Research Article

Occurrence of type A trichothecenes in conventionally and organically produced oats and oat products

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Among cereals, oats are known to be very frequently contaminated with type A trichothecenes and so they can play a major role in the exposition of the consumer to these mycotoxins. Seventy representative oat samples of both conventional and organic production were drawn at mills and at wholesale stage according to Commissions Regulation (EC) No 401/2006 and analyzed for nine type A trichothecenes by LC-MS/MS. High contamination rates were found for most of the toxins in conventional as well as in organic products (*e.g.* 100% for T-2 toxin or 99% for HT-2 toxin). The mean concentration of T-2/HT-2 (sum of the toxins) was 17 ± 18 μg/kg (mean ± SD) in all samples, 27 ± 21 μg/kg in conventional, and 7.6 ± 4.6 μg/kg in organic products, respectively. The highest T-2/HT-2 level has been determined in conventionally produced oat flakes (85 μg/kg). The mean level of T-2 tetraol (9.5 ± 7.7 μg/kg) in all samples was found to be even higher than that of T-2 (5.1 ± 6.0 μg/kg), whereas levels of T-2 triol, 4,15-diacetoxyscirpenol, 15-monoacetoxyscirpenol, and neosolaniol were considerably lower. For oats and oat products from organic farming contamination levels of T-2, HT-2, T-2 triol, T-2 tetraol, and neosolaniol were significantly lower. The results are discussed with respect to possible health risks for the consumer.

Keywords: Mycotoxin / Organic farming / T-2 tetraol / T-2 toxin / Trichothecenes

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1 Introduction

The infection of cereals and corn with *Fusarium* spp. is a worldwide problem and already takes place on the field during the flowering period. Apart from the economic damage caused by *Fusarium*-derived diseases of the plant leading to yield losses or cereals of poor quality, the ability of certain species of this fungus to produce mycotoxins like fumonisins, zearalenone (ZEA) and trichothecenes is a problem concerning food and feed safety. Trichothecenes are cyclic sesquiterpenoids with an epoxid group and are known as a large group of cytotoxic and immunosuppressive mycotoxins [1]. According to their chemical structures they are divided into four groups (A – D), while group A and B are the most important in cereals and cereal-based food. *F. sporotrichiodes* and the newly described species *F. langsethiae* are the main producers of type A trichothecenes like T-2 toxin (T-2) and HT-2 toxin (HT-2) (Fig. 1) in European cereals [2, 3]. Oats and maize are considered the most contaminated with this group of toxins [4]. In 2001, the Scientific Committee on Food has proposed a temporary tolerable daily intake (tTDI) for the sum of T-2 and HT-2 of 0.06 μg/kg body weight [5]. According to mean intake data provided by a study of the Scientific Cooperation the ingestion of type A trichothecenes could exceed this tTDI especially in the group of infants and children by more than 5-fold [4]. So far, the European Commission (EC) has set maximum levels for the *Fusarium*-mycotoxins deoxynivalenol (DON) and ZEA. Already proposed maximum levels for fumonisins shall apply from 1 October 2007 if no other levels will be considered. Concerning T-2 and HT-2, the EC claimed urgently for more occurrence data of these toxins

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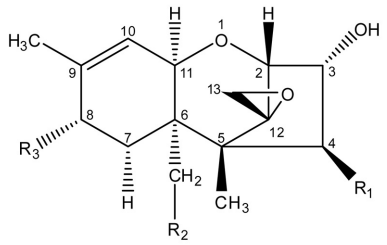
Abbreviations: DacVOL, 4,15-diacetylverrucarol; **DAS**, 4,15-diacetoxyscirpenol; **DON**, deoxynivalenol; **EC**, European Commission; **HT-2**, HT-2 toxin; **MAS**, 15-monoacetoxyscirpenol; **MRM,** multiple reaction monitoring mode; **NEO**, neosolaniol; **T-2**, T-2 toxin; **tTDI**, temporary tolerable daily intake; **VOL**, verrucarol; **ZEA**, zearalenone



Trichothecene type A	MW	R_1	R_2	R_3
T-2 toxin	466	OAc	OAc	$OCOCH_2CH(CH_3)_2$
HT-2 toxin	424	OH	OAc	$OCOCH_2CH(CH_3)_2$
T-2 triol	382	OH	OH	$OCOCH_2CH(CH_3)_2$
T-2 tetraol	298	OH	OH	OH
4,15-Diacetoxyscirpenol	366	OAc	OAc	H
15-Monoacetoxyscirpenol	324	OH	OAc	H
Neosolaniol	382	OAc	OAc	OH
4,15-Diacetylverrucarol	350	OAc	OAc	H
Verrucarol	266	OH	OH	H

Figure 1. Chemical structures of nine type A trichothecenes [1].

especially in oats and oat products and, if necessary, will establish an appropriate maximum level presumably until 1 July 2008 [6].

In this project, data on the contamination rates and levels of type A trichothecenes of representative oat samples are presented. Furthermore, the influence of the agricultural practice on amounts of type A trichothecenes has been examined. The production of organic food in Europe has increased remarkably during the last years [7]. In the first nine months of the year 2006, the expenses of private households in Germany for organic products have grown about 17% compared with the year before. Organic food achieved a market share of 2.7% of the overall food market [8]. In the 1990s, this share was roughly 1% of the total consumption of food [9]. Nevertheless, organic farming and the relation to mycotoxin contamination is still very diversely discussed. While some authors did not observe any effects on *Aspergillus*-, *Penicillium*- or *Fusarium*-mycotoxins, others described higher or lower amounts of mycotoxins in organic products [10]. Data published so far mainly concern the influence on aflatoxins, ochratoxin A, ZEA, and DON, but not on type A trichothecenes like T-2 and HT-2. The results obtained in this study are discussed and the levels of mycotoxin contamination are evaluated with respect to possible health risks for the consumer.

2 Materials and methods

2.1 Sampling

Seventy representative samples (35 from conventional farming, 35 from organic farming) have been analyzed. Since mycotoxins can be distributed very heterogeneously in one lot, a representative sampling in determination of mycotoxins is highly important. Therefore, sampling has been performed according to Commissions Regulation (EC) No 401/2006 [11]. All samples were at least cleaned and de-hulled oats intended for human consumption. Nine samples were oats, 43 oat flakes, eleven oat bran, and seven oat-containing infant food. The samples were drawn at mills and at wholesale stage from the Bavarian market and were all of German origin of the production year 2005.

2.2 Chemicals and reagents

Standards of the type A trichothecenes T-2, HT-2, 4,15-diacetoxyscirpenol (DAS), 15-monoacetoxyscirpenol (MAS), T-2 triol, T-2 tetraol, neosolaniol (NEO), 4,15-diacetylverrucarol (DacVOL), and verrucarol (VOL) were purchased from Sigma (Deisenhofen, Germany). ACN for extraction (HPLC grade) was purchased from Riedel-de Haën (Deisenhofen, Germany). Methanol (Riedel-de Haën) and ammonium formate (Fluka, Deisenhofen, Germany) were used in LC-MS quality. Water was purified on a milli-Q water purification system (Millipore, Schwalbach, Germany).

2.3 Sample preparation

Before analysis, the complete aggregate samples were finely ground and homogenized in the laboratory. The subsamples (20 g) were extracted with 80 mL ACN/water 84/16 v/v by blending at high speed (9500 rpm, 3 min) on an Ultra Turrax homogenizer (IKA-Labortechnik, Staufen, Germany). The extracts were centrifuged (5000 rpm, 10 min) and aliquots of 8 mL were purified with MycoSep® columns (#226, Coring System Diagnostix, Germany). This procedure has been successfully applied for similar LC-MS/MS multi-mycotoxin analyses [12, 13]. Four milliliters of the extracts were evaporated to dryness under a gentle stream of nitrogen at a temperature of 40°C (Barkey, Leopoldshöhe, Germany). For LC-MS/MS analysis the residues were dissolved in 1 mL of methanol/deionized water 5/95 v/v and filtered through a 0.45 µm PTFE syringe filter (Supelco, Deisenhofen, Germany).

2.4 LC-MS/MS analysis

LC-MS/MS analysis was performed with a high pressure gradient HPLC apparatus from Shimadzu (Duisburg, Germany) and a triple quadrupole mass spectrometer API 4000

Table 1. MRM transitions, declustering potentials and collision energies for nine type A trichothecenes

Analyte	Precursor Ion $[M+NH_4]^+$		Fragment Ions		
	Q1	DP (V)	Q3		CE (eV)
T-2	483.9	61	305.2	Quantifier	19
			215.2	Qualifier	25
HT-2	442.4	51	263.2	Quantifier	17
			215.1	Qualifier	19
T-2 triol	400.4	36	281.2	Quantifier	17
			215.1	Qualifier	13
T-2 tetraol	316.2	36	215.2	Quantifier	13
			281.3	Qualifier	11
DAS	384.2	51	307.3	Quantifier	17
			247.1	Qualifier	19
MAS	342.2	41	265.1	Quantifier	13
			107.1	Qualifier	19
NEO	400.2	51	305.3	Quantifier	17
			245.1	Qualifier	25
DacVOL	368.3	51	291.2	Quantifier	7
			249.1	Qualifier	11
VOL	284.2	36	267.2	Quantifier	13
			249.3	Qualifier	21

from Applied Biosystems (Darmstadt, Germany). The LC system consisted of an autosampler (SIL HT-C), two pumps (LC-10ADVP), a degasser (DGU-14A), and a column oven (CTO-10ACVP). As analytical column a Synergi™ polar-RP® 150 × 2 mm, 4 µm (Phenomenex, Aschaffenburg, Germany) was used. For chromatographic separation of the analytes a binary linear gradient was applied which consisted of eluent A (methanol + 5 mmol/L ammonium formate) and eluent B (deionized water) with a flow rate of 0.4 mL/min: 0 min 95% B, 11 min 95% B, 22 min 35% B, 26 min 35% B, 27 min 95% B, 35 min 95% B. The column oven temperature was maintained at 40°C and the injection volume was 20 µL.

For all analytes, MS experiments were carried out in positive ESI mode. The source temperature was set at 500°C and the nitrogen pressure of the nebulizer gas has been optimized with 55 psi. For the other parameters like the curtain gas, the heating gas and the ion spray voltage best sensitivity could be achieved by splitting the method in periods with different optimal values. For T-2 tetraol the pressure of curtain gas has been set at 25 psi, of heating gas at 55 psi and ion spray voltage at 3000 V, while for the other toxins 20 psi, 50 psi and 4500 V were used, respectively. All analytes were measured as adduct ions of ammonium $[M+NH_4]^+$. The toxins were identified in multiple reaction monitoring mode (MRM) by two specific fragment ions of one precursor ion according to Commissions Decision 2002/657/EC [14]. The MRM-transitions, declustering potentials and collision energies of the toxins are shown in Table 1.

2.5 Method validation and mycotoxin quantification

Recovery experiments were carried out using fortified oat samples at three levels (1 µg/kg, 10 µg/kg and 50 µg/kg) with five replicates each. The contamination with the trichothecene standard mix has been accomplished before extraction.

Quantification of the toxins was performed by external calibration. The LOD/LOQ was calculated by the S/N approach and ranged from 0.01/0.04 µg/kg for MAS to 0.3/0.8 µg/kg for VOL. For the evaluation of the results, levels of toxins between LOD and LOQ were calculated with half LOQ. The results were corrected by mean recovery rates obtained from the recovery experiments. Statistical analyses were carried out using SigmaStat 9.0. A two way ANOVA has been applied for the assessment of the influence of the agricultural practice and to consider possible influences of different food commodities.

3 Results and discussion

3.1 Recovery experiments

The recoveries obtained with this method ranged between 91 and 110% for all toxins with a RSD from 0.9 to 6.4%. Only T-2 tetraol showed a lower recovery rate of 60% and a RSD of 5.0%, but all were considered to comply with the requirements of the EC [11].

3.2 Occurrence of type A trichothecenes

Seventy oat samples from conventional and organic farming have been analyzed for nine type A trichothecenes. The contamination rates of conventionally as well as organically produced oats and oat products were very high for most of the analytes. The rates of T-2, HT-2, MAS, and NEO ranged between 97 and 100% in samples from both production types. Incidences higher than 90% were also found for T-2 tetraol and DAS in conventional samples. However, in organic samples, the incidences were lower with 83 and 71%, respectively. T-2 triol was determined in 77% of the conventional and 40% of the organic samples. VOL and DacVOL were not detected in any sample. In SCOOP task 3.2.10, far lower contamination rates of 16% for T-2, 41% for HT-2 or 0% for DAS were reported for 464 oat samples, possibly due to higher LOD's of the methods applied (5 – 30 µg/kg). In the SCOOP-study, no oat samples have been analyzed for T-2 triol, T-2 tetraol, MAS, NEO, VOL or DacVOL [4].

The mean concentration of T-2 and HT-2 (sum of the toxins) in all 70 samples was 17 ± 18 µg/kg (mean ± SD). Twenty-one percent of the samples showed T-2/HT-2 levels higher than 25 µg/kg, 7.1% were higher than 50 µg/kg. Conventionally produced samples had a mean T-2/HT-2

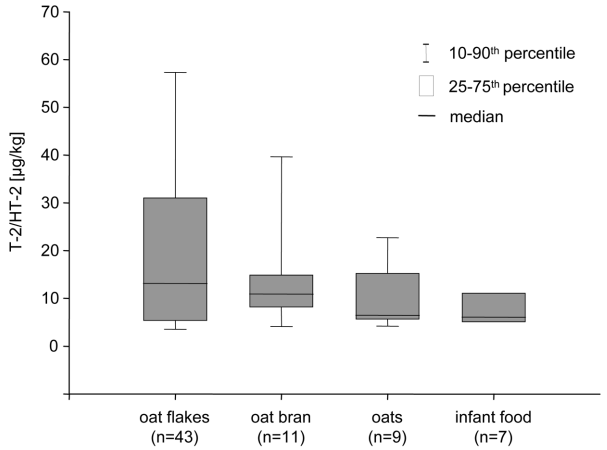


Figure 2. Comparison of contamination levels of oats and oat products with T-2 and HT-2 (sum of the toxins).

contamination of 27 ± 21 µg/kg, samples from organic production were contaminated with 7.6 ± 4.6 µg/kg. It was noticeable that the level of HT-2 usually was about 2-fold as high as that of T-2. In addition, the mean contamination level of T-2 tetraol in all samples (9.5 ± 7.7 µg/kg) was found to be even higher that of T-2 (5.1 ± 6.0 µg/kg). A study from Schollenberger *et al.* [15] showed a similar relation between T-2, HT-2 and T-2 tetraol in oats originating from Germany even though the mean levels were higher. Klötzel *et al.* [12] also highlighted the high contamination levels of T-2 tetraol in oat samples. Compared to the high levels found for T-2, HT-2 and T-2 tetraol, the mean contamination levels for T-2 triol, DAS, MAS, and NEO were all below 1 µg/kg. By contrast, one study from Poland reported a mean burden of oats with DAS of 23 µg/kg with an incidence of 12% [16].

Comparing the different food commodities, the highest contamination levels of all samples were found in oat flakes (Fig. 2) with a T-2/HT-2 mean value of 21 µg/kg and a maximum of 85 µg/kg in a conventional oat flake sample (Table 2). Surprisingly, the bran samples were contaminated with lower values than oat flakes. It could be assumed that oats used for oat flake production may be of lower quality than bran intended for human consumption. The lower levels in the bran indicate that this fraction might have been accurately separated from hulls during oat processing. Other studies examined that the toxin amount of oats is lowered by over 90% after de-hulling the kernel [17, 18]. The samples analyzed in this study, however, were exclusively dehulled oats. Finally, the lowest levels were found in infant food (mean T-2/HT-2 contamination 7.6 µg/kg).

3.3 Influence of the agricultural practice

Equal shares (n=35) of samples originating both from organic and conventional agriculture were analyzed for examining the influence of the agricultural practice. The amount of type A trichothecenes found in organic products differed considerably from that in conventional products. A statistically significant difference has been calculated for

Table 2. Results from 43 samples of oat flakes (25 conventional and 18 organic products) on type A trichothecene contamination

Analyte	Agricultural practice	Contamination rate (%)	Mean value (µg/kg)	Median (µg/kg)	95th percentile (µg/kg)	Maximum value (µg/kg)
T-2	organic	100	1.7	1.2	4.9	5.2
	conventional	100	9.8	8.4	21	34
	both	100	6.4	4.3	17	34
HT-2	organic	100	5.2	4.3	12	15
	conventional	100	21	18	47	51
	both	100	14	8.7	44	51
T-2 + HT-2	organic	100	6.9	5.5	17	20
	conventional	100	31	26	66	85
	both	100	21	13	64	85
T-2 triol	organic	39	0.18	< LOD	0.69	1.1
	conventional	84	0.85	0.91	1.6	2.7
	both	65	0.57	0.25	1.5	2.7
T-2 tetraol	organic	78	5.2	4.2	14	27
	conventional	100	14	13	26	34
	both	91	11	9.4	26	34
DAS	organic	67	0.04	0.04	0.11	0.14
	conventional	100	0.11	0.09	0.21	0.38
	both	86	0.08	0.04	0.17	0.38
MAS	organic	94	0.08	0.07	0.18	0.24
	conventional	96	0.13	0.08	0.28	0.66
	both	95	0.11	0.08	0.24	0.66
NEO	organic	100	0.16	0.07	0.55	1.1
	conventional	100	0.85	0.68	2.0	2.7
	both	100	0.56	0.34	1.9	2.7

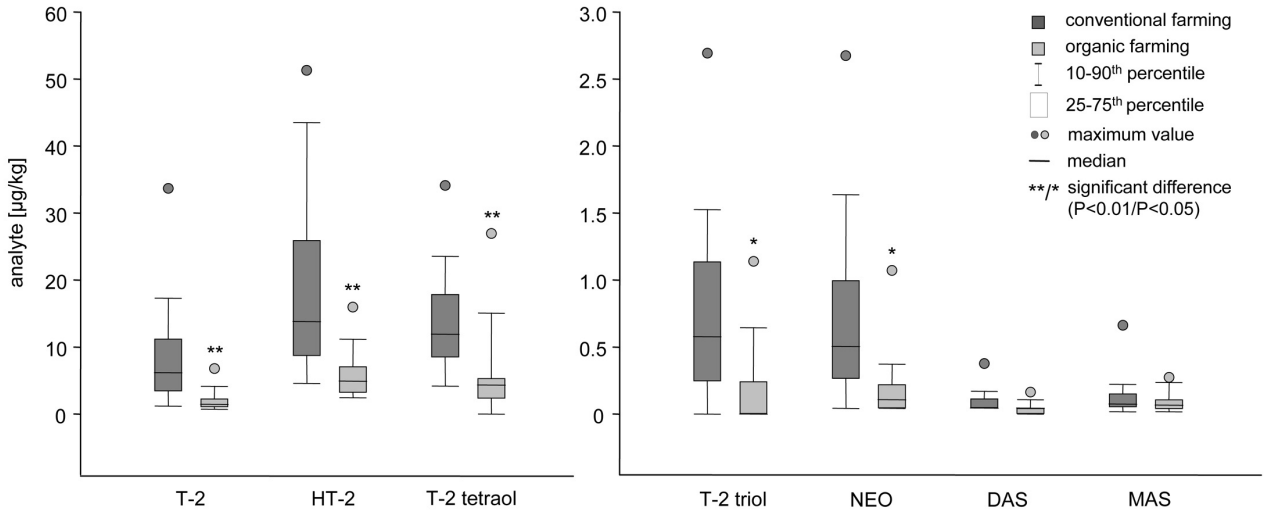


Figure 3. Comparison of contamination levels of type A trichothecenes in oats and oat products originating from conventional (n=35) and organic farming (n=35).

T-2, HT-2, T-2 tetraol ($p < 0.01$), T-2 triol, and NEO ($p < 0.05$) by two way ANOVA also after allowing for effects of differences in food commodities (Fig. 3). Values of DAS and MAS were very low without any significant difference.

The influence of the agricultural practice on mycotoxin contamination of cereals is controversially discussed. Our findings concerning T-2 and HT-2 are supported by the reports of Edwards *et al.* [17] and Pettersson [19]. In addition, Schollenberger *et al.* [20, 21], Schneweis *et al.* [22], Meister [23, 24] and Döll *et al.* [25] also found lower values in organic products when analyzing samples for DON or ZEA. However, these toxins are produced by different *Fusarium* species than type A trichothecenes, but the influence of the agricultural practice on these fungi should be similar. In contrast, Jestoi *et al.* [26], Pussemier *et al.* [27] and Marx *et al.* [28] reported no or negative effects of organic farming on the contamination with DON and ZEA. A study of Birzele *et al.* [29] showed contradictory results of infection rates and mycotoxin levels depending on climatic conditions and fungicide utilization. High humidity during the flowering period of wheat led to high grain infection rates and higher DON levels in organic wheat than in conventionally produced wheat treated by fungicides. In another year with moderate humidity during flowering, a lower DON burden occurred in cereals from the organic farming system.

An examination of the influence of the agricultural practice requires the same environmental factors like temperature, humidity and soil conditions or preceding crops for a proper study. Nevertheless, these requirements usually cannot be fulfilled for market-orientated supply studies or surveys. Schneweis *et al.* [22] conducted a field trial with winter wheat under the same environmental influences and concluded that DON and ZEA concentrations were in fact higher in conventionally than in organically produced wheat. Thus, a direct relation between the agricultural practice and the concentration of certain *Fusarium* toxins could be shown.

In general, an intensive tilling, the compliance with special crop rotations and a less closely sowing as basic principles of organic agriculture practices could be regarded as reasons for lower fungi and mycotoxin burden. Furthermore, it has been observed that the non-application of insecticides and fungicides in organic production did not lead to higher mycotoxin contamination rates or concentrations in foodstuffs [30]. The use of synthetic nitrogen fertilizers in conventional agriculture can also be a reason for higher mycotoxin levels because the increased growth speed comes along with thinner cell walls of the plant, which facilitates a mold infection [31].

3.4 Dietary intake estimation

The toxicity of T-2 and HT-2 has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by the Scientific Committee on Food of the EC. Both of them have calculated a tTDI of 0.06 µg/kg body weight for the sum of T-2 and HT-2 [5, 32]. A problem in performing dietary exposure assessments is always the lack of sufficient consumption data, particularly for certain sub-populations like infants or children. This is the consumer group with the highest risk, because they have an exceptionally high intake in relation to their body weight. According to recently published consumption data for children aged 2.5-years (mean body weight 16.15 kg), the mean consumption of oat flakes by this subpopulation is quoted with 3.1 g/day [33]. T-2/HT-2 intake estimates were conducted as follows for a mean case (average consumption *x* mean toxin concentration) and a bad case scenario (average consumption *x* 95th percentile value of toxin concentration).

Mean case scenario: Assuming the consumption of conventionally produced oat flakes with a mean contamination of 31 µg/kg T-2/HT-2 (Table 2) a total intake of 0.096 µg or 9.9% of the tTDI was calculated. The consumption of organic oat flakes (6.9 µg/kg) resulted in a total intake of 0.021 µg or 2.2% of the tTDI.

Bad case scenario: The consumption of conventionally produced oat flakes with a 95th percentile contamination of 66 µg/kg T-2/HT-2 (Table 2) led to an intake of 0.205 µg corresponding to 21% of the tTDI. For organic products (17 µg/kg) an intake of 0.053 µg or 5.4% of the TDI was calculated. Consequently, the lower toxin burden of organic oat flakes resulted in about 25% of the intake by conventional oat flakes. It can be concluded from SCOOP and JECFA data [4, 32], that oats contribute only to about 15% to the total daily intake of T-2/HT-2. Wheat, however, which was shown to have considerably lower type A trichothecene contamination levels, contributes to more than 60% due to European consumption habits. Anyway, the contribution of oats to the daily exposure especially of the subpopulation of infants and young children should be considered because of its high contamination levels.

4 Concluding remarks

In this study, statistically significant differences between organic and conventional farming could be demonstrated for the first time for specific type A trichothecenes in representative oat samples. All samples which exceeded a level of 50 µg/kg for the sum of T-2 and HT-2 originated from conventional production. In comparison with T-2 and HT-2 levels found in oats originating from countries in Northern Europe [34, 35], the contamination levels obtained in this survey were remarkably lower. These levels, however, referred to de-hulled oats which differ remarkably from levels in raw oats [17, 18]. Anyway, T-2 and HT-2 as well as T-2 tetraol were shown to play a major role in the total trichothecene type A burden of oats and oat products. Since mean levels of T-2 tetraol were found to be even higher than that of T-2, the occurrence of this type A trichothecene should be further studied and evaluated.

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