

SURVEY ON T-2 E HT-2 TOXINS IN SAMPLES OF CEREALS AND PRODUCTS THEREOF FROM NORTH ITALY

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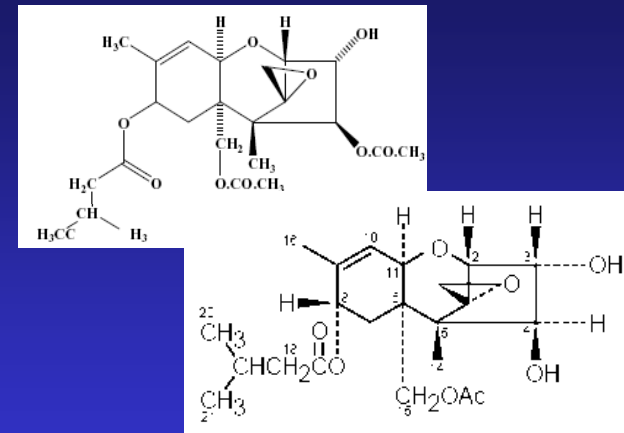


T2 and HT-2 TOXIN

Trichotecenes: contaminants of cereals, mainly in humid and cool climates

Produced by different fungi belonging to genus *Fusarium*.

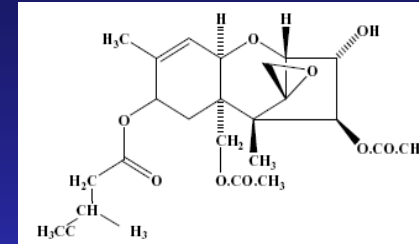
The most interesting specie is *F. sporotrichioides*; in our area it is a weak pathogen, psychrofile and hygrophilus.



	Temp. °C	Aw
Min	- 2	0,88
Opt	22,5 ÷ (27,5)	
Max	35	> 0,99

It produces toxins between 8 and 25°C with opt. T < 15°C and Aw between 0.95 and 0.97. It survives at 30°C but at this temp. it stops toxinogenic activity.

T2 and HT-2 TOXICITY



HUMAN

Toxic aleukia (ATA). Thousands of people poisoned in Siberia during the second world war

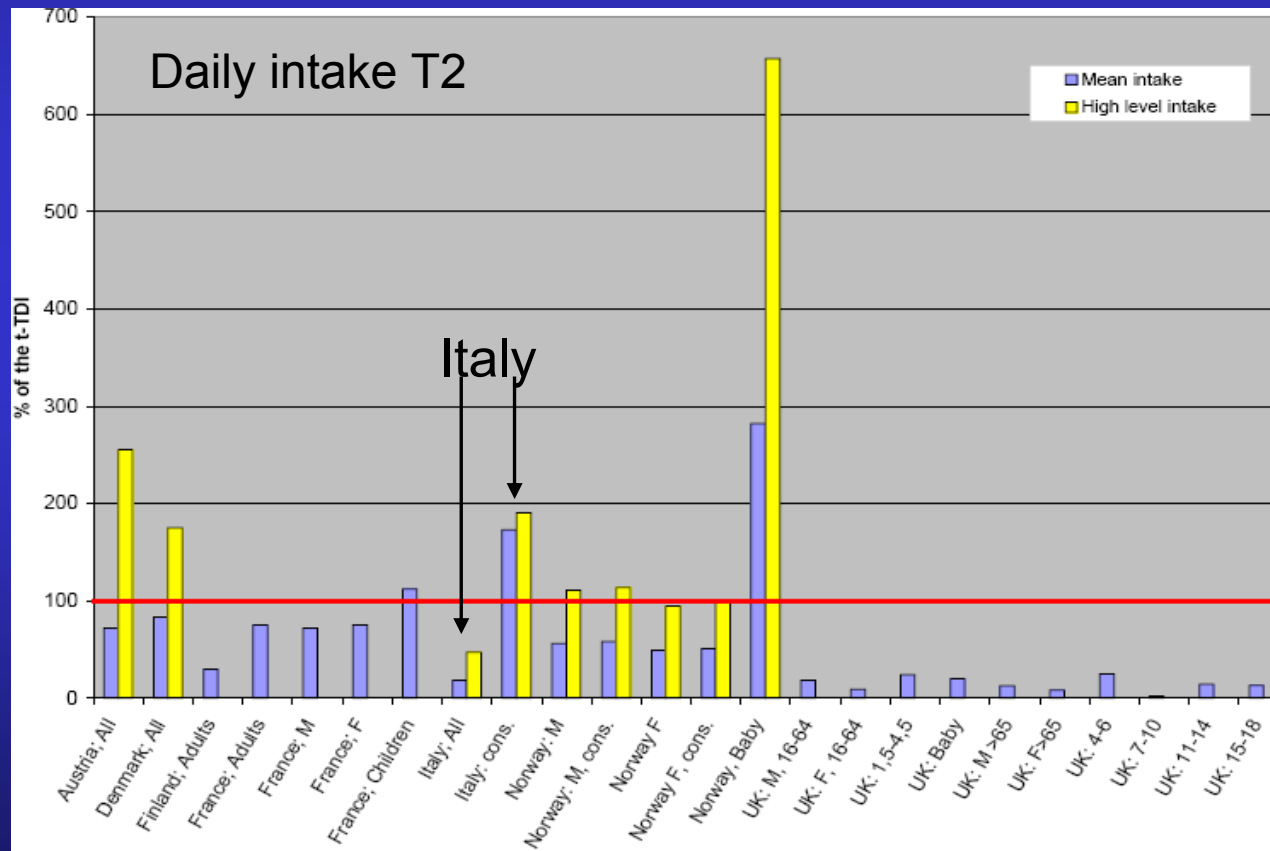
ANIMAL

Hemorrhagic syndrome, oral ulcer, gastrointestinal disease and neurotoxic effects (chicken)

IMMUNODEPRESSION

T2 and HT-2 TOXICITY

T-2 and HT-2 estimated dietary intake exceed in most cases the t-TDI (Final Report SCOOP TASK 3.2.10)



temporary TDI
0.06 $\mu\text{g}/\text{kg bw}$
combined
T2+HT-2

AVAILABLE DATA ON T2 AND HT-2

EU REGULATIONS no. 856/2005 and 1881/2006

In most cases the estimated T-2 and HT-2 dietary intake exceed the t-TDI. However, it has to be remarked that the majority of T-2 and HT-2 occurrence data were obtained by analysis methods with high detection limit (DL). Moreover, considering that less than 20% of the samples has been found above the DL, the dietary intake was strongly influenced by the detection limit of the analysis methods.

FINAL REPORT SCOOP TASK 3.2.10

Few available data on food contamination.

AIMS

To increase knowledge about the contamination by these toxins of wheat, barley and maize samples collected in experimental fields, drying units and mills.

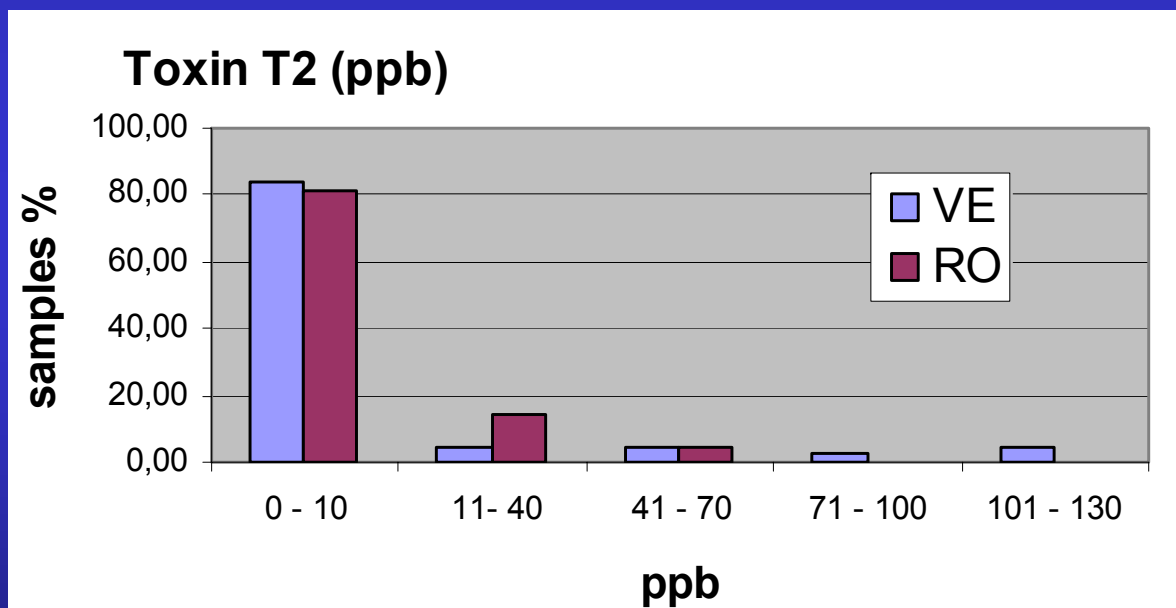


EXPERIMENTAL FIELD TRIAL (Maize - Year 2004)

68 hibryds: (6 Class 300; 5 Class 400; 17 Class 500; 29 Class 600; 11 Class 700)

2 location (VENICE area and ROVIGO area)

4 replications



Loc.	Av. ppb T2
VE	18,42
RO	13,48
Total	15,87

EXPERIMENTAL FIELD TRIAL (Maize - Years 2000-2006)

Isolation of *F. sporotrichioides*

Low frequency (< 1% ; max. value 3,6%)

Sometimes no findings (sporadic presence)

Location and season influence

Most frequently isolated during the first period of vegetative stage (pollen and silk)

ANALYTICAL ASSAYES ON CEREAL SAMPLES

Cereals: 21 wheat samples
17 barley samples
32 maize samples

Method of sampling : Dynamic

Origin: crop areas of North Italy
(drying units, mills and feed stores)

METHOD: ELISA

Kit EZ Quant T-2[®] (Diagnostix) – Or sell -

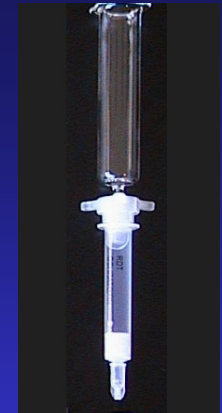
Determination of T2

Range 0,025-0,5 mg/kg

Chemical	% Cross-reactivity
HT-2	38
T-2 Triol	1,6
T-2Tetraol	<0,04
Verrucarol	<0,04

METHOD HPLC (preparation)

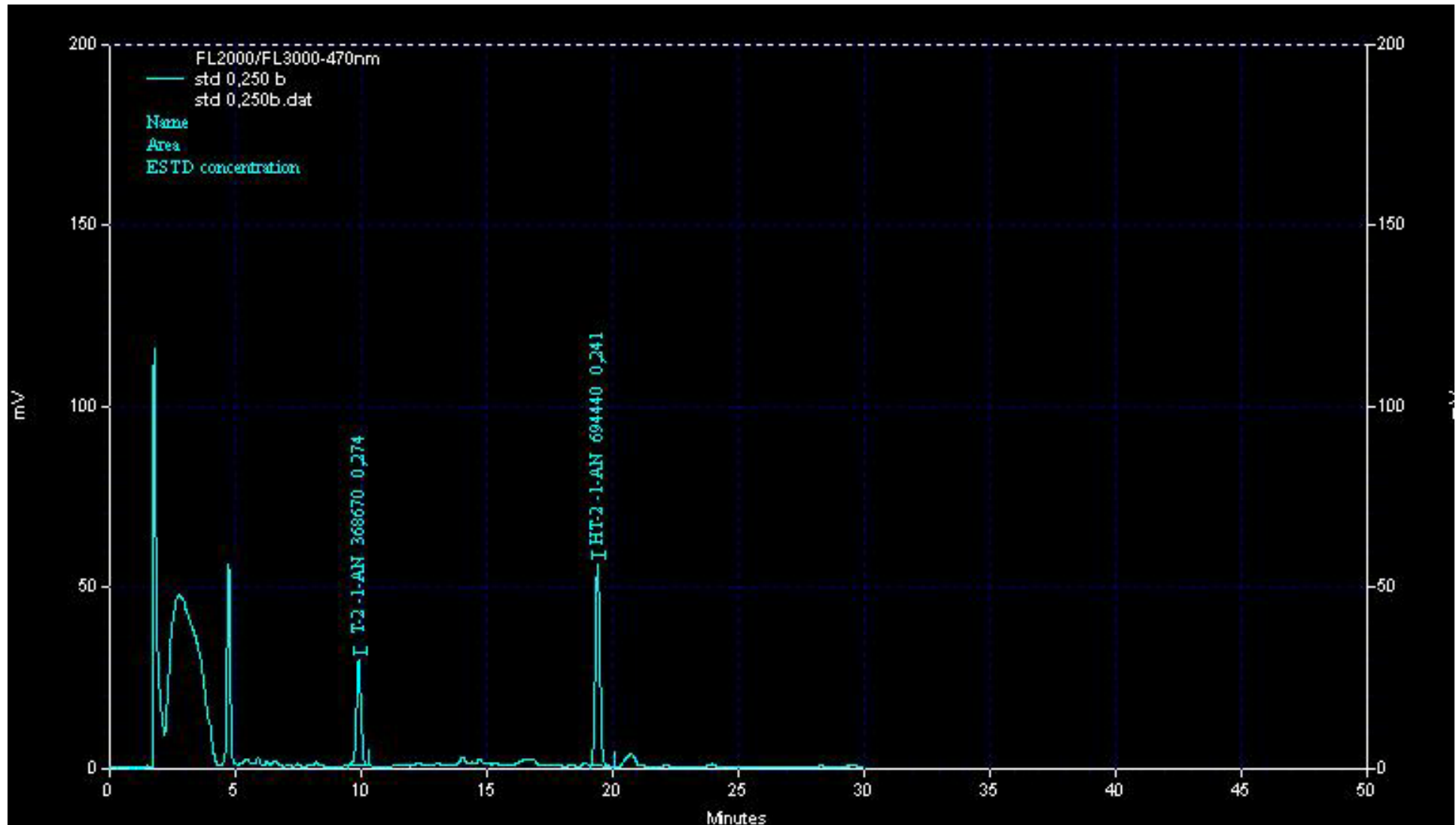
- Method Visconti et al., 2005. LOD 5 μg per T2 e 3 μg per HT2
- 50 g of sample in 250 ml of water-methanol solution (90% methanol)
- extraction
- clean-up on immunoaffinity column with monoclonal antibody T-2 e HT-2 (EASY-EXTRACT T-2 & HT-2, RHONE LTD supplied by OR SELL)
- derivatisation (solution of D-MAP and of 1-AN in toluene)
- dried sample retake with 1 ml of acetonitrile/ water solution – 70/30.



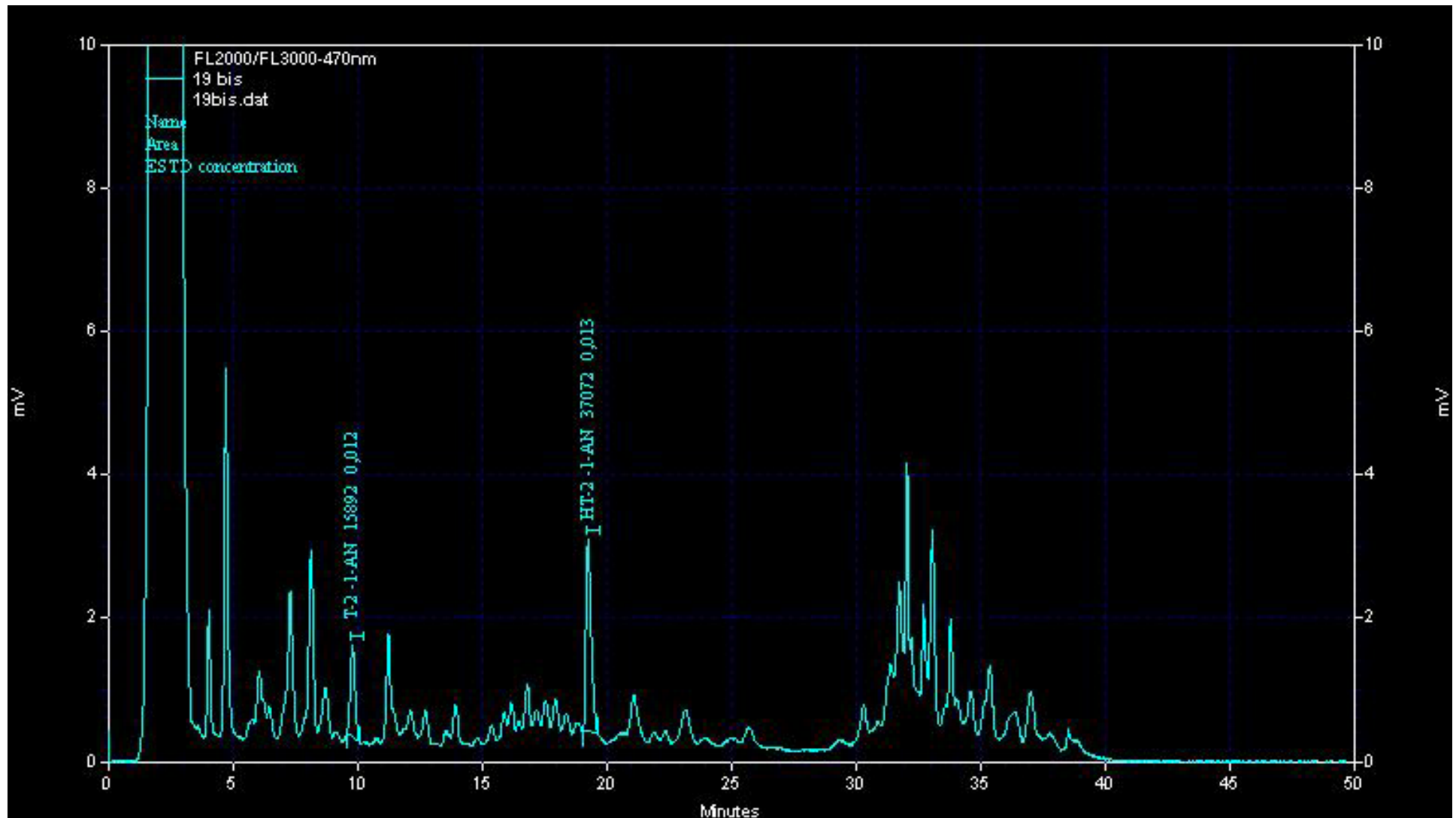
METHOD HPLC (chromatographic condition)

- Column: Gemini 5 μ C6 – Phenyl 110 A (150 X 4.6 mm)
- Mobile phase: binary gradient of water– acetonitrile with a starting composition of 70 % of acetonitrile
- Column temperature: 40°C
- Flow: 1 ml at minute
- Injected volume: 75 μ l
- Wavelength: Excitation: 381 nm
Emission: nm 470

METHOD HPLC Chromatogramm of a STD



METHOD HPLC: Chromatogramm of a real sample



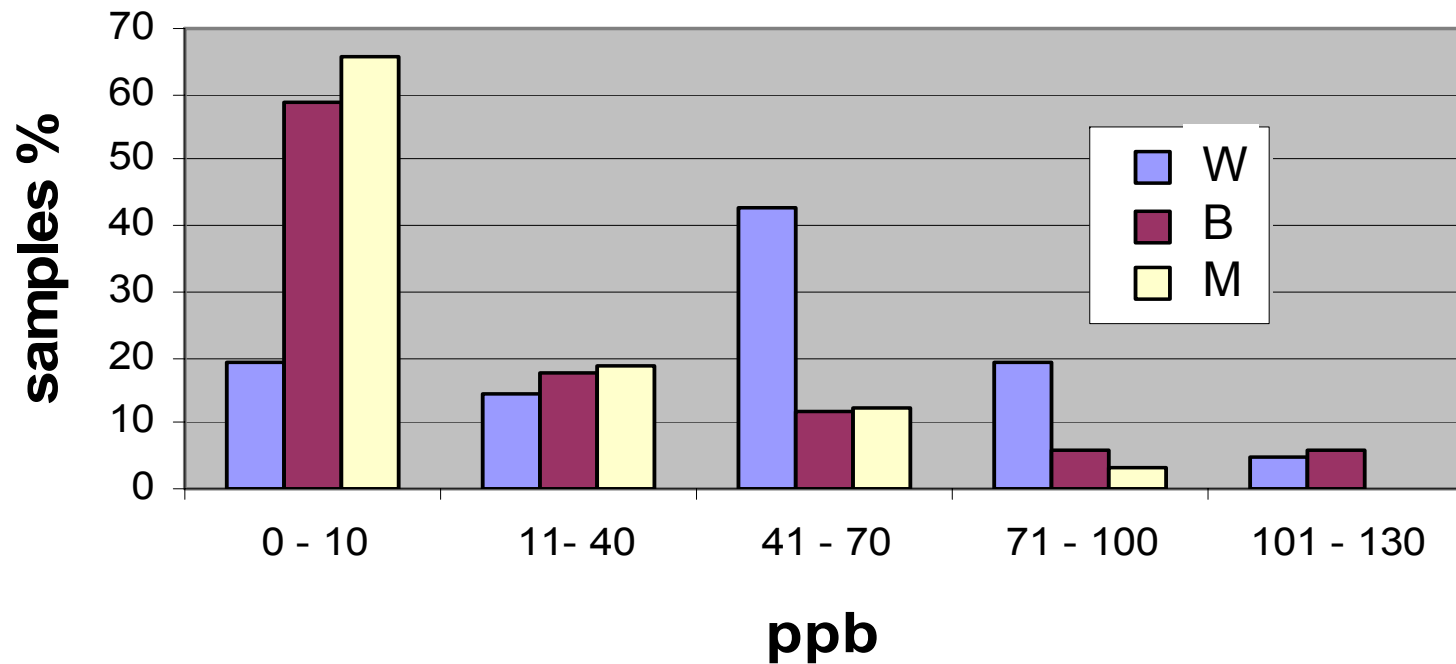
METHOD HPLC (estimation of recovery %)

	T-2	H-T2
Wheat	67.27	97.05
Maize	93.58	96.94
Barley	67.23	84.70

RESULTS

FREQUENCY (Method ELISA)

Toxin T2 (ppb) Method ELISA



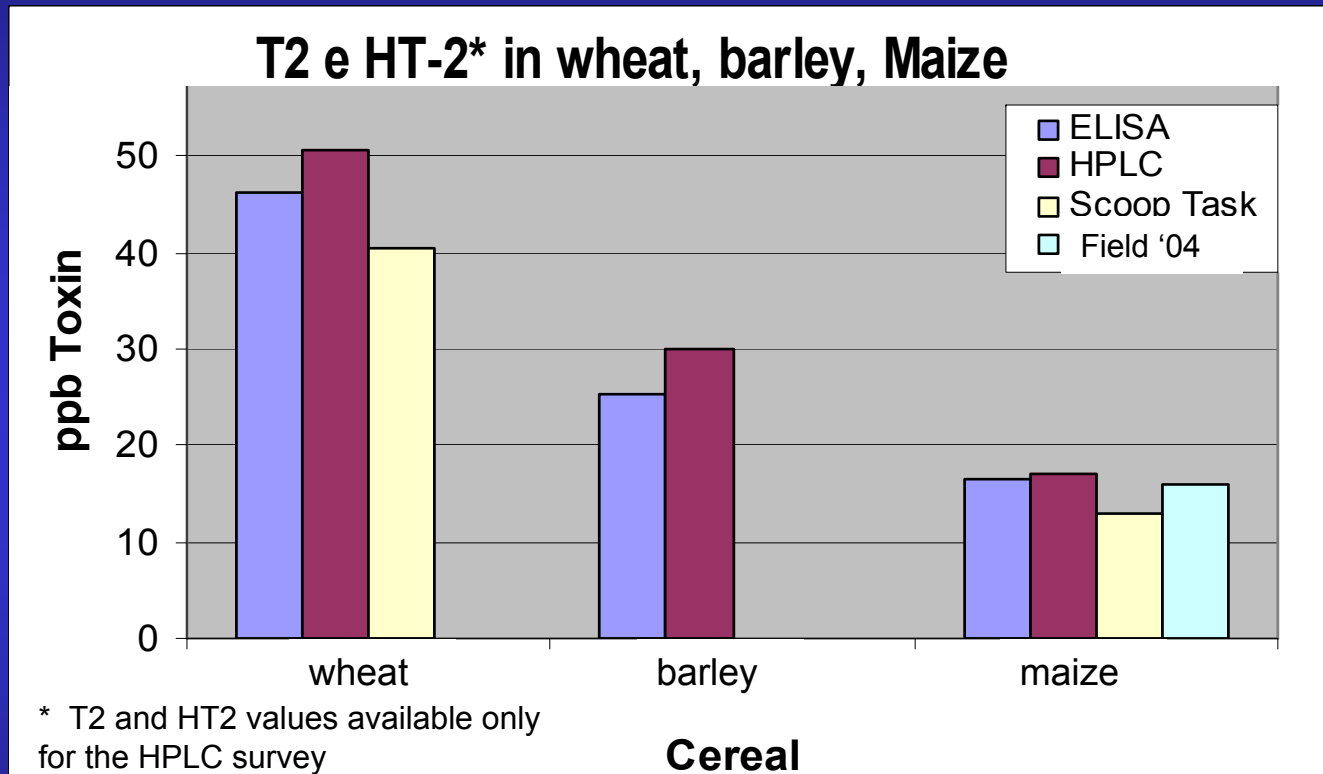
RESULTS

SAMPLES FREQUENCY >LOD (Method HPLC)

Cereal	T - 2	HT - 2
Wheat	10%	15%
Barley	18%	47%
Maize	26%	19%

RESULTS

AVERAGE VALUES, METHODS ELISA AND HPLC



Cer	Elisa	HPLC	Sc.Tsk 3.2.10	Field
W	46,29	50,68	40,4	
B	25,37	30,02		
M	16,59	17,03	12,9	15,87

RESULTS

ISOLATION FREQUENCY *F. sporotrichioides*

Cereal	Samples "HIGH"	Samples "LOW"
Wheat	0,7%	0,18%
Barley	0,24%	0,11%
Maize	---	---

CONCLUSIONS

Different contamination in different cereals (but the influence of the environmental factor must be considered)

In most cases samples have a low level of contamination in comparison with other *Fusarium*-toxins (*F. sporotrichioides* is not very frequent)

Values pointed out are similar to that of Final report SCOOP TASK 3.2.10; values with HPLC are a little higher.

Analytical methods: ELISA overvalue or undervalue? HPLC results to be confirmed (few data over LOD) but it is evident the superposition with ELISA which reacts only with part of HT2.

The situation seems not worrying but...which will be the limits?