National Fusarium Head Blight Forum
St. Louis, Missouri
December 6, 2011

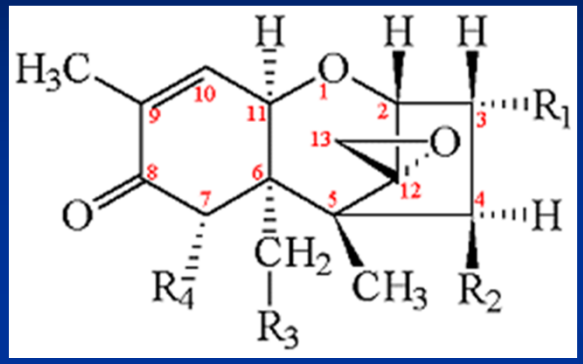
A New Understanding of DON's Mechanisms of Action- Implications for Food Safety and Security

James J. Pestka,
Food Science and Human Nutrition
Microbiology and Molecular Genetics
Center for Integrative Toxicology
Michigan State University

Bottom Line

- DON/Type B's cause illness in animals and humans
- Two animal models devised for predictng risk of anorexia (mouse) and emesis (mink)
  - Anorexia/Emesis are transient/reversible
  - Toxic potencies: DON.≥15-ADON≥3-ADON
  - Anorexia and emesis might result from elevated gut satiety hormone secretion
- Risks posed by DON dependent on population
- Food safety regulations impact food security

Fusarium and the Type B Trichothecenes



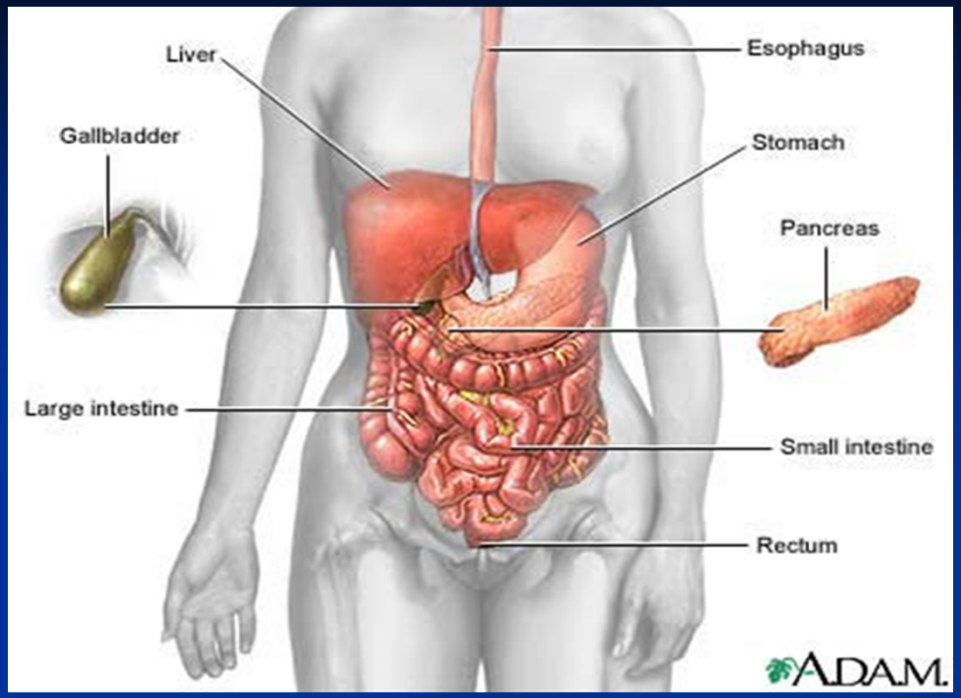
- Sesquiterpenoid metabolites (>200)
- DON ("vomitoxin") is most common
- Bind to ribosome -inhibit translation and activate stress signaling
- Animal and human toxicity

Toxin	Abbr.	R1	R2	R3	R4
Deoxynivalenol	DON	OH	H	OH	OH
3-Acetyl Deoxynivalenol	3ADON	OAc	H	OH	OH
15-Acetyl Deoxynivalenol	15ADON	OH	H	OAc	OH
Nivalenol	NIV	OH	OH	OH	OH
Fusarenon X	FX	OH	OAc	OH	OH

Trichothecenes Associated with Human Gastroenteritis Outbreaks

- USSR (1930's - 1940's)
- Japan,Korea (1940's - 1960's)
- India - Kashmir Valley (1987)
- China (1960s-1990's)

DON and Other Trichothecenes Target GI Function



Acute	Chronic
Anorexia	Growth suppression
Nausea	Weight loss
Vomiting	

Pestka. 2010. Arch Toxic.

JECFA Tolerable Daily Intake is Based on Growth Suppression in Mice

TABLE I. Average Body Weight and Food Consumption for Male and Female B6C3F1 Mice

DON (ppm)	Average body weight* N	Mean (g)	SD (g)	Average daily food consumption N	Mean (g)	SD (g)
Female						
0.00	36	41.54	6.26	22	4.48	0.25
1.00	42	38.71	4.73	24	4.44	0.23
5.00	37	33.76*	3.92	23	4.46	0.26
10.00	38	28.55*	2.08	25	4.34	0.24
Male						
0.00	37	43.85	2.69	24	4.30	0.16
1.00	35	43.51	2.86	24	4.28	0.17
5.00	43	40.03*	3.00	25	4.05*	0.17
10.00	42	35.09*	2.56	25	3.95*	0.24

*Based on intercept values and analyzed on the reciprocal scale.
*Significantly different from control value ($P < 0.001$).

Iverson et. al., 1995. Teratog Carcinog Mutagen.
Canady et. al. 2001. JECFA47 Report.

Current Allowable Levels

United States: FDA	European Union
Guidance Limit	Maximum Level
1000 µg/ kg grain Processed Products	200 - 1250 µg/ kg grain Processed to Unprocessed Products
No Tolerable Daily Intake	TDI = NOEL x Uncertainty Factor TDI = 100 µg/kg bw x (1/10) x (1/10) = 1 µg/kg bw
No Published Risk Assessment	Based on Iverson et. al (1995) and JECFA TDI with X Uncertainty Factor

Canady et. al. 2001. JECFA47 Report.
FDA. 2010. http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/NaturalToxins/UCM217558.pdf

Consumption Data Determine Mycotoxin Regulatory Limits

Modest Regulatory Limit

Stringent Regulatory Limit

Hypothetical Regulatory Limit (mg/k

Undesired area

8
6
4
2
0

10
100
300
400

Grain consumed per day (g)

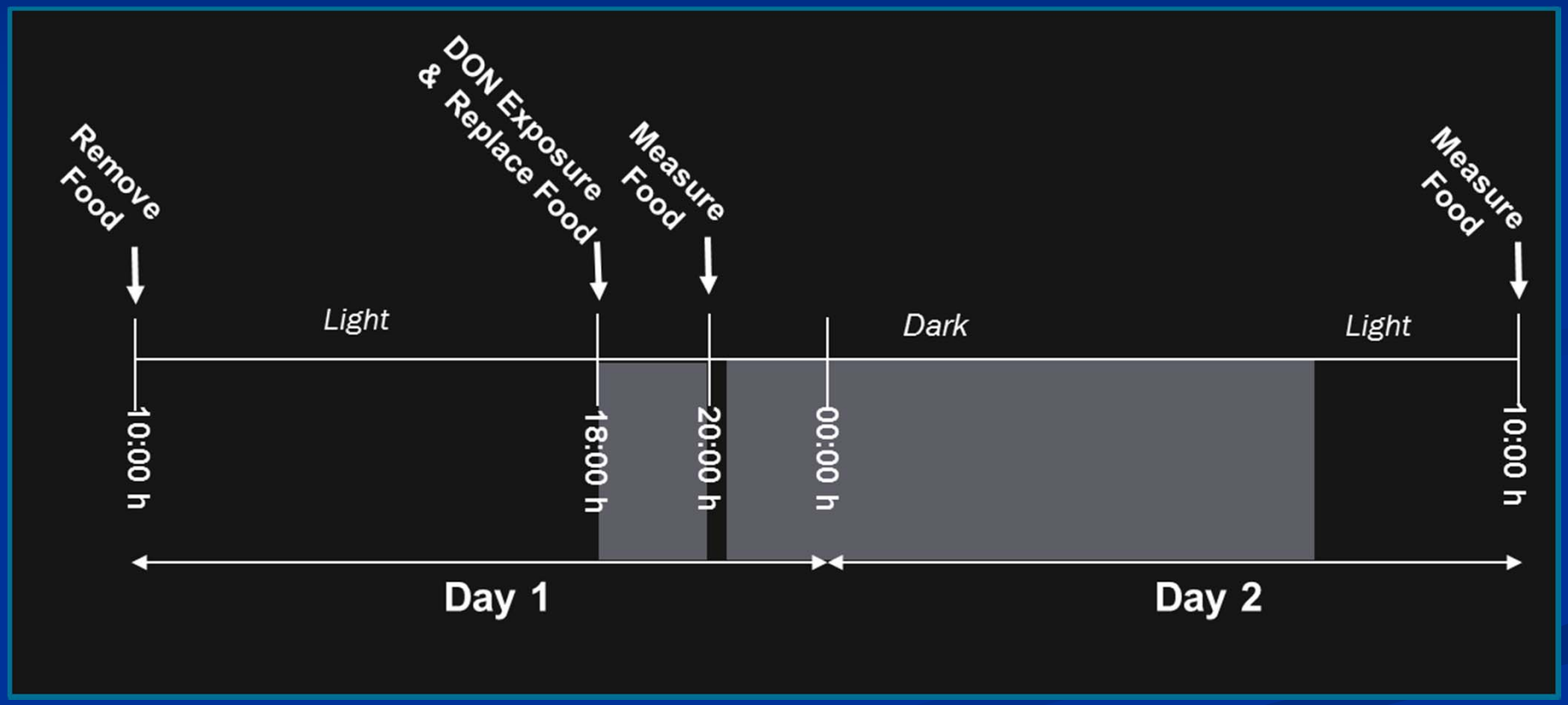
J. Pestka Michigan State University-

Hans van Egmond -RIKILT Institute of Food Safety, Wageningen University

Recent Studies:

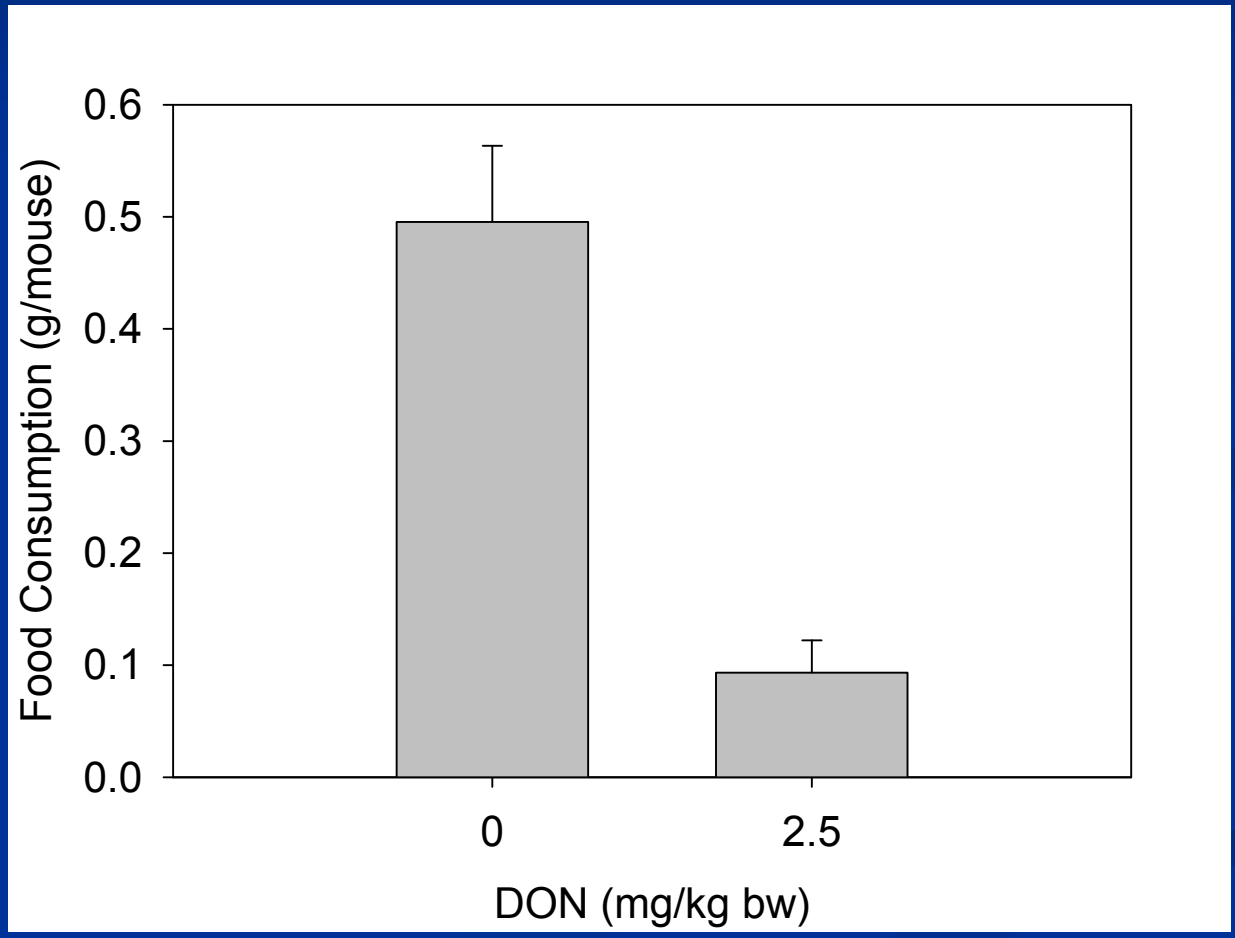
- Establish reliable animal models for DON toxicity
  - Anorexia – Mouse
  - Emesis – Mink
- Determine toxicologic potencies of Type B trichothecenes
- Elucidate mechanisms for DON-induced anorexia and emesis

Model 1: Anorexia (Mouse)

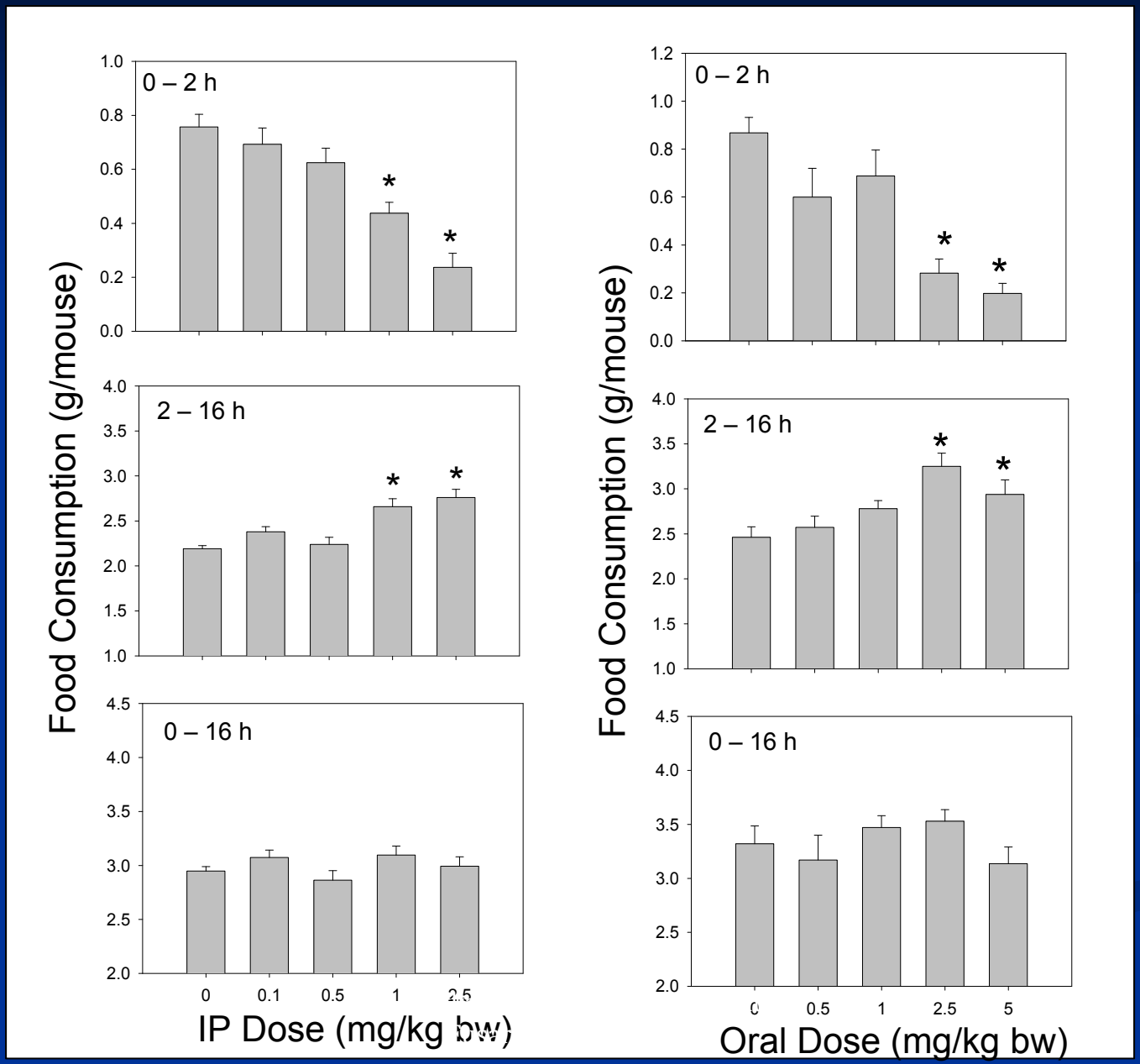


Flannery et. al., 2011. Food Chem Toxicol.

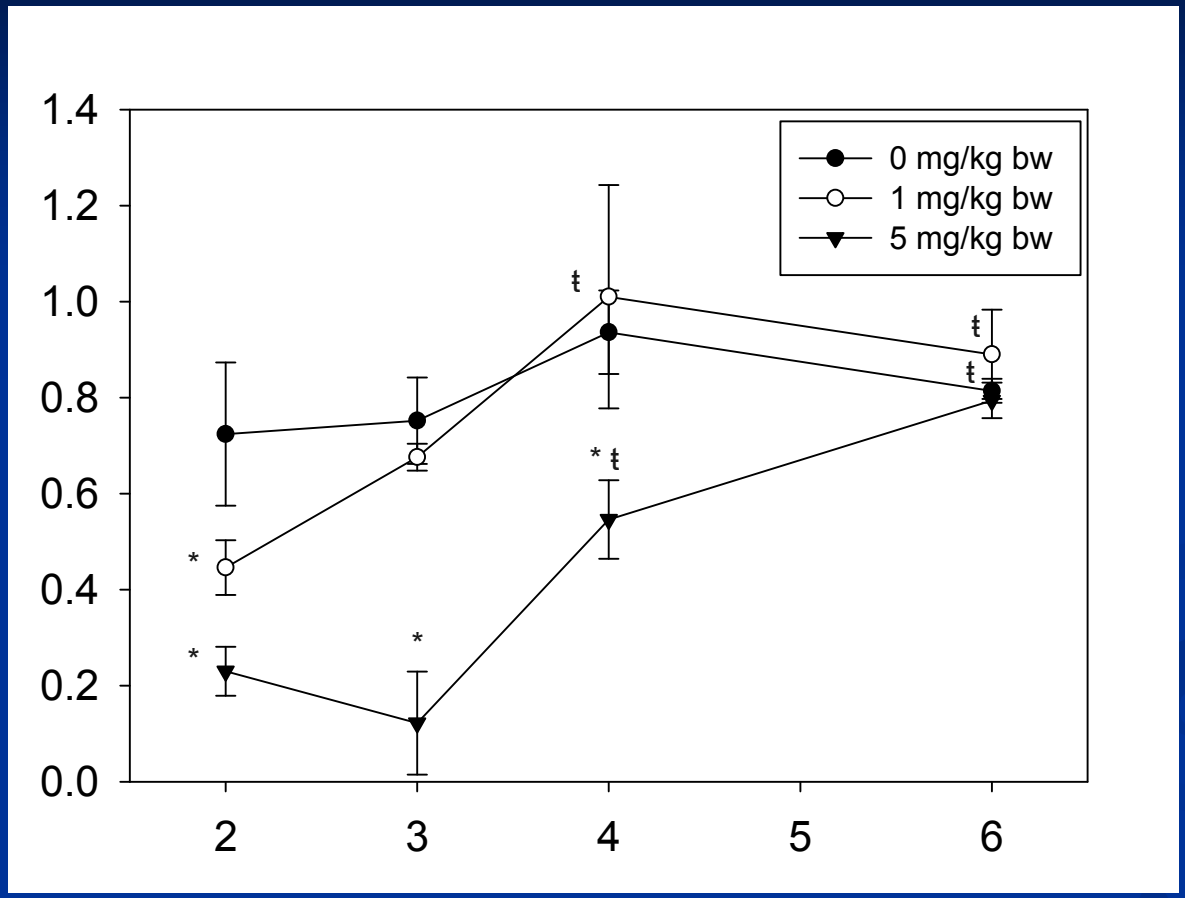
DON-induced anorexia occurs rapidly- 30 min



DON-induced anorexia is transient



DON-Induced Anorexia is Reversible



Flannery et. al., 2011. Food Chem Toxicol.

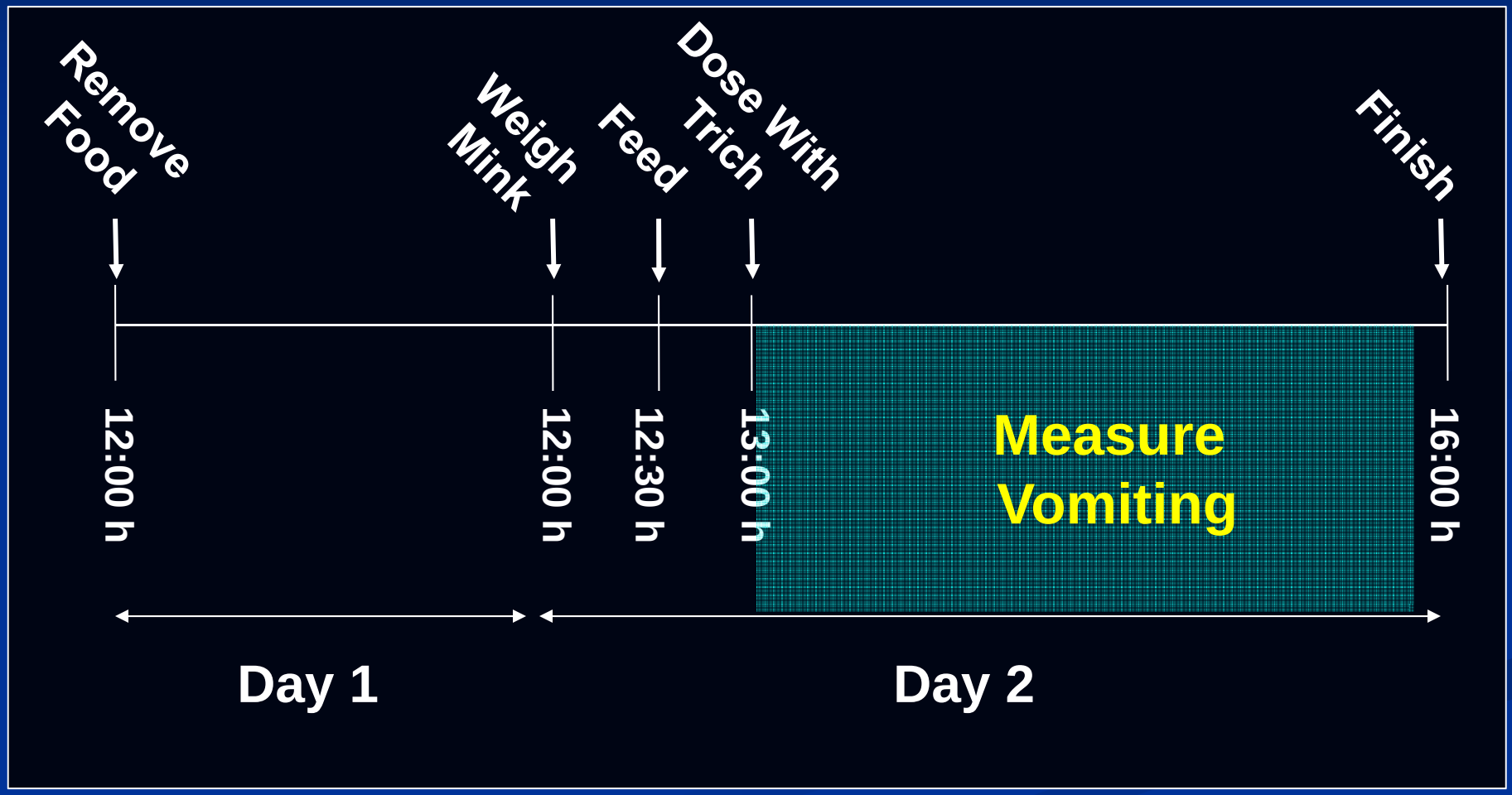
Comparative Anorectic Effects of 8-Ketotrichothecenes in Mouse Model (2 h)

	IP		ORAL	
TOXIN	NOAEL	LOAEL	NOAEL	LOAEL
DON	0.5	1	1	2.5
3-ADON	0.5	1	1	2.5
15-ADON	0.5	1	1	2.5

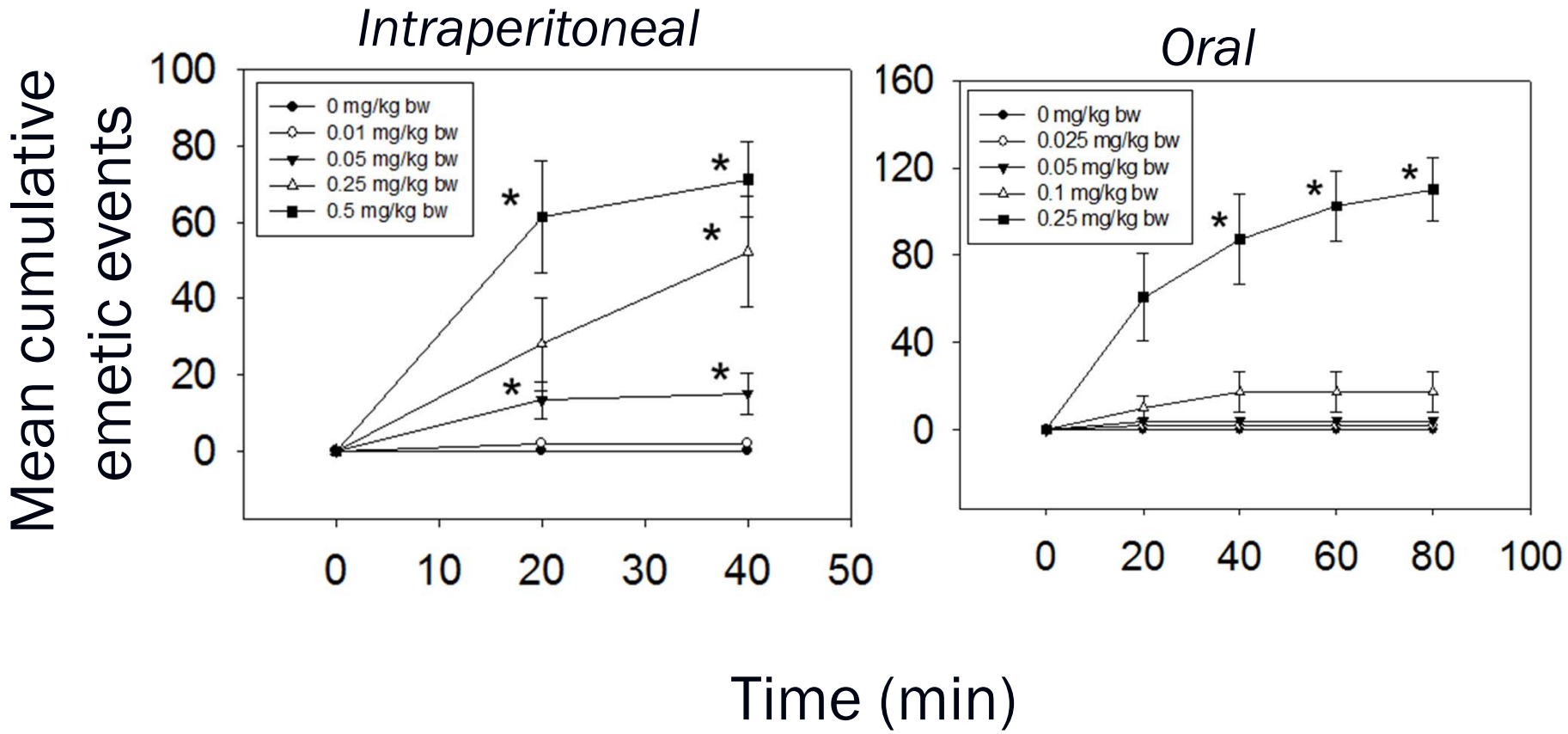
•NOAEL = no observed adverse effect level

•LOAEL = lowest observed adverse effect level

Model 2: Emesis- Mink



DON-induced Emesis in Mink: Kinetics and Dose Response



Comparison of Emetic Responses Type B Trichothecene Oral Exposure

Toxin	Dose (mg/kg bw)	No. of animals	Duration of emesis (min)	Emetic events
Control	0	0/30	-	-
DON	0.01	0/6	-	-
	0.05	5/6	16.2±1.8	15±5.3
	0.25	6/6	23.3±2	52.3±14.7*
	0.5	6/6	25.7±4.4	71.3±10.1*
15-ADON	0.01	0/6	-	-
	0.1	5/6	21.3±4.1	11.2±3.9
	0.5	6/6	21.7±2.5	29.5±5.5*
	1	6/6	31.3±5	32±6.6*
3-ADON	0.05	0/6	-	-
	0.25	1/6	63±0	2±2
	0.5	5/6	65.2±7.9	20±9
	1	6/6	66±9.3	78.5±13.5*

JECFA Tolerable Daily Intake is Based on Growth Suppression in Mice

TABLE I. Average Body Weight and Food Consumption for Male and Female B6C3F1 Mice

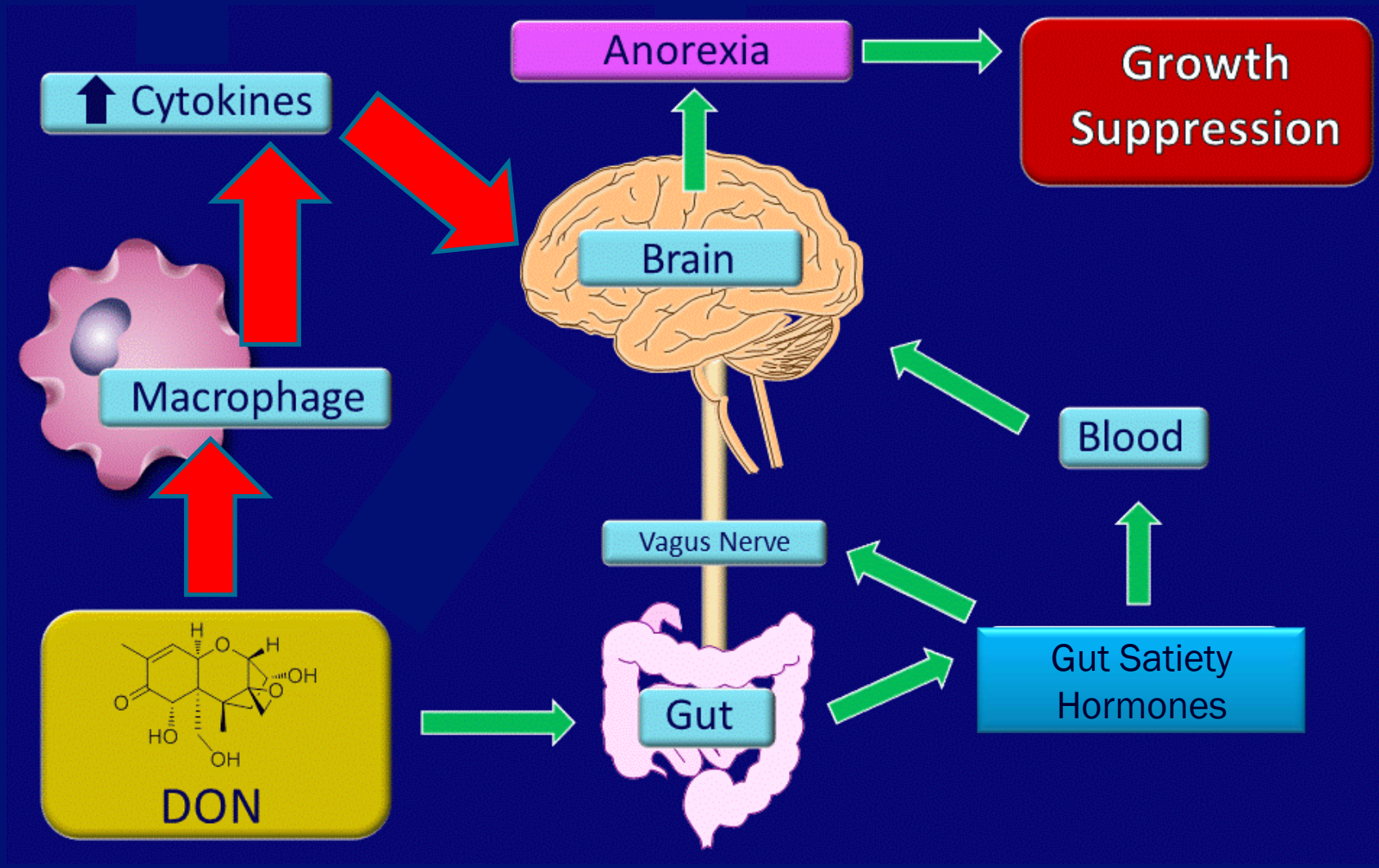
DON (ppm)	Average body weight[a] N	Mean (g)	SD (g)	Average daily food consumption N	Mean (g)	SD (g)
Female						
0.00	36	41.54	6.26	22	4.48	0.25
1.00	42	38.71	4.73	24	4.44	0.23
5.00	37	33.76*	3.92	23	4.46	0.26
10.00	38	28.55*	2.08	25	4.34	0.24
Male						
0.00	37	43.85	2.69	24	4.30	0.16
1.00	35	43.51	2.86	24	4.28	0.17
5.00	43	40.03*	3.00	25	4.05*	0.17
10.00	42	35.09*	2.56	25	3.95*	0.24

[a]Based on intercept values and analyzed on the reciprocal scale.
*Significantly different from control value ($P < 0.001$).

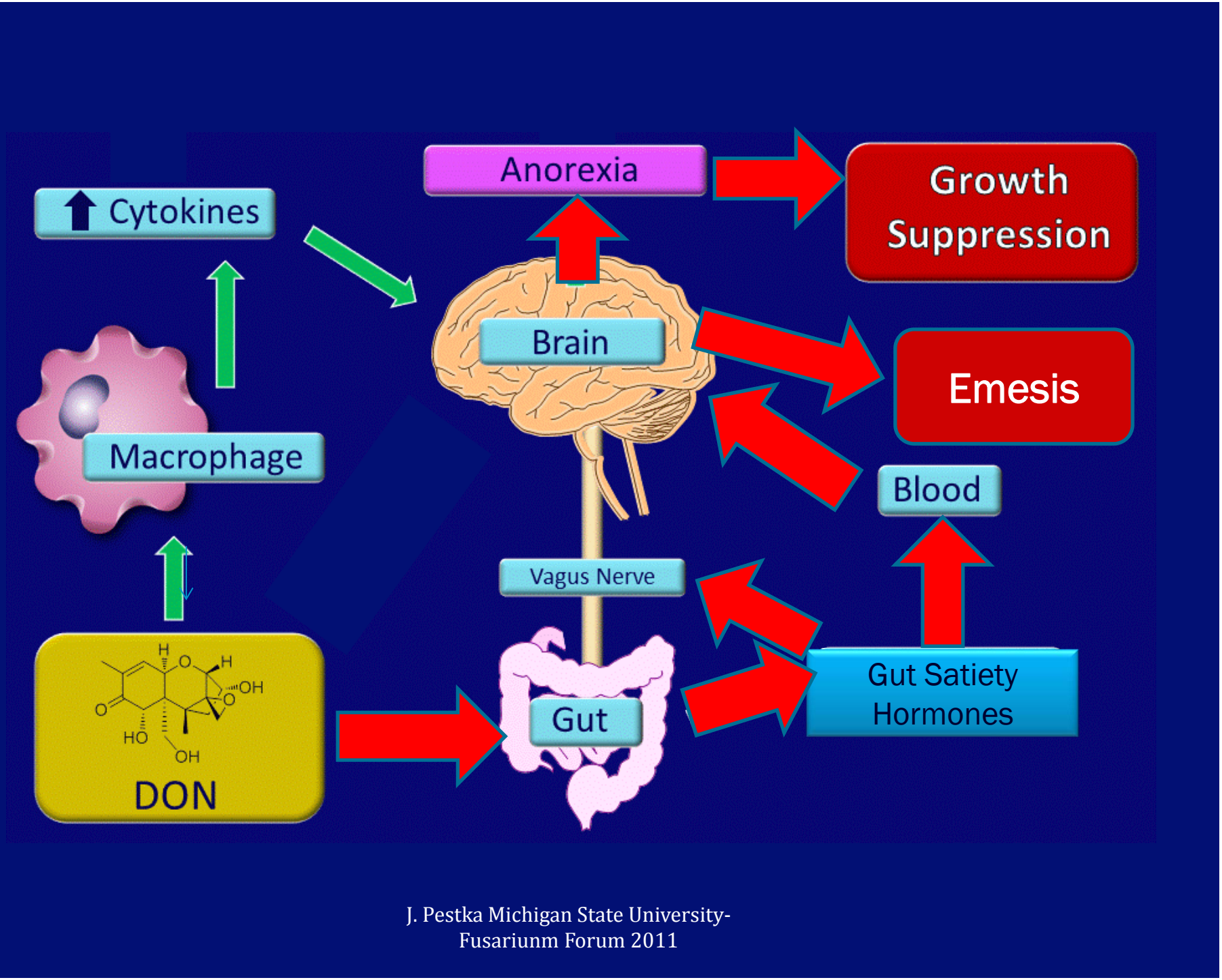
Iverson et. al., 1995. Teratog Carcinog Mutagen.
Canady et. al. 2001. JECFA47 Report.

What causes DON-induced anorexia and growth suppression?

- Proinflammatory Cytokines
- Gut satiety hormones



J. Pestka Michigan State University-
Fusariunm Forum 2011



J. Pestka Michigan State University-
Fusariunm Forum 2011

Vomitoxin- Friend or Foe?

Anorexia (bad)

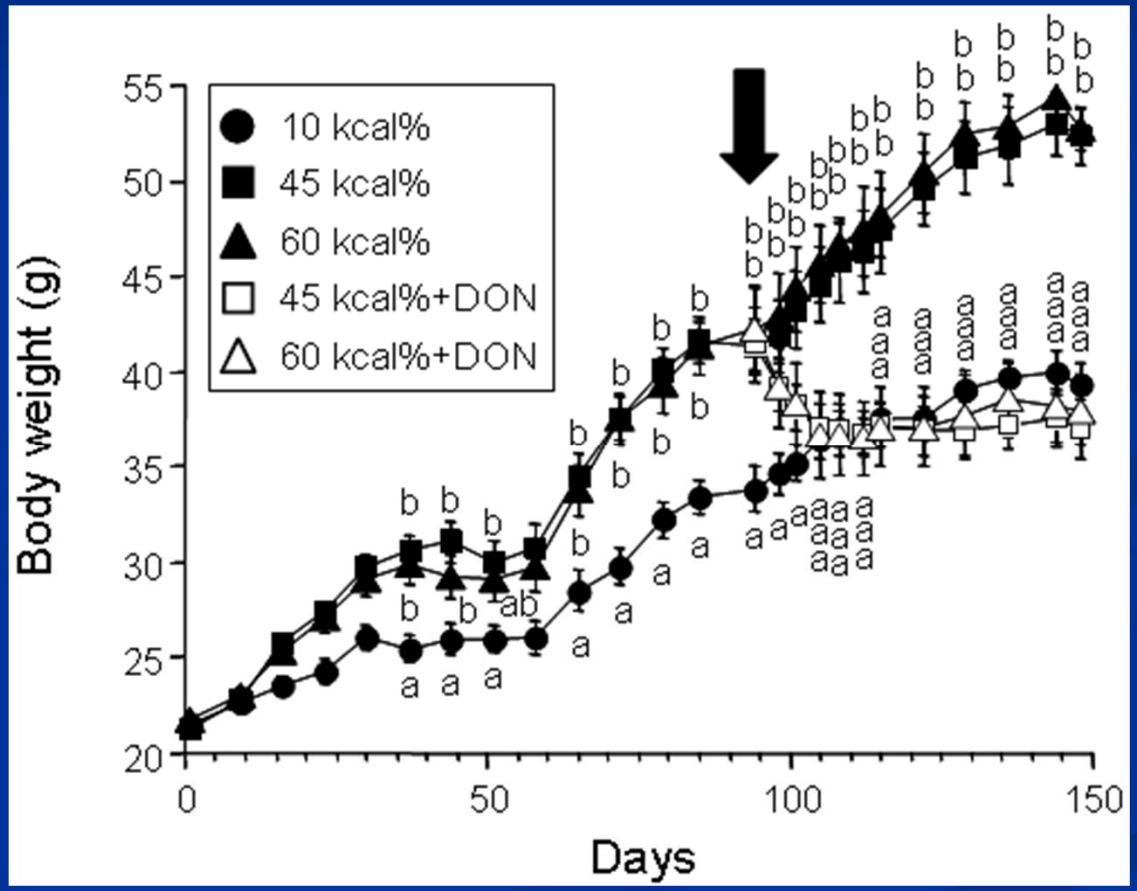
Satiety (good)

Can DON or its analogues be used to treat obesity?

Paracelsus (1493—1541)

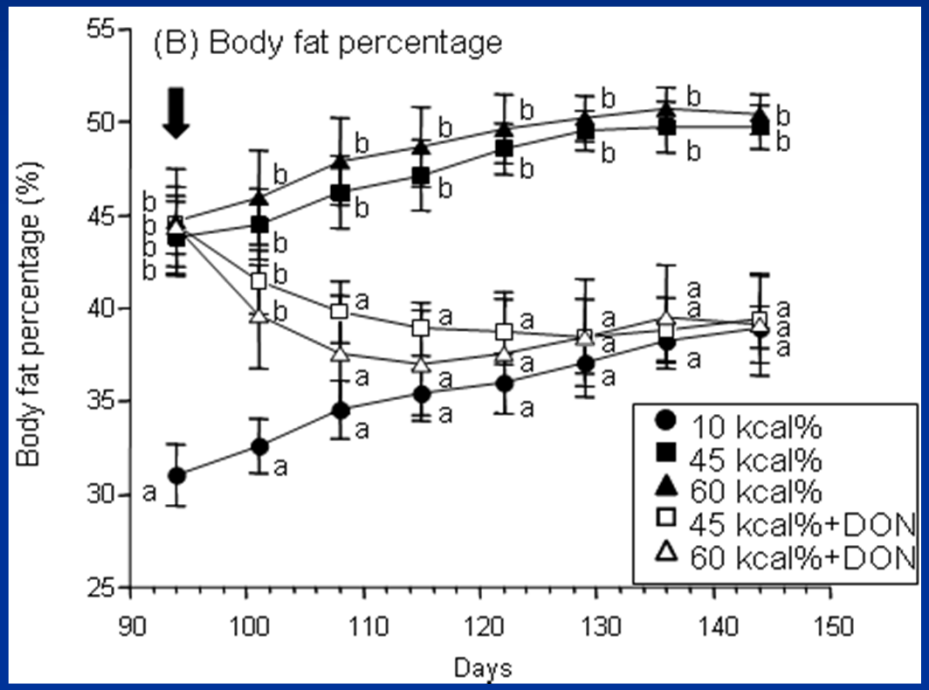
- "All substances are poisons; there is none which is not a poison. The right dose differentiates the poison from a remedy."

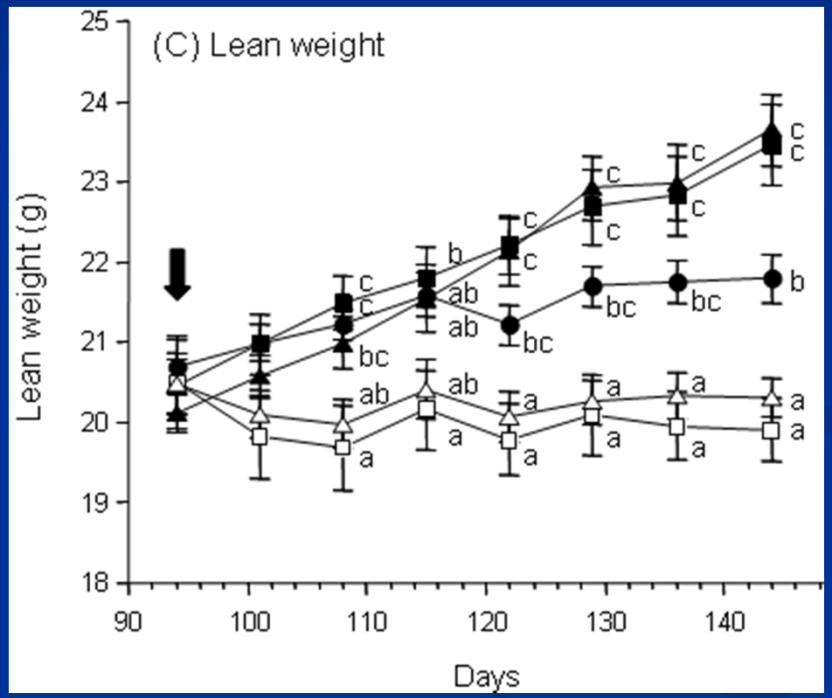
DON impairs food intake and induces weight loss in HF diet-induce d obese mice



Hattori et al . 2011, Mol. Nutr. Food Sci (in press)

DON induces body fat loss without lean weight loss in HF diet-induced obese mice





Hattori et al . 2011, Mol. Nutr. Food Sci (in press)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 July 2002 (18.07.2002)

PCT

(10) Internati
WO 0

(51) International Patent Classification[7]: C07D 493/00

(21) International Application Number: PCT/CA02/00025

(22) International Filing Date: 11 January 2002 (11.01.2002)

(81) Designated States (na
AZ, BA, BB, BG, BR,
CZ, DE, DK, DM, DZ,
GM, HR, HU, ID, IL,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,

NOVEL MULTI-RING ORGANIC COMPOUNDS FOR REGULATING GUT MOTILITY AND FOOD INTAKE

GENERAL FIELD OF THE INVENTION

This invention is generally in the field of treating obesity and regulating food intake. In particular, this invention relates to compositions and methods for regulating gut motility and treating obesity using novel multi-ring organic compounds.

Bottom Line

- DON/Type B's cause illness in animals and humans
- Two animal models devised for predictng risk of anorexia (mouse) and emesis (mink)
  - Anorexia/Emesis are transient/reversible
  - Toxic potencies: DON.≥15-ADON≥3-ADON
  - Anorexia and emesis might result from elevated gut satiety hormone secretion
- Risks posed by DON dependent on population
- Food safety regulations impact food security

Towards Science-Based Risk Assessment

- Develop toxicologic equivalency factors for Type B trichothecenes and metabolites
- Determine potential susceptibility factors (age, gender, genetics) in emesis
- Elucidate mechanisms for gut satiety hormone induction

Acknowledgements

MSU Colleagues

- Dr. Patti Ganey
- Dr. Dale Romsos
- Dr. James Galligan
- Dr. Burisan and Lab

Pestka Lab

- Brenna Flannery
- Wenda Wu
- Dr. Dozie Amuzie
- Dr. Kazuo Hattori
- Xiao Pan
- Kaiyu He
- Mary Rosner
- Dr. Hui-Ren Zhou

Funding

- USDA Wheat and Barley SCAB Initiative
- MSU-AES & FSHN
- MSU CIT NIEHS Training Grant
- PHS Grant DK58833