codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 16 (e) **CX/FAC 05/37/25-Add. 1**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON FOOD ADDITIVES AND CONTAMINANTS

Thirty-seventh Session

The Hague, the Netherlands, 25 – 29 April 2005

DEOXYNIVALENOL (DON) CONTAMINATION IN CEREALS

(INFORMATION SUBMITTED IN RESPONSE TO CL 2004/9-FAC)

The following comments have been received from Cuba, European Community, Japan and USA

Cuba:

We appreciate and are looking forward to the information that the European Union and Japan will put forward at the next session; meanwhile Cuba thinks that a maximum level of 1 mg/kg would be appropriate in both wheat and cereals in general, except for cereal products for infants.

European Community:

Information on deoxynivalenol (DON) Contamination in cereals from the European Community is contained in document CX/FAC 05/37/25. It was further mentioned that the EC would be able to provide in advance of the meeting information on maximum levels, sampling procedures and methods of analysis for consideration at the 37th session of CCFAC

Please find enclosed information on maximum levels, sampling procedures and methods of analysis. These measures will be adopted by the European Commission very shortly and will become applicable in the European Community from 1 July 2006 onwards.

MAXIMUM LEVELS FOR DEOXYNIVALENOL

Product (1)	Maximum level (µg/kg)
Unprocessed cereals (2) other than durum wheat, oats and maize	1250
Unprocessed durum wheat and oats	1750
Unprocessed maize	- (3)
Cereal flour, including maize flour, maize grits ands maize meal (4)	750
Bread, pastries, biscuits, cereal snacks and breakfast cereals	500
Pasta (dry)	750
Processed cereal-based food for infants and young children and baby food (5)	200

(1) For the purpose of the application of maximum levels of deoxynivalenol, rice is not included in cereals" and rice products not included in "cereal products"

(2) The maximum levels set for "unprocessed cereals" applies to cereals placed on the market for first-stage processing.

"First-stage processing" shall mean any physical or thermal treatment, other than drying, of or on the grain. Cleaning, sorting and drying procedures are not considered to be "first stage processing" insofar no physical action is exerted on the grain kernel itself and the whole grain remains intact after cleaning and sorting.

(3) If no specific level is fixed before 1 July 2007, the level of 1750 µg/kg will apply thereafter to maize referred to in this point.

(4) This category includes also similar products otherwise denominated such as semolina.

(5) The maximum level for processed cereal-based foods for infants and young children and baby food refers to the dry matter.

SAMPLING PROCEDURE FOR THE OFFICIAL CONTROL OF DEOXYNIVALENOL

1. Different types of lots

Food commodities may be traded in bulk, containers, or individual packings, such as sacks, bags, retail packings. The sampling procedure may be applied to all the different forms in which the commodities are put on the market.

The following formula may be used as a guide for the sampling of lots traded in individual packs, such as sacks, bags, retail packings.

$$\text{Sampling Frequency (SF) } n = \frac{\text{Weight of the lot} \times \text{Weight of the incremental sample}}{\text{Weight of the aggregate sample} \times \text{Weight of individual packing}}$$

- weight: in kg
- sampling frequency (SF): every nth sack or bag from which an incremental sample must be taken (decimal figures should be rounded to the nearest whole number).

2. Weight of the incremental sample

The weight of the incremental sample must be about 100 grams. In the case of lots in retail packings, the weight of the incremental sample shall depend on the weight of the retail packing.

3. Sampling procedure for cereals and cereal products

3.1. Subdivision of lots into sublots depending on product and lot weight

Commodity	Lot weight (ton)	Weight or number of sublots	No incremental samples	Aggregate sample Weight (kg)
Cereals and cereal products	≥1 500 >300 and < 1 500 ≥ 50 and ≤ 300 < 50	500 tonnes 3 sublots 100 tonnes --	100 100 100 3-100*	10 10 10 1-10

* Depending on the lot weight

3.2. Sampling procedure for cereals and cereal products for lots ≥ 50 tonnes

- On condition that the sublot can be separated physically, each lot must be subdivided into sublots following the table under 3.1. Taking into account that the weight of the lot is not always an exact multiple of the weight of the sublots, the weight of the sublot may exceed the mentioned weight by a maximum of 20 %.
- Each sublot must be sampled separately.
- Number of incremental samples: 100. Weight of the aggregate sample = 10 kg
- If it is not possible to carry out the method of sampling set out in this point because of the commercial consequences resulting from damage to the lot such as packaging forms, means of transport, an alternative method of sampling may be applied provided that it is as representative as possible and is fully described and documented.

3.3. Sampling provisions for cereals and cereal products for lots < 50 tonnes

For lots of cereals and cereal products less than 50 tonnes, the sampling plan must be used with 10 to 100 incremental samples, depending on the lot weight, resulting in an aggregate sample of 1 to 10 kg. For very small lots (≤ 0.5 tonnes) a lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall be also in that case at least 1 kg.
The figures in the table below may be used to determine the number of incremental samples to be taken.
Table: Number of incremental samples to be taken depending on the weight of the lot of cereals and cereal products

Lot weight (tonnes)	No of incremental samples
≤ 0.05	3
> 0.05 - ≤ 0.5	5
> 0.5 - ≤ 1	10
> 1 - ≤ 3	20
> 3 - ≤ 10	40
> 10 - ≤ 20	60
> 20 - ≤ 50	100

3.4 Sampling procedure for foods intended for infants and young children

- The sampling procedure for cereals and cereal products as set out in point 3.3 shall apply to food intended for infants and young children. Accordingly the number of incremental samples to be taken shall depend on the weight of the lot, with a minimum of 10 and a maximum of 100, in accordance with the table under point 3.3. For very small lots (≤ 0.5 tonnes) a lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall be also in that case at least 1 kg.
- weight of the incremental sample must be about 100 grams. In the case of lots in retail packing, the weight of the incremental sample shall depend on the weight of the retail packing and in case of very small lots (≤ 0.5 tonnes) the incremental samples must have a weight as such that uniting the incremental samples results in an aggregate sample of at least 1 kg..
- weight of aggregate sampling = 1-10 kg sufficiently mixed.

3.5 Sampling at retail stage

Sampling of foodstuffs at the retail stage must be done where possible in accordance with the sampling provisions set out in points 3.2. and 3.3. Where that is not possible, other effective sampling procedures at retail stage may be used provided that they ensure sufficient representativeness for the sampled lot.

4. Acceptance of a lot or sublot

- acceptance if the aggregate sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- rejection if the aggregate sample exceeds the maximum limit beyond reasonable doubt taking into account the measurement uncertainty and correction for recovery.

SAMPLE PREPARATION AND CRITERIA FOR METHODS OF ANALYSIS TO BE USED FOR THE OFFICIAL CONTROL OF DEOXYNIVALENOL

1. Precautions

As the distribution of Fusarium toxins is non-homogeneous, samples shall be prepared, and in particular homogenised samples, with extreme care.
All the material received by the laboratory must be used for the preparation of test material.

2. Treatment of the sample as received in the laboratory

Each laboratory sample must be finely grinded and mixed thoroughly using a process that has been demonstrated to achieve complete homogenisation.
In case the maximum level applies to the dry matter, the dry matter content of the product shall be determined on a part of the homogenised sample, using a procedure that has been demonstrated to determine accurately the dry matter content.

3. Subdivision of samples for enforcement and defence purposes

The replicate samples for enforcement, trade (defence) and referee purposes shall be taken from the homogenised material unless such procedure conflicts with Member States' rules on sampling

4. Method of analysis to be used by the laboratory and laboratory control requirements

4.1. Definitions

A number of the most commonly used definitions that the laboratory shall be required to use are the following:

The most commonly quoted precision parameters are repeatability and reproducibility.

r = Repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions, namely same sample, same operator, same apparatus, same laboratory, and short interval of time may be expected to lie within a specific probability (typically 95%) and hence r = 2.8 x s_r.

s_r = Standard deviation, calculated from results generated under repeatability conditions.

RSD_r = Relative standard deviation, calculated from results generated under repeatability conditions [(s_r / $\bar{x}$) x 100].

R = Reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions, namely on identical material obtained by operators in different laboratories, using the standardised test method may be expected to lie within a certain probability (typically 95%); R = 2.8 x s_R.

s_R = Standard deviation, calculated from results under reproducibility conditions.

RSD_R = Relative standard deviation calculated from results generated under reproducibility conditions [(s_R / $\bar{x}$) x 100].

4.3. Performance Criteria

Level µg/kg	Deoxynivalenol		
	RSD_r %	RSD_R %	Recovery %
> 100 - ≤ 500	≤ 20	≤ 40	60 to 110
> 500	≤ 20	≤ 40	70 to 120

The detection limits of the methods used are not stated as the precision values are given at the concentrations of interest

The precision values are calculated from the Horwitz equation:

$$RSD_R = 2^{(1-0.5\log C)}$$

where:

RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions [(s_R / $\bar{x}$) x 100]

C is the concentration ratio (i.e. 1 = 100g/100g, 0.001 = 1,000 mg/kg

That is a generalised precision equation, which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

4.4. Recovery calculation and reporting of results

The analytical result must be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical result corrected for recovery shall be used for checking compliance

The analytical result must be reported as x +/- U whereby x is the analytical result and U is the expanded measurement uncertainty.

U is the expanded uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95%."

Japan:

The 36th Codex Committee on Food Additives and Contaminants (CCFAC) agreed to discontinue the consideration of the maximum levels of deoxynivalenol (DON) and to request information on the occurrence of DON in cereals *etc*. On the basis of this agreement, we submit the results of surveillances (surveillance 1 and 2) on DON in wheat grains and flours to facilitate discussion at the 37th CCFAC.

The Government of Japan has introduced the provisional maximum level of DON for husked wheat at 1.1 mg/kg in May 2002. The reduction of DON level in wheat flour in 2003 (surveillance 2) may be attributed to the introduction of this provisional standard.

Surveillance 1

The surveillance 1 was carried out in Japan from 2003 to 2004. DON was extracted from comminuted husked wheat grains, which were domestically produced, by shaking them in a mixture of acetonitrile and water (85:15) and analyzed with HPLC/MS. The analytical results are summarized in Table 1.

Surveillance 2

The surveillance 2 was conducted for husked wheat (in 2001-2002) and for wheat flour (in 2002-2003). The samples were collected within the country and may include both domestic and imported products. DON was extracted from the samples using acetonitrile:water (85:15) solution and analyzed by GC/MS and LC/MS after one-step solid phase extraction clean up procedure. The results are shown in Table 2 and Figure 1 - 4.

Table 1. Aggregated Data on DON Levels in Domestically Produced Wheat Grains (Surveillance 1)

Field number	Short field name	Descriptive field name	Data
1	SN	Serial no. of the record	
2	CD	Creation date of record	29-Mar-05
3	CC	Country code	JPN
4	FD	Food identifier	GC654
5	OR	Food origin	JPN
6	SP	Sampling period	06/2003-02/2004
7	REP	Sample representativeness	NW
8	NOL	Number of contributing laboratories	1
9	AQA	Analytical quality assurance	IQ

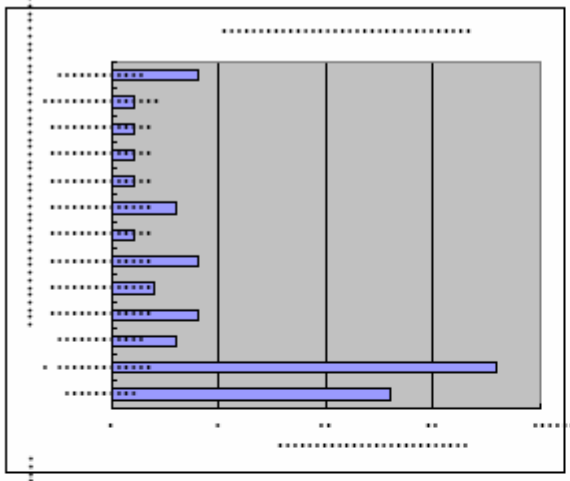
Field number	Short field name	Descriptive field name	Data
10	CON	Contaminant	170
11	DIM	Dimension of results	1
12a	LODMIN	LOD minimum	
12b	LODMAX	LOD maximum	
12c	LOQMIN	LOQ minimum	0.05
12d	LOQMAX	LOQ maximum	0.05
13	BASE	Results based on	A
14	N	Number of samples	213
15	<	Number of samples less than the LOQ	136
16a	MIN	Range - minimum	0.05
16b	MAX	Range - maximum	0.58
17a	X	Mean or best estimate	0.083
17b	XL	Mean - lower bound	0.067
17c	XU	Mean - upper bound	0.099
18	MED	Median or best estimate	
19	90th	90th Percentile	0.26
20	STDDEV	Standard deviation (Optional)	
21	STATUS	Status of data	0
22	REM	Remarks/ References	(1)Food id.:husk removed (2)As LOQ was not reported, X(17a), XL(17b) and XU(17c) were calculated with non-quantified=1/2LOQ(=0.025), 0, LOQ(=0.05) respectively.

Table 2. Aggregate Data on DON Levels in Wheat Grains and Flours (Surveillance 2)

Field number	Short field name	Descriptive field name				
1	SN	Serial no. of the record	TI01001	TI02001	TI02002	TI03001
2	CD	Creation date of record	15-Feb-02	15-Mar-03	15-Mar-03	15-Mar-04
3	CC	Country code	JPN	JPN	JPN	JPN
4	FD	Food identifier	CF1212	CF1212	CF1211	CF1211
5	OR	Food origin	JPN	JPN	JPN	JPN
6	SP	Sampling period	08/2000-02/2001	09/2001-03/2002	09/2001-03/2002	10/2002-03/2003
7	REP	Sample represent ativeness	SW	SW	SW	SW
8	NOL	Number of contributing laboratories	1	1	1	1
9	AQA	Analytical quality assurance	IQ	IQ	IQ	IQ
10	CON	Contamin ant	170	170	170	170
11	DIM	Dimensio n of results	2	2	2	2
12a	LODMIN	LOD minimum	1	5	5	5
12b	LODMAX	LOD maximum	1	5	5	5

12c	LOQMIN	LOQ minimum	1	10	10	10
12d	LOQMAX	LOQ maximum	1	10	10	10
13	BASE	Results based on	A	A	A	A
14	N	Number of samples	56	80	84	80
15	<	Number of samples less than the LOQ	13	4	17	21
16a	MIN	Range minimum	1	10	10	10
16b	MAX	Range - maximum	2248	2452	1147	1620
17a	XL	Mean or best estimate	286	184	138	43
17b	XU	Mean lower bound	285	184	137	42
17c	X	Mean upper bound	286	184	139	45
18	MED	Median or best estimate	6	78	55	17
19	90th	90th Percentile	801	347	431	52
20	STDDEV	Standard deviation (Optional)	535	351	200	180
21	STATUS	Status of data	0	0	0	0
22	REM	Remarks/ References				

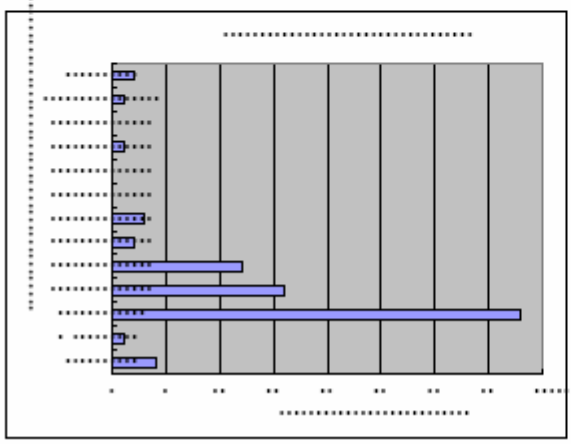
Figure 1. DON Levels in Husked Wheat Grains in 2001



total number of samples	Minimum concentration (ug/kg)	Maximum concentration (ug/kg)	Median (ug/kg)	Average (ug/kg)
56	0.5	2248	6	288

Concentration (mg/kg)	No. of samples	Ratio (%)	cumulative No.	Ratio(%)
<1.0	13	23.21	13	23.21
1.1-10	18	32.14	31	55.36
11-100	3	5.36	34	60.71
101-200	4	7.14	38	67.86
201-300	2	3.57	40	71.43
301-400	4	7.14	44	78.57
401-500	1	1.79	45	80.36
501-600	3	5.36	48	85.71
601-700	1	1.79	49	87.50
701-800	1	1.79	50	89.29
801-900	1	1.79	51	91.07
901-1100	1	1.79	52	92.86
>1.100	4	7.14	56	100.00
.....	56	100.00		

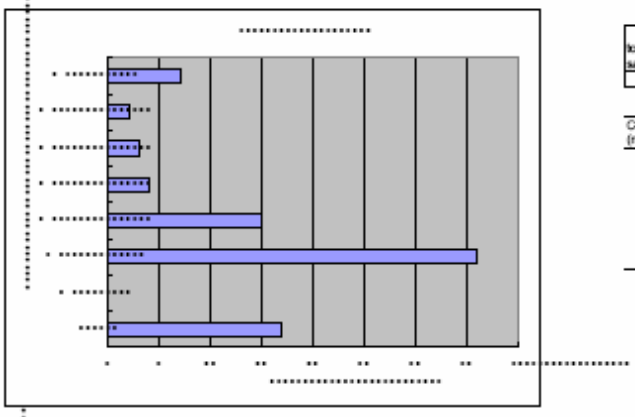
Figure 2. DON Levels in Husked Wheat Grains in 2002



total number of samples	Minimum concentration (ug/kg)	Maximum concentration (ug/kg)	Median (ug/kg)	Average (ug/kg)
80	5	2248	78	184

Concentration (mg/kg)	No. of samples	Ratio (%)	cumulative No.	Ratio(%)
<5.0	4	5.00	4	5.00
6-10	1	1.25	5	6.25
11-100	38	47.50	43	53.75
101-200	16	20.00	59	73.75
201-300	12	15.00	71	88.75
301-400	2	2.50	73	91.25
401-500	3	3.75	76	95.00
501-600	0	0.00	76	95.00
601-700	0	0.00	76	95.00
701-800	1	1.25	77	96.25
801-900	0	0.00	77	96.25
901-1100	1	1.25	78	97.50
>1100	2	2.50	80	100.00
.....	80	100.00		

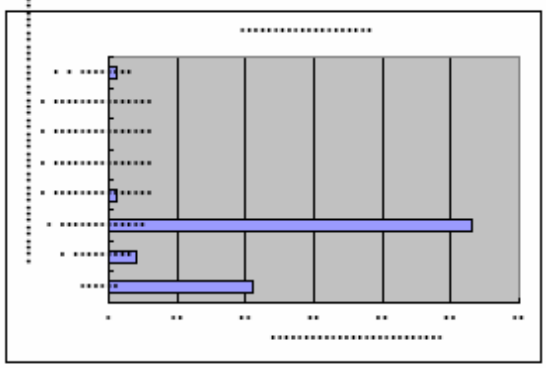
Figure 3. DON Levels in Wheat Flour in 2002



total number of samples	Minimum concentration (ug/kg)	Maximum concentration (ug/kg)	Median (ug/kg)	Average (ug/kg)
84	5	1147	55	138

Concentration (mg/kg)	No. of samples	Ratio (%)	cumulative No.	Ratio(%)
<5	17	20.24	17	20.24
5-10	0	0.00	17	20.24
11-100	36	42.86	53	63.10
101-200	15	17.86	68	80.95
201-300	4	4.76	72	85.71
301-400	3	3.57	75	89.29
401-549	2	2.38	77	91.67
>=550	7	8.33	84	100.00
**********	84	100.00		

Figure 4. DON Levels in Wheat Flour in 2003



total number of samples	Minimum concentration (ug/kg)	Maximum concentration (ug/kg)	Median (ug/kg)	Average (ug/kg)
84	5	1820	17	43

Concentration (mg/kg)	No. of samples	Ratio (%)	cumulative No.	Ratio(%)
<5	21	26.25	21	26.25
5-10	4	5.00	25	31.25
11-100	53	66.25	78	97.50
101-200	1	1.25	79	98.75
201-300	0	0.00	79	98.75
301-400	0	0.00	79	98.75
401-549	0	0.00	79	98.75
>=550	1	1.25	80	100.00
**********	80	100.00		

USA:

This responds to CL 2004/9-FAC which requests information on: the occurrence of deoxynivalenol in cereals; the influence of processing, decontamination, sorting, etc. to lower the level of DON in a lot; national levels or guideline levels for DON; sampling procedures and methods of analysis; etc. for consideration by the next Session of the Committee. The United States of America appreciates the opportunity to provide the following comments for consideration at the forthcoming 37th Session of the Codex Committee on Food Additives and Contaminants (CCFAC).

At the 36th Session of the CCFAC (Rotterdam, The Netherlands, 22-26 March 2004) the Committee agreed to request information on the occurrence of deoxynivalenol in cereals for consideration by the next Session of the Committee (ALINORM 04/27/12, § 158).

The United States (U.S.) is pleased to submit at this time data on the occurrence of deoxynivalenol in raw barley (Table 1) and raw wheat (Table 2) crops in the United States. In the near future, we hope to provide the CCFAC with comprehensive data on the effects of various processing procedures on the level of DON contamination in cereals.

TABLE 1. DEOXYNIVALENOL LEVELS IN UNITED STATES BARLEY CROPS 1993-2003[1,2]							
		DEOXYNIVALENOL RANGE (mg/kg)					
Year	No. of Samples Examined	<0.49	0.49-0.99	0.99-2.99	2.99-4.99	4.99>5.0	Average
1993	173	31(17.9)	9(5.2)	34(19.6)	32(18.5)	67(38.7)	4.5
1994	170	37(21.8)	14(8.2)	29(17.1)	14(8.2)	76(44.7)	9.1
1995	147	23(15.7)	14(9.5)	29(19.7)	10(6.8)	71(48.3)	5.9
1996	194	55(28.4)	33(17.0)	46(23.7)	25(12.9)	35(18.0)	3.2
1997	175	57(32.6)	16(9.1)	28(16.0)	23(13.1)	51(29.1)	4.9
1998	158	45(28.5)	21(13.3)	45(28.5)	24(15.2)	23(14.5)	2.7
1999	162	69(42.6)	43(26.5)	38(23.5)	5(3.1)	7(4.3)	1.1
2000	143	35(24.5)	34(23.8)	38(26.6)	23(16.1)	13(9.1)	2.2
2001	275	108(39.3)	33(12.0)	51(18.6)	41(14.9)	42(15.3)	2.6
2002	243	168((69.1)	36(14.8)	27(11.1)	6(2.5)	6(2.5)	0.7
2003	266	179(67.3)	52(19.6)	32(12.0)	2(0.7)	1(0.4)	0.5

TABLE 2. DEOXYNIVALENOL LEVELS IN UNITED STATES WHEAT CROPS 1994-2003[3]								
		DEOXYNIVALENOL RANGE (ng/g)						
Year	No. of samples Examined.	<500	500-800	800-1000	1000- 3000	3000-6000	>6000	Maximum
1994	10	7	2	1	0			940
1995	3	0	0	0	3	0	0	1,430
1996	68	10	11	9	13	11	14	13,000
1997	23	1	2	18	2	0	0	1,230
1998	15	2	0	10	1	2	0	4,220
1999	1	0	0	0	1	0	0	1,100
2000	11	5	0	6	0	0	0	900
2001	937	297	124	2	461	52	1	6,700
2002	635	298	97	33	191	16	0	5,900
2003	821	409	114	45	252	1	0	5,200

1 These are commercial crop samples; sampling plan and sampling was done by government officials and analyzed in a State University laboratory. The samples were analyzed by GC-ECD; the limit of quantitation was 0.49 mg/kg.

2 **Number in parenthesis refers to percent.**

3 Samples collected and analyzed by HPLC in industrial food laboratories. Limit of quantitation 100 ng/g.