Progress in the accuracy of mycotoxin analysis in the last quarter century

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Summary

Trends in analytical methods used in mycotoxin analyses over the last 25 years are discussed based on results from WHO and FAPAS[®]. In 1978, most determinations of aflatoxin B₁ in maize samples were done utilizing TLC methods. The percentage of TLC methods decreased to 48 % in 1989 and to 7 % in 2002. Alternatively, the use of HPLC has increased from less than 5 % in 1978 to 36 % in 1989 and 77 % in 2002. ELISA based methods have also been used in more recent tests, but the percentage of laboratories using ELISA is not high. The percentage of data falling within $|z| \leq 2$ increased steadily from 50 % in 1978 to 82 % in 2002. Similar trends over time were observed for aflatoxin B₁ in peanut, aflatoxin M₁ in milk and ochratoxin A data.

Key words : mycotoxin, analysis method, progress, proficiency testing

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Introduction

Since food and feedstuffs, such as cereal crops, nuts, spices, dried fruit and fruit juices, can be infected by molds¹⁾ during growth or post harvest handling, mycotoxins can be found as contaminants in these materials²⁾. They may be present in food and feed without any obvious sign of fungal growth, thus, for human and animal health, determination of mycotoxin contamination in various agricultural commodities is important. For this purpose, much effort has been made to improve analytical methods for each mycotoxin in different commodities and many analytical methods have been developed for the determination of mycotoxins^{3,4)}. Methods used for such analyses have changed over time. For example, fluorescence detection has been used for aflatoxin determination since the 1960's, however, separation procedures have progressed from TLC to HPLC.

Public demand for accuracy of data has been growing and there is therefore a continuing need for laboratories to demonstrate performance and reliability in laboratory analysis. It is now widely accepted that there are three essential elements to laboratory quality assurance. These elements are, use of validated methods, participation in laboratory proficiency testing and accreditation to ISO/IEC 17025. The second of these, proficiency testing is an external check of quality of analysis. It provides an independent and unbiased assessment of the performance of all aspects of laboratory. Since the

1970's IARC, WHO have organised check sample programs for mycotoxins similar to more recent proficiency testing schemes such as those provided by FAPAS[®], CSL, UK.

In this report, we discuss trends in analytical methods used in mycotoxin analyses and improvement of accuracy of data over the last 25 years based on the results from WHO and FAPAS[®]. In order to compare results over this period, all statistical procedures were carried out according to the FAPAS[®] protocol.

Materials and methods

Source of results of proficiency testing Results from ten check studies/proficiency tests were chosen for this study. These tests were organised by WHO/IARC or FAPAS[®]/CSL. These are six tests for aflatoxin B₁ in maize and peanut samples, two for aflatoxin M₁ in milk and two for ochratoxin A in wheat. A summary of these tests and samples used were listed in Table 1.

Classification of the determination methods In all reports of the proficiency tests, determination methods used by participants were recorded. However, in the test reports produced by WHO/IARC, a considerable amount of the participants' data was not listed into specific methods. Re-allocation of these data was carried out based on the data descriptions provided in the reports.

Statistical method In order to compare results from different programs it was necessary to re-analyse older results according to the FAPAS[®] Protocol⁹⁾. This procedure is recommended in the IUPAC/ISO/AOAC International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories⁶⁾.

1) Calculation of the assigned value The assigned value, $\bar{X}$, which is the best estimate of the true concentration of analyte, was derived as the consensus of the analytical results submitted by participants. The following steps were followed:

1. Results reported as 0 µg/kg or more than 1000 times greater than the majority of submitted data were removed. Those results in FAPAS[®] tests not quoting a recovery value or results stated by

Table 1. Description of analytical tests

analyte	sample	name of test	year	assigned value µg/kg ^a	total no. of scores	satisfactory % ^b
aflatoxin B ₁	maize	WHO No. 78-2	1978	14.9	111	50
		WHO No. 89-1	1989	111	221	62
		FAPAS 0446	2002	6.78	71	82
	peanut	WHO No. 78-1	1978	8.44	109	60
		WHO No. 89-2	1989	13.0	201	62
		FAPAS 0447	2002	4.19	101	77
aflatoxin M ₁	lyophilized milk	WHO No. 89-3	1989	0.49	117	43
		FAPAS 0445	2002	0.26	74	74
ochratoxin A	wheat	WHO No. 87-4	1988	182	103	57
		FAPAS 1721	2003	6.61	85	85

^a The values estimated from the participants' data using Huber's proposal 2⁷⁾

^b Percentage of z-scores in the satisfactory range, $|z| \leq 2$

participants as uncorrected were also removed. Since the concept of the “recovery value” was introduced around 2000, this information was not reported by WHO/IARC.

2. A robust statistical procedure⁷⁾ was used to derive the mean value to minimize the influence of outliers. The robust mean (Huber’s proposal 2) was calculated using the function Hubers in the public domain R program version 1.70.⁸⁾

After these steps the robust mean calculated was set as the assigned value.

2) Estimation of the target standard deviation Estimation of the target standard deviation was carried out based on the Horwitz equation⁹⁾ which predicts a standard deviation from a given concentration. The equation has three forms according to the analyte levels:

- i) for analyte concentrations <120 µg/kg

$$\sigma_p = \frac{0.22c}{mr}$$

- ii) for analyte concentrations $\geq 120 \mu\text{g/kg}$ and $\leq 13.8 \%$

$$\sigma_p = \frac{0.02c^{0.8495}}{mr}$$

- iii) for analyte concentrations >13.8 %

$$\sigma_p = \frac{0.01c^{0.5}}{mr}$$

where, in all three cases, c = concentration, i.e. the assigned value, $\hat{X}$, expressed as a dimensionless mass ratio, e.g. 1 mg/kg 10^{-6} or % 10^{-2}
and mr = dimensionless mass ratio, e.g. 1 mg/kg 10^{-6} or % 10^{-2}

3) Calculation of z-scores For all analytical results submitted by participants z-scores were calculated as:

$$z = \frac{(x - \hat{X})}{\sigma_p}$$

where x = the participant’s reported result

$\hat{X}$ = the assigned value

and σ_p = the target value for standard deviation

Results and discussion

Determination of aflatoxin B₁ In programs carried out in 1978 (i.e. WHO No. 78-1 and 78-2) 87 and 90 % of participants used TLC methods for separation and quantitation (Fig. 1). The use of TLC decreased to 48 and 43 % in 1989, whereas the use of HPLC was 5 % at 1978 increased to 36 and 37 % at 1989. In 2002, the use of HPLC amounted to 77 and 85 %, and only 7 % of results were reported by TLC. ELISA for aflatoxin analysis was introduced in 1980 by Pestka *et al*¹⁰⁾ and approximately 5 % of

Fig. 1. Transition of Methods Used in Aflatoxin B₁ Analysis.
 ■ : TLC, ■ : HPLC, □ : ELISA, □ : Others (including mini columns and unspecified).

participants reported their results by ELISA in 1989, however, there was no evidence of a significant increase in the use of ELISA in 2002.

In addition to the trends in the use of different determination methods, the numbers of participants reporting satisfactory results increased over time. For example, in the three programs for maize samples, the percentage of the data falling in the “ $|z| \leq 2$ ” category increased from 50 % in 1978 to 62 % in 1989 (Table 1). In 2002, the percentage of satisfactory results increased to more than 80 % given that the concentration of the analyte (i.e. 6.78 µg/kg) was considerably lower than the concentration in the previous program (i.e. 111 µg/kg in 1989). A similar trend was observed in the programs for peanut samples. This indicates that the percentage of laboratories reporting results outside the satisfactory range, $|z| > 2$, has reduced by half over the last 25 years; and the precision of aflatoxin B₁ analysis has improved remarkably.

Determination of aflatoxin M₁ and ochratoxin A Changes in the analytical methods used for the analysis of aflatoxin M₁ and ochratoxin A over time were shown in Fig. 2. In the case of aflatoxin M₁, 35 % of participants used HPLC method in 1989. The use of HPLC has increased to 89 % in 2002, and TLC method was seldom used in 2002 (4 %). For ochratoxin A, the trend in methods used are comparable to that seen for aflatoxin M₁; the use of HPLC increased from 27 % to 88 % and the use of TLC decreased from 58 % to 5 %. The immunological method, ELISA, was also used in the quantification of aflatoxin M₁ and ochratoxin A, though the proportion was 11 % or less.

The number of satisfactory results submitted from participants for these analytes increased over time. For aflatoxin M₁ analysis, the percentage of satisfactory data increased from 43 to 74 during the period between 1989 and 2002 while the assigned value decreased from 0.49 to 0.26 µg/kg. For ochratoxin A analysis, the number of satisfactory results increased from 57 to 85, while the assigned value decreased markedly from 182 to 6.61 µg/kg (Table 1).

During the last 25 years, the majority of analytical methods used have changed from TLC to

Fig. 2. Transition of Methods Used in Mycotoxins Analysis.

▨ : TLC, ■ : HPLC, □ : ELISA, □ : Others (including mini columns and unspecified).

HPLC for these mycotoxins. This has coincided with an improvement in the accuracy of analysis. For example, even though the level of aflatoxin B₁ (in maize) and ochratoxin A in the FAPAS[®] testings are obviously lower than in the tests in 1989 and 1988, the percentage of unsatisfactory results decreased from 40 % to less than 20 % in both cases. Albeit the reasons for these changes may in part be due to the differing laboratories that participated in these programs, it is thought that the main reasons for this change may be attributed to the technological improvements such as clean-up and determination methods.

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ここ 25 年間におけるマイコトキシン分析の手法の変化と精度

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WHO と FAPAS による分析試験の結果に基づき, ここ 25 年間におけるマイコトキシン分析の手法の変遷と分析精度の変化を解析した. トウモロコシ試料中のアフラトキシン B₁ の分析に関しては, 1978 年には TLC による分析法がほとんどであったが, その割合は 1989 年には 48%, 2002 年には 7%へと低下した. その一方で, HPLC をベースにした手法の割合が 1978 年の 5%から, 36% (1989 年), 77% (2002 年) と増加した. 近年においては ELISA を用いた分析法も使用されているが, その割合は 5%程度にとどまっている. 分析精度に関しては, $|z|$ が 2 以下となるデータの割合が, 1978 年の 50%から 2002 年の 82%へと次第に増大しており, 分析精度が向上してきたことが明らかとなった. 落花生中のアフラトキシン B₁, 乳中のアフラトキシン M₁, 小麦中のオクラトキシン A の分析についても, 分析手法と精度が同様に変化してきたことを明らかにした.

キーワード: マイコトキシン, 分析手法, 進歩, プロフィシエンシーテスト