VI Congresso Nazionale: Micotossine e Tossine Vegetali

10-12 June 2019

EFSA's recent assessments of fusarium toxins and their modified forms

Katleen Baert

Scientific Officer

BIOCONTAM Unit

Trusted science for safe food

European Food Safety Authority

Recent assessments fusarium toxins

Appropriate to set group HBGV incl modified forms	• **Zearalenone (ZEN; 2016)** • **T-2 and HT-2 toxin (2017)** • **Nivalenol (NIV; 2017)** • **Fumonisins B (FBs; 2018)**
Generic opinion feed	• *ZEN (2017)* • **FBs (2018)**
Generic opinion food and feed	• *Deoxynivalenol (DON), metabolites of DON and masked DON (2017)* • *Moniliformin (2017)* • *Diacetoxyscirpenol (2018)*

HBGV: health-based guidance value (e.g. TDI, ARfD)

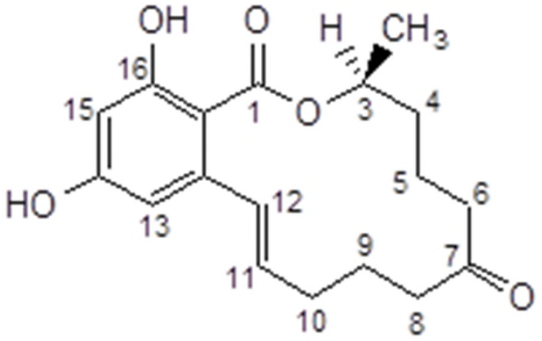
Modified mycotoxins

Metabolites formed in plant or fungus by phase I and phase II metabolism or as a consequence of food processing or transfer from feed to livestock

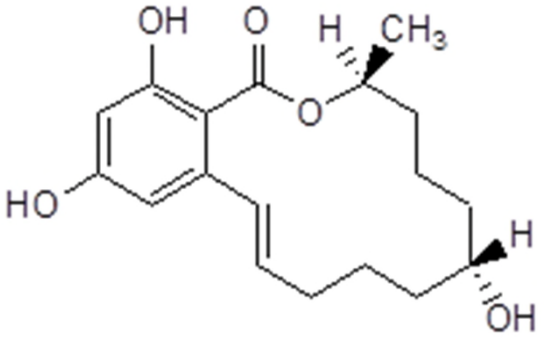
ZEARALENONE & modified forms

- Found in maize, wheat, barley, sorghum, rye
- Few occurrence data on modified zearalenone (ZEN) (cereal-based products)
- Modified forms may add up to 100% relative to ZEN.

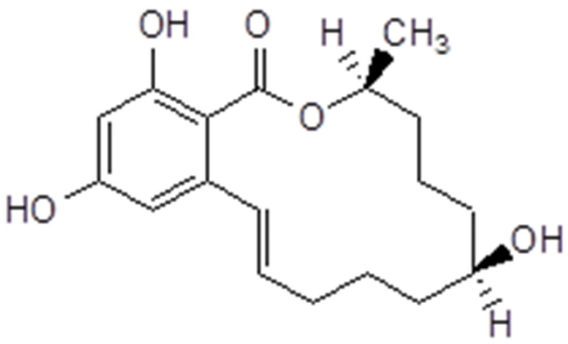
ZEN and its Phase I metabolites



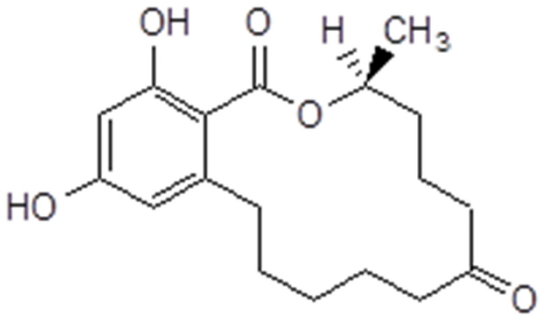
Zearalenone (ZEN)



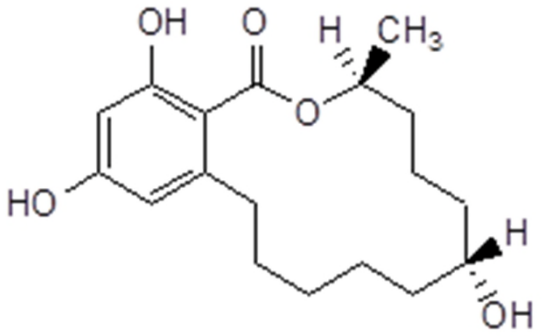
α-Zearalenol (α-ZEL)



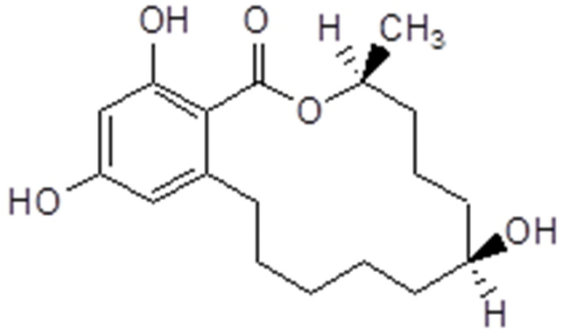
β-Zearalenol (ß-ZEL)



Zearalanone (ZAN)

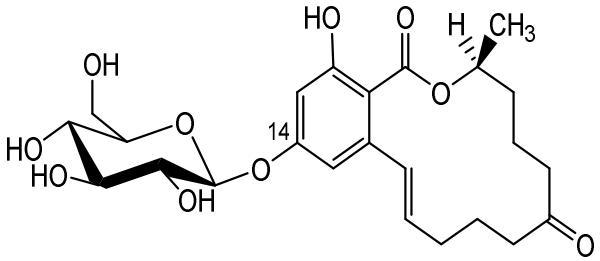


α-Zearalanol (α-ZAL)

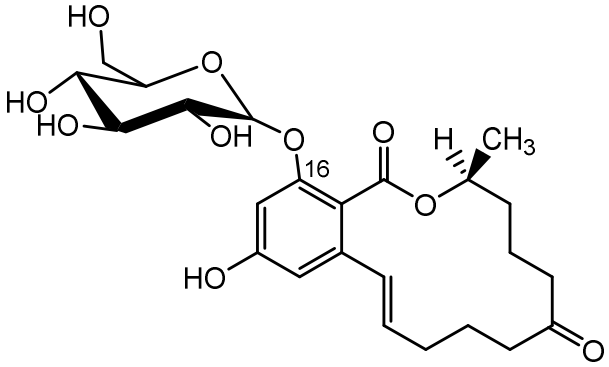


β-Zearalanol (ß-ZAL)

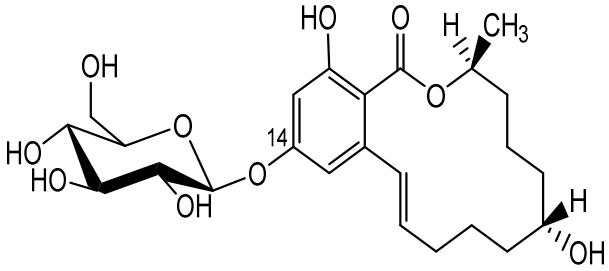
ZEN and its Phase II metabolites



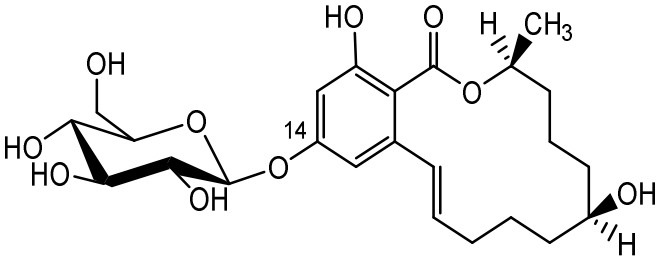
ZEN14ßDGlc*p*



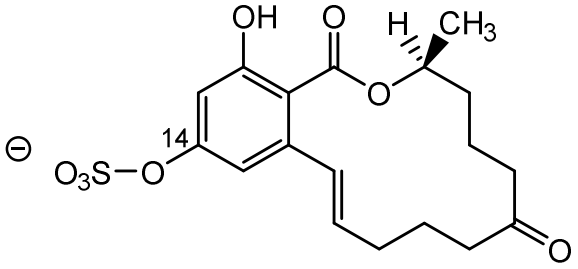
ZEN16ßDGlc*p*



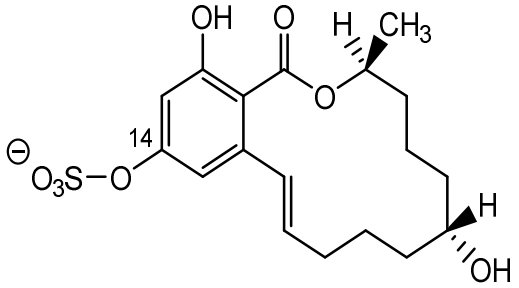
αZEL14ßDGlc*p*



ßZEL14ßDGlc*p*



ZEN14Sulf



αZEL14Sulf

Toxicity

- Low acute toxicity - no acute reference dose (ARfD) needed.
- Critical chronic toxic effect: Oestrogenic activity. NOEL: 10.4 µg/kg bw per day in pigs.
- TDI: 0.25 µg/kg bw per day (UF of 40).
- Modified ZEN:
  - No data to identify NOAEL/LOAELs
  - Likely same mode of action
  - Modified ZEN can be included in a group TDI with ZEN adding relative potency factors (RPFs) and assuming dose addition
  - RPFs for phase I metabolites derived from *in vivo* uterotrophic assays in mice and rats
  - RPFs to be applied for phase II metabolites - complete cleavage of ZEN/metabolites assumed

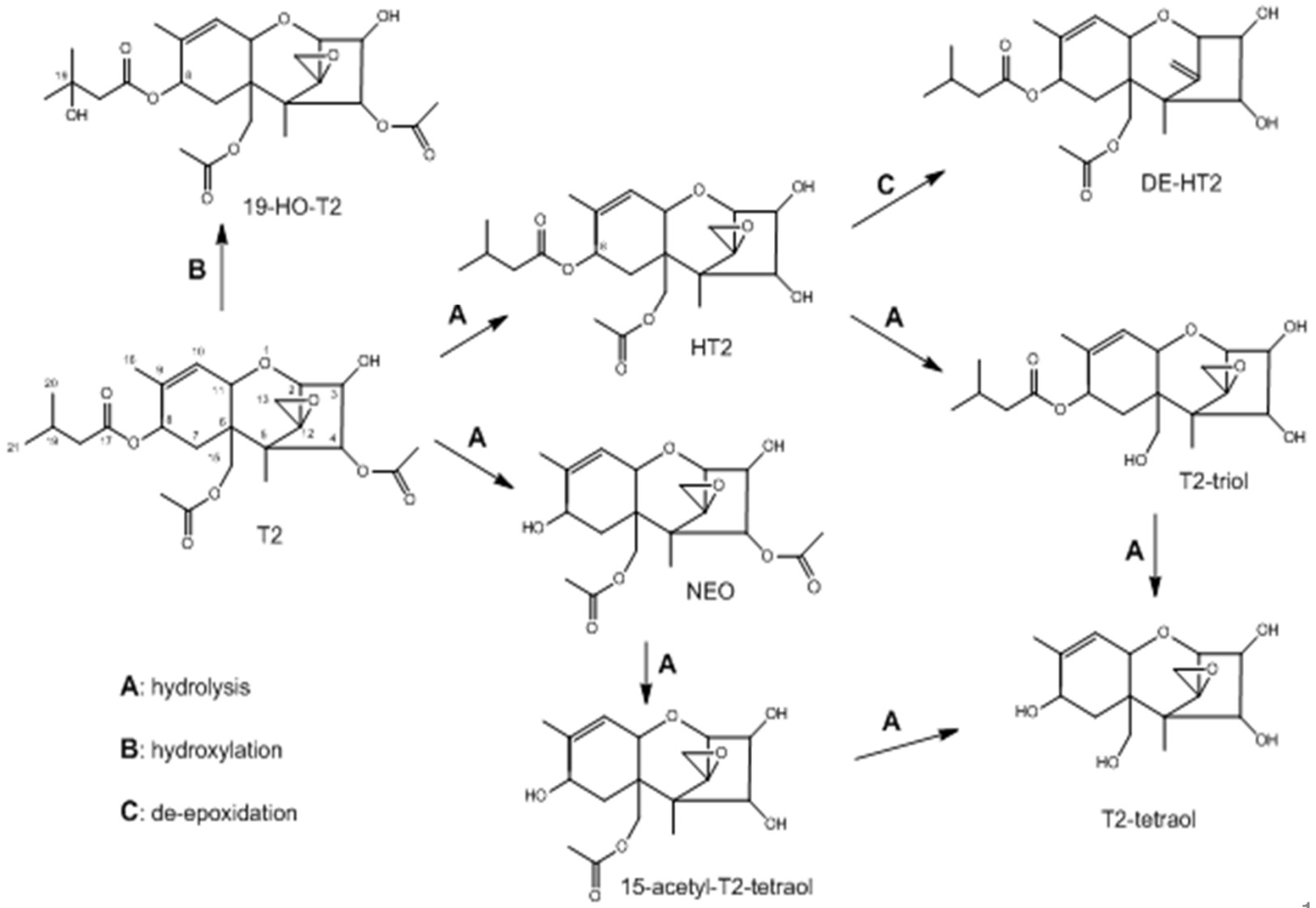
RPFs for Modified ZEN to be included in a Group TDI

Compound	Proposed RPF
ZEN, ZENGlcs, ZENSulfs	1.0
α-ZEL, α-ZELGlcs, α-ZELSulfs	60
β-ZEL, β-ZELGlcs, β-ZELSulfs	0.2
ZAN, ZANGlcs, ZANSulfs	1.5
α-ZAL, α -ZALGlcs, α -ZALSulfs	4.0
β-ZAL, β-ZALGlcs, β-ZALSulfs	2.0
cis-ZEN, cis-ZENGlcs, cis-ZENSulfs	1.0
cis-α-ZEL, cis-α-ZELGlcs, cis-α-ZELSulfs	8.0
cis-β-ZEL, cis-β-ZELGlcs, cis-β-ZELSulfs	1.0

T2/HT2 and their modified forms

- **Found in oats, barley, wheat and maize**
- **Modified forms may add up to 40% relative to T2/HT2.**

Phase I metabolites of T2/HT2



NEO: neosolaniol

Toxicity of T2/HT2

Chronic toxicity

- ✓T2 induces haemato- and myelotoxicity
- ✓T2 is rapidly metabolised to HT2 => toxicity of T2 might partly be attributed to HT2
- ✓$BMDL_{10}$ of 3.3 µg/kg bw per day for T2 for ↓ leukocytes in rats
- ✓Group TDI for T2/HT2: 0.02 µg/kg bw; UF of 200.

Acute toxicity

- ✓T2/HT2 induces anorectic effects upon short-term exposure
- ✓$BMDL_{10}$ of 2.97 µg/kg bw for emetic events seen in mink
- ✓Group ARfD for T2/HT2: 0.3 µg/kg bw; UF of 10.

Toxicity of modified T2/HT2

- ✓ No data for setting NOAELs/LOAELs available
- ✓ *In vitro/in vivo* data show that phase I metabolites have same MoA for critical **chronic effect** (haematotoxicity)
- ✓ RPFs for phase I metabolites derived from comparative *in vitro/in vivo* assays
- ✓ RPFs for T2/HT2 and its phase I metabolites to be applied for their phase II metabolites, as complete cleavage can be assumed
- ✓ **Acute toxicity**: NEO showed equal emetic potency



RPFs for modified T2 and HT2

Chronic toxicity (group TDI)

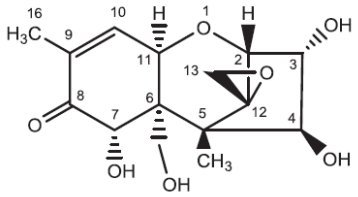
Compound	RPF
T2, T2-3-Glc, T2-3-diGlc, T2-3-Sulf, T2-3-GlcA, 3-Ac-T2, 3-Fer-T2, 19-HO-T2	1.0
HT2, HT2-3-Glc, HT2-diGlc, HT2-GlcA, HT2-MalGlc	1.0
19-HO-HT2	0.3
NEO, NEO-Glc	0.3
T2-triol, T2-triol-Glc	0.1
T2-tetraol, T2-tetraol-Glc	0.1

Acute toxicity (group ARfD)

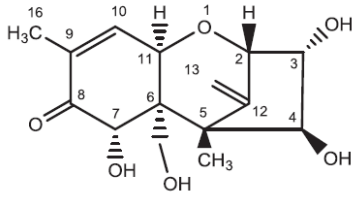
Compound	RPF
T2, HT2, NEO, NEO-Glc	1.0

NIVALENOL (NIV) & modified forms

- Found in cereal crops (e.g. wheat, maize, barley, oats)
- Few occurrence data on modified NIV (wheat, barley and oats)
- Modified forms may add up to 50% relative to NIV.



Nivalenol



De-epoxy-nivalenol

Phase II: NIV-3-glucoside

Toxicity of NIV and modified forms

Chronic toxicity

- ✓ Immuno/haematotoxic (similar MoA as T2)
- ✓ $BMDL_{10}$ 0.35 mg/kg bw per day for ↓ leukocyte counts in rats
- ✓ TDI: 1.2 µg/kg bw; UF of 300

Acute toxicity

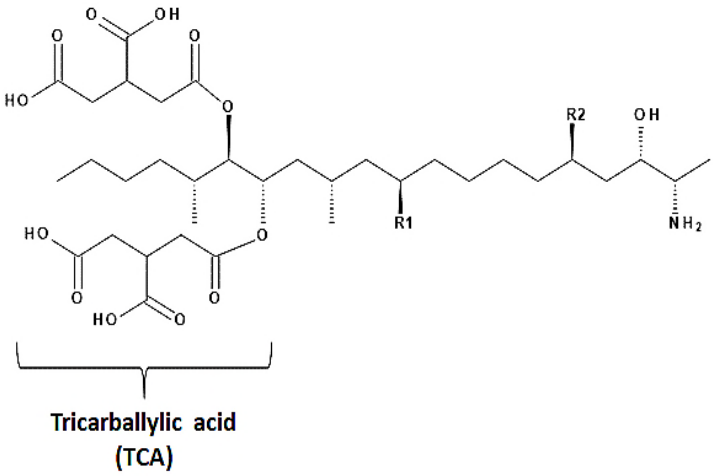
- ✓ NIV causes anorectic effects upon short term exposure (likely same MoA as T2/HT2)
- ✓ $BMDL_{10}$ of 0.14 µg/kg bw for emetic events in mink
- ✓ ARfD: 14 µg/kg bw., UF of 10

NIV-3-ß-Glc to be included in group TDI and ARfD with NIV with the same potency (cleavage to NIV assumed)

De-epoxy-NIV: lack of significant toxicity

Fumonisins

Common contaminants of maize, and to a lesser extent of wheat and other cereals



Tricarballylic acid (TCA)

	R1	R2	Polar surface	logP
FB1	OH	OH	288.51	-0.044
FB2	H	OH	268.27	1.3169
FB3	OH	H	268.27	1.3169
FB4	H	H	248.04	2.5538

FBs 1-4 and modified FBs 1-4

Fumonisins B_{1-4}	**FB_{1-4}**
Hydrolysed fumonisin B_{1-4}	**HFB_{1-4}**
Partially hydrolysed fumonisin B_{1-2}	**$pHFB_{1-2}$a,b**
N-(carboxymethyl) fumonisin B_1	**NCM-FB_1**
N-(1-deoxy-D-fructos-1-yl)-fumonisin B_{1-3}	**NDF-FB_{1-3}**
O-fatty acyl fumonisin B_1	**O-fatty acyl FB_1**
N-fatty acyl fumonisin B_1	**N-fatty acyl FB_1**
N-fatty acyl hydrolysed fumonisin B_{1-2}	**N-fatty acyl HFB_{1-2}**
N-palmitoyl hydrolysed fumonisin B_1	**N-palmitoyl HFB_1**
N-acetyl fumonisin B_1	**FA_1**

Hidden Fumonisins

- Non-covalent binding products with food/feed matrix (e.g. starch, proteins, lipids)
- No change in chemical structure
- Not considered for HBGV opinion
- Included in animal exposure assessment

Toxicity FBs & Modified Forms

- FB1 causes liver and kidney toxicity
- $BMDL_{10}$ of 0.1 mg/kg bw per day for an increase in megalocytic hepatocytes in mice (Bondy et al., 2015)
- **TDI** for FB_1: 1.0 µg/kg bw; UF of 100
- $FB_{2\text{-}4}$ should be included in **group TDI** based on structural similarity, and data indicating similar toxic profile and toxic potencies
- Data on modified FBs suggest that they also block ceramide synthases and have a similar or rather lower toxicological potency but data are too limited to include also modified forms in a group TDI with FB1-4

Recommendations

- More data on occurrence of modified forms of ZEN, T2/HT2 and FB_{2-6} in food and feed
- Standards/calibrants for modified form of ZEN, T2/HT2, NIV and FB_{2-6}
- More data on toxicokinetics and toxicity of modified mycotoxins
  - Comparative oestrogenicity studies with α-ZEL in pigs
  - TK studies on NIV3Glc
  - Toxicity studies with FB_{2-6} /any modified FBs using pure compounds
- Verification of dose addition assumption for ZEN and T2/HT2 and their modified forms

Acknowledgements

- Members of the **WG on HBGV** for mycotoxins and their modified forms
- Members of the **WG fumonisins in feed**
- Members of the **WG on zearalenone in feed**
- Members of the **WG on fusarium toxins**
- Members of the **CONTAM Panel**
- **EFSA staff** from the DATA and BIOCONTAM Units
- **Member States - European countries**
- **Stakeholders**
  - ✓ occurrence data
  - ✓ consumption data

Thank you for your attention

Stay connected!

Subscribe to
www.efsa.europa.eu/en/news/newsletters
www.efsa.europa.eu/en/rss

Engage with careers

Follow us on Twitter
@efsa_eu
@plants_efsa
@methods_efsa