



Assessment of urinary deoxynivalenol as an exposure biomarker

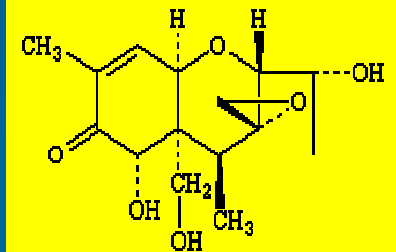
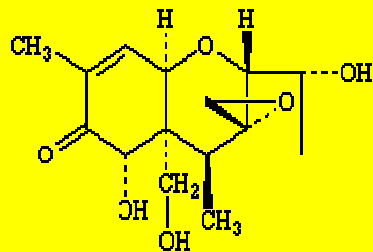
Funded by UK Food Standards Agency

Paul C. Turner

**Kay White, Victoria Burley, Richard Hopton, Anita Rajendram
Julie Fisher, Janet Cade, Christopher Wild**

Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics,
Leeds Institute of Genetics Health and Therapeutics,

Faculty of Medicine and Health, University of Leeds, UK



Human – acute

China (Luo 1994)

Nausea, vomiting, abdominal pain, diarrhea, headache, dizziness, fever

1961-1981 scabby/moldy cereal

1984-1991 DON in food

Anhui 130,141 people affected.

India tens of thousands of individuals (Bhat et al., 1989)

USA – poisoning incidences that may be related to DON (CDC)

Human – chronic (Pestka and Smolinski 2005)

Unknown – - gastroenteritis, growth faltering and immune toxicity???

Fusarium mycotoxins possibly linked to oesophageal cancer?

The problem

The lack of a validated exposure biomarker hampers exposure assessment at the individual level.

The development and validation of an exposure biomarker should better inform epidemiological studies of possible health effects

Response

Can we develop a useful biological measure of exposure

PhD studentship funded by Egyptian Government – Dr F. Meko

Development of a urinary biomarker of human exposure to deoxynivalenol

F.A. Meko^a, P.C. Turner^a, A.E. Ashcroft^b, J.D. Miller^c, Y.-L. Qiao^d, M.J. Roth^e,
C.P. Wild^{a,*}

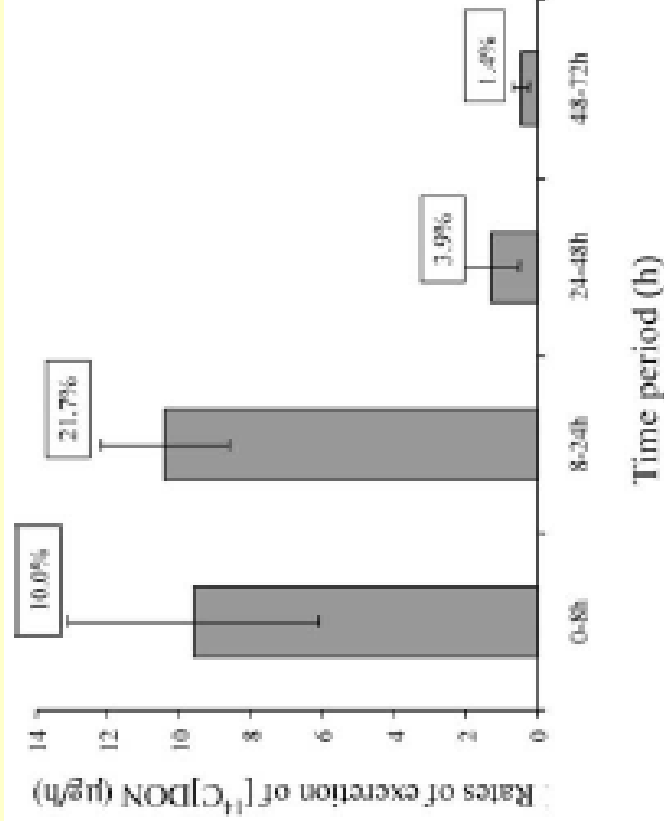


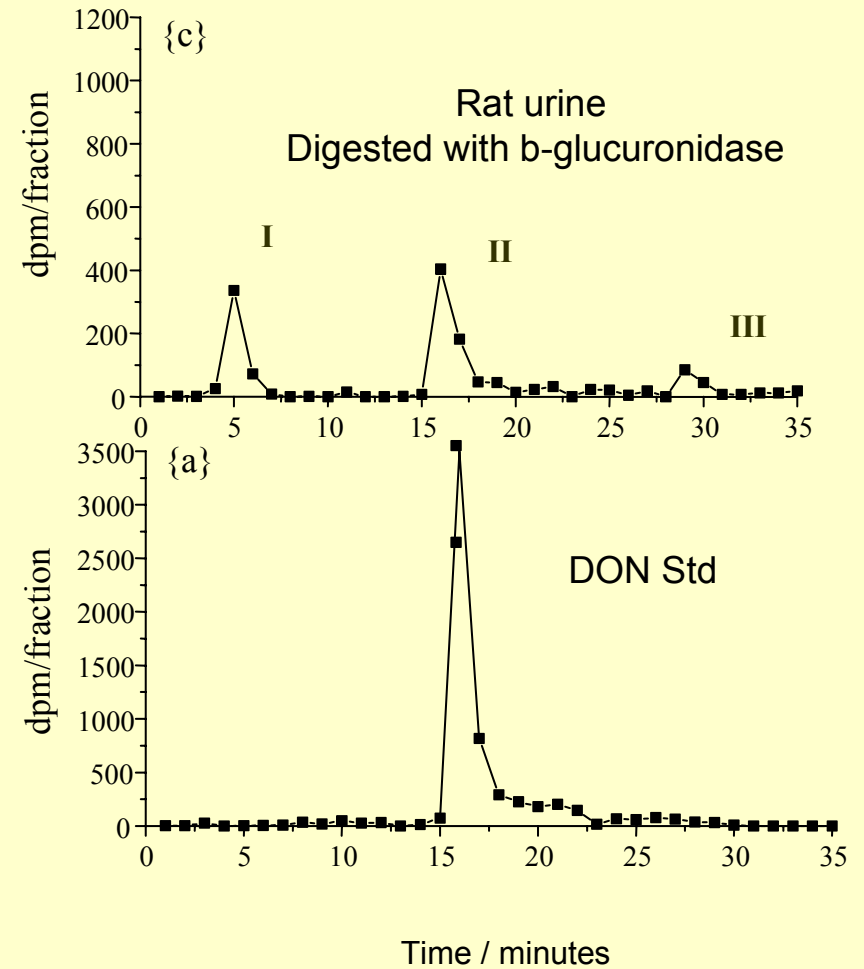
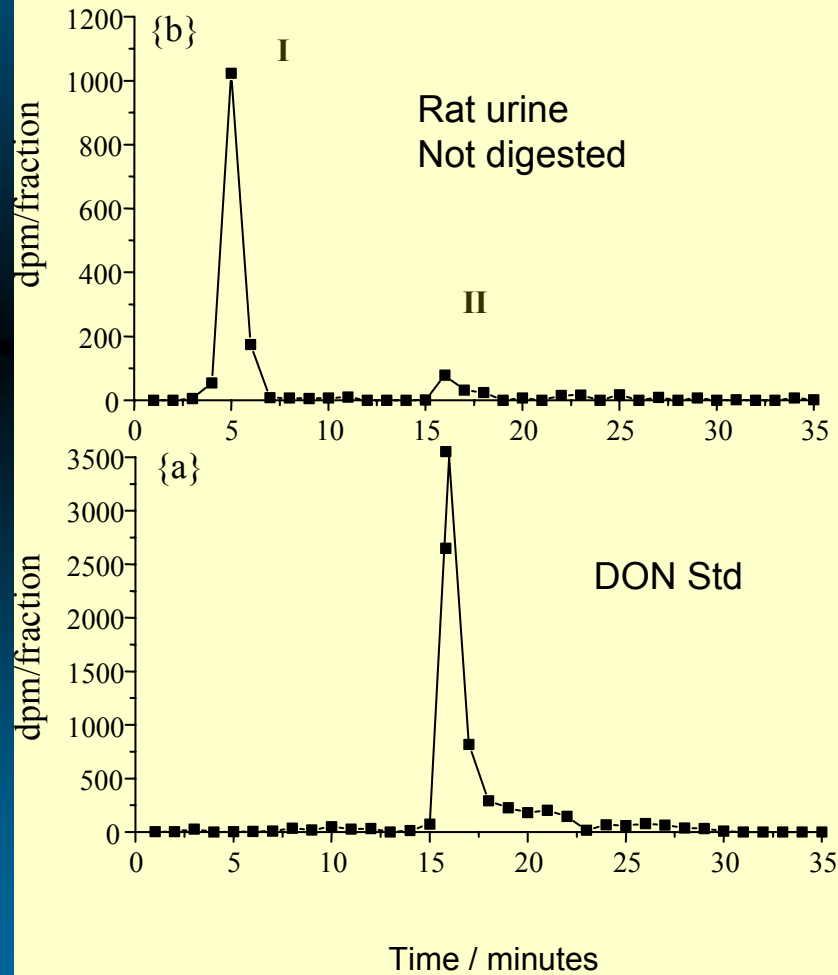
Fig. 1. Excretion profile of radioactivity in the urine of Sprague-Dawley rats following a single oral dose of [¹⁴C]DON (5 mg/kg; 5.5 µCi/kg). Data points from 0–8, 8–24, 24–48, 48–72 h are the mean ± S.D. of the DON excreted per hour. The percentage of the administered dose excreted over each time period is given in boxes for each period.

Rat study HPLC radio-chromatograms of urine

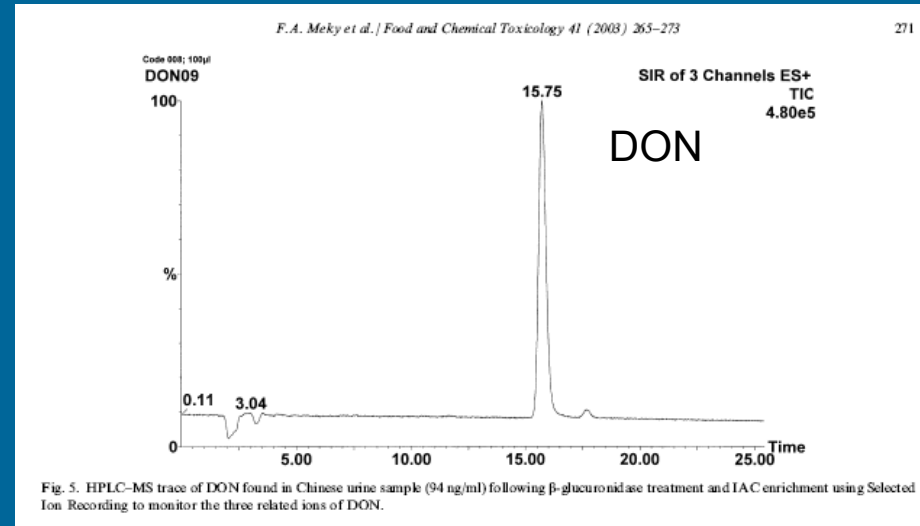
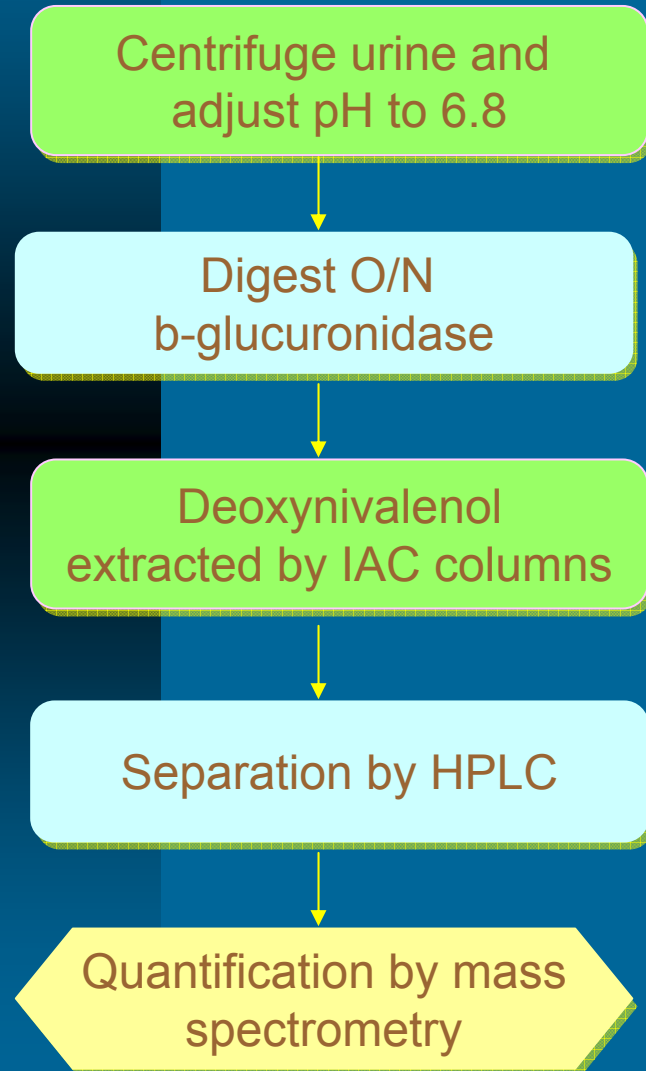
Meky et al., 2003

Rat dosed with 5mg/kg (bw) ¹⁴C-DON

- I - DON-polar metabolites
- II - DON
- III - DOM-1



Extraction and LC-MS analysis of DON Pilot study in Chinese females



Fifteen Chinese females are resident in Linxian Co., P.R.China

- * 11 from a high-risk region (dietary staple corn and wheat).
- * 4 from a low risk region (dietary staple rice).

Urinary deoxynivalenol from 15 Chinese females

Meky et al., 2003

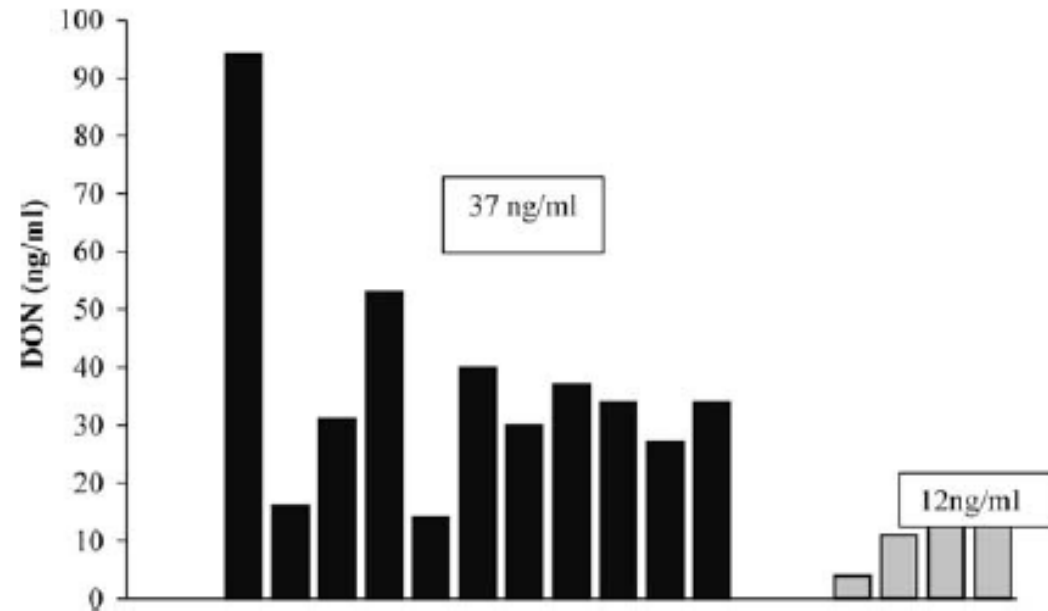


Fig. 6. The concentration of DON ng/ml urine detected by HPLC-MS from 15 Chinese; 11 from a high risk (black bars) and four from a low risk area (shaded bars), following β -glucuronidase treatment. Mean levels of DON for the two groups are given in the boxes. DON was extracted using IAC and injected onto HPLC-MS with SIR monitoring.

What is the frequency and level of urinary DON in the UK

UK FSA funded – Wild, Turner, Cade

UK adults - National Diet and Nutrition Survey (NDNS)

An assessment of the prevalence of exposure to DON in the UK using biomarkers.

- A stratified analysis of urinary DON by dietary intake of cereals in the UK population.
- The adult NDNS provides access to 24-hr urine samples from ~ 1500 individuals across the UK aged 19-64 years.
- For each individual there is a dietary interview on usual food consumption patterns and a detailed 7-day weighed dietary record.
 - ◆ low cereal intake, 2nd / 3rd deciles
 - ◆ medium cereal intake, 5th / 6th deciles
 - ◆ high cereal intake, 9th deciles.
- In each of these three groups we will then randomly select 50 males and 50 females to provide a total of 100 subjects per group.

Extraction and LC-MS analysis of DON

IS – ^{13}C -DON

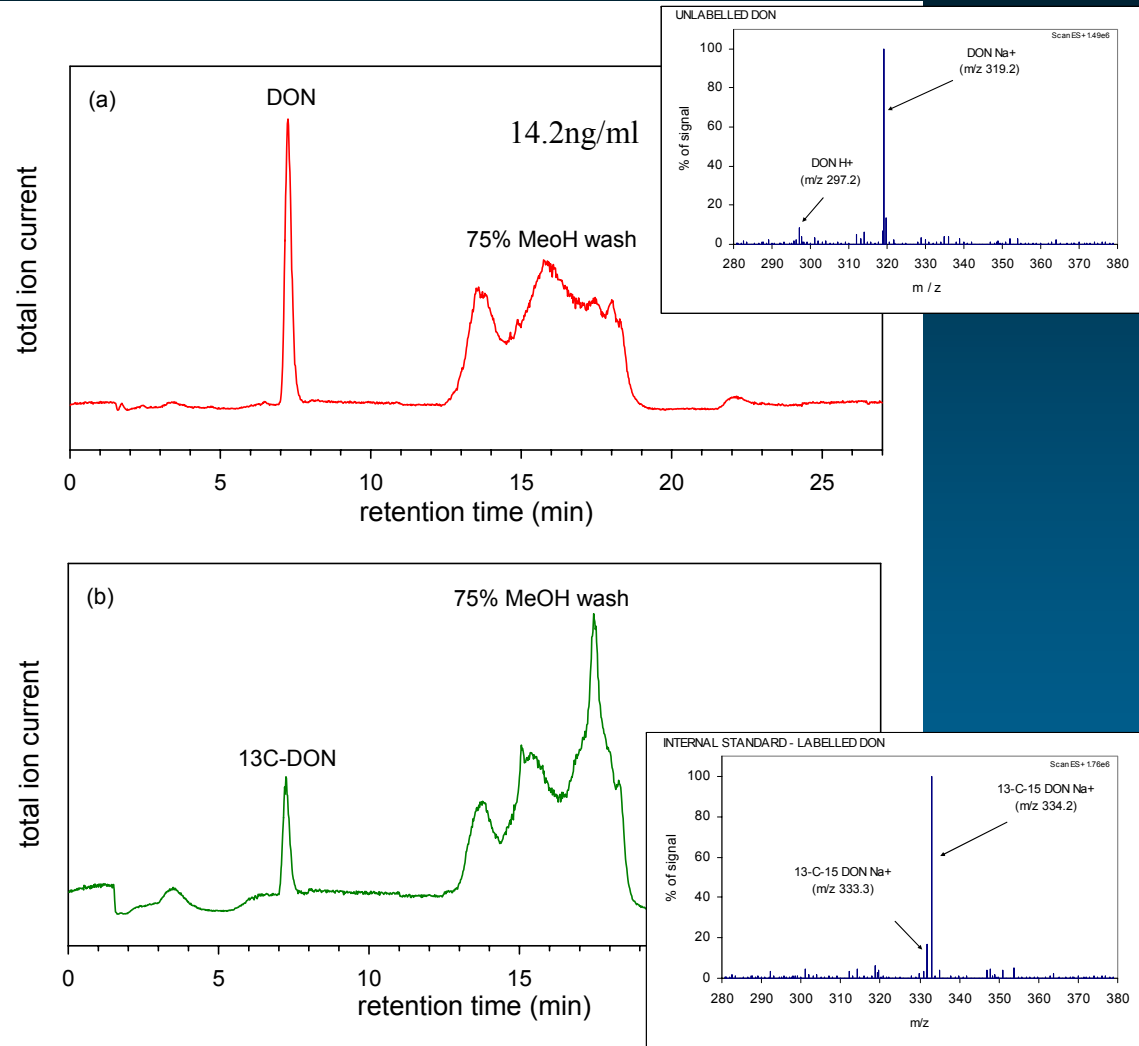
Centrifuge urine and
adjust pH to 6.8

Digest O/N
 β -glucuronidase

Deoxynivalenol
extracted by IAC columns

Separation by HPLC

Quantification by mass
spectrometry



Urinary DON by cereal intake group

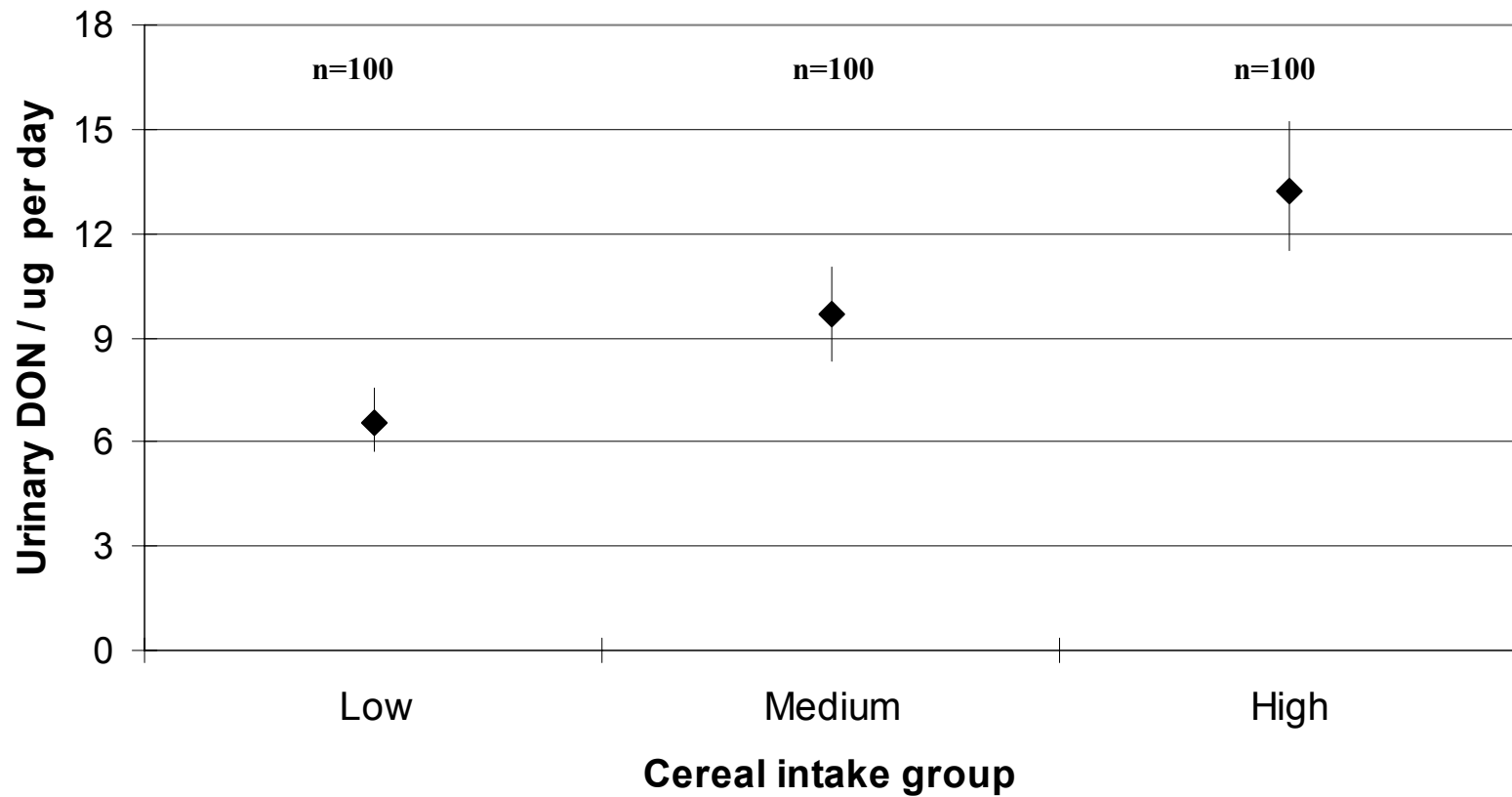
Geometric mean and 95% confidence intervals

DON was detectable in urine samples from the UK

296/300 samples positive

mean 7.5ng/ml; range nd – 56.4ng/ml urine

geometric mean: 9.4ug DON/day; range: non detectable to 66.0ug/day



$P < 0.001$

What would be the effect of a simple wheat restriction dietary intervention on the levels of urinary DON

NIEHS funded – Wild and Turner

Intervention study for DON exposure in the UK

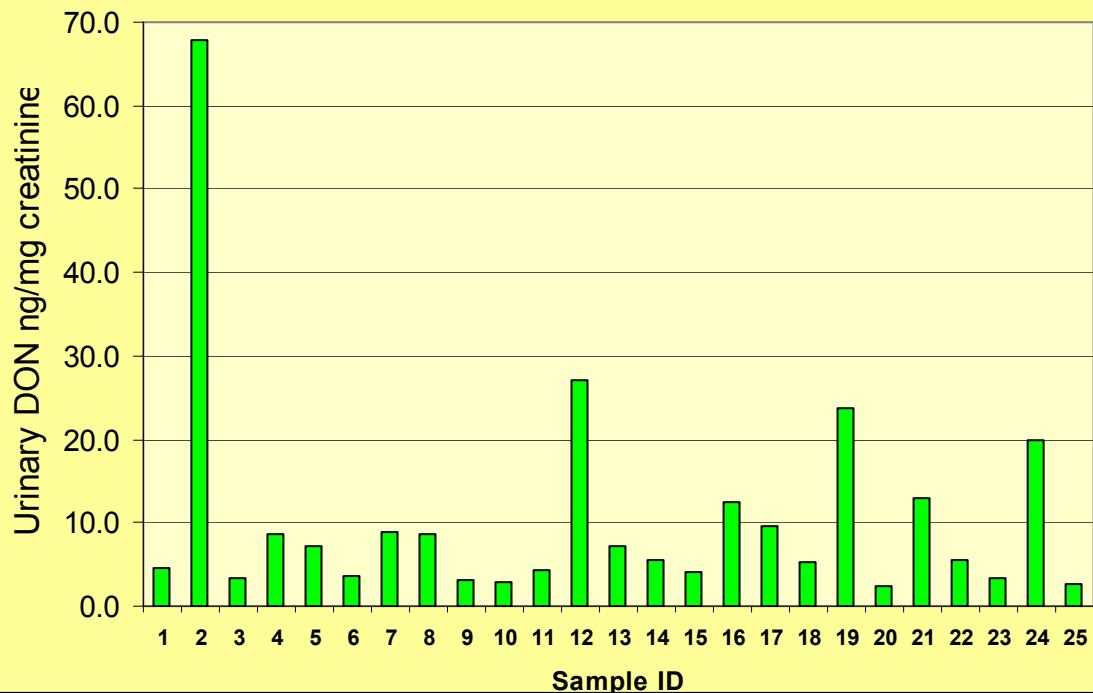
If the major source is from wheat based products, would a dietary intervention to restrict wheat impact on :

- a) the frequency of detection
- b) The levels of detection.

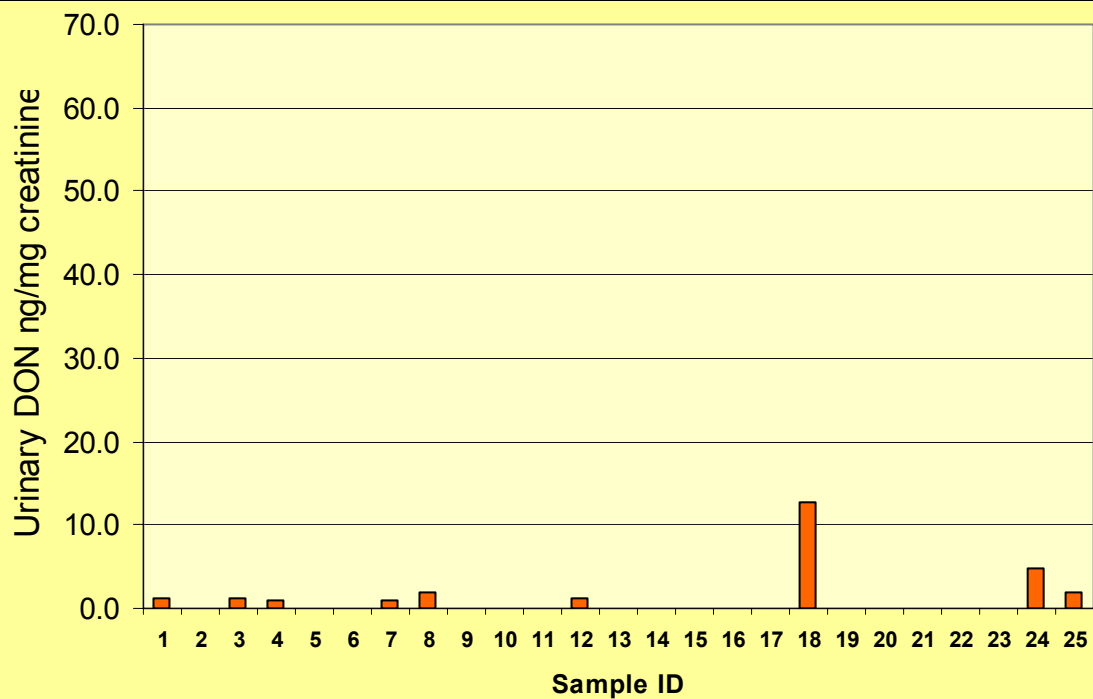
Intervention – restricted bread, wheat based breakfast cereal, pasta, cakes

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
		Pre-Breakfast Urine				Pre-Breakfast Urine
Normal Diet	Normal Diet	Intervention	Intervention	Intervention	Intervention	Normal Diet
Diary	Diary			Diary	Diary	

**NORMAL DIET
ADJUSTED FOR
CREATININE**



**INTERVENTION
ADJUSTED FOR
CREATININE**



Summary Table

Turner et al.,2008 J. Exp.Sci. Environ. Epidemiol.

	n	Normal Diet	Intervention	p-value
Urinary DON ng / mg creatinine	25	7.2 (4.9 – 10.5)	0.6 (0.4 – 0.9)	<0.001
Wheat based food intake Grams / day (range)	25	322 (131 – 542)	26 (0 – 159)	<0.001

- During consumption of the normal diet DON exposure is frequent
- Within four days of restricting wheat in the diet, DON exposure is reduced to <10%

Can we make estimates of intake?

- **300 individuals NDNS**
- 50% transfer of DON to urine ?????
- Individually MEAN INTAKE ESTIMATE - 450ng/kg(bw)/day
- 21 of 300 individuals (7.0%) would be predicted to exceed the TDI using the above calculation
 - ◆ Most in high group
 - ◆ 9th decile

Further validation of urinary DON as an exposure biomarker

FSA funded – Wild, Turner, Cade

Q1. What is the temporal nature of this urinary DON measure

Q2. What are the kinetics of clearance of urinary DON

Q3. What is the relationship between total DON (DON–glucuronide plus free DON) and free DON in urine.

Q4. Is DON intake correlated with urinary DON

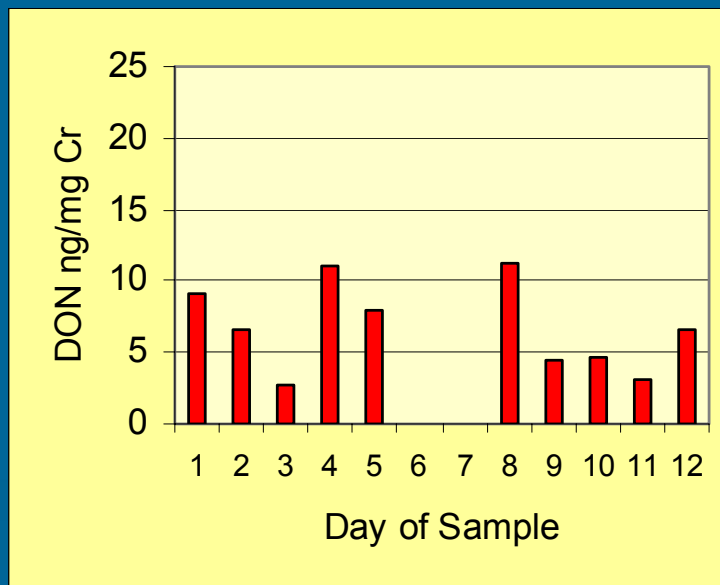
Q5. Can we estimate DON intake based on the biomarker

Study Design

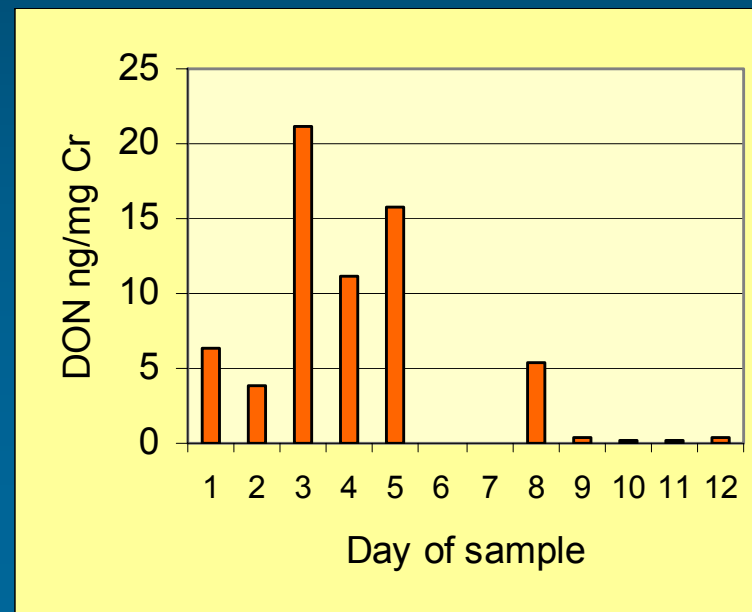
		W E E K O N E							W E E K T W O						
		Sun	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon	Tue	Wed	Thur	Fri	Sat
GROUP 1	Diet	N O R M A L							P A R T I A L						
		D I E T							I N T E R V E N T I O N						
	Diary	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	Urine	X	√	√	√	√	√	X	X	√	√	√	√	√	√
	Food	X	X	X	X	X	X	X	X	√	√	√	√	√	√
GROUP 2	Diet	N O R M A L							F U L L						
		D I E T							I N T E R V E N T I O N						
	Diary	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	Urine	X	√	√	√	√	√	X	X	√	√	√	√	√	√
	Food	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Sun	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon	Tue	Wed	Thur	Fri	Sat
		W E E K O N E							W E E K T W O						

Study Design

		W E E K O N E							W E E K T W O						
		Sun	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon	Tue	Wed	Thur	Fri	Sat
GROUP 1	Diet	N O R M A L							P A R T I A L						
		D I E T							I N T E R V E N T I O N						
	Diary	√	√	√	√	√	√	√	√	√	√	√	√	√	√
	Urine	X	√	√	√	√	√	X	X	√	√	√	√	√	√
	Food	X	X	X	X	X	X	X	X	√	√	√	√	√	√



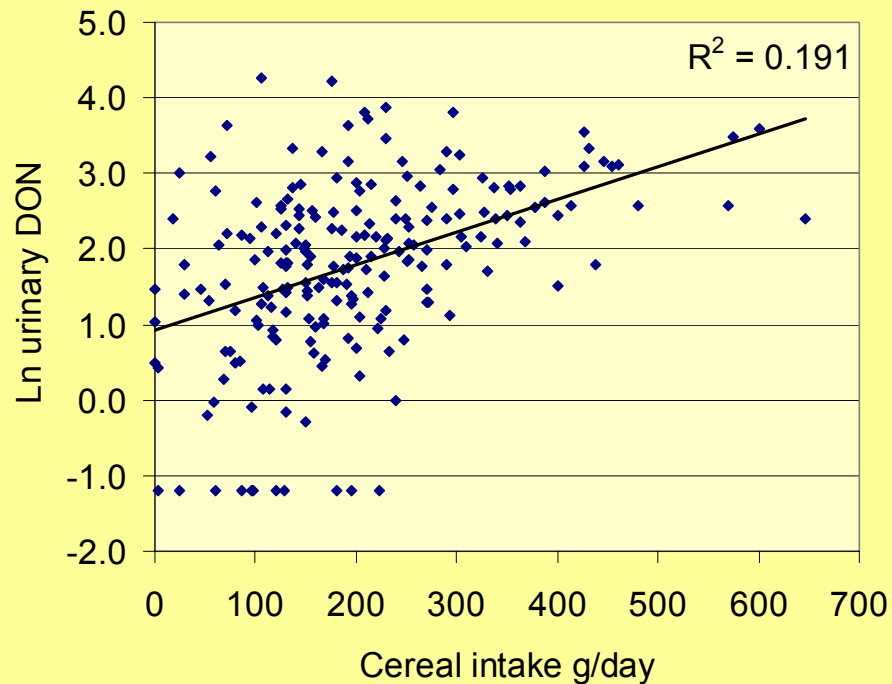
GROUP 2	Diet	N O R M A L							F U L L																		
		D I E T							I N T E R V E N T I O N																		
	Diary	√	√	√	√	√	√	√	√	√	√	√															
	Urine	X	√	√	√	√	√	X	X	√	√	√	√	√													
	Food	X	X	X	X	X	X	X	X	X	X	X	X														
		Sun	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mon	Tue	Wed	Thur	Fri	Sat												
		W E E K							O N E							W E E K						T W O					



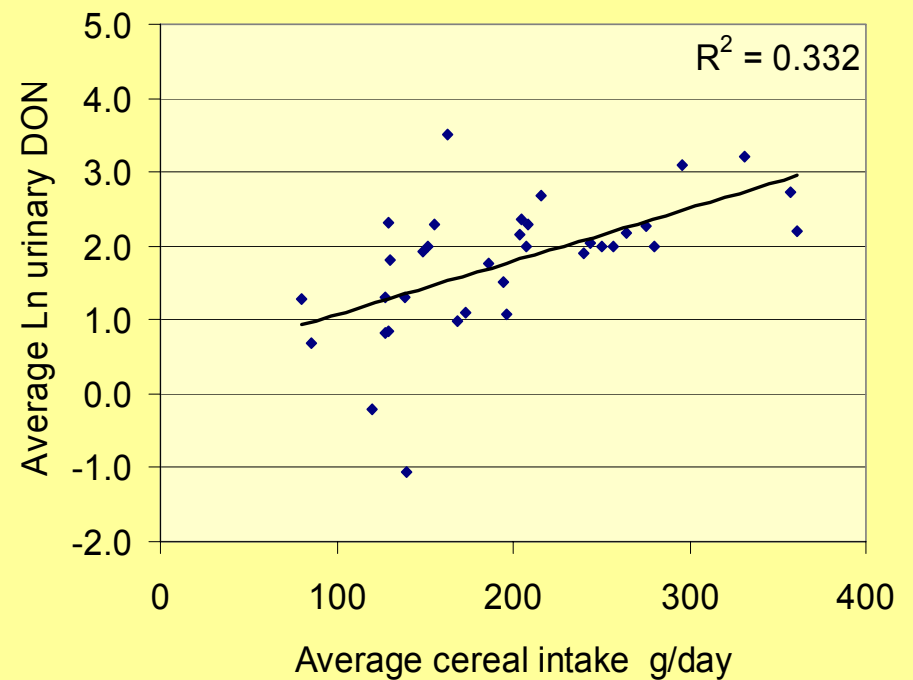
Summary of urinary DON data

Urinary DON ng / mg creatinine				
	Normal	Intervention	% of normal	p-value
Partial	9.9 (nd -70.7)	5.9 (nd - 28.4)	59.6	<0.001
Full	10.6 (nd - 44.9)	0.7 (nd - 3.2)	6.6	<0.001

Scatter-plot of cereal intake versus urinary DON – normal diet ($p < 0.001$)



All data points



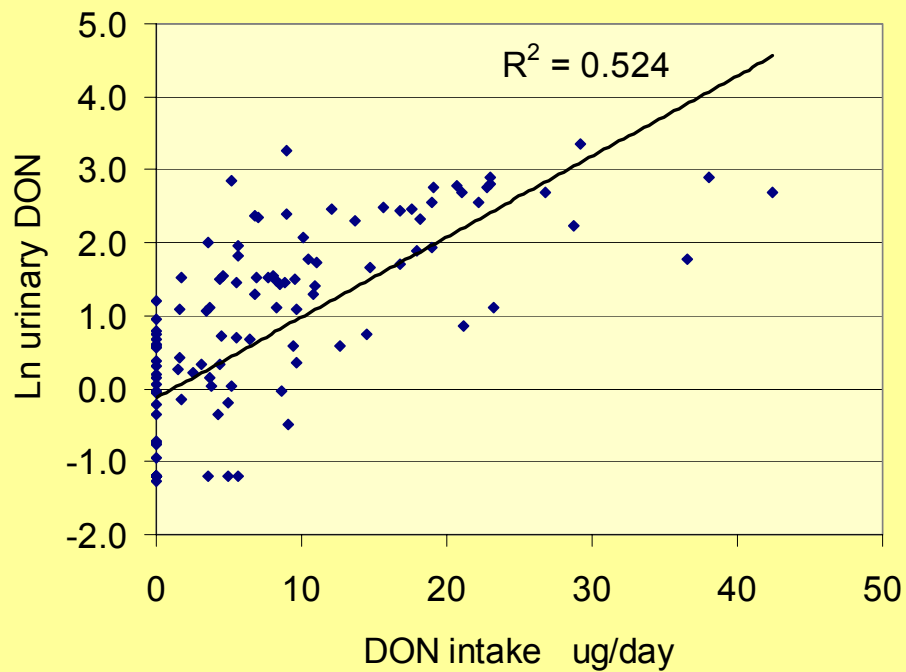
Average by person

Analysis of bread – collected from intervention

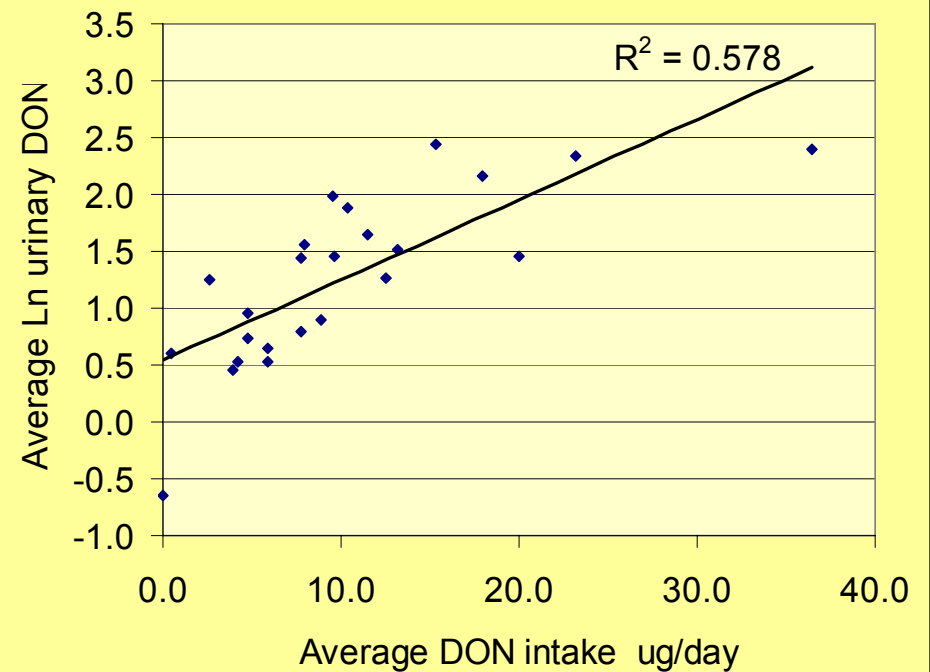
RHM Technology, Premier Foods, UK
Claire Hazel, Sue Patel

- 118 bread samples, over four days from 25 individuals
- 82 distinct samples identified.
- DON was detected in all 82/82 (100%) of samples
- Mean 74µg/kg; range 20 – 316µg/kg. LOD 5µg/kg
- Accurate measure of intake based on bread consumption.
- No individuals exceeded the TDI during the bread only intake phase
 - ◆ Maximum intake was 582ng/kg bw/day

Scatter-plot of DON intake versus urinary DON – intervention ($p < 0.001$)



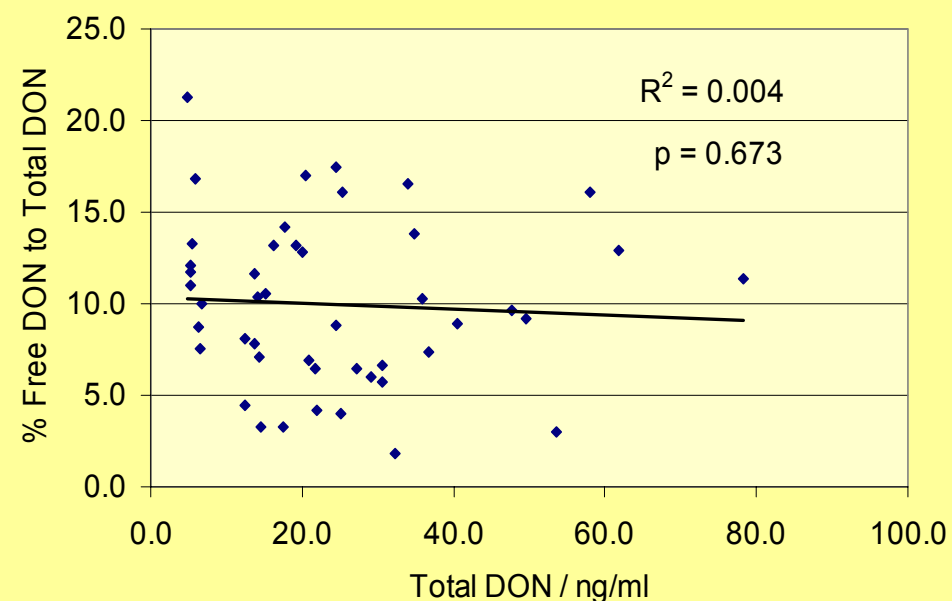
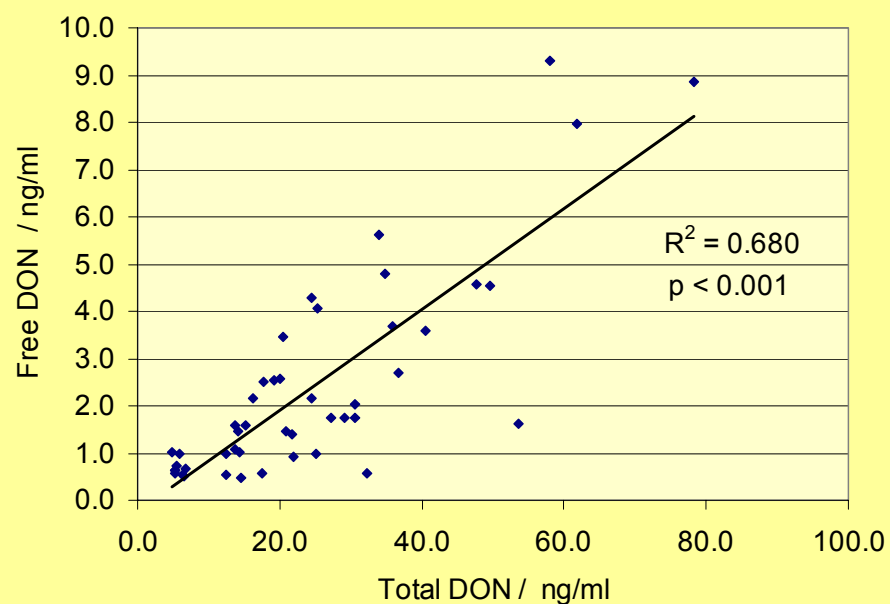
All data points



Average by person

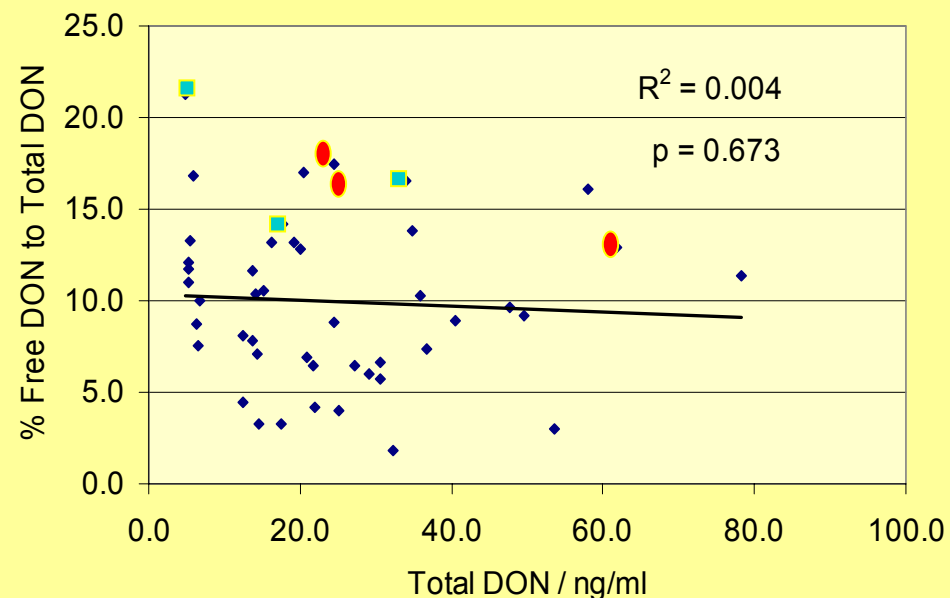
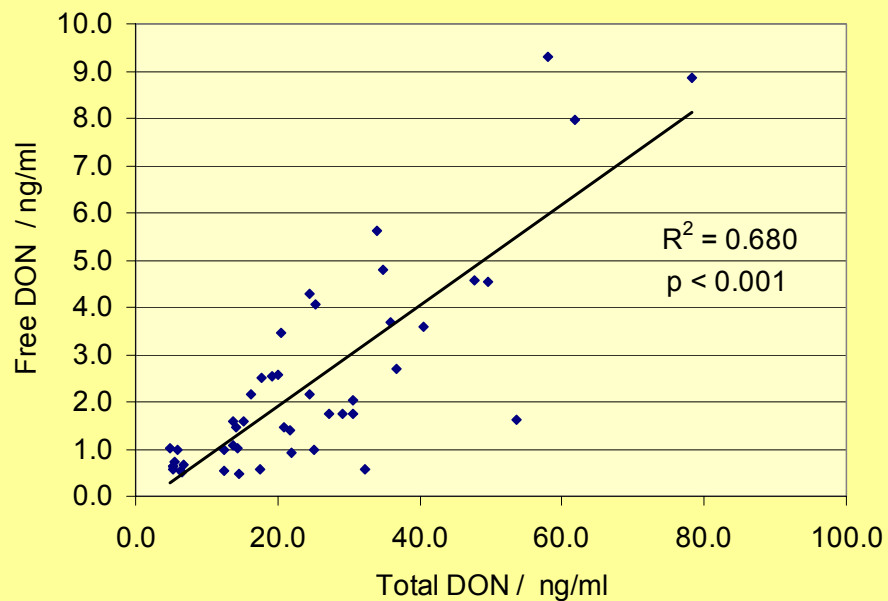
Free DON and DON-glucuronide

- Urine contains free DON and DON-glucuronide
- The proportion of free may be a phenotypic measure of susceptibility to toxicity
- 100 urines (total DON >5ng/ml) were used to assess the % of free DON



DON and DON-glucuronide

- Urine contains free DON and DON-glucuronide
- The proportion of free DON may be a phenotypic measure of susceptibility
- 100 urines (total DON >5ng/ml) were used to assess the % of free DON



DON intake estimates – normal diet?

- The current recommended TDI DON is 1000ng/kg bw/day).
- No individuals exceeded the TDI during the bread only intake phase
 - ◆ Maximum intake was 582ng/kg bw day
- The average DON intake compared with average urinary DON.
- An estimated transfer to urine of **64.3% (95% CI: 48.7, 79.9%)**.
- Based on this transfer: DON intake was estimated for the normal phase.
- Mean intake was **264ng/kg bw/day (95%CI: 213, 349ng/kg bw/day)**
- **Range 11 – 2,046ng/kg bw/day.**

DON intake estimates

- During consumption of the normal diet an estimated 3% (95%CI: 2, 7%) of the samples measured indicated that for that day the TDI was exceeded.

- Based on the highest level of bread contamination (316ug/Kg) consumption of
 - ◆ The average intake of bread - DON intake = **671 ng/kg bw/day**
 - ◆ 95th centile intake of bread - DON intake = **1,282 ng/kg bw day**
 - ◆ Highest intake of bread - DON intake = **2,415 ng/kg bw day**

Summary -1

- We have developed a robust and sensitive assay to measure urinary DON.
- Based on this measure DON exposure was common in the UK
- Urinary DON was significantly associated with cereal intake
- Bread consumption in the UK was a major contributor to urinary DON
- DON was present in UK urine predominantly as DON-glucuronide
 - ◆ Variation may be related to individual rather than overall exposure level
- **Urinary DON was strongly correlated with DON intake**

Summary - 2

- Average estimated DON intake in the UK was below the TDI, but intake was highly variable
 - ◆ The TDI was exceeded for an estimated 3% (95%CI: 2-7%) of samples.

- To date we have only sampled a relatively small cross section of the UK adult population
 - ◆ NDNS n=300 and 1 sample per person – 300 urines
 - ◆ Pilot intervention n= 25 and 2 samples per person – 50 urines
 - ◆ Detailed intervention n=35 and 10 samples per person – 350 urines

- DON exposure levels in CHILDREN are likely to exceed those for adults.

- DON exposure in some other parts of the EU are predicted to be higher

Future

- Explore exposure patterns in children
- Assess exposure patterns in other regions
 - ◆ Ongoing – Egypt, France, USA
 - ◆ Planned – Iran, China – cohorts linked to esophageal cancer
- Link to health outcomes – options within New generis (Born in Bradford)
- Develop biomarkers to other tricothecenes
- Investigate potentially longer term biomarkers of exposure eg protein binding
- Investigate metabolomic approaches for biomarker development