WHEAT FACTS

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HOW TO SAMPLE WHEAT TO ACCURATELY DETERMINE VOMITOXIN LEVELS

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Wheat, the only economic winter cereal grown in Michigan, is an important rotational crop that provides Michigan farmers with economic and agricultural diversity. Several properties distinguish wheat from the other major agronomic crops grown in Michigan:

— As a fall-sown, cool-season grass, wheat possesses a spectrum of ecological properties that can be exploited to manage diseases, insects and weeds in spring-sown crops.

—Wheat provides invaluable resistance to soil erosion during the winter months, and its early fall and midsummer main work periods fall between the peak times for most other crops.

— Winter wheat also acts to retard water pollution from nitrates and pesticides.

— Recent research identifies winter wheat as a valuable rotation tool that adds as much as 18 percent to subsequent corn yields, compared with corn monoculture.

In economic impact, wheat differs from corn and soybeans in that the majority of the value it adds to Michigan's economy occurs after the grain leaves the farm. Michigan's milling and wheat processing industry is very well established and plays a significant role in Michigan's overall economy.

Gibberella zeae, also known as *Fusarium graminearum*, is a plant pathogenic fungus that causes scab in wheat and ear mold and stalk rot in corn. Another common name for wheat scab is Fusarium head blight. *G. zeae* can infect wheat when there are several consecutive days of wet weather. Widespread and severe epidemics can occur when the rainy period coincides with flowering.

During the infection process, *G. zeae* produces a toxic secondary substance called vomitoxin. Other names for vomitoxin are deoxynivalenol and DON. Vomitoxin is a mammalian toxin. The most distinctive symptoms occur in swine, which will vomit when they ingest vomitoxin-contaminated grain. If the levels of vomitoxin are high enough, they will refuse to eat the grain.

These and other toxic properties of vomitoxin prompted the Food and Drug Administration to issue an advisory on acceptable levels of vomitoxin in wheat. The advisory levels are:

— 1 part per million (ppm) of vomitoxin for bran, flour and germ intended for human consumption.

— 10 ppm of vomitoxin in grain and grain byproducts destined for ruminating beef and feedlot cattle older than 4 months and for chickens, with the added recommendation that these ingredients not exceed 50 percent of the diet of cattle or chickens.

— 5 ppm of vomitoxin in grains and grain byproducts destined for swine and all other animals, with the added recommendation that these ingredients not exceed 20 percent of the diet for swine and 40 percent of the diet for other animals. Sampling and testing of wheat are necessary to determine that vomitoxin is below these levels in wheat products, and to ensure the safety of the food and feed supply.

Accurate and precise assessment of the vomitoxin concentration in wheat requires that a sample of wheat kernels or ground wheat be obtained from a particular storage unit (truck or storage bin) and then analyzed. The sample design must be a carefully chosen combination of probabilistic sampling strategy and intensity to properly represent the vomitoxin distribution and to provide precise estimates of vomitoxin concentrations with a high level of confidence. Analytical methods to test for vomitoxin should be accurate, simple to use, rapid and inexpensive. Analytical tests that use thin layer chromatograms, gas chromatography and mass spectroscopy are accurate but require specialists to run the tests, and they are not rapid or inexpensive. The enzyme

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linked immunosorbant assay (ELISA) does meet these criteria. ELISAs that detect vomitoxin have been validated by federal agencies, and they are used extensively by commercial wheat handlers. ELISAs are also flexible in that they can be used as a quantitative test to determine levels of vomitoxin or as a qualitative test to screen samples for the presence or absence of vomitoxin.

A visual examination of wheat kernels may not provide a reliable estimate of the potential for the grain to contain vomitoxin. Infected kernels may be shriveled and appear somewhat bleached to red in color, but not all infected kernels display these symptoms. A visual analysis may suggest that a grain sample with "scabby" kernels contains a higher level of vomitoxin than samples with no kernels having these symptoms, but samples with zero or low levels of vomitoxin cannot be distinguished from samples with moderate levels. Therefore, an analytical test to screen all samples to separate vomitoxin-positive samples from negative samples may be necessary, and sampling issues gain importance to assure reliability and confidence.

The sample collection recommendations made here are based on a sampling study of vomitoxin in wheat collected from various sized trucks or hoppers used to transport freshly harvested wheat from the field to the elevator or storage bin. This study, conducted in 1996, occurred during a year when wheat scab was epidemic. The sampling protocol may not be as reliable during a year when scab occurs at less than epidemic levels.

Samples of whole wheat must be collected with a probe 6 feet long, and each probe sample should weigh between 1 and 2 pounds. Alternatively, stream samples could be collected as the truck is being unloaded if they are taken randomly. Using either method, a minimum number of samples per truck must be randomly collected (Table 1), and the samples must represent spatially distinct areas of the truck. After the wheat has been collected, all of the wheat must be thoroughly ground in a mill to obtain flour. A subsample of the wheat equivalent to 5 to 15 percent of the total weight can be collected during milling and used for analysis, or the entire milled sample can be analyzed. In the 1996 study, each probe sample was analyzed separately by ELISA to determine the vomitoxin level. It is possible to thoroughly mix together each of the milled samples and test only one subsample from the mixture. However, the mixed sample should be blended to obtain as homogeneous a mixture as possible, and the analyzed sample should be equivalent to at least 10 percent of the total sample weight.

Table 1 lists the number of probes necessary to ensure that a certain range of vomitoxin levels covers the unknown, true level with 95 percent confidence. For example, to be within 0.86 to 1.0 ppm of the upper limit of the mean vomitoxin level (one-sided 95 percent) would require an analysis of four probes if either the whole milled sample (bulk) or a subsample (substream) were analyzed. On the other hand, to be within 1.0 ppm on either side of the mean (two-sided 95 percent) would require analysis of five probes. Analyzing more or fewer probe or stream samples can improve or reduce accuracy. Generally, bounding the upper limit of possible vomitoxin levels is probably preferable in most instances and requires one less probe or stream sample to be analyzed.

Table 1. Relationship between the number of sample probes and the one- or two-sided boundaries for vomitoxin concentration at the 95 percent confidence level.

	ELISA analysis performed on			
	Bulk sample of ground wheat		Substream sample of ground wheat	
No. of probes	Two-sided 95%	One-sided 95%	Two-sided 95%	One-sided 95%
2	15.30	3.80	13.18	3.27
3	4.23	1.43	3.64	1.24
4	2.71	1.00	2.33	0.86
5	2.11	0.81	1.82	0.70
6	1.79	0.70	1.54	0.60
7	1.57	0.63	1.36	0.54
8	1.42	0.57	1.23	0.49
9	1.31	0.53	1.13	0.45
10	1.22	0.49	1.05	0.43

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