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OCHRATOXIN A IN GRAIN DUST – ESTIMATED EXPOSURE AND RELATIONS TO AGRICULTURAL PRACTICES IN GRAIN PRODUCTION

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Abstract: Ochratoxin A (OTA) is a nephrotoxin frequently contaminating grains. OTA inhalation during grain handling may therefore represent a health risk to farmers, and was the subject of this study. Airborne and settled grain dust was collected during grain work on 84 Norwegian farms. Climate and agricultural practices on each farm were registered. *Penicillium* spp., *Aspergillus* spp. and OTA in settled dust were measured. Settled dust contained median 4 µg OTA/kg dust (range 2-128), correlating with *Penicillium* spp. (median 40 cfu/mg; range 0-32000, r_s=0.33; p<0.01). Similar levels were found across grain species, districts and agricultural practices. *Penicillium* levels, but not OTA levels, were higher in storage than in threshing dust (p=0.003), and increased with storage time (r_s=0.51, p<0.001). Farmers were exposed to median 1 mg/m^3 (range 0.2-15) dust during threshing and median 7 mg/m^3 (range 1-110) dust during storage work, equalling median 3.7 pg/m^3 (range 0.6-200) and median 40 pg/m^3 (range 2-14000) OTA, respectively (p<0.001). Agricultural practices could not predict OTA, *Penicillium* or *Aspergillus* contamination. Compared to oral intake of OTA, the inhalant exposure during grain work was low, although varying by more than 1,000-fold. However, the farmers may occasionally be highly exposed, particularly during handling of stored grain.

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INTRODUCTION

The mycotoxin ochratoxin A (OTA) frequently contaminates cereal grains [17, 49, 55], beans, coffee, nuts, olives, cheese, fish, pork, milk powder, wine, beer and bread [51, 52, 54]. In Norwegian and imported grain used for human consumption, median 0.3 µg OTA/kg (range 0-20 µg/kg) was found [50]. In Northern Europe, OTA is mainly produced by the moulds *Penicillium verrucosum* and *Aspergillus ochraceus* during storage of cereal grain [12, 18, 27, 29, 30, 35].

Mould growth and mycotoxin formation is mainly affected by moisture, temperature and time in laboratory cultures [3]. However, mycotoxin contamination of cereal grains in storage may be affected by a large number of environmental and cultivation variables, both pre- and post harvest. Both inoculum carried over from soil particles, plant debris, and residues from harvesting

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equipment, as well as crop rotation, ploughing, use of fungicides and growth regulators [44, 56], lodging of the grain in the field [31], rapidity of drying during storage, rewetting, ambient humidity and mechanical injury all affect the mould growth and mycotoxin formation [3].

OTA contamination has mainly been associated with post harvest conditions [1, 2, 37]. An experimental study of ambient air flow through stored wet grain, demonstrated good conditions for fungal growth and OTA production if airflow was too low, resulting in as high as 43 µg OTA/kg grain in bins with stagnant ambient air [32].

Gastrointestinal exposure to high OTA levels in humans has been associated with the potentially fatal kidney disease Balkan endemic nephropathy. Chronic exposure may induce kidney cancers [39, 40]. In animal models OTA is carcinogenic [25], teratogenic [23], embryotoxic and immunosuppressive [21, 22, 27]. OTA is classified as a potential human carcinogen group 2b by IARC [25, 52].

Information on the effects of OTA inhalation is limited, but it has been shown to lead to renal failure in both human beings and animals [13, 27]. Inhalation of dust during handling and processing of contaminated products can thus represent a health risk for occupationally exposed workers [6, 20, 26], including grain farmers [28].

The aim of this study was to examine farmers' exposure to OTA contaminated dust during grain handling, and to investigate adapted agricultural practices and climate as determinants for mould growth and OTA formation. The level of OTA, *Penicillium* spp. and *Aspergillus* spp. in settled dust from barley, oats and spring wheat on 84 farms is presented. Dust exposure and estimated OTA exposure during threshing and storage work is also shown.

MATERIALS AND METHODS

Grain dust sampling. Samples of settled (n=99) and airborne (n=96) grain dust were collected from 84 farms in 11 Norwegian municipalities in 3 climatically different districts during threshing and storage handling of barley, oats and spring wheat produced in 1999 and 2000. The storage samples (n=68) were obtained from grain with 17-40% water content (wc) at harvest (n=38); 11-22% wc at sampling time (n=63), and had been stored 2-27 weeks. Samples of 0.1-3 g settled dust were obtained using dustbusters (Black & Decker HC431, Dustbuster Turbo, Berkshire, UK) equipped with dust collector nozzles (ALK Abelló, Horsholm, Denmark) containing paper filter cassettes (ALK Abelló) covered with a 440 µm mesh (National Institute of Occupational Health, Oslo, Norway) to avoid the collection of large particles. Samples were transferred to sterile polystyrene tubes (Nalge Nunc International Corp., Naperville, USA) and stored at –20ºC until analysis. Inhalable grain dust was collected on polycarbonate filters (pore size 0.8 µm, Poretics, Osmonics, Livermore, USA) by personal sampling using PAS-6 cassettes [53] with a flow rate of 2 l/min in 6-60 (median 25) minutes.

Table 1. Grain production details predefined as possible mycotoxin determinants.

Variables	N	%
Growth season		
1999	19	19
2000	80	81
District		
District 1 (River Glomma)	34	34
District 2 (Lake Mjoesa)	20	20
District 3 (Trondhjem Fjord)	45	46
Cereal species		
Barley	58	59
Oats	28	28
Spring wheat	13	13
Ploughing		
In autumn only	50	51
In spring only	27	27
In spring and autumn on same grain batch	14	14
No ploughing	7	7
Production last year		
Same species as preceding season	47	48
Cereals, but other species preceding season	33	33
Potato, oil seed, cabbage or no crop	10	10
Field fungicide or growth regulator		
Fungicides, all types	46	47
Growth regulators	17	17
Farmers observation		
Lodged grain on >10% of crop	57	58
Visible mould damage	35	35
Problems with drying[a]	20	20
Work operation		
Threshing	31	31
Storage (grain mixing and ventilation)	36	36
Bin emptying	32	32
Grain storage technology[a]		
Cold air grain dryer	40	59
Heated air grain dryer	28	41

[a] Storage samples only (N=68)

Information on grain production and climatic conditions. The farmers gave information on the grain species, cultivation and production details predefined as possible grain dust mycotoxin determinants (Tab. 1). Meteorological data (temperature, humidity and rainfall) from April–September of each growth season were obtained from 8 regional meteorological stations of The Norwegian Crop Research Institute. Regional meteorological measurements were allocated to the respective farms in each municipality. Climatic charcteristics for each district are given in Figure 1. The location of the farms according to altitude and distance to the nearest river, lake or fjord in each district is shown in Table 2.

OTA extraction. OTA was extracted from the settled dust samples by adding 10 ml 70% methanol per gram

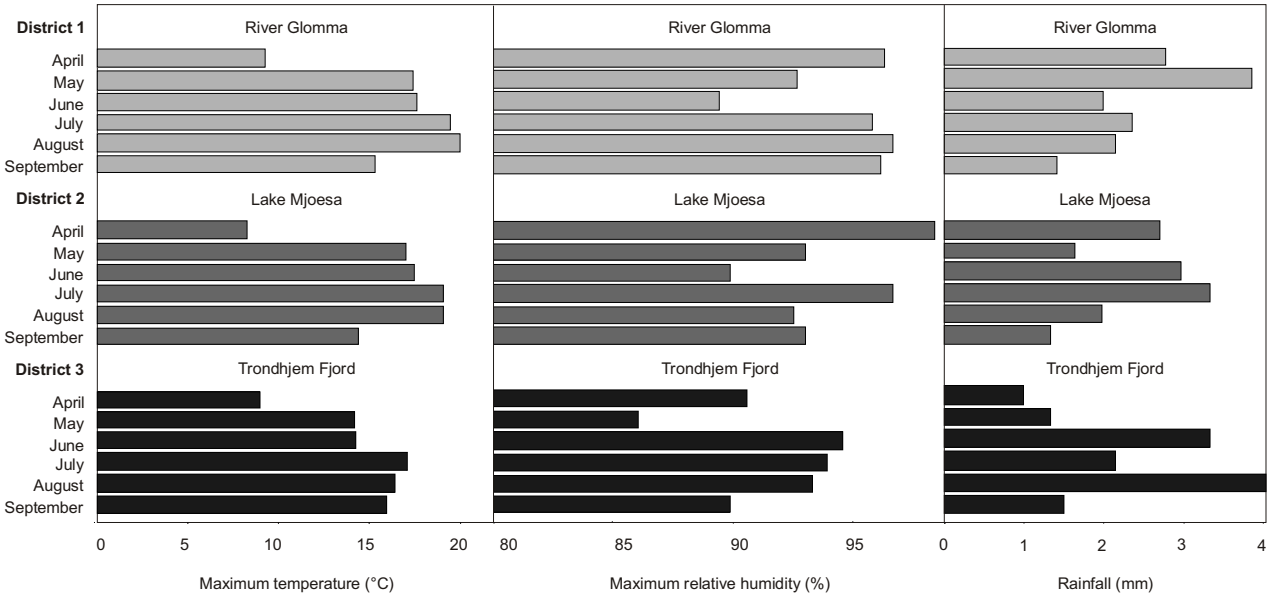

Figure 1. Bar-charts showing maximum daily air temperature, maximum daily air humidity and daily rainfall April–September in 1999 and 2000 in the 3 sampling districts: River Glomma (district 1), Lake Mjoesa (district 2) and Trondhjem Fjord (district 3). The average values for each month are shown.

dust, mixed by vortexing (Labinco L46 Vortex mixer, Breda, The Netherlands) for 3 minutes at max. speed followed by centrifugation for 10 minutes at 3,000 rpm. The supernatants were transferred to centrifuge tubes containing a 0.22 µm filter device (Hydrofile Durapore, Millipore, Massachusetts, USA), and filtrated by centrifugation for 10 minutes at 3,000 rpm. Extracts were stored at 4ºC, and diluted to contain 50% methanol before analysis.

Quantification of ochratoxin by competitive direct ELISA. Ochratoxin in the dust extracts was quantified by a competitive direct ELISA in microwell format (Veratox® quantitative test kits, Neogen Corp., Lansing, MI, USA) originally developed for feed and grains. In brief, free ochratoxin in samples and controls was allowed to compete with enzyme-labelled ochratoxin for the antibody binding sites in precoated microwells for 5–10 minutes. After a wash step, substrate was added to react 5–10 minutes with the bound conjugate and produce blue colour before stop solution was added. Optical density was measured in a microwell reader (Fusion™, Packard BioScience, Meriden, Connecticut, USA) at 650 nm within 20 minutes of completion of the test. The optical densities of the samples were plotted against a standard curve to calculate the exact concentration of the mycotoxin (Fusion™ Data Analysis Software, Packard BioScience, Meriden, Connecticut, USA). The test had a detection limit of 1 µg/kg and a quantitation limit of 2 µg/kg.

Table 2. Topographic characteristics of farms and storage time of grain in each district.

	Altitude (m)			Distance to nearest lake, river or fjord (km)			Storage time[1] (weeks)		
	Min	Median	Max	Min	Median	Max	Min	Median	Max
District 1 (n=34)	20	145	210	0	1.4	7.5	3	5	27
District 2 (n=20)	123	180	323	0	1.5	12	2	6	27
District 3 (n=45)	0	58	166	0	1	5	2	21	27

[1] Storage samples only.

Table 3. OTA, *Penicillium* spp., *Aspergillus* spp. in grain dust.

	N	% positive	Detection limit	Minimum	Median	Maximum
Settled dust						
OTA (mg/kg)	99	100	1	2	4	128
Penicillium spp. (cfu/mg)	99	52	40	0	40	32 000
Aspergillus spp. (cfu/mg)	99	20	40	0	0	5 300
Inhalable dust						
Total dust (mg/m^3)	96	100		0.2	5	108
OTA (pg/m^3)[a]	96	100		0.6	20	14 000

[a] Estimated from [OTA] in settled dust times [inhalable total dust].

Theoretically the antibody had 100% reactivity towards ochratoxin A and 18% crossreactivity with ochratoxin B.

Cultivation of *Penicillium* and *Aspergillus*. For each sample, 10 mg grain dust was added to 10 ml of sterile water and mixed by vortexing in a small bottle for 15 seconds (Fisons whirlimixer, WM/220/F, Rich-Mond, Wigan, UK). The suspension was diluted to 1% and 500 µl of each suspension was plated on each of 5 Petri dishes (90 mm) with Czapek-Dox agar containing 50 µg chlortetracycline/l. The plates were incubated for 7 days at 25ºC in cycles of 12 hours black light/cold daylight and 12 hours darkness. The number of fungal colonies was counted and presented as the number of colony forming units/mg dust (cfu/mg). The isolates were identified to genus level.

Table 4. Associations between grain production variables and OTA, *Penicillium* spp. or *Aspergillus* spp.

	N	OTA (µg/kg)			*Aspergillus* spp. (cfu/mg)			*Penicillium* spp. (cfu/mg)		
Variables		median	max.	p[a]	median	max.	p	median	max.	p
Growth season				n.s.			n.s.			*
1999	19	5	75		0	200		160	27,000	
2000	80	4	128		0	5,300		0	32,000	
District				n.s.			n.s.			n.s
District 1 (Glomma)	34	3	28		0	5,300		40	27,000	
District 2 (Lake Mjoesa)	20	4	52		0	2,200		40	4,400	
District 3 (Trondhjem Fjord)	45	4	128		0	1,800		0	32,000	
Cereal species				n.s.			n.s.			n.s.
Barley	58	3	128		0	5,300		40	32,000	
Oats	28	5	75		0	1,800		40	27,000	
Spring wheat	13	4	13		0	800		0	720	
Barley subspecies				(*)			n.s.			n.s.
- 2 row	37	3	54		0	5,300		40	1,000	
- 6 row	21	4	128		0	1,600		40	32,000	
Autumn ploughing[b]				n.s.			*			n.s.
- yes	64	4	128		0	2,200		40	32,000	
- no	35	4	75		0	5,300		40	4,400	
Same species as preceding season				n.s.			n.s.			n.s.
- yes	47	4	128		0	2,200		40	32,000	
- no	52	4	75		0	5,300		40	27,000	
Field fungicide				n.s.			n.s.			n.s.
- yes	46	3	128		0	5,300		0	32,000	
- no	53	5	75		0	1,800		40	27,000	
Growth regulator				n.s.			n.s.			n.s.
- yes	17	3	128		0	5,300		40	32,000	
- no	82	4	75		0	2,200		40	27,000	
Lodging				n.s.			n.s.			n.s.
- yes	58	4	29		0	1,800		20	4,000	
- no	41	4	128		0	5,300		40	32,000	
Visible mould damage				n.s.			n.s.			n.s.
- yes	35	3	128		0	2,200		0	32,000	
- no	64	4	75		0	5,300		40	27,000	
Work operation				n.s.			*			**
- threshing	31	3	13		0	120		0	4,800	
- storage	68	4	128		0	5,300		40	32,000	
Grain storage technology[c]				(*)			n.s.			n.s.
- ambient air dryer	40	3	75		0	5,300		80	4,400	
- heated air dryer	28	5	128		0	800		40	32,000	

[a] Categories were compared by Mann-Whitney tests (2 categories) or Kruskal-Wallis tests (>2 categories), n.s. (not significant), $p>0.1$; (*) $p\leq0.1$; * $p<0.05$; ** $p<0.01$; [b] Autumn ploughing includes whole (N=50) and partly (N=14) autumn ploughed fields; [c] Storage samples only (N=68).

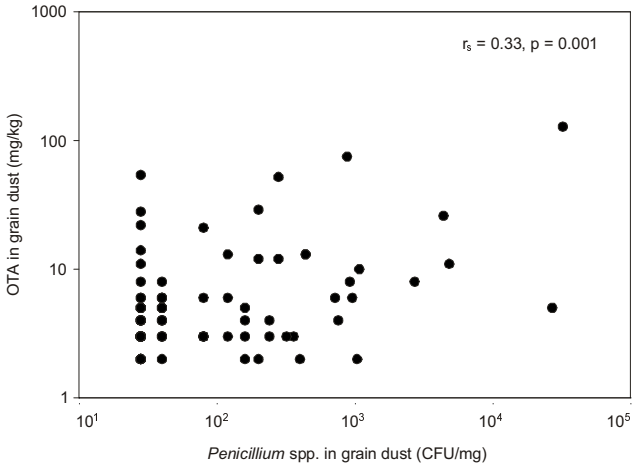

Figure 2. Scatter plot showing the correlation between ochratoxin A and *Penicillium* spp. in settled grain dust. Spearman correlation coefficient and significance level is included. Measurements of *Penicillium* spp. below the detection limit are substituted with the lowest value (40 cfu/mg) divided by the square root of 2.

OTA exposure estimates. Personal sampling of the aerosol yielded median 0.2 mg (range 0.01-6.5 mg) dust gross weight. As measurements of mycotoxins in grain dust require larger amounts of dust (≥200 mg), we could not measure OTA in the collected aerosol. Therefore, we estimated OTA exposure by multiplying the inhalable dust exposure with the OTA concentration in the corresponding settled dust samples, presuming settled dust composition to be similar to that of airborne dust. As no maximum limit for OTA inhalation exists, the exposure estimates were compared with the tolerably daily intake (TDI) of OTA accepted for oral intake; 5 ng/kg bw [48]. This was done by calculating inhaled OTA per kg bodyweight (bw), assuming that the farmer weighed 80 kg, worked at a moderate physical activity, and breathed 30 l of air per minute. Inhaled OTA during 8 hrs work were given as percentage of the TDI of OTA.

Table 6. Inhalable dust and estimated OTA exposure during grain handling compared to the TDI[a] of OTA.

Work task	N	Inhalable dust (mg/m^3)	Inhalable OTA (pg/m^3)	% of TDI (8 hrs work)
Threshing	31	1.2 (0.2-15)	4 (0.6-200)	0.01 (0.002-0.7)
Storage	34	5.9 (1.1-32)	40 (2-600)	0.14 (0.007-2.2)
Emptying	31	7.5 (1.1-110)	37 (3-14 000)	0.14 (0.01-50)

[a] TDI; tolerably daily intake of OTA is set to 5 ng/kg bw [48]. Values in table are calculated assuming the farmers' body weight to be 80 kg and moderate physical activity with airflow 30 l/min.

Statistical analysis. The median, minimum and maximum values are given for inhalable dust and OTA concentrations, and for OTA and cultivated *Penicillium* and *Aspergillus* in settled grain dust. The percentage of samples positive for cultivated fungi is also given. Measurements of *Penicillium* below the detection limit were arbitrarily substituted with the value of the lowest value divided by the square root of 2 in scatter plots and box plots [15]. Categories were compared by the Mann-Whitney (2 categories) or Kruskal-Wallis tests (more than 2 categories). Associations were studied by non-parametric Spearman rank correlation coefficients (r_s). A p-value of 0.05 or less was considered statistically significant. Selected univariate associations with OTA and *Penicillium* spp. are shown in box plots. The data had

Table 5. Climatic predictors for OTA, *Penicillium* spp. and *Aspergillus* spp. contamination in the field[a].

Climatic predictor	OTA		*Penicillium* spp.		*Aspergillus* spp.	
	r[b]	p[c]	r	p	r	p
Temperature[d] in April	0.22	n.s.	0.47	**	-0.24	n.s.
May	0.34	(*)	0.47	**	-0.02	n.s.
June	0.37	*	0.37	*	-0.02	n.s.
July	0.30	(*)	0.45	*	0.10	n.s.
August	0.21	n.s.	0.35	(*)	-0.04	n.s.
September	-0.18	n.s.	0.04	n.s.	-0.25	n.s.
Humidity[e] in April	0.21	n.s.	0.27	n.s.	0.22	n.s.
May	-0.08	n.s.	0.24	n.s.	0.21	n.s.
June	-0.27	n.s.	-0.25	n.s.	-0.09	n.s.
July	0.10	n.s.	0.29	n.s.	0.01	n.s.
August	-0.14	n.s.	0.13	n.s.	-0.33	(*)
September	0.28	n.s.	0.37	*	-0.15	n.s.
Rain[f] in April	-0.03	n.s.	0.10	n.s.	0.35	(*)
May	0.16	n.s.	0.30	(*)	0.02	n.s.
June	-0.03	n.s.	-0.18	n.s.	-0.27	n.s.
July	0.45	*	0.43	*	0.03	n.s.
August	-0.21	n.s.	-0.14	n.s.	-0.19	n.s.
September	-0.31	(*)	-0.28	n.s.	0.01	n.s.

[a] Threshing samples only (n=31); [b] Spearman correlation coefficient; [c] n.s. (not significant), p>0.1; (*), p≤0.1; *, p<0.05; **, p<0.01; [d] Maximum daily air temperature (ºC); [e] Maximum daily air humidity (%); [f] Average daily rainfall (mm).

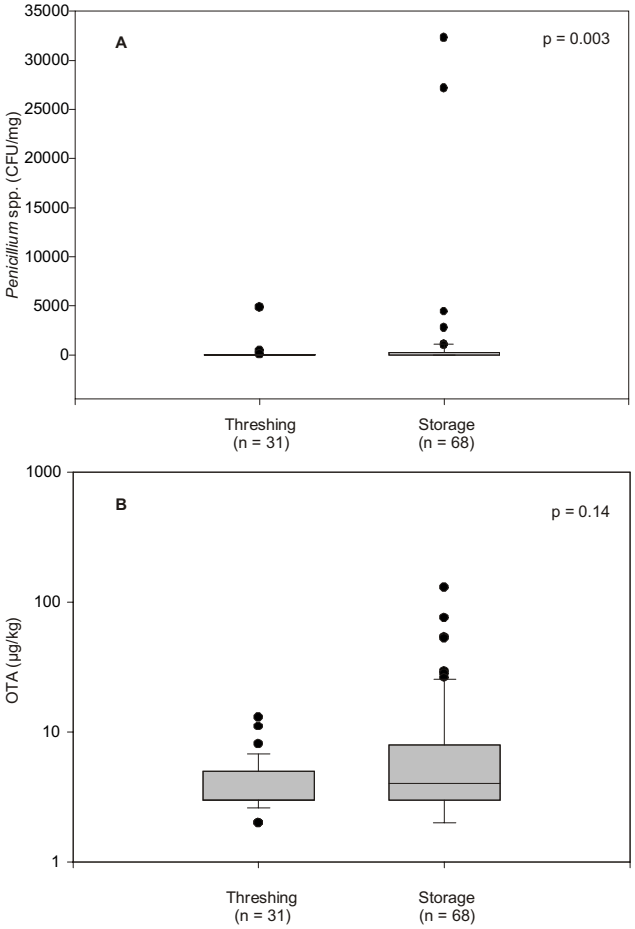

Figure 3. Box plots showing the distribution of *Penicillium* spp. (A) and ochratoxin A (B) in threshing and storage dust. Data within the 25 and 75 percentiles are boxed. Spikes indicate the 10–90 percentiles and filled circles indicate outliers. The p-value of the Mann-Whitney t-test is included.

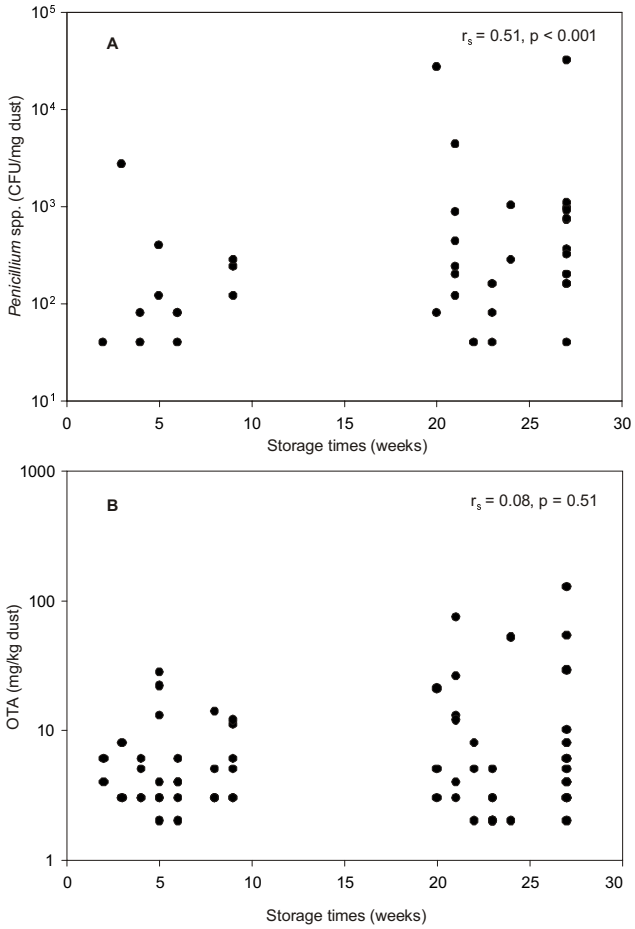

Figure 4. Scatter plots showing the correlation of *Penicillium* spp. (A) and ochratoxin A (B) concentration with storage time in settled dust from storage (n=68). Spearman correlation coefficients and significance levels are included. Measurements of *Penicillium* spp. below the detection limit are substituted with the lowest value (40 cfu/mg) divided by the square root of 2.

skewed distributions and were plotted on a log-scale. Statistical analysis was performed with software package SPSS, version 11.5 for Windows. Graphic presentations were prepared with software package SigmaPlot 2001.

RESULTS

Ochratoxin, *Penicillium* spp. and *Aspergillus* spp. in settled grain dust. All samples contained OTA. The median OTA concentration was 4 µg/kg (range 2-128 µg/kg, Tab. 3). Thirteen percent of the samples had 3-30 times higher OTA levels than the median (range 13-128 µg/kg). The grain dust contained median 40 cfu of *Penicillium* spp./mg dust (range 0-32,000 cfu/mg dust) and median 0 (range 0-5,300 cfu/mg dust) *Aspergillus* spp. (Tab. 3). Culturable *Penicillium* spp. and *Aspergillus* spp. were detected in 52% (n=51) and 20% (n=20) of the samples, respectively. OTA correlated with *Penicillium* spp. (r_s=0.33, p<0.001; Fig. 2), but not with *Aspergillus* spp. OTA and *Penicillium* spp. were significantly correlated in all grain species and in 2 out of 3 districts (r_s=0.52, p=0.02 in District 2 and r_s=0.38, p=0.01 in District 3).

Associations between agricultural variables, mould and OTA. There where similar levels of OTA and moulds in settled dust from barley, oats and spring wheat. Similar levels were also found across districts (Tab. 4). Storage dust contained significantly more *Penicillium* spp. (Fig. 3a, p<0.01, Tab. 4) and *Aspergillus* spp. (p<0.05) than threshing dust. A similar trend was found for OTA. However, the difference was not significant (Fig. 3b, Tab. 4). Although the amount of *Penicillium* spp. increased significantly during storage (r_s=0.51, p<0.001, Fig. 4a), neither OTA (r_s=0.08, n.s., Fig. 4b) nor *Aspergillus* spp. (r_s=0.11, n.s.) did so. Similar results were obtained for all grain species and in 2 out of 3 districts. Correlations between *Penicillium* spp. and storage time were r_s=0.80, p>0.001 in District 2 and r_s=0.57, p>0.001 in District 3. Moreover, in storage samples of 6-row barley (n=12), both OTA and *Penicillium* concentrations increased with storage time (r_s=0.61, p=0.03 for both), and a strong correlation between OTA and *Penicillium* was observed in these samples (r_s=0.83, p=0.001). Altitude and OTA concentration were correlated (r_s=0.39, p=0.03) in threshing samples. There was no correlation with distance

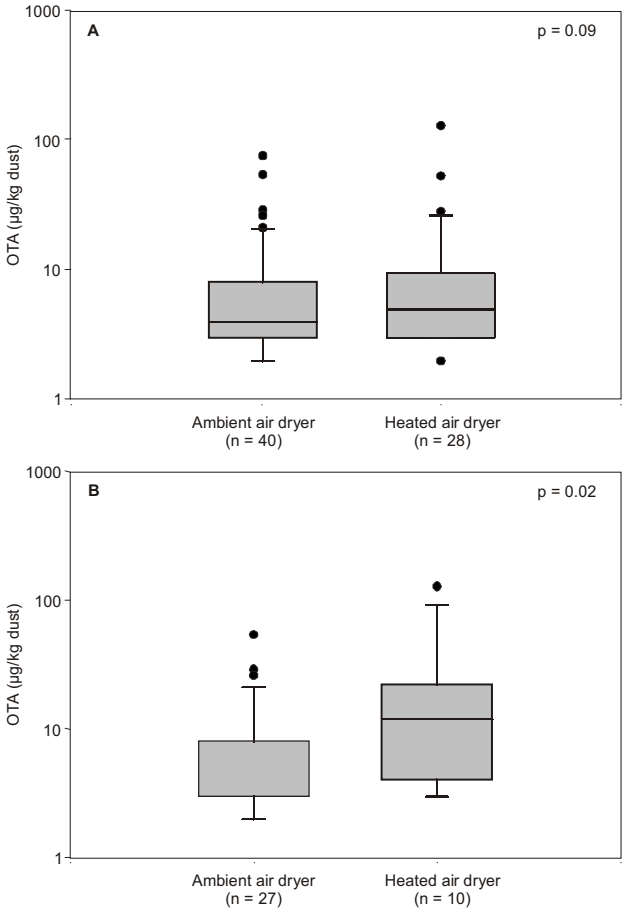

Figure 5. Box plots showing distribution of ochratoxin A in settled dust from grain dried in ambient and heated air driers. All storage samples (n=68) (A) and stored barley (n=37) (B). Data within the 25 and 75 percentiles are boxed. Spikes indicate the 10–90 percentiles and filled circles indicate outliers. The p-value of the Mann-Whitney t-test is included.

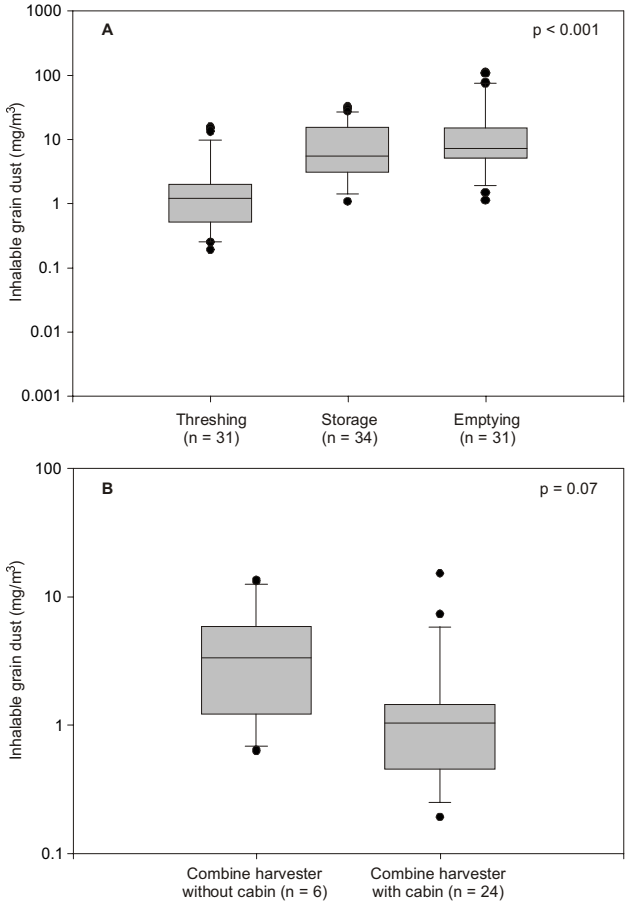

Figure 6. Box plots showing distribution of inhalable grain dust during threshing, storage and emptying (A), and during threshing with and without cabin on the combine harvester (B). Data within the 25 and 75 percentiles are boxed. Spikes indicate the 10–90 percentiles and filled circles indicate outliers. The p-value of the Kruskal-Wallis test is included.

to the nearest lake, river or fjord, water content of grain, or thickness of grain layer in storage bins.

Grain dust from heated air driers tended to have higher concentrations of OTA (median 5 µg/kg) than dust from ambient air driers (median 3 µg/kg) (Fig. 5a, p=0.09, Tab. 4), whereas the *Penicillium* spp. concentration was lower (median 80 cfu/mg, range 0-4,400 cfu/mg and median 40 cfu/mg, range 0-32,200 cfu/mg, respectively). Moreover, in storage samples of barley, the OTA concentration was significantly different in heated driers compared to ambient driers (Fig. 5b, p=0.02).

Climatic predictors for mould growth and OTA production. The maximum daily temperature during May–July and rainfall in July was near-significantly and significantly correlated with OTA (r_s=0.30-0.45, p<0.1-p<0.05) in grain dust from threshing (Tab. 5). The maximum daily temperature during April–August, maximum air humidity in September and rainfall in July were significantly correlated with *Penicillium* spp. (Tab. 5). *Aspergillus* spp. were near-significantly and negatively correlated with maximum air humidity in August and positively with rain in April (Tab. 5). No correlations with OTA or *Penicillium* spp. were seen in storage samples. However, in storage samples, *Aspergillus* spp. was weakly correlated with maximum temperature in May (r_s=0.23, p=0.06), maximum air humidity in June (r_s=0.36, p=0.003) and rainfall in May (r_s=0.27, p=0.03).

OTA containing grain dust exposure. The farmers inhaled median 5 mg grain dust/m^3 air (range 0.2-108 mg/m^3), corresponding to median 20 pg OTA/m^3 (range 0.6-14,000 pg/m^3) (Tab. 6). Exposure to inhalable dust and OTA was significantly higher during storage work compared to threshing, emptying of the bin, giving the highest exposure (Fig. 6a, p<0.001, Tab. 6). A cabin on the combine harvester reduced the dust exposure by 70% compared with no cabin, although the difference was of borderline significance only [median 1.0 mg/m^3 (range 0.19-15.1) and median 3.4 mg/m^3 (range 0.63-13.3), respectively, p=0.07, Fig. 6b].

Inhalable OTA compared to the TDI of OTA. As no maximum limit for OTA inhalation exists, estimated

inhalable OTA levels were compared with the TDI recommended for maximum oral intake of OTA [48]. Therefore, threshing, storage and emptying constituted less than 0.7% (Tab. 6). However, the highest observed OTA measurement amounted to 50% of the TDI (Tab. 6).

DISCUSSION

This study describes the presence of OTA, *Penicillium* spp. and *Aspergillus* spp. in dust from threshing and storage work. The main findings show that of all the studied agricultural practices, only the use of heated driers, to a certain degree, was associated with OTA contamination of the dust. Temperature and rain seemed to predict *Penicillium* spp. growth and OTA production in the field. Although OTA levels in grain dust increased with storage time only when heated air driers had been used for grain drying, the exposure levels were overall higher during drying and storage work than during threshing. The variations in exposure levels were dependent on whether the combine harvester had a cabin or not, and on the kind of on-farm drying system. The findings will be discussed below.

Although OTA contamination has mainly been associated to storage conditions, OTA was found in all dust samples collected during threshing. Hökby *et al.* [24] have also found OTA in grain on the field. They reported 20 µg/kg OTA in 2 of 10 areas of barley 2 weeks before harvest. *P. verrucosum* is an integral part of the soil ecosystem [16] and OTA has also been detected in soil [38]. Contamination of the grain in the field is thus possible, and it seemed also to occur in our study. Moreover, similar OTA levels in settled dust from threshing and storage suggested that OTA formation mainly occurred in the field, and that it did not increase during storage under given conditions. Temperature and rain seemed to predict mould growth and OTA production in the field, as demonstrated by correlations with OTA and *Penicillium* spp. levels in threshing samples. However, Sayer *et al.* [46] found that while higher rainfall and temperature at anthesis increased the incidence of *Fusarium* spp., no such effect was found on *Aspergillus* spp. or *Penicillium* spp. In a recent Swedish study, Lindblad *et al.* concluded that the content of OTA increased with increasing levels of *Penicillium verrucosum*, and with increased water activity in stored grain, while no effect of the temperature was found on toxin production [34]. On the other hand, both moisture and temperature affect the growth of *Penicillium* spp. and *Aspergillus* spp. [43, 45]. Higher temperatures provided favourable conditions for *Penicillium* spp. growth in the field. Significant correlation between *Penicillium* spp. and air humidity in September only seemed plausible as the soil moisture is expected to influence the *Penicillium* spp. growth more than the air humidity before the grain matures. This may also explain the weak correlations with rainfall. However, both OTA and *Pencillium* in dust collected during threshing were significantly correlated with rainfall in July, the start of grain maturation. Splash dispersal of the spores during rainfall at this time may have caused the initial penicillium contamination of the grain [19]. Observed annual variations in *Penicillium* spp. and OTA levels were expected due to annual meteorological variations. Altitude seemed to predict OTA in threshing dust. However, this was probably indirectly due to the temperature, rain and humidity. Indeed, the altitude was strongly correlated with the climatic variables (data not shown).

Although OTA did not correlate with storage time, *Penicillium* spp. growth during storage was suggested by significant concentration differences between threshing and storage, and by a significant increase in cfu/mg with storage time. As OTA levels did not increase with storage time, the growing *Penicillium* spp. was presumably not OTA producing. Sporulation as a last step of penicillium development after growth on the grain in the field may also explain a higher concentration of *Penicillium* spp. observed in the storage samples. Wind dispersal of the spores in the field may have added further to this difference. Identification and quantification of moulds by cultivation may have some limitations, such as competitive inhibition between species and the fact that non-cultivable OTA-producers are not detected. It is therefore likely that the level of moulds identified and quantified in this study were underestimated.

The grain in the present study contained 17-40% water at harvest, similar to previous findings in Norway [32]. It may take weeks to dry wet grain sufficiently if only ambient air is used for drying. Meanwhile, the fungi may continue to grow and produce mycotoxins [5, 7, 8, 30]. *P. verrucosum* and OTA have been most frequently found in grain dried with ambient air [32], which was used on 59% of the farms in the present study. It was therefore unexpected to find higher concentrations of OTA and *Penicillium* spp. in dust from grain dried with heated air compared to ambient air. OTA levels also increased with storage time when heated air driers had been used for grain drying. When the grain is wet at harvest, the farmer may choose to use heated air driers to dry the grain quickly. Grain already at risk of mould growth and mycotoxin formation may thus be selected for heated air drying. In fact, we observed a significant difference in OTA levels between threshing dust and dust from heated driers. This may also be due to favourable conditions for OTA-producing *Penicillium* in the beginning of the heated drying, resulting in mould growth and OTA production. *P. verrucosum* is able to grow at 0-31ºC [12, 42] with a temperature range for OTA production of 4-31ºC [33]. In comparison, *A. ochraceus* is able to grow at 8-37ºC and has a temperature range for OTA production of 12-37ºC, optimum 31-37ºC [33]. The higher temperature optimum of *Aspergillus* spp. compared to *Penicillium* spp. was probably the reason for the low concentration of *Aspergillus* spp. observed in grain dust. Whereas *Penicillium* spp. may thrive in a temperate climate like Norway [12], *Aspergillus* spp. may be more important in areas with warmer climate [33].

Several components in the grain dust may exert health effects [14]. Grain farmers reported work-related irritation symptoms in eyes, nose, throat and lower airways at dust exposure levels similar to the levels presented here [36]. Corey *et al.* [9] reported work related changes in pulmonary function and dose-effect relationships with exposure to 0.6-15 mg/m^3 dust in grain elevator workers.

During threshing, the farmer seemed to be protected by the cabin on the combine harvester. However, the protective value of a cabin may also be dependent on the filter efficiency of the air intake. Moreover, the necessary opening of the cabin door during threshing limits the protective function. Nevertheless, total dust levels were reduced by 70%, which is similar to a study of shotcreting operators where a cab on the rig reduced exposure levels by 72-77% [4].

Storage work generated on average more than 5 times more dust than threshing. Expectedly, storage work was found to be an important exposure determinant. The 100-fold difference in dust exposure between different storage technologies implicates, however, that protective actions must be evaluated individually. As only 25% used respiratory protection (results not shown), their work could possibly represent a health risk.

Studies on exposure to airborne OTA on workplaces are limited. A small study among food industry workers reported 0.003-8 ng OTA/m^3 in the production of black pepper, nutmeg and cocoa beans, with black pepper production showing the highest levels [6, 26]. No occupational exposure limit for OTA inhalation exists. However, based on suggestions from expert groups from Canada and the Nordic and European countries, the European Community has established a tolerably daily oral OTA intake (TDI) of 5 ng/kg bw [48]. Estimation of inhaled OTA during 8 hours grain work and comparison with the TDI, revealed only minor levels compared to the daily Norwegian OTA intake derived from cereals, the latter comprising 12-40% of the TDI. However, some grain dust samples had up to 30 times more OTA than the median, the inhaled dose amounting up to 50% of the TDI. Moreover, inhalation of mycotoxins may be at least 10 times more toxic than dermal [47], oral or intraperitoneal exposure [10, 11] due to higher bioavailability [41]. Therefore, the health risk by inhalation is probably higher than by oral exposure at a similar level. Thus, inhalation of the grain dust may occasionally represent a health risk for the farmer, depending on the grain production technology in use. The exposure level may even be substantially higher when working with clearly mouldy grain.

CONCLUSIONS

OTA and Penicillium contamination of grain dust occurs in the field, although *Penicillium* spp. may continue to grow during storage. Rainfall in July and maximum temperatures throughout the grain growth season were associated with penicillium growth and OTA contamination. However, there were no associations with agricultural practices. Through insight into the agro-climatic niches in their fields and knowledge of resistant grain species, Norwegian farmers are able to obtain crops of good quality. In most cases, the actions taken in this respect seemed to be effective to limit mould growth and mycotoxin formation. Nevertheless, the present study shows the need for more focus on mycotoxin contamination. Drying and storage work were identified as significant determinants for farmers' exposure to OTA-containing dust, although the exposure levels were highly variable depending on the type of grain dryer. The estimated inhaled OTA was low compared to normal oral intake. However, considering higher bioavailability of OTA through inhalation, grain handling may constitute a health risk, especially when working with mouldy grain.

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