CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **CX/CF 16/10/3-Add.1**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

Tenth Session

Rotterdam, The Netherlands, 4 – 8 April 2016

MATTERS OF INTEREST ARISING FROM FAO AND WHO

FURTHER ANAYSIS OF DATA PROVIDED BY THE FAO/WHO PROJECT ON MYCOTOXINS IN SORGHUM

Introduction

1. The FAO/WHO project on mycotoxins in sorghum was implemented from 2012 to 2014 in Burkina Faso, Ethiopia, Mali and Sudan. The objective of the project was to provide mycotoxin occurrence data, as well as to inform on farming and production practices along the value chain. This project resulted from discussions in the Committee on Contaminants in Foods (CCCF) on the potential need for a Maximum Level on mycotoxins in sorghum. A final status report on the project (ref. CX/CF 15/9/3-Add.1) was submitted to the 9th session of the CCCF, communicating the main results from the project to the Committee.

2. At its 9th session, the Committee considered this final status report and concluded as follows: "*The Committee agreed to ask FAO and WHO to analyse the data and provide recommendations to the Committee at its next session as regards the mycotoxins of importance and the feasibility to establish MLs for these mycotoxins and to propose changes to the Code of Practice for the Reduction and Prevention of Mycotoxin Contamination in Cereals.*" (REP15/CF, para

3. In response to this request, this paper summarizes the results of further statistical analysis performed on the data collected as well as an analysis of the information collected on good practices during the project, with respect to the current content of the proposed draft revision of the code of practice for the prevention and reduction of mycotoxins contamination in cereals (CAC/RCP 51-2003) and its draft annexes.

Main results from the sorghum project

4. A total of 1533 samples of sorghum were collected in the four countries between December 2012 and December 2013, in three rounds (immediately after harvest; immediately prior to wet season and before yearly stocks end). They were analysed for a total of 23 mycotoxins[1] using a validated multi-analyte LC-MS/MS method[2] in an ISO 17025 accredited laboratory (Laboratory of Food Analysis at the University of Ghent).

5. Out of the 23 mycotoxins analysed, 16 different mycotoxins were detected in at least one sample at levels above the LOQ (Aflatoxins, Altenuene, Fumonisins, HT-2 toxin, Ochratoxin A, Deoxynivalenol, Zearalenone, Alternariol, Alternariol monoethylether Sterigmatocystin, Diacetoxyscirpenol,). The table below shows a summary of the results:

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1 Nivalenol, Deoxynivalenol, Fusarenon X, Neosolaniol, 3-Acetyldeoxynivalenol, 15 Acetyldeoxynivalenol, Aflatoxin G2, Aflatoxin G1, Aflatoxin B2, Aflatoxin B1, Diacetoxyscirpenol, Altenuene, Roquefortin C, HT-2 toxin, Fumonisin B1, Fumonisin B2, Fumonisin B3, Alternariol, T-2 toxin, Ochratoxin A, Zearalenone, Sterigmatocystin and Alternariol- Monomethylether

2 E. Njumbe Ediage, J. Diana Di Mavungu, C. Van Peteghem, S. De Saeger. (2011). A validated multi-analyte LC–MS/MS method for the quantification of 25 mycotoxins in cassava flour, peanut cake and maize samples. Journal of Agricultural and Food Chemistry. 59, 5173–5180

Mycotoxins	**LOQs (µg/kg)**	MAX (µg/kg)	% samples>LOQ
Aflatoxins B1	**7.5**	359	7.11
Aflatoxins B2	**2.5**	49	3.59
Aflatoxins G1	**2.5**	714	3.06
Aflatoxins G2	**7.5**	32	0.39
Altenuene	**25**	44	0.06
Fumonisins B1	**25**	3419	11.87
Fumonisins B2	**35**	1606	3.78
Fumonisins B3	**40**	589	1.82
HT-2 toxin	**10**	11.9	0.06
Ochratoxin A	**3**	163	2.15
Deoxynivalenol	**40**	112	0.45
Zearalenone	**6.5**	382	2.74
Alternariol	**80**	1090	3.06
AlternariolMonomethylether	**10**	257	2.34
Diacetoxyscirpenol	**2.5**	109	11.29
Sterigmatocystin	**2.5**	1189	16.05

Table 1: Summary data on analytical results of mycotoxins identified in sorghum samples.

6. In order to perform further statistical analysis for the purpose of the FAO/WHO response to CCCF request, only mycotoxins of importance were selected.

7. Mycotoxins of importance are defined as those mycotoxins that were detected in at least one percent of all samples. These mycotoxins are: Aflatoxins; Fumonisins; Sterigmatocystin; Diacetoxyscirpenol; Zearalenone; Ochratoxin A, Alternariol and Alternariol monomethylether.

8. Statistical analysis was performed as follows:

   - The following parameters were analysed: Percentage of samples with contamination level above LOQ for a particular mycotoxin, Minimum level, Arithmetic mean, Standard deviation, Median, 95th percentile, 97.5th percentile, Maximum level.

   - These calculations were performed over all samples including those with results below LOQ; for the latter, the result <LOQ was replaced by the numerical value of the LOQ for the mycotoxin considered (upper bound approach). While it is noted that this approach overestimates the mean and median value, for the purpose of setting up maximum levels, it is necessary to determine 95th or 97.5th percentile of the sample distribution and this approach has no effect on the numerical values of these relevant percentile measures.

   - All aflatoxin B1, B2,G1,G2 were summed as “total aflatoxins” and fumonisins 1, 2 and 3 were summed as “total fumonisins” to facilitate data analysis. The LOQ for total fumonisins was found to be 100µg/kg and LOQ for total aflatoxins was found to be 20µg/kg.

9. It should be noted that:

- No significant difference was observed for mycotoxin levels within and between countries. Therefore, all data from the four countries were pooled together and processed for statistical analysis for each mycotoxin.
- Diacetoxyscirpenol is the only mycotoxin for which there was a statistical difference observed on the levels between rounds 1 and 3, in only one country. No causal effect from practices could be determined.

Table 2 below summarizes the main results for ease of consultation by CCCF:

Mycotoxins	**95th percentile**	**97.5th percentile**	**Max**	**LOQ**
Total Aflatoxins	27.6	46	1092	20
Total Fumonisins	181	383	5421	100
Sterigmatocystin	25	56.6	1189	2.5
Diacetoxyscirpenol[3]	4.7	7.7	109	2.5
Zearalenone	6.5	11.8	382	6.5
Ochratoxin A	3	3	163	3
Alternariol	80	93.6	1090	80
Alternariol monomethylether	10	10	257	10

Table 2: summary of key statistical results on the distribution parameters (all values in µg/kg)

10. Full details on statistical distribution parameters for each mycotoxin are provided in tables and statistical distribution curves in the annex of this paper.

Information from the value chain studies and good practices for production of sorghum

11. The project gathered information related to practices along the sorghum value chain in the four countries. These practices were presented at the 9th CCCF in CX/CF 15/9/3-Add.1. A further review of these practices was undertaken to compare them with provisions of draft revised code of practice for the prevention and reduction of mycotoxins contamination in cereals (CAC/RCP 51-2003) and its draft annexes.

12. The following findings are not currently reflected in the current draft revised Code of Practice (which is not sorghum specific):

- **Seeds**: preliminary findings suggest that high tannin and red genotypes may be associated to higher resistance to fungal infestation.
- **Immediate postharvest practices**: additional/improved winnowing may be suitable to lower the mycotoxins concentration in sorghum by further cleaning the grains.

13. It is noted that the lack of statistical differences in observed contamination levels between rounds (except in one country for diacetoxyscirpenol), could suggest that additional intervention to improve mid and long term post-harvest practices may have little influence on contamination and that a strong focus should be given to pre-harvest and immediate postharvest practices.

Conclusions

14. In response to the 9th CCCF request, the project's results and subsequent statistical analysis are a good basis to note that:

[3] For the purpose of this table only summary values are provided

- **Mycotoxins of importance** were defined as those that occurred at levels above the LOQ in at least one percent of all samples tested. These mycotoxins were: Total Aflatoxins; Total Fumonisins; Sterigmatocystin; Diacetoxyscirpenol; Zearalenone; Ochratoxin A, Alternariol and Alternariol monether.

- Diacetoxyscirpenol and Sterigmatocystin were the mycotoxins most frequently found at levels above the LOQ and Fumonisins, Sterigmatocystin and Alternariol were found at the numerically highest levels of concentration. It is noted that in the absence of a risk assessment, these measures of prevalence and concentrations cannot be correlated to any risk. It is further noted that aflatoxins, fumonisins, sterigmatocsytin and diacetoxyscirpenol will be reviewed during the upcoming 83rd JECFA meeting (November 2016).

  Regarding the specific question on **feasibility of setting up maximum levels**, a number of factors need to be considered, including information generated by the upcoming 83rd JECFA meeting. For those mycotoxins where there was evidence of occurrence above 1%, levels at the end of the distribution curve demonstrated a ratio (maximum level/95th percentile) ranging from 8 to 60 (see tables in annex). Further work should be undertaken to assess the effective exposure and risk for consumers, including in the 4 project countries, to understand dietary exposure estimates to these different mycotoxins. Indeed, any work on establishing maximum levels should take into account the risk ascertained through risk assessment as per Policy of the Committee on Contaminants in Foods for exposure assessment of contaminants and toxins in foods or food groups (Procedural Manual, CAC).

- From the cross review of the findings from value chain studies and the draft code of practice, CCCF may consider two sorghum-specific consideration related to seeds and immediate postharvest practices, as indicated in paragraph 12.

Annex

Table 3: statistical distribution parameters for Total Aflatoxins (sum of B1, B2, G1, G2, ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio max/95th
1533	116	7.6	20	23.5	33.6	20	27.6	46	1092	40

Fig 1: Distribution curve for total Aflatoxins (N=1533, 4 countries, ND=LOQ, in µg/kg)

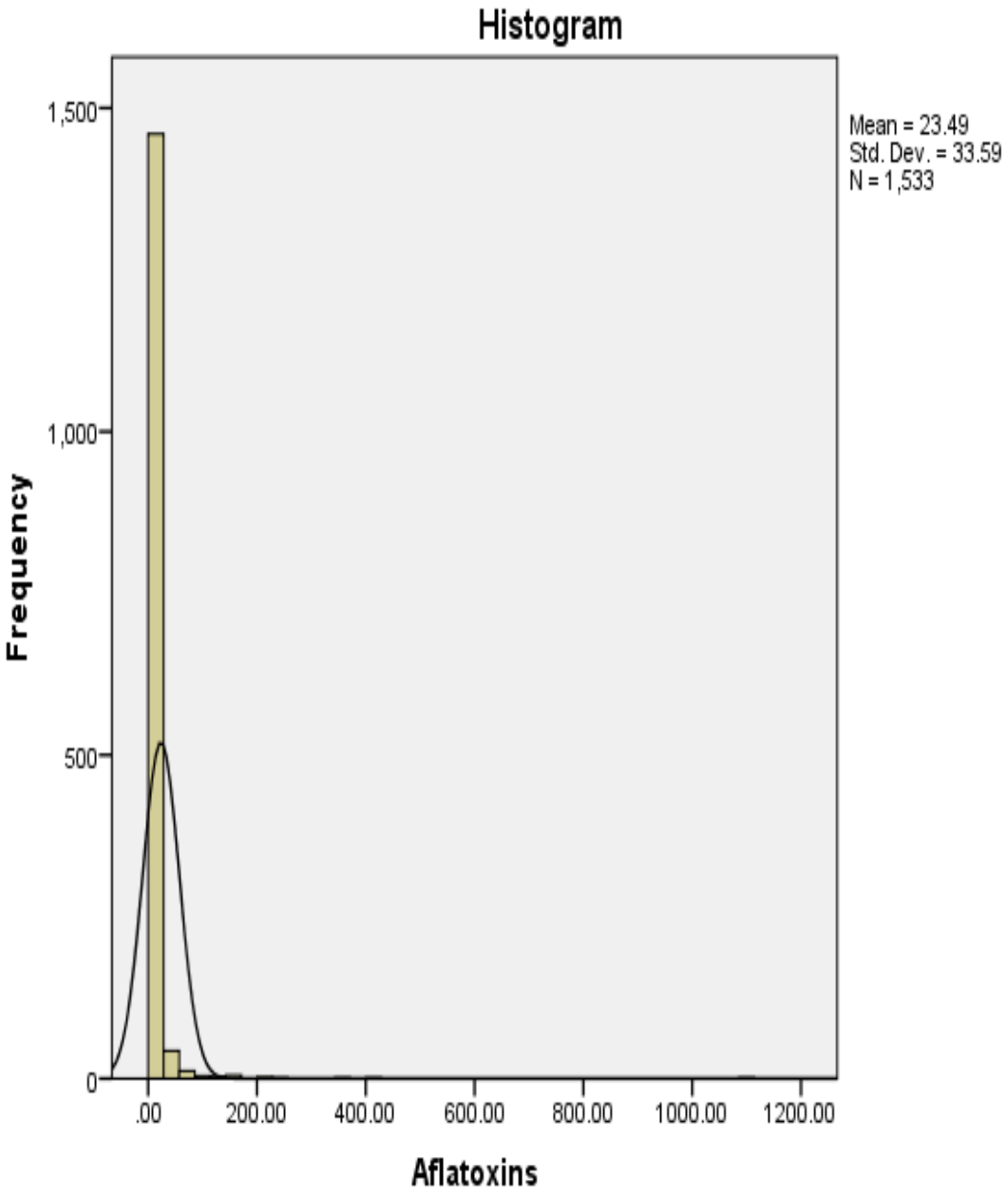


Table 4: statistical distribution parameters for Total Fumonisins (sum of BI, B2, B3, ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97-5th	Max	Ratio Max/95th
1533	179	11.7	95	137.5	300	100	181	383	5421	30

Fig 2: Distribution curve for total Fumonisins (N=1533, 4 countries, ND=LOQ, in µg/kg)

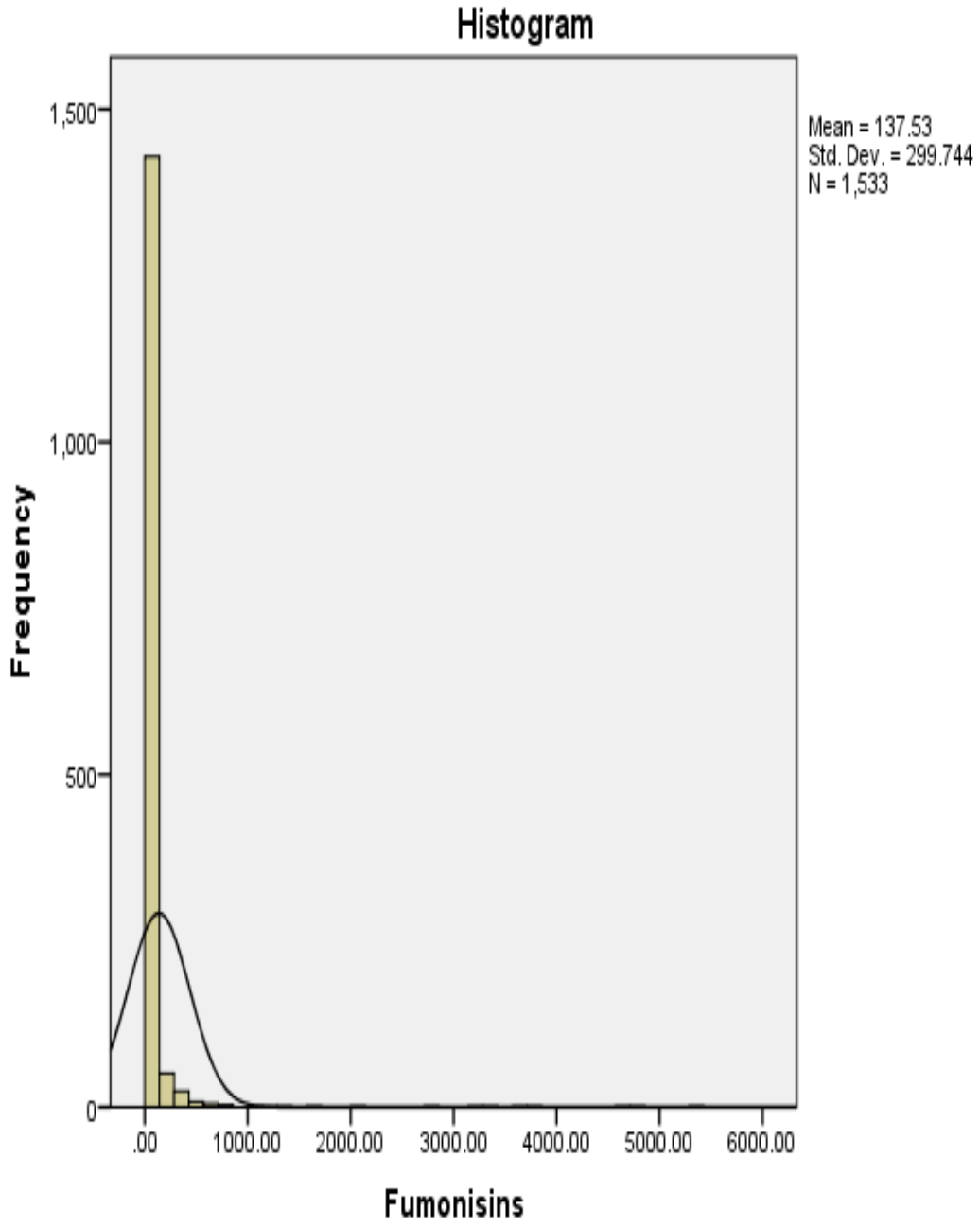


Table 5: statistical distribution parameters for Sterigmatocystin (ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio Max/95th
1533	246	16.1	2.5	11.1	64.9	2.5	25.0	56.6	1189	50

Fig 3: Distribution curve for Sterigmatocystin (STC) (N=1533, 4 countries, ND=LOQ, in µg/kg)

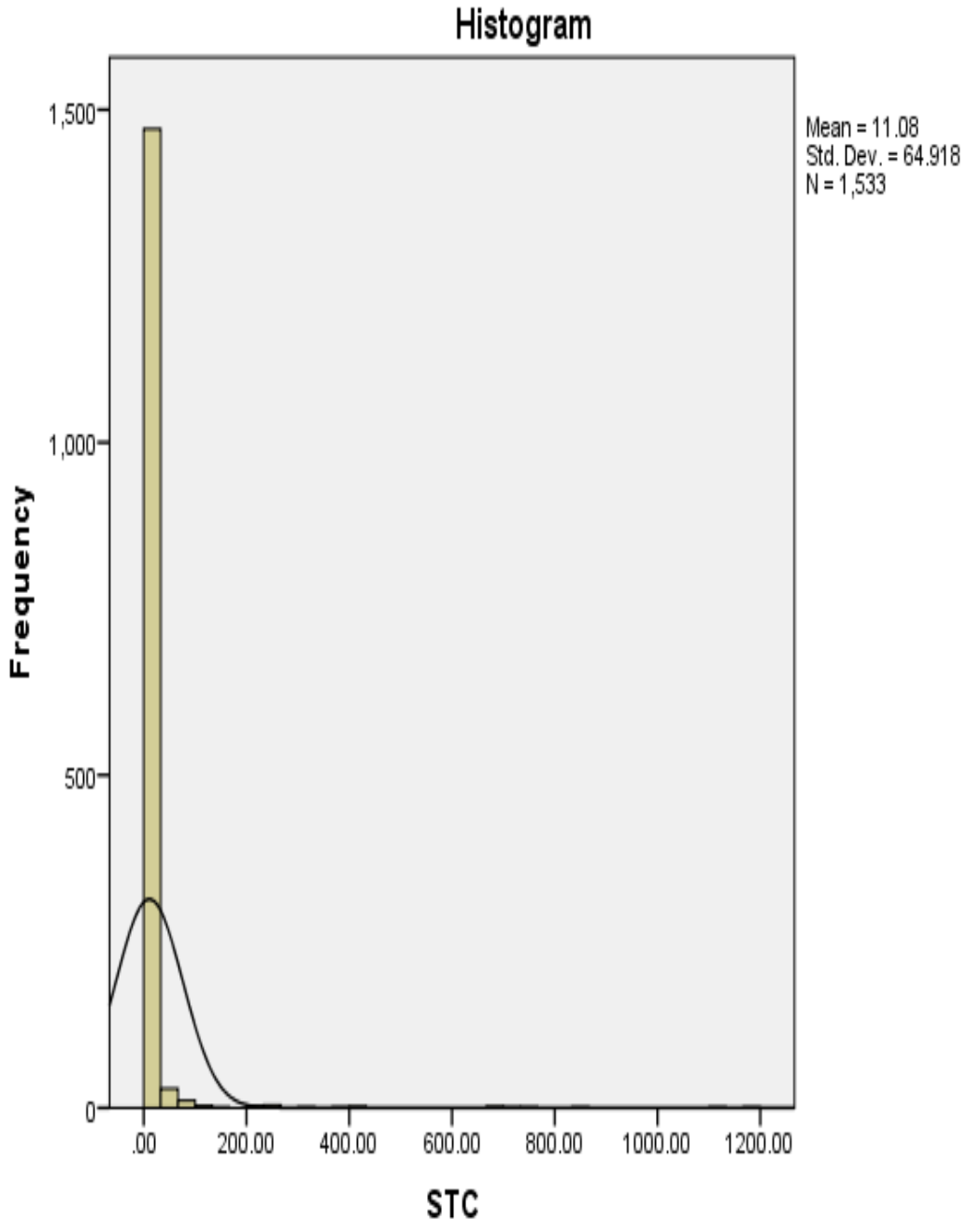
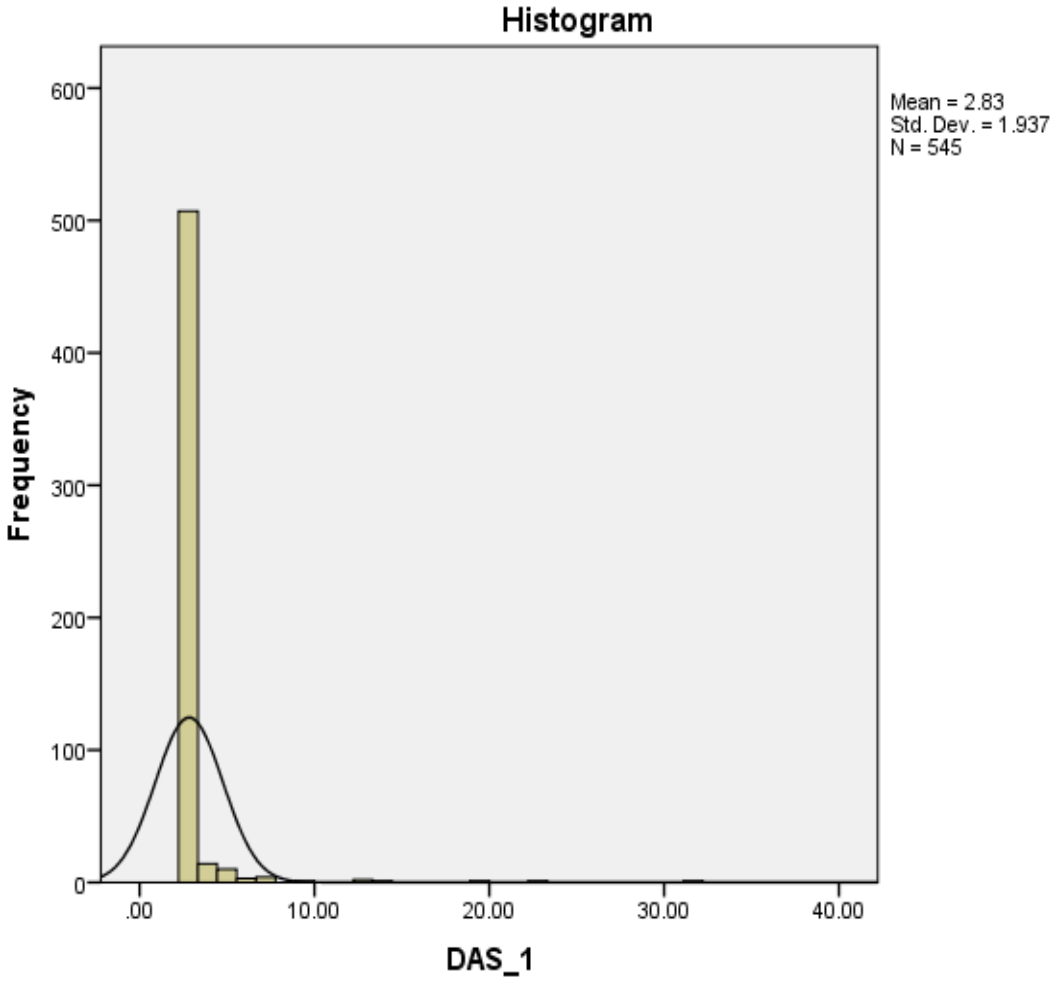


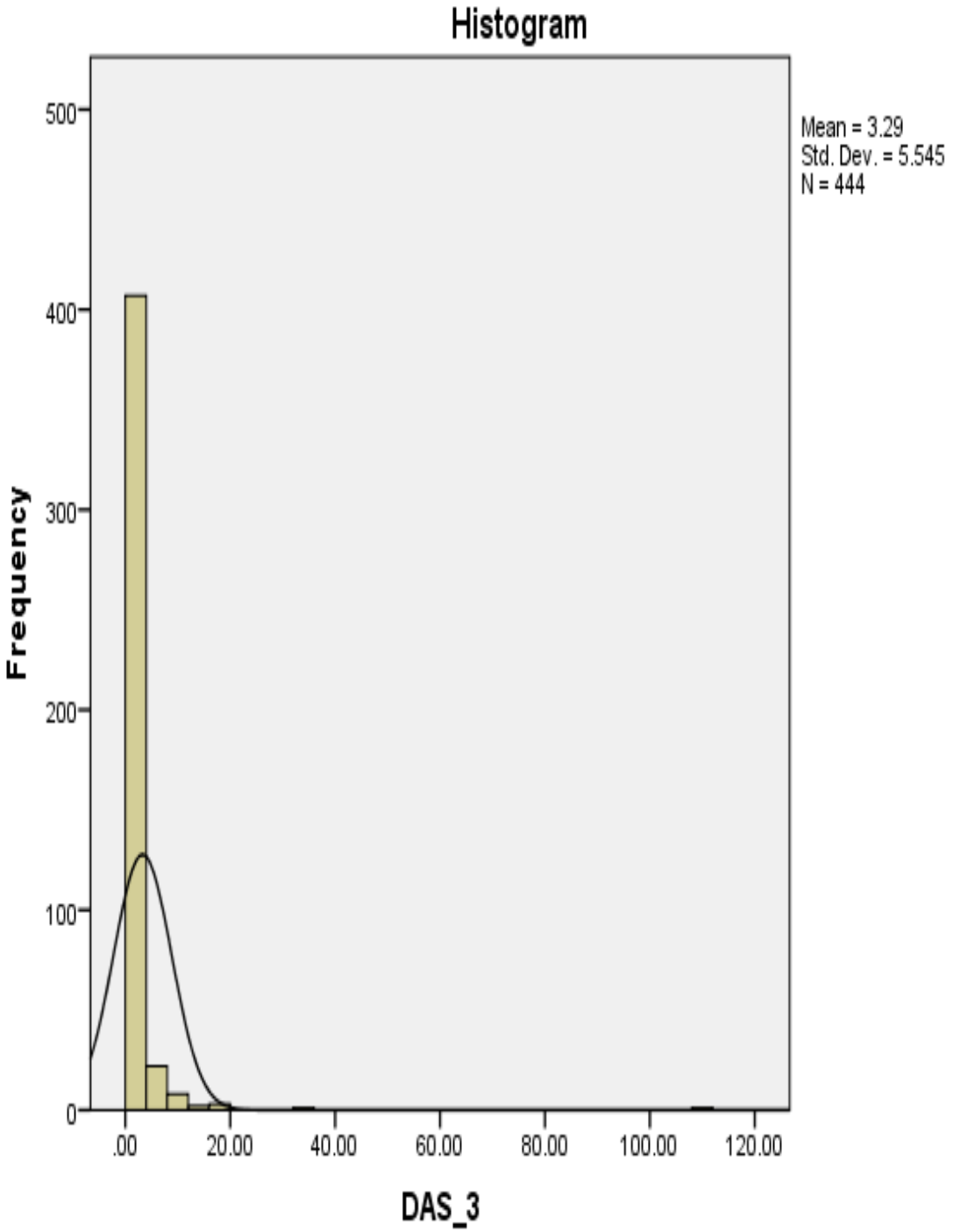
Table 6: statistical distribution parameters for Diacetoxyscirpenol (DAS) (ND=LOQ, µg/kg)

Rounds	N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio Max/95th
Round 1	545	44	8.1	2.5	2.83†	1.9	2.5	4.1	5.8	31.3	8
Round 2	544	65	12	2.5	2.89	2.1	2.5	4.4	7.3	33.9	8
Round 3	444	63	14.2	2.5	3.29†	5.5	2.5	7.8	8.8	109	14
Total	1533	172	11.2	2.5	2.98	3.4	2.5	4.7	7.7	109	23

†Significant difference in the mean level of DAS between round 1 & round 3 ($p<0.037$)

Fig 4: Distribution curves for Diacetoxyscirpenol (DAS) for rounds 1 and 3 (ND=LOQ, in µg/kg)

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Std. Dev. = 5.545
N = 444
Frequency
500
400
300
200
100
0
.00
20.00
40.00
60.00
80.00
100.00
120.00
DAS_3

Table 7: statistical distribution parameters for Zearalenone (ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio Max/95th
1533	41	2.67	6.5	9.0	22.4	6.5	6.5	11.8	382	60

Fig 5: Distribution curve for Zearalenone (ZEA) (N=1533, 4 countries, ND=LOQ, in µg/kg)

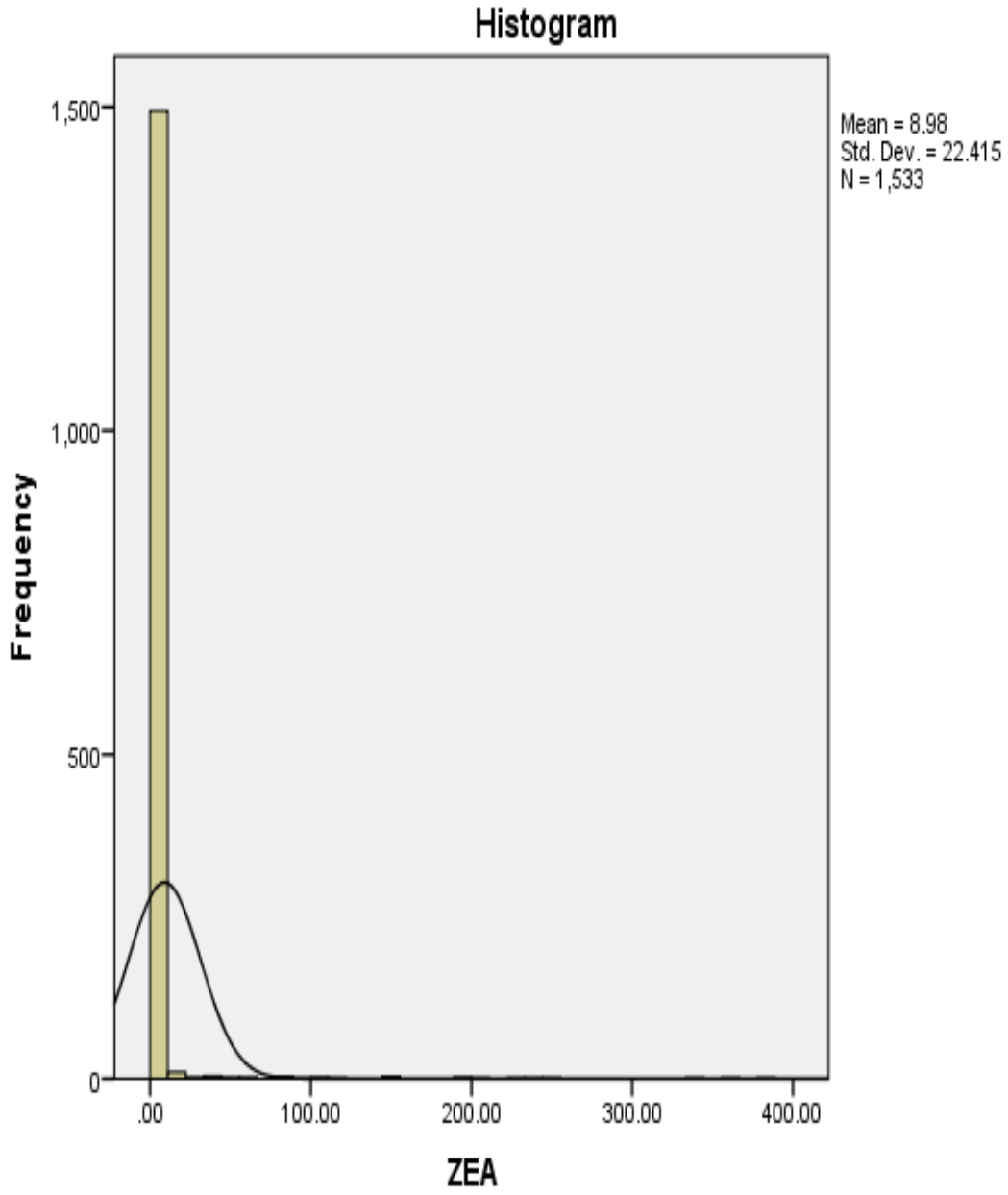

Table 8: statistical distribution parameters for Ochratoxin A (ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio Max/95th
1533	33	2.15	3.0	3.5	6.1	3.0	3.0	3.0	163	50

Fig 6: Distribution curve for Ochratoxine A (OTA) (N=1533, 4 countries, ND=LOQ, in µg/kg)

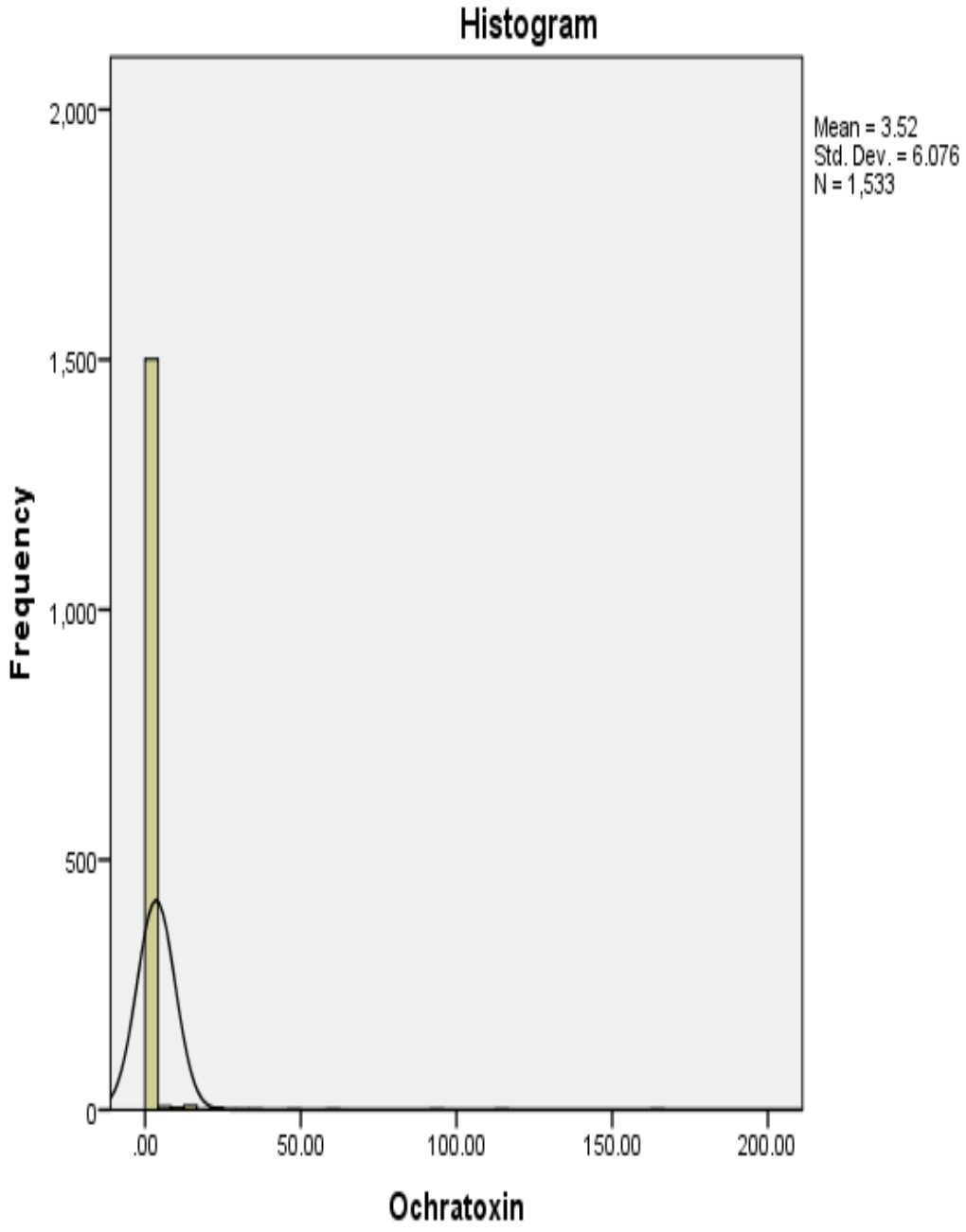

Table 9: statistical distribution parameters for Alternariol (ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio Max/95th
1533	47	3.1	80.0	84.2	41.3	80.0	80.0	93.6	1090	14

Fig 7: Distribution curve for Alternariol (N=1533, 4 countries, ND=LOQ, in µg/kg)

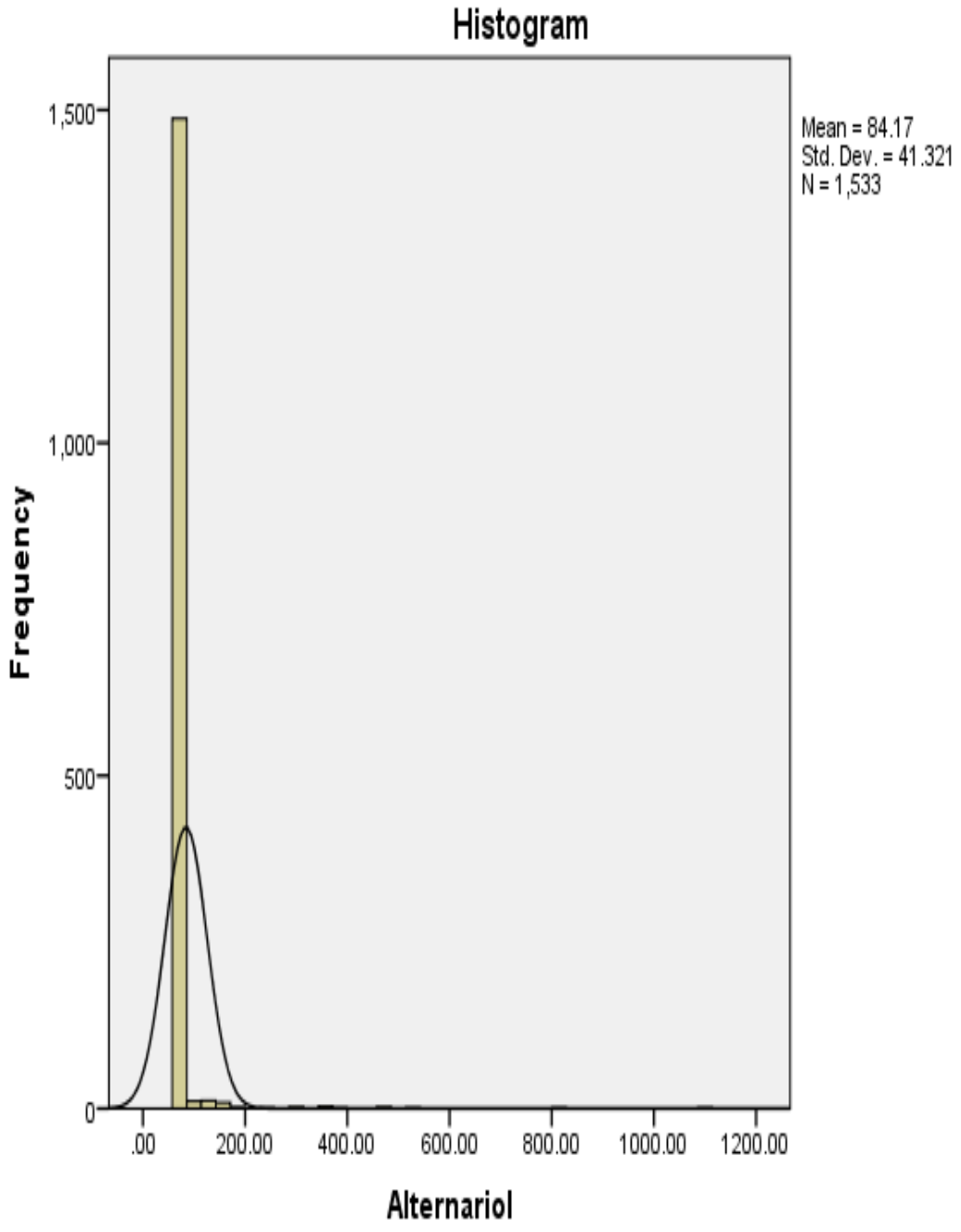

Table 10: statistical distribution parameters for Alternariol monoethylether (ND=LOQ, µg/kg)

N	N>LOQ	Perc (%)	Min	Mean	STD	Median	95th	97.5th	Max	Ratio Max/95th
1533	35	2.3	10.0	11.3	12.5	10.0	10.0	10.0	257	26

Fig 8: Distribution curve for Alternariol monoethylether (N=1533, 4 countries, ND=LOQ, in µg/kg)