Joint Research Project

„Improvement and Validation of Methods of Analysis for Type A Trichothecenes (T-2 Toxin and HT-2 Toxin), and Occurrence of these Mycotoxins in Foods in Germany“

Project Status Quo Report January 2008

at the

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Joint Research Project „Improvement and Validation of Methods of Analysis for Type A Trichothecenes (T-2 Toxin and HT-2 Toxin), and Occurrence of these Mycotoxins in Foods in Germany“ 01.01.2006-31.12.2008

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Main Objectives of the Project
1. Develop new and/or improve existing analytical methods for T-2 toxin and HT-2 toxin. Methods should be sufficiently sensitive and broadly applicable for food analysis, considering the requirements of the TDI for these toxins (0.06 ng/kg b.w.). Methods should be useful for various purposes: - Quantitative Screening Methods - Quantitative Routine Methods - Quantitative Reference Methods
2. Perform a multi-year survey (retail shops) to calculate T-2/HT-2 toxin levels as present in food purchased by the German consumer. This includes matrices known to possibly contain T-2/HT-2 toxin, als well as „search analyses“ in other matrices
3. Calculate the T-2/HT-2 toxin intake of the German consumer

What detection limits are necessary for T-2/HT-2 in aspects of food safety and consumers‘ protection? (Child, 16.15 kg b.w.)

Assumed LOD 20 µg/kg, negative results expressed as 10 µg/kg, all results <LOD

	Intake, g	Reported toxin level, µg/kg	Calculated toxin intake, µg	Toxin intake, µg/kg b.w.	Toxin intake as % of TDI
Oats long term intake	3.3	0.01	0.033	0.0017	2.8
Oats short term intake	165.3	0.01	1.653	0.0827	137.8
Maize long term intake	2.4	0.01	0.024	0.0012	2.0
Maize short term intake	168.6	0.01	1.686	0.0843	140.5
Wheat long term intake	66.4	0.01	0.664	0.0332	55.3
Wheat short term intake	228.6	0.01	2.286	0.1143	190.5
Rye long term intake	12.8	0.01	0.128	0.0064	10.7
Rye short term intake	97.5	0.01	0.975	0.0488	81.3
Rice long term intake	4.3	0.01	0.043	0.0022	3.6
Rice short term intake	187.8	0.01	1.878	0.0939	156.5
% usage of the TDI at long term intake					**74.3**
% usage of TDI at short term intake					**706.5**

=> Even if all samples would be negative, no useful information could be obtained concerning toxin intake at the TDI level with a LOD of 20 µg/kg.

What detection limits are necessary for T-2/HT-2 in aspects of food safety and consumers‘ protection? (Child, 16.15 kg b.w.)

Assumed LOD 2 µg/kg, negative results expressed as 1 µg/kg, all results <LOD

	Intake, g	Reported toxin level, µg/kg	Calculated toxin intake, µg	Toxin intake, µg/kg b.w.	Toxin intake as % of TDI
Oats long term intake	3.3	0.001	0.0033	0.0002	0.3
Oats short term intake	165.3	0.001	0.1653	0.0083	13.8
Maize long term intake	2.4	0.001	0.0024	0.0001	0.2
Maize short term intake	168.6	0.001	0.1686	0.0084	14.1
Wheat long term intake	66.4	0.001	0.0664	0.0033	5.5
Wheat short term intake	228.6	0.001	0.2286	0.0114	19.1
Rye long term intake	12.8	0.001	0.0128	0.0006	1.1
Rye short term intake	97.5	0.001	0.0975	0.0049	8.1
Rice long term intake	4.3	0.001	0.0043	0.0002	0.4
Rice short term intake	187.8	0.001	0.1878	0.0094	15.7
% usage of the TDI at long term intake					**7.4**
% usage of TDI at short term intake					**70.7**

=> To obtain useful information concerning the T-2/HT-2 intake of the German consumer, LODs in the range of 2 µg/kg (or better) have to be achieved.

Analytical Methodology

Quantitative Screening Method:

Enzyme Immunoassay (Recognizes T-2 and HT-2 Toxins)

Quantitative Routine/Reference Methods:

GC-Electron Capture Detection (IAC)
LC-MS/MS (SPE, Bond Elut Mycotoxin)
LC-MS/MS (ASE)

Method development:

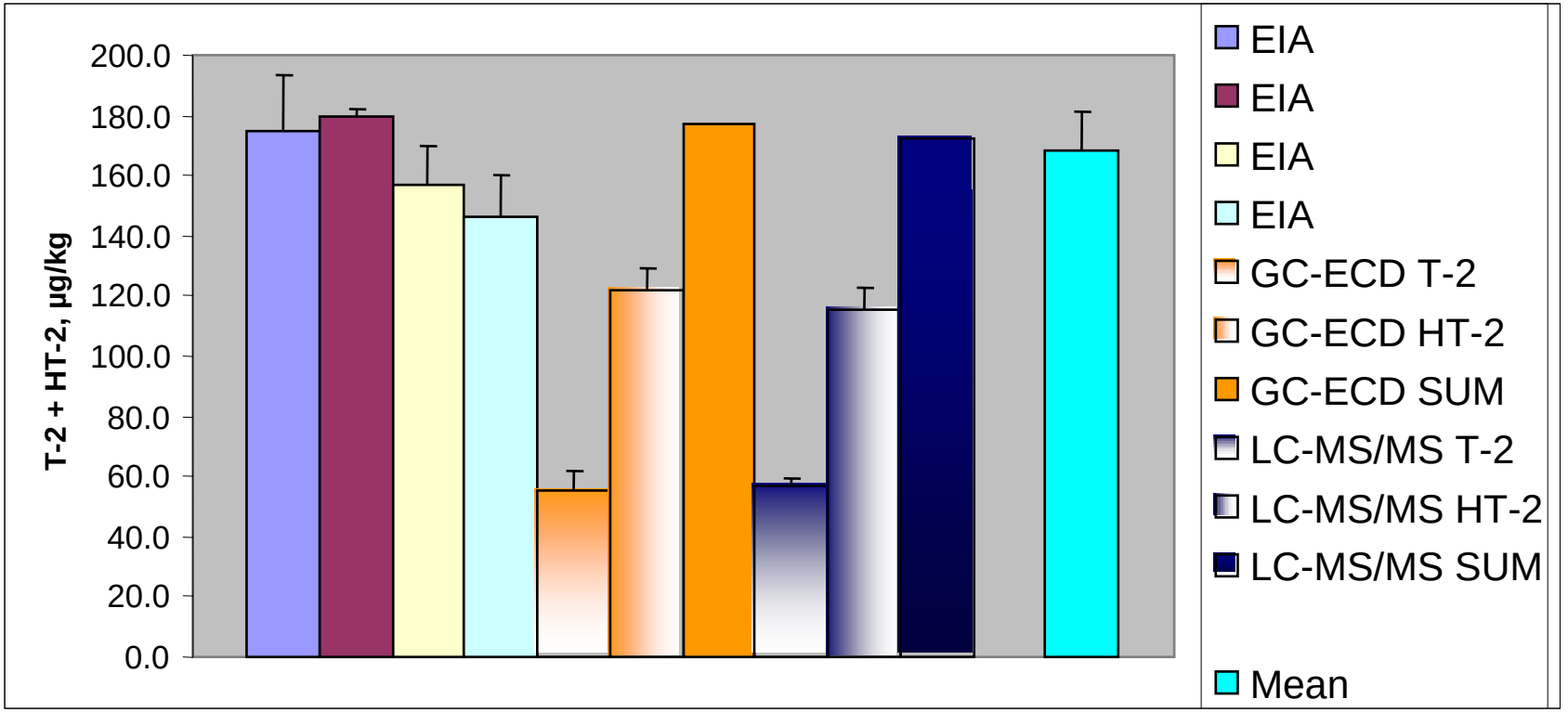
HPLC-Fluorescence detection (IAC, Precolumn derivatization)
HPLC-Immunoassay

Main methods, performance characteristics

	EIA	**CG-ECD**	**LC-MS/MS**
LOD, µg/kg	(0.2-)0.5 Sum of Toxins	1.1-1.7 T-2 1.5-2.3 HT-2	0.14-0.25 T-2 1.5-4 HT-2
Recovery data summary			
Measured mean recoveries (levels 1-50 µg/kg)	81-91	59-116	85-100
Acceptable recoveries (at 50-200 µg/kg)*	60-130		
RSD_r of mean, %	6-14	6-20	2-7
Acceptable U*f* ** range expressed as RSD, %	18-25	18-30	18-30

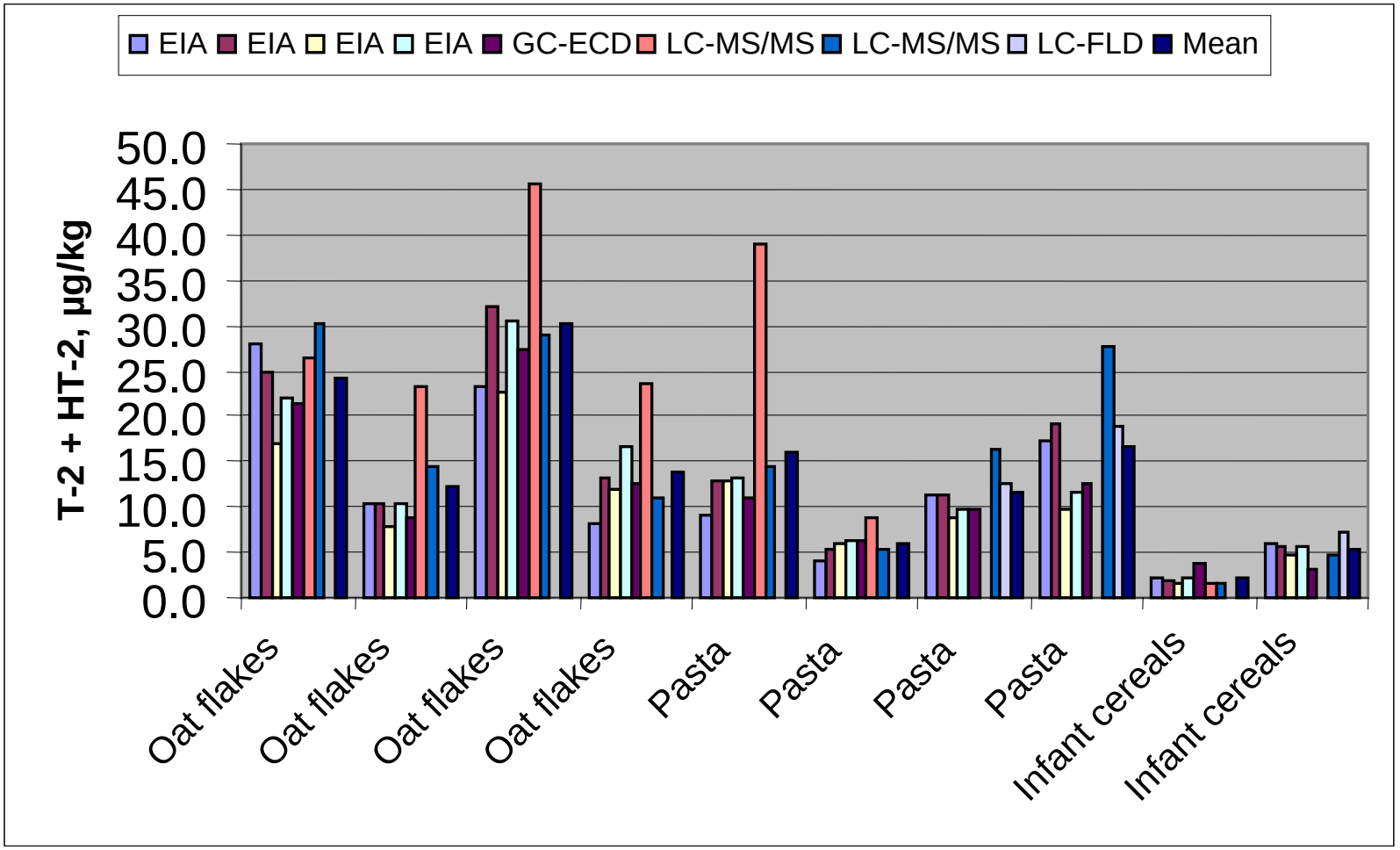
*performance criteria for T-2 and HT-2 Toxin
* „Maximum standard uncertainty“ according to the fitness-for-purpose approach Regulation 401/2006

Main methods, interlaboratory comparison studies



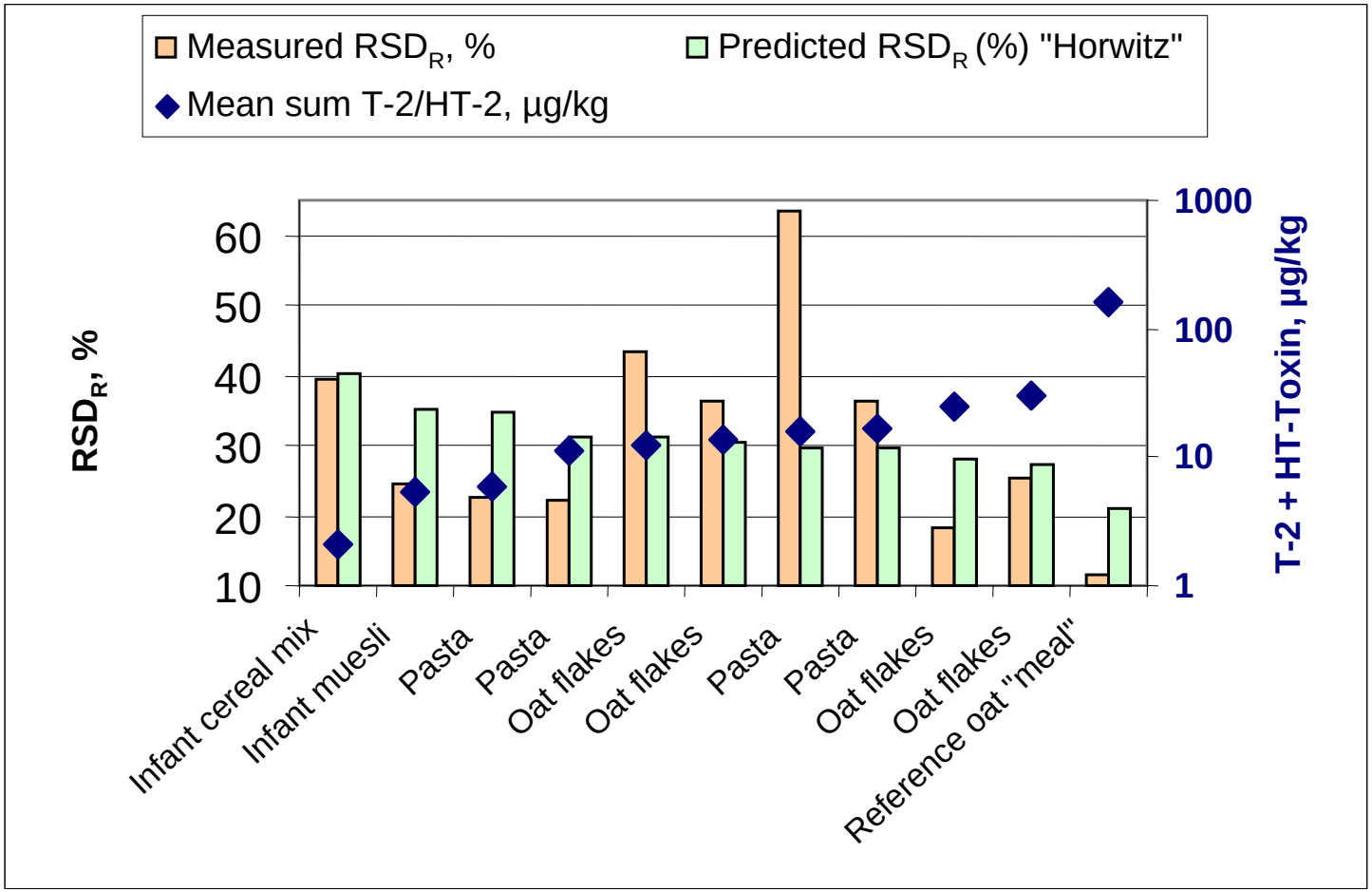
Internal reference material (oat „meal“,
prepared from naturally contaminated oat)
for validation at **high toxin levels**

Main methods, interlaboratory comparison studies



Interlaboratory comparisons using naturally contaminated material for validation at **low/medium toxin levels**

Main methods, interlaboratory comparison studies



Interlaboratory comparisons using naturally contaminated material for validation at **low/medium toxin levels**

Conclusion - analytical methods

- Analytical method have been established which are suitable to detect T-2 and HT-2 toxin in the range of interest (1 µg/kg).

- Validation studies show performance criteria which are substantially better than the requirements set by Regulation 401/2006

Food Survey

- Samples were purchased from retail shops, discounters, organic farming shops as offered.

- Total number of samples: 2895
- 2006: 1466
- 2007: 1429

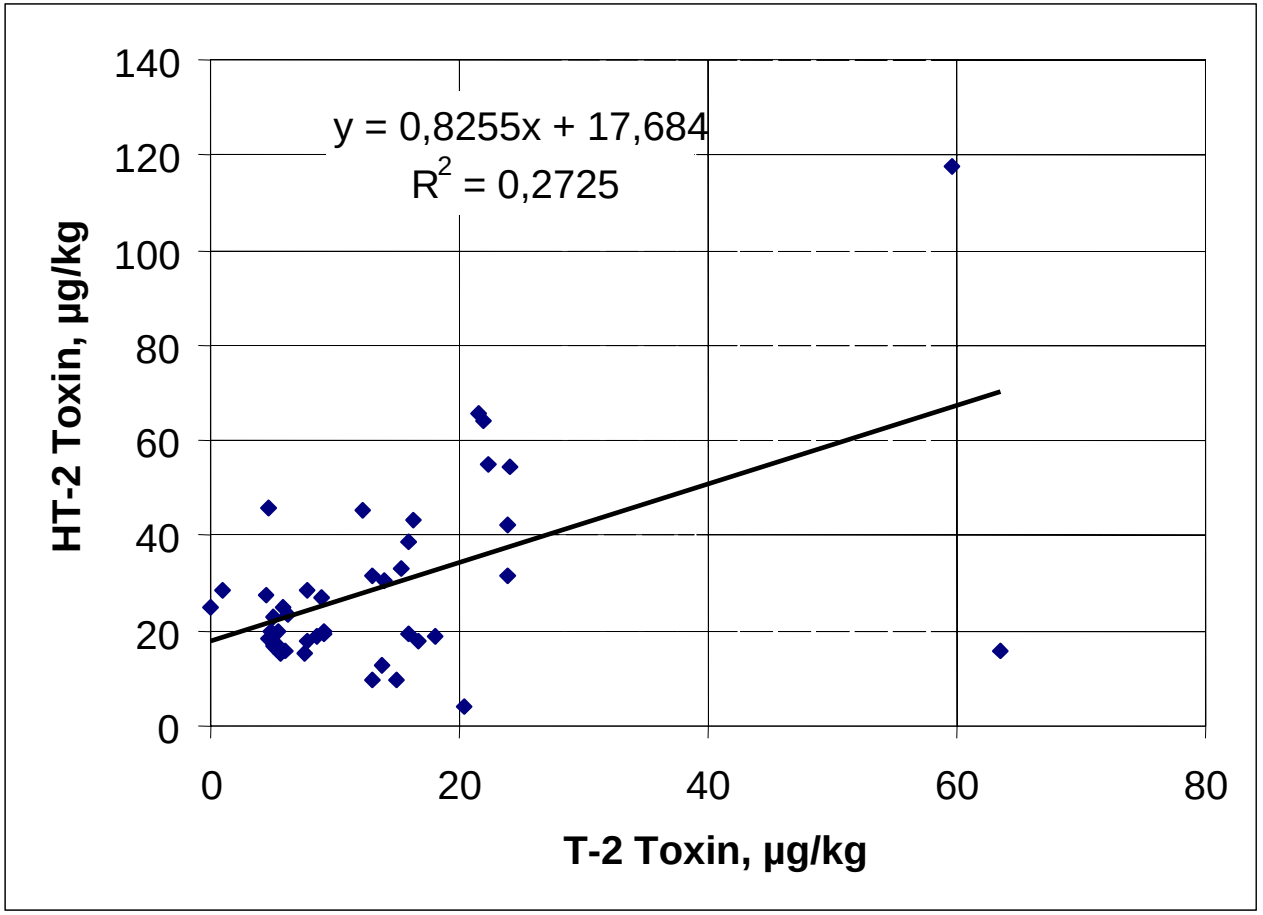
Food Survey

- Grouping of samples according to the 6-digit German food code (Matrix code). Important food groups included:
- **15xxxx:** Cereals for direct consumption (**n=213**)
- **16xxxx:** Cereal products, including 1601xx Cereal flour, 1602xx Cereal grits, 1606xx + 1611xx Breakfast cereals and muesli (**n=1056**)
- **1 7xxxx:** Bread and rolls (**n=238**)
- **18xxxx:** Fine bakery products (**n=294**)
- **22xxxx:** Pasta (**n=354**)
- **48xxxx:** Infant foods (>4 months) containing cereals (**n=354**)
- Plus 386 samples of other food matrices, including non-alcoholic beverages, beer, seeds, nuts, chocolate, spices, potatoes, vegetables...

Food Survey

- For evaluation, the sum of T-2 and HT-2 was used
- At n=1016, both toxins were detected in 49% of positive samples. T-2 only was detected in 13%, and HT-2 only was detected in 38% of all samples.
- Negative samples (<LOD) were calculated as 0.5 x LOD, typically 0.2-0.5 µg/kg

T-2 and/or HT-2 in foods



Comparison of T-2 toxin und HT-2 toxin levels in highly contaminated samples from the German market (>20 µg/kg total toxins; n=43).
The mean contribution of T-2 toxin to the total toxin burden was at 31.2 +/- 17% 0,2-84%). There is only a very weak correlation between the two toxins.

Food Survey

GENERAL RESULTS

- **High frequency** of T-2 and HT-2 in cereals:
  a total of 2270 samples (78%) was positive for T-2 and/or HT-2 toxin

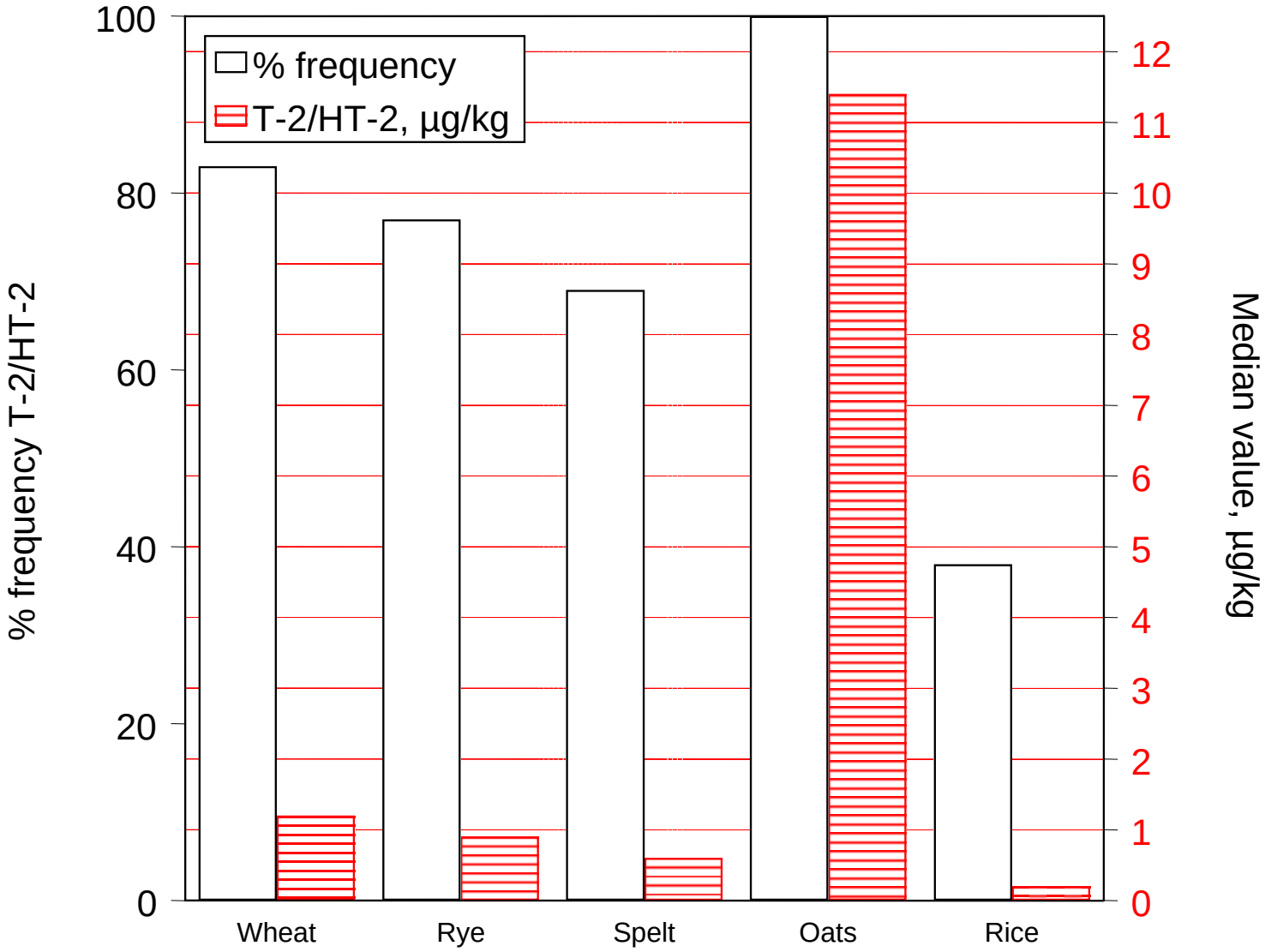
- **Low levels** of T-2 and HT-2 in cereals:
  the overall median value in all samples was at 1.1 µg/kg
  the 95% percentile was 15 µg/kg in 2006 and 13 µg/kg in 2007

- The most relevant commodities were wheat, oats, and products thereof

Food Survey, 2006

Food type	Matrix code	n	T-2 + HT-2, µg/kg						
			positive	mean	max	min	Percentiles		
			%				50 P median	75 P	90 P	95 P
Cereals for direct consumption	**15…**	**93**	**81**	**4,50**	**177**	**0.20**	1.2	**2.0**	**6.8**	**11.4**
Wheat	1501-00, -01	29	83	2.02	7.9	0.25	1.2	2.0	4.2	7,5
Rye	1502-00,-01	13	77	1.49	5.0	0.25	0.9	1.9	3.8	4,5
Spelt	150103	13	69	0.89	2.5	0.25	0.6	1.4	1.6	1,9
Oats	1504-00, -01	9	100	30.18	177	0.94	11.4	11.5	71.0	124,1
Rice	150600	14	38	0.22	0.3	0.20	0.2	0.2	0.3	0.3

Food Survey, 2006



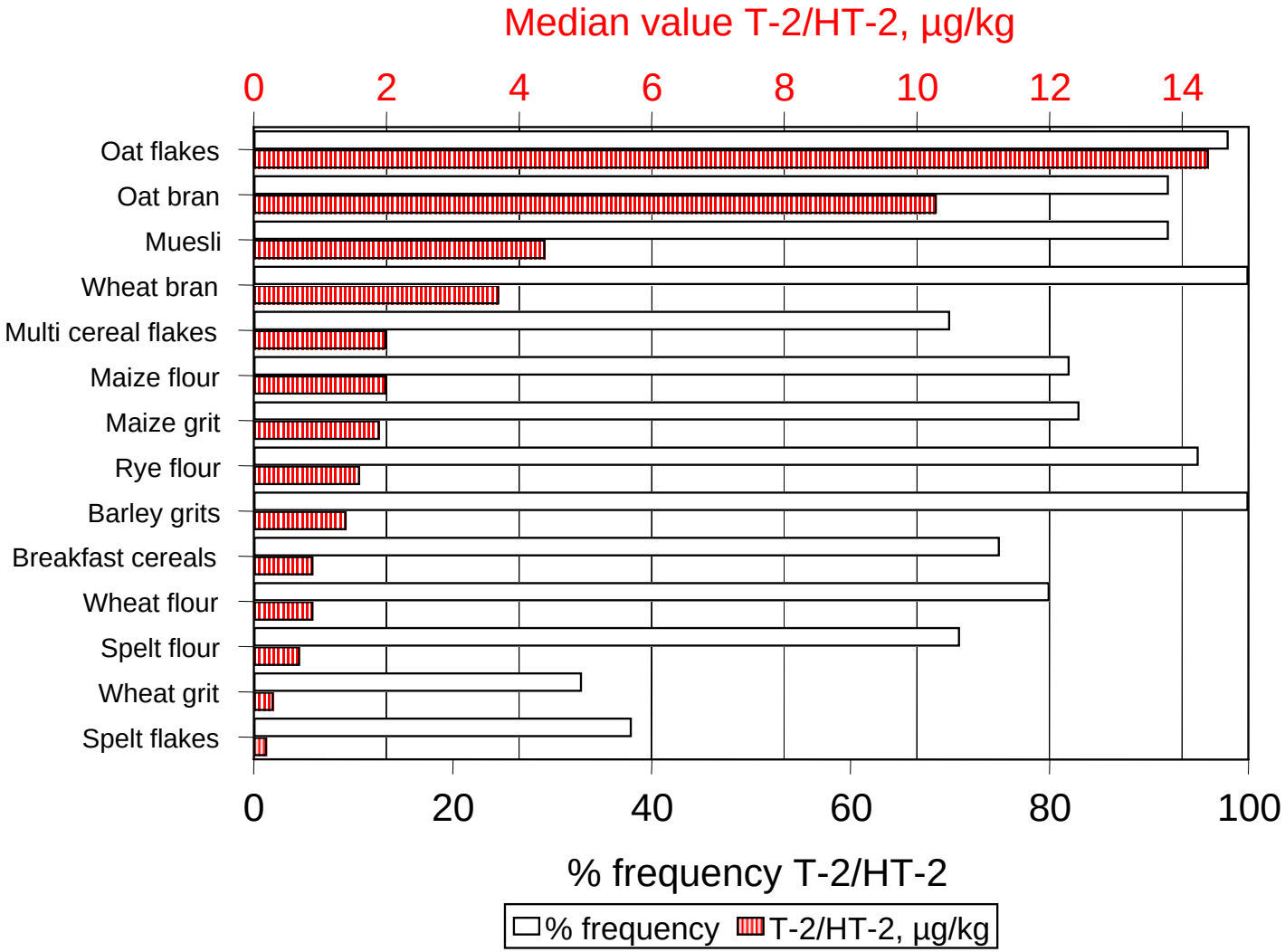
Food Survey, 2006 vs. 2007

Food type	Matrix code	n	T-2 + HT-2, µg/kg							
			positive	mean	max	min	Percentiles			
			%				50 P median	75 P	90 P	95 P
Wheat for direct consumption 2006	1501-00, -01	29	83	2.02	7.9	0.25	1.2	2.0	4.2	7.5
2007	1501-00, -01	37	81	2.15	13.2	0.09	0.8	2.5	6.4	7.8
Oats for direct consumption 2006	1504-00, -01	9	100	30.18	177	0.94	11.4	11.5	71.0	124.1
2007	1504-00, -01	14	100	5.41	22	1.34	3.4	4.8	13.7	18.7

Food Survey, 2006

Food type	Matrix code	n	T-2 + HT-2, µg/kg						
			positive	mean	max	min	Percentiles		
			%				50 P median	75 P	90 P	95 P
Cereal "grits", Cornflakes, Breakfast cereals	16060-0, -1, -5, -6	61	75	5.1	87.3	0.20	0.9	3.3	6.0	9.8
Wheat bran	160801	13	100	6.4	26.1	1.27	3.7	5.0	13.3	18.9
Oat bran	160805	24	92	13.2	29.2	0,20	10.3	21.3	25.9	27.3
Oat flakes	160907	81	98	20.5	79.5	0,20	14.4	28.5	44.8	55.6
Barley grits	16090-8, -9	7	100	1.3	1.7	0.75	1.4	1.6	1.7	1.7
Spelt flakes	160916	8	38	0.6	1.9	0.20	0.2	0.5	1.5	1.7
Multi cereal flakes	160998	10	70	2.3	5.4	0.20	2.0	3,3	5,2	5.3
Cereals mixed with other ingredients (Muesli type)	1611-08, -13, -14, -15, -17, -18	65	92	6.7	48.6	0.20	4.4	9.0	13.1	16.1

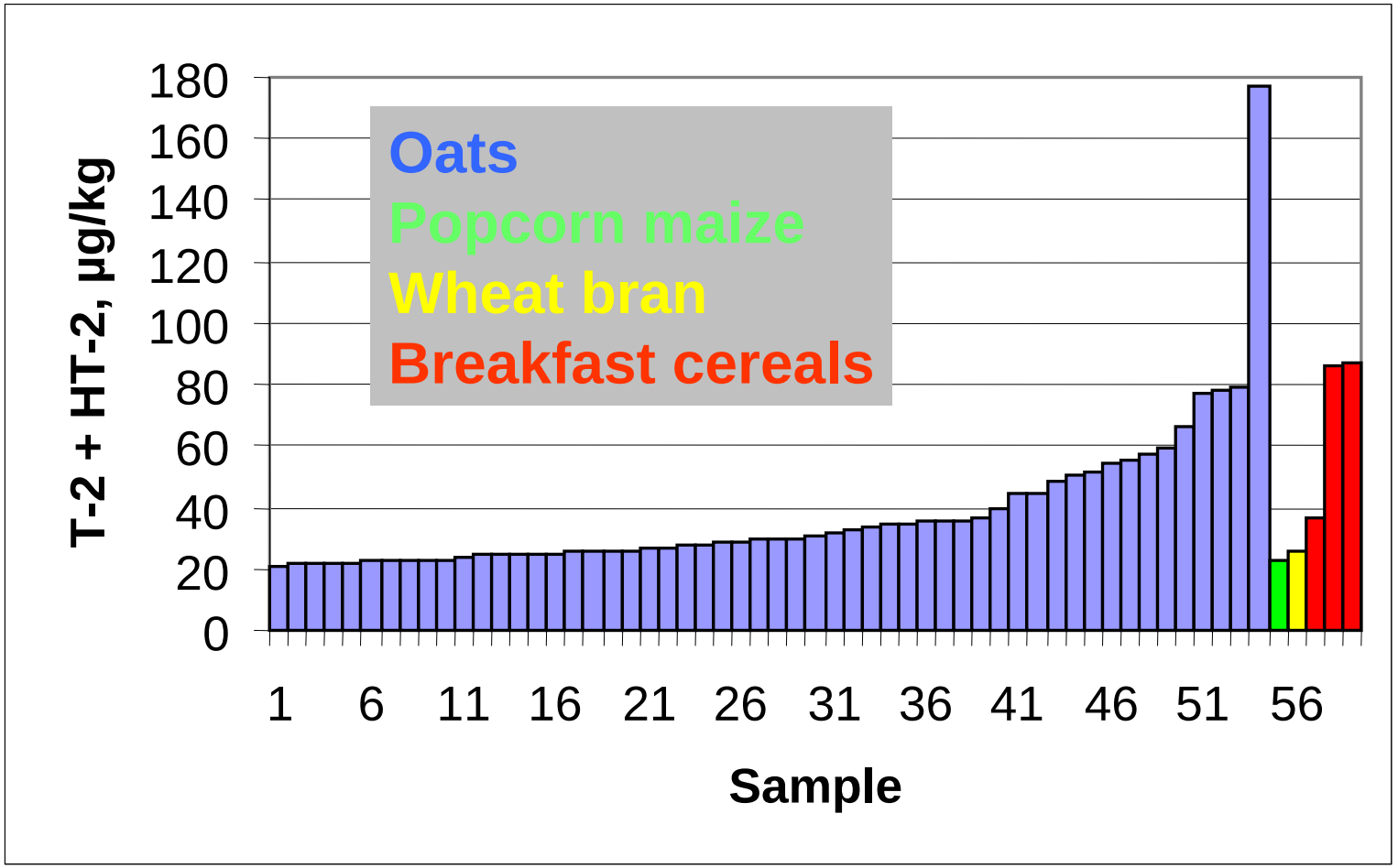
Food Survey, 2006



Food survey, 2006 vs. 2007

Food type	Matrix code	n	T-2 + HT-2, µg/kg						
			positive				Percentiles		
			%	mean	max	min	50 P median	75 P	90 P	95 P
Cereal "grits", Cornflakes, Breakfast cereals 2006	16060-0, -1, -5, -6	61	75	5.1	87.3	0.20	0.9	3.3	6.0	9.8
Cereal "grits", Cornflakes, Breakfast cereals 2007	16060-0, -1, -5, -6	36	75	2.8	34.5	0.20	1.2	3.2	5.3	7.1
Oat flakes 2006	160907	81	98	20.5	79.5	0,20	14.4	28.5	44.8	55.6
Oat flakes 2007	160907	54	100	14.4	50.7	1.01	12.6	18.0	26.0	31.7
Cereals mixed with other ingredients (Muesli type) 2006	1611-08, -13,-14, -15, -17, -18	65	92	6.7	48.6	0.20	4.4	9.0	13.1	16.1
Cereals mixed with other ingredients (Muesli type) 2007	1611-08, -13, -14, -15, -17, -18	115	95	5.3	28.1	0.20	3.7	6.7	11.2	16.6

Food survey 2006



„Highest“ 2006 samples (>20 µg/kg, n=59): 54 were plain oats products

Food survey, 2006 vs. 2007
Cereal products and infant foods

Food type	Matrix code	Year	n	% positives	median, µg/kg	P95 µg/kg	max µg/kg
Bread and rolls	17xxxx	2006	162	72	0.75	3.6	10.3
		2007	76	80	0.8	1.9	3.8
Fine bakery products	18xxxx	2006	117	84	1.2	5.7	66.2
		2007	177	84	1.4	8.4	26.7
Pasta	22xxxx	2006	223	89	1.3	6.2	14.4
		2007	132	80	1.5	5.6	16.9
Infant foods containing cereals	48xxxx	2006	163	83	1.8	12.9	30.6
		2007	134	72	0.9	7.8	23.8

Food survey 2007
Selected other products

Food type	Matrix code	n	% positives	median, µg/kg	P95 µg/kg	max µg/kg
Beer, malt drinks	36xxxx	33	79	0.6	1.0	1.5
Confectionery, sweets (cont. cereals)	40xxxx	24	96	1.4	3.0	5.6
Soy products	23xxxx	32	56	0.6	3.3	4.4

Consumption of T-2/HT-2 via Food: How far is the TDI?

Necessessary daily consumption of some food commodities, which would result in a 10% exhaustion of the TDI (0.06 µg/kg b.w.) by an adult (70 kg). Calculation based on the median value (T-2 toxin + HT-2 toxin) as found in foods in 2006.

Commodity	Median (T-2 Toxin + HT-2 Toxin), µg/kg	Daily consumption resulting in 10% of the TDI
Oat flakes	14.4	29 g
Muesli	4.4	95 g
Bread, rolls	0.7	600 g
Breakfast cereals	0.9	470 g
Fine bakery products	1.3	320 g
Pasta	1.3	320 g

T-2/HT-2 in Food: „Preliminary Conclusions“

Low levels of T-2 and/or HT-2 occur in the vast majority of cereal containing foods. Median values are typically at around 1 µg/kg, except for oats and oat products, with are about 3-10 times higher.

Highly contaminated samples typically contained T-2/HT-2 at levels of 10-20 µg/kg, only very few samples exceeded a value of 20 µg/kg. Maximum values in oats and products thereof were at 100 µg/kg.

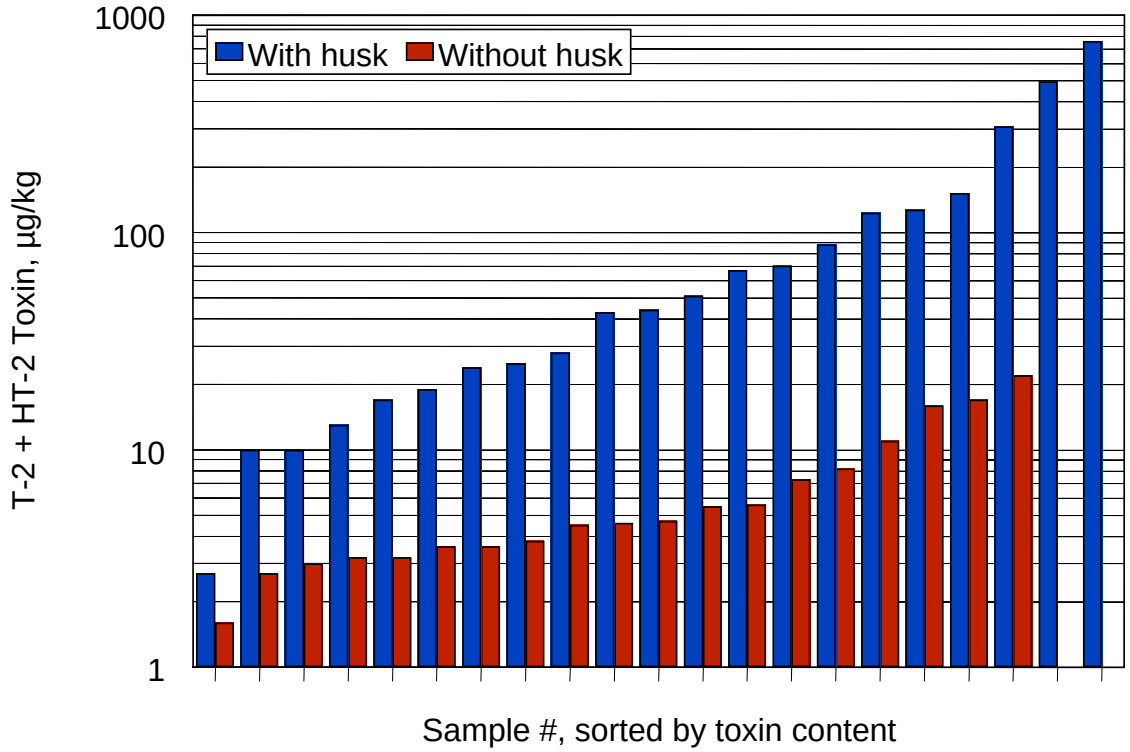
Since the consumption of wheat and wheat products far exceeds that of oats, the low levels found in bread, fine bakery products and pasta, are not irrelevant.

In a mean case scenario (median toxin levels, median consumption) the intake of T-2/HT-2 toxin by the German consumer is much lower than the TDI.

Special Harvest Survey 2005

Dehulled oats for human consumption vs. oats with husk for animal feeding

- Preliminary data -



	Without husk	With husk
n	19	21
T-2/HT-2, mean +/- SD, µg/kg	6.9 +/- 5.6	118 +/- 180
T-2/HT-2, median, µg/kg	4.6	44

=> About 90% of the T-2/HT-2 seems to go with dehulling.