

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

Hans Pettersson

Dept. Animal Nutrition and Management
Swedish University of Agricultural Sciences

P. Box 7024, SE-750 07 Uppsala, Sweden

E-mail: Hans.Pettersson@huv.slu.se



CEEREAL T-2 and HT-2 Oat Project

Aim and Scope

- Evaluation level of T-2/HT-2 in oats and oat products
 - Distribution in raw oats, oat products and by-products
 - Reduction from oat to oat flakes
 - Levels in flakes of different origin

Plan of CEERREAL Study

- 700 samples/year over 2 years → total 1.400 samples
 - 630 samples from UK
 - 630 samples from Germany
 - 100 samples from Finland
 - 40 samples from Ireland
- Sampling protocol based on EU Reg. 401/2006
- Analysis method: LC-MS/MS
- Analyzing lab: Gesellschaft für Bioanalytik Hamburg GmbH (GBA)

Summary CEEREAL T-2 and HT-2 Analyses in Oats and Oat Products 2006 and 2007 (Analyses prior Dec 2007)

Product	Number of Samples	Percentage of Samples			Mean (µg/kg)	Median (µg/kg)	90th % ile (µg/kg)	Max (µg/kg)
		>50 ppb	>200 ppb	>500 ppb				
T-2								
Oats raw	20	45	10	0	71	42	189	228
Oat products	185	0	0	0	6	5	11	29
Oat by-product	20	90	50	30	299	246	563	595
HT-2								
Oats raw	20	75	25	10	175	105	521	530
Oat products	185	2	0	0	16	13	34	83
Oat by-product	20	100	80	40	464	337	909	963
T-2 + HT-2								
Oats raw	20	80	45	15	246	165	688	758
Oat products	185	9	0	0	22	16	43	112
Oat by-product	20	100	85	60	763	578	1382	1558

CEEREAL T-2 and HT-2 Analyses in Oats and Oat Products Difference between Harvest Year

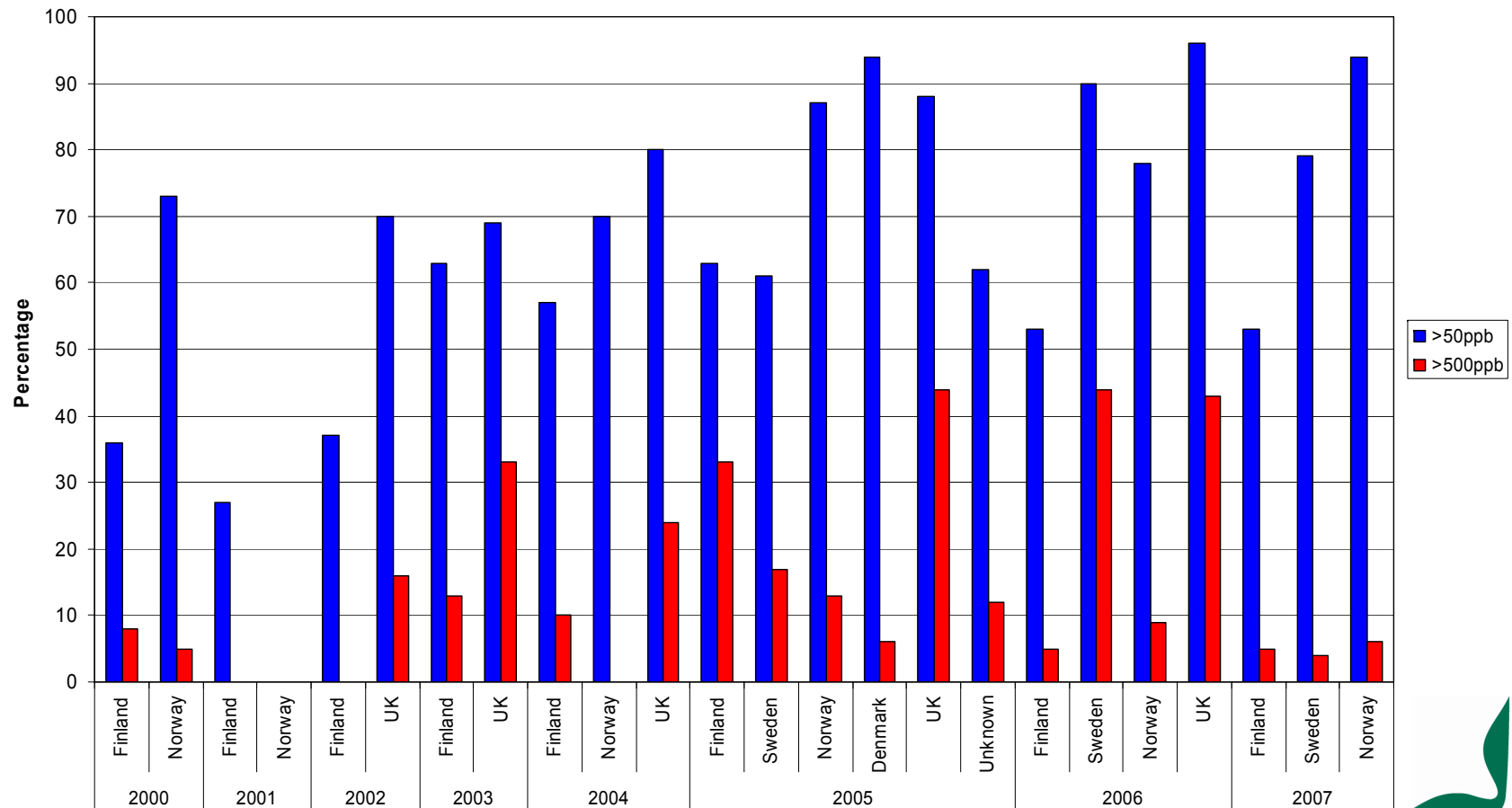
Year	Product	Number of Samples	Percentage of Samples			Mean (µg/kg)	Median (µg/kg)	Max (µg/kg)
			>50 ppb	>200 ppb	>500 ppb			
2006	Oats raw	12	100	58	25	340	235	758
2007	Oats raw	8	50	25	0	106	45	369
2006	Oat products	56	21	0	0	29	24	74
2007	Oat products	31	10	0	0	21	14	112
2006	By-product	10	100	100	90	1144	1281	1558
2007	By-product	9	100	67	22	360	437	659

Sum T-2 and HT-2 in Raw Oats Surveys 2000-2007

Year	Country	Number of Samples	Percentage of Samples		Mean (µg/kg)	Median (µg/kg)	Max (µg/kg)	Reference
			>50ppb	>500ppb				
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	Unknown	42	62	12	814	91	14640	Biselli 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	79	4	158	130	517	Pettersson 2007
	Norway	32	94	6	217	177	980	Clasen 2008

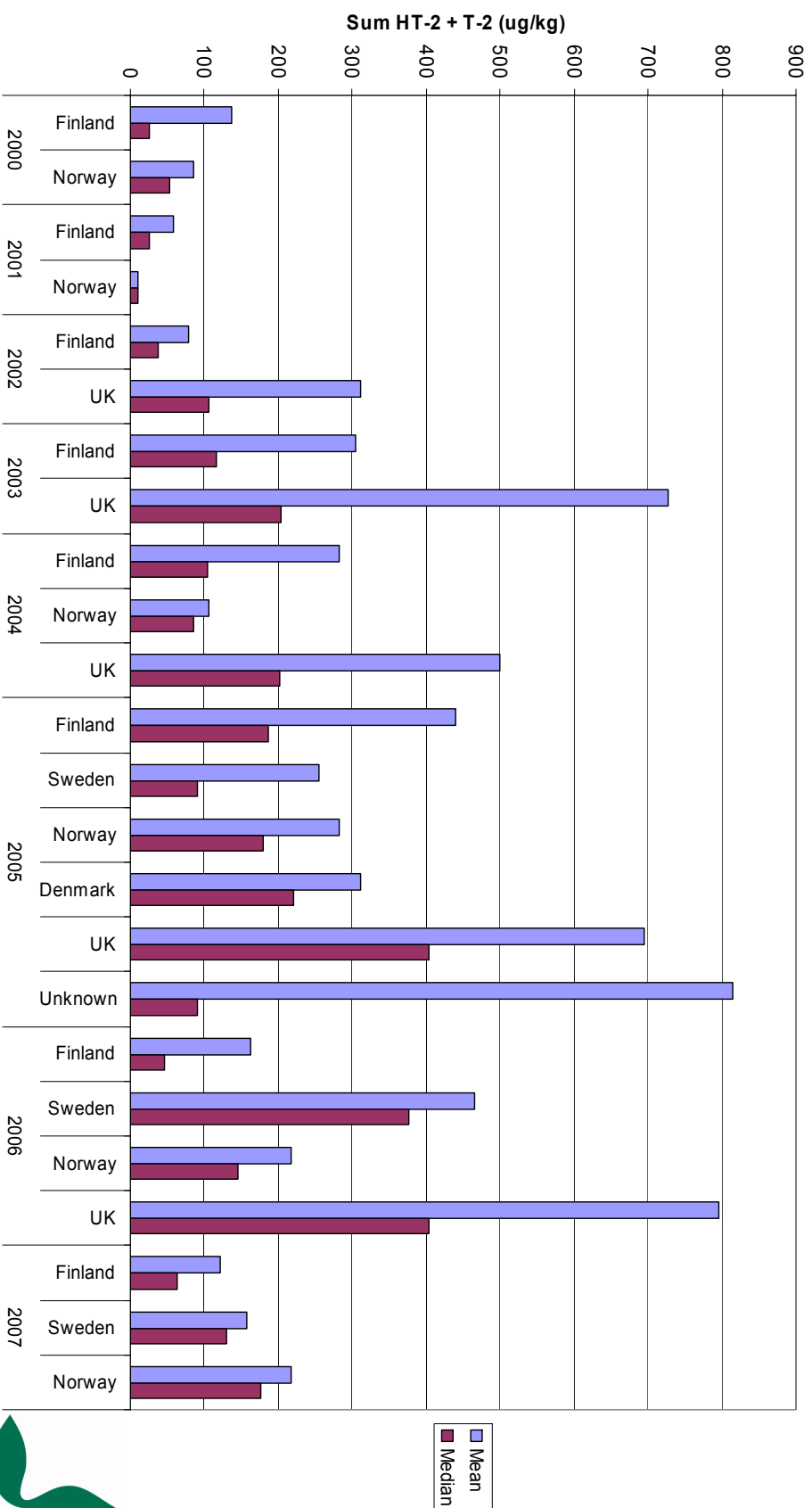
Sum of T-2 and HT-2 in Raw Oats Surveys 2000-2007

Percentage of Samples Above Certain Levels



Sum of T-2 and HT-2 in Raw Oats Surveys 2000-2007

Mean and Median Levels



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

Results reported through CEEREAL

Year	Country	Number of Samples	Percentage of Samples			Mean (µg/kg)	Median (µg/kg)	Max (µg/kg)
			>50ppb	>200ppb	>500ppb			
2004-2006	Scandinavia	31	74	35	6	254	133	2343
2004-2006	UK	22	91	77	45	765	436	3528
2003-2007	UK	19	79	58	21	606	223	3530
2006-2007	CEEREAL	20	80	45	15	246	165	758

Average Levels of T-2 and HT-2 toxins in Raw Oats 2000-2007

	Number of Samples	Percentage of Samples		Mean (µg/kg)	Median (µg/kg)	Max (µg/kg)
		>50ppb	>500ppb			
Survey Mean	1467	66	15	317	154	2891
Survey Median	1467	70	10	282	130	1532
Mills Mean	92	81	22	468	239	2540
Mills Median	92	80	18	430	194	2936

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

Year	Country	Number of Samples	Percentage of Samples			
			>500 ppb	> 750 ppb	>1000 ppb	>2000 ppb
2003-2007	Europa	92	21	14	11	11
2003	Finland	30	17	13	7	
	UK	104	33		18	6
2004	Finland	30	10	10	7	
	Norway	41	0	0	0	0
	UK	128	24		13	5
2005	Finland	60	33	20	10	10
	Sweden	41	17	12	2	0
	Norway	126	14	9	4	1
	UK	134	44		25	9
2006	Finland	59	5		2	
	Sweden	71	44	17	13	0
	Norway	102	9	4	2	0
	UK	100	43			
2007	Finland	80	5			0
	Norway	32	6	6	0	0
	Sweden	24	4	0	0	0
2003-2007 Median			17	10	7	0

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

Oat product	Year	Country	Number of Samples	Percentage of Samples			Mean (µg/kg)	Median (µg/kg)	Max (µg/kg)	Reference
				>50ppb	>200ppb	>500ppb				
Oat flakes	2000-2001	Norway	18	0	0	0	20	20	20	Clasen 2003
Oat groat	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 flake/groats	2005	Unknown	29	6	3	3	49	18	607	Biselli 2006
Oat products	2006-2007	CEEREAL	185	9	0	0	22	16	112	CEEREAL 2007
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	2006-2007	CEEREAL	20	100	85	60	763	578	1558	CEEREAL 2007
Oat bran	2000-2001	Norway	23	0	0	0	20	20	20	Clasen 2003
Oat meal	2000-2001	Norway	5	0	0	0	20	20	20	Clasen 2003

Low levels in Oat flakes and Oat meal!

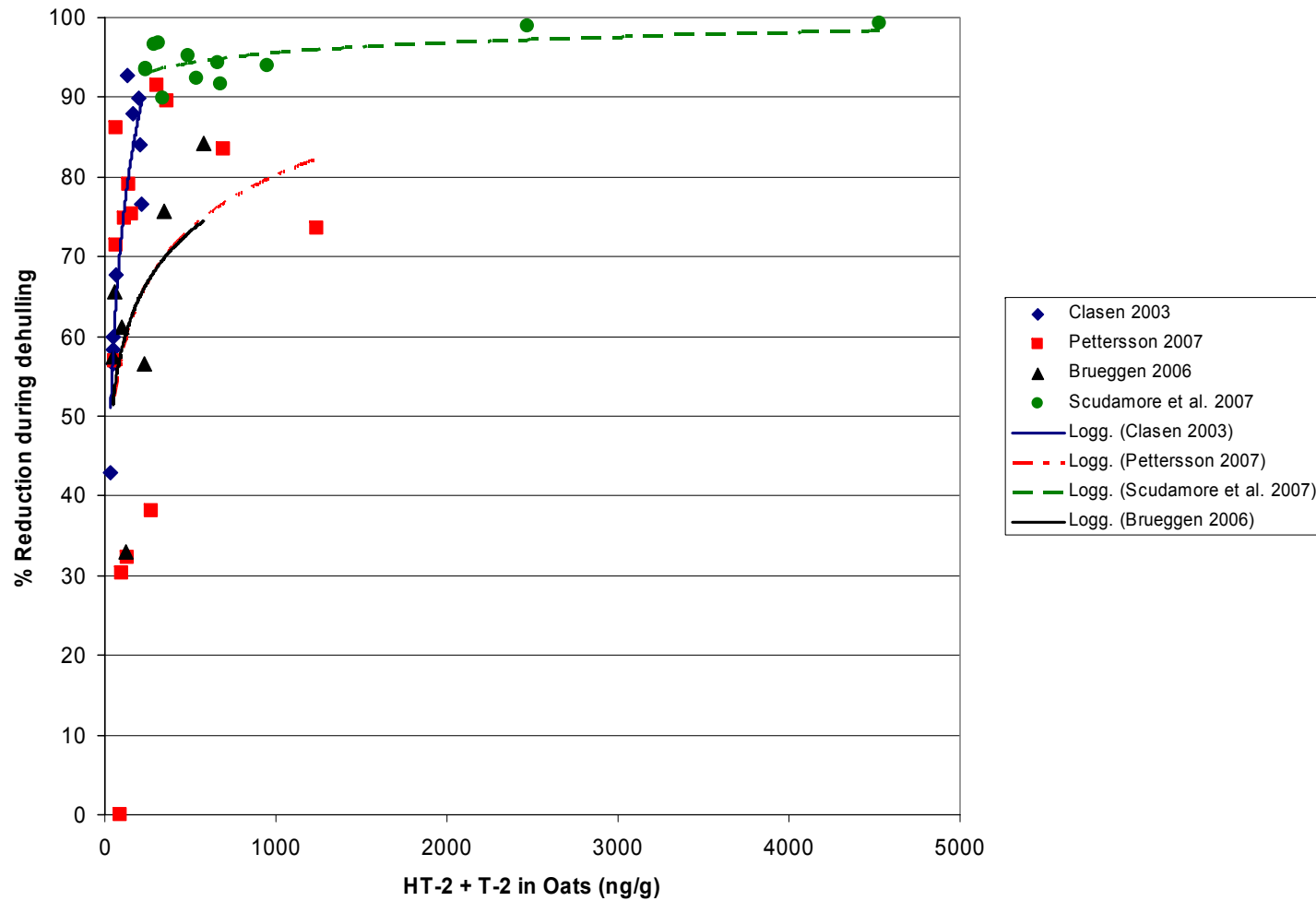
High levels in Oat by-products!

Oat Processing Reduce 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
 - Lower reduction at low levels
- Sortex cleaning of dehulled oats – discoloured fraction higher in HT-2/T-2

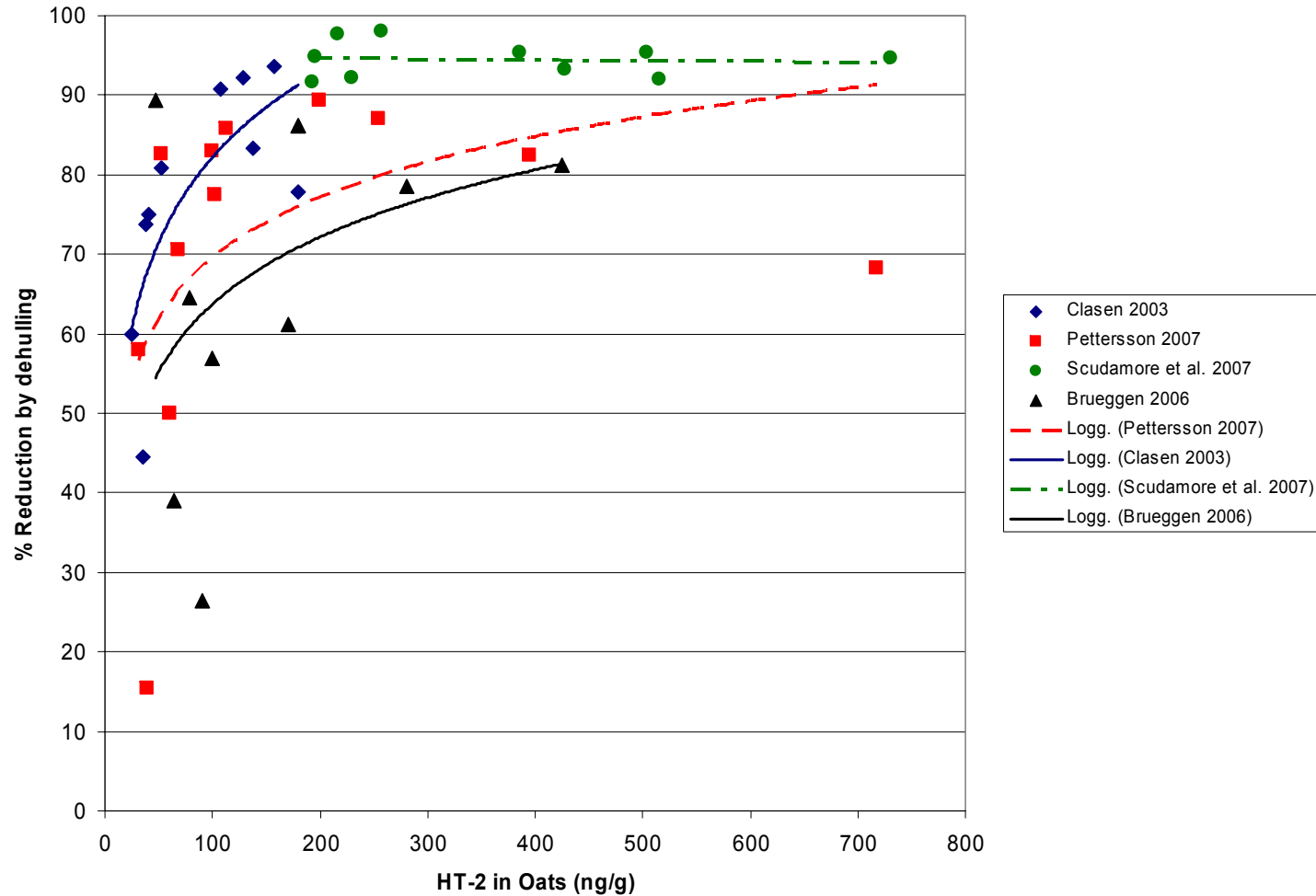
Reduction in T-2+HT-2 During Dehulling

Results mainly from Mill Processing

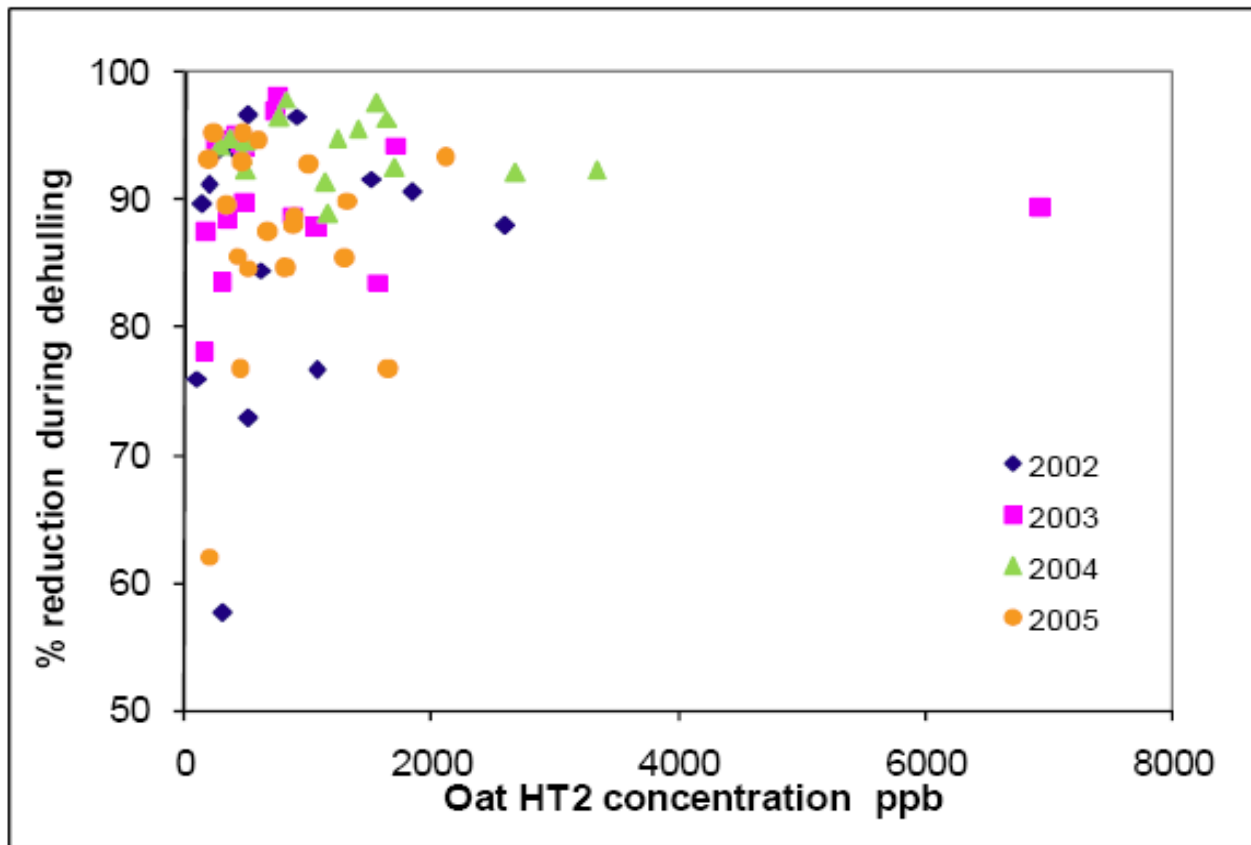


Reduction in HT-2 During Dehulling

Results mainly from Mill Processing



Percentage Reduction in HT-2 during Dehulling, UK lab scale (from Edwards 2007)



Summary Reduction in T-2 + HT-2 by 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**

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!

Analytical Methods

T-2 + HT-2 toxins

- Difficult analyses – Improvements needed
 - → 50% variation between laboratories
 - matrix effects
- No Standardized method
 - GC-MS → LC-MS/MS
- Rapid method needed for own control
- Calibration
 - Calibrant concentration
 - Internal labelled standards
 - Certified Reference Materials lacking
 - Control samples + Proficiency testing
- Extraction efficiency for T-2 in natural contaminated Oats
 - Low for methanol-water?

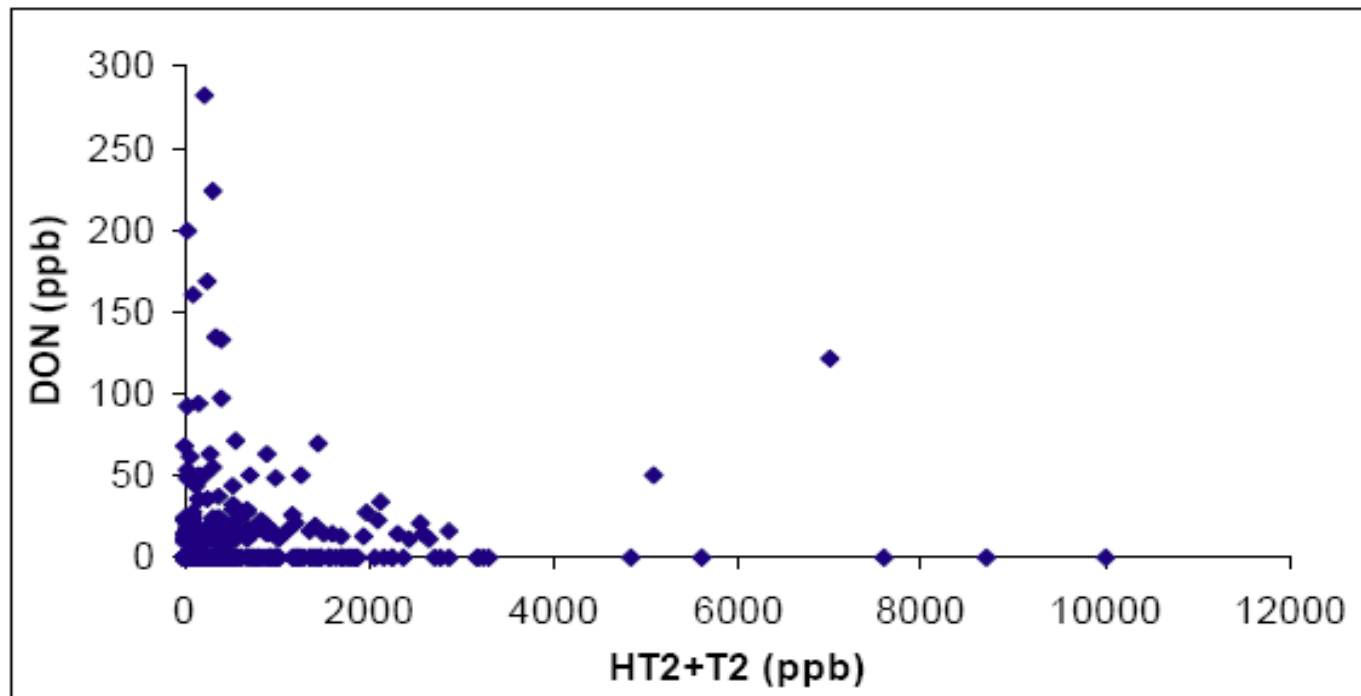
Toxicology + Risk Assessment

- Risk Assessment Food
 - Nordic (1998), Scientific Committee Food (2001), JECFA (2001)
 - Similar tTDI 0,06 µg/kg bw, day
 - Deficiency in tox database. Long term studies.
 - High (500) safety factor used
- Risk Assessment Feed
 - Need to be done
 - Little tox information on highly exposed animals (horses, ruminants)

Agricultural Practice to Reduce T-2 + HT-2 in Oats

- Best way for control! But how?
- EU Recommendation on the prevention and reduction of fusarium toxins in cereals not applicable for T-2 + HT-2 in oats!
- Different Fusarium fungi (*F. langsethiae*) with different biology and toxin production
- Time and way of infection unknown!

DON against HT-2 + T-2 conc. in UK Oat samples 2002-2005



(n=458)

After Edwards 2007

Agronomy Factors T-2 and HT-2 in Oats

- Organic cultured oats lower in T-2 and HT-2 (Gottschalk 2007; Edwards 2007; Biselli 2007)
- Differences between varieties (Edwards 2007; Hietaniemi 2007; Pettersson 2007)
- No or small effect of fungicides (Comet, Amistar, Tilt Top, Proline) (Pettersson 2007)
- Previous crop may have effect (Edwards 2007)
- Cultivation – Ploughing no effect? (Edwards 2007)
- Fertilization
- Weather - Climate dry and warm increase?

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



Thanks for Your Attention!

and

Thanks to All Contributing
Laboratories



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