# Biomarkers and risk assessment for Deoxynivalenol

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# Md. Receives Federal Crop Disaster Designation for 10 Counties

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(Photo: WBOC)



**ANNAPOLIS**, Md.- State agriculture officials say 10 counties have received federal disaster designation for crop losses because of vomitoxin contamination resulting from wet weather that occurred in May and June 2009.

Vomitoxin (deoxynivalenol toxin) may be produced in wheat and barley grain infected by the fungus, Fusarium head blight or scab. The Nov. 13 letter from U.S. Secretary of Agriculture Tom Vilsack to Maryland Gov. Martin O'Malley stated that there were sufficient production losses in Baltimore, Carroll, Cecil, Harford, Howard, Kent, Montgomery, Queen Anne's, Talbot and Washington counties to warrant a disaster designation.

"Because farmers throughout most of Maryland experienced significant crop losses, we requested a disaster designation and thank Secretary Vilsack for granting it," O'Malley said. "It is our hope that the designation will provide relief to the farmers who need it and help them prepare for the next growing season."

"Persistent spring rain throughout most of the state caused significant contamination in wheat and barley harvest in the form of vomitoxin, which can make the crop unmarketable or unusable as feed," said Buddy Hance, secretary of the Maryland Department of Agriculture. "Farmers in the disaster designation areas experienced market value losses ranging from 30 to 55 percent."

This designation makes farm operators in the 10 primary counties as well as contiguous counties – Allegany, Anne Arundel, Caroline, Dorchester, Frederick and Prince George's counties - eligible to be considered for assistance from the USDA Farm Service Agency, provided eligibility requirements are met.

# Outline

- Overview of Deoxynivalenol and its toxicity
- Uncertainty in risk assessment of Deoxynivalenol
- Use of biomarkers to reduce uncertainty
- Identification of a biomarker for Deoxynivalenol's effects
- Potential application of the present biomarker

# Deoxynivalenol (DON)

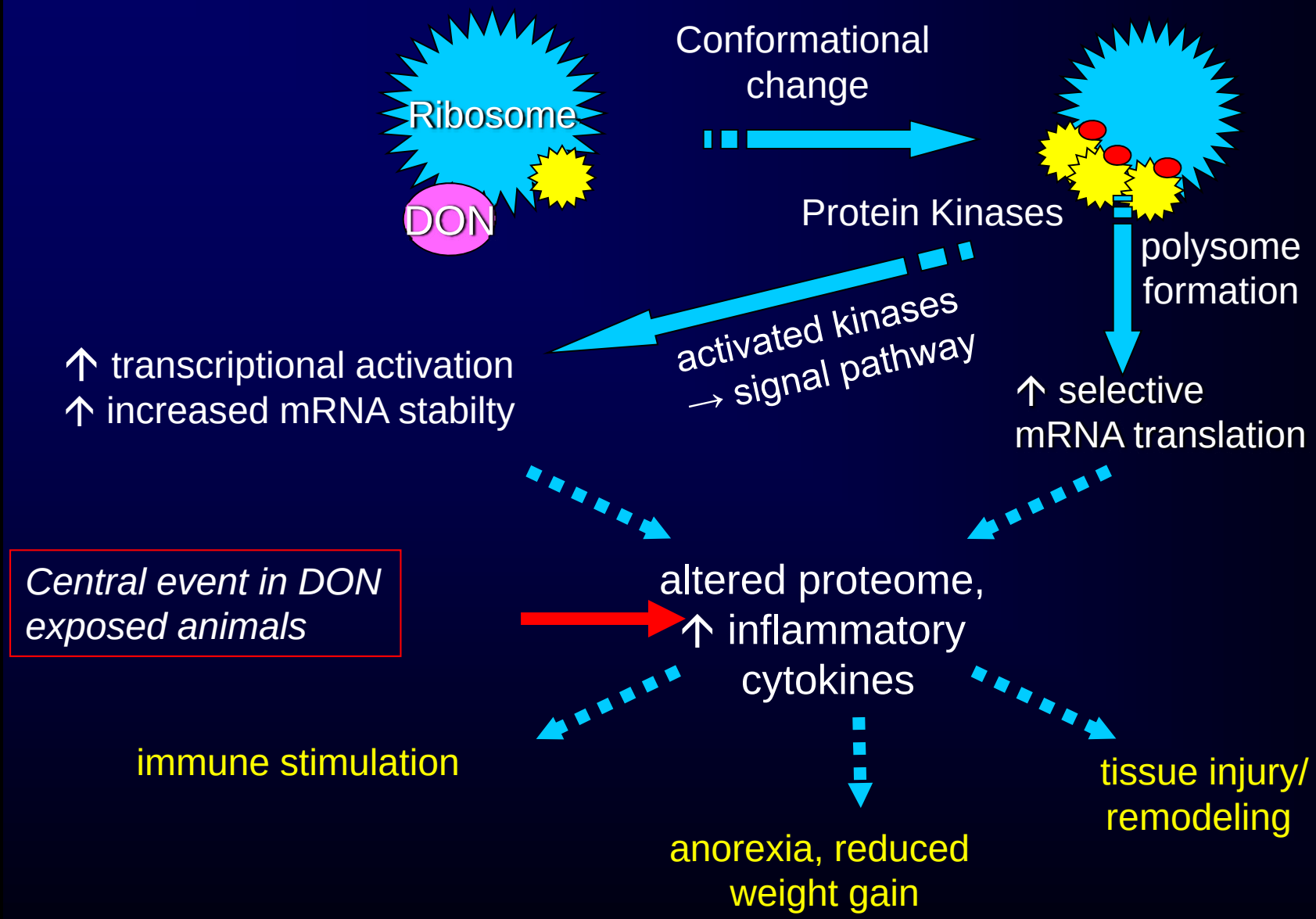
- A trichothecene compound
  - secondary metabolite of fungi
  - mostly elaborated by *Fusarium sp*

- In food
  - commonly detected in grains
  - not destroyed by milling processes
  - regular contaminant of processed food

# Effects of DON

- Kinetics
  - rapidly absorbed and distributes to many tissues
  - detoxified by glucuronidation and de-epoxidation
- Acute effects
  - vomiting
  - nausea
- Chronic effects
  - feed refusal
  - Weight gain reduction (growth retardation)

# Proposed molecular mechanism of DON toxicity






adapted from Bae, Hee Kyong

# Risk assessment of DON

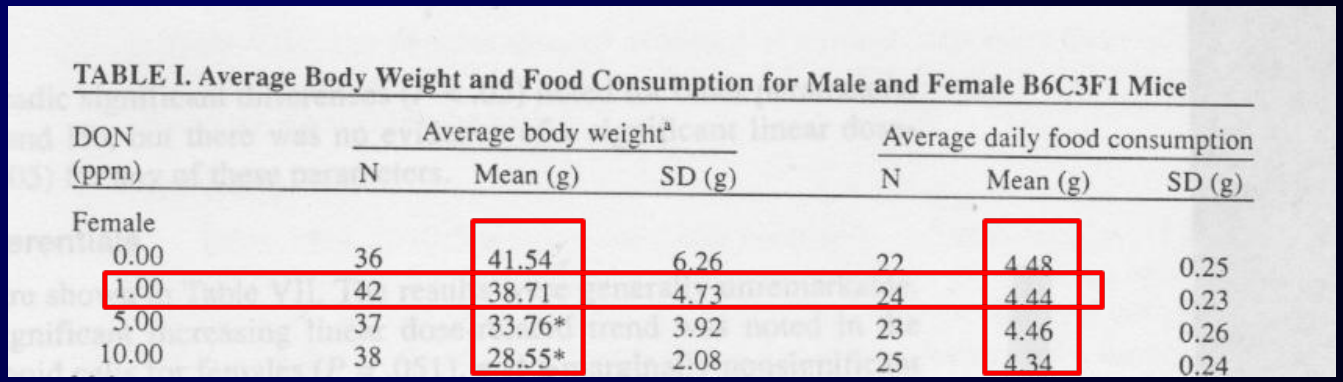


TABLE I. Average Body Weight and Food Consumption for Male and Female B6C3F1 Mice

| 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 |

Iverson, F., et al , 1995. Teratog Carcinog Mutagen 15:283-306.

| Compound | Critical effect: Growth retardation | Uncertainty/ Safety factor | Tolerable daily intake |
|---|---|---|---|
| DON | 0.1 (mg/kg bw/day) (NOAEL) | 100 | 1 (ug/kg bw/day) |

Tritscher A.M (2004) Toxicol Lett. 153(1):155-63

NOAEL=No observed adverse effect level

# Why 100 fold safety factor?

"This factor of 100 appears to be high enough to reduce the hazard of food…."

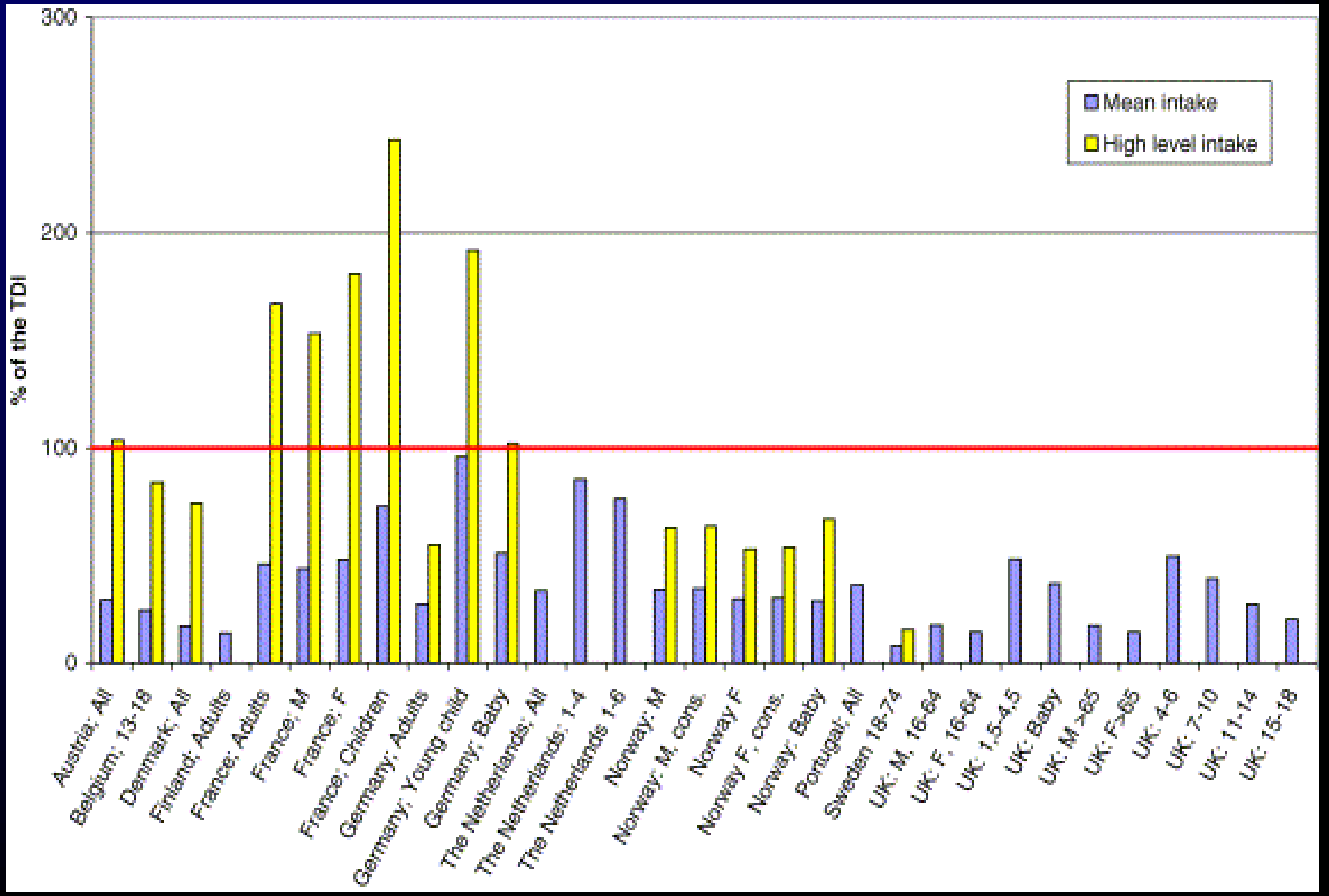
Lehman, A. and Fitzhugh, O. (1954). Food Drug Officials of the US Quarterly Bulletin XVIII (1):33-35.

Inherent assumptions

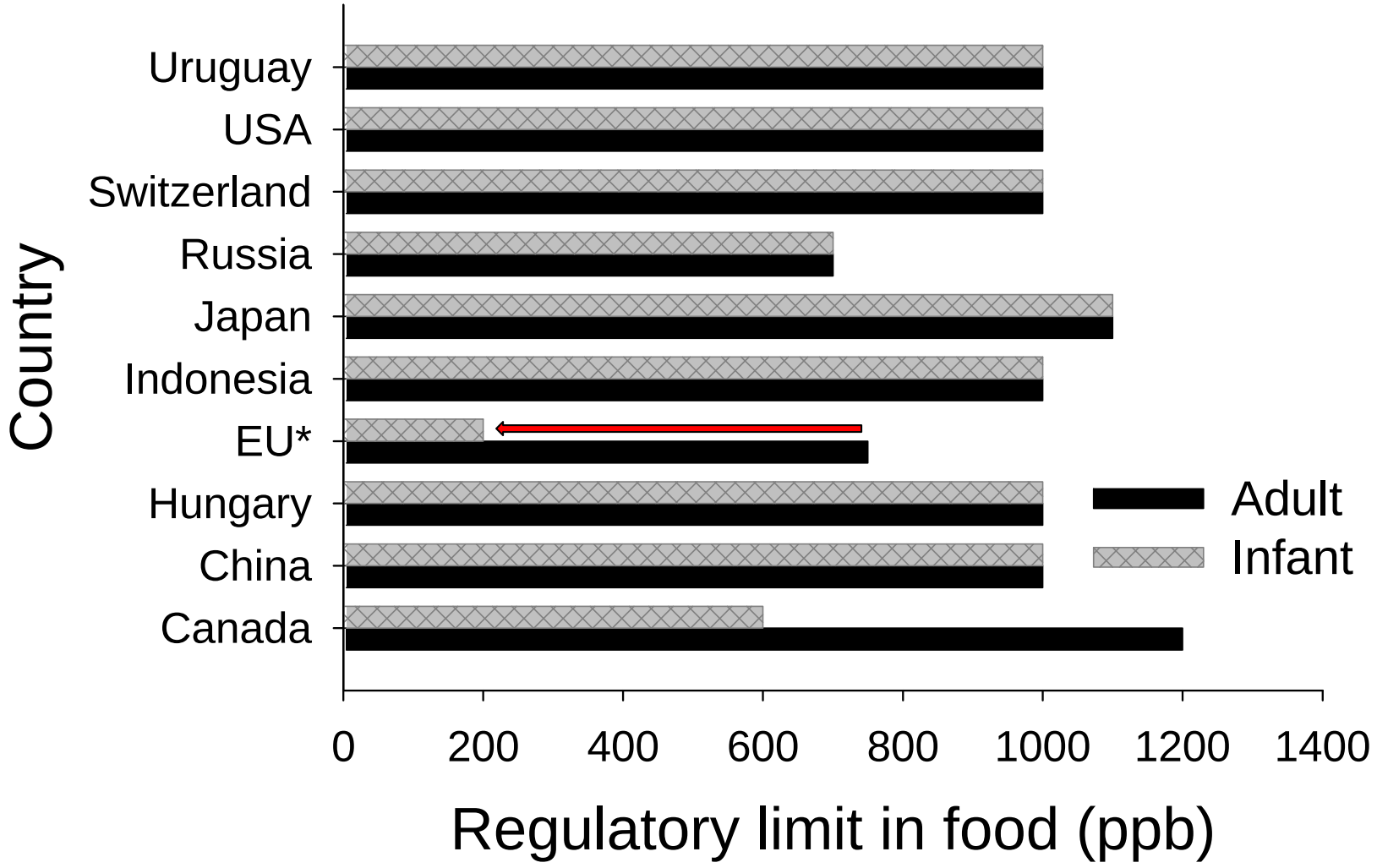
Similar effects occur in humans and laboratory animals

Humans are 100 times more sensitive to toxins than laboratory animals

# How much DON is hazardous in diets?

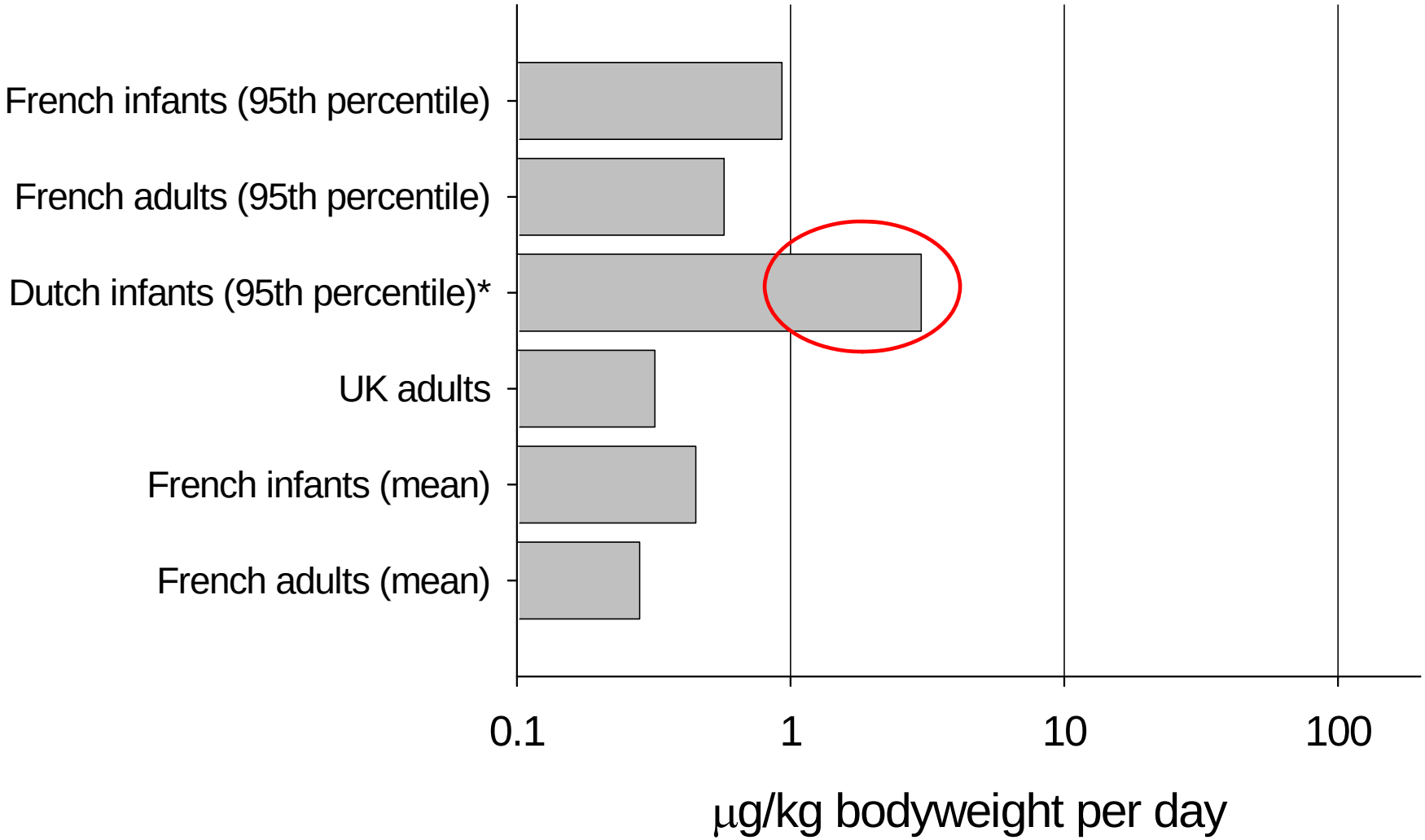


# World Deoxynivalenol limits




FAO , 2003 Worldwide regulations for mycotoxins in food and feed in 2003. Food and Nutrition Paper No.81

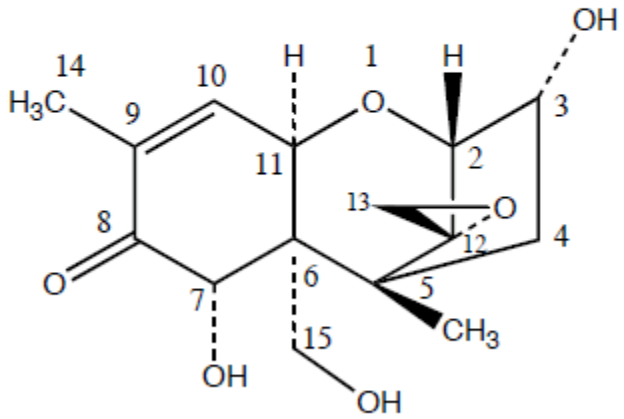
# Relationship between Tolerable daily intake and NOAEL



# Risk communication is very critical

**NTP Research Concept: Deoxynivalenol**

Deoxynivalenol (Vomitoxin;12,13-Epoxy-3,7,15-trihydroxy-(3α,7α)-trichothec-9-en-8-one; CAS RN 51481-10-8)




**Project Leader:**

concentrations In the Netherlands, 80% of one-year-old children exceeded the provisional maximum tolerable daily intake (PMTDI), and 20% of them had twice the value. Porridges were a significant source of DON intake in these children. A PMTDI of

# Risk communication is very critical

RIVM report 388802 022

**Risk Assessment of Deoxynivalenol in Food**
An assessment of exposure and effects in the Netherlands

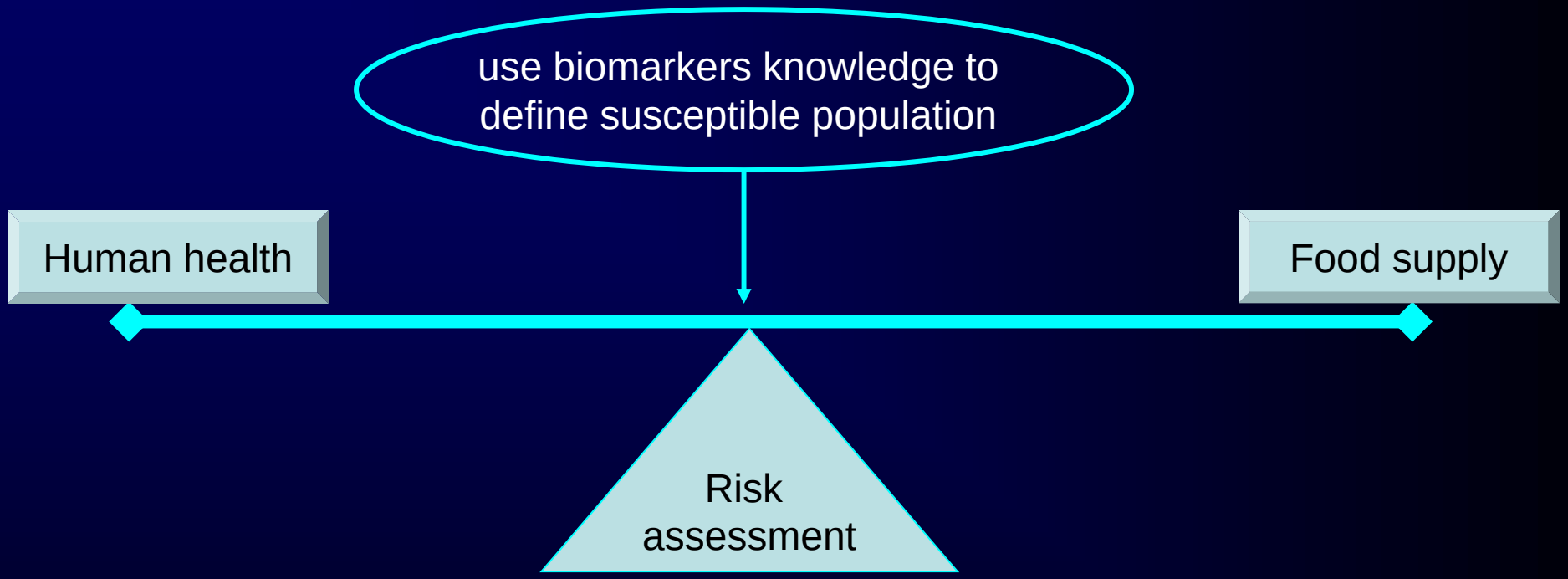
M.N. Pieters, J. Freijer, A.J. Baars, W. Slob

March 2001

**CONCLUSIONS**

- The dietary intake of DON in the Netherlands (September 1998 – January 2000) exceeded the provisional TDI of 1.1 µg/kg bw, especially in children. Eighty percent of the one-year-olds had a DON intake above the provisional TDI and 20% of these children exceeded twice the provisional TDI.
- Monitoring data show that the average DON concentration in wheat was 446 µg/kg ($n$ = 219, sampling period September 1998-January 2000). The major source of DON intake is bread. For one-year-olds porridges also contribute significantly. The DON-intake through other grains is negligible in the Netherlands since the consumption of these grains is low.
- At these exposure levels of DON health effects might occur in children. Considering the 95$^{th}$ percentile of DON intake of one-year-olds, the suppressive effects on body weights (growth retardation) and relative liver weight are estimated at 2.2 and 2.7%. However, the large confidence intervals around these estimates indicate that the magnitudes of these effects are uncertain. Whether the estimated effect levels are considered (un)acceptable, is a matter of debate and forms part of the risk management process. The probabilistic effect assessment is based on extrapolation of observations in animal experiments to humans. At present, there is no evidence (yet) that the estimated effects will occur in the human population.

# Risk assessment of DON




*Biomarkers are parameters of injury or toxicity in animals or patients that help diagnose or monitor a disease process, predict outcome or evaluate therapeutic intervention (Lock and Bonventre 2008)*

# Risk assessment paradigms

## Traditional

Mice exposed to DON

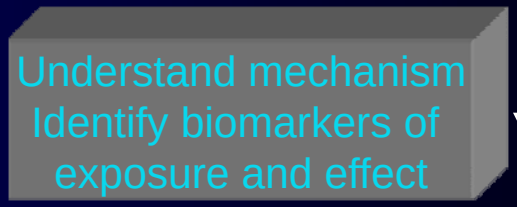

Critical effect and dose determined

Critical dose divided by safety factor (100)

Exposure assessment

## Mechanism-based

Mice exposed to DON




Critical effect and dose determined

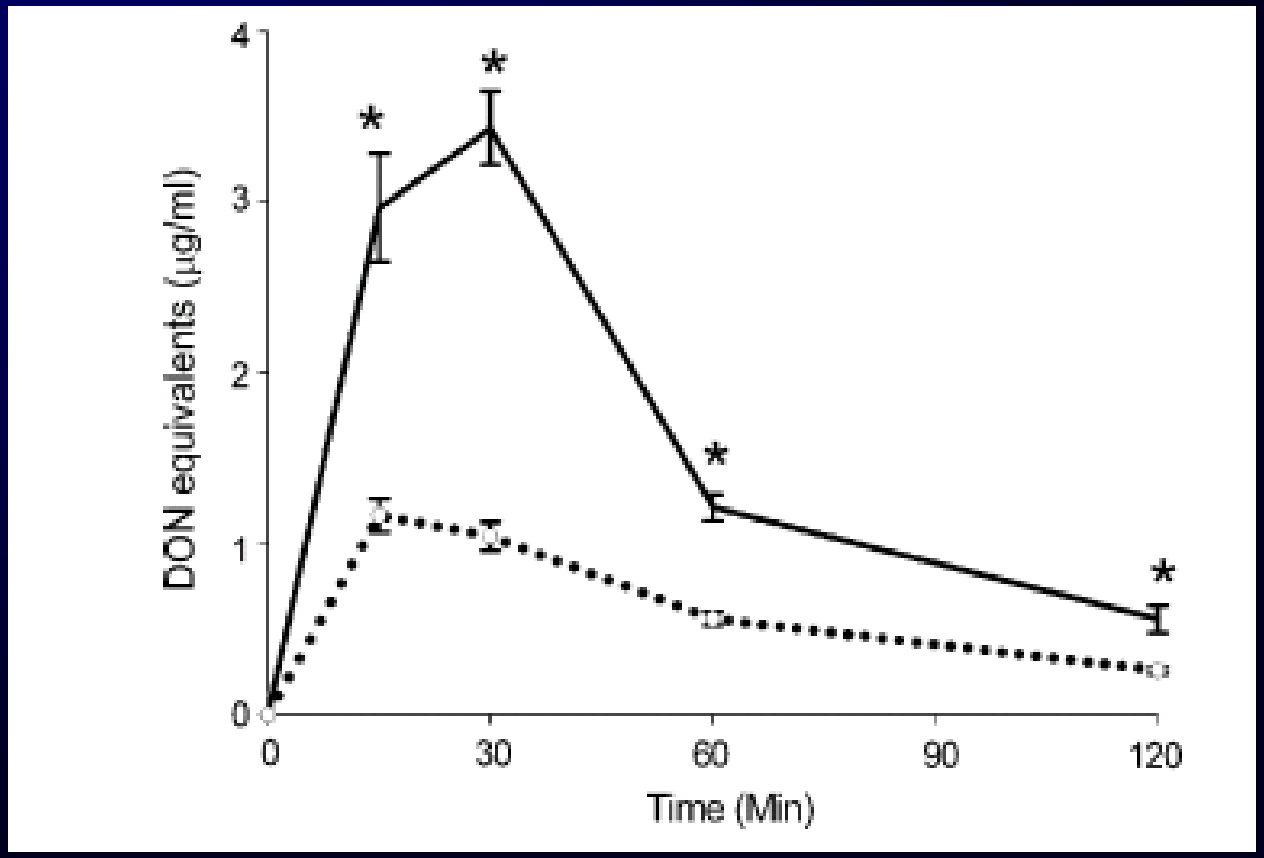
Critical dose divided by chemical-specific safety factor

Exposure assessment linked with biomarkers of effect

Does critical effect occur in humans?
If so, at what dose?

Regulatory limit

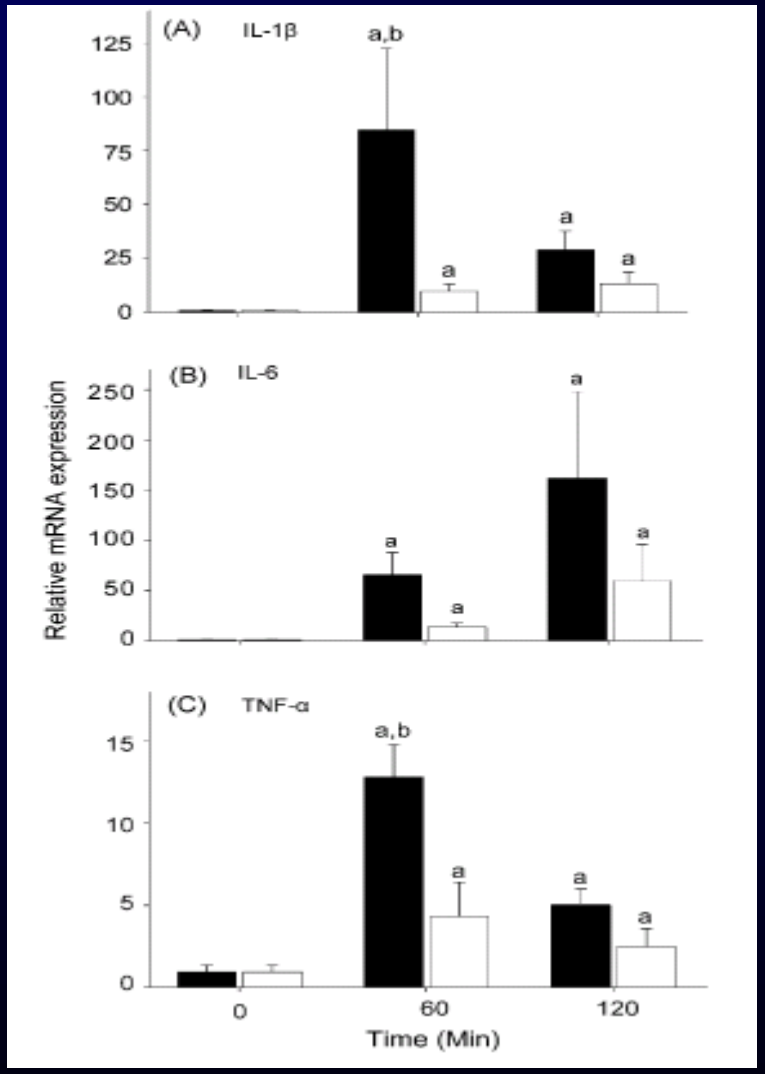
# Plasma DON as biomarker of DON exposure






Amuzie, C.J *et al* 2008, Toxicology 248, 39-44

# Cytokines as transient biomarkers



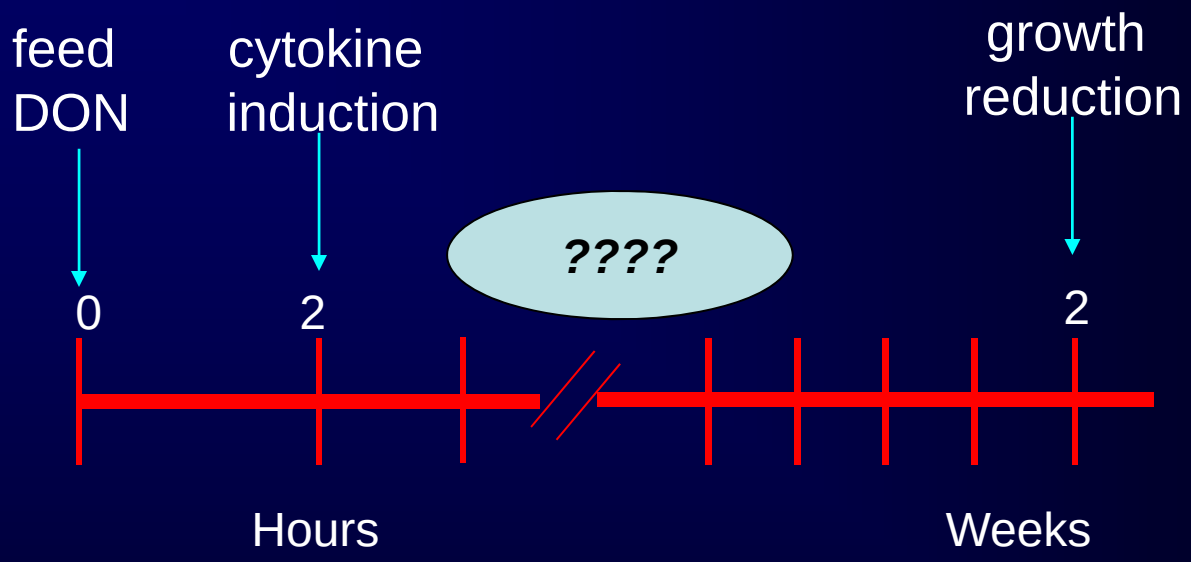



Amuzie, C.J *et al* 2008, Toxicology **248,** 39-44

# Conclusion from cytokine studies

- Regardless of exposure route, DON
  - is distributed to many tissues
  - induces proinflammatory cytokines in many tissues
- Nasal exposure results in greater cytokine upregulation and tissue DON concentration
- Proinflammatory cytokine upregulation is a transient marker of DONs effect

# Identification of biomarker of DON's effect




Goals

Identify potential markers of effect

Understand mechanism of action

Integrate acute and subchronic observations

# Growth hormone resistance

- Reduced growth rate  despite growth hormone sufficiency
  - congenital (gene mutation)
  - sepsis (endotoxin)
- Foodborne mycotoxins  impair growth
  - Aflatoxins associated with impaired growth in children
  - Deoxynivalenol  impairs growth in several species
    - Swine are very sensitive

# Review of growth hormone signaling

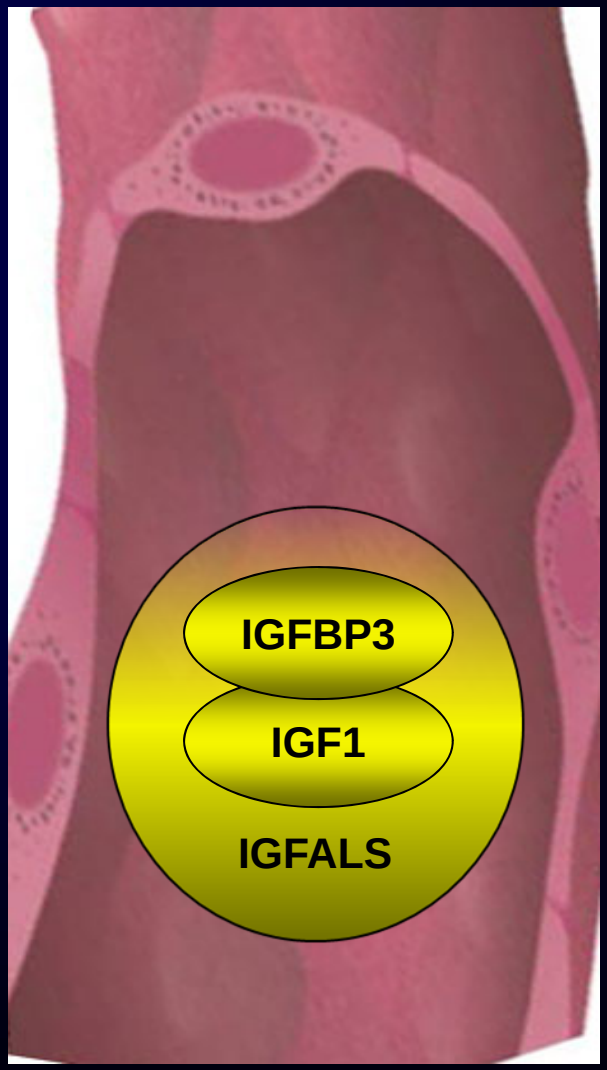
Anterior pituitary

↓

Liver and other targets

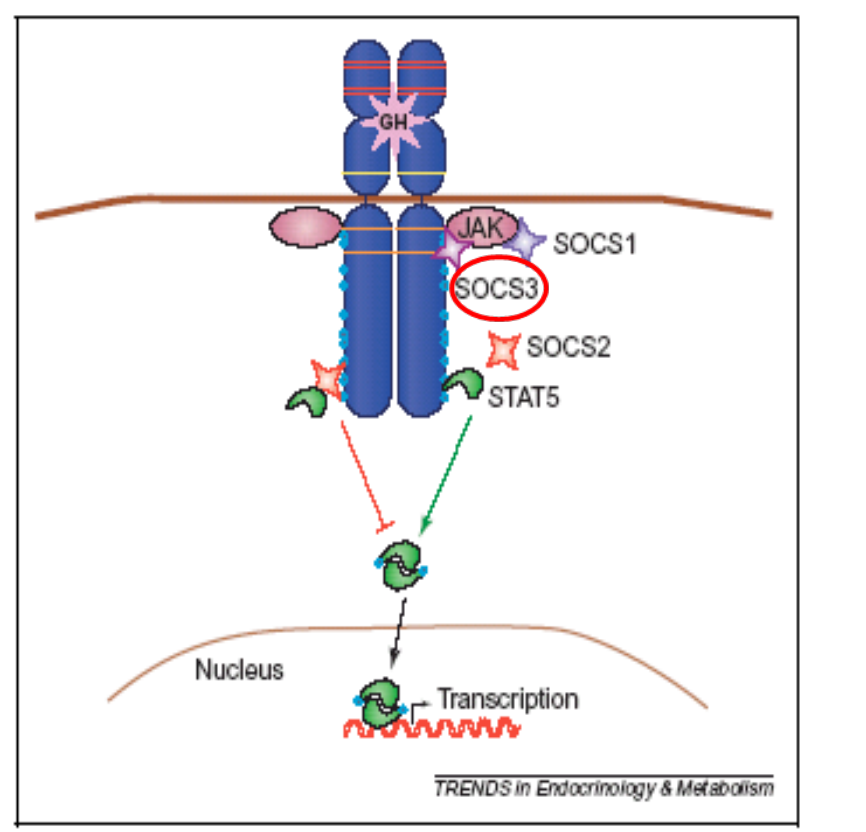
↓

Insulin-like growth factor (IGF1) and binding partners



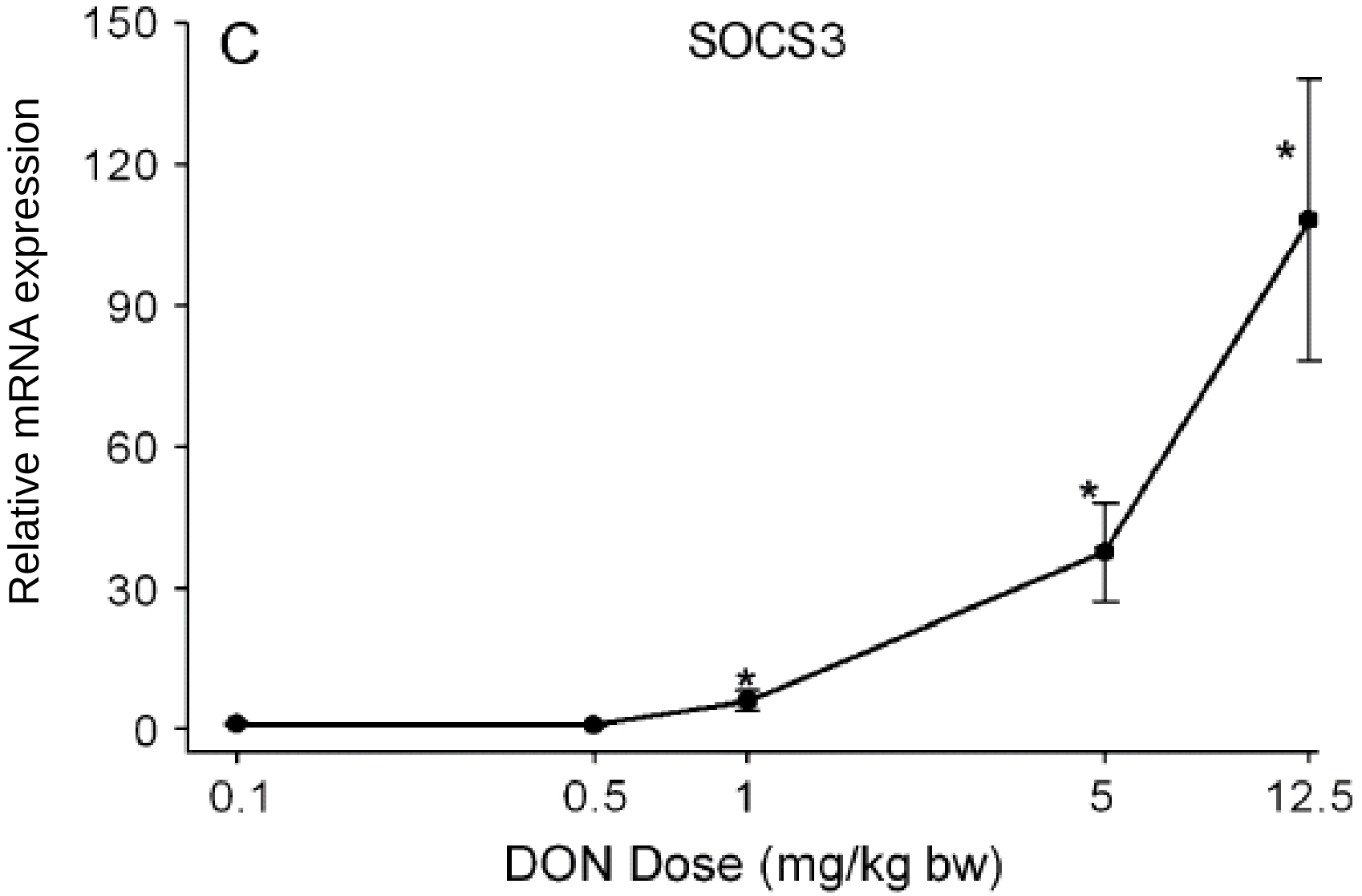
# Suppressors of cytokine signaling (SOCS)

- Cytokine-inducible suppressors of signaling
  - inhibit GH-induced STAT phosphorylation
  - impair GH-induced gene expression



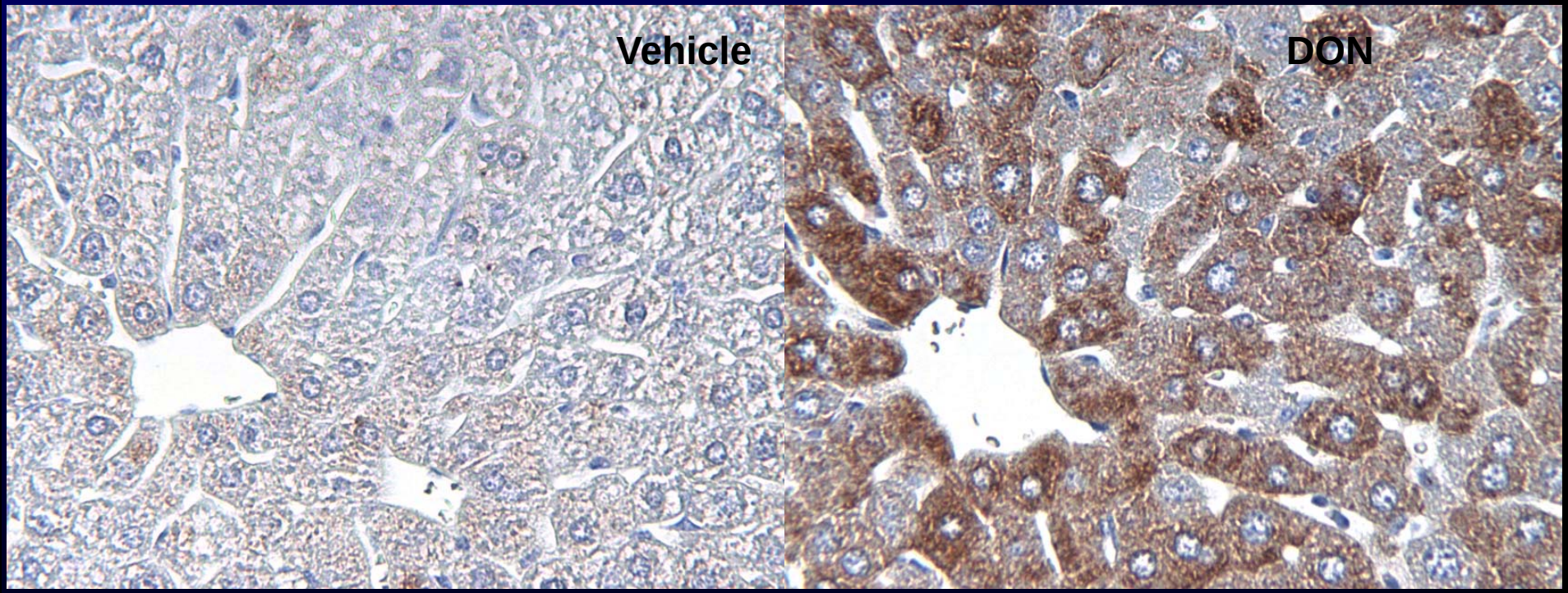

Turnley AM, 2005. Trends in Endo. and Metabolism 16 (2): 53-58

# DON induces cytokine suppressors of cytokine signaling 3 in hepatocytes

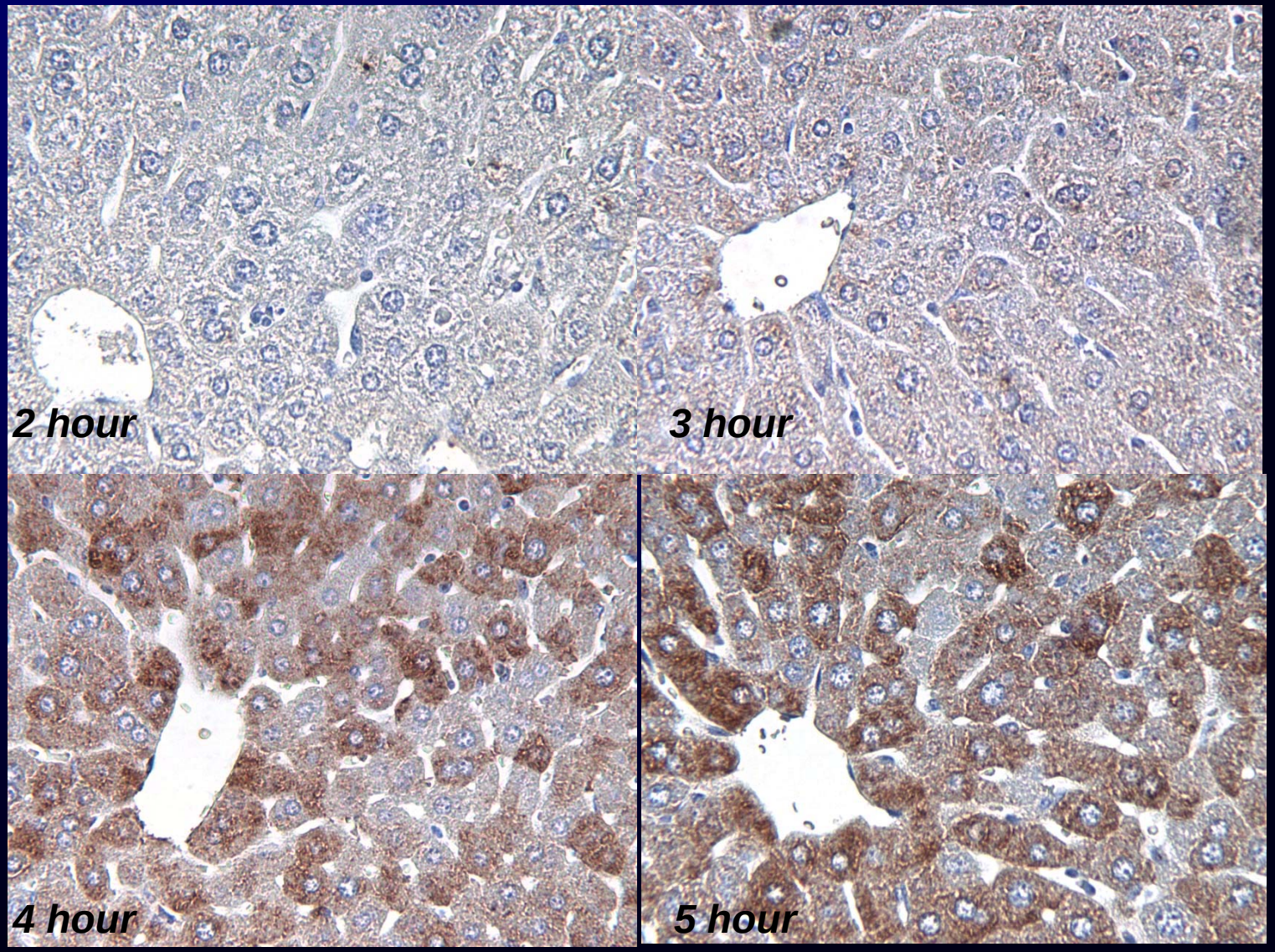



Amuzie, C.J., et al 2009, Toxicol Sci. *Oct;111(2):277-87*

# DON induces cytokine suppressors of cytokine signaling 3 in hepatocytes

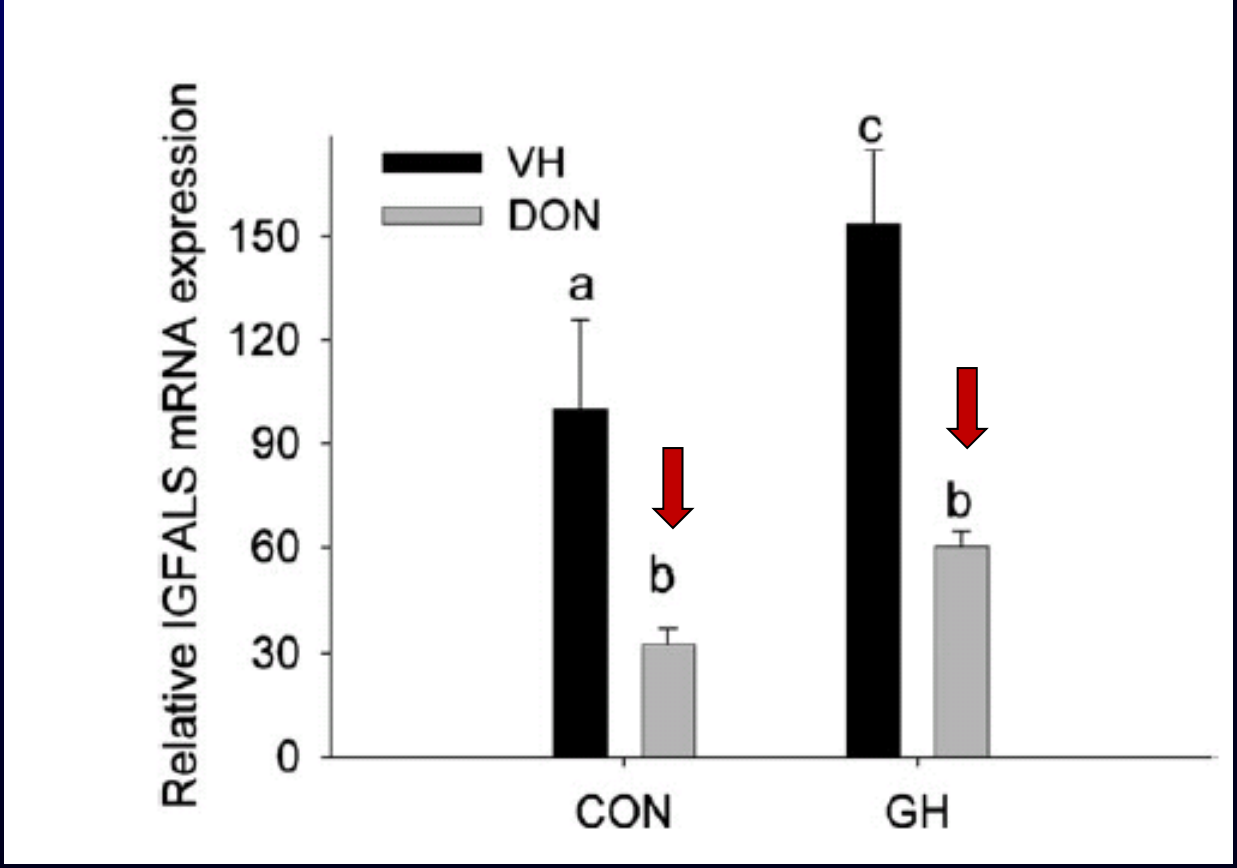


# Time course of SOCS3 induction in liver




DON 12.5 mg/kg bw oral gavage
staining with anti-SOCS3 antibody

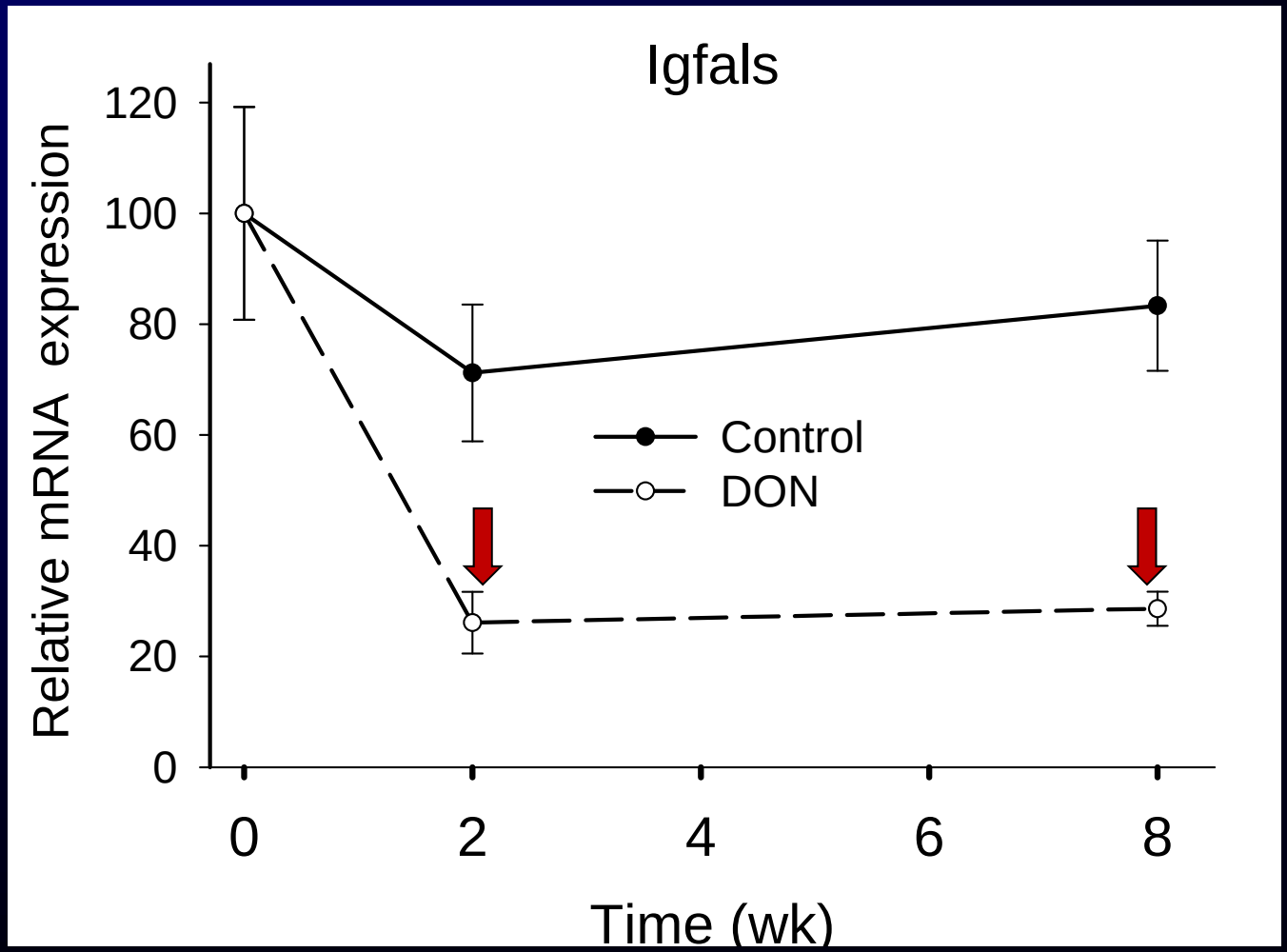
# DON suppresses IGFALS



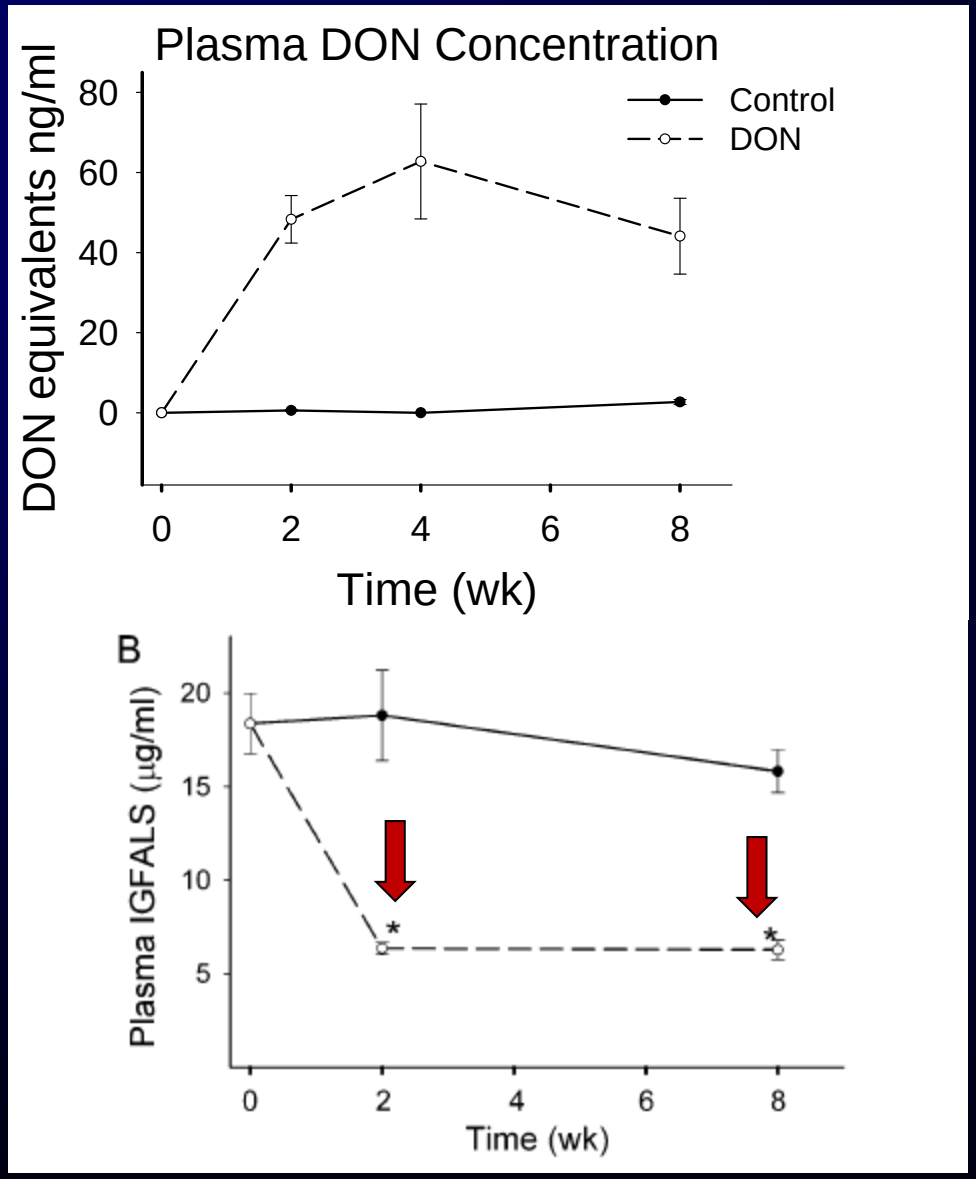

Amuzie, C.J., et al 2009, Toxicol Sci. 2009 Oct 4 (epub)

IGFALS=Insulin-like growth factor acid labile subunit

# Dietary DON suppresses IGFALS mRNA

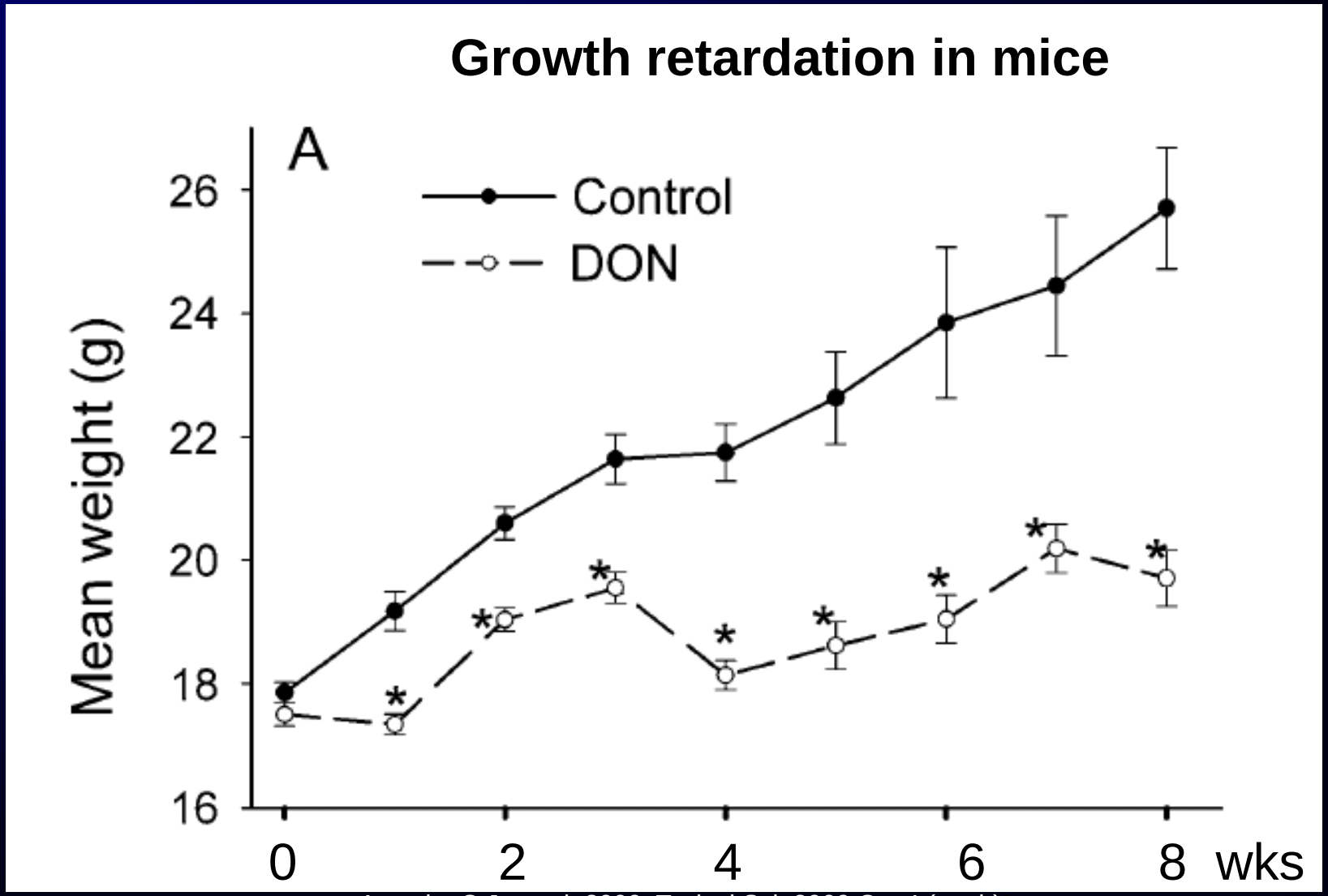


# DON suppresses circulating IGF1 and IGFALS



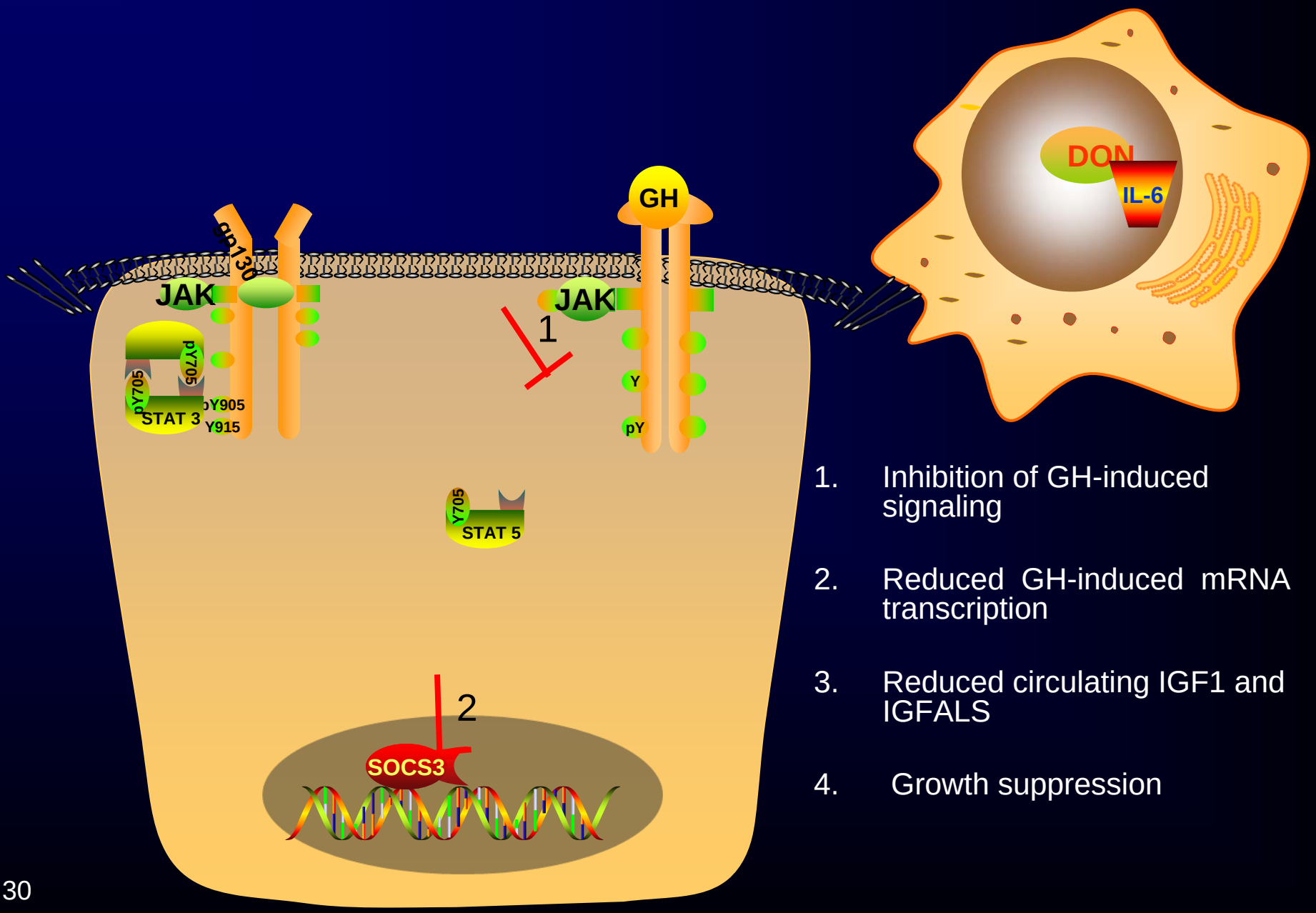

Amuzie, C.J., et al 2009, Toxicol Sci. 2009 Oct 4 (epub)

# DON suppresses growth in mice




Amuzie, C.J., et al 2009, Toxicol Sci. 2009 Oct 4 (epub)

# Summary of DON's biomarker of effect pathway

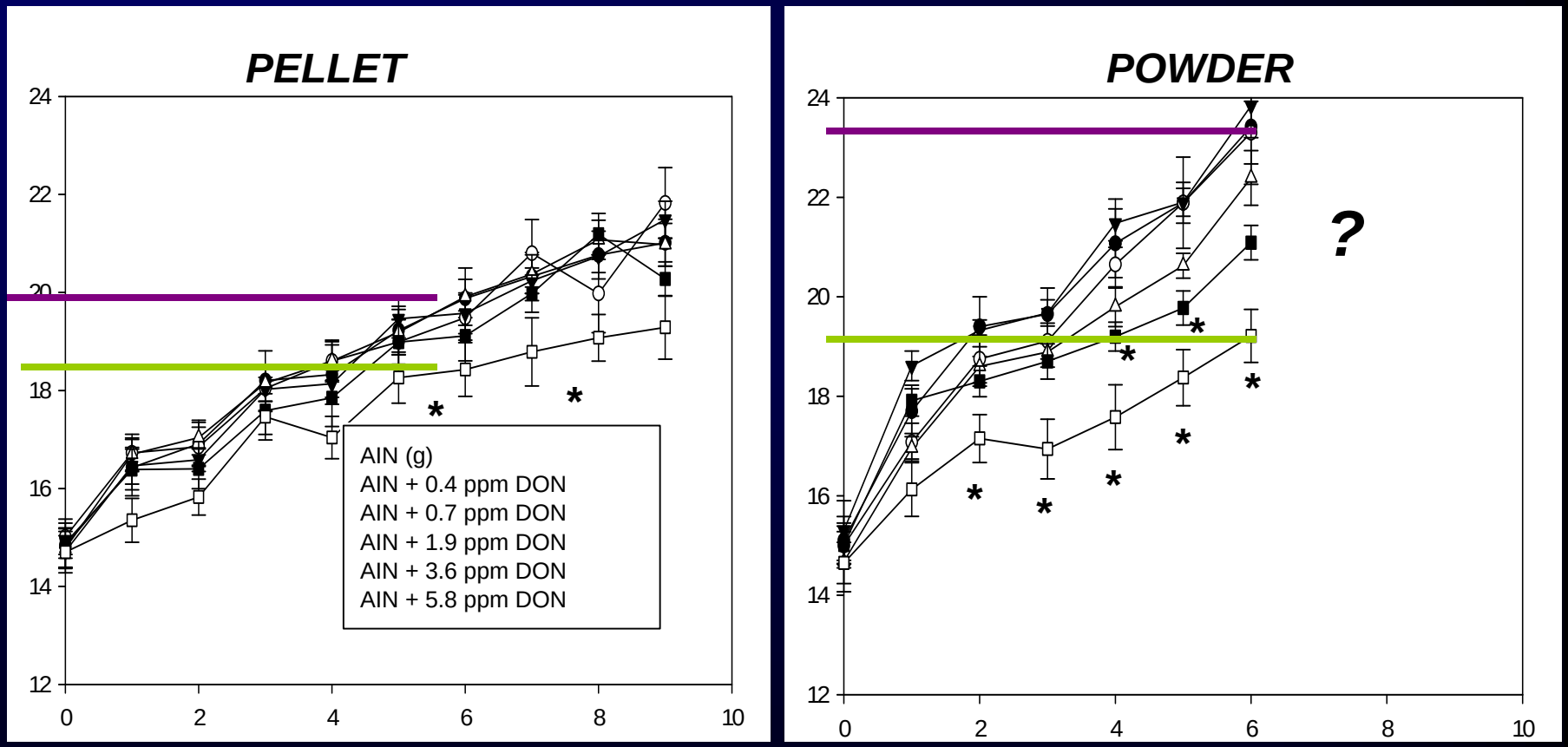



1. Inhibition of GH-induced signaling
2. Reduced GH-induced mRNA transcription
3. Reduced circulating IGF1 and IGFALS
4. Growth suppression

# Potential application of DON biomarker

- Measure IGFALS and IGF1 in high risk DON exposure groups
- Correlate with plasma/urinary DON levels
- Correlation (yes/no) will inform regulatory policy

# Diet forms affect NOAEL and LOAEL




Preliminary data from Flannery

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