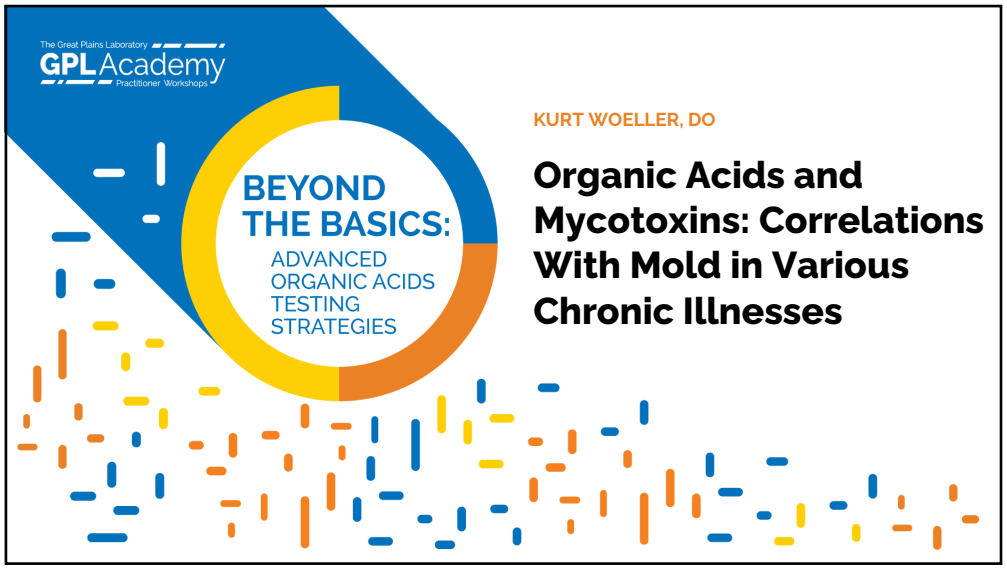
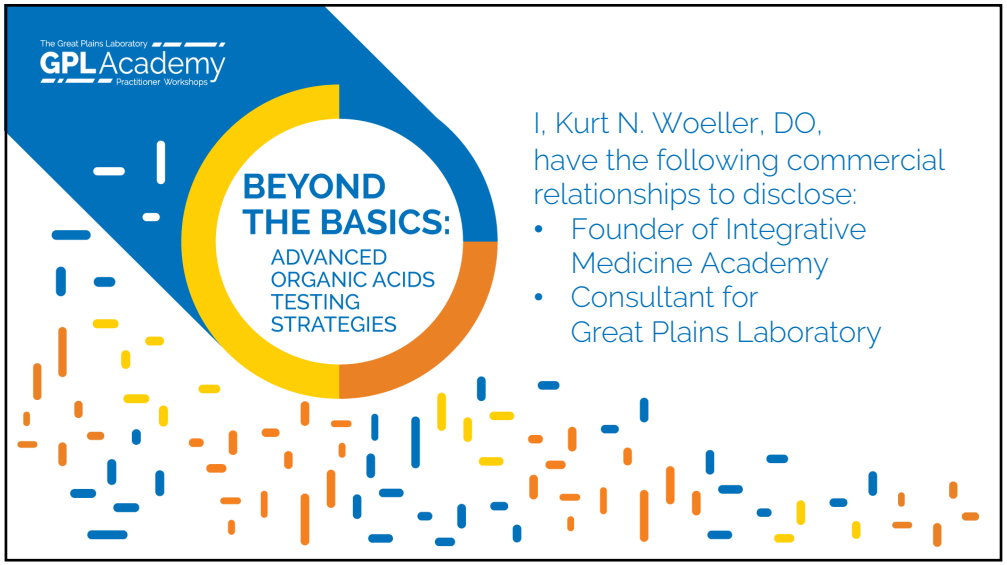

The Great Plains Laboratory
GPLAcademy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
KURT WOELLER, DO
Organic Acids and Mycotoxins: Correlations With Mold in Various Chronic Illnesses

1


The Great Plains Laboratory
GPLAcademy
Practitioner Workshops
BEYOND THE BASICS:
ADVANCED ORGANIC ACIDS TESTING STRATEGIES
I, Kurt N. Woeller, DO,
have the following commercial relationships to disclose:
• Founder of Integrative Medicine Academy
• Consultant for Great Plains Laboratory

2

Disclaimer

- The material contained within this presentation is not intended to replace the services and/or medical advice of your personal licensed health care professional.
- This material is for educational purposes only
- This information is not meant to encourage diagnosis and treatment of disease.
- Any application of suggestions set forth in the following portions of this presentation is at the reader's discretion.
- Implementation and/or experimentation with any supplements, herbs, dietary changes, medications, and/or lifestyle changes, etc., is done so at your sole risk and responsibility.

3

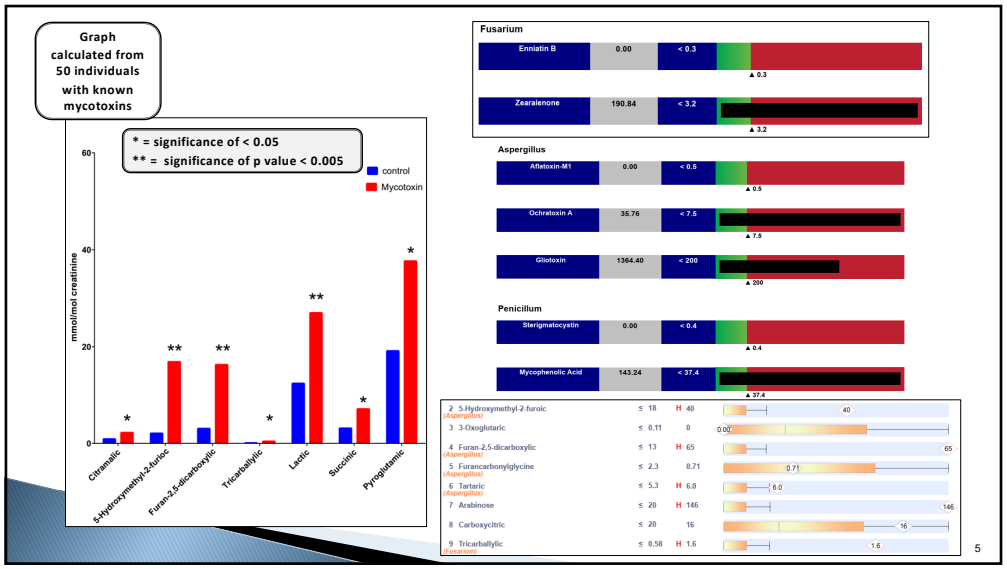
3

Goals of this lecture

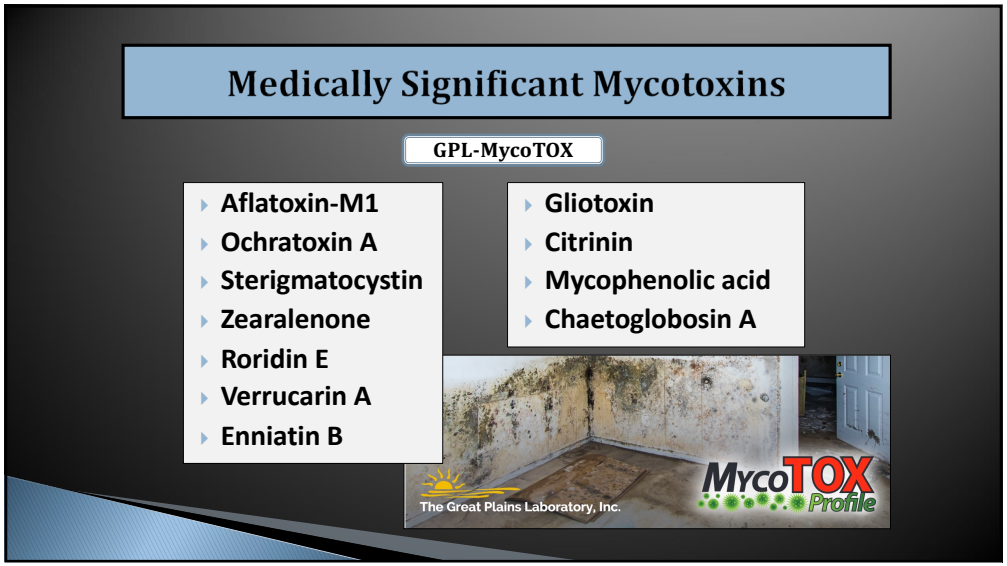
1. Deepen our understanding of various mycotoxins with regards to their toxicity and certain pathogenicity mechanisms, including mitochondrial damage, and various illnesses.
2. Discuss some examples of specific chronic health problems linked to mold and mycotoxin exposures.
3. Correlate information from the Organic Acids Test (OAT) to mold exposure and the possibility of mycotoxin accumulation.
4. Discuss, in brief, some intervention strategies for mold and mycotoxin exposures.

4

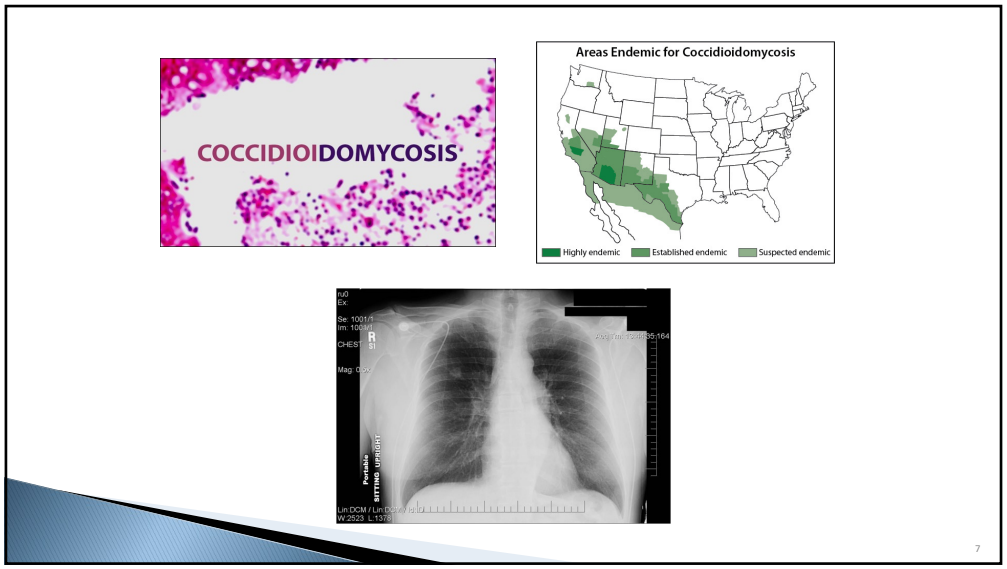
4


Graph calculated from 50 individuals with known mycotoxins
* = significance of < 0.05
** = significance of p value < 0.005
control
Mycotoxin
mmol/mol creatinine
Citramalic
5-Hydroxymethyl-2-furoic
Furan-2,5-dicarboxylic
Tricarballylic
Lactic
Succinic
Pyroglutamic
Fusarium
Enniatin B
Zearalenone
Aspergillus
Aflatoxin-M1
Ochratoxin A
Gliotoxin
Penicillum
Sterigmatocystin
Mycophenolic Acid
5

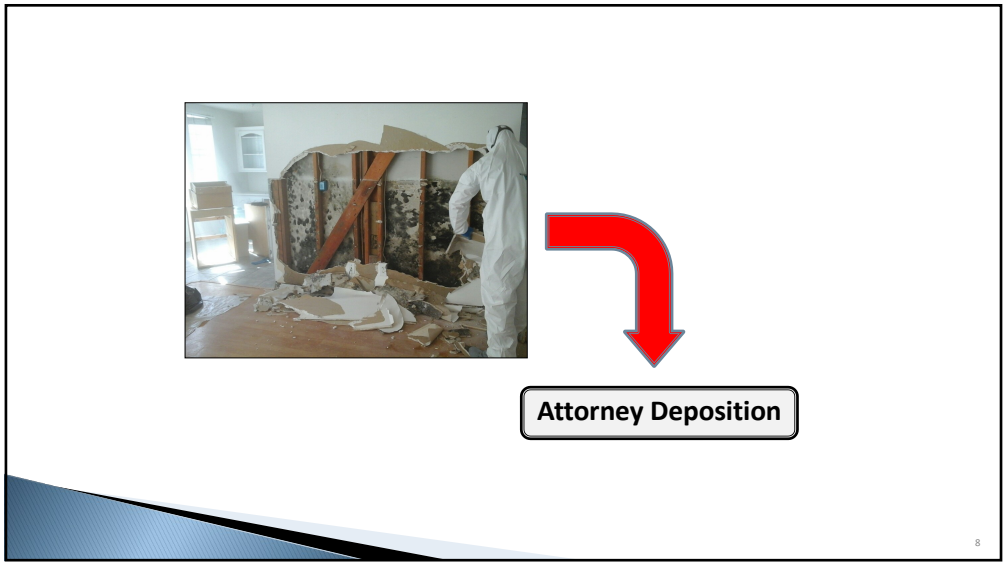
5


Medically Significant Mycotoxins
GPL-MycoTOX
Aflatoxin-M1
Ochratoxin A
Sterigmatocystin
Zearalenone
Roridin E
Verrucarin A
Enniatin B
Gliotoxin
Citrinin
Mycophenolic acid
Chaetoglobosin A
The Great Plains Laboratory, Inc.
MycoTOX Profile

6


COCCIDIOIDOMYCOSIS
Areas Endemic for Coccidioidomycosis
Highly endemic
Established endemic
Suspected endemic
7

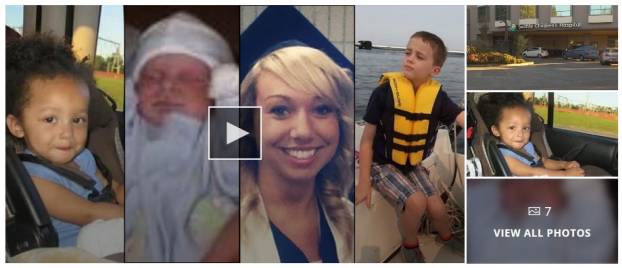
7


Attorney Deposition
8

8

Seattle Children's Hospital knew of deadly mold danger since 2005, lawsuit says

by KOMO News Staff | Monday, December 2nd 2019



Mold infection victims (left to right) Aiden Wills, Logan Shaffer, Whitney Stettler and Ian Gunnell (Photo: Stritmatter Kessler Koehler Moore)

SEATTLE – Seattle Children's Hospital was hit with two lawsuits Monday over Aspergillus mold that has sickened and killed children on and off for 18 years.

9

November 18, 2019 11:39 AM

Seattle Children's: Mold outbreak linked to infections since 2001

Seattle Children's Hospital said Monday it believes a mold outbreak that has sickened seven patients, one fatally, since summer 2018 is linked to mold-related infections dating back nearly two decades.

Five children died from infections linked to Aspergillus mold in operating rooms at Children's from 2001 to 2014, and two others became ill, Dr. Jeff Sperring, the hospital's chief executive, said in a written statement.

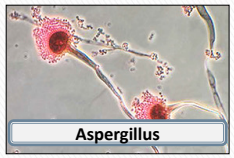
Those cases were thought at the time to be isolated, but officials now believe they and the more recent infections are all linked to inadequate air filtration, he said.

10

What Are Molds?

- Molds are fungi that can be found in both indoor and outdoor environments.
- Exact numbers of fungi species don't exist, but estimates are tens to hundreds of thousands.
- Molds grow best in warm, damp and humid conditions:
  - *They can survive in harsh, dry environments too.*
- Molds spread and reproduce via mold spores.

Fungi
(Biological Group Of Organisms)



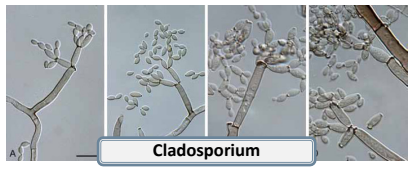
- **Yeast** – ex: *Candida, Saccharomyces.*
- **Mold** – ex: *Aspergillus, Penicillium.*
- **Mushrooms**

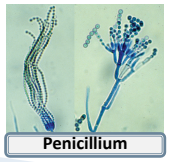
11

11

Common Indoor Molds

- *Cladosporium*
- *Penicillium*
- *Alternaria*
- *Aspergillus*





12

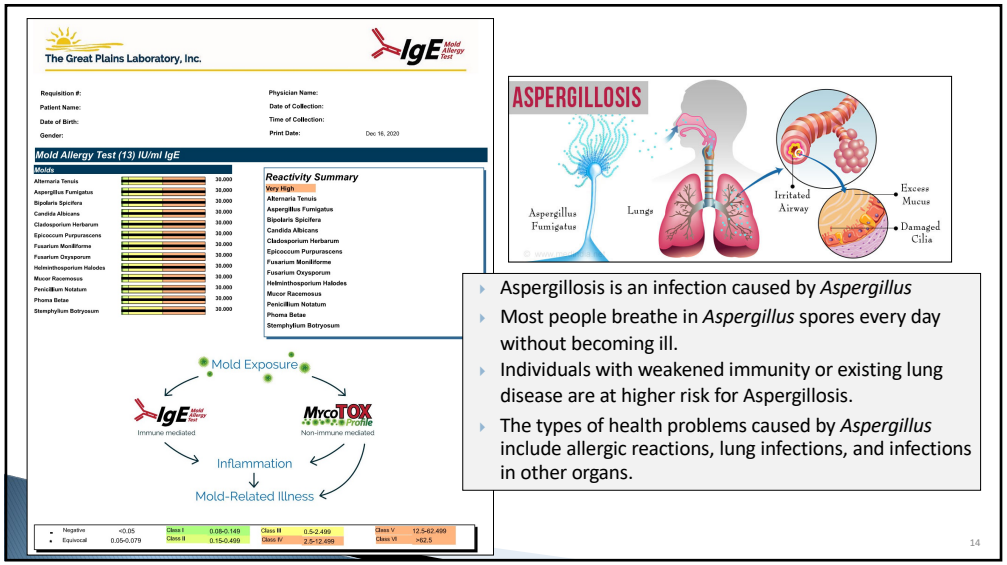
12

Route of Exposure

- Ingestion/skin contact/inhalations
- Blood stream and lymphatic system
- Inhibit protein synthesis
- Damage macrophage system
- Inhibit particle clearage of the lungs

13

13



- Aspergillosis is an infection caused by *Aspergillus*
- Most people breathe in *Aspergillus* spores every day without becoming ill.
- Individuals with weakened immunity or existing lung disease are at higher risk for Aspergillosis.
- The types of health problems caused by *Aspergillus* include allergic reactions, lung infections, and infections in other organs.

14

14

Usual Adult Dose for Aspergillosis - Aspergilloma

Loading dose: 200 mg orally 3 times a day for the first 3 days of therapy
Maintenance dose: 200 mg orally once or twice a day
Duration of therapy: At least 3 months and until clinical parameters and laboratory tests indicate the active fungal infection has subsided

Comments:
-Capsule formulation
-A loading dose should be used in life-threatening situations.

Use: For the treatment of aspergillosis (pulmonary and extrapulmonary) in immunocompromised and non-immunocompromised patients intolerant of, or refractory to, amphotericin B

IDSA Recommendations:
Invasive aspergillosis: 200 mg orally 3 times a day for 3 days, then 200 mg orally twice a day
Empirical and preemptive antifungal therapy: 200 mg orally twice a day
Prophylaxis against invasive aspergillosis: 200 mg orally twice a day

Source: *https://www.drugs.com/dosage/itraconazole*

Willian Shaw, PhD

15

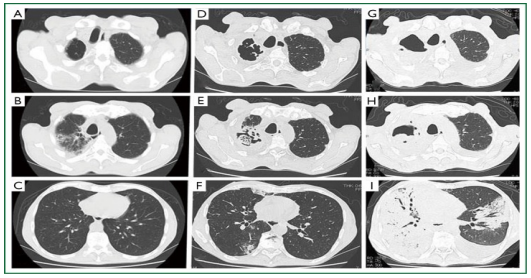
Respiratory Diseases

- **Allergic bronchopulmonary aspergillosis** – wheezing, shortness of breath, cough and fever *(in rare cases).*
- **Allergic Aspergillus sinusitis** – stuffiness, runny nose, headache, reduced ability to smell.
- **Chronic pulmonary aspergillosis** - weight loss, cough, coughing up blood, fatigue, shortness of breath.
- **Aspergilloma** ("fungus ball") – cough, coughing up blood, shortness of breath.
- **Invasive Aspergillosis** – infection spreads to other parts of the body.

16

16

Calcium oxalate crystal deposition in a patient with Aspergilloma due to *Aspergillus niger*



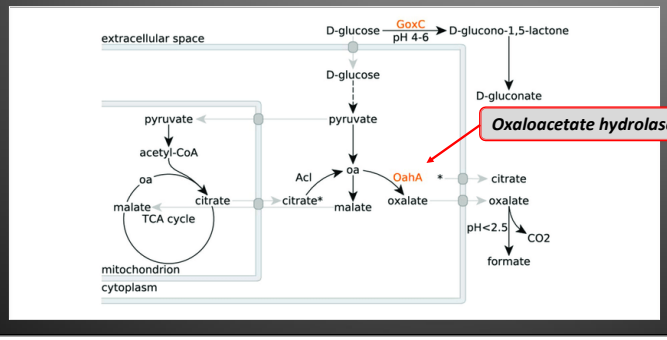
Thoracic CT 9 months (A, B, C) and 3 weeks (D, E, F) before arrival at our hospital and upon admission (G, H, I). Old inflammatory and cystic changes in right upper lobe (A and B); Fungal, ball-like lesion 2.5 cm in diameter with air space consolidation in right upper lobe (D and E) and mild consolidation in right lower lobe (F); Right lung (G) and left middle lobe (H) are replaced by massive consolidation with air and bilateral pleural effusion is evident (I).

Source: J Thorac Dis. 2013 Aug; 5(4): E174–E178

17

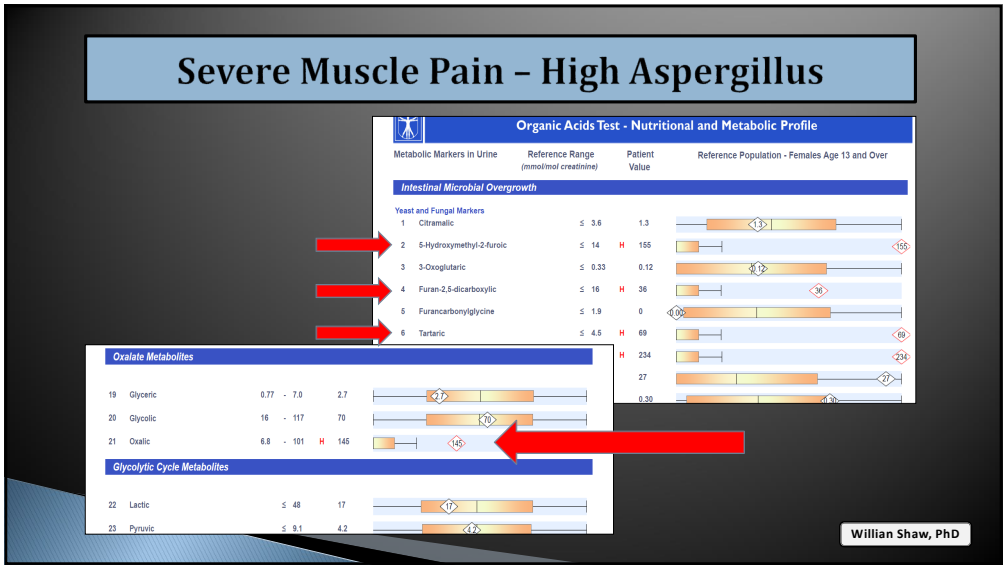
17

Aspergillus niger



Source: *Aspergillus niger Secretes Citrate to Increase Iron Bioavailability - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Organic-acid-production-in-A-niger-N402-and-its-derivatives-Metabolic-routes-for_fig1_318858213.*

18



19

Aspergillus Toxicity

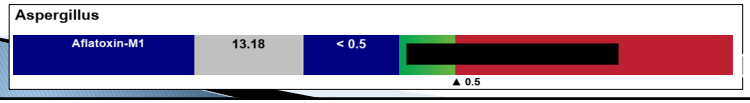
- Most prevalent mold group in the environment and has caused billions of dollars in damage to crops and livestock.
- Most common *Aspergillus* mycotoxins are **aflatoxin**, **ochratoxin**, patuli, and fumagillin.
- *Aspergillus* toxins have been found in all major cereal crops, e.g., peanuts, corn, rice.
- They can also be found in eggs, milk and meat from animals fed contaminated grains.
- There are approximately 180 species of *Aspergillus*, but fewer than 40 of them are known to cause infections in humans.
- *Aspergillus fumigatus* is the most common cause of human *Aspergillus* infections. Other common species include *A. flavus*, *A. terreus*, and *A. niger.*
- The liver is the main target of these toxins.

20

20

Wojciech Blonski, David S Kotlyar, Kimberly A Forde Non-viral causes of hepatocellular carcinoma *World J Gastroenterol*, 2010 August 7; 16(29): 3603-3615

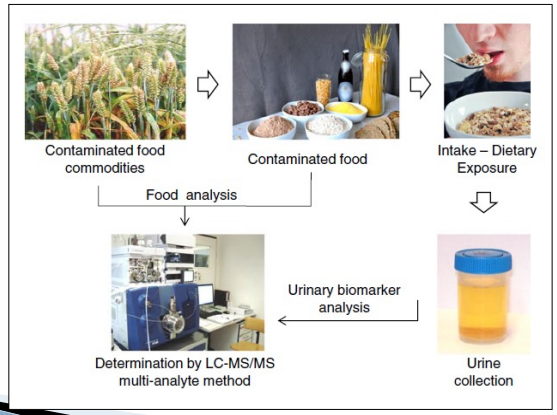
- Aflatoxin B1 (AFB1) is independent of the risk conferred by Hepatitis B Virus (HBV).
- However, concomitant exposure to both HBV and AFB1 markedly increases the risk of hepatocellular carcinoma (HCC).
- When compared to those without HBV infection and absence of urinary AFB1 markers, the risk of HCC was 60 times higher in patients with HBV infection and a concomitant elevation of urinary AFB1 markers.



Willian Shaw, PhD

21

Determination of Mycotoxins



Willian Shaw, PhD

22

22

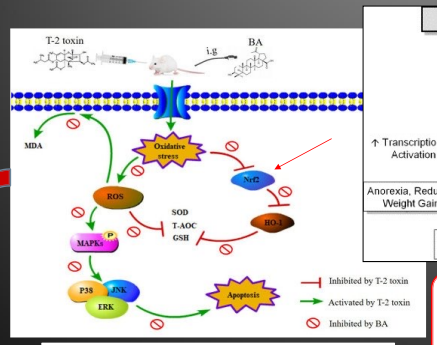
What are Mycotoxins?
(myco = of fungal origin)

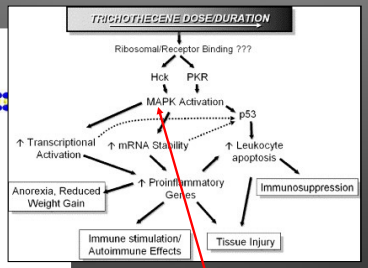
- Mycotoxins are chemicals produced by molds that cause toxic results.
- Hundreds of fungi that can produce toxic compounds.
- Most mycotoxin exposures are through food ingestion or airborne exposure.
- Mycotoxins are resistant to heat and many processing procedures.
- Cause mitochondrial damage and deplete glutathione.
- Mycotoxins can disrupt cellular processes such as protein production and degradation, DNA and RNA synthesis and repair.

23

23

T-2 Toxicity Example



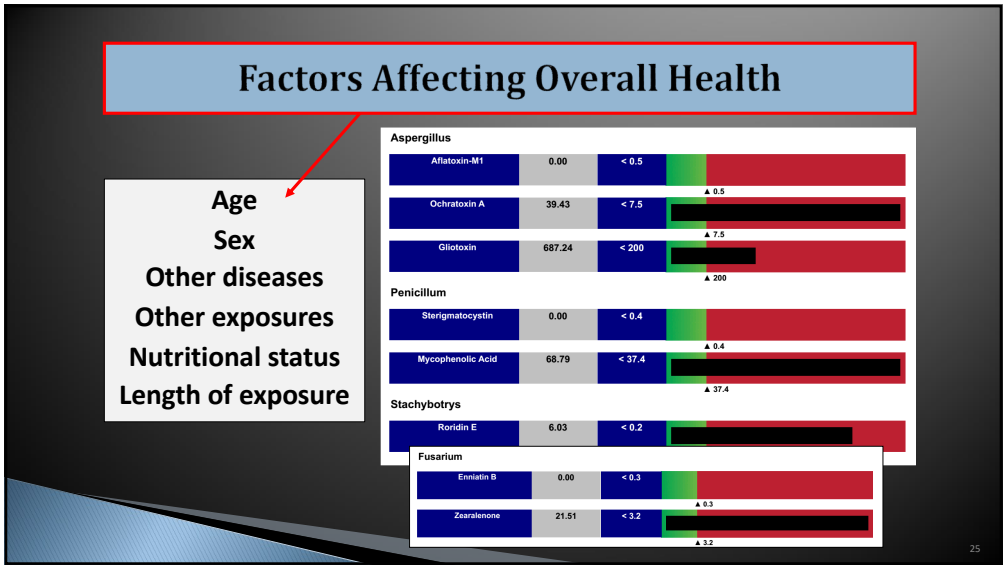


Source: *https://www.mdpi.com/2072-6651/12/9/540*

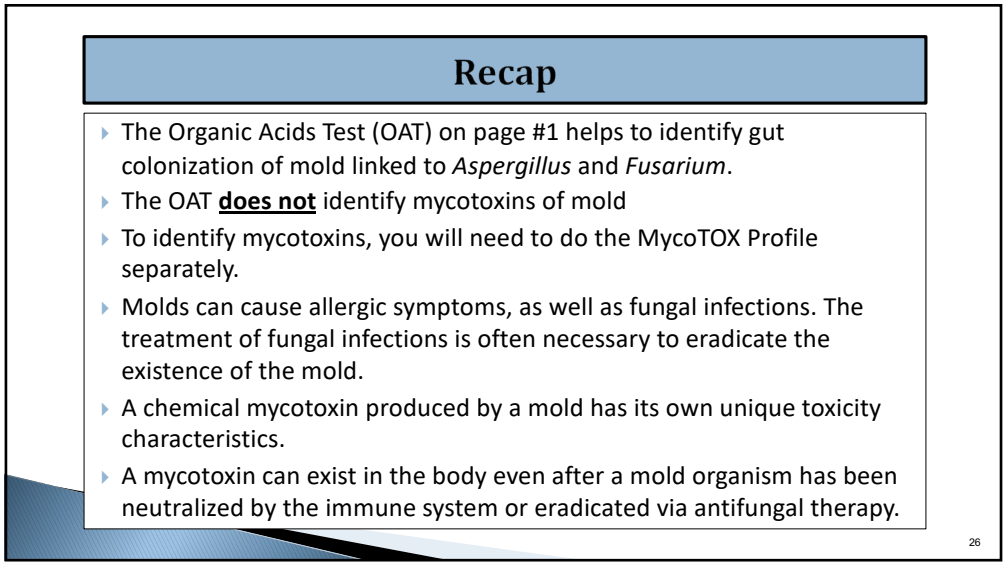
Mitogen-activated protein kinase **(MAPK) are three sequentially activated protein kinases that are key components of a series of vital signal transduction pathways that regulate processes such as cell proliferation, cell differentiation, and cell death.**

"The multifunctional regulator nuclear factor erythroid 2-related factor (Nrf2) is considered not only as a cytoprotective factor regulating the expression of genes coding for antioxidants, anti-inflammatory and detoxifying proteins, but it is also a powerful modulator of species longevity."

24


Factors Affecting Overall Health
Age
Sex
Other diseases
Other exposures
Nutritional status
Length of exposure
Aspergillus
Aflatoxin-M1 0.00 < 0.5
Ochratoxin A 39.43 < 7.5
Gliotoxin 687.24 < 200
Penicillum
Sterigmatocystin 0.00 < 0.4
Mycophenolic Acid 68.79 < 37.4
Stachybotrys
Roridin E 6.03 < 0.2
Fusarium
Enniatin B 0.00 < 0.3
Zearalenone 21.51 < 3.2
25

25


Recap
The Organic Acids Test (OAT) on page #1 helps to identify gut colonization of mold linked to *Aspergillus* and *Fusarium*.
The OAT **does not** identify mycotoxins of mold
To identify mycotoxins, you will need to do the MycoTOX Profile separately.
Molds can cause allergic symptoms, as well as fungal infections. The treatment of fungal infections is often necessary to eradicate the existence of the mold.
A chemical mycotoxin produced by a mold has its own unique toxicity characteristics.
A mycotoxin can exist in the body even after a mold organism has been neutralized by the immune system or eradicated via antifungal therapy.
26

26

Medically Significant Mycotoxins

GPL-MycoTOX

- Aflatoxin-M1
- Ochratoxin A
- Sterigmatocystin
- Zearalenone
- Roridin E
- Verrucarin A
- Enniatin B
- Gliotoxin
- Citrinin
- Mycophenolic acid
- Chaetoglobosin A

27

Ochratoxin A

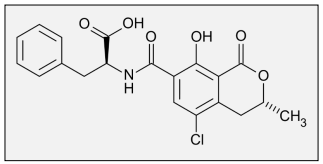
Common mycotoxin from Aspergillus mold

28

28

Ochratoxin A (OTA)

- Species: *P. Verrucosum and A. ochraceus.*
- Most common mycotoxin in urine.
- Carcinogenic
- Neurological problems
- Affects kidney function:
  - Inhibits synthesis of proteins
  - Disrupts DNA and RNA
  - Inhibits enzymes in the kidney



Ochratoxin A	249.42	4 - 20

▲ 4 20 ▲

29

29

Ochratoxin A

- Ochratoxin A (OTA) is a nephrotoxic, immunotoxic, and carcinogenic mycotoxin.
- Produced by *Aspergillus* and *Penicillium*.
- Exposure is primarily through contaminated foods such as cereals, grape juices, dairy, spices, wine, dried vine fruit and coffee.

- Can come from inhalation exposure in water-damaged buildings.
- OTA can lead to kidney disease and adverse neurological effects.
- Studies have shown that OTA can cause significant oxidative damage to multiple brain regions.
- **Dopamine levels in the brain have been shown to be decreased after exposure to OTA.**

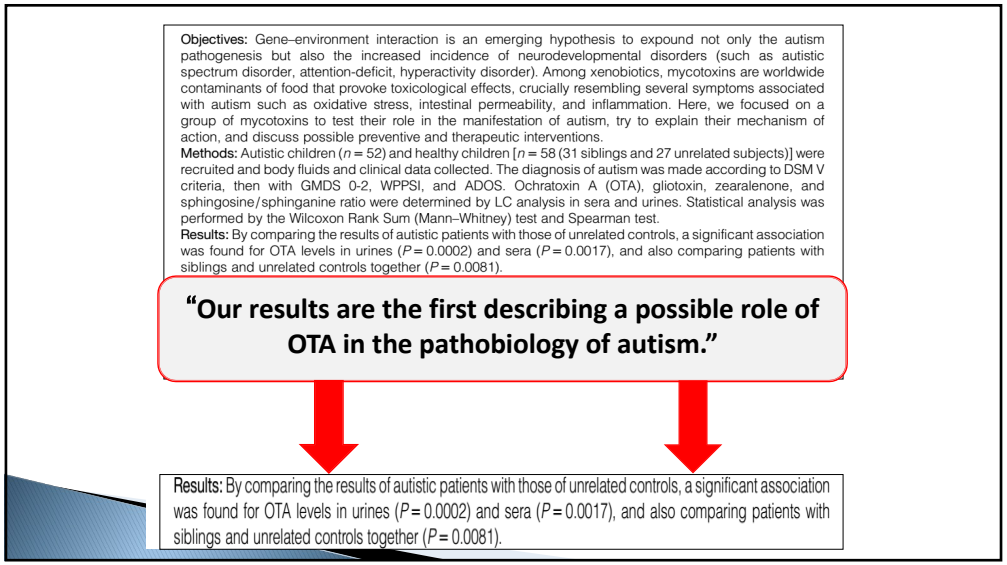
Low HVA on the OAT?

30

30


Nutritional Neuroscience
An International Journal on Nutrition, Diet and Nervous System
ISSN: 1028-415X (Print) 1476-8305 (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20
Role of mycotoxins in the pathobiology of autism: A first evidence
Barbara De Santis, Carlo Brera, Alessandra Mezzelani, Sabina Soricelli, Francesca Ciceri, Giorgio Moretti, Francesca Debegnach, Maria Clara Bonaglia, Laura Villa, Massimo Molteni & Maria Elisabetta Raggi
To cite this article: Barbara De Santis, Carlo Brera, Alessandra Mezzelani, Sabina Soricelli, Francesca Ciceri, Giorgio Moretti, Francesca Debegnach, Maria Clara Bonaglia, Laura Villa, Massimo Molteni & Maria Elisabetta Raggi (2019) Role of mycotoxins in the pathobiology of autism: A first evidence, Nutritional Neuroscience, 22:2, 132-144, DOI: 10.1080/1028415X.2017.1357793
To link to this article: https://doi.org/10.1080/1028415X.2017.1357793
Ochratoxin A
249.42
4 - 20
4
20

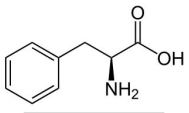
31


Objectives: Gene–environment interaction is an emerging hypothesis to expound not only the autism pathogenesis but also the increased incidence of neurodevelopmental disorders (such as autistic spectrum disorder, attention-deficit, hyperactivity disorder). Among xenobiotics, mycotoxins are worldwide contaminants of food that provoke toxicological effects, crucially resembling several symptoms associated with autism such as oxidative stress, intestinal permeability, and inflammation. Here, we focused on a group of mycotoxins to test their role in the manifestation of autism, try to explain their mechanism of action, and discuss possible preventive and therapeutic interventions.
Methods: Autistic children (n = 52) and healthy children [n = 58 (31 siblings and 27 unrelated subjects)] were recruited and body fluids and clinical data collected. The diagnosis of autism was made according to DSM V criteria, then with GMDS 0-2, WPPSI, and ADOS. Ochratoxin A (OTA), gliotoxin, zearalenone, and sphingosine/sphinganine ratio were determined by LC analysis in sera and urines. Statistical analysis was performed by the Wilcoxon Rank Sum (Mann–Whitney) test and Spearman test.
Results: By comparing the results of autistic patients with those of unrelated controls, a significant association was found for OTA levels in urines (P = 0.0002) and sera (P = 0.0017), and also comparing patients with siblings and unrelated controls together (P = 0.0081).
"Our results are the first describing a possible role of OTA in the pathobiology of autism."
Results: By comparing the results of autistic patients with those of unrelated controls, a significant association was found for OTA levels in urines (P = 0.0002) and sera (P = 0.0017), and also comparing patients with siblings and unrelated controls together (P = 0.0081).

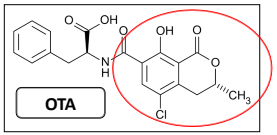
32

OTA is a small organic molecule, consisting of an isocoumarin moiety linked to a phenylalanine (phe) molecule. The phe of OTA competes with phe in binding to the phe-metabolizing enzymes phenylalanine hydroxylase (PAH), thus causing its inhibition.[26] In support of this finding, a simultaneous

OTA competes with Phenylalanine which is involved in dopamine production. Dopamine is needed for attention and mental focus.



Phenylalanine



OTA

administration of OTA and an antagonist, such as aspartame,[26] reduces the inhibition of PAH restoring the physiological activity of the enzyme and reversing the mycotoxin toxicity. In physiological condition, PAH converts phe into the amino acid tyrosine necessary for the biosynthesis of catecholamines, i.e. the neurotransmitters dopamine, noradrenaline, and adrenaline. Dopamine is, moreover, involved in social behaviour,[27] since it interacts with oxytocin (OT), a hormone with a key role in social communication; thus, PAH inhibition leads to a decrease in catecholamines and consequent interfering in OT activity. Interestingly, autistic children have significant lower level of OT in plasma and are characterized by

Dopamine is also involved in social behavior and interacts with Oxytocin.

33

Parkinson's Foundation

Understanding Parkinson's | Living with Parkinson's | Expert Care | Research | Get Involved | Ways to Give

A A A

Home › Living With Parkinson's › Parkinson's Today Blog
› What's Hot in PD? Could Fungus and Mold be an Important Contributor to Parkinson's Disease?

Top Blog Posts

1 Looking Ahead: Parkinson's, COVID-19 & the New Normal

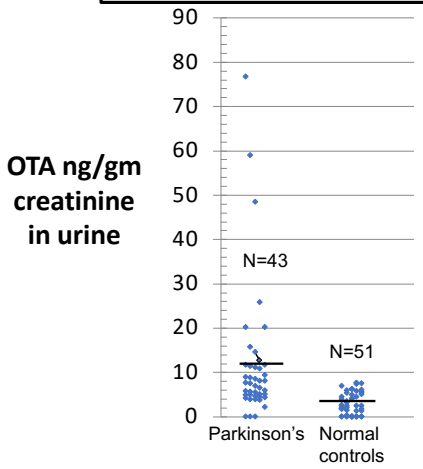
What's Hot in PD? Could Fungus and Mold be an Important Contributor to Parkinson's Disease?

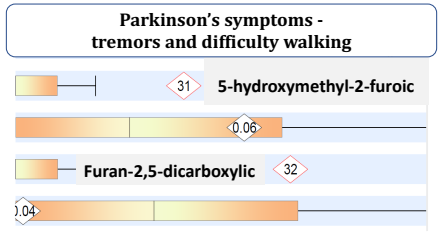
There are many great mysteries and unanswered questions surrounding the potential causes of Parkinson's disease. One of the most important and most studied is, "why

Willian Shaw, PhD

34

Ochratoxin A (OTA) in Parkinson's disease





— mean

T-test, p<0.0002

Willian Shaw, PhD

35

Mycotoxin-assisted mitochondrial dysfunction and cytotoxicity: Unexploited tools against proliferative disorders

Muhammad Torequl Islam, Siddhartha Kumar Mishra, Swati Tripathi, Marcus Vinícius Oliveira Barros de Alencar, João Marcelo de Castro e Sousa, Hercília Maria Lins Rolim ... See all authors

First published: 04 September 2018 | https://doi.org/10.1002/iub.1932 | Citations: 8

cultured adrenocortical tumor cells. ACTH increases the basal steroidogenic rate in cultured adrenocortical tumor cells while moderate to high doses of cytochalasin B inhibited both basal and ACTH-induced steroidogenesis 16. Ochratoxin A obtained from *Aspergillus ochraceus* is a nephrotoxic, hepatotoxic, teratogenic, immunotoxic, and phytotoxic mycotoxin. Ochratoxin A (0.5 and 1 mM) and oosporein (0.25–1 mM) caused nephrotoxicity through either mitochondrial dysfunction or by lipid peroxidation. Furthermore, ochratoxin A caused mitochondrial dysfunction and induced proximal tubule cell death in isolated rat renal proximal tubules 17. On the other hand, it caused disturbance of Ca^{2+} homeostasis in rat trachea, possibly by inducing toxicity through mitochondrial enzyme inhibition 18. Moreover, in *Arabidopsis thaliana*, this mycotoxin increased reactive oxygen species (ROS) and caused structural damage and mitochondrial dysfunction 19. In a similar fashion, ochratoxin A triggered accelerated respiration, increased production of mitochondrial ROS, which caused an opening of ROS-dependent mitochondrial permeability transition pores. This led to the decrease in mitochondrial membrane potential and release of cytochrome *c* into the cytosol 20, and leading to the induction of mitochondrial intrinsic apoptosis.

Ochratoxin A	0.01, 0.25 mM in *Arabidopsis thaliana*	↑ROS generation

36

Ochratoxin Contaminated Foods

DOI 10.1002/mnfr.200700266 Mol. Nutr. Food Res. 2008, 52, 496 – 501

Research Article

Maternal dietary habits and mycotoxin occurrence in human mature milk

Fabio Galvano[1,4], Amedeo Pietri[2], Terenzio Bertuzzi[2], Luigi Gagliardi[3], Sabina Ciotti[2], Stefano Luisi[4], Matteo Bognanno[1], Luca La Fauci[1], Anna Maria Iacopino[1], Francesco Nigro[1], Giovanni Li Volti[5], Luca Vanella[6], Giuseppe Giammanco[7], Gabriella Lucia Tina[6] and Diego Gazzolo[4,7]

[1] STAFA Department, Mediterranean University of Reggio Calabria, Reggio Calabria, Italy
[2] ISAN, Università Cattolica del Sacro Cuore, Piacenza, Italy
[3] Division of Pediatrics and Neonatology, Ospedale "Venzilla", Lido di Camaiore, Italy
[4] Department of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Italy
[5] Department of Maternal, Fetal and Neonatal Health, G. Garibaldi Hospital, Catania
[6] Department of Biological Chemistry, Medical Chemistry and Molecular Biology, University of Catania, Italy
[7] Department of Pediatrics, G. Gaslini Children's University Hospital, Genoa, Italy

Table 2: Median Concentrations for Ochratoxin A in Different Food Groups

Food group	Median concentration (µg/kg)
Cereals and cereal products	0.032
Legumes, pulses and their products	0.025
Meat, poultry and their products	0.042
Chocolate	0.142
Dried fruits	0.299
Juice drinks	0.004
Coffee & tea	0.003

(Note: The value of 1/2 LOD was assigned to non-detects and results below quantification)

Chocolate and Dried Fruits

Willian Shaw, PhD

37

37

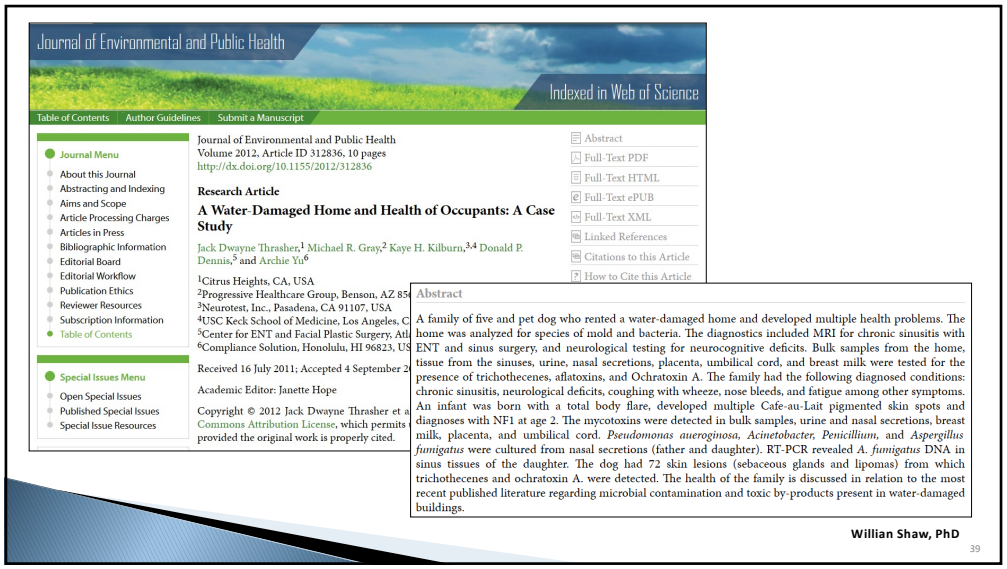
Family Case Study

Father (40 years old)
Mother (39 years old)
Son & Daughter (child age)
Newborn
Dog (? Years old)

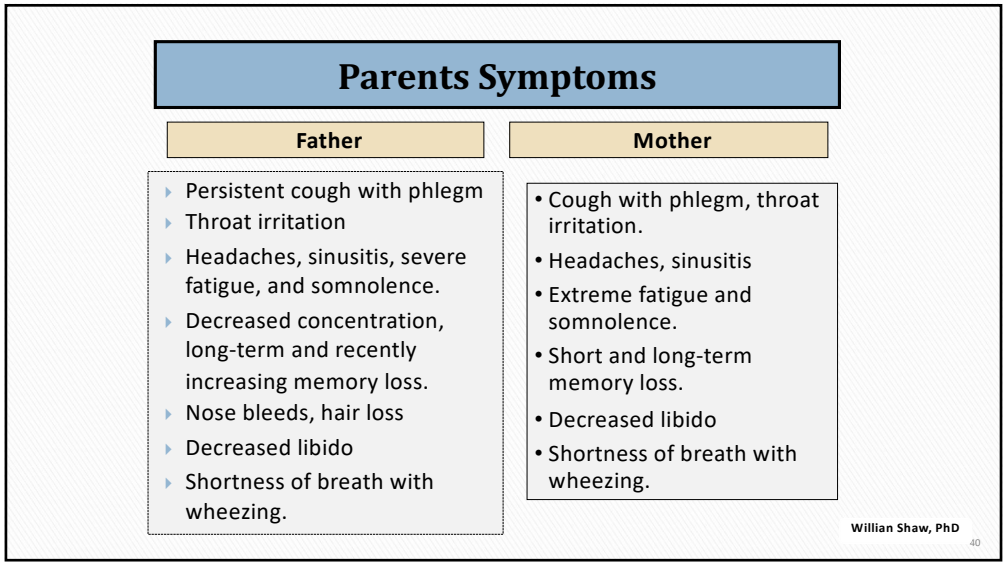
Willian Shaw, PhD

38

38


Journal of Environmental and Public Health
Indexed in Web of Science
Table of Contents
Author Guidelines
Submit a Manuscript
Journal Menu
About this Journal
Abstracting and Indexing
Aims and Scope
Article Processing Charges
Articles in Press
Bibliographic Information
Editorial Board
Editorial Workflow
Publication Ethics
Reviewer Resources
Subscription Information
Table of Contents
Special Issues Menu
Open Special Issues
Published Special Issues
Special Issue Resources
Journal of Environmental and Public Health
Volume 2012, Article ID 312836, 10 pages
http://dx.doi.org/10.1155/2012/312836
Research Article
A Water-Damaged Home and Health of Occupants: A Case Study
Jack Dwayne Thrasher,[1] Michael R. Gray,[2] Kaye H. Kilburn,[3,4] Donald P. Dennis,[5] and Archie Yu[6]
[1]Citrus Heights, CA, USA
[2]Progressive Healthcare Group, Benson, AZ 85
[3]Neurotest, Inc., Pasadena, CA 91107, USA
[4]USC Keck School of Medicine, Los Angeles, C
[5]Center for ENT and Facial Plastic Surgery, Atl
[6]Compliance Solution, Honolulu, HI 96823, US
Received 16 July 2011; Accepted 4 September 2
Academic Editor: Janette Hope
Copyright © 2012 Jack Dwayne Thrasher et a
Commons Attribution License, which permits
provided the original work is properly cited.
Abstract
Full-Text PDF
Full-Text HTML
Full-Text ePUB
Full-Text XML
Linked References
Citations to this Article
How to Cite this Article
Abstract
A family of five and pet dog who rented a water-damaged home and developed multiple health problems. The home was analyzed for species of mold and bacteria. The diagnostics included MRI for chronic sinusitis with ENT and sinus surgery, and neurological testing for neurocognitive deficits. Bulk samples from the home, tissue from the sinuses, urine, nasal secretions, placenta, umbilical cord, and breast milk were tested for the presence of trichothecenes, aflatoxins, and Ochratoxin A. The family had the following diagnosed conditions: chronic sinusitis, neurological deficits, coughing with wheeze, nose bleeds, and fatigue among other symptoms. An infant was born with a total body flare, developed multiple Cafe-au-Lait pigmented skin spots and diagnoses with NF1 at age 2. The mycotoxins were detected in bulk samples, urine and nasal secretions, breast milk, placenta, and umbilical cord. Pseudomonas aueroginosa, Acinetobacter, Penicillium, and Aspergillus fumigatus were cultured from nasal secretions (father and daughter). RT-PCR revealed A. fumigatus DNA in sinus tissues of the daughter. The dog had 72 skin lesions (sebaceous glands and lipomas) from which trichothecenes and ochratoxin A. were detected. The health of the family is discussed in relation to the most recent published literature regarding microbial contamination and toxic by-products present in water-damaged buildings.
Willian Shaw, PhD
39

39


Parents Symptoms
Father
Persistent cough with phlegm
Throat irritation
Headaches, sinusitis, severe fatigue, and somnolence.
Decreased concentration, long-term and recently increasing memory loss.
Nose bleeds, hair loss
Decreased libido
Shortness of breath with wheezing.
Mother
• Cough with phlegm, throat irritation.
• Headaches, sinusitis
• Extreme fatigue and somnolence.
• Short and long-term memory loss.
• Decreased libido
• Shortness of breath with wheezing.
Willian Shaw, PhD
40

40

Kids Symptoms

- Headaches
- Fatigue
- Nasal congestion and nose bleeds
- Throat irritation
- Shortness of breath with mild wheezing
- Decreased attention in classroom activities

Willian Shaw, PhD
41

41

Café au Lait Spots and Total Body Flare on Newborn



Willian Shaw, PhD
42

42

Dog

- The pet dog had approximately 72 skin lesions on its legs, trunk and ears.
- The lesions were surgically removed.
- Pathology of the ear mass described it as a sebaceous gland.
- The other lesions were lipomas.

Willian Shaw, PhD

43

43

Patient specimen	Trichothecenes (ppb)	Aflatoxins (ppb)	Ochratoxin (ppb)
Father-Urine	NP	NP	18.2
Father-Nasal[1] Secretion	NP	0.5 11.2	13 7.7
Mother-Urine	NP	NP	18.2
Mother-Nasal Secretion	1.02	1.2	1.6
Daughter-Urine	0.23	NP	28.0
Daughter-Nasal[2] Secretion	4.68	NP	3.8
Son-Urine	0.2	NP	18.9
Son-Nasal Secretion	ND	ND	ND

Source: J. Env Public Health vol 2012 article 312836

Willian Shaw, PhD

44

Patient specimen	Trichothecenes (ppb)	Aflatoxins (ppb)	Ochratoxin (ppb)
Breast Milk	0.18	0.9	2.7
Placenta	NP	NP	4.2
Umbilical Cord	NP	NP	7
New Born-Urine	NP	NP	NP
Dog-Urine	1.49	NP	25.9
Dog-Ear Mass	23.07	0	2.2
Dog-Lipoma	20.9	0	1.4

Source: J. Env Public Health vol 2012 article 312836

Willian Shaw, PhD

45

Gliotoxin

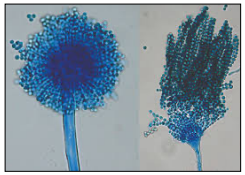
Disruptor of Innate and Adaptive Immunity

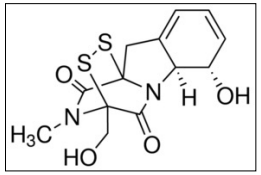
46

46

Gliotoxin

- Produced by *Aspergillus* mold
- Conidia are released which can infiltrate the alveolar airways.





Gliotoxin	139304.00	< 200

▲ 200

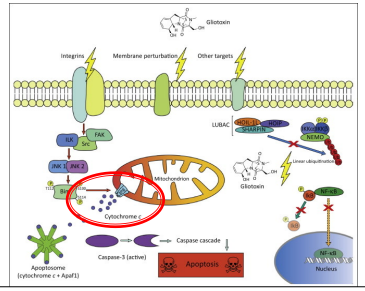
47

47

Gliotoxin

Gliotoxin: Gliotoxin (GTX) is produced by the mold genus Aspergillus. Aspergillus spreads in the environment by releasing conidia which are capable of infiltrating the small alveolar airways of individuals. In order to evade the body's defenses Aspergillus releases Gliotoxin to inhibit the immune system. One of the targets of Gliotoxin is PtdIns (3,4,5) P3. This results in the downregulation of phagocytic immune defense, which can lead to the exacerbation of polymicrobial infections. Gliotoxin impairs the activation of T-cells and induces apoptosis in monocytes and in monocyte-derived dendritic cells. These impairments can lead to multiple neurological syndromes. (PMID: 16712786, 27048806, 21575912, 23278106)

Gliotoxin can lead to a downregulation in phagocytic immune function, as well as T-cell activation.

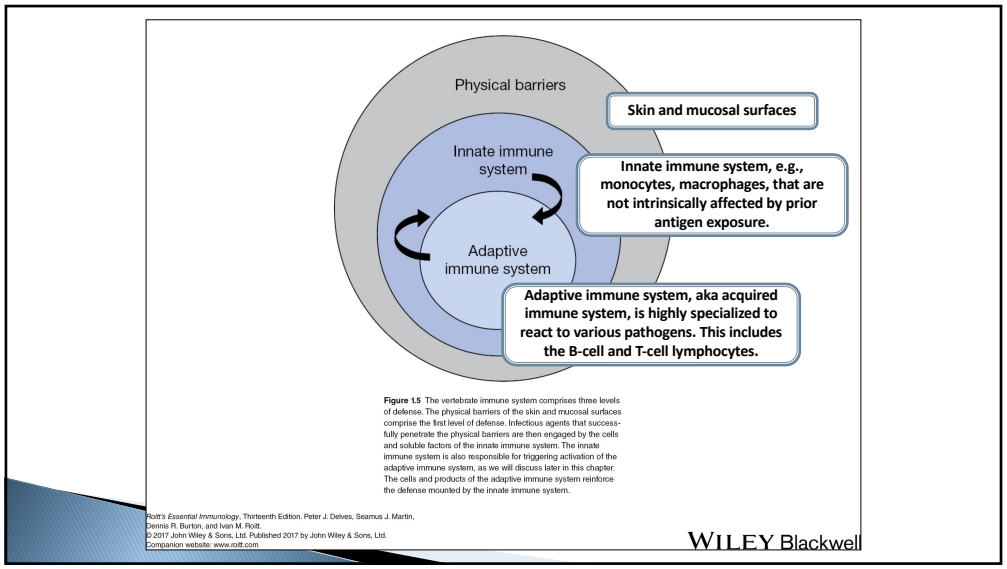


Gliotoxin	39752.21	200 - 2000

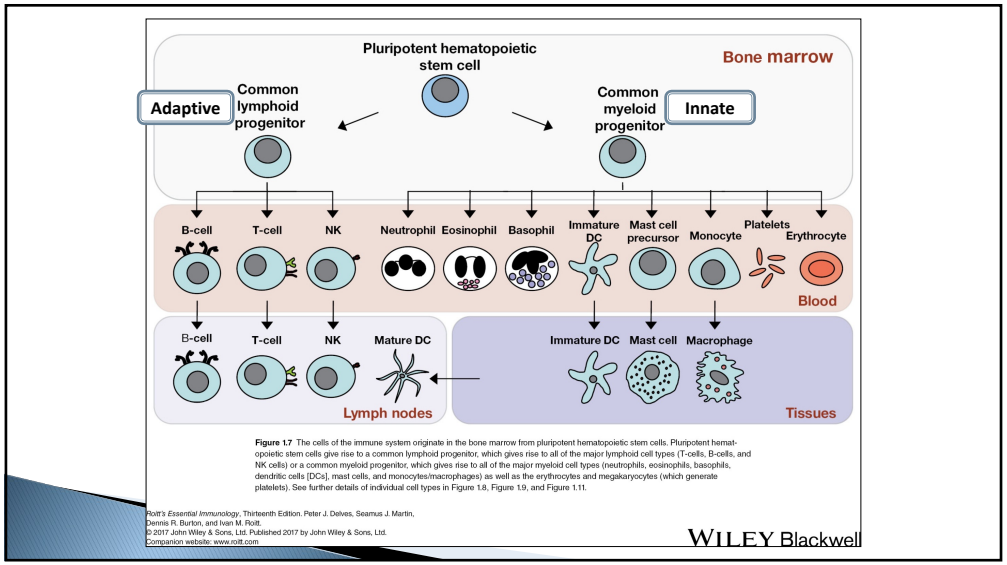
▲ 200 2000 ▲

48

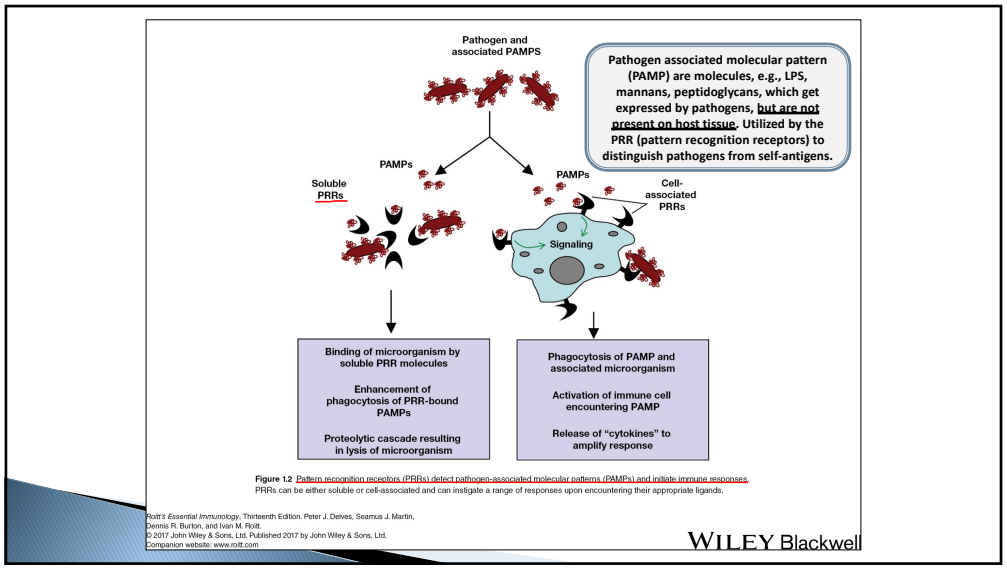
48


Physical barriers
Skin and mucosal surfaces
Innate immune system
Innate immune system, e.g., monocytes, macrophages, that are not intrinsically affected by prior antigen exposure.
Adaptive immune system
Adaptive immune system, aka acquired immune system, is highly specialized to react to various pathogens. This includes the B-cell and T-cell lymphocytes.
Figure 1.5 The vertebrate immune system comprises three levels of defense. The physical barriers of the skin and mucosal surfaces comprise the first level of defense. Infectious agents that successfully penetrate the physical barriers are then engaged by the cells and soluble factors of the innate immune system. The innate immune system is also responsible for triggering activation of the adaptive immune system, as we will discuss later in this chapter. The cells and products of the adaptive immune system reinforce the defense mounted by the innate immune system.
Roitt's Essential Immunology, Thirteenth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, and Ivan M. Roitt.
© 2017 John Wiley & Sons, Ltd. Published 2017 by John Wiley & Sons, Ltd.
Companion website: www.roitt.com
WILEY Blackwell

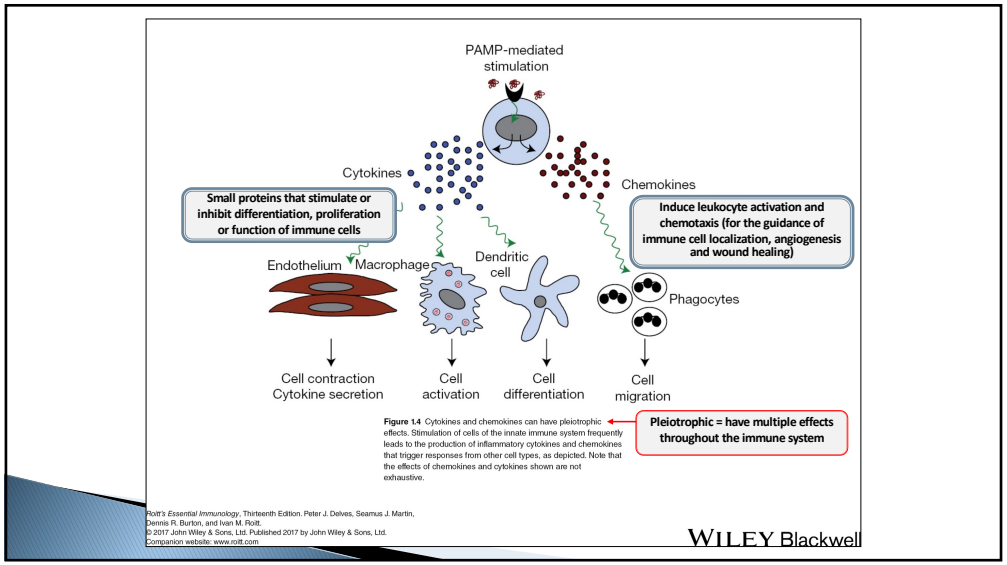
49


Pluripotent hematopoietic stem cell
Bone marrow
Adaptive
Common lymphoid progenitor
Common myeloid progenitor
Innate
B-cell
T-cell
NK
Neutrophil
Eosinophil
Basophil
Immature DC
Mast cell precursor
Monocyte
Platelets
Erythrocyte
Blood
B-cell
T-cell
NK
Mature DC
Lymph nodes
Immature DC
Mast cell
Macrophage
Tissues
Figure 1.7 The cells of the immune system originate in the bone marrow from pluripotent hematopoietic stem cells. Pluripotent hematopoietic stem cells give rise to a common lymphoid progenitor, which gives rise to all of the major lymphoid cell types (T-cells, B-cells, and NK cells) or a common myeloid progenitor, which gives rise to all of the major myeloid cell types (neutrophils, eosinophils, basophils, dendritic cells [DCs], mast cells, and monocytes/macrophages) as well as the erythrocytes and megakaryocytes (which generate platelets). See further details of individual cell types in Figure 1.8, Figure 1.9, and Figure 1.11.
Roitt's Essential Immunology, Thirteenth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, and Ivan M. Roitt.
© 2017 John Wiley & Sons, Ltd. Published 2017 by John Wiley & Sons, Ltd.
Companion website: www.roitt.com
WILEY Blackwell

50

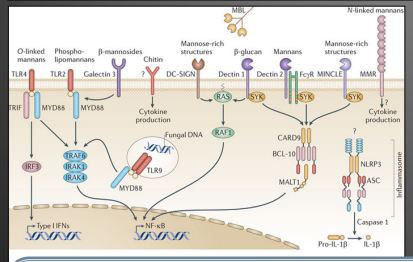

Pathogen and associated PAMPS
Pathogen associated molecular pattern (PAMP) are molecules, e.g., LPS, mannans, peptidoglycans, which get expressed by pathogens, but are not present on host tissue. Utilized by the PRR (pattern recognition receptors) to distinguish pathogens from self-antigens.
PAMPs
PAMPs
Soluble PRRs
Cell-associated PRRs
Signaling
Binding of microorganism by soluble PRR molecules
Enhancement of phagocytosis of PRR-bound PAMPs
Proteolytic cascade resulting in lysis of microorganism
Phagocytosis of PAMP and associated microorganism
Activation of immune cell encountering PAMP
Release of "cytokines" to amplify response
Figure 1.2 Pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs) and initiate immune responses. PRRs can be either soluble or cell-associated and can instigate a range of responses upon encountering their appropriate ligands.
Roitt's Essential Immunology, Thirteenth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, and Ivan M. Roitt.
© 2017 John Wiley & Sons, Ltd. Published 2017 by John Wiley & Sons, Ltd.
Companion website: www.roitt.com
WILEY Blackwell

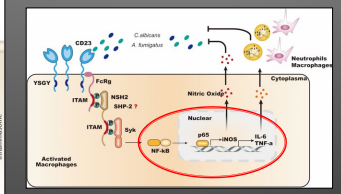
51


PAMP-mediated stimulation
Cytokines
Small proteins that stimulate or inhibit differentiation, proliferation or function of immune cells
Chemokines
Induce leukocyte activation and chemotaxis (for the guidance of immune cell localization, angiogenesis and wound healing)
Endothelium
Macrophage
Dendritic cell
Phagocytes
Cell contraction Cytokine secretion
Cell activation
Cell differentiation
Cell migration
Figure 1.4 Cytokines and chemokines can have pleiotrophic effects. Stimulation of cells of the innate immune system frequently leads to the production of inflammatory cytokines and chemokines that trigger responses from other cell types, as depicted. Note that the effects of chemokines and cytokines shown are not exhaustive.
Pleiotrophic = have multiple effects throughout the immune system
Roitt's Essential Immunology, Thirteenth Edition. Peter J. Delves, Seamus J. Martin, Dennis R. Burton, and Ivan M. Roitt.
© 2017 John Wiley & Sons, Ltd. Published 2017 by John Wiley & Sons, Ltd.
Companion website: www.roitt.com
WILEY Blackwell

52

Pattern Recognition Receptors (PRR) for *Candida albicans*



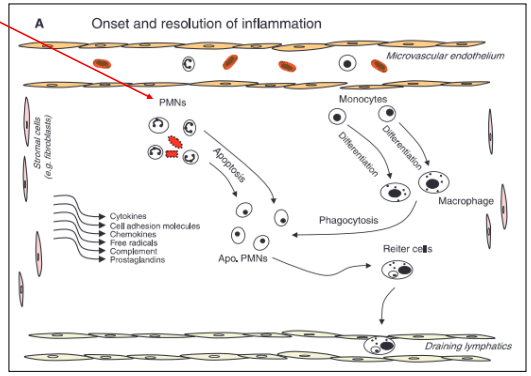


Soluble and membrane bound PRRs for various components of *Candida albicans*. Ultimately, the recognition of the existence of *candida* as a commensal or pathogenic organism takes place through adherence and receptor initiation of secondary immune responses.

Source: ***Candida albicans morphogenesis and host defense: Discriminating invasion from colonization - https://www.researchgate.net/figure/The-main-pattern-recognition-receptors-involved-in-recognizing-Candida-albicansThe_fig1_51868409***

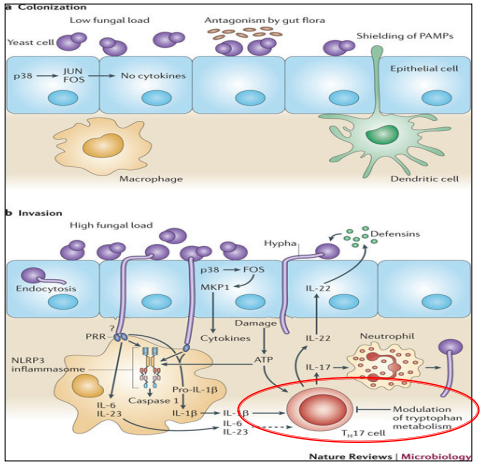
53

PMN - Polymorphonuclear Leukocytes (basophils, eosinophils, neutrophils, and mast cells)



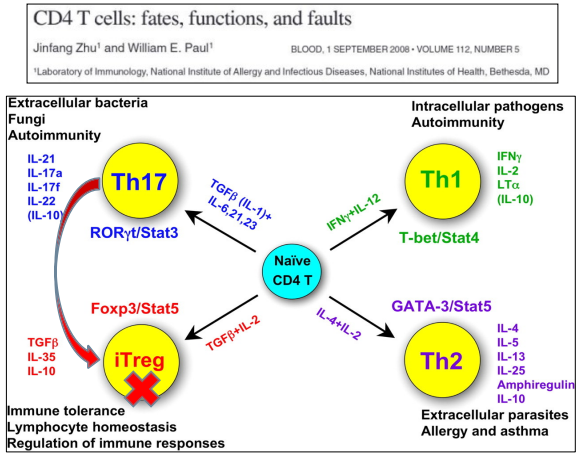
Source: ***Chronic inflammation: a failure of resolution? Int J Experimental Path. 2006. Volume: 88, Issue: 2, 85-94.***

54



Source: *Nature Reviews/Microbiology*

55



56

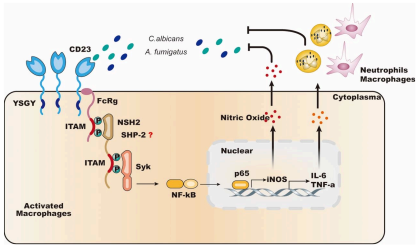
The Immunosuppressive Fungal Metabolite Gliotoxin Specifically Inhibits Transcription Factor NF-κB

By Heike L. Pahl,* Beate Krauß,* Klaus Schulze-Osthoff,* Thomas Decker,‡ E. Britta-Mareen Traenckner,* Markus Vogt,* Christina Myers,§ Tom Parks,§ Paul Warring,‖ Arno Mühlbacher,‖ Armin-Peter Czernilofsky,¶ and Patrick A. Baeuerle*

*From the *Institute of Biochemistry and Molecular Biology, Albert-Ludwigs-University, D-79104 Freiburg, Germany; ‡Vienna Biocenter, Institute for Microbiology and Genetics, A-1030 Vienna, Austria; the §Department of Inflammatory Diseases, Boehringer Ingelheim, Ridgefield, Connecticut 06877; the ‖Division of Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory 2601, Australia; and ¶Boehringer Ingelheim Research Vienna, A-1120 Vienna, Austria*

Summary

Opportunistic infections, such as aspergillosis, are among the most serious complications suffered by immunocompromised patients. *Aspergillus fumigatus* and other pathogenic fungi synthesize a toxic epipolythiodioxopiperazine metabolite called gliotoxin. Gliotoxin exhibits profound immunosuppressive activity in vivo. It induces apoptosis in thymocytes, splenocytes, and mesenteric lymph node cells and can selectively deplete bone marrow of mature lymphocytes. The molecular mechanism by which gliotoxin exerts these effects remains unknown. Here, we report that nanomolar concentrations of gliotoxin inhibited the activation of transcription factor NF-κB in response to a variety of stimuli in T and B cells. The effect of gliotoxin was specific because, at the same concentrations, the toxin did not affect activation of the transcription factor NF-AT or of interferon-responsive signal transducers and activators of transcription. Likewise, the activity of the constitutively DNA-binding transcription factors Oct-1 and cyclic AMP response element binding protein (CREB), as well as the activation of protein tyrosine kinases $p56^{lck}$ and $p59^{fyn}$, was not altered by gliotoxin. Very high concentrations of gliotoxin prevented NF-κB DNA binding in vitro. However, in intact cells, inhibition of NF-κB did not occur at the level of DNA binding; rather, the toxin appeared to prevent degradation of IκB-α, NF-κB's inhibitory subunit. Our data raise the possibility that the immunosuppression observed during aspergillosis results in part from gliotoxin-mediated NF-κB inhibition.



"It induces apoptosis in thymocytes, splenocytes, and mesenteric lymph node cells and can selectively deplete bone marrow of mature lymphocytes."

57

Toxicol Sci. 2000 Mar;54(1):194-202. doi: 10.1093/toxsci/54.1.194.

Gliotoxin-induced cytotoxicity proceeds via apoptosis and is mediated by caspases and reactive oxygen species in LLC-PK1 cells

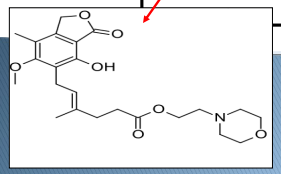
X Zhou [1], A Zhao, G Goping, P Hirszel

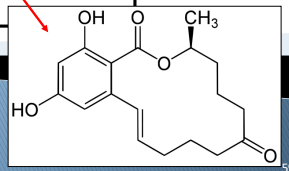
dose-dependent manner. Further analyses of DNA fragmentation, hypodiploid nuclei, mitochondrial membrane potential, and plasma membrane integrity revealed that cell death proceeded via apoptosis. Gliotoxin-induced apoptosis was associated with dose-dependent and time-dependent activation of caspase-3-like proteases. Boc-aspartyl (OMe)-fluoromethylketone attenuated the killing effect. Gliotoxin also increased the intracellular levels of reactive oxygen species as measured by flow cytometry. N-acetylcysteine, a well-known antioxidant, completely abolished the gliotoxin-induced caspase-3-like activity, cytotoxicity, and reactive oxygen species. In conclusion, (1) gliotoxin at 100 ng/ml unmasks the ability of TNF-alpha-induced apoptosis, and the effect of TNF-alpha is mediated by caspase-3-like proteases; and (2) at higher concentrations gliotoxin itself induces cell death, which is via apoptosis and dependent on caspase-3-like activity and reactive oxygen species.

58

Mycophenolic acid & Zearalenone

Penicillium and Fusarium Mold Derived Mycotoxins



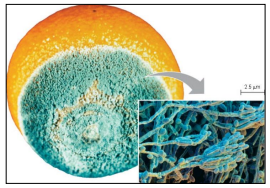


59

59

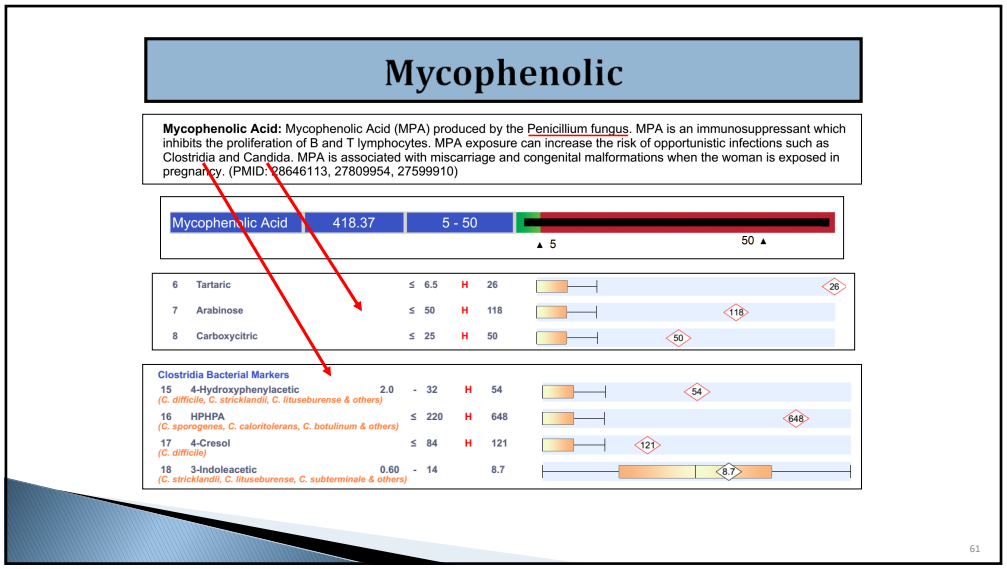
Penicillium

- There are over 200 known species of *Penicillium*.
- *Penicillium chrysogenum* is the most common of these species.
- It is commonly found in indoor environments and is responsible for many allergic reactions.
- *Penicillium* can also contaminate different food items:
  - *Citrus fruits can become contaminated with Penicillium, as well as seeds and grains.*

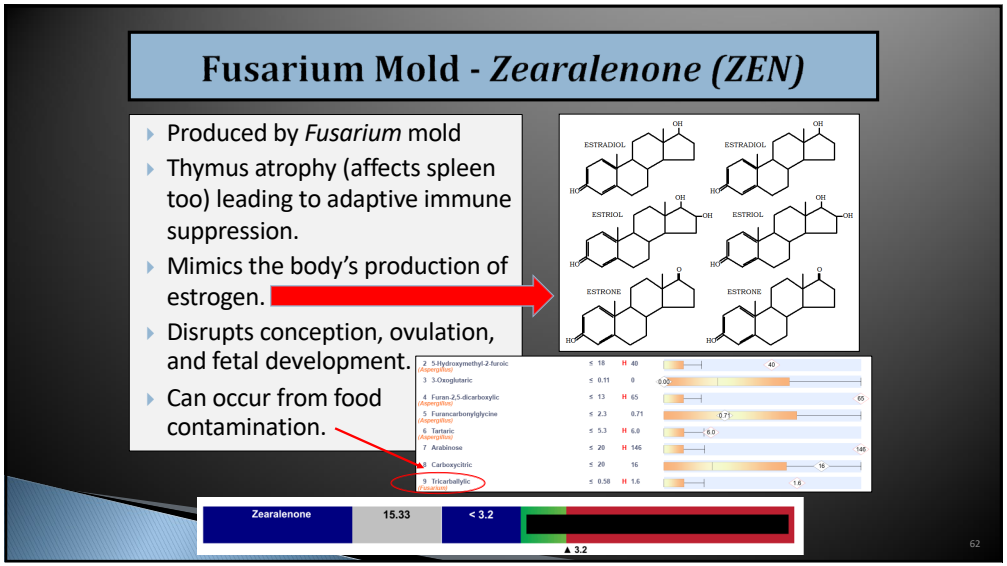


60

60


Mycophenolic
Mycophenolic Acid: Mycophenolic Acid (MPA) produced by the Penicillium fungus. MPA is an immunosuppressant which inhibits the proliferation of B and T lymphocytes. MPA exposure can increase the risk of opportunistic infections such as Clostridia and Candida. MPA is associated with miscarriage and congenital malformations when the woman is exposed in pregnancy. (PMID: 28646113, 27809954, 27599910)
Mycophenolic Acid 418.37 5 - 50
▲ 5
50 ▲
6 Tartaric ≤ 6.5 H 26
7 Arabinose ≤ 50 H 118
8 Carboxycitric ≤ 25 H 50
Clostridia Bacterial Markers
15 4-Hydroxyphenylacetic 2.0 - 32 H 54
(C. difficile, C. stricklandii, C. lituseburense & others)
16 HPHPA ≤ 220 H 648
(C. sporogenes, C. caloritolerans, C. botulinum & others)
17 4-Cresol ≤ 84 H 121
(C. difficile)
18 3-Indoleacetic 0.60 - 14 8.7
(C. stricklandii, C. lituseburense, C. subterminale & others)
61

61


Fusarium Mold - Zearalenone (ZEN)
Produced by Fusarium mold
Thymus atrophy (affects spleen too) leading to adaptive immune suppression.
Mimics the body's production of estrogen.
Disrupts conception, ovulation, and fetal development.
Can occur from food contamination.
ESTRADIOL
ESTRIOL
ESTRONE
Zearalenone 15.33 < 3.2
▲ 3.2
62

62

J Biochem Mol Toxicol. 2017 Oct;31(10). doi: 10.1002/jbt.21944. Epub 2017 Jun 13.

Zearalenone induces ROS-mediated mitochondrial damage in porcine IPEC-J2 cells

Wentao Fan [1], Tongtong Shen [1], Qiaoqi Ding [1], Yanan Lv [1], Li Li [1], Kehe Huang [1], Liping Yan [1 2], Suquan Song [1]

In this study, the mitochondrial damage effect and mechanism of zearalenone (ZEA) in swine small intestine IPEC-J2 cells in vitro were comprehensively characterized. The analyses revealed that ZEA at high doses (8 and 7 μg/mL) can significantly increase $P < 0.05$ the malondialdehyde levels and decrease antioxidant enzymes activities after 48 h of exposure. Meanwhile, the reactive oxygen species (ROS) accumulation increased in high dose ZEA-treated groups after 2 h treatment, but decreased due to the ROS-induced mitochondrial damage and the caused cell apoptosis after 48 h of high does ZEA treatment. Moreover, the decreasing of mitochondrial membrane potential (MMP; ΔΨ) in high dose ZEA exposure was observed in line with the increasing ROS production in mitochondria. Results suggest that ZEA exposure can induce mitochondrial damage by reducing antioxidant enzyme activities, accumulation of ROS, and decreasing MMP. The mitochondrial damage had a dramatic concentration-effects relationship with ZEA.

Zearalenone	10 μM in H295R cells	↓oxidative phosphorylation

63

Anal Biochem. 2018 Jul 1; 552: 50–59.
Published online 2017 Jul 12. doi: 10.1016/j.ab.2017.07.009

Mitochondrial membrane potential

Ljubava D. Zorova,[a,b] Vasily A. Popkov,[a,c] Egor Y. Plotnikov,[a] Denis N. Silachev,[a] Irina B. Pevzner,[a] Stanislovas S. Jankauskas,[a] Valentina A. Babenko,[a,c] Savva D. Zorov,[c] Anastasia V. Balakireva,[d] Magdalena Juhaszova,[e] Steven J. Sollott,[e] and Dmitry B. Zorov[a,e,*]

The mitochondrial membrane potential (ΔΨm) generated by proton pumps (Complexes I, III and IV) is an essential component in the process of energy storage during oxidative phosphorylation. Together with the proton gradient (ΔpH), ΔΨm forms the transmembrane potential of hydrogen ions which is harnessed to make ATP. The levels of ΔΨm and ATP in the cell are kept relatively stable although there are limited fluctuations of both these factors that can occur reflecting normal physiological activity. However, sustained changes in both factors may be deleterious. A long-lasting drop or rise of ΔΨmvs normal levels may induce unwanted loss of cell viability and be a cause of various pathologies. Among other factors, ΔΨm plays a key role in mitochondrial homeostasis through selective elimination of dysfunctional mitochondria. It is also a driving force for transport of ions (other than H^+) and proteins which are necessary for healthy mitochondrial functioning. We propose additional potential mechanisms for which ΔΨm is essential for maintenance of cellular health and viability and provide recommendations how to accurately measure ΔΨm in a cell and discuss potential sources of artifacts.

Zearalenone	10 μM in H295R cells	↓oxidative phosphorylation

64

Recap

- Various mycotoxins have immune suppressive effects, as well as damaging mitochondria, other cellular structures, etc.
- Ochratoxin is the most common mycotoxin seen on urine testing. It can lead to brain abnormalities and is carcinogenic and nephrotoxic.
- Gliotoxin is a potent immunotoxin. It can disrupt both the innate and adaptive immune systems.
- Zearalenone and mycophenolic acid have their own immune toxic properties. Like other mycotoxins they can cause mitochondrial problems.
- Innate and adaptive immunity are linked through various cellular mechanisms such as pattern recognition receptors (PRRs) responding to pathogen associated molecular patterns (PAMPs).
- Fungal infections can trigger induction of Th-17 cells which can have a suppressive effect on T-regulatory cells which leads to loss of immune tolerance with an increased potential for autoimmunity.

65

65

Toxicity Examples of Trichothecenes

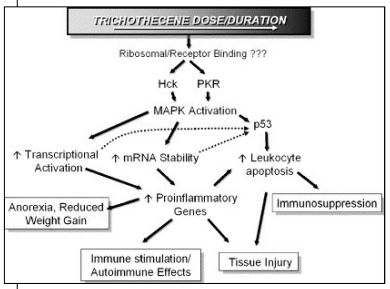
Roridin E and Verrucarin A mycotoxins of Stachybotrys, Fusarium, and other molds

66

66

Trichothecenes (TCT)

- Trichothecenes (TCT) are a large family of fungal metabolites, aka mycotoxins.
- Trichothecene producing molds include *Cephalosporium*, *Fusarium*, *Myrothecium*, *Spicellum*, *Stachybotrys*, *Trichoderma, and others.*
- Source of mold exposure can occur from (examples):
  - Damp indoor environments, e.g., *Stachybotrys chartarum.*
  - Barley, corn, oats, wheat, e.g., *Fusarium* species
  - Poisonous mushroom producing Roridin and/or Verrucarin mycotoxins.

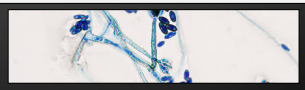


67

Roridin E and Verrucarin A

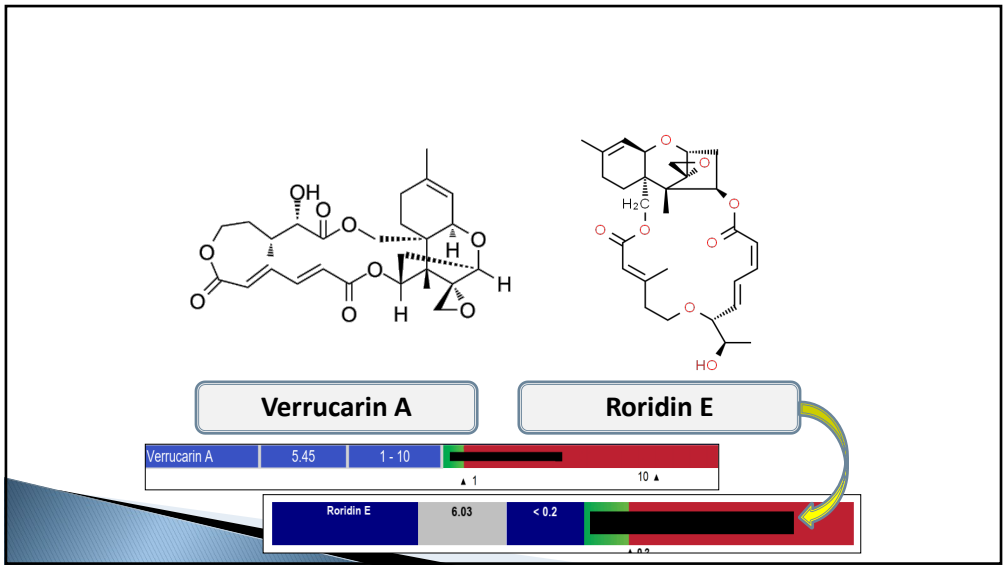
- Both are macrocyclic trichothecenes produced by the *Fusarium*, *Myrothecium*, and *Stachybotrys*.
- Trichothecenes are frequently found in buildings with water damage but can also be found in contaminated grain.

- Trichothecenes are considered extremely toxic and have been used as biological warfare agents.
- Low levels of exposure to trichothecenes can lead to serious neurological problems, immune, cardiovascular, gastrointestinal and endocrine disruption.

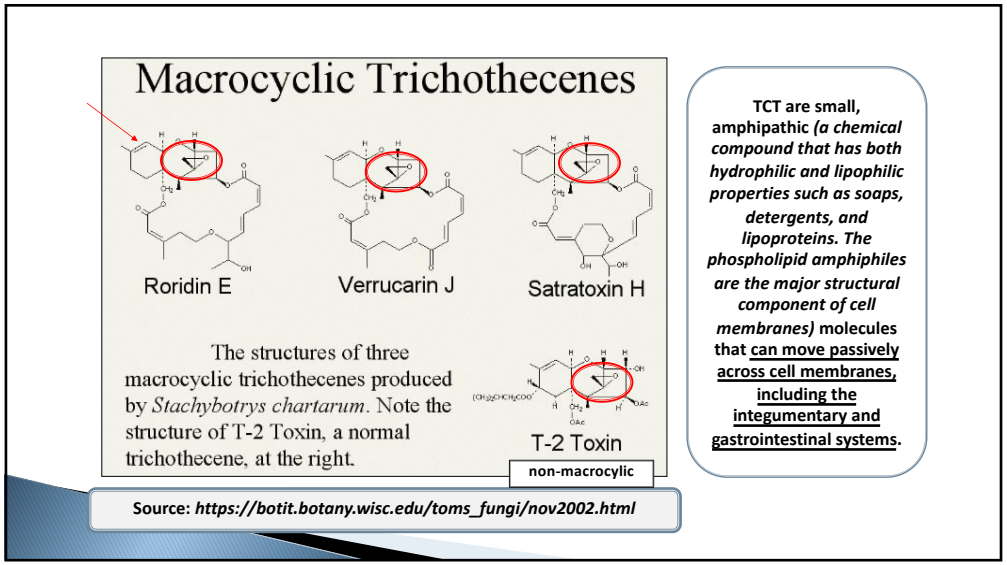


68

68


Verrucarin A
Roridin E
Verrucarin A
5.45
1 - 10
1
10
Roridin E
6.03
< 0.2

69

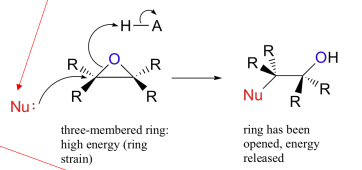

Macrocyclic Trichothecenes
Roridin E
Verrucarin J
Satratoxin H
The structures of three macrocyclic trichothecenes produced by Stachybotrys chartarum. Note the structure of T-2 Toxin, a normal trichothecene, at the right.
T-2 Toxin
non-macrocylic
Source: https://botit.botany.wisc.edu/toms_fungi/nov2002.html
TCT are small, amphipathic (a chemical compound that has both hydrophilic and lipophilic properties such as soaps, detergents, and lipoproteins. The phospholipid amphiphiles are the major structural component of cell membranes) molecules that can move passively across cell membranes, including the integumentary and gastrointestinal systems.

70

Epoxide

- Most epoxides are toxic because they are highly reactive.
- This reactivity in biological systems makes them mutagenic.
- Common nucleophiles are OH^- and -SH and are involved in mechanisms that the body uses to eliminate epoxides.
- Epoxide reactivity with nucleic acids can interfere with G-C pairing.

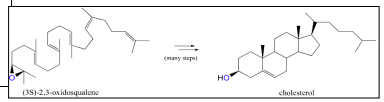
Nu = Nucleophile = "Nucleus Loving"
Nucleophiles provide electrons for creating a new chemical bond.



three-membered ring: high energy (ring strain)

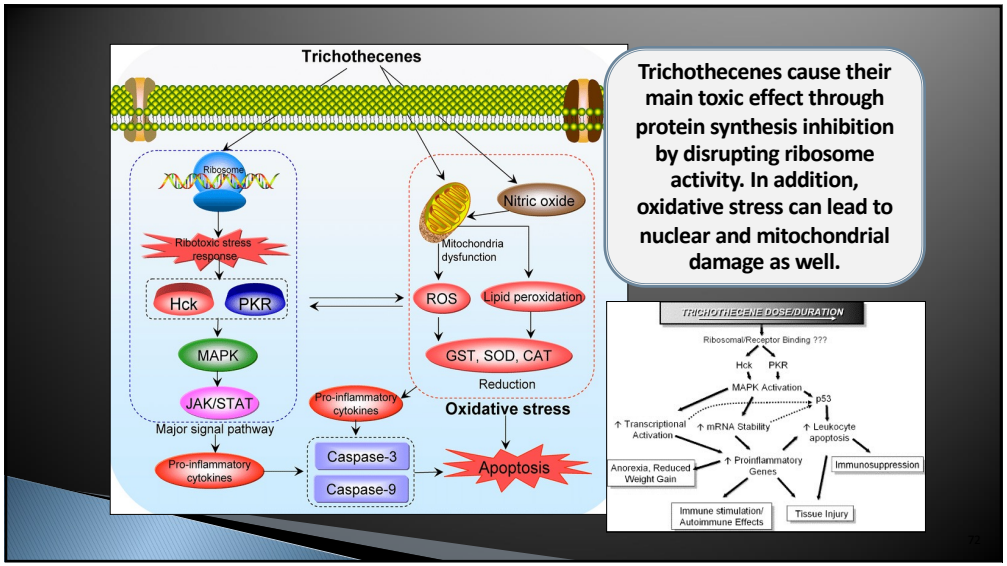
ring has been opened, energy released

OH^- = Hydroxide & -SH = Thiol (aka sulfhydryl)



https://chem.libretexts.org

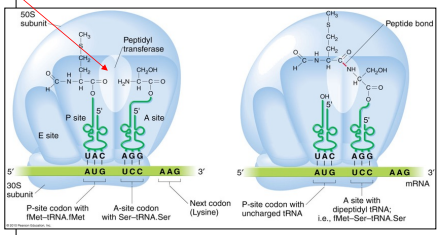
71



72

TCT Toxicity Examples

- Disrupts a ribosomal subunit activity by binding to *peptidyl transferase* center.
- Affects polypeptide chain initiation or elongation.
- Disrupt mitochondrial protein synthesis.
- Interact with protein sulfhydryl groups.
- Generative oxidative stress and free radicals.



Source: *https://www.mun.ca/biology/scarr/iGen3_06-18.html*

73

molecules MDPI

Review

T-2 Toxin—The Most Toxic Trichothecene Mycotoxin: Metabolism, Toxicity, and Decontamination Strategies

Edyta Janik [1], Marcin Niemcewicz [1], Marcin Podogrocki [1], Michal Ceremuga [2], Maksymilian Stela [3] and Michal Bijak [1,*]

Abstract: Among trichothecenes, T-2 toxin is the most toxic fungal secondary metabolite produced by different *Fusarium* species. Moreover, T-2 is the most common cause of poisoning that results from the consumption of contaminated cereal-based food and feed reported among humans and animals. The food and feed most contaminated with T-2 toxin is made from wheat, barley, rye, oats, and maize. After exposition or ingestion, T-2 is immediately absorbed from the alimentary tract or through the respiratory mucosal membranes and transported to the liver as a primary organ responsible for toxin's metabolism. Depending on the age, way of exposure, and dosage, intoxication manifests by vomiting, feed refusal, stomach necrosis, and skin irritation, which is rarely observed in case of mycotoxins intoxication. In order to eliminate T-2 toxin, various decontamination techniques have been found to mitigate the concentration of T-2 toxin in agricultural commodities. However, it is believed that 100% degradation of this toxin could be not possible. In this review, T-2 toxin toxicity, metabolism, and decontamination strategies are presented and discussed.

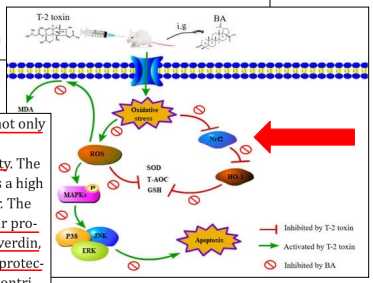
Molecules **2021**, *26*, 6868. https://doi.org/10.3390/molecules26226868

74

Cell Mol Life Sci. 2016; 73: 3221–3247.
Published online 2016 Apr 21. doi: 10.1007/s00018-016-2223-0
PMCID: PMC4967105
PMID: 27100828

Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism

Agnieszka Loboda,[1,2] Milena Damulewicz,[3] Elzbieta Pyza,[3] Alicja Jozkowicz,[1]



The multifunctional regulator nuclear factor erythroid 2-related factor (Nrf2) is considered not only as a cytoprotective factor regulating the expression of genes coding for anti-oxidant, anti-inflammatory and detoxifying proteins, but it is also a powerful modulator of species longevity. The vertebrate Nrf2 belongs to Cap 'n' Collar (Cnc) bZIP family of transcription factors and shares a high homology with SKN-1 from *Caenorhabditis elegans or* CncC found in *Drosophila melanogaster*. The major characteristics of Nrf2 are to some extent mimicked by Nrf2-dependent genes and their proteins including heme oxygenase-1 (HO-1), which besides removing toxic heme, produces biliverdin, iron ions and carbon monoxide. HO-1 and their products exert beneficial effects through the protection against oxidative injury, regulation of apoptosis, modulation of inflammation as well as contribution to angiogenesis. On the other hand, the disturbances in the proper HO-1 level are associated with the pathogenesis of some age-dependent disorders, including neurodegeneration, cancer or macular degeneration. This review summarizes our knowledge about Nrf2 and HO-1 across different phyla suggesting their conservative role as stress-protective and anti-aging factors.

75

T-2 Toxin—The Most Toxic Trichothecene Mycotoxin: Metabolism, Toxicity, and Decontamination Strategies

by Edyta Janik [1], Marcin Niemcewicz [1], Marcin Podogrocki [1], Michal Ceremuga [2], Maksymilian Stela [3] and Michal Bijak [1,*]

Molecules **2021**, *26*(22), 6868; https://doi.org/10.3390/molecules26226868

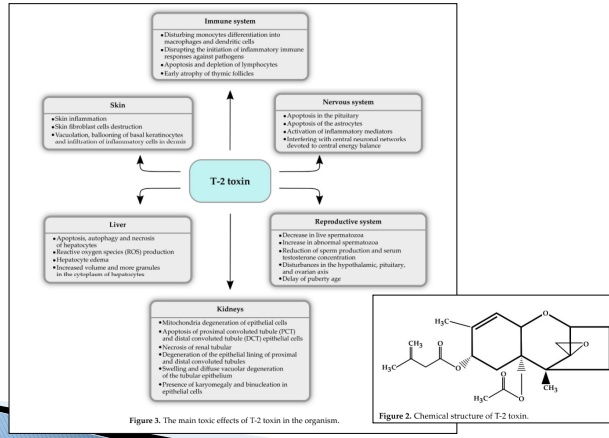


Figure 3. The main toxic effects of T-2 toxin in the organism.

Figure 2. Chemical structure of T-2 toxin.

76

Other Mycotoxins on GPL Profile

Chaetoglobosin A

Chaetoglobosin A: Chaetoglobosin A (CHA) is produced by the mold Chaetomium globosum (CG). CG is commonly found is homes that have experienced water damage. Up to 49% of water-damaged buildings have been found to have CG. CHA is highly toxic, even at minimal doses. CHA disrupts cellular division and movement. Most exposure to CG is through the mycotoxins because the spores tend not to aerosolize. Exposure to CHA has been linked to neuronal damage, peritonitis, and cutaneous lesions. The use of binders is recommended, take 1-2 capsules of G.I. Detox™*, 1-2x daily,1 hour apart from food, supplements and medication as needed. To treat possible fungal infections caused by mold exposure patients can take pharmaceutical medications such as itraconazole or nystatin. Patients can also take 2 capsules of Candida Formula* 2x daily with food for 3 months, 2 hours apart from probiotics. Retesting is recommended after 3-6 months of treatment.

Citrinin

Citrinin (Dihydrocitrinone DHC): Citrinin (CTN) is a mycotoxin that is produced by the mold genera Aspergillus, Penicillium, and Monascus. CTN exposure can lead to nephropathy, because of its ability to increase permeability of mitochondrial membranes in the kidneys. The three most common exposure routes are through ingestion, inhalation, and skin contact. CTN has been shown to be carcinogenic in rat studies. Multiple studies have linked CTN exposure to a suppression of the immune response. Retesting is recommended after 3-6 months of treatment.

77

77

Other Mycotoxins on GPL Profile

Enniatin B

Enniatin B: Enniatin B (ENB) is a fungal metabolite categorized as cyclohexa depsipeptides toxin produced by the fungus Fusarium. The main cause of exposure is from water damaged buildings, although this strain of fungus is one of the most common cereal contaminants. Grains in many different countries have recently been contaminated with high levels of enniatins The toxic effects of Enniatin are caused by the inhibition of the acyl-CoA cholesterol acyltransferase, depolarization of mitochondria, and inhibition of osteoclastic bone resorption. Enniatin has antibiotic properties and chronic exposure may lead to weight loss, fatigue, and liver disease. Sequestrants bind to mycotoxins in the GI tract making them unavailable for reabsorption. These agents are not absorbed and work best for patients with GI symptoms or those whose toxin exposure is coming from food. Activated charcoal, clay, chlorophyll, and cholestyramine have all been shown to bind mycotoxins. (PMID: 18274964, 16730043, 21622627, 23710148)

Sterigmatocystin

Sterigmatocystin: Sterigmatocystin (STC) is a mycotoxin that is closely related to aflatoxin. STC is produced from several species of mold such as Aspergillus, Penicillium, and Bipolaris. STC is considered to be carcinogenic, particularly in the cells of the GI tract and liver. STC has been found in the dust from damp carpets. It is also a contaminant of many foods including grains, corn, bread, cheese, spices, coffee beans, soybeans, pistachio nuts, and animal feed. In cases of lung aspergilloma, STC has been found in human tissue specimens. The toxicity of STC affects the liver, kidneys, and immune system. Tumors have been found in the lungs of rodents that were exposed to STC. Oxidative stress becomes measurably elevated during STC exposure which causes a depletion of antioxidants such as glutathione, particularly in the liver. Because STC is structurally similarity to Aflatoxin, many of the same therapies will be effective. A diet of carrots, parsnips, celery, and parsley may reduce the carcinogenic effects of STC. Bentonite or zeolite clay is reported to reduce the absorption of multiple mycotoxins found in food, including STC. Supplementation with chlorophyllin, zinc, and vitamins A, E, and C has been used to treat exposure to STC. Retesting is recommended after 3-6 months of treatment.

78

78

Intervention Strategies For TCT and Other Mycotoxins

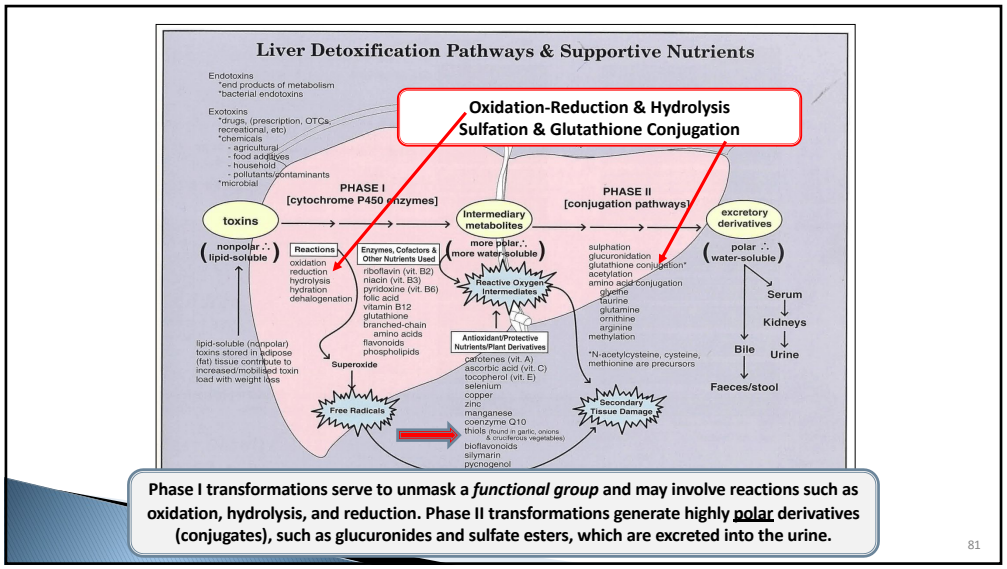
79

79

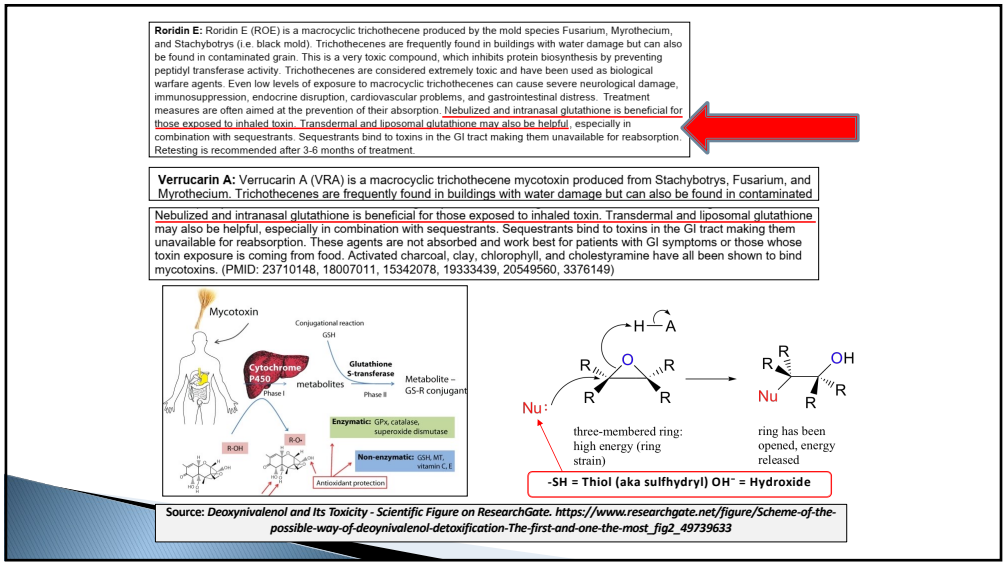
Strategies for dealing with TCT and other mycotoxins

- Find the source of exposure
- Removal from building, e.g., school, office, home, if currently mold contaminated.
- Optimal nutrition through whole food organic and non-GMO based diet.
- Foundational nutrients, e.g., vitamins, minerals, essential fatty acid, antioxidants.
- Improve diversity of microbiome, i.e., consume 12 to 15 plant-based foods daily.
- Probiotics, e.g., L casei, L. rhamnosis *(https://youarethehealer.org/mold-and-toxins/moldy-people/healing-from-mold/types-of-toxin-mycotoxin-binders/probiotics-to-biotransform-toxins/).*

80


Liver Detoxification Pathways & Supportive Nutrients
Endotoxins
*end products of metabolism
*bacterial endotoxins
Exotoxins
*drugs, (prescription, OTCs, recreational, etc)
*chemicals
- agricultural
- food additives
- household
- pollutants/contaminants
*microbial
Oxidation-Reduction & Hydrolysis
Sulfation & Glutathione Conjugation
PHASE I
[cytochrome P450 enzymes]
PHASE II
[conjugation pathways]
toxins
nonpolar ∴ lipid-soluble
Reactions
oxidation
reduction
hydrolysis
hydration
dehalogenation
Enzymes, Cofactors & Other Nutrients Used
riboflavin (vit. B2)
niacin (vit. B3)
pyridoxine (vit. B6)
folic acid
vitamin B12
glutathione
branched-chain amino acids
flavonoids
phospholipids
intermediary metabolites
more polar ∴ more water-soluble
Reactive Oxygen Intermediates
Superoxide
Free Radicals
Antioxidant/Protective Nutrients/Plant Derivatives
carotenes (vit. A)
ascorbic acid (vit. C)
tocopherol (vit. E)
selenium
copper
zinc
manganese
coenzyme Q10
thiols (found in garlic, onions & cruciferous vegetables)
bioflavonoids
silymarin
pycnogenol
sulphation
glucuronidation
glutathione conjugation*
acetylation
amino acid conjugation
glycine
taurine
glutamine
ornithine
arginine
methylation
*N-acetylcysteine, cysteine, methionine are precursors
Secondary Tissue Damage
excretory derivatives
polar ∴ water-soluble
Serum
Kidneys
Bile
Urine
Faeces/stool
lipid-soluble (nonpolar) toxins stored in adipose (fat) tissue contribute to increased/mobilized toxin load with weight loss
Phase I transformations serve to unmask a functional group and may involve reactions such as oxidation, hydrolysis, and reduction. Phase II transformations generate highly polar derivatives (conjugates), such as glucuronides and sulfate esters, which are excreted into the urine.
81

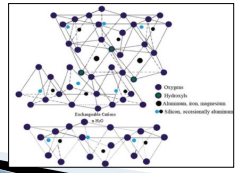
81

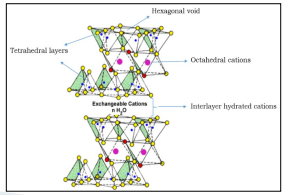

Roridin E: Roridin E (ROE) is a macrocyclic trichothecene produced by the mold species Fusarium, Myrothecium, and Stachybotrys (i.e. black mold). Trichothecenes are frequently found in buildings with water damage but can also be found in contaminated grain. This is a very toxic compound, which inhibits protein biosynthesis by preventing peptidyl transferase activity. Trichothecenes are considered extremely toxic and have been used as biological warfare agents. Even low levels of exposure to macrocyclic trichothecenes can cause severe neurological damage, immunosuppression, endocrine disruption, cardiovascular problems, and gastrointestinal distress. Treatment measures are often aimed at the prevention of their absorption. Nebulized and intranasal glutathione is beneficial for those exposed to inhaled toxin. Transdermal and liposomal glutathione may also be helpful, especially in combination with sequestrants. Sequestrants bind to toxins in the GI tract making them unavailable for reabsorption. Retesting is recommended after 3-6 months of treatment.
Verrucarin A: Verrucarin A (VRA) is a macrocyclic trichothecene mycotoxin produced from Stachybotrys, Fusarium, and Myrothecium. Trichothecenes are frequently found in buildings with water damage but can also be found in contaminated
Nebulized and intranasal glutathione is beneficial for those exposed to inhaled toxin. Transdermal and liposomal glutathione may also be helpful, especially in combination with sequestrants. Sequestrants bind to toxins in the GI tract making them unavailable for reabsorption. These agents are not absorbed and work best for patients with GI symptoms or those whose toxin exposure is coming from food. Activated charcoal, clay, chlorophyll, and cholestyramine have all been shown to bind mycotoxins. (PMID: 23710148, 18007011, 15342078, 19333439, 20549560, 3376149)
Mycotoxin
Conjugational reaction
GSH
Cytochrome P450
Phase I
metabolites
Glutathione S-transferase
Phase II
Metabolite – GS-R conjugant
Enzymatic: GPx, catalase, superoxide dismutase
Non-enzymatic: GSH, MT, vitamin C, E
Antioxidant protection
R-OH
R-O•
Nu:
three-membered ring: high energy (ring strain)
ring has been opened, energy released
-SH = Thiol (aka sulfhydryl) OH⁻ = Hydroxide
Source: Deoxynivalenol and Its Toxicity - Scientific Figure on ResearchGate. https://www.researchgate.net/figure/Scheme-of-the-possible-way-of-deoynivalenol-detoxification-The-first-and-one-the-most_fig2_49739633

82

Binders

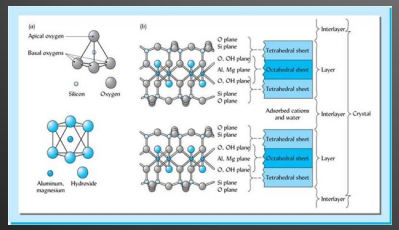
- One of the main treatment goals for any mycotoxin is to prevent absorption from the digestive system.
- Sequestrants bind to toxins in the gastrointestinal tract and prevent their absorption or reabsorption.
- Because of the chemical nature of trichothecenes (and other mycotoxins), clay binders may effective.

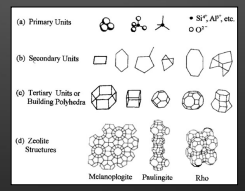




83

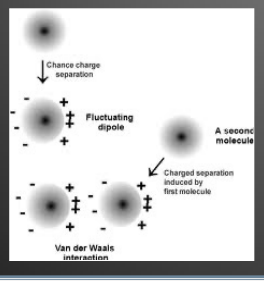
Clay (ex: Bentonite, Pyrophyllite, Zeolite)





84

Charcoal (aka activated charcoal)



- Activated charcoal is a type of carbon made from vegetables, wood and other materials.
- Appears as a fine black powder
- Unique manufacturing technique results in highly porous charcoals.
- Surface area up to 2,000 square meters per gram.

Charcoals are used to absorb *(or attached to it by chemical attraction)* substances/toxins in the digestive system.

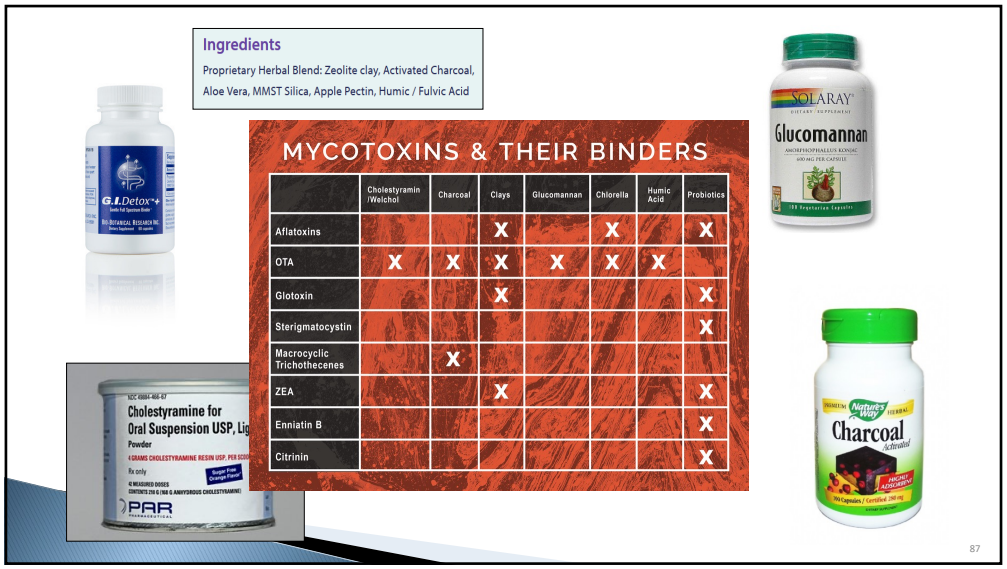
85

Townsends Letter – 2014

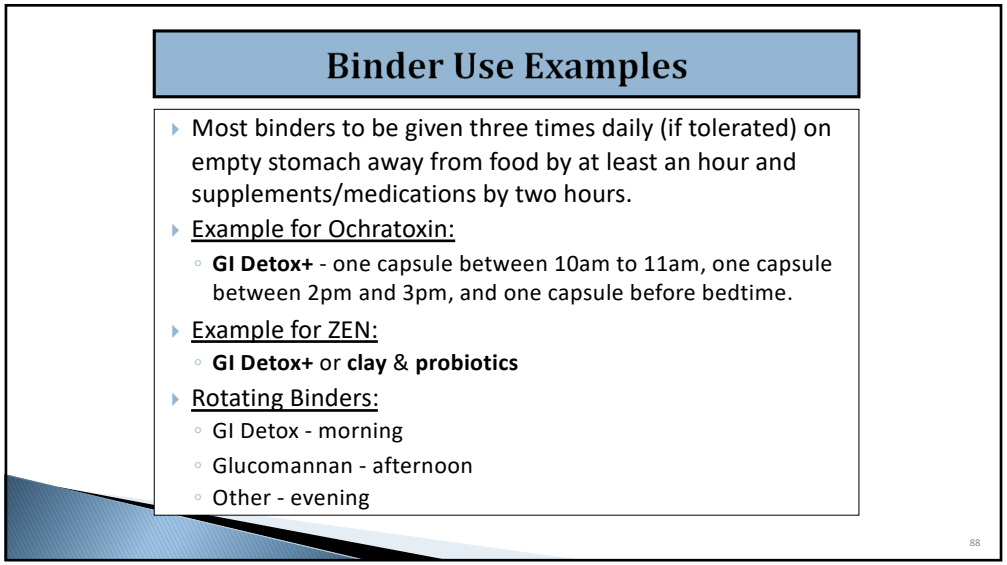
Mycotoxin	Associated Molds	Example Binders	Potential Food Sources
Aflatoxin	• *Aspergillus flavus* • *Aspergillus parasiticus*	• Clays (bentonite, montmorillonite) • Charcoals • Zeolites • Glucomannan • Diatomaceous earth	Milk, cheese, eggs, meat (contaminated feed), cereals, wheat, spices, tree nuts, peanuts, pistachios, Brazil nuts, chilies, oilseeds, corn, spices, black pepper, dried fruit, figs, dried coconut
Ochratoxin	• *Aspergillus albertensis* • *Aspergillus alliaceus* • *Aspergillus auricomus* • *Aspergillus carbonarius* • *Aspergillus niger* • *Aspergillus ochraceus* • *Aspergillus sclerotiorum* • *Aspergillus sulphureus* • *Aspergillus wentii* • *Penicillium nordicum* • *Penicillium viridicatum* • *Penicillium verrucosum*	• Cholestyramine • Zeolites • Glucomannan • Diatomaceous earth	Cereals, wheat, corn, oats, coffee, dried fruit, wine, beer, cocoa, nuts, beans, peas, bread, rice, cheese, meats (contaminated feed, especially pork and poultry), dried and smoked fish, soybeans, garbanzo beans
Trichothecene	• *Cephalosporium* • *Fusarium* • *Myrothecium* • *Stachybotrys* • *Trichoderma* • *Trichothecium* • *Verticimonosporium*	• Clays (bentonite; montmorillonite) • Charcoals • Zeolites • Glucomannan • Diatomaceous earth	Grains, cereals, wheat, barley, oats, corn, rye, durum, soybeans, potatoes, sunflower seeds, peanuts, bananas

This table is a partial listing of organisms that may produce mycotoxins. The focus is on the specific mycotoxins tested via urinary mycotoxin testing from RealTime Laboratories. Additional sources of mycotoxins or mycotoxin binders may not be listed in this table. Some of the binders mentioned above are from veterinary literature, as mycotoxins are a serious concern in the production of animal products such as milk, eggs, and meat.

86


Ingredients
Proprietary Herbal Blend: Zeolite clay, Activated Charcoal, Aloe Vera, MMST Silica, Apple Pectin, Humic / Fulvic Acid
MYCOTOXINS & THEIR BINDERS
Cholestyramin /Welchol
Charcoal
Clays
Glucomannan
Chlorella
Humic Acid
Probiotics
Aflatoxins
OTA
Glotoxin
Sterigmatocystin
Macrocyclic Trichothecenes
ZEA
Enniatin B
Citrinin
Cholestyramine for Oral Suspension USP, Lig
Powder
PAR
SOLARAY
Glucomannan
Charcoal
87

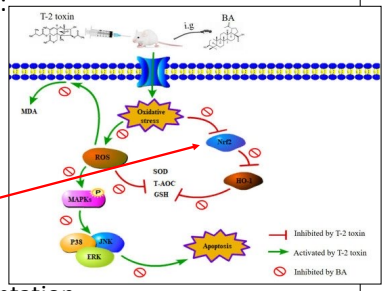
87


Binder Use Examples
Most binders to be given three times daily (if tolerated) on empty stomach away from food by at least an hour and supplements/medications by two hours.
Example for Ochratoxin:
GI Detox+ - one capsule between 10am to 11am, one capsule between 2pm and 3pm, and one capsule before bedtime.
Example for ZEN:
GI Detox+ or clay & probiotics
Rotating Binders:
GI Detox - morning
Glucomannan - afternoon
Other - evening
88

88

Additional Intervention Considerations

- Mitochondrial support:
  - *Ubiquinol*
  - *Carnitine*
  - *PQQ*
- Various antioxidants:
  - *Curcumin*
  - *Resveratrol*
  - ***Sulforaphane***
- Liver and bile support
- Glutathione supplementation
- Infra-red sauna, ION Cleanse, etc.

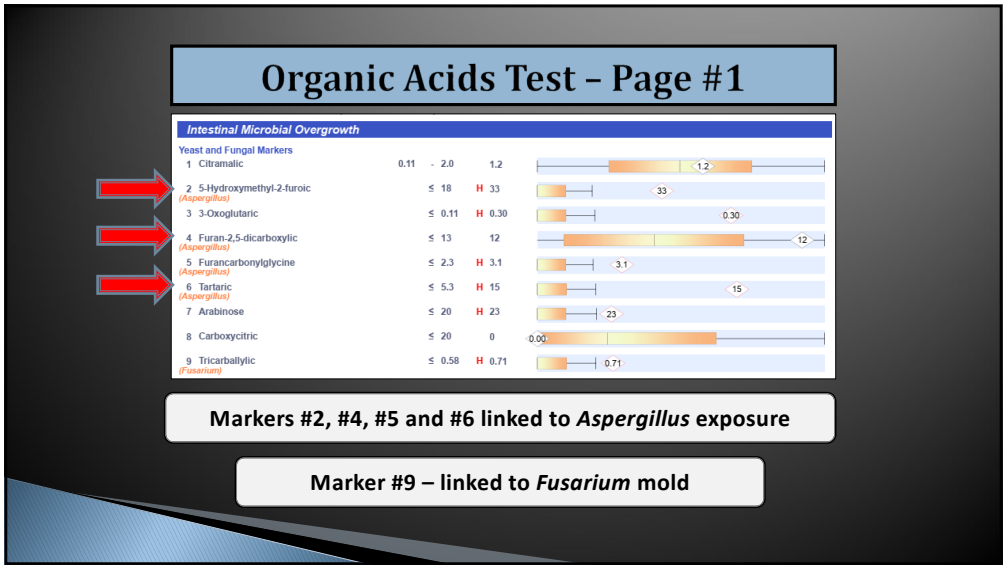


89

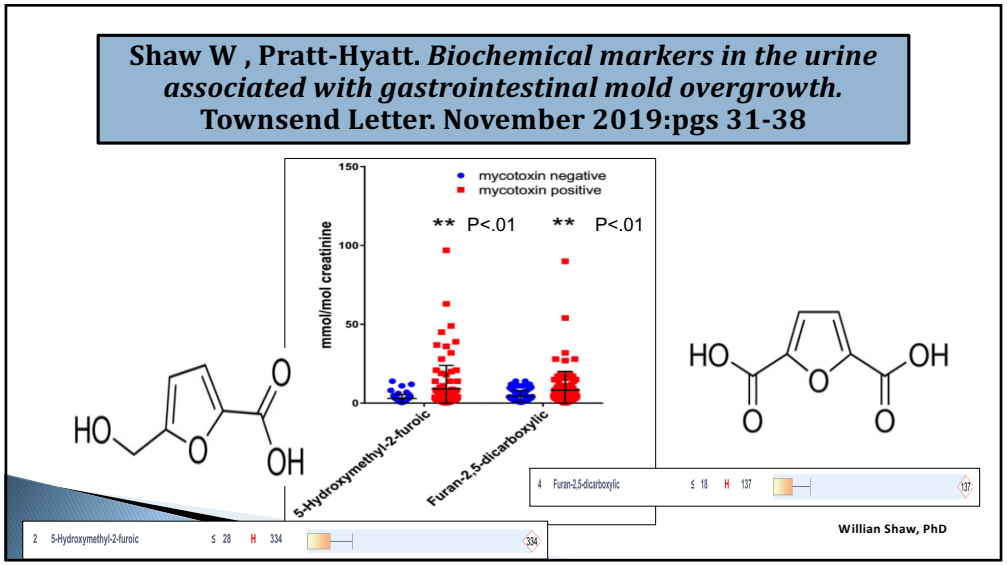
Complementary Use of the Organic Acids Test and Mycotoxin Profile

90

90

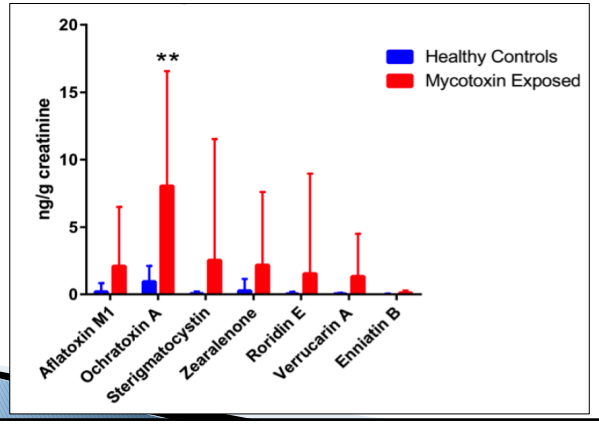

Organic Acids Test – Page #1
Intestinal Microbial Overgrowth
Yeast and Fungal Markers
1 Citramalic 0.11 - 2.0 1.2
2 5-Hydroxymethyl-2-furoic (Aspergillus) ≤ 18 H 33
3 3-Oxoglutaric ≤ 0.11 H 0.30
4 Furan-2,5-dicarboxylic (Aspergillus) ≤ 13 12
5 Furancarbonylglycine (Aspergillus) ≤ 2.3 H 3.1
6 Tartaric (Aspergillus) ≤ 5.3 H 15
7 Arabinose ≤ 20 H 23
8 Carboxycitric ≤ 20 0
9 Tricarballylic (Fusarium) ≤ 0.58 H 0.71
Markers #2, #4, #5 and #6 linked to Aspergillus exposure
Marker #9 – linked to Fusarium mold

91


Shaw W , Pratt-Hyatt. Biochemical markers in the urine associated with gastrointestinal mold overgrowth.
Townsend Letter. November 2019:pgs 31-38
mycotoxin negative
mycotoxin positive
** P<.01
** P<.01
mmol/mol creatinine
150
100
50
0
5-Hydroxymethyl-2-furoic
Furan-2,5-dicarboxylic
HO
O
OH
4 Furan-2,5-dicarboxylic ≤ 18 H 137
2 5-Hydroxymethyl-2-furoic ≤ 28 H 334
Willian Shaw, PhD

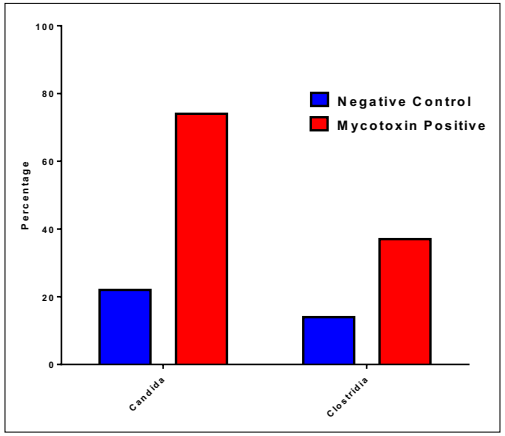
92

Shaw W , Pratt-Hyatt. *Biochemical markers in the urine associated with gastrointestinal mold overgrowth.* Townsend Letter. November 2019:pgs 31-38



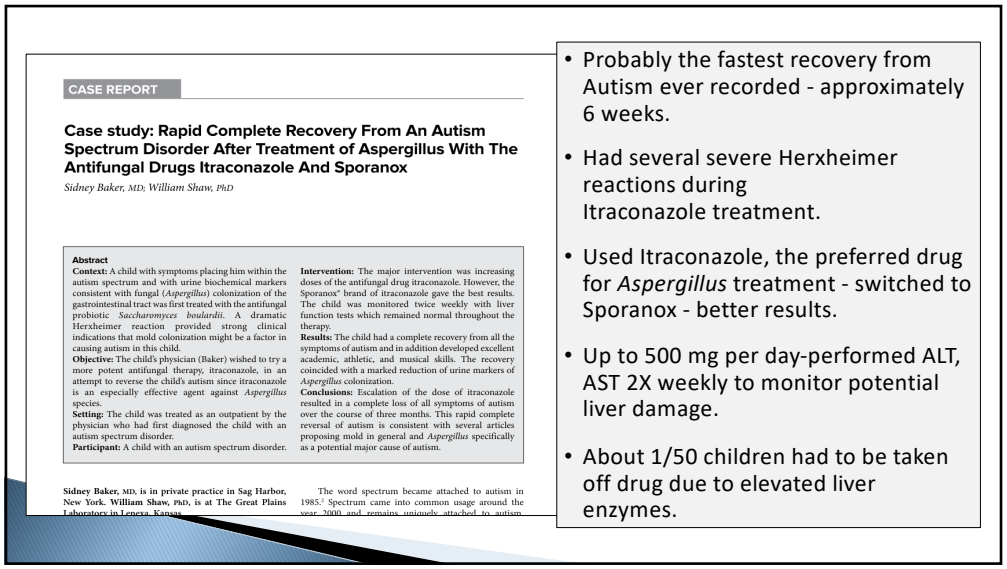
Willian Shaw, PhD

93



94

94



CASE REPORT

Case study: Rapid Complete Recovery From An Autism Spectrum Disorder After Treatment of Aspergillus With The Antifungal Drugs Itraconazole And Sporanox

Sidney Baker, MD; William Shaw, PhD

Abstract

Context: A child with symptoms placing him within the autism spectrum and with urine biochemical markers consistent with fungal (*Aspergillus*) colonization of the gastrointestinal tract was first treated with the antifungal probiotic *Saccharomyces boulardii*. A dramatic Herxheimer reaction provided strong clinical indications that mold colonization might be a factor in causing autism in this child.

Objective: The child's physician (Baker) wished to try a more potent antifungal therapy, itraconazole, in an attempt to reverse the child's autism since itraconazole is an especially effective agent against *Aspergillus* species.

Setting: The child was treated as an outpatient by the physician who had first diagnosed the child with an autism spectrum disorder.

Participant: A child with an autism spectrum disorder.

Intervention: The major intervention was increasing doses of the antifungal drug itraconazole. However, the Sporanox® brand of itraconazole gave the best results. The child was monitored twice weekly with liver function tests which remained normal throughout the therapy.

Results: The child had a complete recovery from all the symptoms of autism and in addition developed excellent academic, athletic, and musical skills. The recovery coincided with a marked reduction of urine markers of *Aspergillus* colonization.

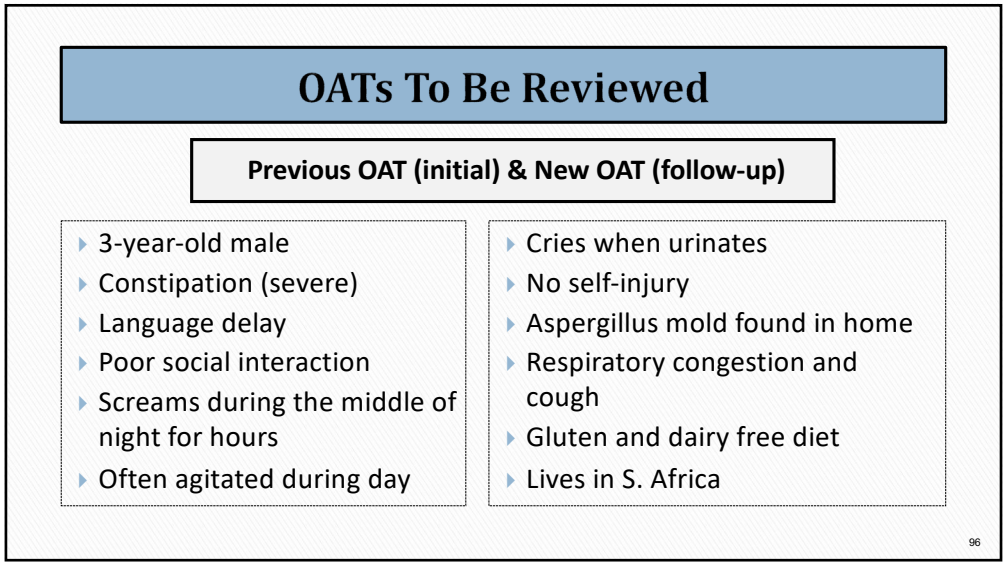
Conclusions: Escalation of the dose of itraconazole resulted in a complete loss of all symptoms of autism over the course of three months. This rapid complete reversal of autism is consistent with several articles proposing mold in general and *Aspergillus* specifically as a potential major cause of autism.

Sidney Baker, MD, is in private practice in Sag Harbor, New York. William Shaw, PhD, is at The Great Plains Laboratory in Lenexa, Kansas.

The word spectrum became attached to autism in 1985.[2] Spectrum came into common usage around the year 2000 and remains uniquely attached to autism

- Probably the fastest recovery from Autism ever recorded - approximately 6 weeks.
- Had several severe Herxheimer reactions during Itraconazole treatment.
- Used Itraconazole, the preferred drug for *Aspergillus* treatment - switched to Sporanox - better results.
- Up to 500 mg per day-performed ALT, AST 2X weekly to monitor potential liver damage.
- About 1/50 children had to be taken off drug due to elevated liver enzymes.

95



OATs To Be Reviewed

Previous OAT (initial) & New OAT (follow-up)

- 3-year-old male
- Constipation (severe)
- Language delay
- Poor social interaction
- Screams during the middle of night for hours
- Often agitated during day

- Cries when urinates
- No self-injury
- Aspergillus mold found in home
- Respiratory congestion and cough
- Gluten and dairy free diet
- Lives in S. Africa

96

96

Treatment Program

Flagyl (metronidazole):

- Flagyl – One dose given three times daily for 10 days straight. Then, a cycling dose for an additional 3 weeks.
- Cycling dose – one dose given three times per treatment day. A treatment day is done every 72 hours following the completion of the initial 10 days listed above. For example, if the last day of the initial 7 days ends on a Sunday, the cycling dose would be given on Wednesday, then again Saturday and so forth until completed.
- The cycling phase helps to reduce the recurrence of *clostridia* bacteria and the initial 10 days is to significantly reduce the overall amounts of bacteria present.

97

97

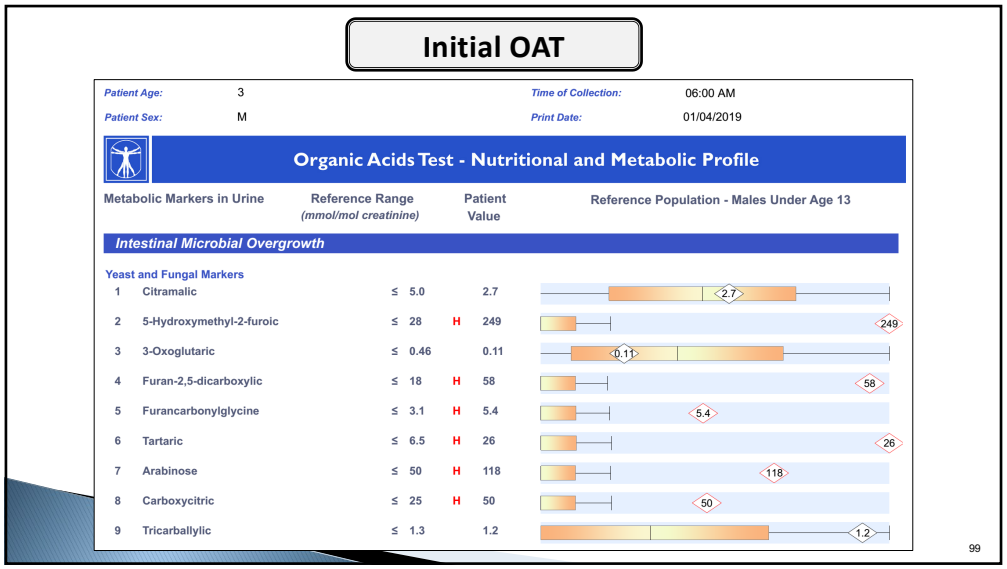
Treatment Program

Additional Support:

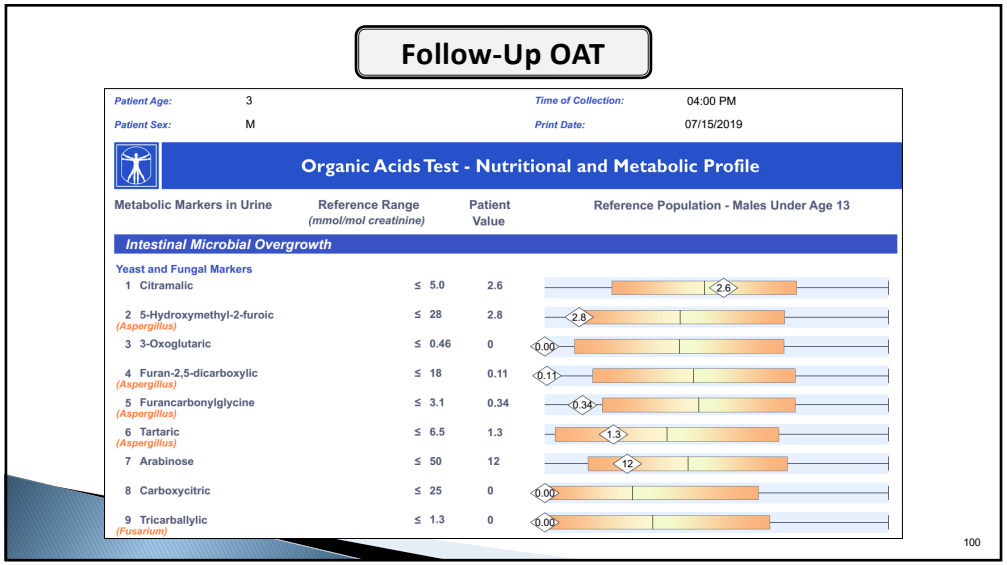
- Multivitamin/multimineral/antioxidant
- Biocidin (liquid) – 5 drops three times daily
- Probiotic (soil-based organism) – two capsules nightly before bed
- Oxypowder – one to three capsules nightly for constipation
- Calcium citrate with meals
- Nystatin *(oral suspension)* – 5 ml (one teaspoon) TID for 4 weeks, then switched to Diflucan for 4 weeks, then switched to Sporanox.
- GI Detox+ - one capsule twice daily

98

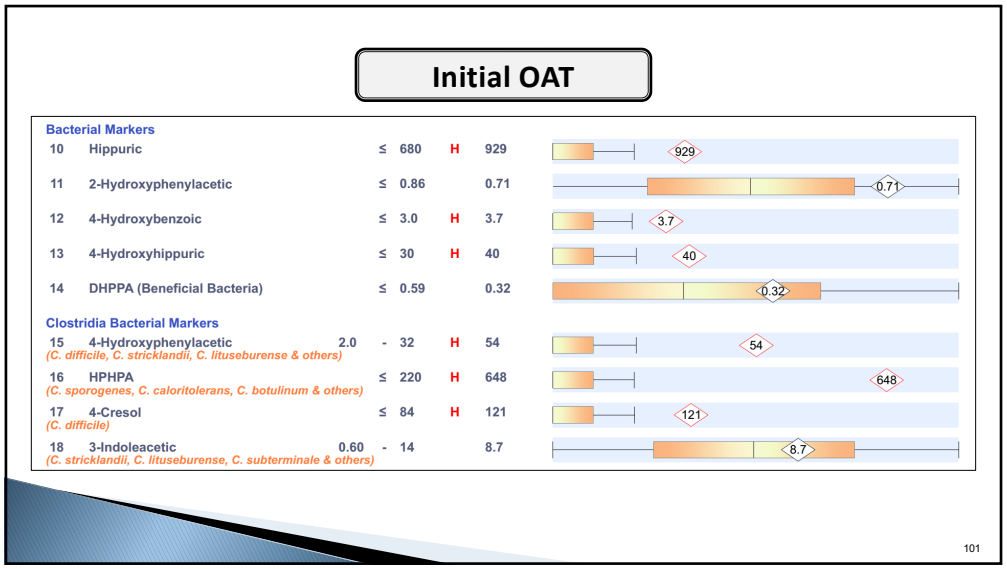
98


Initial OAT
Patient Age: 3
Patient Sex: M
Time of Collection: 06:00 AM
Print Date: 01/04/2019
Organic Acids Test - Nutritional and Metabolic Profile
Metabolic Markers in Urine
Reference Range (mmol/mol creatinine)
Patient Value
Reference Population - Males Under Age 13
Intestinal Microbial Overgrowth
Yeast and Fungal Markers
1 Citramalic ≤ 5.0 2.7
2 5-Hydroxymethyl-2-furoic ≤ 28 H 249
3 3-Oxoglutaric ≤ 0.46 0.11
4 Furan-2,5-dicarboxylic ≤ 18 H 58
5 Furancarbonylglycine ≤ 3.1 H 5.4
6 Tartaric ≤ 6.5 H 26
7 Arabinose ≤ 50 H 118
8 Carboxycitric ≤ 25 H 50
9 Tricarballylic ≤ 1.3 1.2
99

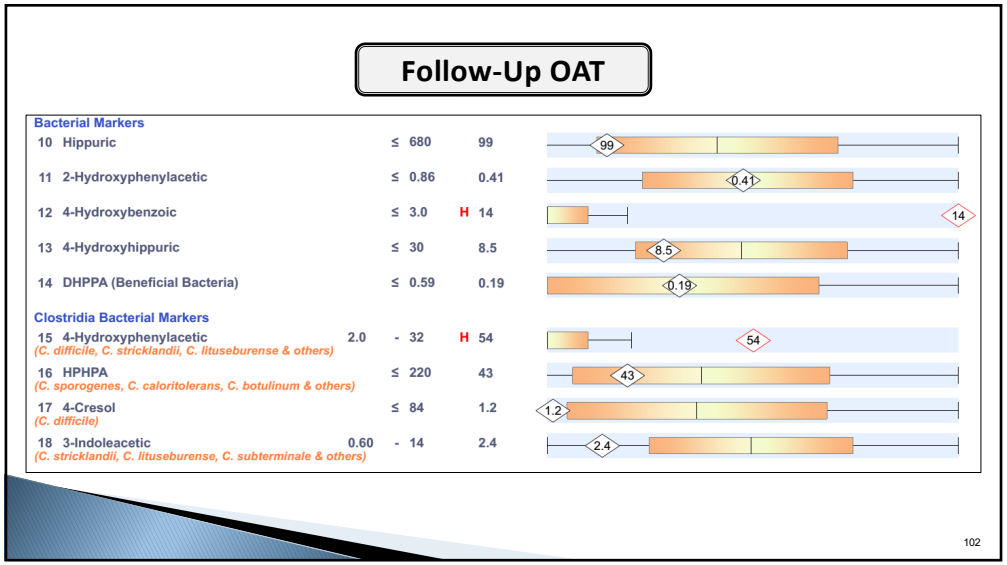
99


Follow-Up OAT
Patient Age: 3
Patient Sex: M
Time of Collection: 04:00 PM
Print Date: 07/15/2019
Organic Acids Test - Nutritional and Metabolic Profile
Metabolic Markers in Urine
Reference Range (mmol/mol creatinine)
Patient Value
Reference Population - Males Under Age 13
Intestinal Microbial Overgrowth
Yeast and Fungal Markers
1 Citramalic ≤ 5.0 2.6
2 5-Hydroxymethyl-2-furoic (Aspergillus) ≤ 28 2.8
3 3-Oxoglutaric ≤ 0.46 0
4 Furan-2,5-dicarboxylic (Aspergillus) ≤ 18 0.11
5 Furancarbonylglycine (Aspergillus) ≤ 3.1 0.34
6 Tartaric (Aspergillus) ≤ 6.5 1.3
7 Arabinose ≤ 50 12
8 Carboxycitric ≤ 25 0
9 Tricarballylic (Fusarium) ≤ 1.3 0
100

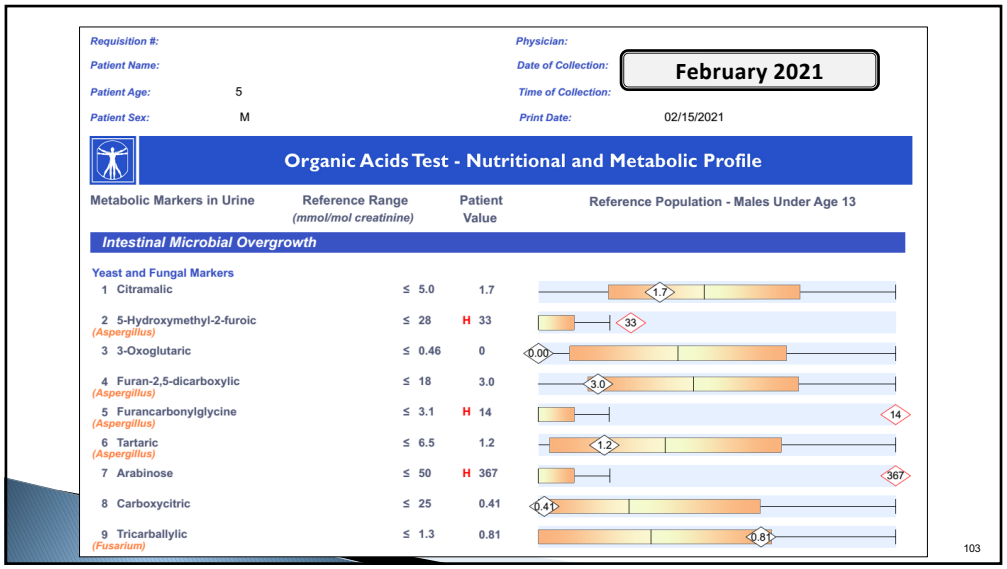
100


Initial OAT
Bacterial Markers
10 Hippuric ≤ 680 H 929
11 2-Hydroxyphenylacetic ≤ 0.86 0.71
12 4-Hydroxybenzoic ≤ 3.0 H 3.7
13 4-Hydroxyhippuric ≤ 30 H 40
14 DHPPA (Beneficial Bacteria) ≤ 0.59 0.32
Clostridia Bacterial Markers
15 4-Hydroxyphenylacetic 2.0 - 32 H 54
(C. difficile, C. stricklandii, C. lituseburense & others)
16 HPHPA ≤ 220 H 648
(C. sporogenes, C. caloritolerans, C. botulinum & others)
17 4-Cresol ≤ 84 H 121
(C. difficile)
18 3-Indoleacetic 0.60 - 14 8.7
(C. stricklandii, C. lituseburense, C. subterminale & others)
929
0.71
3.7
40
0.32
54
648
121
8.7
101

101


Follow-Up OAT
Bacterial Markers
10 Hippuric ≤ 680 99
11 2-Hydroxyphenylacetic ≤ 0.86 0.41
12 4-Hydroxybenzoic ≤ 3.0 H 14
13 4-Hydroxyhippuric ≤ 30 8.5
14 DHPPA (Beneficial Bacteria) ≤ 0.59 0.19
Clostridia Bacterial Markers
15 4-Hydroxyphenylacetic 2.0 - 32 H 54
(C. difficile, C. stricklandii, C. lituseburense & others)
16 HPHPA ≤ 220 43
(C. sporogenes, C. caloritolerans, C. botulinum & others)
17 4-Cresol ≤ 84 1.2
(C. difficile)
18 3-Indoleacetic 0.60 - 14 2.4
(C. stricklandii, C. lituseburense, C. subterminale & others)
99
0.41
14
8.5
0.19
54
43
1.2
2.4
102

102



Requisition #:
Physician:
Patient Name:
Date of Collection: February 2021
Patient Age: 5
Time of Collection:
Patient Sex: M
Print Date: 02/15/2021

Organic Acids Test - Nutritional and Metabolic Profile

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Males Under Age 13
Intestinal Microbial Overgrowth			
Yeast and Fungal Markers			
1 Citramalic	≤ 5.0	1.7	1.7
2 5-Hydroxymethyl-2-furoic (Aspergillus)	≤ 28	H 33	33
3 3-Oxoglutaric	≤ 0.46	0	0.00
4 Furan-2,5-dicarboxylic (Aspergillus)	≤ 18	3.0	3.0
5 Furancarbonylglycine (Aspergillus)	≤ 3.1	H 14	14
6 Tartaric (Aspergillus)	≤ 6.5	1.2	1.2
7 Arabinose	≤ 50	H 367	367
8 Carboxycitric	≤ 25	0.41	0.41
9 Tricarballylic (Fusarium)	≤ 1.3	0.81	0.81

103

103

CASE REPORT

Case study: Rapid Complete Recovery From An Autism Spectrum Disorder After Treatment of Aspergillus With The Antifungal Drugs Itraconazole And Sporanox

Sidney Baker, MD; William Shaw, PhD

Sporanox Program:

As we know, Sporanox is an effective medication against Aspergillus mold. It is also a systemic antifungal which gets metabolized through the liver so there is always the possibility of liver stress with continued use. Up to know, has done well with this medication at 150mg daily without any negative side effects. Based on the recent case study report by William Shaw, Ph.D. and Sydney Baker, M.D. (who used a maximum dose of 600mg daily) I believe increasing his dose of Sporanox could be beneficial. However, it will require monitoring of his liver enzymes through blood testing. Here are my recommendations.

1. Restart Sporanox at 150mg daily for 7 days. This was the dose he was on before and he was fine with it.
2. After one week back on 150mg of Sporanox, increase dose to 300mg daily. This dose is to be split up 150mg twice daily.
3. After 7 days of 300mg daily dosing obtain blood test for liver enzyme function to include AST (SGOT), ALT (SGPT), GGTP (GGT) and Alkaline Phosphatase (Alk. Phos.).
4. Before considering an increase in the dose from 300mg (perhaps to 450mg), we need to make sure his liver enzymes are not elevated, as well as determining how well is doing on current dose of Sporanox.
5. Once Sporanox starts, discontinue any Nystatin he might be taking.
6. If Milan is still taking high dose Vitamin A, I would suggest discontinuing that now.

Please let me know if you have any questions. Also, keep detailed notes/observations on with regards to reintroduction of Sporanox at 150mg and the increase to 300mg.

that there are some sources of generic itraconazole lack efficacy and that a diagnostic trial of Sporanox® is most efficiently done with an increase to 600 mg over a period of seven to ten days.

We cite as an example, a patient whose identical twin's autism responded to treatments from the protocols of the Defeat Autism Now! consensus document to become a young man with a job. His twin suffered with severe ongoing symptoms that were only partially relieved with antifungal, and a long list of "alternative" and conventional therapies. After Baker's experience with M, he simply suggested that he take 600 mg of Brand Name itraconazole daily while monitoring his AST, ALT and GGT. His dramatic response surpassed any outcome from

104

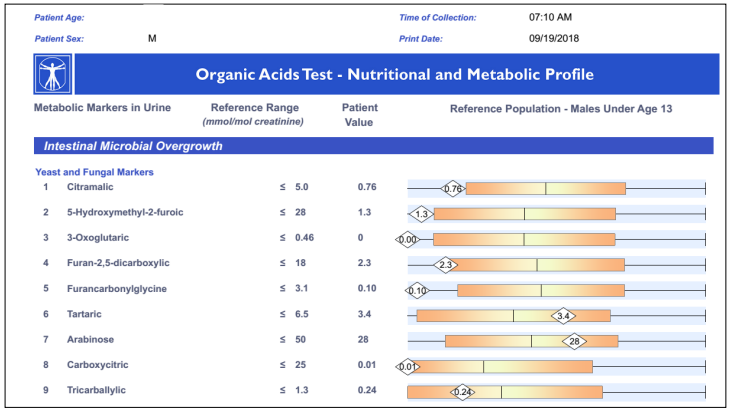
104

Recap

- TCT has unique chemical characteristics that contribute to its toxicity effects at the cellular level.
- Most mycotoxins discussed will have immune dysregulation properties and certain mycotoxins such as gliotoxin and mycophenolic can increase the potential for other opportunistic infections, e.g., candida, clostridia.
- Intervention strategies need to be focused on removing the exposure source, binders for mycotoxins, nutritional support, glutathione/NAC, and treating active fungus if present.
- In many cases, mycotoxins are not going to disappear on their own if digestive system colonization exists.
- Don't rely on the OAT for all your information about mold exposure and mycotoxins.

105

105



Patient Age:
Patient Sex: M
Time of Collection: 07:10 AM
Print Date: 09/19/2018

Organic Acids Test - Nutritional and Metabolic Profile

	Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)	Patient Value	Reference Population - Males Under Age 13
Intestinal Microbial Overgrowth				
Yeast and Fungal Markers				
1	Citramalic	≤ 5.0	0.76	0.76
2	5-Hydroxymethyl-2-furoic	≤ 28	1.3	1.3
3	3-Oxoglutaric	≤ 0.46	0	0.00
4	Furan-2,5-dicarboxylic	≤ 18	2.3	2.3
5	Furancarbonylglycine	≤ 3.1	0.10	0.10
6	Tartaric	≤ 6.5	3.4	3.4
7	Arabinose	≤ 50	28	28
8	Carboxycitric	≤ 25	0.01	0.01
9	Tricarballylic	≤ 1.3	0.24	0.24

106

Verrucarin A	4.95	0.5 - 1.2

▲ 0.5 1.2 ▲

Verrucarin A (VRA) is a macrocyclic trichothecene mycotoxin produced from Stachybotrys, Fusarium, and Myrothecium. Trichothecenes are frequently found in buildings with water damage but can also be found in contaminated grain. VRA is a small, amphipathic molecule that can move passively across cell membranes. The primary tissues affected by VRA are intestinal and gastric mucosa, bone marrow, and spleen. VRA causes damage to human cells by inhibiting protein and DNA synthesis, disrupting mitochondrial functions, and by producing oxidative stress (due to generation of free radicals). Exposure to VRA can cause immunological problems, vomiting, skin dermatitis, and hemorrhagic lesions. Nebulized and intranasal glutathione is beneficial for those exposed to inhaled toxin. Transdermal and liposomal glutathione may also be helpful, especially in combination with sequestrants. Sequestrants bind to toxins in the GI tract making them unavailable for reabsorption. These agents are not absorbed and work best for patients with GI symptoms or those whose toxin exposure is coming from food. Activated charcoal, clay, chlorophyll, and cholestyramine have all been shown to bind mycotoxins. (PMID: 23710148, 18007011, 15342078, 19333439, 20549560, 3376149)

Metabolite	Results (ng/g creatinine)	Common Range of Positive Results
Enniatin B	0.514	0.09 - 0.45

▲ 0.09 0.45 ▲

Enniatin B (ENB) is a fungal metabolite categorized as cyclohexa depsipeptides toxin produced by the fungus Fusarium. This strain of fungus is one of the most common cereal contaminants. Grains in many different countries have recently been contaminated with high levels of enniatins The toxic effects of Enniatin are caused by the inhibition of the acyl-CoA cholesterol acyltransferase, depolarization of mitochondria, and inhibition of osteoclastic bone resorption. Enniatin has antibiotic properties and chronic exposure may lead to weight loss, fatigue, and liver disease. Sequestrants bind to mycotoxins in the GI tract making them unavailable for reabsorption. These agents are not absorbed and work best for patients with GI symptoms or those whose toxin exposure is coming from food. Activated charcoal, clay, chlorophyll, and cholestyramine have all been shown to bind mycotoxins. (PMID: 18274964, 16730043, 21622627, 23710148)

107

The Great Plains Laboratory, Inc.

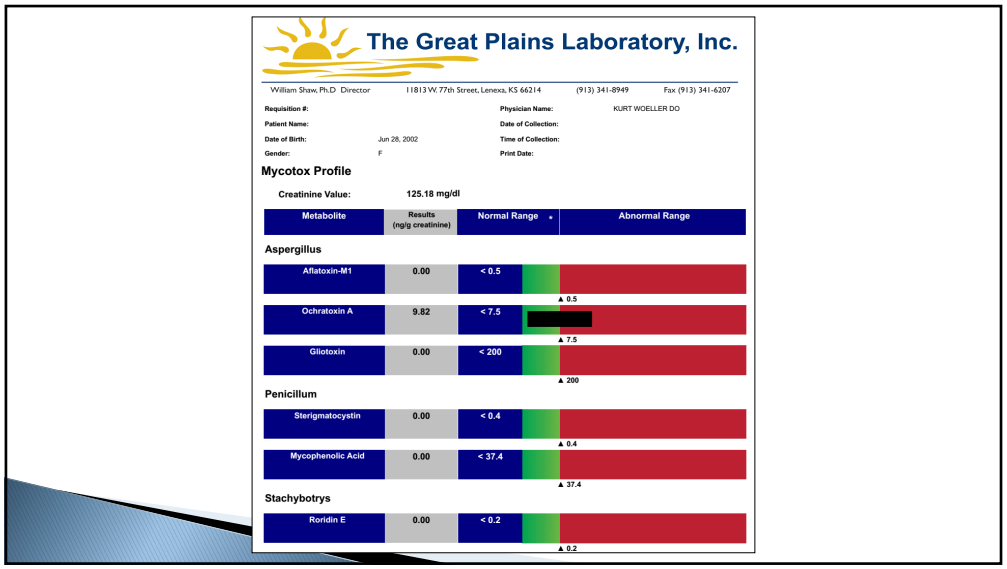
William Shaw, Ph.D., Director | 11813 West 77th Street, Lenexa, KS 66214 | (913) 341-8949 | Fax (913) 341-6207

Requisition #:		*Physician:*	KURT WOELLER DO
Patient Name:		*Date of Collection:*	
Patient Age:	17	*Time of Collection:*	
Patient Sex:	F	*Print Date:*	

Organic Acids Test - Nutritional and Metabolic Profile

Metabolic Markers in Urine	Reference Range (mmol/mol creatinine)		Patient Value	Reference Population - Females Age 13 and Over
Intestinal Microbial Overgrowth				
Yeast and Fungal Markers				
1 Citramalic	≤ 3.6		1.2	1.2
2 5-Hydroxymethyl-2-furoic (Aspergillus)	≤ 14	H	100	100
3 3-Oxoglutaric	≤ 0.33		0.20	0.20
4 Furan-2,5-dicarboxylic (Aspergillus)	≤ 16	H	53	53
5 Furancarbonylglycine (Aspergillus)	≤ 1.9		0.19	0.19
6 Tartaric (Aspergillus)	≤ 4.5		3.8	3.8
7 Arabinose	≤ 29	H	54	54
8 Carboxycitric	≤ 29		5.5	5.5
9 Tricarballylic (Fusarium)	≤ 0.44	H	0.55	0.55

108



The Great Plains Laboratory, Inc.

William Shaw, Ph.D Director 11813 W. 77th Street, Lenexa, KS 66214 (913) 341-8949 Fax (913) 341-6207

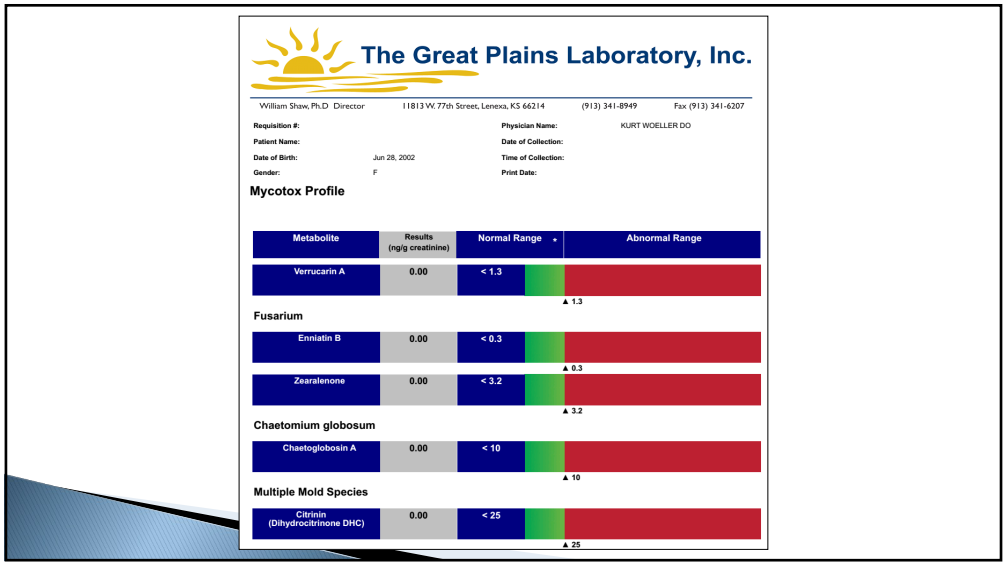
Requisition #: Physician Name: KURT WOELLER DO
Patient Name: Date of Collection:
Date of Birth: Jun 28, 2002 Time of Collection:
Gender: F Print Date:

Mycotox Profile

Creatinine Value: 125.18 mg/dl

Metabolite	Results (ng/g creatinine)	Normal Range *	Abnormal Range
Aspergillus			
Aflatoxin-M1	0.00	< 0.5	▲ 0.5
Ochratoxin A	9.82	< 7.5	▲ 7.5
Gliotoxin	0.00	< 200	▲ 200
Penicillum			
Sterigmatocystin	0.00	< 0.4	▲ 0.4
Mycophenolic Acid	0.00	< 37.4	▲ 37.4
Stachybotrys			
Roridin E	0.00	< 0.2	▲ 0.2

109



The Great Plains Laboratory, Inc.

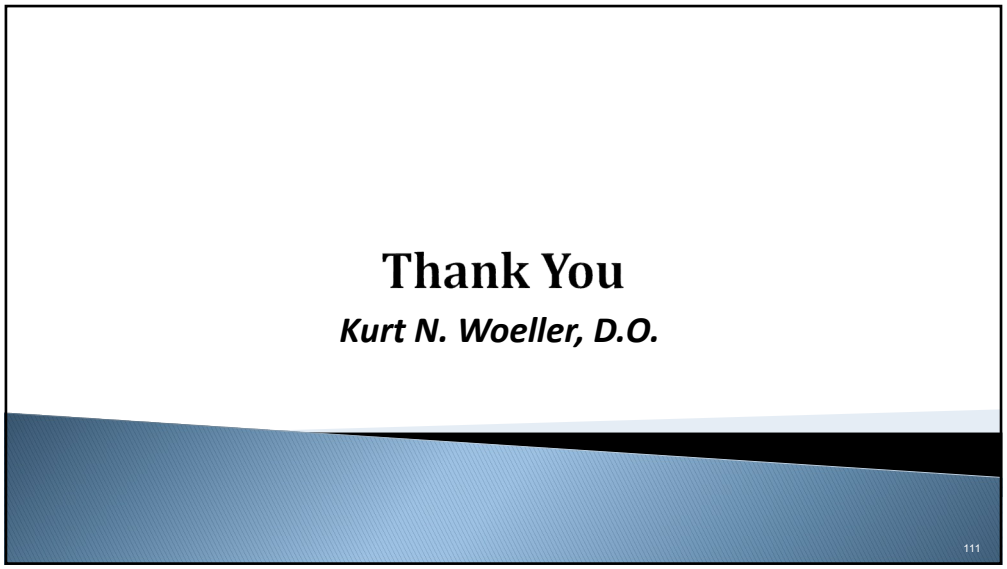
William Shaw, Ph.D Director 11813 W. 77th Street, Lenexa, KS 66214 (913) 341-8949 Fax (913) 341-6207

Requisition #: Physician Name: KURT WOELLER DO
Patient Name: Date of Collection:
Date of Birth: Jun 28, 2002 Time of Collection:
Gender: F Print Date:

Mycotox Profile

Metabolite	Results (ng/g creatinine)	Normal Range *	Abnormal Range
Verrucarin A	0.00	< 1.3	▲ 1.3
Fusarium			
Enniatin B	0.00	< 0.3	▲ 0.3
Zearalenone	0.00	< 3.2	▲ 3.2
Chaetomium globosum			
Chaetoglobosin A	0.00	< 10	▲ 10
Multiple Mold Species			
Citrinin (Dihydrocitrinone DHC)	0.00	< 25	▲ 25

110



111