calculated by simulating sampling on a computer. (Calculation of the corresponding buyer’s risk of accepting a bad lot, as defined in ref *9*, requires additional knowledge of the expected probability of submission of bad lots by the seller. As this is not known here, this calculation will be avoided here.) The method has been described (*9*) and follows the Monte Carlo approach first suggested by Whitaker et al. (*10*). The risk is computed here using the European Union (EU) protocol for lot acceptance. This requires that a tree nut lot will be accepted if three separate 10 kg samples each test at ≤4 ng/g total aflatoxin (and ≤2 ng/g B_1 only). Should this fail, the lot may be reprocessed (re-sorted, not blended) if the average of three 10 kg samples test at ≤10 ng/g total (and ≤5 ng/g B_1). If this fails as well, the lot is to be seized. It was noted above that almost all aflatoxin, on average, was B_1. This makes the B_1 test much more restrictive, and it will govern acceptance. The risk calculations are based on B_1 content.

Given a lot with a known distribution function $p_i(c_i)$, one can compute a sample distribution $P_i(C_i)$ (e.g., by Monte Carlo) and from this derive the probability of acceptance, reprocessing, or rejection according to the EU protocol. These probabilities (risks) are associated with the average lot concentration $\langle c\rangle = \sum_i p_i c_i = \sum_i P_i C_i$. If one wishes to ascertain the corresponding risks for a lot of a higher (or lower) contamination, but having a probability distribution of the same shape, one simply multiplies each individual probability p_i by a fixed factor *k*, yielding $kp_i(c_i)$, and repeats the Monte Carlo calculation to obtain new risks, associated with a new lot average, $k \times \langle c\rangle$. By carrying out this calculation for a range of *k* values, one may construct a risk curve for a range of averages $\langle c\rangle$, provided only that the underlying probability distributions all have the same shape. This was carried out for both probability distributions A and B separately and yielded the risk curves shown in Figures 3 and 4. (The points $\langle c\rangle$ = 19.1 and 3.14 ng/g for distributions A and B, respectively, correspond to *k* = 1.) In each case, calculations were carried out in steps

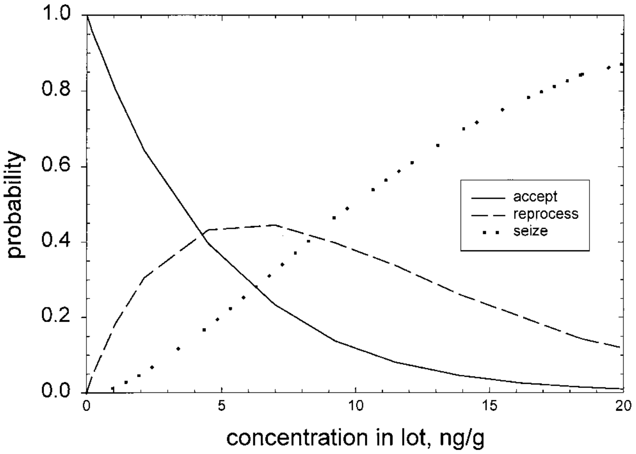

Figure 3. Computed risk of required reprocessing and seizure in the EU, based on distribution A: partially insect damaged Ne Plus almonds.

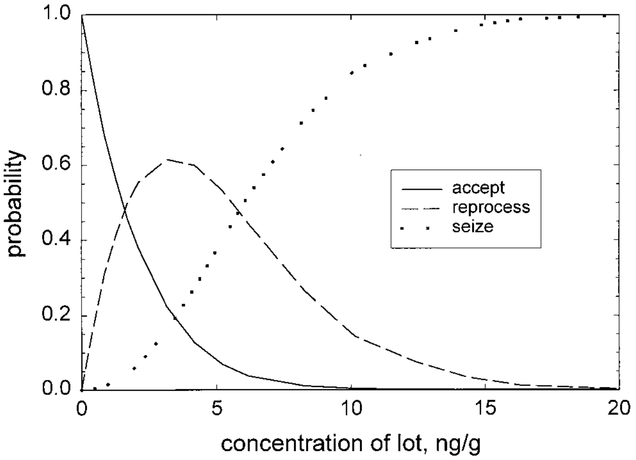

Figure 4. Computed risk of required reprocessing and seizure in the EU, based on distribution B: partially insect damaged Nonpareil almonds.

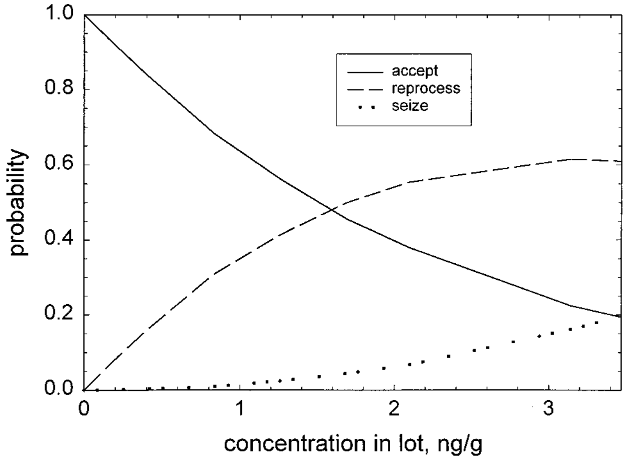

Figure 5. Computed risk of required reprocessing and seizure in the EU, based on distribution B: partially insect damaged Nonpareil almonds. Rescaled to $0 < k < 1$.

of ~0.5 in k to cover the range 0−20 ng/g in the lot mean, $k \times \langle c \rangle$. For easier viewing, an expanded version of Figure 4 is shown in Figure 5.

DISCUSSION

Processors indicate that pinhole damage is notoriously difficult to detect visually, particularly in natural (in-the-skin) almonds (*11*). Considerable research effort (*12*, *13*) has been expended to develop pinhole detectors based on X-ray absorption and infrared scattering. It now appears that from a practical point of view this effort may have been wasted, although it may have some scientific interest in imaging. The major, and unexpected, result obtained here is that pinhole damage appears to carry little or no aflatoxin. The average raw (not binned) aflatoxin level of the pinhole sublot, not counting the single high sample, amounts to but 0.0003 of total aflatoxin ng/g (1.18 ng/g for all 200 samples). In practical terms this means that a great deal of product which is currently considered to be harmful may simply be unsanitary (insect damaged) but have no adverse health effects.

The source of the single highly contaminated pinhole damaged nut is unclear. One possibility is that such nuts occur naturally among pinhole populations. This, however, would lead to a very unusual distribution function, zero everywhere (for $c > 60$ ng/g), but for a single high bin. Not only has such a distribution never been observed in tree or ground nuts, and probably other commodities as well, it would be very difficult to explain on theoretical grounds. A more likely source is a feeding or gross damaged almond that was inadvertently not sorted out from the pinhole sublot or dropped in later by accident. It should be pointed out that a single such nut in a 10 kg sample would, by itself, result in a 4.9 ng/g aflatoxin measurement for this sample, enough to force reprocessing by the EU standard. In what follows, it is assumed that such highly contaminated nuts are not present in pinhole damaged sublots. It is noted from Tables 3 and 4 that the low end of the distribution function, for $c \leq 100$ ng/g, is quite similar to the feeding + gross distribution, suggesting a common source.

Distribution Functions. Consideration of curves A and B in Figures 1 and 2 reveals some significant differences. Curve B has a distinct shape, similar to that noted previously for pistachios (*8*), consisting of a broad peak at higher c, adjoined to a rapidly rising probability at lower c. At first glance, this is quite different from curve A, accepting the argument regarding scatter presented above. In curve B this peak ranges from about 360 to 100000ng/g, with a maximum around 4000 ng/g. It has been argued that the log c axis of a distribution function, as in Figure 1, could be viewed as a time axis, assuming mold growth (strictly aflatoxin production) was exponential (*14*). Some evidence exists for this. In the case of pistachios, the 10^6 ng/g point could be related to the initiation of hull splitting 6 weeks prior to harvest, whereas 5000 ng/g corresponded to 2 weeks prior to harvest, the end of hull splitting. This yielded a scale factor relating aflatoxin concentration to time prior to harvest. A very similar shape is now seen in curve B. Carrying over the parameters obtained from pistachios, the peak appears to occur from 4 weeks prior to harvest to 1 day before harvest. Indeed, hull splitting in almonds begins ~4 weeks before harvest, followed eventually by a slow drying of the nut until harvest, which would arrest further aflatoxin production. It thus appears the broad maximum in the distribution function in both tree nuts corresponds to the same phenomenon, hull splitting, which allows access to the kernel. Hull splitting in pistachios may allow access to mold spores, whereas in almonds splitting allows access by insects, against which spraying is used. Note that details, such as are seen in curve B, involve the time of contamination by the precursor to aflatoxin production, not the aflatoxin level itself. If one were to simply measure aflatoxin levels (which would be considerably simpler than distributions, as is done here), one would need to time differentiate the results and accuracy would be difficult to achieve.

Curve A differs from curve B in several respects. First, it is higher, which may simply reflect a less rigorous sort (and thus a more contaminated reject stream). Second, the minimum in probability of contamination between the broad peak and the rapid rise toward lower concentration is missing in curve A. One possibility is that the Ne Plus cultivar behaves differently from the Nonpareil in terms of insect damage. In particular, it appears to indicate additional feeding and gross damage around harvest time, which, in turn, may arise from the later harvest of the Ne Plus nuts. Alternatively, because curve A is derived from a number of smaller growers, details such as are seen in curve B may have been averaged out. Possibly, some additional nuts in the "oil stock" contributed, although this is thought to be unlikely. Finally, curve A extends to a higher value of c than curve B. Because these high values of c dominate the mean as well as broaden the distribution, this has profound implications on the mean and risk values. In the case at hand, the ~12-fold increase in average concentration, $\langle c \rangle$, from B to A, is partially (3−4-fold) accounted by the higher level, the remainder arising from this broadening, as does the need to use a less contaminated mix to achieve similar risk values.

Means. The mean of a pistachio lot is strongly dependent on the aflatoxin distribution among the nuts. In general, the mean is given by $\langle c \rangle = \int p(c)c\, dc$, which can be approximated by $\sum p_i \times c_i$. The higher terms generally dominate because c_i increases much more rapidly than p_i decreases (the term $i = 0$ is always zero because $c_0 = 0$). For almonds the raw (not binned) values for total aflatoxin (100% feeding and gross damage) were $\langle c \rangle = 31.7$ ng/g for sublot A and 3.47 ng/g for sublot B. Calculations, using the sum, yield 41.2 and 4.4 ng/g, respectively. (Most samples fell in the lower part of their respective bins.) For aflatoxin B_1 only, raw values were 19.1 and 3.14 ng/g for A and B, respectively.

Risk. As indicated, the primary risk of lot rejection is borne by the seller, but this risk is of interest to the buyer as well. A lot is typically subjected to repeated retesting as it passes through sales channels, first in the country of origin, then in the country of consumption, by customs, health authorities, and even consumer groups. Rejection at a late stage of the sales chain can result in very high costs of recall and permanent loss of market. (This example is not hypothetical, but has occurred on several occasions in the EU.) As a result, a buyer is commonly less interested in whether a lot has passed an acceptance test, but rather in the probability that it will pass further tests. Such probabilities can be read directly from the risk curves. We can then relate insect damage directly to aflatoxin levels and likelihood of acceptance. We will illustrate this by an example.

Suppose a buyer requires that a lot has a >95% probability of passing an acceptance test. Given that one knows the individual nut aflatoxin distribution, this 95% probability can be read directly from the probability of acceptance curves, shown in Figures 3 and 5. For a lot with a distribution proportional to A in Figure 2, one requires a lot mean $\langle c \rangle \leq 0.225$ ng/g (Figure 3); for a distribution proportional to B, $\langle c \rangle \leq 0.12$ ng/g is required (Figure 5). Because lot A yielded an average of $\langle c \rangle = 19.1$ ng/g as received ($k = 1$), the k required to obtain 0.225 ng/g amounts to 0.225/19.1 = 1.2%. The corresponding value for a lot of type B is $k = 0.12/3.14 = 3.8\%$. Lots with $k < 1$ can be prepared by starting with a lot consisting solely of feeding and gross pickouts ($k = 1$) and diluting it with a clean (no insect damage, $k = 0$) lot so that the blend contains the desired k value. However, it would clearly not be economical to start with an unsorted (field run) lot, remove all insect damaged nuts by sorting to obtain a clean lot, and then blend back enough pickouts to obtain the desired k value. Instead, one would sort to reduce the (feeding and gross) insect damaged fraction to 1.2 or 3.8%. Generally speaking, a seller would have enough information about his or her product to have a fairly good idea about the underlying aflatoxin probability distribution to know which distribution applies or whether an intermediate distribution is called for. (The actual distribution may depend on cultivar, possibly on crop year, and almost certainly on postharvest handling when the crop is on the ground and subject to insect attack. Such studies are not included here. In any event, the underlying probability distribution could always be measured, once and for all, for the type of material to be shipped.) A few points should be noted. The values given for insect damage are basis gross and feeding damage only. We are told by processors that the vast majority of insect damage is gross and feeding damage (*11*); hence, using a total insect damage basis would be close. As pointed out, pinhole damage has no effect in any case. Furthermore, the aflatoxin contributions of nuts below 0.33 ng/g have been neglected. These have a negligible effect in that they simply change the acceptance standard from 2 ng/g B_1 to ~1.9 ng/g. In summary, using this approach, one may establish the maximum insect damage fraction that can be accepted to satisfy any required reject rate, an important marketing result.

SAFETY

Aflatoxin is a highly toxic material that should be handled with care. Solid aflatoxin for calibration should be handled in a biohood, using a nose and mouth mask. Nuts with possible contamination should be handled with surgical gloves and in a biohood if feasible.

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