Zeitschrift: Mitteilungen aus Lebensmitteluntersuchungen und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 96 (2005)

Heft: 6

Artikel: Estrogenic mycotoxins in the environment

Autor: Bucheli, Thomas D. / Erbs, Marianne / Hartmann, Niccolo

DOI: https://doi.org/10.5169/seals-981962

Estrogenic mycotoxins in the environment*

Thomas D. Bucheli, Marianne Erbs, Niccolo Hartmann, Susanne Vogelgsang, Felix E. Wettstein, Hans-Rudolf Forrer

Introduction

Zearalenone (ZON) is the most prominent of the resorcyclic acid lactones (RALs) produced by a variety of *Fusarium* fungi that grow on corn, wheat and other cereals. ZON has been known to be a potent natural estrogen for almost half a century, and plenty of effort has been made to understand the occurrence and behavior of RALs in food, feed and domestic animals. However, the environmental exposure to RALs has not yet been investigated in greater detail. This article aims at 1) summarizing the current limited knowledge on the presence of estrogenic mycotoxins in the environment, and 2) presenting preliminary results of our ongoing research activities on the environmental fate and behavior of estrogenic mycotoxins. The former part is subject to an extended Introduction, whereas the latter one will be presented in the Results and Discussion section.

The mycoestrogen and -toxin producing fungi *Fusarium* spp.: Mycotoxins are naturally occurring secondary metabolites of fungi growing on a wide variety of crops such as small grain cereals and corn. The problem of infestation of crops with mycotoxigenic fungi largely emerged with the increasing agricultural industrialization. In recent years the problem has worsened probably due to changed cultivation techniques, such as the increasing use of soil conservation instead of plough tillage and simplified crop rotations with a high fraction of cereals and corn, and, possibly, climate change. Among the most important mycotoxigenic fungi today are *Fusarium* spp. (1). They pose a severe economical threat, which in the US wheat and barley production of the 1990s led to losses of some three billion US$ (2). No exact figures are available for Europe, but Germany faced some severe losses of crops in 1987, 1993, and 1998 (3). In Switzerland, both yield and quality losses have increased in recent years (4). The current climate change towards warmer and moister conditions might provide even more fertile ground for infection by these pathogens in the future.

*Lecture presented at the 117th annual conference of the society of Food and Environmental Chemistry on 8 and 9 September 2005

The most important mycoestrogen-producing fungus, *Fusarium graminearum*, is the world's major causal agent for red ear rot in corn or *Fusarium* head blight in wheat, barley, rye, and oats (1, 5). Airborne ascospores of *F. graminearum* infect the corn ear via the silk channel, and small grain cereals via flowering ears. In cereals, the fungus may then appear as a pink to red mould along the edge of the glumes or at the base of the spikelets. In case of severe infections, other organs such as knots and leave sheets are affected as well. In corn, the cob, husks, and shank are attacked. The pathogen survives during the winter on plant residues such as wheat stubbles or corn stalks left on the field after harvest (6, 7). In general, however, little is known about the relative distribution of fungus and mycotoxin content on different plant organs. Hecker and co-workers found higher concentrations of the mycotoxin deoxynivalenol (DON) in wheat straw than in wheat corn (8), indicating that important emission sources of mycotoxins might hitherto have been overlooked.

The actual extent of *Fusarium* infestation – and, thus, possibly mycotoxin exposure – is determined by a variety of factors, the most important of which are: 1) climatic conditions (rainfall, air humidity, and temperature), 2) crop rotation, in particular wheat after corn, 3) reduced or no tillage (direct seeding) as opposed to ploughing, and 4) susceptibility of wheat varieties (9–11). Variations in wheat kernel content of DON under the investigated conditions can easily reach factors of ten and more (5, 8, 9, 11).

Fusarium species were occasionally also detected in building materials (12), and even sediments (13). The consequences of this potential occurrence of such fungi in other environmental compartments for their exposure with mycoestrogens and -toxins remain largely unclear.

Resorcyclic acid lactones (RALs): The isolation of an estrogenically active substance from cultures of *Gibberella zeae* (the sexual stage of *Fusarium graminearum)* was reported by *Stob et al.* (14). Later, *Christensen et al.* (15) isolated an estrogenic metabolite from an unidentified *Fusarium* sp. Its chemical structure was determined by *Urry et al.* (16) and later named zearalenone (ZON). ZON belongs to the chemical group of the RALs and is one of the world-wide most common mycotoxins

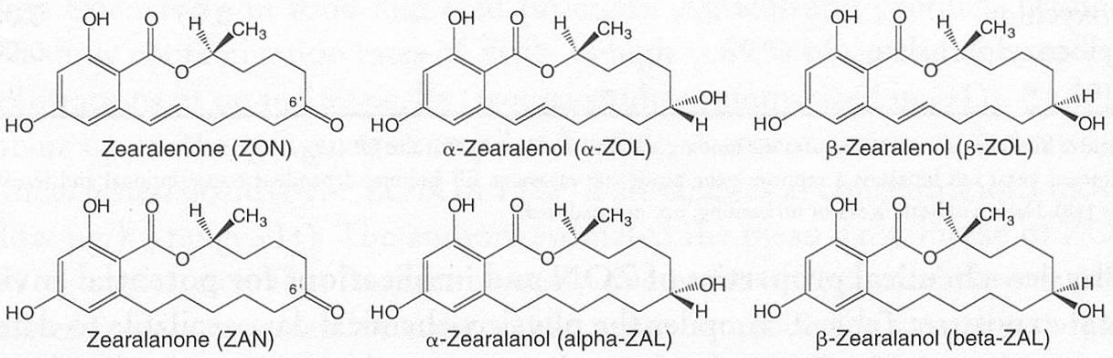

Figure 1 **Molecular structures of resorcyclic acid lactones (RALs)**

(e.g., (1)). Several RALs that can co-occur in fungal exudates of *F. graminearum* (and to a lesser extent *F. culmorum, F. crookwellense* and *F. equiseti),* or metabolites of ZON, are depicted in Figure 1. To date, RALs are the only known class of estrogenic mycotoxins. However, other structural classes might exist.

Estrogenic potential of RALs: The estrogenic activity of RALs is comparable with that of natural estrogens (e.g., (17)). In comparison with many notorious synthetic endocrine disruptors, their estrogen receptor (ER) binding affinities and their estrogenic potencies are several orders of magnitude higher (18, 19, Table 1). For a more detailed overview of respective *in vitro* studies, see (20). Due to its tremendous estrogenic potential, α-zearalanol (α-ZAL; commercial names: Zerapel, Ralgro, Figure 1), a ZON metabolite, is licensed as a growth promoter for ruminants in the USA and Canada (sales > 20 million US$ in 2000; (21)). In the EU, the product has been banned since 1988. In fact, the prevalent toxic effects of RALs are caused by their remarkable estrogenic activity and anabolic properties. They can lead to hyperestrogenism and severe reproductive and infertility problems in husbandry animals (e.g., (22, 23)).

Table 1
Relative ER binding affinities (RBAs) and relative estrogenic potencies (RP) of natural and synthetic estrogenic chemicals

	RBAs[a]	*RP*[b]		
ER from species:	*human*	*mouse*	*rainbow trout*	*human*
natural estrogenic steroids				
17β-estradiol	100	100	100	100
estrone	45	28	14	9.6
estriol	28	13	3.7	0.63
RALs				
ZON	9.3	12	82	0.26
α-ZOL	48	53	267	8.7
β-ZOL	13	11	91	0.066
Phytoestrogens				
coumestrol	0.81	0.33	0.24	0.67
genistein	0.46	0.33	0.44	0.049
synthetic endocrine disruptors				
bisphenol A	0.0080	0.0086	0.21	0.005
o,p'-DDT	–	0.0076	0.43	0.00011
methoxychlor	–	–	0.95	0.0033
buthylbenzylphthalate	–	–	–	0.0004
atrazine	–	–	–	n.a.

[a]competitive binding assay that measures the binding affinity of a chemical for the ER (19).

[b]recombinant yeast cell bioassay, a reporter gene assay that measures ER binding-dependent transcriptional and translational activity (18). Dashes indicate weak or no binding; n.a: not analysed.

Physico-chemical properties of ZON and implications for potential environmental exposure: Table 2 compiles the physico-chemical data available to date and compares them with those of other relevant organic compounds. Fundamental physico-chemical parameters such as boiling point and vapor pressure are – to the

best of our knowledge – lacking for the RALs. From the limited information available, ZON should be classified as only moderately water soluble and rather hydrophobic. Its aqueous solubility and octanol-water partition coefficient (K_{ow}) are in the same order of magnitude as those of estradiol and its derivatives (24). Test results of different cereal washing procedures revealed that, despite its limited water solubility, up to 61 % of ZON (and up to the 69 % of the more water soluble DON) is desorbed from contaminated corn kernels into distilled water (25). This indicates some environmental aqueous phase mobility. Simultaneously, with an estimated log K_{ow} of 3.6 (Table 2), ZON very likely exhibits a certain potential for sorption and retention in soil systems.

Table 2
Chemico-physical properties of selected mycotoxins and other organic pollutants

compound	*aqueous solubility (mg/L)*	*log Kow (–)*	*pKa (–)*	*vapor pressure (Pa)*	*Henry constant ($atmLmol^{-1}$)*
ZON	2.6–4.8[a]	3.7[a]	7.6[a]	n.a.	n.a.
deoxynivalenol (DON)	n.a.	–0.7[b]	–	n.a.	n.a.
17β-estradiol	3.6[b], 13[c]	3.1–4.0[d]	–	1.7E–6[b]	1.3E–6[e]
atrazine	33[f]	2.5[f]	–	3.9E–5[f]	2.7E–6[e]

[a](42). [b]Syracuse Research Corporation: PHYSPROP database (http://www.syrres.com/esc/physprop.htm). [c](24). [d](43). [e]calculated from aqueous solubility and vapor pressure. [f](44). n.a.: not available.

As the (abiotic) stability of ZON during milling, food processing, heating, etc. is considerably high (e.g., (26)), it is reasonable to assume that this compound is rather persistent in the environment as well. Data on biotic transformation of ZON generally indicate as a main metabolization pathway the reduction of the 6'-keton to yield α- and β-zearalenol (ZOL; e.g., (27); Figure 1). The fractions of the two isomers are species-dependent. This is of relevance for the overall estrogenic activity, as in general the estrogenic potential of RALs decreases in the following order: α-ZOL >ZON>β-ZOL. Mammalian excretion of RALs is reported to occur to a significant extent via urine (28–30).

Occurrence of ZON in food and feed: Owing to their considerable risks to human and animal health and the economy, the occurrence of mycotoxins has been studied extensively in food and feed products. Agricultural products around the world show contamination rates of ZON as high as 69 % of the tested samples with concentrations of up to 180 µg/kg (several studies summarized in (31)). Even larger numbers up to 21 mg/kg were compiled by (32). A recent overview in Swiss cereal products tested positive for ZON in 13% of all samples at concentrations mostly in the low µg/kg range (31). The authors estimated the mean daily intake of ZON to be <1 µg/capita/day. This is approximately one order of magnitude lower than the evaluated temporary tolerable daily intake value issued by the scientific committee on food of the European Commission (33). In general, the results point to a frequent and global occurrence of RALs in food and feed products. However, as the

Table 3
Compilation of RALs detected in wastewater treatment plant (WWTP) and surface waters

concentration (ng/L)			*sample number*	*detected in x samples*	*sampling period*	*sample type*	*location*	*remarks*	*reference*
minimum	*median/ mean*	*maximum*							
ZON									
1	4 (mean)	7	10	4	March–July 2000	WWTP effluent	Genzano-RM, Italy	–	(35)
n.d.	n.d.	36	21	1	August 1998–December 1999	WWTP effluents	Hechingen, discharge into River Neckar, Germany	detected October–December 1999	(37)
<10 (LOQ)	<10 (LOQ)	60	10	1	1998–1999	river water	various rivers in Hessen, Germany	detected in River Kinzig at Hanau	(38)
3	15 (median)	18	7	n.a.	March–May 2002	WWTP influent	Rome, Italy	–	(34)
3	7 (median)	10	7	n.a.	March–May 2002	WWTP effluent	Rome, Italy	–	(34)
2	3 (median)	5	7	n.a.	March–May 2002	river water	River Tiber, Rome, Italy	downstream of the WWTP	(34)
α-ZOL									
1	7 (mean)	11	10	5	March–July 2000	WWTP effluent	Genzano-RM, Italy	–	(35)
<10 (LOQ)	<10 (LOQ)	31	24	2	1998–1999	WWTP effluents	various WWTP in Hessen and Niedersachsen, Germany	location of positive samples not specified	(38)
<10 (LOQ)	<10 (LOQ)	30	10	3	1998–1999	river water	various rivers in Hessen, Germany	detected in River Kinzig at Hanau, River Schwarzbach at Taunus and Astheim	(38)
–	–	–	30	1	1	January 2000	filtered WWTP effluents	discharge into River Neckar	–	(36)
–	–	–	20	1	1	January 2000	filtered WWTP effluents	discharge into River Rhine	–	(36)

concentration (ng/L)			sample number	detected in x samples	sampling period	sample type	location	remarks	reference
minimum	*median/ mean*	*maximum*							
α-ZAL									
n.d.	3 (median)	10	7	n.a.	March–May 2002	WWTP influent	Rome, Italy	–	(34)
n.d.	3 (median)	7	7	n.a.	March–May 2002	WWTP effluent	Rome, Italy	–	(34)
n.d.	1 (median)	3	7	n.a.	March–May 2002	river water	River Tiber, Rome, Italy	downstream of the WWTP	(34)
β-ZAL									
n.d.	3 (median)	8	7	n.a.	March–May 2002	WWTP influent	Rome, Italy	–	(34)
n.d.	2 (median)	5	7	n.a.	March–May 2002	WWTP effluent	Rome, Italy	–	(34)
n.d.	1 (median)	3	7	n.a.	March–May 2002	river water	River Tiber, Rome, Italy	downstream of the WWTP	(34)

n.d.: not detected; n.a.: no information; LOQ: limit of quantification

contamination of, e.g., wheat and corn, is most likely not limited to the edible plant organ (6–8), such surveys probably only consider the "tip of the iceberg" of the overall RAL exposure.

Occurrence of ZON in surface waters: Several recent publications reported the occurrence of RALs in surface waters (34–38). These data are compiled in Table 3. Concentrations of ZON, as well as its derivatives α-ZOL, α-ZAL and β-ZAL, ranged from not detected up to 36 ng/L. At one occasion, even a number of 60 ng/L was reported for ZON. All of these four RALs were found in wastewater treatment plant (WWTP) influents and effluents, as well as in river waters (Table 3). Two other RALs, namely β-ZOL and ZAN, were either never detected (35, 38), or not analyzed (34, 36, 37). The compounds appear to be present in surface waters throughout the year. However, the limited data available does not allow for a distinction of seasonal patterns. To address the ecotoxicological significance of the presence of RALs in such waters, it is useful to compare their concentrations at the low ng/L level with such of other endocrine disruptors. Specifically, numbers in the same order of magnitude were found for the natural estrogenic steroids (20, 39). Hence, given the comparable hormone activity of the non-steroidal RALs and natural estrogens (Table 1), the former might contribute substantially to the overall estrogenic activity in such waters.

Unfortunately, in a majority of the papers listed in Table 3, the authors did not elucidate the potential emission sources of the detected RALs, although they went to great lengths to analyze them at trace concentrations. Only *Lagana et al.* (34) suspected the presence of RALs to be primarily caused by cattle excretion of growth promoting residues.

Possible pathways into the environment: The above outlined physico-chemical properties, together with recent results from our research institution and the few findings from environmental studies, suggest that these compounds are very likely to be emitted into the environment. Several pathways appear plausible and are illustrated in Figure 2:

1) toxins released from *Fusarium*-infested plants might
   a. contaminate and penetrate the soil and infiltrate into groundwater,
   b. elute by surface runoff or subsurface drainage to surface waters or WWTPs,
   c. evaporate or drift off on air-borne fungal spores or soil particles,
2) toxin residues in excrements of exposed livestock might enter agricultural soils and local waters directly or via application of manure,
3) food-industry wastewater and/or human excretions might introduce toxin residues via sewer systems into surface waters.

Our current research activities within the National Research Program 50 "Endocrine disruptors: Relevance to Humans, Animals, and Ecosystems" have so far focused on the first of the above indicated pathways and preliminary results are presented below.

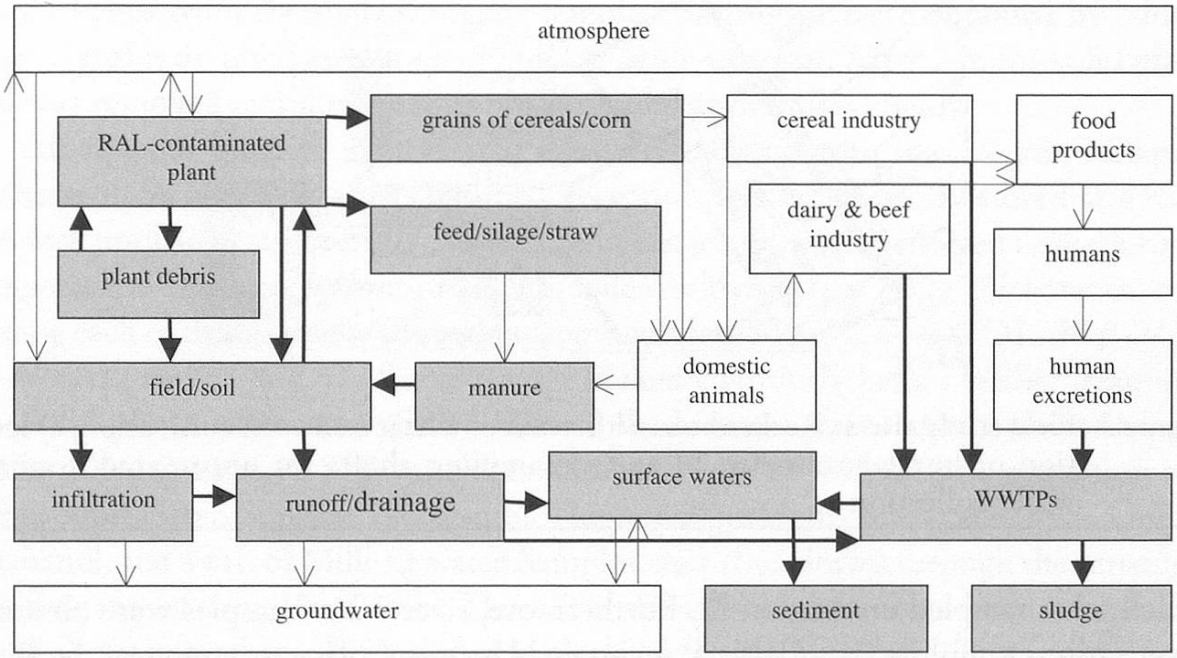

Figure 2 **Hypothesized environmental distribution of RALs.** Grey boxes and thick arrows indicate possible relevant compartments and pathways, respectively, and are subject to primary investigation

Methods

Materials: 3-, and 15-acetyldeoxynivalenol (Ac-DON), diacetoxyscirpenol (DAS), DON, fusarenone X (FUS-X), HT-2 toxin (HT-2), nivalenol (NIV), T-2 toxin (T-2), zearalanone (ZAN), α-ZAL, β-zearalanol (β-ZAL), α-ZOL, β-ZOL, and ZON were obtained from Sigma-Aldrich (Buchs, Switzerland). The deuterated internal standards d4-α-ZAL, d4-β-ZAL d4-α-ZOL, and d4-β-ZOL were from RIVM (Bilthoven, The Netherlands). The d6-ZON was produced in our laboratory by base catalyzed hydrogen-deuterium exchange on native ZON following the procedure described in (40). Purity of the deuterated product was controlled with LC-MS analysis. Methanol (99.98 %) and acetonitrile (99.99 %) were purchased from Scharlau (Barcelona, Spain), and ammonium acetate was from Merck AG (Dietikon, Switzerland). Deionized water was further purified with a Milli-Q gradient A10 water purification system (Millipore, Volketswil, Switzerland). Nitrogen (4.5) was from PanGas (Dagmarsellen, Switzerland).

Samples: Drainage water samples were taken during July 2005 from our field study site at Zürich Reckenholz (Figure 3), using several ISCO samplers (Teledyne Isco Inc., Lincoln NE, U.S.A.). The waters consisted in essence of rain water percolating a 20 acre winter wheat plot artificially infected with a mixture of five different *Fusarium graminearum* isolates on June 9 2005 (resulting in approximately 74 % visually infected individual plants as determined on June 29 2005). The heavy rainfalls during the second half of August resulted in the formation of surface water pools,

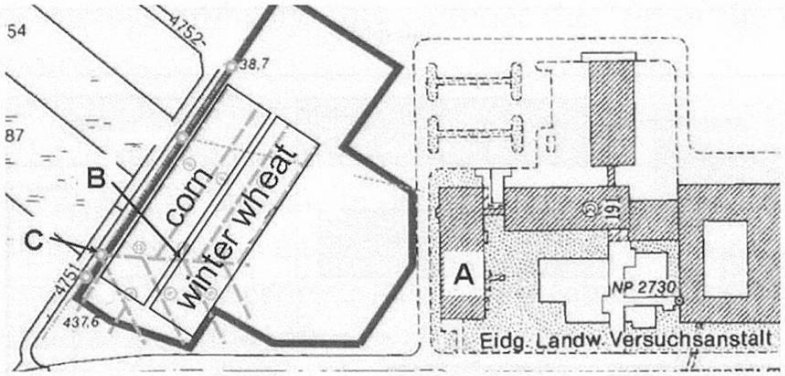

Figure 3 **Field study site at Reckenholz with areas of wheat and corn cultivation; a) location of our laboratories; b) and c) sampling shafts for automated drainage water collection**

which were sampled on August 22. Furthermore, river water samples were obtained from various sampling sites (Table 4) maintained by monitoring programs of the Swiss government (National Long-Term Surveillance of Swiss Rivers; NADUF) and the Canton of Zürich (Office for Waste, Water, Energy, and Air; AWEL).

Table 4
Surface water monitoring sites of the NADUF and AWEL monitoring program

	average flow Q (m^3s^{-1})	*approx. area of wheat cultivated in the catchment* (km^2)	*ratio*
River Töss[a]			
Zell	3.2	1.1	0.4
Winterthur (Kempt)	1.1	3.9	3.5
Wülflingen (Eulach)	0.8	5.0	6.1
Freienstein	8.4	15.3	1.8
Rämismühle	3.2	1.1	0.4
River Glatt[a]			
Mönchaltorf (Aabach)	0.9	1.8	1.9
Niederuster (Aa)	1.5	3.1	2.0
Fällanden	3.8	6.0	1.6
Rümlang	5.6	11.4	2.0
Rheinsfelden	7.1	17.4	2.5
River Thur at Andelfingen	60.2	31.6	0.5
River Saane at Gümmenen	54	–	–
River Klein-Emme at Littau	16	–	–
River Aare at Brugg	319	–	–
River Reuss at Mellingen	137	–	–
River Rhein at Rekingen	436	71.9	0.16

[a]tributaries in parentheses; –: not known; NADUF: National Long-Term Surveillance of Swiss Rivers; AWEL: Office for Waste, Water, Energy, and Air, Canton Zürich

Ears, leaves, and stalks were sampled several times during winter wheat cultivation. Eluates from these plant organs sampled on July 11 were obtained by adding 3 to 10 g of individual samples to 50 mL of Milli-Q water. After 2 h, the solid material was removed and the aqueous phase treated as described below.

All aqueous samples were filtered (glass fibre filters, pore size 1.2 µm, Millipore Volketswil, Switzerland) after collection/reception and subsequently stored at 4 °C in the dark until analysis. Prior to analysis, the water samples were allowed to reach room temperature. The exact volume of 1 L was spiked with 50 µL of a 2 µg/mL solution containing each of the following deuterated compounds: d6-ZON, d4-α-ZOL, d4-β-ZOL, d4-α-ZAL, and d4-β-ZAL. Samples were shaken vigorously before further treatment.

Solid phase extraction (SPE): SPE of the water samples was performed using 6 mL, 500 mg, Envi-18 SPE cartridges mounted on a 12-fold vacuum extraction box (all products from Supelco, Bellafonte). The cartridges were conditioned with 5 mL of methanol, and 4 mL of Milli-Q water. Samples were then drawn through the cartridges at a flow rate of max. 10 mL/min. Thereafter, the solid phase was washed with 2 mL of Milli-Q water/methanol (95:5 v/v), and air-dried for at least 10 min. The analytes were then eluted with 1.8 mL of methanol. The eluate was concentrated by evaporating the solvent to dryness using a gentle stream of nitrogen for approx. 30 min. The dried extract was reconstituted in 300 µL of Milli-Q water/methanol (80/20 v:v).

Analysis: Liquid chromatography/tandem mass spectrometry (LC/MSMS) was performed with a Varian 1200L LC-MS System (VarianInc, Walnut Creek, CA). Separation of the RALs was achieved by injecting 50 µL of the extract onto a Polaris Amide C18 column (3 µm, 150×2.0 mm; VarianInc, Walnut Creek, CA) and applying the following elution gradient: 3 min at 100 % eluent A, to 40 % eluent B in 1 min, to 80 % eluent B in 30.5 min, to 100 % eluent B in 0.5 min, 4 min at 100 % eluent B; with eluent A consisting of Milli-Q water/acetonitrile (95/5 v:v) and eluent B of Milli-Q water/acetonitrile (5/95 v:v). Both eluents were buffered with 10 mM ammoniumacetate. The mobile phase flow rate was 0.2 mL/min. Retention times are indicated in Table 5. Interface parameters of the LC-MSMS were as follows: needle voltage: –2000 V, nebulizing gas: 61 psi, capillary voltage: –54 V, drying gas: 200 °C and 23 psi, shield voltage: –600 V. Detection of the RALs was performed in the ESI- mode using the ions specified in Table 5. The CID gas pressure was 1.5 mTorr. The detector voltage was set to 2000 V. Method detection limits (signal to noise ratio of three) were around 1 ng/L for each of the investigated RALs, and linearities were confirmed up to 500 ng/L. The further analytical figures of merit are currently determined in our laboratories. Although the method is not thoroughly validated to this end, the use of deuterated RALs as internal standards assures its robustness.

Selected extracts obtained as described above were also analyzed for trichothecenes. Separation of these compounds was achieved by injecting 50 µl of the extract onto a Polaris C18-A (3 µm, 50×2.0 mm; VarianInc. Walnut Creek, CA) applying the following gradient: 2 min at 5 % eluent B, to 100 % B in 15 min, 18 min at 100 % B; with eluent A consisting of Milli-Q water/methanol (95/5 v:v) and elu-

Table 5
Precursor and multiple reaction mode daughter ions of the investigated mycotoxins and their deuterated internal standards

compound	*ionization mode*	*retention time (min)*	*molecular weight (Da)*	*precursor ion (m/z)*	*daughter ions*			
					quantifier (m/z)	*collision cell voltage (V)*	*qualifier (m/z)*	*collision cell voltage (V)*
RALs								
d4-β-ZAL	ESI–	19.0	326.4	325	281	25	263	20
β-ZAL	ESI–	19.1	322.4	321	277	15	303	20
d4-β-ZOL	ESI–	19.6	324.4	323	279	20	174	30
β-ZOL	ESI–	19.7	320.4	319	275	20	160	30
d4-α-ZAL	ESI–	21.9	326.4	325	281	25	163	20
α-ZAL	ESI–	22.0	322.4	321	277	15	259	25
d4-α-ZOL	ESI–	23.1	324.4	323	280	25	133	30
α-ZOL	ESI–	23.2	320.4	319	160	25	188	25
ZAN	ESI–	25.4	320.4	319	275	20	205	25
d6-ZON	ESI–	25.9	324.4	323	134	30	279	20
ZON	ESI–	26.1	328.4	317	131	26	175	20
trichothecenes								
NIV	APCI–	1.4	312.3	371	311	7	281	7
DON	APCI–	2.5	296.3	355	295	9	265	9
FUS-X	APCI–	5.2	354.4	413	263	11	205	11
Ac-DON	APCI–	7.1	338.4	337	307	20	217	20
DAS	APCI+	9.8	366.4	384	307	–12	247	–12
HT-2	APCI+	11.6	424.5	442	263	–12	215	–12
T-2	APCI+	12.7	466.5	484	215	–18	185	–18

ent B of Milli-Q water/methanol (5/95 v:v). Both eluents contained 20 mM ammoniumacetate. The mobile phase flow rate was 0.2 mL/min. Detection of the trichothecenes was performed with the APCI-interface using negative and positive modes and ions as specified in Table 5. Interface parameters were as follows: housing temperature: 50 °C, corona current: –10 µA (negative mode) and +7.5 µA (positive mode), spray gas: 300 °C and 59 psi, drying gas 200 °C and 18 psi, shield voltage –600 V (negative mode) and +525 V (positive mode). The CID gas pressure was 1.5 mTorr. The detector voltage was set to 1800 V. Note that the method has not yet been validated for trichothecenes and all data must be considered semi-quantitative.

Results and Discussion

Presence of ZON in different aqueous field compartments: Figure 4 presents the ZON concentrations found in different wheat organ eluates, a surface water pond on the field site at Reckenholz, as well as the mean observed in its drainage water during July 2005. Hereby, the organ eluates concentrations might be considered as "worst-case". They might represent the total amount of ZON that is available for wash-off, for instance in situations of prolonged and intense rainfall, or over the total crop season from infection to harvest. In contrast, the surface water sample gives a direct indication of maximum concentrations to be expected in local

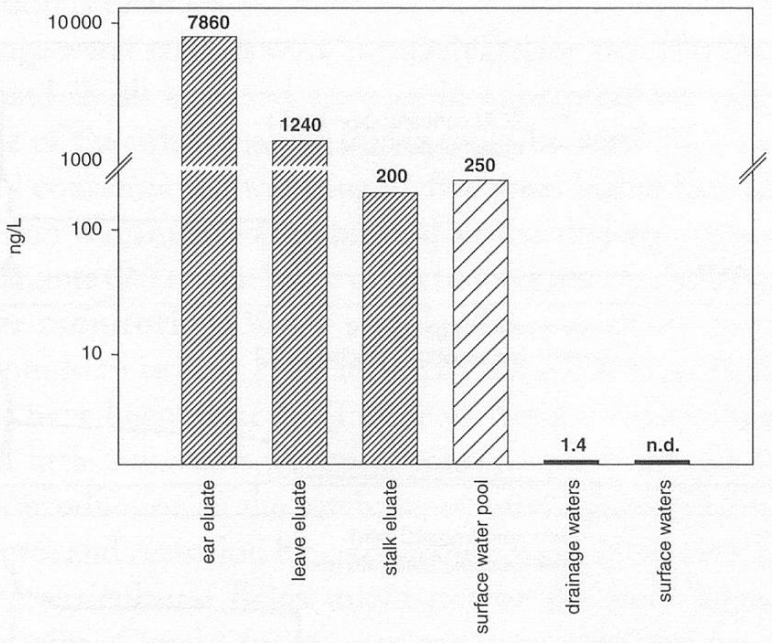

Figure 4 **Concentrations of ZON measured in wheat organ eluates (sampled July 11; n = 1 for each organ), a surface water pool (sampled August 22, n = 1), and drainage waters (July 7–28, mean value as determined by division of total ZON load by total water discharge) of the field study site at Reckenholz. ZON was never detected in the various surface waters sampled weekly from May to July 2005 (for locations, see Table 4)**

catchments of *Fusarium*-infected fields that are prone to surface runoff. The concentration range observed in drainage water stands for situations where surface runoff, and probably preferential flow into the subsurface, is limited. Note that there is more than one order of magnitude concentration difference between different wheat organ eluates and the surface water pool, as well as between surface and drainage waters. To this end, it remains unclear whether the considerable concentration gap between the latter two might be caused by dilution of percolating water with drainage water originating from adjacent areas that were not infected with *Fusarium*, efficient sorption of ZON to soil, or simply different periods of investigation (July (pre-harvest) vs. August (post-harvest)).

Figure 5 depicts the temporal development of precipitation onto the field site at Reckenholz (a), the resulting drainage water discharge from the field (b), and the ZON concentrations in these waters (c) during July 2005, encompassing three major rain events. The first one around July, 11 deposited some 25 mm of rain water. As a consequence of the dry and sunny weather situation during the weeks before, this water was almost fully absorbed by the dry soil. The second rain event around July 19 (Figure 5a) with some 30 mm of precipitation resulted in an at least partial water saturation of the soil, and hence, the initiation of substantial runoff via the drainage system. This situation was even more pronounced during July 25–26, where an equal amount of rain water caused a three-fold increase of discharged drainage water (Figure 5b). During the whole period, ZON was detected in the drainage water at concentrations between one

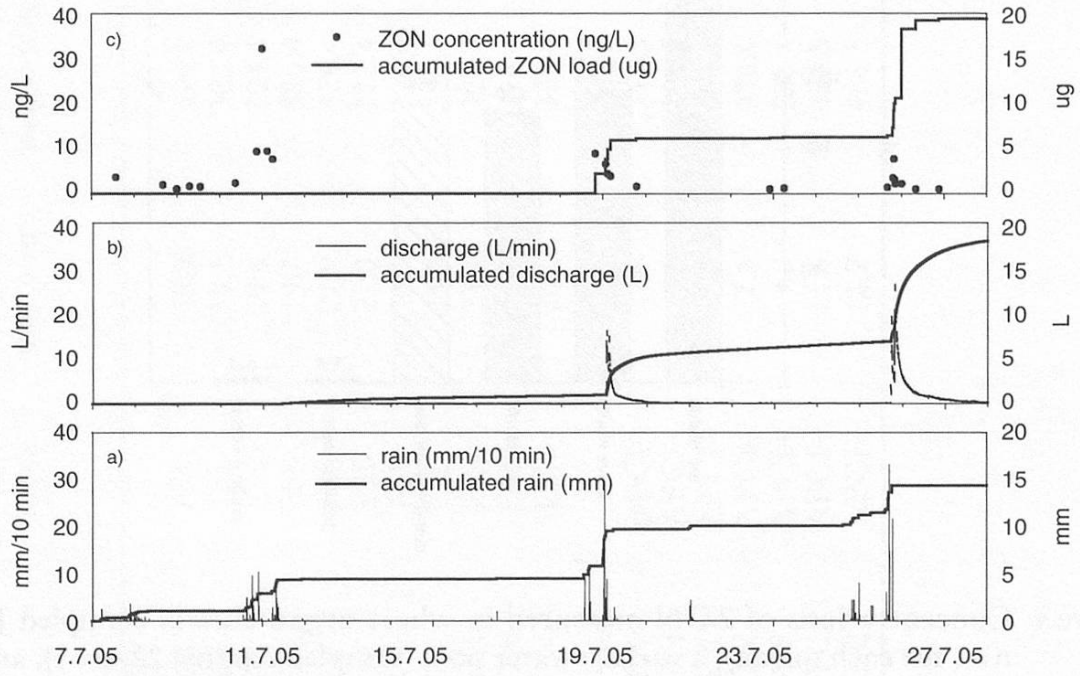

Figure 5 **Precipitation (a), drainage water discharge (b) and ZON concentrations and cumulative load in drainage waters (c) during July 2005 at the field study site at Reckenholz**

to 32 ng/L (Figure 5c). Generally, concentrations were elevated at the onset of the rain events, whereas some dilution occurred later on. It remains to be investigated what key mechanisms determine the dynamics of ZON in such waters. Presumably, the "classical" environmental processes which control, e.g., pesticide behavior, such as hydrodynamics and water-solid phase distribution, might be superimposed by additional mycological processes such as mycotoxin production and release. None of the other RALs were detected in drainage waters. In summary, some 100 mm of precipitation (Figure 5a, right axis) caused a drainage water discharge of roughly 10000 L (Figure 5b, right axis), in which a total load of about 20 µg of ZON (Figure 5c, right axis) was introduced during July 2005 into the surface waters downstream of the field. It is illustrative to compare this amount of about 0.7 µg/d, e.g., with the input of human natural steroid hormones into surface waters. In the case of 17β-estradiol, an average excretion of 3.3 µg/capita/d was estimated to reach the sewer. With an estimated removal of 82 % in activated sludge plants, this would result in an input of some 0.6 µg/capita/d into surface waters (41).

The temporal development of RALs in drainage waters from the field study site at Reckenholz will be further monitored to investigate their emission caused by other agricultural activities under the wheat-corn crop rotation. Specifically, corn is host of *F. graminearum* and infected fields could be a more severe source for environmental ZON contaminations. Contrary to wheat where the straw is collected and used in animal husbandry, huge amounts of straw remain in the field when corn for grain production is produced.

Selected drainage water samples were also analyzed for a set of trichothecenes. DON was regularly found in all analyzed samples in concentrations ranging from 10 to 60 ng/L, but none of the other trichothecenes could be detected. In a majority of the samples, the DON concentration was roughly five times higher than that of ZON, and similar concentration dynamics were observed in the drainage water. This is to our knowledge the first time that the presence of trichothecenes in natural waters is reported.

Surface water monitoring: Water samples from locations specified in Table 4 were collected from May to July 2005 and analyzed for RALs. To this end, none of these compounds have been detected (Figure 4). Several reasons might account for this, such as: too little *Fusarium* infection rates possibly due to dry weather and hence little ZON production in the catchments, too large water dilution factors, or efficient sorption to, and retention by, agricultural soils. Moreover, on a larger scale, *Fusarium*-infected agricultural fields might not be the only important source of estrogenic mycotoxins. Clearly, future research must take into consideration alternative sources of RALs as outlined in the Introduction Section to account for the occasionally observed concentrations in the low ng/L range in surface waters.

Acknowledgments

This project is part of the National Research Program 50 "Endocrine disruptors: Relevance to Humans, Animals, and Ecosystems" and financed by the Swiss

National Science Foundation. We are grateful to Irene Bänziger, Andi Hecker, Effi Jenny, Tomke Musa, Hans-Heinrich Rast, and Karl Roth for their invaluable advice and support with field work, crop cultivation and *Fusarium* inoculum production and infection. Bernhard Luder, Adrian Jakob (Swiss Federal Office for Water and Geology), Barbara Känel, Walo Meier, Peter Spohn (all from the Office for Waste, Water, Energy, and Air of the Canton of Zürich), and Jörg Zobrist (EAWAG) are kindly acknowledged for their collaboration in surface water monitoring. We thank Chistian Stamm, Heinz Singer, and Alfred Lück (EAWAG) for collaboration on the field site at Reckenholz. Thanks due to Jean Paul for the French translation of the Summary.

Summary

Fungal pathogens of the genus *Fusarium* attack cereals such as corn or wheat und produce toxic metabolites, so-called mycotoxins. Globally, reports on *Fusarium* infection and mycotoxin contamination of cereals have increased. Possible causes are shifts in crop cultivation practices as well as climate change. Some *Fusarium* fungi produce also zearalenone (ZON) and similar metabolites. These compounds are potent natural estrogens. Their risk for human and animal health has been recognized for several decades, hence, food and feed products are frequently analyzed for ZON and other mycotoxins. However, it is likely that mycotoxins such as ZON are also introduced into the environment. Possible pathways are runoff from contaminated fields, wastewaters from the food and feed industry, the application of a ZON-metabolite as a growth promoter for ruminants, as well as human and animal excretions. In this article, we summarize the currently limited knowledge on the occurrence of estrogenic mycotoxins in the environment, and present some preliminary results of our ongoing research activities in this area. Specifically, we provide first-time evidence for the introduction of estrogenic mycotoxins into surface waters in the low ng/L concentration range via drainage waters from a field with *Fusarium*-infested wheat.

Zusammenfassung

Pilze der Gattung *Fusarium* befallen viele Kulturpflanzen wie Mais, Weizen oder andere Getreide und bilden giftige Stoffwechselprodukte, sogenannte Mykotoxine. Weltweit wird zunehmend von *Fusarium*-Infektionen und Pilzgiftbelastungen bei Getreide berichtet. Mögliche Ursachen könnten veränderte Anbautechniken, aber auch der Klimawandel sein. Neben anderen Pilzgiften produzieren einzelne *Fusarium*-Arten auch das östrogen wirksame Zearalenon (ZON), dessen Gefahr für Mensch und Tier seit Jahrzehnten bekannt ist. Nahrungs- und Futtermittel werden deshalb immer wieder auf ZON und andere Pilzgifte untersucht. Darüber hinaus könnten diese Substanzen aber auch in die Umwelt gelangen. Mögliche Eintragspfade sind die Abschwemmung aus Feldern mit pilzbefallenem Getreide, Abwässer der Nahrungs- und Futtermittelindustrie, der Einsatz eines ZON-Metaboliten als

Wachstumsförderer in der Viehzucht oder menschliche und tierische Ausscheidungen. Die vorliegende Arbeit bietet einen ersten Einblick über das Auftreten von östrogenen Mykotoxinen in der Umwelt und berichtet über erste Resultate unserer aktuellen Untersuchungen zu diesem Thema. Im Besonderen konnten wir erstmals den Eintrag von östrogenen Mykotoxinen in Oberflächengewässer über Drainagewässer belegen: in einem Feld mit *Fusarium*-infiziertem Weizen haben wir regelmässig ZON im tiefen ng/L Konzentrationsbereich nachgewiesen.

Résumé

Des pathogènes fongiques de l'espèce *Fusarium* attaquent les céréales tels le maïs et le blé et produisent des métabolites toxiques appelés mycotoxines. Globalement, le nombre de rapports sur l'infection et la contamination des céréales par le *Fusarium* ont augmenté. Les causes possibles sont des changements climatiques et des mesures culturales. Certains champignons de *Fusarium* produisent aussi zéaralénone (ZON) et des métabolites similaires. Les risques résultants pour les populations humaines et animales one été reconnus depuis plusieurs décades; c'est la raison pour laquelle les produits céréaliers et de fourrage sont souvent analysés pour ZON et autres mycotoxines. Cependant, il est fort probable que des mycotoxines comme ZON sont aussi introduites dans l'environnement. Des voies possibles sont les eaux de ruissellement en provenance de champs contaminés, les eaux usées des industries alimentaires et fourragères, l'application d'un métabolite ZON afin de favoriser la croissance des ruminants et finalement les excréments humains et animaux. Dans cet article, nous résumons le savoir limité actuel sur l'occurrence des mycotoxines estrogènes dans l'environnement, de même que de la recherche en cours effectuée dans nos laboratoires. Spécifiquement, nous avons pu démontrer pour la première fois l'introduction de mycotoxines estrogènes, au niveau de concentration des faibles ng/L, dans les eaux de ruissellement en provenance du drainage d'un champ de blé infesté par le *Fusarium.*

Key words

Endocrine disruptors, resorcyclic acid lactones, deoxynivalenol, runoff, field study

Literature

1 *Chelkowski J.:* Distribution of *Fusarium* species and their mycotoxins in cereal grains, in: Mycotoxins in agriculture and food safety. Marcel Dekker, Inc., New York, 45–64 (1998)

2 *Windels C.E.:* Economic and social impacts of *Fusarium* head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology, **90**, 17–21 (2000)

3 *Verreet J.A. und Aumann J.:* Mykotoxinproblematik in der Pflanzenproduktion. Schriftenreihe der Agrar- und Ernährungswissenschaftlichen Fakultät der Universität Kiel, **94**, 12–17 (2002)

4 *Jenny E., Hecker A., Kessler P., Külling C. und Forrer H.R.:* Getreidefusariosen: Sortenresistenz und Toxingehalte. Agrarforschung, **7**, 270–273 (2000)

5 *Parry D.W., Jenkinson P. and McLeod L.: Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol, **44**, 207–238 (1995)

6 *Sutton J.C.:* Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum.* Can J Plant Pathol, **4**, 195–209 (1982)
7 CAST, Mycotoxins: risks in plant, animal, and human systems. Task Force Report No. 139. Council for Agricultural Science and Technology, Ames, Iowa, USA, (2003)
8 *Hecker A., Bänziger I., Jenny E., Forrer H.R. und Vogelgsang S.:* Weniger Fusarien-Toxin durch geeignete Sortenwahl? Agrarforschung, **11**, 384–389 (2004)
9 *Krebs H., Dubois D., Külling C. und Forrer H.R.:* Fusarien- und Toxinbelastung des Weizens bei Direktsaat. Agrarforschung, **7**, 264–268 (2000)
10 *Schachermayr G. und Fried P.M.:* Problemkreis Fusarien und ihre Mykotoxine. Agrarforschung, **7**, 252–257 (2000)
11 *Tischner H. und Doleschel P.:* Einflussfaktoren auf den Befall und die Toxinbildung durch Ährenfusarien an Weizen. Getreidemagazin, **8**, 68–74 (2003)
12 *Tuomi T., Reijula K., Johnsson T., Hemminki K., Hintikka E.L., Lindroos O., Kalso S., Koukila-Kahkola P., Mussalo-Rauhamaa H. and Haahtela T.:* Mycotoxins in crude building materials from water-damaged buildings. Appl Environ Microbiol, **66**, 1899–1904 (2000)
13 *Ueno Y., Ishii K., Sawano M., Ohtsubo K., Matsuda Y., Tanaka T., Kurata H. and Ichinoe M.:* Toxicological approaches to metabolites of Fusaria.11. Trichothecenes and zearalenone from Fusarium species isolated from river sediments. Jpn J Exp Med, **47**, 177–184 (1977)
14 *Stob M., Baldwin R.S., Gillette K.G., Andrews F.N. and Tuite J.:* Isolation of an anabolic, uterotrophic compound from corn infected with *Gibberella zeae.* Nature, **196**, 1318 (1962)
15 *Christensen C.M., Nelson G.H. and Mirocha C.J.:* Effect on White Rat uterus of a toxic substance isolated from *Fusarium.* Appl Microbiol, **13**, 653–659 (1965)
16 *Urry W.H., Wehrmeister H.L., Hodge E.B. and Hidy P.H.:* Structure of zearalenone. Tetrahedron Lett, **7**, 3109–3114 (1966)
17 *Le Guevel R. and Pakdel F.:* Assessment of oestrogenic potency of chemicals used as growth promoter by in-vitro methods. Hum Reprod, **16**, 1030–1036 (2001)
18 *Coldham N.G., Dave M., Sivapathasundaram S., Mcdonnell D.P., Connor C. and Sauer M.J.:* Evaluation of a recombinant yeast cell estrogen screening assay. Environ Health Persp, **105**, 734–742 (1997)
19 *Matthews J., Celius T., Halgren R. and Zacharewski T.:* Differential estrogen receptor binding of estrogenic substances: a species comparison. J Steroid Biochem, **74**, 223–234 (2000)
20 *Bätscher R., Studer C. und Fent K.:* Umweltgefährdende Stoffe: Stoffe mit endokriner Wirkung in der Umwelt. Schriftenreihe Umwelt Nr. 308. Bundesamt für Umwelt, Wald und Landschaft (BUWAL), Bern, (1999)
21 *Smith R.:* Top 20 animal health and nutrition products. Changes continue going into 2001. Feedstuffs. The weekly newspaper for agribusiness, **73**, April 23 (2001)
22 *Kuiper-Goodman T., Scott P.M. and Watanabe H.:* Risk assessment of the mycotoxin zearalenone. Regul Toxicol Pharm, **7**, 253–306 (1987)
23 *Gaumy J.L., Bailly J.D., Burgat V. and Guerre P.:* Zearalenone: properties and experimental toxicity. Rev Med Vet-Toulouse, **152**, 219–234 (2001)
24 *Lai K.M., Johnson K.L., Scrimshaw M.D. and Lester J.N.:* Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. Environ Sci Technol, **34**, 3890–3894 (2000)
25 *Trenholm H.L., Charmley L.L., Prelusky D.B. and Warner R.M.:* Washing procedures using water or sodium-carbonate solutions for the decontamination of 3 cereals contaminated with deoxynivalenol and zearalenone. J Agr Food Chem, **40**, 2147–2151 (1992)
26 *Ryu D., Hanna M.A., Eskridge K.M. and Bullerman L.B.:* Heat stability of zearalenone in an aqueous buffered model system. J Agr Food Chem, **51**, 1746–1748 (2003)
27 *Galtier P.:* Biotransformation and fate of mycotoxins. J Toxicol-Toxin Rev, **18**, 295–312 (1999)
28 *Fitzpatrick D.W., Arbuckle L.D. and Hassen A.M.:* Zearalenone metabolism and excretion in the rat – effect of different doses. J Environ Sci Heal B, **23**, 343–354 (1988)

29 *Kleinova M., Zollner P., Kahlbacher H., Hochsteiner W. and Lindner W.:* Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers. J Agr Food Chem, **50**, 4769–4776 (2002)
30 *Danicke S., Swiech E., Buraczewska L. and Ueberschar K.H.:* Kinetics and metabolism of zearalenone in young female pigs. J Anim Physiol An N, **89**, 268–276 (2005)
31 *Rhyn P. and Zoller O.:* Zearalenone in cereals for human nutrition: relevant data for the Swiss population. Eur Food Res Technol, **216**, 319–322 (2003)
32 *Pittet A.:* Natural occurrence of mycotoxins in foods and feeds – an updated review. Rev Med Vet-Toulouse, **149**, 479–492 (1998)
33 European Commission, Opinion of the scientific committee on food on *fusarium* toxins. Part 2: Zearalenone. Health and consumer protection directorate-general; Directorate C – Scientific Opinions; C3-Management of scientific committees II; scientific co-operation and networks, **SCF/CS/CNTM/MYC/22 Rev 3 Final**, (2000)
34 *Lagana A., Bacaloni A., De Leva I., Faberi A., Fago G. and Marino A.:* Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. Anal Chim Acta, **501**, 79–88 (2004)
35 *Lagana A., Fago G., Marino A. and Santarelli D.:* Development of an analytical system for the simultaneous determination of anabolic macrocyclic lactones in aquatic environmental samples. Rapid Commun Mass Sp, **15**, 304–310 (2001)
36 *Pawlowski S., Ternes T.A., Bonerz M., Rastall A.C., Erdinger L. and Braunbeck T.:* Estrogenicity of solid phase-extracted water samples from two municipal sewage treatment plant effluents and river Rhine water using the yeast estrogen screen. Toxicol In Vitro, **18**, 129–138 (2004)
37 *Spengler P.:* Identifizierung und Quantifizierung von Verbindungen mit östrogener Wirkung im Abwasser. Ph. D. Thesis. Institut für Siedlungswasserbau, Wassergüte und Abfallwirtschaft. Universität Stuttgart. Germany, (2001)
38 *Ternes T.A., Eggert T. und Meisenheimer M.:* Pflanzliche hormonell wirksame Stoffe in der aquatischen Umwelt und deren Verhalten bei der Trinkwasseraufbereitung. Abschlussbericht. ESWE-Institut für Wasserforschung und Wassertechnologie GmbH. Wiesbaden. Germany, (2001)
39 *Ying G.G., Kookana R.S. and Ru Y.J.:* Occurrence and fate of hormone steroids in the environment. Environ Int, **28**, 545–551 (2002)
40 *Miles C.O., Erasmuson A.F., Wilkins A.L., Towers N.R., Smith B.L., Garthwaite I., Scahill B.G. and Hansen R.P.:* Ovine metabolism of zearalenone to alpha-zearalanol (zeranol). J Agr Food Chem, **44**, 3244–3250 (1996)
41 *Johnson A.C. and Williams R.J.:* A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Environ Sci Technol, **38**, 3649–3658 (2004)
42 *Lemke S.L., Grant P.G. and Phillips T.D.:* Adsorption of zearalenone by organophilic montmorillonite clay. J Agr Food Chem, **46**, 3789–3796 (1998)
43 *Lee L.S., Strock T.J., Sarmah A.K. and Rao P.S.C.:* Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. Environ Sci Technol, **37**, 4098–4105 (2003)
44 *Tomlin C.: The pesticide manual: a world compendium.* 10th Edition 10. Farnham (UK), Thornton Heath, (1994)

Corresponding address: Thomas D. Bucheli, Agroscope FAL Reckenholz,
Swiss Federal Research Station for Agroecology and Agriculture (FAL),
Reckenholzstrasse 191, CH-8046 Zürich, Switzerland.
E-mail: thomas.bucheli@fal.admin.ch