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General introduction to mycotoxin biomarkers

Arnau Vidal, Liesel Claeys, Sarah De Saeger & Marthe De Boevre

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1. Biomarkers introduction

2. Biomarkers determination

3. Mycotoxin biomarkers

4. Conclusions

Comprehensive REVIEWS in Food Science and Food Safety

Mycotoxin Biomarkers of Exposure: A Comprehensive Review

Arnau Vidal, Marcel Mengelers, Shupeng Yang, Sarah De Saeger, and Marthe De Boevre

Abstract: To date, the use of biomarkers has become generally accepted. Biomarker-driven research has been proposed as a successful method to assess the exposure to xenobiotics by using concentrations of the parent compounds and/or metabolites in biological matrices such as urine or blood. However, the identification and validation of biomarkers of exposure remain a challenge. Recent advances in high-resolution mass spectrometry along with new analytical (post-acquisition data-mining) techniques will improve the quality and output of the biomarker identification process. Chronic or even acute exposure to mycotoxins remains a daily fact, and therefore it is crucial that the mycotoxins' metabolism is unravelled so more knowledge on biomarkers in humans and animals is acquired. This review aims to provide the scientific community with a comprehensive overview of reported *in vitro* and *in vivo* mycotoxin metabolism studies in relation to biomarkers of exposure for deoxynivalenol, nivalenol, fusarenon-X, T-2 toxin, diacetoxyscirpenol, ochratoxin A, citrinin, fumonisins, zearalenone, aflatoxins, and sterigmatocystin.

Keywords: biomarkers, exposure, human, *in vitro*, *in vivo*, metabolism, mycotoxin

- **Biomarker:** *biological marker; a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.*

  - ✓ **Biomarker of exposure:** *a characteristic to assess the exposure of xenobiotics of individuals through an estimation of their metabolites in biological fluids.*

  - ✓ **Biomarker of effect:** *a characteristic measured through a biochemical, physiological, behavioural, or other alternation within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease.*

  - ✓ **Biomarker of susceptibility:** *an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance.*

- Biomarker-driven research to assess the exposure to xenobiotics:
  - Based on concentrations of **the parent compounds.**
  - Based on concentrations of the **metabolites.**

- Identification and validation of biomarkers of exposure.

Biological fluids to analyze biomarkers:

- *Urine*
- *Blood and/or plasma/serum*
- *Feces*
- *Breast milk*
- *Hair*

BIOMARKERS: DETERMINATION

- Mycotoxin screening methods
- Confirmatory methods

Mycotoxin biomarker screening methods

- Quantitative

  - ✓ Enzyme-Linked ImmunoSorbent Assay (ELISA)
  - ✓ Fluorescent Labelled ImmunoSorbent Assay (FLISA)
  - ✓ Fluorescent Polarization ImmunoAssay (FPIA)
  - ✓ Biosensors

- Qualitative/Semi-quantitative

  - ✓ Membrane/paper tests
    - Lateral flow/dipstick

Confirmatory methods

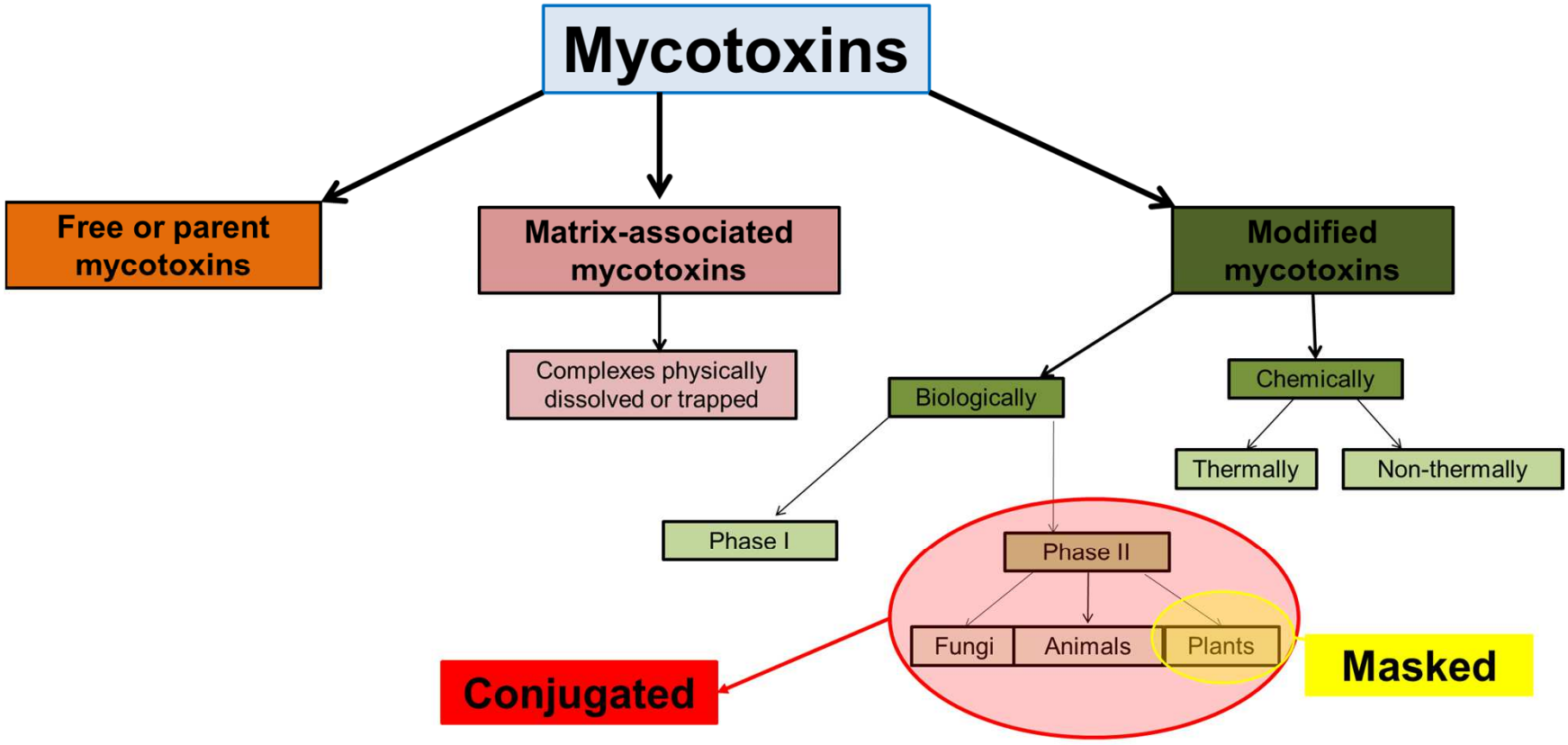
- Liquid chromatography:
  - ✓ Mass-spectrometry (MS)
    - Multi-mycotoxins
    - Low limit of detection
  - ✓ High Resolution MS (HRMS)
    - Identify and detect new biomarkers - structural elucidation.
    - Untargeted analysis or screening.
    - Screening method to simultaneously detect a large number of compounds.

Mycotoxins: Toxic fungal secondary metabolites

- Contaminate agricultural commodities during cultivation, harvesting, transport, processing and storage.

- Most important producing genera:
  - ✓ *Aspergillus*
  - ✓ *Fusarium*
  - ✓ *Penicillium*

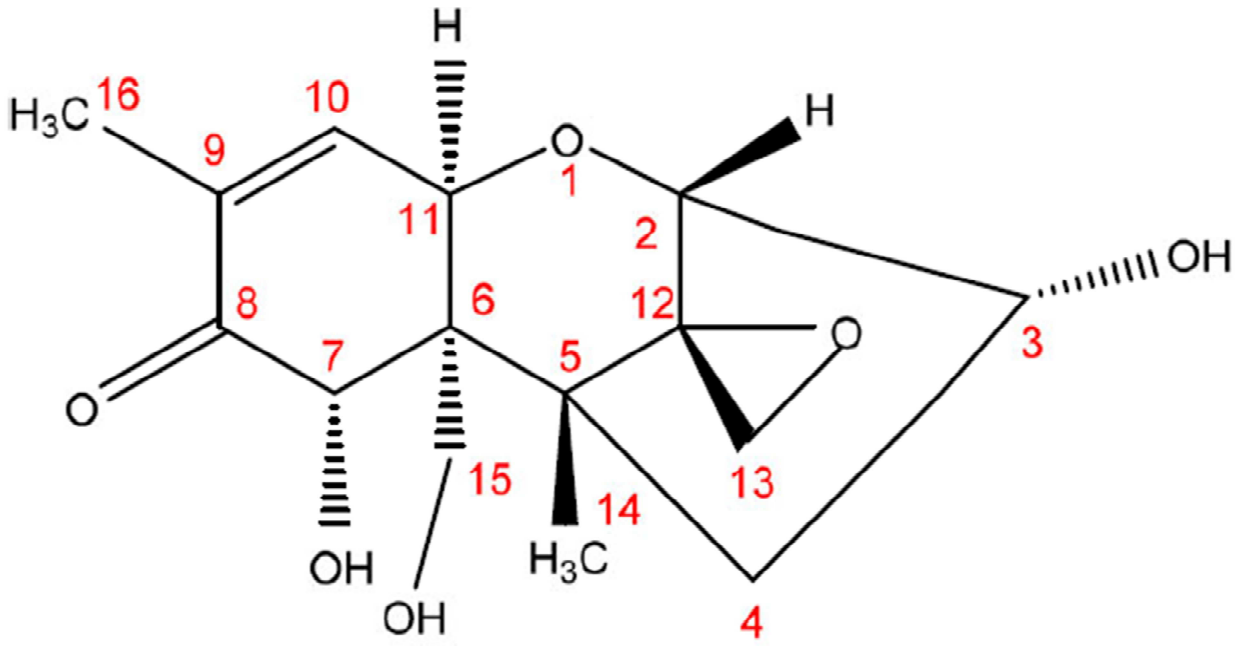
Mycotoxins: classification



Reference: Rychlik *et al.* (2014)

- International Agency for Research on Cancer (IARC)
- Classification according to evidence of carcinogenicity to humans

Group	Classification	Mycotoxins
1	Carcinogenic to humans	**aflatoxins**
2A	Probably carcinogenic to humans	/
2B	Possibly carcinogenic to humans	**ochratoxin A, sterigmatocystin and fumonisins**
3	Not classifiable as to its carcinogenicity to humans	**deoxynivalenol, nivalenol, T-2 toxin, diacetoxyscirpenol, zearalenone, citrinin and fusarenon-X**
4	Probably not carcinogenic to humans	/



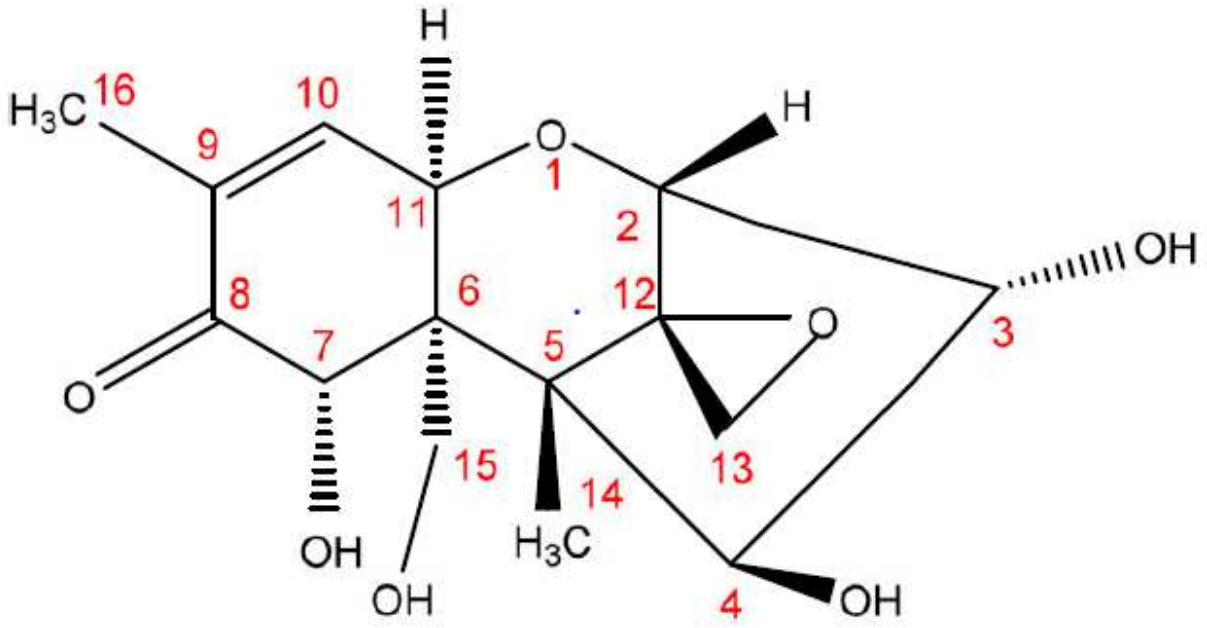
Deoxynivalenol (DON)

- Produced by *Fusarium* species and type B trichothecene.
- Highly common in cereals and cereal-based products (bread, pasta, beer, …).
- Group 3 carcinogen.
- Modified or masked DON:
  - ✓ Deoxynivalenol-3-glucoside (DON-3-glucoside): even more present than DON after food processing.
  - ✓ 3- and 15-acetyldeoxynivalenol (3- and 15-ADON).

- DON is fast and mainly excreted by urine:
  - ✓ DON glucuronides form are the main metabolites (DON-15-glucuronide and DON-3-glucuronide) followed by DON.
  - ✓ Next presentation on human intervention trial.
- Deepoxy-DON in feces

- Animals produce more DON metabolites than humans

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
10-deepoxy-deoxynivalenol-1-sulfonate	10-DOM-1-sulfonate	$C_{15}H_{21}O_8S$	Rats	*In vivo*: urine	II	(Wan et al., 2014)
Deepoxy deoxynivalenol	DOM-1	$C_{15}H_{20}O_5$	Rats	*In vivo*: urine & feces	-	(Nagl et al., 2012)
			Cows	*In vivo*: urine & feces		(Cote, Dahlem, Yoshizawa, Swanson, & Buck, 1986)
			Swines	*In vivo*: urine & feces		(Nagl et al., 2014)
			Humans	*In vivo*: urine		(Vidal et al., 2018)
Deepoxy-deoxynivalenol-15-glucuronide	DOM-15-glucuronide	$C_{21}H_{28}O_{11}$	Humans, rats, swines, cows	*In vivo*: urine	II	(Schwartz-Zimmermann et al., 2017)
Deepoxy-deoxynivalenol-3-glucuronide	DOM-3-glucuronide	$C_{21}H_{28}O_{11}$	Humans, rats, cows	*In vivo*: urine	II	(Schwartz-Zimmermann et al., 2017)
Deoxynivalenol sulfonate 1	DON S1	$C_{15}H_{19}O_9S$	Rats	*In vivo*: feces	II	(Schwartz-Zimmermann et al., 2017)
Deoxynivalenol sulfonate 2	DON S2	$C_{15}H_{19}O_9S$	Rats	*In vivo*: feces	II	(Schwartz-Zimmermann et al., 2017)
Deoxynivalenol sulfonate 3	DON S3	$C_{15}H_{19}O_9S$	Rats	*In vivo*: feces	II	(Schwartz-Zimmermann et al., 2017)
Deoxynivalenol-15-glucuronide	DON-15-glucuronide	$C_{21}H_{28}O_{12}$	Humans	*In vivo*: urine	II	(Heyndrickx et al., 2015)
			Humans	*In vitro*: liver		(Schwartz-Zimmermann et al., 2017)
Deoxynivalenol-15-sulfate	DON-15-sulfate	$C_{15}H_{19}O_9S$	Rats	*In vivo*: urine	II	(Pestka et al., 2017)
			Rats	*In vitro*: liver		
Deoxynivalenol-3-glucuronide	DON-3-glucuronide	$C_{21}H_{28}O_{12}$	Humans	*In vivo*: urine	II	(Heyndrickx et al., 2015; Schwartz-Zimmermann et al., 2017)
			Rats, swines, cows, humans	*In vitro*: liver		Schwartz-Zimmermann et al. (2017)
Deoxynivalenol-3-sulfate	DON-3-sulfate	$C_{15}H_{19}O_9S$	Humans	*In vivo*: urine	II	(Warth et al., 2016)
			Chickens	*In vivo*: urine		(Wan et al., 2014)
			Chickens & turkeys	*In vivo*: urine		(Schwartz-Zimmermann et al., 2015)
			Rats	*In vivo*: urine		(Pestka et al., 2017)
Deoxynivalenol-8,15-hemiketal-8-glucuronide	DON-8,15-hemiketal-8-glucuronide	$C_{21}H_{29}O_{13}$	Rats	*In vitro*: liver	II	(Uhlig, Ivanova, & Fæste, 2016)
			Rats	*In vitro*: liver		(Schwartz-Zimmermann et al., 2017; Uhlig, Ivanova, & Fæste, 2013)
			Rats	*In vivo*: urine		
Iso-deepoxydeoxynivalenol	Iso-DOM	$C_{15}H_{20}O_5$	Bacterial strain BBSH 797	*In vitro*: incubation	-	(Fuchs et al., 2002)
Iso-deepoxy-deoxynivalenol-15-glucuronide	iso-DOM-15-glucuronide	$C_{21}H_{30}O_{11}$	Rats	*In vitro*: liver	II	(Schwartz-Zimmermann et al., 2017)
			Humans	*In vitro*: liver		
Iso-deepoxy deoxynivalenol-3-glucuronide	Iso-DOM-3-glucuronide	$C_{21}H_{30}O_{11}$	Rats & cows	*In vivo*: urine	-	(Uhlig et al., 2016)
Iso-deepoxy-deoxynivalenol-8-glucuronide	iso-DOM-8-glucuronide	$C_{21}H_{30}O_{11}$	Rats	*In vitro*: liver	II	(Schwartz-Zimmermann et al., 2017)
Iso-deoxynivalenol	Iso-DON	$C_{15}H_{20}O_6$	Rats	*In vivo*: urine	-	(Schwartz-Zimmermann et al., 2017)
Iso-deoxynivalenol-15-glucuronide	iso-DON-15-glucuronide	$C_{21}H_{30}O_{11}$	Rats & humans	*In vitro*: liver	II	(Schwartz-Zimmermann et al., 2017)
Iso-deoxynivalenol-3-glucuronide (previously deoxynivalenol-7-glucuronide)	iso-DON-3-glucuronide	$C_{21}H_{30}O_{11}$	Rats	*In vivo*: urine	II	(Schwartz-Zimmermann et al., 2017)
			Rats	*In vitro*: liver		
Iso-deoxynivalenol-8-glucuronide	iso-DON-8-glucuronide	$C_{21}H_{30}O_{11}$	Rats	*In vitro*: liver	II	(Schwartz-Zimmermann et al., 2017)

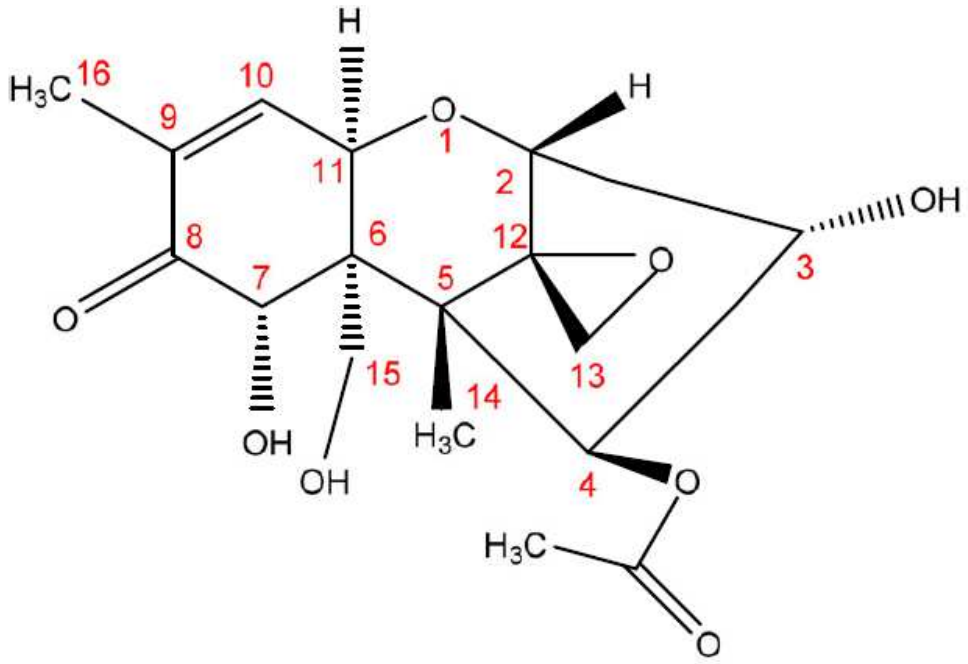


Nivalenol (NIV)

- Produced by *F. cerealis, F. poae, F. graminearum, and F. culmorum and* type B trichothecene.
- Observed in cereals, especially in wheat products.
- Group 3 carcinogen.
- It has demonstrated immuno-, hemato-, myelotoxicity, and developmental and reproductive toxicity.

- The metabolism of NIV has been scarcely investigated.
- A study was not able to detect NIV in human urine.
- The non-detection of NIV could be assigned to the fact that NIV was probably predominantly excreted in the glucuronidated form, similar to DON.
- Deepoxy-NIV is a predominant compound in feces

(Hedman & Pettersson, 1997).

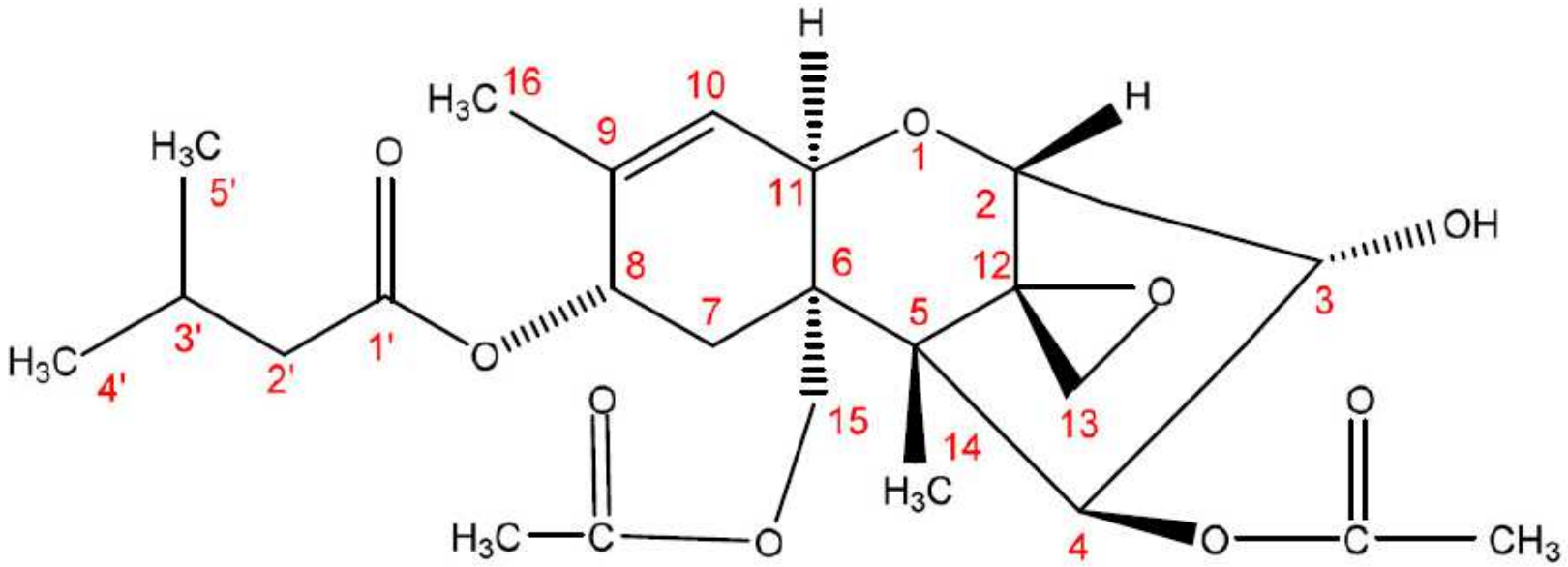


Fusarenon-X (FUS-X)

- Produced by different *Fusarium species and* type B trichothecene.
- Mainly found in cereals and co-occurs with DON and NIV.
- Group 3 carcinogen.
- Exerts intestinal inflammation, inhibits protein synthesis, induces apoptosis, and alters genetic material.

- FUS-X is highly converted to NIV in liver and kidney.
- Focus on quantification of NIV both in urine and plasma.

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
Nivalenol	NIV	$C_{15}H_{20}O_7$	Duck	*In vitro*: liver	I	(Poapolathep et al., 2008)
			Broiler	*In vitro*: liver		(Ohta et al., 1978; Poapolathep et al., 2003)
			Rabit	*In vitro*: liver		
			Mice	*In vitro*: liver		(Saengtienchai et al., 2014)
			Pig	*In vitro*: liver		(Poapolathep et al., 2003)
			Pig	*In vivo*: urine		(Poapolathep et al., 2008)
			Mice	*In vivo*: urine & feces		
			Broiler	*In vivo*: plasma		
			Duck	*In vivo*: plasma		


H
H3C 16
10
9
O
1
H
H3C
5'
O
11
2
OH
8
6
12
O
3
7
5
H3C
4'
3'
2'
1'
O
15
13
14
O
O
H3C
C
O
H3C
4
O
C
CH3

T-2 toxin (T-2)

- Produced by various *Fusarium* type A trichothecene.
- Detected in cereals and cereal-based products.
- Group 3 carcinogen.
- Act as a potent **inhibitor of protein synthesis** and **mitochondrial function**; immunosuppressive and cytotoxic effects.
- HT-2 and T-2-glucoside as modified T-2.
- JECFA concluded that the toxic effects of T-2 and HT-2 cannot be differentiated.

- HT-2 is the predominant compound during *in vitro* and *in vivo* studies, and should therefore be considered as the main T-2 biomarker in urine and in plasma.

- There are more T-2 metabolites which have not been detected in human urine.

- Differences among animals.

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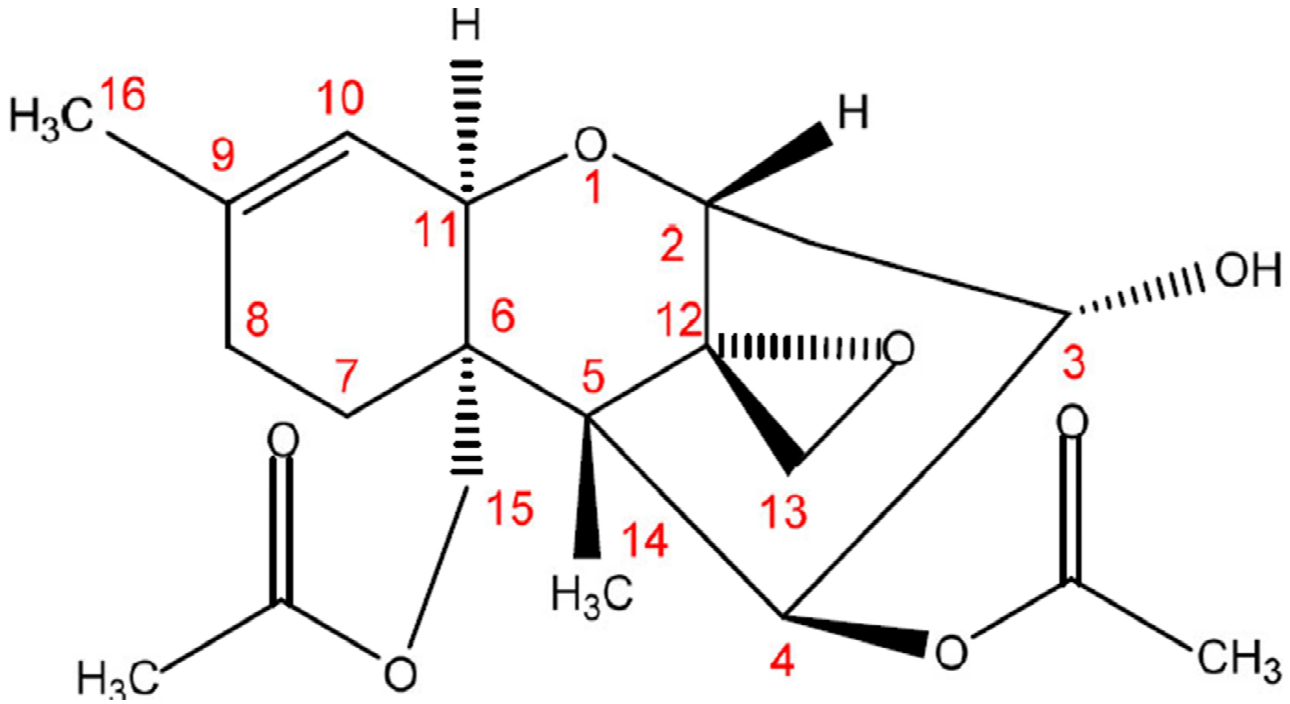
T-2 toxin

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
15-deacetylneosolaniol	15-deacetylneosolaniol	$C_{17}H_{24}O_7$	Rats	*In vivo*: urine & feces	II	Yang, Li, ... (2013)
15-deacetyl-T-2	15-deacetyl-T-2	$C_{22}H_{32}O_8$	Rats	*In vivo*: urine & feces	II	Yang, Li, ... (2013)
3'-7-dihydroxy-HT-2	3'-7-diOH-HT-2	$C_{22}H_{32}O_{10}$	Rats	*In vivo*: urine & feces	I	Yang, Li, ... (2013)
3'-7-dihydroxy-HT-2 (isomer)	3'-7-diOH-HT-2 (isomer)	$C_{22}H_{32}O_{10}$	Rats	*In vivo*: urine & feces	I	Yang, Li, ... (2013)
3'-7-dihydroxy-T-2	3'-7-diOH-T-2	$C_{24}H_{34}O_{11}$	Rats	*In vivo*: urine & feces	I	Yang, Li, ... (2013)
3'-hydroxy-9-hydroxy-T-2	3'-OH-9-OH-T-2	$C_{24}H_{36}O_{11}$	Rats	*In vivo*: urine & feces	I	Yang, Li, ... (2013)
3'-hydroxy-T-2-glucoside	3'-OH-T-2-glucoside	$C_{30}H_{44}O_{15}$	Rats Human	*In vitro*: liver *In vitro*: liver	I	Yang, S., Van Poucke, C., ... (2017)
3-4-dihydroxy-T-2	3',4'-di-OH-T-2	$C_{22}H_{32}O_8$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
3-4-dihydroxy-T-2 (isomer)	3',4'-di-OH-T-2 (isomer)	$C_{22}H_{32}O_8$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
3-hydroxy-15-deacetyl-T-2	3-OH-15-deacetyl-T-2	$C_{22}H_{32}O_9$	Rats	In vivo: urine & feces	II	Yang, Li, ... (2013)
3-hydroxy-HT-2(also known as T-2 triol)	3'-OH-HT-2	$C_{20}H_{30}O_7$	Rats, chickens, swines, goats, cows, humans. Chickens Rats Cows Chickens Humans	*In vitro*: liver *In vivo*: feces & bile *In vivo*: urine & feces *In vivo*: urine *In vivo*: plasma *In vivo*: milk	I	Yang, S., De Boevre, M. (2017) (Sun et al., 2015) (Rubert et al., 2014) (Yoshizawa, Sakamoto., Ayano, & Mirocha, 1982) (Sun et al., 2015)) (Rubert et al., 2014)
3-hydroxy-HT-2-3-sulfate	3'-OH-HT-2 3-SO_3H	$C_{24}H_{39}O_{11}S$	Chickens	*In vivo*: feces & bile	II	Yang, S., De Boevre, M. (2017)
3-hydroxy-T-2	3'-OH-T-2	$C_{24}H_{34}O_{10}$	Rats, chickens, swines, goats, cows, humans Chickens Rats Cows	*In vitro*: liver *In vivo*: feces & bile *In vivo*: urine & feces *In vivo*: urine	I	Yang, S., De Boevre, M. (2017) Yang, S., De Boevre, M. (2017) Yang, Li, ... (2013); Yoshizawa, Sakamoto, & Kuwamura, 1985) (Yoshizawa et al., 1982)
3-hydroxy-T-2-3-sulfate	3'-OH-T-2 3-SO_3H	$C_{26}H_{41}O_{12}S$	Chickens	*In vivo*: feces & bile	II	Yang, S., De Boevre, M. (2017)
4'-carboxyl-3'-hydroxy-T-2	4'-COOH-3'-OH-T-2	$C_{17}H_{24}O_7$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
4'-carboxyl-3'-hydroxy-T-2 (isomer)	4'-COOH-3'-OH-T-2 (isomer)	$C_{17}H_{24}O_7$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
4'-carboxyl-HT-2	4'-COOH-HT-2	$C_{22}H_{32}O_8$	Chickens	*In vivo*: feces	I	Yang, S., De Boevre, M. (2017)
4'-carboxyl-HT-2 (isomer)	4'-COOH-HT-2 (isomer)	$C_{22}H_{32}O_8$	Chickens	*In vivo*: feces	I	Yang, S., De Boevre, M. (2017)

4'-hydroxy-T-2-glucoside	4'-OH-T-2-glucoside	$C_{30}H_{44}O_{15}$	Rats Human	*In vitro*: liver *In vitro*: liver	I	Yang, S., Van Poucke, C. (2017)
4'-hydroxy-T-2-glucoside (isomer)	4'-OH-T-2-glucoside (isomer)	$C_{30}H_{44}O_{15}$	Rats Human	*In vitro*: liver *In vitro*: liver	I	Yang, S., Van Poucke, C. (2017)
4-4-dihydroxy-T-2	4',4'-di-OH-T-2	$C_{22}H_{32}O_8$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
4-deacetylneosolaniol	4-deAc-NEO	$C_{17}H_{24}O_7$	Rats, chickens, swines, goats, cows, humans Chickens Rats	*In vitro*: liver *In vivo*: feces *In vivo*: urine & feces	II	Yang, S., De Boevre, M. (2017) Yang, S., De Boevre, M. (2017) Yang, Li, … (2013)
4-hydroxy-HT-2	4'-OH-HT-2	$C_{22}H_{32}O_9$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
4-hydroxy-HT-2 (isomer)	4'-OH-HT-2 (isomer)	$C_{22}H_{32}O_9$	Chickens	*In vivo*: feces & bile	I	Yang, S., De Boevre, M. (2017)
7-hydroxy-HT-2	7-OH-HT-2	$C_{28}H_{42}O_{14}$	Rats	*In vivo*: urine & feces	I	Yang, Li, … (2013)
7-hydroxy-HT-2 (isomer)	7-OH-HT-2 (isomer)	$C_{28}H_{42}O_{14}$	Rats	*In vivo*: urine & feces	I	Yang, Li, … (2013)
9-hydroxyl-T-2	9-OH-T-2	$C_{24}H_{36}O_{10}$	Rats	*In vivo*: urine & feces	I	Yang, Li, … (2013)
De-epoxy-3',7-dihydroxy-HT-2	De-epoxy-3',7-diOH-HT-2	$C_{22}H_{32}O_9$	Rats	*In vivo*: urine & feces	-	Yang, Li, … (2013)
De-epoxy-3'-hydroxy-HT-2	De-epoxy-3'-OH-HT-2	$C_{22}H_{32}O_8$	Rats	*In vivo*: urine & feces	-	Yang, Li, … (2013)
De-epoxy-3'-hydroxy-T-2 triol	De-epoxy-3'-OH-T-2 triol	$C_{24}H_{34}O_9$	Rats	*In vivo*: urine	-	(Yoshizawa et al., 1985)
De-epoxy-HT-2	De-epoxy-HT-2	$C_{22}H_{32}O_7$	Rats	*In vivo*: urine & feces	-	Yang, Li, … (2013)
HT-2 toxin	HT-2	$C_{22}H_{32}O_8$	Rats, chickens, swines, goats, cows, humans Chickens Rats Humans	*In vitro*: liver *In vivo*: feces & bile *In vivo*: urine & feces *In vivo*: milk & urine	I	Yang, S., De Boevre, M. (2017)Yang, S., VAn Poucke, C. (2017)Yang, Li, … (2013)(Rubert et al., 2014) (Rodriguez-Carrasco et al., 2014)
HT-2-3-glucuronide	HT-2-3-glucuronide	$C_{28}H_{40}O_{14}$	Rats, chickens, swines, goats, cows, humans Chickens	*In vitro*: liver *In vivo*: feces	II	Yang, S., De Boevre, M. (2017) Yang, S., De Boevre, M. (2017)
HT-2-4-glucuronide	HT-2-4-glucuronide	$C_{28}H_{40}O_{14}$	Rats, chickens, swines, goats, cows, humans Chickens Humans	*In vitro*: liver *In vivo*: feces *In vivo*: urine	II	Yang, S., De Boevre, M. (2017) (Gerding, Cramer, & Humpf, 2014; Gerding et al., 2015) (Gerding et al., 2015)
HT-2-glucoside	HT-2-glucoside	$C_{28}H_{42}O_{13}$	Rats	In vitro: liver	I	Yang, S., Van Poucke, C.



Diacetoxyscirpenol (DAS)

Diacetoxyscirpenol

- Mainly produced by *Fusarium* species type A trichothecene.
- Main food group contributing to the occurrence of 4,15-DAS is cereals, and most reports are on sorghum, wheat, rice, and maize.
- Group 3 carcinogen.
- Shows immuno- and hematotoxic effects, pulmonary disorders growth retardation, and cardiovascular effects.

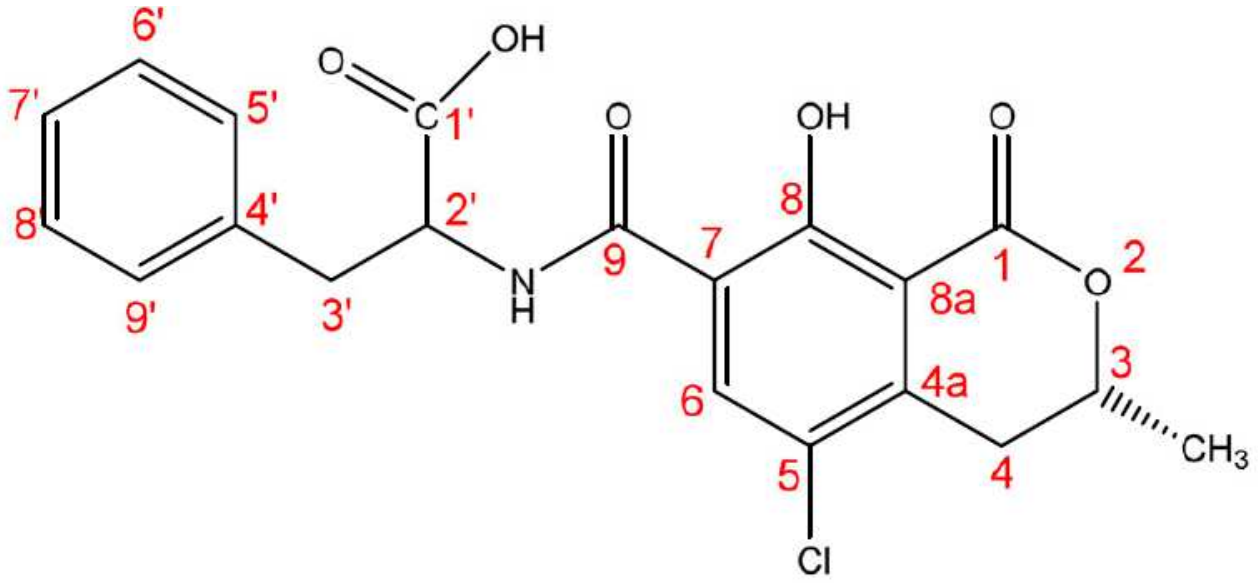
- DAS is metabolized in a wide range of metabolites, but its metabolism is species-dependent.

- Main DAS biomarker:
  - In urine and feces: **15-monoacetoxyscirpenol (15-MAS)**
  - In plasma: **scirpentriol (SCP)**

- Future research need to reveal if glucuronidated DAS can be assigned as relevant DAS biomarkers in urine.

Diacetoxyscirpenol

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
15- monoacetoxyscirpenol-3-glucuronide	15-MAS-3-glucuronide	$C_{23}H_{32}O_{12}$	Rats, swines, goats cows, humans	*In vitro*: liver	II	(Yang et al., 2015)
15-monoacetoxyscirpenol	15-MAS	$C_{17}H_{24}O_6$	Rats, chickens, swines, goats, cows, humans Rats Chickens Swines	*In vitro*: liver *In vivo*: urine & feces *In vivo*: feces *In vivo*: plasma	I	(Yang et al., 2015) (Yang et al., 2015) (Sakamoto et al., 1986) (Yang et al., 2015) (Bauer et al., 1985)
15-monoacetoxyscirpenol-4-glucuronide	15-MAS-4-glucuronide	$C_{23}H_{32}O_{12}$	Swines, goats, cows, humans	*In vitro*: liver	II	(Yang et al., 2015)
4-monoacetoxyscirpenol	4-MAS	$C_{17}H_{24}O_6$	Rats Rats	*In vitro*: liver *In vivo*: urine	I	(Yang et al., 2015) (Yang et al., 2015)
7-hydroxy-diacetoxyscirpenol	7-OH-DAS	$C_{19}H_{26}O_8$	Rats, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
7-hydroxy-diacetoxyscirpenol (isomer)	7-OH-DAS (isomerr)	$C_{19}H_{26}O_8$	Rats, chickens, swines, goats, cows, humans Rats Chickens	*In vitro*: liver *In vivo*: urine & feces *In vivo*: feces	I	(Yang et al., 2015)
8β-hydroxy-diacetoxyscirpenol	8β-OH-DAS	$C_{19}H_{26}O_8$	Rats, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
Deepoxy-15-monoacetoxyscirpenol	Deepoxy-15-MAS	$C_{17}H_{24}O_6$	Rats, swines, cows Rats	*In vitro*: feces *In vivo*: urine & feces	I	(Swanson et al., 1988) (Sakamoto et al., 1986; Swanson et al., 1988)
Deepoxy-scirpentriol	Deepoxy-SCP	$C_{15}H_{22}O_5$	Rats, swines, cows Rats	*In vitro*: feces *In vivo*: urine & feces	I	(Swanson et al., 1988) (Sakamoto et al., 1986; Swanson et al., 1988)
diacetoxyscirpenol-3-glucuronide	DAS-3-glucuronide	$C_{25}H_{34}O_{13}$	Rats, swines, goats, cows, humans	*In vitro*: liver	II	(Yang et al., 2015)
Neosolaniol	NEO	$C_{19}H_{26}O_8$	Rats, swines, goats, cows, humans Rats	*In vitro*: liver *In vivo*: urine	I	(Yang et al., 2015)
Scirpentriol	SCP	$C_{15}H_{22}O_5$	Rats Swines Rats, swines, goats	*In vivo*: urine *In vivo*: plasma *In vitro*: liver	I	(Swanson et al., 1988) (Sakamoto et al., 1986) (Yang et al., 2015)



Ochratoxin A (OTA)

- Mainly produced by *Penicillium* and *Aspergillus*.
- Observed in cereals and cereal-based products, coffee, grapes, and nuts.
- Group **2B carcinogen**
- Exerts **nephrotoxicity** and possesses carcinogenic, teratogenic, immunotoxic, and neurotoxic properties.

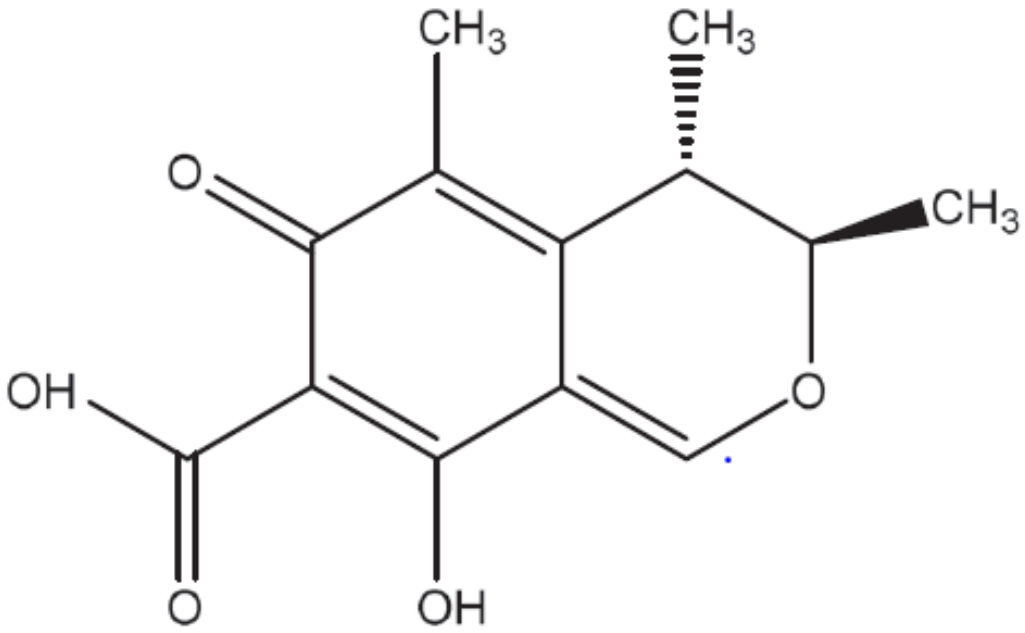
- OTA low level of metabolization

- OTA, OTB (the dechlorinated form of OTA), OTα (formed by the cleavage of the phenylalanine moiety of OTA), and their glucuronides are suggested to be the most prevailing fraction of total excreted OTA.

- Use these metabolites as OTA-biomarkers of exposure both in urine and plasma.

Ochratoxin A

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
(4R)-hydroxyochratoxin A	(4R)-OH-OTA	$C_{20}H_{18}ClNO_7$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
			Rabbits	*In vitro*: liver		(Størmer et al., 1983)
			Rats	*In vivo*: urine & feces		(Yang et al., 2015)
			Chickens	*In vivo*: feces		(Yang et al., 2015)
(4S)-hydroxyochratoxin A	(4S)-OH-OTA	$C_{20}H_{18}ClNO_7$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
			Rabbits	*In vitro*: liver		(Størmer et al., 1983)
			Rats	*In vivo*: urine		(Yang et al., 2015)
			Chickens	*In vivo*: feces		(Yang et al., 2015)
4(R)-hydroxyochratoxin B	4(R)-OH-OTB	$C_{20}H_{19}NO_7$	Chickens	*In vivo*: feces	I	(Yang et al., 2015)
4(S)-hydroxochratoxin B	4(S)-OH-OTB	$C_{20}H_{19}NO_7$	Chickens	*In vivo*: faeces	I	(Yang et al., 2015)
5-hydroxyochratoxin A	5'-OH-OTA	$C_{20}H_{18}ClNO_7$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
			Rats	*In vivo*: urine		(Yang et al., 2015)
			Chickens	*In vivo*: feces		(Yang et al., 2015)
7-hydroxyochratoxin A	7'-OH-OTA	$C_{20}H_{18}ClNO_7$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
			Rats	*In vivo*: urine		
			Chickens	*In vivo*: feces		
9-hydroxyochratoxin A	9'-OH-OTA	$C_{20}H_{18}ClNO_7$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
			Rats	*In vivo*: urine		
			Chickens	*In vivo*: feces		
Ochratoxin A-8-β-glucuronide	OTA-8-glucuronide	$C_{26}H_{27}NO_{13}$	Chickens	*In vivo*: feces	II	(Bordini, Rossi, Ono, Hirooka, & Sataque Ono, 2017)
Ochratoxin alpha	OTα	$C_{11}H_9ClO_5$	Sheeps	*In vivo*: urine	I	(Schaut et al., 2008)
			Humans	*In vivo*: plasma & plasma		(Ali, Muñoz, & Degen, 2017)
			Rats	*In vivo*: urine & plasma		(Abbas, Blank, Wein, & Wolffram, 2013)
Ochratoxin B	OTB	$C_{20}H_{19}NO_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	(Yang et al., 2015)
			Rats	*In vivo*: urine & feces		
			Chickens	*In vivo*: feces		
Open lactone-ochratoxin A-8- β-glucuronide	Lactone-OTA-8-glucuronide	$C_{26}H_{25}NO_{12}$	Chickens	*In vivo*: feces	II	(Bordini et al., 2017)

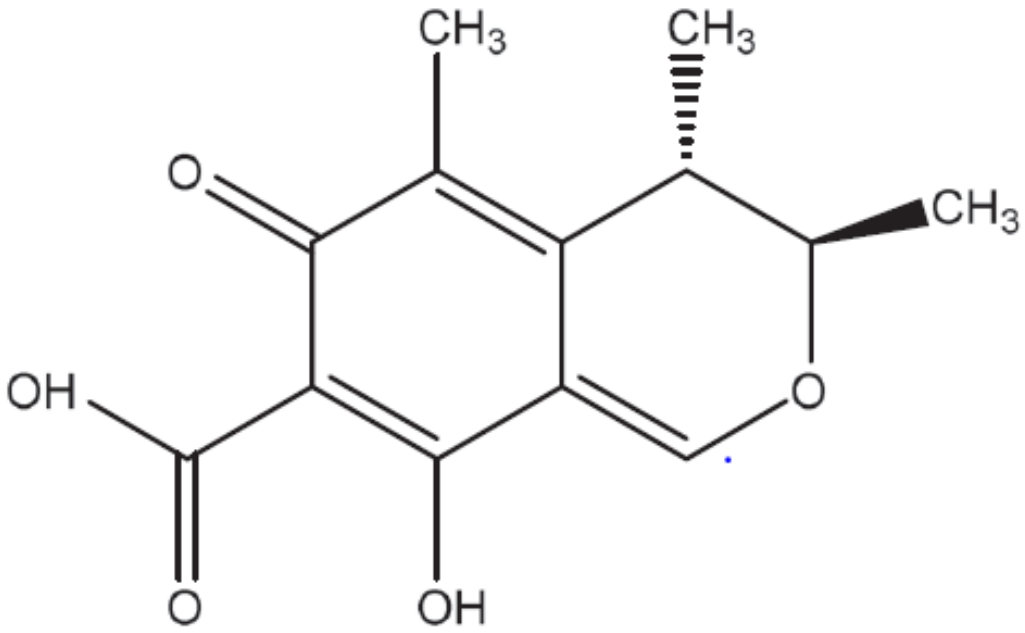


Citrinin (CIT)

- Produced by *Penicillium* and *Aspergillus.*
- Occurs mainly in cereals and cereal-based products.
- Group 3 carcinogen.
- Affects the kidney function in different species, but it appears to be considerably less toxic than OTA, results in necrosis of the distal tubule epithelium in the kidneys.

- Dihydro-citrinone (DH-CIT) should be considered as the most relevant metabolite in urine (84%).
- The lack of information on other metabolites can lead to an underestimation of CIT-exposure as possibly relevant other CIT-biomarkers have not yet been identified.
- Fast excretion in urine after 22.5 h (Degen et al., 2018).

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
Dihydrocitrinone	DH-CIT	$C_{13}H_{14}O_6$	Humans Rats	*In vivo*: urine *In vivo*: urine	I	(Heyndrickx et al., 2015) (Dunn et al., 1983)



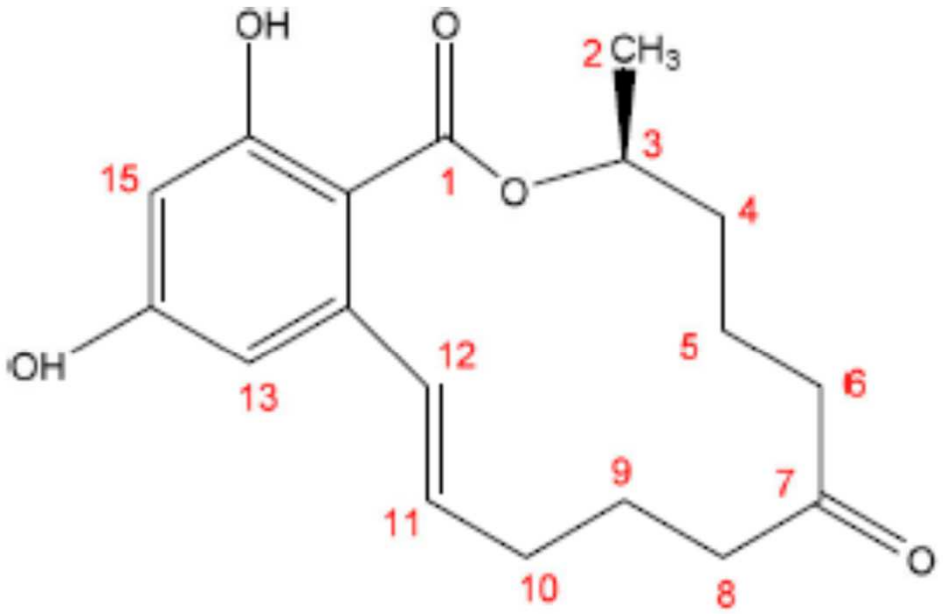
Fumonisins (Fb)

- Produced by *F. verticilloioides, F. proliferatum,* and *F. nygama.*
- Observed in maize.
- 12 fumonisins with the most important being fumonisin B1 (FB1), fumonisin 2 (FB2) and fumonisin 3 (FB3).
- FB1 is classified as a Group 2B carcinogen.
- Causes hepato-, nephron-, cytotoxic effects, and carcinogenic effects.

- FB low level of metabolization, mainly excreted as free form (>90%).
- FB have low absorption and are mainly excreted via the fecal route (> 90%). The level of FB detected in human urine is low.
- The accumulation of FBs in hair evidenced that FB1 in hair could be used as a biomarker for a long-term dietary exposure.

- In urine FB in free form, and N-acyl-fumonisin 1 (NAFB1) and N-acyl-hydrolysed fumonisin 1 (NAHFB1).
- Ration spinganine to sphingosine functional FB biomarker in animals.

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
Hydrolyzed FB1	HFB1	$C_{34}H_{59}NO_{13}$	Swines Humans	*In vitro*: liver *In vitro*: feces	I	(Gazzotti et al., 2011) (Cirlini et al., 2015; Hahn, et al., 2015)
Hydrolyzed FB2	HFB2	$C_{34}H_{59}NO_{12}$	Swines	*In vitro*: liver	I	(Gazzotti et al., 2011)
N-acyl-fumonisin 1	NAFB1	$C_{46}H_{85}NO_{13}$	Humans	*In vitro*: liver	I	(Harrer et al., 2013)
N-acyl-hydrolyzed fumonisin	NAHFB1	$C_{46}H_{81}NO_{14}$	Humans	*In vitro*: liver	I	(Harrer et al., 2013)
Partially hydrolyzed fumonisin B1	pHFB1a	$C_{40}H_{60}NO_{18}$	Swines Humans	*In vitro*: feces *In vitro*: feces	I	(Fodor et al., 2007) (Cirlini et al., 2015; Hahn, et al., 2015)



Zearalenone (ZEN)

- Produced *by F. graminearum, F. culmorum, F. equiseti,* and *F. verticilliodes.*
- Occurs in cereals.
- Group 3 carcinogen.
- A powerful estrogenic activity as its hormonal action exceeds that of most other naturally-occurring non-steroidal estrogens.
- Stimulation of the growth of human breast cancer cells

- Reduction, hydroxylation and glucuronidation are the major metabolic pathways of ZEN.

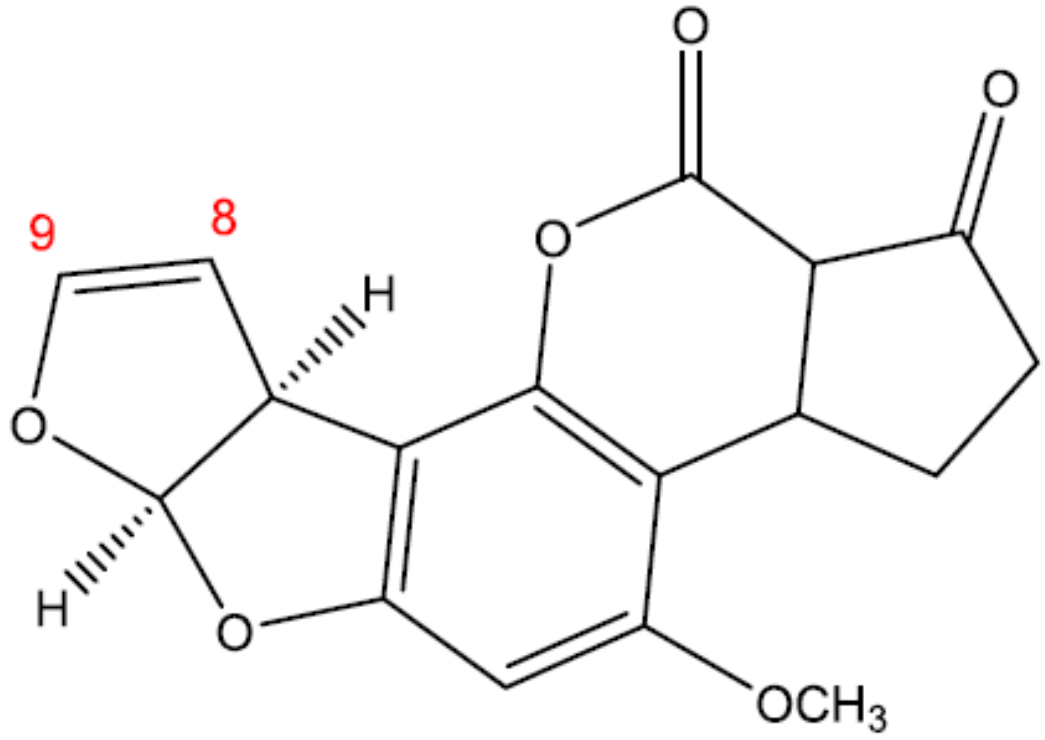
- Biomarker-analysis in urine should focus on free ZEN, α-zearalenol (α-ZEL), β-ZEL, and some of the most common hydroxylation and glucuronidation products like 8-hydroxy-zearalenone (8-OH-ZEN), 13-OH-ZEN, 15-OH-ZEN, and ZEN-14-glucuronide.

Zearalenone

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
10-hydroxy-zearalenone	10-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
13-hydroxy-zearalenone	13-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
15-hydroxy-zearalenone	15-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
2-hydroxy-zearalenone	2-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
3-hydroxy-zearalenone	3-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
4-hydroxy-zearalenone	4-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans Rats	*In vitro*: liver *In vivo*: urine	I	Yang, S., Zhang, H. (2017)
4-hydroxy-zearalenone (isomer)	4-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
5-hydroxy-zearalenone	5-OH-ZEN	$C_{18}H_{22}O_6$	Rats & chickens Rats	*In vitro*: liver *In vivo*: urine	I	Yang, S., Zhang, H. (2017)
5-hydroxy-zearalenone (isomer)	5-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
6-hydroxy-zearalenone	6-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
6-hydroxy-zearalenone (isomer)	6-OH-ZEN	$C_{18}H_{22}O_6$	Swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
8-hydroxy-zearalenone	8-OH-ZEN	$C_{18}H_{22}O_6$	Rats, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
8-hydroxy-zearalenone (isomer)	8-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
9-hydroxy-zearalenone	9-OH-ZEN	$C_{18}H_{22}O_6$	Chickens, goats, cows	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)

Zearalenone

9-hydroxy-zearalenone	9-OH-ZEN	$C_{18}H_{22}O_6$	Chickens, goats, cows	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
9-hydroxy-zearalenone (isomer)	9-OH-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	I	Yang, S., Zhang, H. (2017)
deepoxy-zearalenone	Deepoxy-ZEN	$C_{18}H_{22}O_6$	Cows & humans	*In vitro*: liver	-	Yang, S., Zhang, H. (2017)
deepoxy-zearalenone (isomer)	Deepoxy-ZEN	$C_{18}H_{22}O_6$	Rats, chickens, swines, goats, cows, humans	*In vitro*: liver	-	Yang, S., Zhang, H. (2017)
Hydroxy-zearalenone-glucuronide	OH-ZEN-glucuronide	$C_{24}H_{30}O_{12}$	Rats	*In vivo*: urine & feces	II	Yang, S., Zhang, H. (2017)
Zearalenone-14,16-di-glucuronide	ZEN-14,16-di-glucuronide	$C_{30}H_{38}O_{17}$	Swines, goats, humans	*In vitro*: liver	II	Yang, S., Zhang, H. (2017)
Zearalenone-14-2-di-glucuronide	ZEN-14-2-di-glucuronide	$C_{30}H_{38}O_{17}$	Rats, goats, cows	*In vitro*: liver	II	Yang, S., Zhang, H. (2017)
Zearalenone-14-glucuronide	ZEN-14-glucuronide	$C_{24}H_{30}O_{11}$	Rats, chickens, swines, goats, cows, humans Swines Chicken & rats	*In vitro*: liver *In vivo*: feces *In vivo*: urine	II	Yang, S., Zhang, H. (2017) (Binder et al., 2017) Yang, S., Zhang, H. (2017)
Zearalenone-14-sulphate	ZEN-14-SO_3H	$C_{18}H_{22}SO_8$	Chickens	*In vivo*: feces	II	Yang, S., Zhang, H. (2017)
Zearalenone-16-glucuronide	ZEN-16-glucuronide	$C_{24}H_{30}O_{11}$	Rats, chickens, swines, goats, cows, humans Chicken & rats	*In vitro*: liver *In vivo*: urine	II	Yang, S., Zhang, H. (2017)
α-zearalenol / β-zearalenol	α-ZEL/β-ZEL	$C_{18}H_{24}O_5$	Rats, chickens, swines, goats, cows, humans Rats & chickens Humans Swines	*In vitro*: liver *In vivo*: urine *In vivo*: urine *In vivo*: urine	I	Yang, S., Zhang, H. (2017) (Heyndrickx et al., 2015) (Binder et al., 2017)
α-zearalenol/β-zearalenol-14-glucuronide	α-ZEL/β-ZEL-14-glucuronide	$C_{24}H_{32}O_{11}$	Rats & chickens	*In vivo*: urine & feces	II	Yang, S., Zhang, H. (2017)
α-zearalenol/β-zearalenol-14-sulphate	α-ZEL/β-ZEL-14-SO_3H	$C_{18}H_{24}SO_8$	Chickens	*In vivo*: feces	II	Yang, S., Zhang, H. (2017)
α-zearalenol/β-zearalenol-16-glucuronide	α-ZEL/β-ZEL-16-glucuronide	$C_{24}H_{32}O_{11}$	Rats & chickens Humans	*In vivo*: urine & feces *In vivo*: urine	II	Yang, S., Zhang, H. (2017) (Heyndrickx et al., 2015)



Aflatoxins (AF)

- Produced by *Aspergillus flavus, A. parasiticus* and *A. nomius.*
- Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2).
- Occur in cereals, dairy products, spices and dried fruits.
- Group 1 carcinogen.
- Major risk factor for hepatocellular carcinoma, other effects: immunosuppression, reduced growth rate, lowered milk and egg production, reduced reproductivity, reduced feed utilization and efficiency, and anaemia.

- Focus needs to be set towards the urinary analysis of AFB1, AFB2, AFG1, AFG2, AFM1, AFQ1, AFP1 and AF guanine.

- AFB1-lysine is a validated biomarker of chronic exposure in plasma.

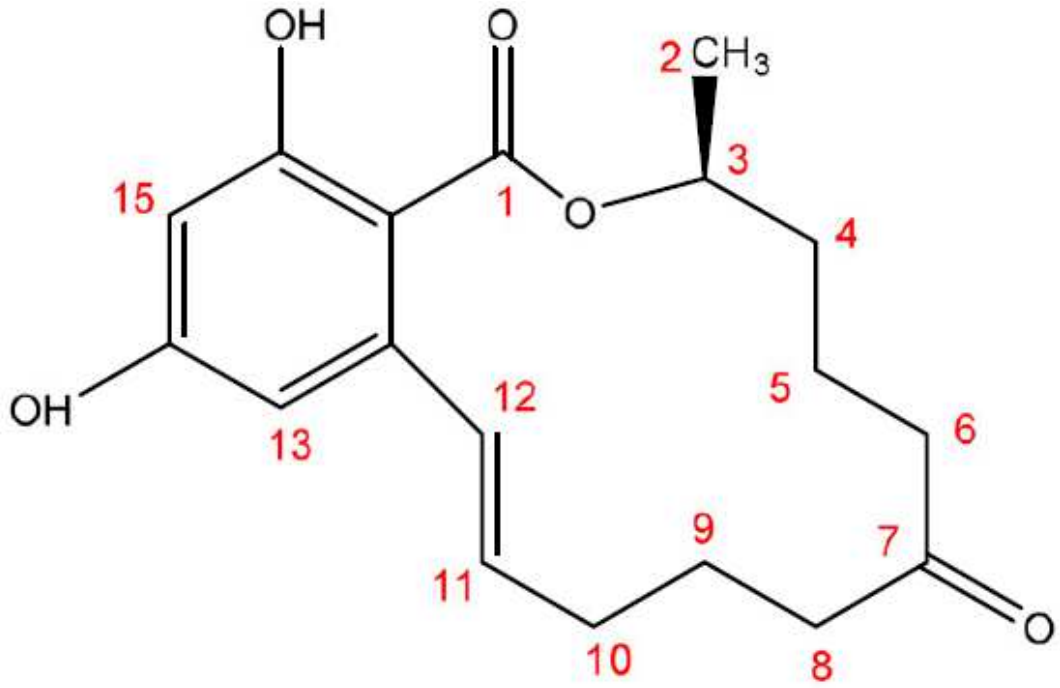
- More knowledge on AFB2, AFG1, and AFG2 metabolism is necessary.

Aflatoxins

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
Aflatoxicol	AFL	$C_{17}H_{14}O_6$	Cows Goats Rabbits, fish, swines, monkeys, rats, humans Chicken, turkey and ducks	*In vivo*: plasma & milk *In vivo*: milk, urine and feces *In vitro*: liver *In vitro*: liver	I	(Trucksess, Richard, Stoloff, McDonald, & Brumley, 1983) (Helferich et al., 1986) (Salhab & Edwards, 1977) (Lozano & Diaz, 2006)
Aflatoxin B1 8,9-dihydrodiol	AFB1 8,9-dihydrodiol	$C_{17}H_{14}O_8$	Humans	*In vitro*: liver	I	(Neal et al., 1998)
Aflatoxin B1-glutathione conjugate	AFB1-GSH	$C_{27}H_{29}N_3O_{13}S$	Bovines	*In vitro*: liver	II	(Kuilman et al., 2000)
Aflatoxin B1-8,9-epoxide	AFBO	$C_{17}H_{12}O_7$	Ducks & turkeys Rats Humans	*In vitro*: liver *In vitro*: liver *In vitro*: liver	-	(Lozano & Diaz, 2006) (Hayes, Judah, Mc Lellan, & Neal, 1991) (Johnson, Yamazaki, Shimada, Ueng, & Guengerich, 1997)
Aflatoxin B1-albumin	AFB1-albumin		Humans Rats	*In vivo*: plasma *In vivo*: plasma	II	(Turner et al., 2005) (Dirr & Schabort, 1986)
Aflatoxin B1-lysine	AFB1-lysine	$C_{23}H_{25}N_2O_8$	Humans Swines Rats	*In vivo*: plasma *In vivo*: plasma *In vivo*: plasma	II	(McMillan, 2018) (Di Gregorio et al., 2017) (Xue, Cai, Tang, & Wang, 2016)
Aflatoxin B-N7-guanine	AFB-N7 -guanine	$C_{22}H_{16}N_5O_7$	Humans Rats	*In vivo*: feces & urine *In vivo*: urine	II	(Mykkänen et al., 2005) (Groopman, Donahue, & Zhu, 1985)
Aflatoxin M1	AFM1	$C_{17}H_{12}O_7$	Cows Donkeys Humans Humans Rats Rats Goats	*In vivo*: milk *In vivo*: milk *In vivo*: urine & feces *In vivo*: milk *In vitro*: liver *In vivo*: urine *In vivo*: milk, urine and feces	I	(Britzi et al., 2013) (Tozzi et al., 2016) (Ferri et al., 2017) (Altun, Gurbuz, & Ayag, 2017) (Gurtoo & Motycka, 1976) (Groopman et al., 1985) (Helferich et al., 1986)

Aflatoxins

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
Aflatoxin M1	AFM1	$C_{17}H_{12}O_7$	Humans	*In vitro*: liver	I	(Neal et al., 1998)
8,9-dihydrodiol	d8,9-dihydrodiol	$C_{17}H_{14}O_8$				
Aflatoxin M2	AFM2	$C_{17}H_{14}O_7$	Donkeys Cows	*In vivo*: milk *In vivo*: milk	I	(Tozzi et al., 2016) (Sartori, de Mattos, de Moraes, & da Nobrega, 2015)
Aflatoxin P1	AFP1	$C_{16}H_{10}O_6$	Humans Rats	*In vivo*: urine & feces *In vivo*: urine	I	(Groopman et al., 1992) (Groopman et al., 1985)
Aflatoxin Q1	AFQ1	$C_{17}H_{12}O_7$	Rats Humans Goats	*In vitro*: liver *In vivo*: urine & feces *In vivo*: milk, urine and faeces	I	(Gurtoo & Motycka, 1976) (Mykkänen et al., 2005) (Helferich et al., 1986)
Aflatoxin B2a	AFB2a	$C_{17}H_{14}O_7$	Rabbits Humans	*In vitro*: liver *In vivo*: plasma	I	(Hatem, Hassab, Al-Rahman, El-Deeb, & El-Sayed Ahmed, 2005)



Sterigmatocystin (STC)

- Biochemical precursor of aflatoxins and produced by several *Aspergillus* species.
- Regularly detected in food, feed, but also in indoor environments, such as carpet and building materials.
- Group 2B carcinogen.
- Induces lung adenocarcinoma in mice and malignant trans-formations in human foetal lung tissue.

- Lack of information on STC metabolites.
- STC glucuronides could be the predominant metabolites from STC.
- More information is necessary regarding STC.

Metabolite	Abbreviation	Composition	Species	Method	Metabolic Phase	Ref
11-hydroxy-sterigmatocystin	11-OH-STERIG	$C_{18}H_{12}O_6$	Humans Rats	*In vitro*: liver	I	(Pfeiffer et al., 2014)
11,12c-dihydroxy-sterigmatocystin	11,12c-diOH-STERIG	$C_{18}H_{13}O_7$	Humans Rats		I	(Pfeiffer et al., 2014)
12c-hydroxy-sterigmatocystin	12c-OH-STERIG	$C_{18}H_{12}O_6$	Humans Rats		I	(Pfeiffer et al., 2014)
9-hydroxy-sterigmatocystin	9-OH-STERIG	$C_{18}H_{12}O_6$	Humans Rats		I	(Pfeiffer et al., 2014)
9,11-dihydroxy-sterigmatocystin	9,11-diOH-STERIG	$C_{18}H_{13}O_7$	Humans Rats		I	(Pfeiffer et al., 2014)
9,12c-dihydroxy-sterigmatocystin	9,12c-diOH-STERIG	$C_{18}H_{13}O_7$	Humans Rats		I	(Pfeiffer et al., 2014)
Sterigmatocystin-1,2-oxide	STERIG-1,2-oxide	$C_{18}H_{12}O_7$	Humans Rats		I	(Pfeiffer et al., 2014)
Sterigmatocystin-1,2-dihydrodiol	STERIG-1,2-dihydrodiol	$C_{18}H_{14}O_8$	Humans Rats		I	(Pfeiffer et al., 2014)

- Every mycotoxin is different: different metabolites and different excretion.
- Every specie is different: different metabolites and different excretion.
- *Lack of research:*
  - *In vitro*: to **elucidate metabolites.**
  - *In vivo*: to **elucidate excretion profile.**
- Needed for identification and validation of mycotoxin biomarkers of exposure in different matrices.
- Multiple biomarker-driven explorations.

Comprehensive REVIEWS in Food Science and Food Safety

Mycotoxin Biomarkers of Exposure: A Comprehensive Review

Arnau Vidal, Marcel Mengelers, Shupeng Yang, Sarah De Saeger, and Marthe De Boevre

Abstract: To date, the use of biomarkers has become generally accepted. Biomarker-driven research has been proposed as a successful method to assess the exposure to xenobiotics by using concentrations of the parent compounds and/or metabolites in biological matrices such as urine or blood. However, the identification and validation of biomarkers of exposure remain a challenge. Recent advances in high-resolution mass spectrometry along with new analytical (post-acquisition data-mining) techniques will improve the quality and output of the biomarker identification process. Chronic or even acute exposure to mycotoxins remains a daily fact, and therefore it is crucial that the mycotoxins' metabolism is unravelled so more knowledge on biomarkers in humans and animals is acquired. This review aims to provide the scientific community with a comprehensive overview of reported *in vitro* and *in vivo* mycotoxin metabolism studies in relation to biomarkers of exposure for deoxynivalenol, nivalenol, fusarenon-X, T-2 toxin, diacetoxyscirpenol, ochratoxin A, citrinin, fumonisins, zearalenone, aflatoxins, and sterigmatocystin.

Keywords: biomarkers, exposure, human, *in vitro*, *in vivo*, metabolism, mycotoxin

Contacts

Arnau Vidal

Arnau Vidal PhD works as a FWO PostDoc at the Centre of Excellence on Mycotoxicology and Public Health, Ghent University, Belgium.

Email:

Arnau.vidalcorominas@ugent.be

Marthe.deboevre@ugent.be

Sarah.desaeger@ugent.be

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