

DEPARTMENT OF AGRICULTURE

Agricultural Marketing Service

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Proposed Change to Evaluation of Mycotoxin Test Kit Accuracy

To evaluate test kit accuracy, replicate analyses of reference materials are performed at different concentrations across the conformance range. At least 95% of these individual results must be within the acceptable range for each of the tested concentrations to meet AMS accuracy requirements. Since the Horwitz-Thompson equation¹ has become the international standard² by which analytical method precision is evaluated, AMS proposes to use this equation to set the acceptable ranges.

The maximum relative standard deviation (RSD_{\max}) used to determine the acceptable ranges would be calculated using equation (1).

$$RSD_{\max} = 2/3 \cdot PRSD_R \quad (1)$$

where $PRSD_R$ is the relative standard deviation predicted by the Horwitz-Thompson equation under interlaboratory conditions. According to the Horwitz-Thompson equation, $PRSD_R = 22\%$ for concentrations less than or equal to 120 ppb and $PRSD_R = 2 \cdot C^{-0.1505}$ for a concentration greater than 120 ppb, where C is the concentration expressed as a mass fraction (Example: 5.0 ppb aflatoxins = $5.0 \cdot 10^{-9}$ g aflatoxins per g sample).

¹ Thompson, M. The Characteristic Function A Method-Specific Alternative to the Horwitz Function. *J. AOAC Int.* **2012**, 95, 1803-1806.

² Procedural Manual of the Codex Alimentarius Commission 26th edition [Online]; Food and Agriculture Organization of the United Nations, Rome, Italy, 2018; pp 82–83.
<http://www.fao.org/documents/card/en/c/l8608EN/> (accessed 9/18/2020).

The multiplier of 2/3 will be used to evaluate analytical method precision under single-laboratory conditions³.

Under the proposed procedure, the acceptable range would be the 95% confidence interval calculated by equation (2).

$$\text{Acceptable Range} = \text{CRV} \pm (\text{CRV} \cdot 2.086 \cdot \text{RSD}_{\text{max}} \div 100); \quad (2)$$

where CRV is the certified reference value (i.e., mean concentration determined by the reference method). The factor of 2.086 represents the appropriate multiplier for a 95% confidence interval using the Student's t-distribution with 20 degrees of freedom. At least three significant figures will be used in calculations leading to the acceptable range. Only the final upper and lower range limits will be rounded to two significant figures.

Tables 1–5 compare the current maximum RSDs and acceptable ranges with the values determined from the proposed process.

Table 1 – Aflatoxins

Aflatoxins (ppb)	RSD _{max} (%)		Acceptable Range (ppb)	
	Current	Proposed	Current	Proposed
5.0	25	15	2.5–7.5	3.5–6.5
20	20	15	12–28	14–26
100	16	15	68–130	69–130
300	16	13	200–400	220–380

Table 2 – Deoxynivalenol

Deoxynivalenol (ppm)	RSD _{max} (%)		Acceptable Range (ppm)	
	Current	Proposed	Current	Proposed
0.50	20	12	0.30–0.70	0.38–0.62
2.0	12	9.6	1.5–2.5	1.6–2.4
5.0	10	8.4	4.0–6.0	4.1–5.9
30	10	6.4	24–36	26–34

³ Guidelines for Standard Method Performance Requirements [Online]. *Official Methods of Analysis*. AOAC International, Rockville, MD, 2018; Appendix F, Section 2.1, p 14. https://www.aoac.org/wp-content/uploads/2019/08/app_f.pdf (accessed 9/18/2020).

Table 3 – Fumonisin

Fumonisin (ppm)	RSD _{max} (%)		Acceptable Range (ppm)	
	Current	Proposed	Current	Proposed
0.50	18	12	0.32–0.68	0.38–0.62
2.0	14	9.6	1.4–2.6	1.6–2.4
5.0	13	8.4	3.7–6.3	4.1–5.9
30	13	6.4	22–38	26–34
100	13	5.3	74–130	89–110

Table 4 – Ochratoxin A

Ochratoxin A (ppb)	RSD _{max} (%)		Acceptable Range (ppb)	
	Current	Proposed	Current	Proposed
1.0	-	15	-	0.69–1.3
5.0	20	15	3.0–7.0	3.5–6.5
20	20	15	12–28	14–26
100	20	15	60–140	69–130

Table 5 – Zearalenone

Zearalenone (ppb)	RSD _{max} (%)		Acceptable Range (ppb)	
	Current	Proposed	Current	Proposed
20	-	15	-	14–26
100	25	15	50–150	69–130
250	20	13	150–350	180–320
1000	20	11	600–1400	780–1200