T-2 and HT-2 toxins in Oats and Oat Products

Update from CEEREAL on the status of the research activities of the European Oat Milling industry

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Fusarium Toxin Forum

This presentation will cover:

- Responses to the Commission's questions where we have data and competence
- An update on CEEREAL's work to build knowledge of mycotoxins in oats and oat products
- A summary of CEEREAL's position statement and its commitment going forward

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CEEREAL / OMC

- CEEREAL is the trade association for the EU breakfast cereal and oat milling industries
- CEEREAL's members are the national breakfast cereal and oat milling associations in 12 EU member states
- Through its Oat Millers Committee it represents the oat milling industry in Finland, Sweden, Denmark, Germany, Ireland and UK

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The Oat Millers are committed to:

- Producing safe and nutritious food
- Safeguarding the growing of oats in member states
- Working with other agencies on building knowledge on fusarium mycotoxins
- Supporting work on providing prevention at source

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Safe and Nutritious Food

- Therefore oats are an important part of the diets in member states but other cereals are consumed in larger daily quantities
- Oats are subjected to only a small amount of processing thereby retaining their natural properties
- Oats and oat porridge are recognised as being helpful to heart health as part of a healthy diet

	Norway	**Great Britain**	**Germany**
	(16-29 years)	(16-64 years)	
	(Langseth, 2000)	(Gregory et al. 1990	(DLG, Ern.ber. 2008, fig. 2006)
oat:	7,5 g/day	12 g/day	5 g/day
rye:	15 g/day	7,4 g/day	23 g/day
wheat:	280 g/day	130 g/day	170 g/day

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Oat growing in Member States

- Oats rank 6th in world production following wheat, rice, maize, barley and sorghum
- It's value is around 6% of global cereal production
- USA and Russia are the largest producers
- Oats are particularly adapted to the colder climates of northern countries in Europe

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Oat growing in Member States

- EU oat crop in 2007 was approximately 8 million tonnes
- Largest producers are Finland and Poland each producing around 1 million tonnes
- Traditionally about 70% of output was used in animal feed and the balance for human foods
- Due to quality criteria not every raw oat may be used for milling purposes
- However usage has been increasing in most EU states and the UK for example now uses around 60% of it's production for human foods

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Quality criteria

Oat milling requirements (Zechner, 2001)

- high value of entire oat (riddle sorting > 2,0 mm)
- low value of hulls
- easy to dehull
- light colour

Quality components (Schönberger and Kropf, 2000)

• value of entire oat:	min. 99 %
• weight of hectolitre:	min. 54 kg
• weight of thousand grains:	min. 30 g
• value of hulls:	under 26 %
• humidity of grains:	max. 15 %

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Oat growing in Member States

- Oats are an important crop within the EU and in some member states they are critically important
- However oat millers take steps to maintain production of mill quality in the face of more marketable crops such as wheat and barley
- Cross member state movement of oats is an essential requirement

CEEREAL T-2/HT-2 Project

- Launched by the Oat Millers' Committee
- Supported by more than 25 mills and associations
- Set up to collect samples of raw oats, oat flakes and by-products in key producing or processing countries
- Started from harvest 2007 through to include harvest 2009

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CEEREAL T-2/HT-2 Project

Aim and Scope of Study

- Evaluation of the level of T-2/HT-2 in oats, oat flakes and oat by-products
- Distribution in oats, oat flakes and by-products
- Substantiate the reduction rate during processing
- Level of T2/HT2 in flakes from different origin

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CEEREAL Sampling Plan

- 1200 samples = 400 pa over 3 years from the UK, Ireland, Finland and Germany
- Analysing Lab : Gesellschaft fur Bioanalytik Hamburg mbh (GBA)
- Method of analysis : LC-MS/MS
- Sampling protocol based on Reg. 401/2006 Sampling for flakes (A.4 of Annex)

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T-2 and HT-2 toxins in Oats and Oat Products

Update from CEEREAL at

Fusarium toxin Forum, Brussels 9-10th February 2009

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Oat Processing

Raw Oat ← Samples

↓

Cleaning

↓

Dehulling

Grain/Groats → Flakes ← Samples

Husk/ By-products → Pellets ← Samples

Summary CEEREAL T-2 and HT-2 Analyses in Oats and Oat Products 2005 - 2008
(Analyses prior Jan 2009)

Product	Number of Samples	Percentage of Samples >200 ppb	>500 ppb	>1000ppb	Mean (µg/kg)	Median (µg/kg)	90th % ile (µg/kg)	Max (µg/kg)
T-2								
Oats raw	138	14	3	0	32	10	93	269
Oat flakes	381	0	0	0	5	3	11	38
Oat by-product	80	13	8	0	122	66	312	595
HT-2								
Oats raw	138	8	2	0	67	15	163	572
Oat flakes	381	0	0	0	14	9	32	159
Oat by-product	80	33	10	0	196	110	484	963
T-2 + HT-2								
Oats raw	138	14	3	0	99	24	264	841
Oat flakes	381	0	0	0	19	12	44	197
Oat by-product	80	49	15	1	319	174	712	1558

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CEEREAL T-2 and HT-2 Analyses in Oats and Oat Products

Difference between Harvest Year

Year	Product	Number of Samples	Percentage of Samples			Mean	Median	90th %ile	Max
			>200ppb	>500ppb	>1000ppb	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)
2006	Oats raw	32	44	13	0	261	189	664	841
2007	Oats raw	23	22	0	0	129	102	305	415
2008	Oats raw	82	0	0	0	28	15	66	283
2006-2008	Oats raw	138	14	3	0	99	24	264	841
2005	Oat flakes	30	0	0	0	48	46	83	96
2006	Oat flakes	70	0	0	0	25	17	54	74
2006/2007	Oat flakes	57	0	0	0	12	10	22	31
2007	Oat flakes	61	0	0	0	24	16	38	197
2008	Oat flakes	142	0	0	0	10	8	21	42
2005-2008	Oat flakes	381	0	0	0	19	12	44	197
2006	Oat by-product	10	100	90	80	1144	1280	1462	1558
2007	Oat by-product	22	68	9	0	313	161	491	659

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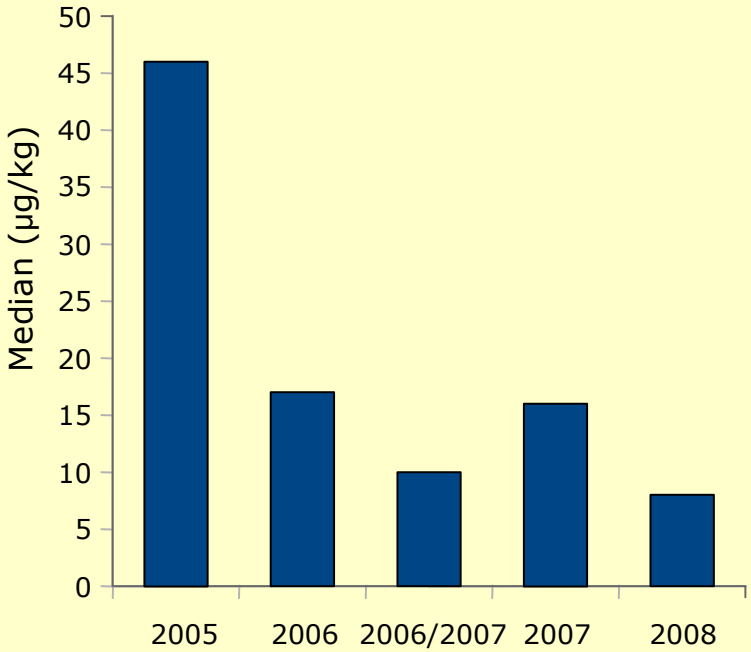
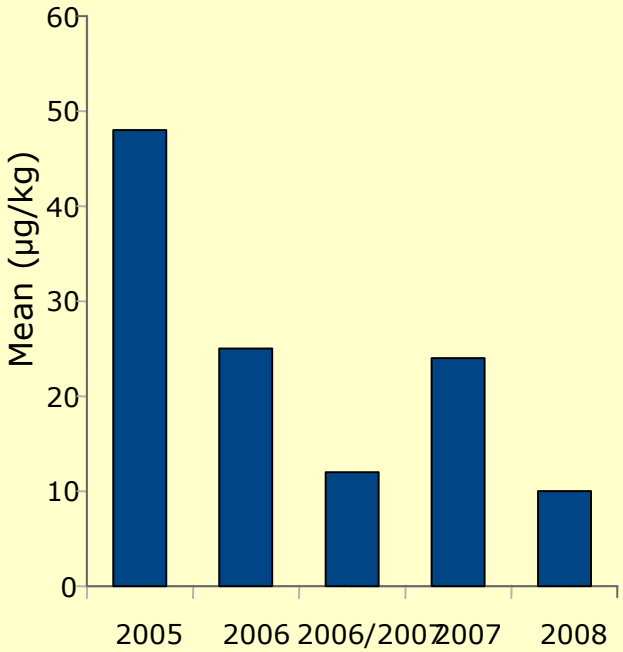
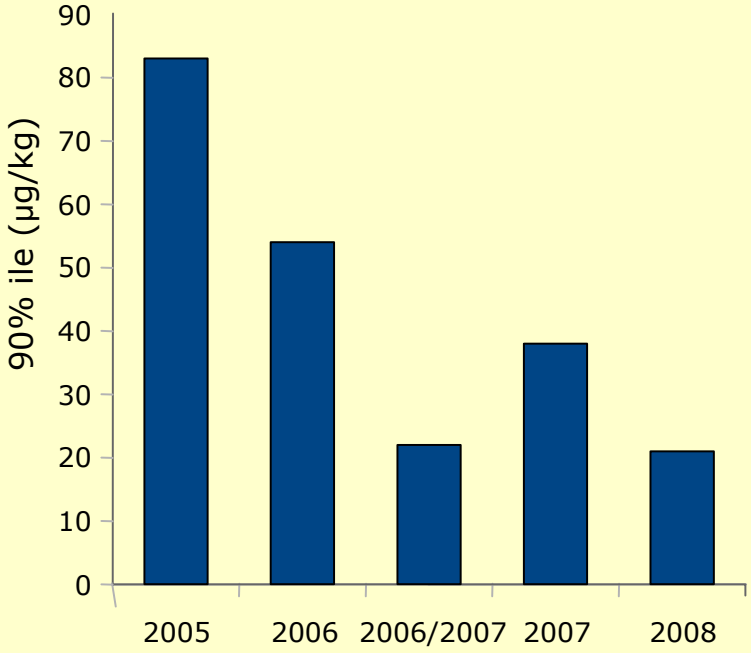
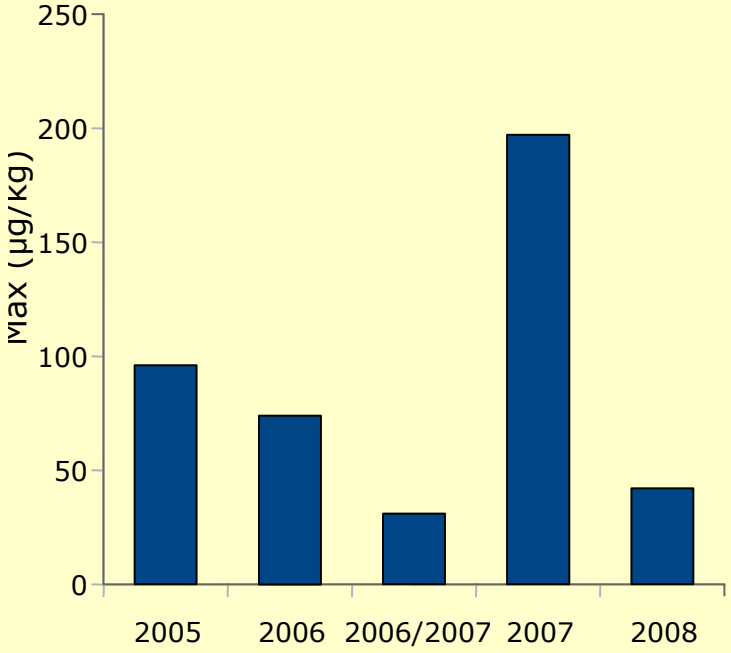
Difference between Harvest Year









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CEEREAL T-2 and HT-2 Analyses in Oats and Oat Products

Difference due to Country Origin

Country Oat origin	Product	Number of Samples	Percentage of Samples			Mean (µg/kg)	Median (µg/kg)	90th %ile (µg/kg)	Max (µg/kg)
			>200ppb	>500ppb	>1000ppb				
UK	Oats raw	81	12	4	0	77	16	208	758
Finland	Oats raw	17	12	0	0	90	71	169	283
Ireland	Oats raw	22	32	5	0	201	173	360	841
Sweden	Oats raw	8	13	0	0	103	98	177	302
UK	Oat flakes	260	0	0	0	18	10	46	197
Finland	Oat flakes	102	0	0	0	21	16	41	96
Sweden	Oat flakes	11	0	0	0	28	20	40	87

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T-2 and HT-2 toxin in Raw Oats delivered to Mills

Results reported through CEEREAL (Different Sampling Protocol and Analytical Method)

Year	Country	Number of Samples	Percentage of Samples			Mean	Median	Max
			>200ppb	>500ppb	>1000 ppb	(µg/kg)	(µg/kg)	(µg/kg)
2004-2006	Scandinavia	31	35	6	3	254	133	2343
2004-2006	UK	22	77	45	23	765	436	3528
2003-2007	UK	19	58	21	21	606	223	3530
2007-2008	Sweden	24	1	0	0	48	26	270

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T-2 and HT-2 toxin levels in Oat products

Oat product	Year	Country	Number of Samples	Percentage of Samples >50ppb	Percentage of Samples >200ppb	Percentage of Samples >500ppb	Mean (µg/kg)	Median (µg/kg)	Max (µg/kg)	Ref
Oat flakes	2000-2001	Norway	18	0	0	0	20	20	20	Clasen 2003
Oat groats	2000-2001	Norway	39	5	0	0	23	20	54	Clasen 2003
Oat flakes	2004-2006	UK	15	20	0	0	38	34	105	BOBMA 2007
Oat flakes	2003-2007	UK	22	18	0	0	35	22	137	Morning 2007
Oat flakes org	2005	Germany	18	0	0	0	7	6	20	Gottschalk et al. 2007
Oat flakes conv	2005	Germany	25		0	0	31	26	85	Gottschalk et al. 2007
Oat flakes/meal	2005-2006	Mix	8	0	0	0	17		35	Meister 2008
Oat groats	2005-2006	Mix	54		0	0	30		174	Meister 2008
Oat groats	2005	Germany	19	0	0	0	7	5		Usleber 2008
Oat flakes	2006	Germany	81		0	0	21	14	80	Usleber 2008
Oat flakes	2007	Germany	54		0	0	14	13	51	Usleber 2008
Oat flakes/groats	2005	Unknown	29	6	3	3	49	18	607	Biselli 2006
Oat flakes	2005-2008	CEEREAL	381	8	0	0	19	12	197	CEEREAL 2009
Oat hull	2000-2001	Norway	39	90	54	15	449	249	3147	Clasen 2003
Oat by-product	2004-2006	UK	27	100	96	89	2711	5643	29700	BOBMA 2007
Oat by-product	2003-2007	UK	14	100	93	93	1540	1004	4540	Morning 2007
Oat by-product	2006-2008	CEEREAL	80	86	49	15	319	174	1558	CEEREAL 2009
Oat bran	2000-2001	Norway	23	0	0	0	20	20	20	Clasen 2003
Oat bran	2006	Germany	24	0	0	0	13	10	29	Usleber 2008
Oat meal	2000-2001	Norway	5	0	0	0	20	20	20	Clasen 2003
Oat meal	2006-2007	CEEREAL	20	0	0	0	27	28	40	CEEREAL 2009

Low levels in Oat flakes!

Higher levels in by-products!

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Sum T-2 and HT-2 in Raw Oats
Surveys 2000-2008

Year	Country	Number of Samples	Percentage of Samples		Mean	Median	Max	Reference
			>50ppb	>500ppb	(µg/kg)	(µg/kg)	(µg/kg)	
2000	Finland	25	36	8	137	25	1369	Hietaniemi 2006
	Norway	22	73	5	86	53	564	SCOOP 2003
2001	Finland	37	27	0	59	25	273	Hietaniemi 2006
	Norway	24	0	0	10	10	10	SCOOP 2003
2002	Finland	30	37	0	78	38	427	Hietaniemi 2006
	UK	92	70	16	311	106	4844	Edwards 2006
2003	Finland	30	63	13	305	116	1647	Hietaniemi 2006
	UK	104	69	33	727	204	9990	Edwards 2006
2004	Finland	30	57	10	282	104	2850	Hietaniemi 2006
	Norway	56	70	0	106	86	334	Clasen 2006
	UK	128	80	24	500	202	6997	Edwards 2006
2005	Finland	60	63	33	440	186	3500	Hietaniemi 2006
	Sweden	41	61	17	255	90	1165	Pettersson 2006
	Norway	126	87	13	283	180	2041	Clasen 2006
	Denmark	18	94	6	312	221	2560	Biselli 2006
	UK	134	88	44	694	403	3188	Edwards 2006
2006	Finland	59	53	5	163	47	1283	Hietaniemi 2007
	Sweden	71	90	44	465	376	1416	Pettersson 2007
	Norway	102	78	9	218	145	1675	Clasen 2006
	UK	100	96	43	795	404	6261	Edwards 2007
2007	Finland	80	53	5	121	64	863	Hietaniemi 2007
	Sweden	24	91	10	244	215	1170	Pettersson 2008
	Norway	32	94	6	217	177	980	Clasen 2008
	UK	103	77	18	438	169	8399	Edwards 2008
2008	Finland	80	38	5	100	32	1932	Hietaniemi 2009
	Norway	33	64	0	62	55	145	Clasen 2009
	UK	60		7	117	47		Edwards 2009

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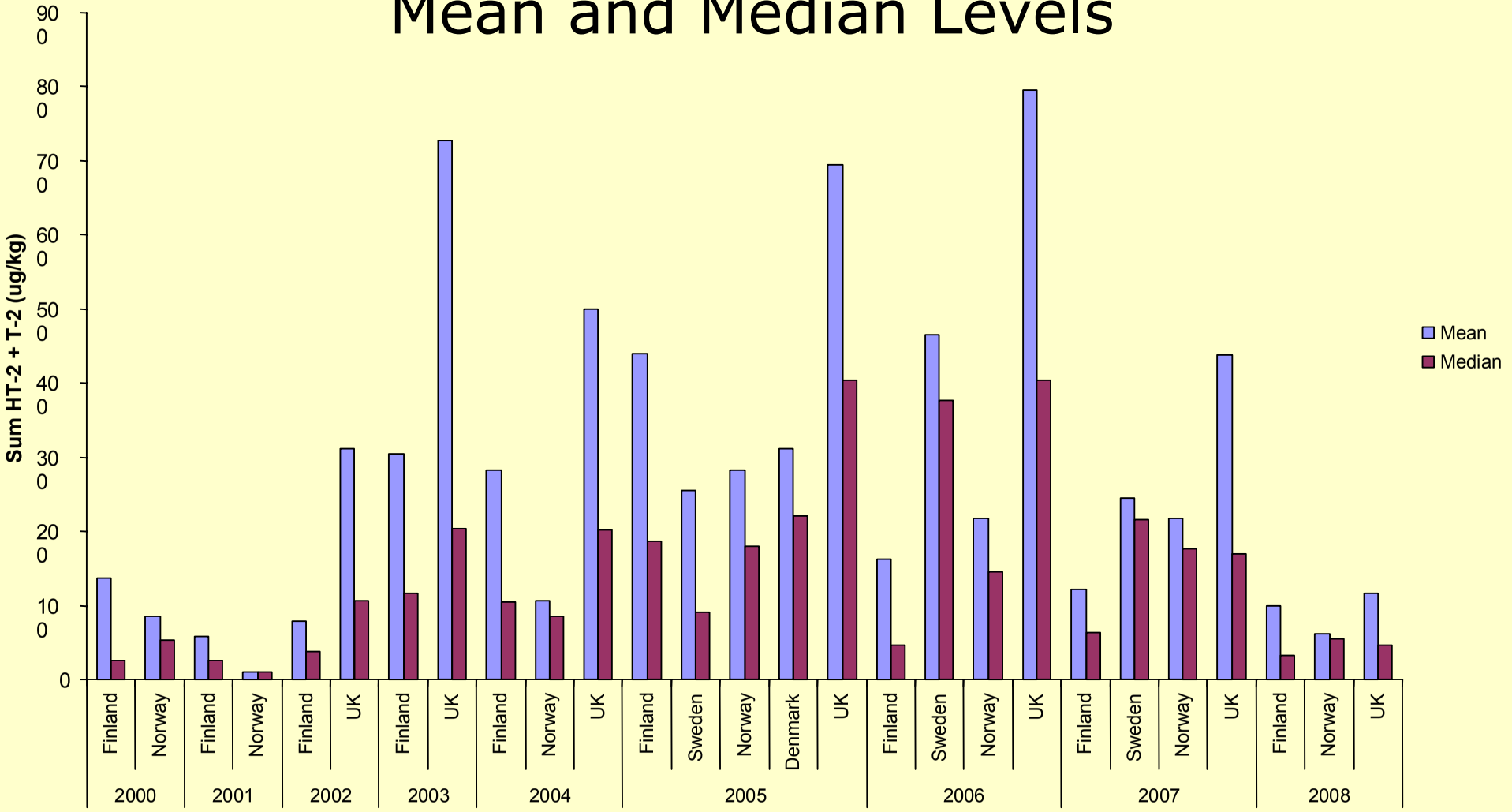
Sum of T-2 and HT-2 in Raw Oats
Surveys 2000-2008
Mean and Median Levels



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Conclusions from Survey Results

Yearly variation within countries

- Increase from 2002 and decrease in 2007 + 2008

Frequent occurrence in all survey countries

Levels not comparable between countries

- Different sampling and analytical methods

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Raw Oats Samples Exceeding Certain T-2 + HT-2 Levels

Year	Country	Number of Samples	Percentage of Samples >500 ppb	>1000 ppb	>2000 ppb
2003	Finland	30	17	7	
	UK	104	41	29	8
2004	Finland	30	10	7	
	Norway	41	0	0	0
	UK	128	27	15	6
2005	Finland	60	33	10	10
	Sweden	41	17	2	0
	Norway	126	14	4	1
	UK	134	51	30	10
2006	Finland	59	5	2	
	Sweden	71	44	13	0
	Norway	102	9	2	0
	UK	100	43	21	9
	CEEREAL	32	13	0	0
2007	Finland	80	5		0
	Sweden	58	10	0	0
	Norway	32	6	0	0
	UK	103	18	8	5
	CEEREAL	23	0	0	0
2008	Finland	80	5		0
	Norway	33	0	0	0
	UK	60	7		0
	CEEREAL	82	0	0	0
2003-2008	Median		10	3	0

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T-2 and HT-2 in raw Oats
Regional Differences?

Survey Results may indicate higher levels in Scandinavian and UK oats

- Due to not sufficient milling oats only very few analytical results from other producing countries (Poland, Germany, France, Spain, Austria)
- Survey country levels not comparable (sampling and method differences)
- No or slight indication of regional differences within survey countries

Climate and Agronomic may cause regional differences

- Dry and warm weather
- Variety choice

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Oat Processing
Reduces T-2 and HT-2 levels

Harvest and Delivery cleaning

- first reduction

Sorting- sieving at the Mill

- Kernel fraction <2.1 mm higher in HT-2/T-2

Dehulling

- Reduction 70 – 95 % at high levels (depends on different oat mill diagramms)
- Lower reduction at low levels

Sortex cleaning of dehulled oats

- discoloured fraction higher in HT-2/T-2

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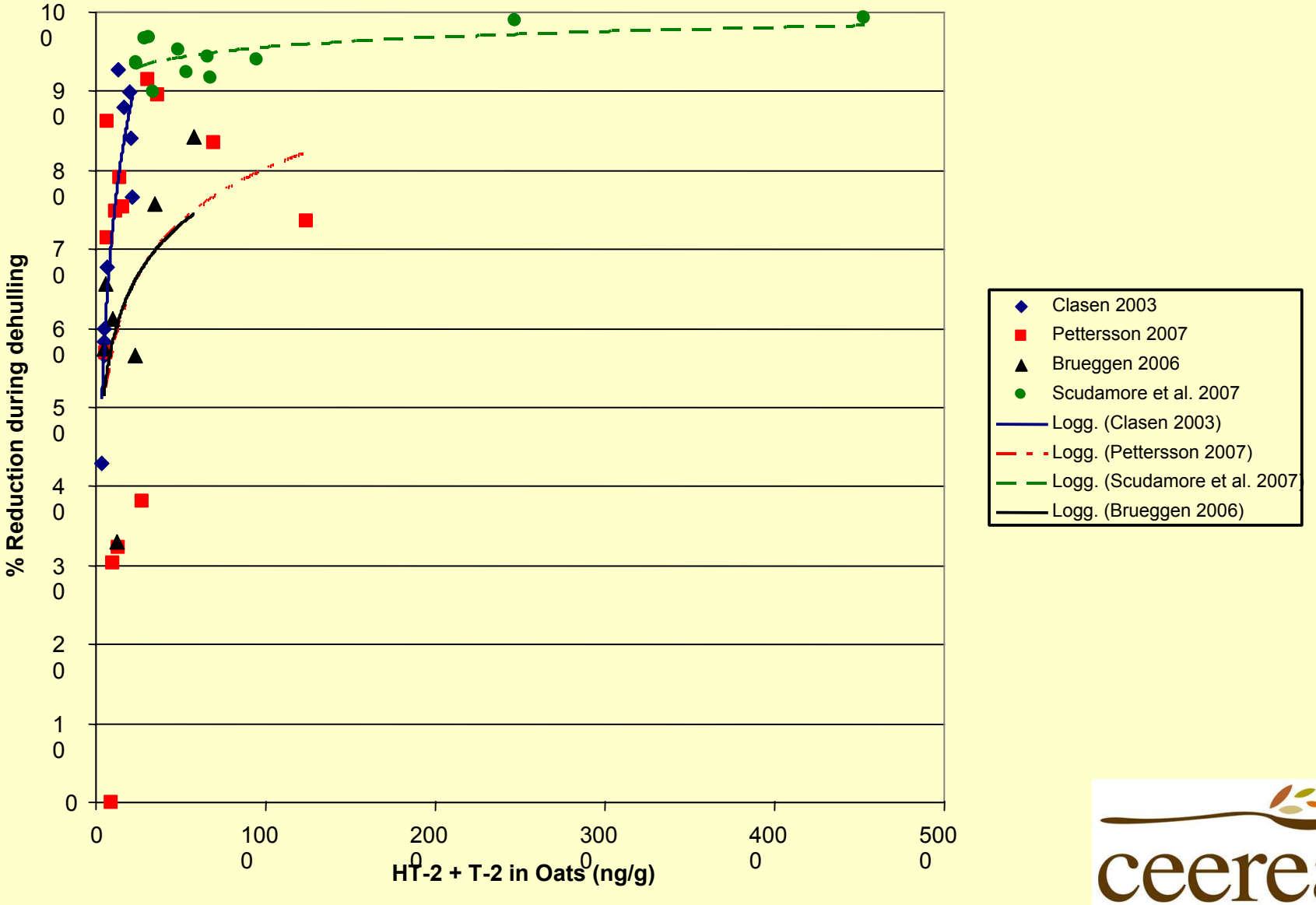
Reduction in T-2+HT-2 During Dehulling
Results mainly from Mill Processing



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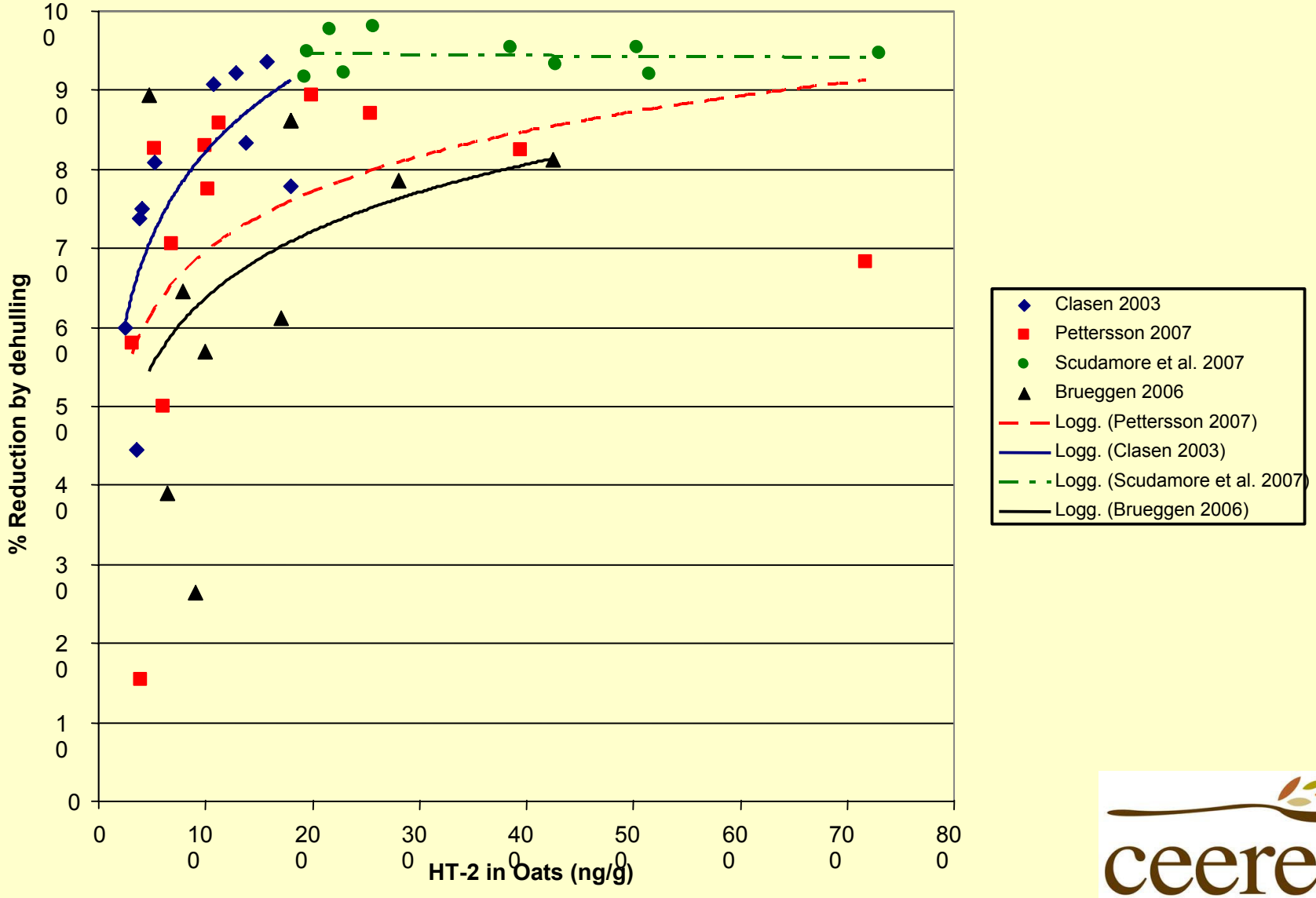
Reduction in HT-2 During Dehulling
Results mainly from Mill Processing



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Summary of Reduction in T-2 + HT-2 by Processing mainly Dehulling

Trichothecene	Number of Trials	% Reduction		
		Mean	sd	Median
HT-2+T-2	47	75	23	84
HT-2	50	79	19	85

Processing will substantially reduce T-2 + HT-2 in oat products for human consumption

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Increased T-2 + HT-2 in Oat By-product

Trichothecene	Number of Trials	% Increase		
		Mean	sd	Median
HT-2+T-2	40	420	343	310
HT-2	48	350	331	280

Oat by-products are used in feed for ruminants and horses!

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Analytical Methods
T-2 + HT-2 toxins

Difficult analyses – Improvements needed

Earlier → 50% variation between laboratories

matrix effects

No Standardized method yet

Discussions in CEN

GC-MS method - interlaboratory validated (IRRM, Geel)

LC-MS method in interlaboratory validation (BfR, Berlin)

Rapid method needed for own control

- Available ELISA-kits only valid for T-2
- A new lateral flow strip test kit for both T-2 and HT-2 introduced (Charm, ROSA)

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Analytical Methods
T-2 + HT-2 toxins

Quality Factors – Problems for all methods to be solved

Milling

Most sample mills using sieves produce non-homogeneous material from raw oat. Husk debris!

Extraction efficiency for T-2 in natural contaminated raw Oats

Low for 70% methanol-water? Used in immunobased methods.

Phase concentrations for acetonitrile-water (84+16)? Used in most chemical methods.

Calibration

Calibrant purity and concentration may differ

Certified Reference Materials lacking

Internal labelled standards will improve for mass-detection methods

Proficiency testing important - naturally contaminated oat samples not spiked

Control samples

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Organisation, Persons and Laboratories Communicating Analytical Results on HT-2 and T-2 toxins in Oats

Person/organisation	Laboratory
CEEREAL	GBA, Hamburg, Germany
Biselli, Scarlett	Eurofins, Hamburg, Germany
Clasen, Per-Erik	Veterinary Institute, Oslo, Norway
Edwards, Simon	Harper Adams University College, Newport, UK
Hietaniemi, Veli	MTT Agrifood Research Finland, Jokionnen, Finland
Pettersson, Hans	Swedish University of Agricultural Sciences, Uppsala, Sweden

Thanks to all of them and their financiers

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Thanks for Your Attention!

and

Thanks to All Contributing Laboratories

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CEEREAL Position

Fully supports the Commission's and Council's work in assessing the situation with regard to T2 and HT2 in oats.

However we would ask that :

- Priority is focussed on prevention of the formation
- There should be a thorough risk-benefit assessment particularly as oats are recognised for their significant contribution to a healthy diet

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CEEREAL Position

- CEEREAL member companies are already and are willing to continue to be active partners in agricultural studies in certain member states.
- CEEREAL member companies can assist in providing data on consumer consumption in certain member states.
- CEEREAL will continue to build data on oats and oat products as part of its study.

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CEEREAL Position

However some points of concern are:

- No approved rapid test method
- There is a shortage of scientific data
- The risk to the consumers has been insufficiently assessed

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Conclusion

Should the experts conclude that risk management requires maximum limits then:

- There should be an entry level for raw oats and a second level for finished products and
- There needs to be a consistent link between the two, taking account of the reduction rates during processing

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Fusarium Toxin Forum 2009

Thank you for your attention

And please note that copies of CEEREAL's position paper are available at this forum.

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