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A survey on the presence of undesirable botanic substances in feed in the European Union

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Summary

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed lists a range of substances from botanic origin (weed seeds) and additionally some chemical compounds directly originating from specific weeds. In order to examine the actual status of enforcement and of the present occurrence of these botanic substances, a survey was carried out. A questionnaire was sent to 103 laboratories, including official control labs from all member states of the European Union. The results, indicating the frequency of occurrence as far as reported, are compared to the publications of the EU Rapid Alert System for Food and Feed (RASFF).
A total of 44 questionnaires were returned (42.7 %) from 22 member states. Ten member states predominantly from north-western Europe appeared to have an active monitoring of botanic undesirable substances. The questionnaire results did not indicate that the other member states enforce this part of Directive 2002/32/EC. Reports on the frequency of occurrence include: a few to 25-50 % of the samples contain traces of ergot (eight member states), a few to 24 % contain at least some traces of thorn apple (six member states), zero to 17 % contain some castor oil plant seeds (three member states), zero to a few samples contain *Crotalaria* seeds (three member states), and zero to 6 % contain traces of sareptian mustard (four member states). A few traces were found of the other mustard species, of croton and of *Lolium temulentum*. One member state conducted extra surveillance since several cases of animal intoxifications have been reported. In some cases a coincidence with undesirable botanic substances was found.

Conclusions and recommendations:
The current survey indicates that botanic undesirable substances do occur in certain frequencies. The occurrence in feed is supported by the reported cases of animal intoxifications. More specific data is desired. In order to evaluate the extent of the occurrence of the undesirable botanic substances, an effective monitoring of these substances in all member states for at least one or two years is recommended. Such a survey would need to include both macroscopic and microscopic examination to be ascertain that no material will be missed.
As far as the results of the current survey are considered, the following species could be included in a future list of undesirable botanic substances: rye ergot (*Claviceps purpurea*), thorn apple (*Datura stramonium*), castor oil plant (*Ricinus communis*), *Crotalaria* spp., purghera (*Jatropha curcas*), and croton (*Croton tiglium*). Some of these species might be included in a list of species containing alkaloids, if such a list will be raised. The problem of ragwort (*Senecio jacobaea*), whether or not as part of such a list, deserves special attention.
Besides these species a risk assessment of the other species currently on the list would be appropriate. The respondents considered it reasonable that some of these species can be deleted from the list. Additional species, such as *Galium aparine*, *Polygonum* spp. and *Ambrosia* spp. could be included in a risk assessment.
Microscopy is an effective technique for examination at macroscopic as well as microscopic level. The development and application of chemical detection methods of the toxic compounds deserves encouragement. However, microscopic examination has its advantage for monitoring of emerging risks of new weed seeds and for all those listed seeds for which no chemical detection is available.
Four samples from the years 2005 and 2006 were mentioned in the questionnaires for rye ergot and for thorn apple with amounts at or over the legal limit (in both cases 1000 ppm). These findings are not

listed in the RASFF notifications. The prevalence and origin of differences between actual results and RASFF listings require attention.
There is a desire for improving knowledge levels for the identification of botanic undesirable substances. This can be achieved by organizing training sessions, raising colleague networks as well as the development of dedicated expert systems.

Contents

1 Introduction

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed lists a range of substances from botanic origin (weed seeds) and additionally some chemical compounds directly originating from specific weeds. The Scientific Committee on Animal nutrition has published an opinion (SCAN, 2003) on the entire list of substances mentioned in this directive, including those of botanic origin (indicated as "Botanical impurities"). SCAN concluded that the current practice of microscopic screening for whole seeds or parts thereof should be replaced by a quantitative chemical analysis of the harmful substances contained in these seeds. The majority of the listed species is considered to have only historical interest or poses no real hazard. It was therefore advised to rephrase the current specific listing to a more general statement for the prevention of these unwanted seeds, in order to accommodate for changes in agricultural practice. Besides the desire for dedicated chemical analysis, SCAN concludes that microscopy should be the primary method for the detection of botanical contamination for its flexibility and possibilities to handle emerging problems. Directive 2002/32/EC was not updated until now (January 2007) concerning the botanic substances.
A survey of the occurrence of undesirable substances of botanical origin in feeds can only be based on data from visual inspection, including microscopy. The current amount of data is scarce, at first glance. From networks of microscopists it can be concluded that not every member state of the European Union enforces actively the monitoring of these undesirable substances, although this is requested by Directive 95/53/EC. Before drawing conclusions on the occurrence or on being (almost) extinct of certain weed species, more information is desired on the status of monitoring programs and of their results.
In this report a survey is presented on the current status of monitoring for undesirable substances of botanical origin in feeds, and on the results of these programs. The results, indicating the frequency of occurrence as far as available, are compared to the publications of the EU Rapid Alert System for Food and Feed, in which alert and information notifications are published from the competent authorities on a weekly basis.

2 Methodology and organisation

During Spring 2006 a questionnaire was developed for collecting information on the status, scope and results of the monitoring program, if available on the laboratory of the addressee. This questionnaire consisted of three sections:

1. Questions on the existence and scope of an active monitoring program for visual examination for undesirable substances of botanical origin. If no monitoring program was carried out, the respondent was asked to skip section 2.
2. Tables for registering results and additional remarks for every individual weed seed.
3. Questions concerning proposed deletions from or additions to Directive 2002/32/EC, and concerning required activities for maintaining or increasing expertise.

This questionnaire was presented at the annual meeting of the IAG working group Microscopy in Rostock, Germany, June 2006. After updating the text, it was sent to a total of 103 laboratories known to have microscopic expertise (see Appendix 1 for distribution over member states). At the closing date of 1 December 2006 a total of 44 returned questionnaires was received at RIKILT.

The lists of the EU Rapid Alert System for Food and Feed (RASFF) are based on Regulation EC/178/2002, and are weekly published at: http://ec.europa.eu/food/food/rapidalert/index_en.htm. The lists of 2005 and 2006 were examined for any notification concerning undesirable substances of botanical origin.

3 Results and discussion

3.1 Response

44 laboratories returned their questionnaires, i.e. a response of 42.7% (Appendix 1). Only three member states out of 25 did not return a questionnaire. There is good dispersion among member states.
Almost 80% of the questionnaires were returned by official control laboratories. Private laboratories do not have an official task according to Directive 882/2005/EC (formerly 95/53/EC), but can maintain programs at the request of private companies. In order to get an overview of the occurrence of the specific species undesirable substances of botanical origin, the results from these two categories have been pooled.

3.2 Active maintenance of monitoring

There were hardly any results reported from monitoring in southern or eastern European countries (PT, ES, I, GR, M, CY, SLO, SLK, CZ, EST, LT, LV). One respondent from ES actively examined feed samples, but did not send in the results. Respondents gave estimates for the number of samples evaluated under the requirements of Directive 882/2005/EC (detailed numbers are listed in Appendix 2). It was not always obvious if these numbers indicated total amounts, or numbers per year. In some cases the total number of samples is low, or identical to the number evaluated for undesirable substances. This might be due to the situation that only the samples sent in to the own department are reported. On the other hand, some labs reported high numbers of samples per year, which might be an indication of the number of analyses. It can be concluded that these numbers do not reflect the actual status of the maintenance of monitoring under Directive 882/2005/EC.
Twenty-seven out of 44 laboratories stated to have an active monitoring for undesirable substances of botanical origin. Of these laboratories, nine indicated to have zero samples examined, which means that no effective monitoring is carried out. A total of 18 laboratories out of 44 respondents have actually examined samples of feeds and/or feed raw materials and eventually found undesirable botanic substances. One laboratory provided the results of a survey separately. The information provided by these labs in section 2 of the questionnaire will be analyzed in more detail. All results of the three sections of the questionnaire are listed in Appendix 2.
The mentioned 18 labs reporting effective monitoring originate from 10 member states: FI, P, HU, A, D, DK, NL, B, IRL, ES. The results from the evaluations carried out by IRL and ES are not yet available for this survey. This means that actual results can be discussed in this report from eight member states. Some of these eight member states have organized the maintenance of the monitoring at regional levels. It appears in these cases that a part of these regions perform monitoring, whereas other regions in the same member states did not evaluate samples for the presence of undesirable substances. Some results are reported from B directly related to animal health incidences. The results of these targeted sampling situations are excluded from the overview.
Directive 2002/32/EC requires macroscopic (by eye or at low magnifications) or microscopic (at high magnifications) evaluation of the samples, depending on the type of prohibition: either as whole seeds only or after processing as well. All reporting 18 laboratories stated that macroscopic examinations are

carried out. Three of these laboratories did not perform microscopic examinations. In this way several botanic undesirable substances can almost not be encountered, especially the mustard species.

3.3 Occurrence of undesirable substances

In the following paragraphs some of the botanic undesirable substances as listed in Directive 2002/32/EC will be discussed in more detail. One respondent mentioned that a part of the positive reports for several undesirable substances are based on a surveillance of a limited number of extra samples. These extra samples have been collected after indications of animal intoxifications. Correction for these amounts might result in a modification of the figures, but detailed information is not available. On the other hand, the situation that animal intoxifications are reported might indicate that toxic levels of these botanic seeds occur.

Rye ergot - *Claviceps purpurea*. Ergot is the most frequently mentioned undesirable substance: 12 laboratories reported either numbers of evaluated samples, or frequencies of occurrence, or both (Appendix 2, section 2). A frequency of occurrence of < 5 % for two samples (member state A) means effectively an occurrence of 0 %. Two member states reported no results (member states IRL and ES). In all other cases (nine labs) ergot appears to be present in low or relevant frequencies, up to 25-50 %. The remark was made that ergot occurrence seems to have increased in recent years. The European Commission recognized this problem and invited research project proposals in the first call of the seventh framework program (2007). As far as reported in the survey, a considerable amount of rye samples appeared to be contaminated. Some other cereal grains as well as compound feeds can contain ergot as well. Only one laboratory proposed to delete ergot from the list of undesirable substances provided that a solid chemical test for control can take its place. More precise information on occurrence of *Claviceps* in feed materials, of ergot alkaloid distribution and on toxic effects is desired. This research need is also indicated in the opinion of the Scientific Panel on Contaminants in Food Chain on ergots (EFSA, 2005).

In the questionnaire one laboratory reported three samples for 2005 and 2006 with amounts exceeding the legal limit according to Directive 2002/32/EC (1000 ppm). These samples were not (yet) reported in the lists of the RASFF system.

Thorn apple - *Datura stramonium*. Eight laboratories reported data on the occurrence of thorn apple. In all cases it was found at least occasionally, with varying frequencies over the years. A variety of feed ingredients that can pose threats for the presence of thorn apple were mentioned: wheat, maize, soybean, linseed, sunflower, rapeseed and compound feeds. Some respondents provided detailed lists of results. One respondent reported the occurrence of thorn apple in maize grits at a level of 0,1 % in 2006, which is at the legal limit of 1000 ppm.

The lists of RASFF include notifications for the presence of thorn apple during the Autumn of 2006: it was found six times in millet samples (*Uroclora ramosa*, 5 samples) and canned green beans (1 sample) originating from HU and A, all for human consumption (figure 1). Thorn apple at a high concentration level was reported once in red millet seeds for feeding purposes by D originating from HU. The absence of this report in the returned questionnaires can be due to the fact that some German laboratories did not respond. In addition, atropine and scopolamine, the main alkaloids, were found twice in buckwheat flour during summer 2006. In the same periods of 2005 no reports were made in the RASFF listings. This absence in the notifications can be due to differing natural occurrences over the years, or to the absence of active monitoring in 2005. The occurrence of thorn apple in maize grit as mentioned in one questionnaire was not reported in the RASFF system. The presence in materials for human consumption

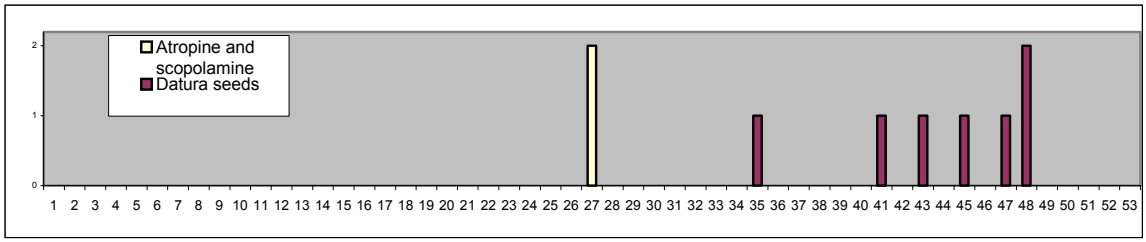

Figure 1. Occurrence of Datura seeds or of the toxic compounds in samples from food and feed monitoring programs (RASFF reporting) in weeks for the year 2006.

can obviously not be reported in monitoring programs for feeds, but the combined data for all materials (food and feed) indicate that thorn apple is certainly not eradicated. Its occurrence and toxicity imply the need for a legal limit.

Castor oil plant - *Ricinus communis* and for *Crotalaria* spp. Only four laboratories indicated to perform monitoring for ricinus or castor oil plant, and *Cotalaria* species. Material of these species is reported to occur occasionally by some countries. Although castor oil beans are usually very easy to recognise, seeds of *Cotalaria* species are much more difficult to detect. Respondents propose to keep these species in the list of undesirable substances because of their high toxicity. From one member state (in the years 1996 and 1998) two occurrences of *Ricinus communis* were found coincided with animal health incidences.

Mustard species – *Brassica* spp. Two to four laboratories reported to search for seeds of mustard species, depending on the species. Reported occurrences were (much) below 5 %, except for one report from B (17 %) for sareptian mustard (*Brassica juncea* ssp. *juncea*). Frequencies below 5% in an amount of 2-3 samples per year mean actually that no mustard seeds were found. It can be questioned whether some of these mustard seeds would need to be included on the list of undesirable substances, since they are used as spices for human consumption and for their moderate toxicity. From one member state two occurrences in the year 1999 of *Brassica juncea* ssp. *juncea* were found coincided with chicken death incidences.

Purghera - *Jatropha curcas* and **Croton -** *Croton tiglium*. Only two laboratories reported active control for these species. From these two species, only croton was found very occasionally. Notwithstanding these very low frequencies of occurrence, it was proposed to keep them in the list of undesirable substances because of their high toxicity.

Other species not listed in Directive 2002/32/EC. The responding laboratory from HU reported a series of additional toxic or noxious weeds in 25 samples examined in 2006. Among the latter category, *Galium aparine* (seven samples, 28 %), *Polygonum* spp. (three different species present in 10 samples, 40%, in six samples in combination with *Galium aparine*), *Ambrosia elatior* (four samples, 16 %) and *Cannabis sativus* (one sample, 4 %) were the most predominant. The *Galium* and *Ambrosia* species were also suggested as possible additions to the list of undesirable substances by other respondents.

3.4 Requirements for modifying Directive 2002/32/EC

Proposals for deletion. Some respondents propose to delete the following species from the list in Directive 2002/32/EC: mowrah (*Madhuca longifolia* and *M. indica*), *Lolium temulentum* and *L. remotum*, apricots (*Prunus armeniaca*), bitter almond (*Prunus dulcis*), beech mast (*Fagus silvatica*) and camelina (*Camelina sativa*). Arguments are listed in sections 2 and 3 of Appendix 2. One laboratory mentioned that species can be deleted only when not found over the last 25 years, and after an indication of no risk according to EFSA. On the other hand, six laboratories proposed to keep all current species on the list of 2002/32/EC. Mustard species can be deleted as well in the view of one respondent, whereas another laboratory proposed to keep them as one item: mustard seeds.
Proposals for addition. There is a range of proposals for additions to the list of undesirable substances (see section 3 of Appendix 2). Highlights are *Ambrosia* spp. (5 laboratories), a list of alkaloid containing seeds (two laboratories) and *Galium aparine* (two laboratories). Member state HU maintains a large list of weed seeds which are prohibited according to national legislation. *Ambrosia* and *Galium aparine* are part of this list. Another species listed is *Datura ferox*, which can hardly be distinguished from *D. stramonium. D. ferox* is proposed to be added to the list by the Hungarian respondent.
The RASFF lists for 2006 contain a notification of seeds of the neurotoxic grass pea (*Lathyrus sativus*). This species is not listed in the proposal of the member state HU for additions of Directive 2002/32/EC. The survey was focusing on weed seeds as category of undesirable substances according to the Directive. Other weed contaminants such as common ragwort (*Senecio jacobaea*) can pose threats in certain feed sources (e.g. fodder, grass meal). Control measures for the increasing problem of ragwort can be set as an addition to item 14 ("Weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination including": followed by a list of three species) of Directive 2002/32/EC. The absence of any discussion in this survey does not imply that these weeds are not relevant in this context.

3.5 Expertise maintenance and improvement

The respondents maintain their level of expertise in several ways. Textbooks and internet as sources for information are most frequently mentioned (25 and 23 indications, respectively). Ten respondents report the implementation of knowledge or expert systems, without any further comments. It could be possible that these indications point to the use of this type of systems in general. It is known that only three of these ten respondents use the expert system ARIES for identification of animal by-products. A survey for collecting further information on the type of systems used would be favourable.
Training on the identification of botanic undesirable substances is indicated as the most important source of expertise development (28 respondents). A network of colleagues (24 respondents) is indicated as almost evenly important. Knowledge or expert systems for increasing knowledge are mentioned by ten laboratories. Dedicated software for the identification of weed seeds is in development at RIKILT. Combination of different activities is proven to be profitable, e.g. training by using software programs.

4 Conclusions and recommendations

The enforcement of the control according to Directive 882/2005/EC is not consistent among the EU member states. In the current survey there are hardly any results reported by respondents from southern and eastern European member states. In order to have a proper evaluation of the list of botanic undesirable substances in Directive 2002/32/EC, an effective monitoring in all member states for at least one or two years is recommended. The results of this monitoring together with risk assessments by EFSA [1] would allow the composition of a new updated list with undesirable botanic substances in feed.
The current list of undesirable botanic substances can be divided in four parts, based on the results of the returned questionnaires in the current study. Parameters for this division are the frequency of occurrence as far as active control is enforced, and the general level of toxicity:

- Moderate or frequent occurrence, moderate to high toxicity: rye ergot (*Claviceps purpurea*), thorn apple (*Datura stramonium*), castor oil plant (*Ricinus communis*) and *Crotalaria* spp.
- Low occurrence, high toxicity: purghera (*Jatropha curcas*), and croton (*Croton tiglium*).
- Low occurrence, low to moderate toxicity: mowrah (*Madhuca longifolia* and *M. indica*), *Lolium temulentum* and *L. remotum*, apricots (*Prunus armeniaca*), bitter almond (*Prunus dulcis*), beech mast (*Fagus silvatica*) and camelina (*Camelina sativa*).
- Variable occurrence, insufficiently known toxicity: mustard species (*Brassica* spp.).

Based on the results presented in the current study, the first two categories would be worth considering for a future new list. The third category can be considered as candidates for deletion, but only after a proper risk assessment. The in- or exclusion of the mustard species and the newly proposed species such *as Galium aparine*, *Polygonum* spp. and *Ambrosia* spp. are recommended for an extensive risk assessment.
Microscopy is an effective technique for detection at macroscopic as well as microscopic level. The development and application of validated chemical detection methods of the toxic compounds should be encouraged. However, microscopic examination is still considered valuable for monitoring of emerging risks of new weed seeds and for all those listed seeds for which no chemical detection is available, as is also concluded in the SCAN report (SCAN, 2003).
Four occurrences of rye ergot and thorn apple with amounts at or over the legal limit in 2005 and 2006 were reported in the survey. These findings were not included in the RASFF notifications. On the other hand, one report of thorn apple in red millet seed was not mentioned in the returned questionnaires. The laboratory that reported this occurrence to the RASFF might not have sent in its questionnaire for the current survey.
There is a need for improving knowledge levels. This can be achieved by organizing training sessions, raising colleague networks and the development of dedicated expert systems, or a combination of these three activities.

[1] Risk assessments for Glucosinolates, Ricin, Tropane alkaloids, Hydrocyanic acid and Pyrrolizidine alkaloids, the basic toxic substances of the undesirable substances as listed in Directive 2002/32/EC, are expected to be published in 2007.

5 Acknowledgements

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6 Literature

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SCAN, 2003. Opinion of the Scientific Committee on Animal Nutrition on undesirable substances in feed. European Commission, Health & Consumer Protection Directorate-General.

Annex 1 Response

Send and returned questionnaires

	Send		Returned	
	Official control laboratories	Other	Official control laboratories	Other
Austria	1	2	1	
Belgium	2	1	2	
Cyprus	1	0	1	
Czech Republic	1	0	1	
Denmark	1	0	1	
Estonia	1	0	1	
Finland	1	0	1	
France	5	2	2	2
Germany	18	7	8	3
Greece	1	1		1
Hungary	1	1	1	
Ireland	1	0	1	
Italy	15	0	2	
Latvia	2	0	1	
Lithuania	1	0		
Luxemburg	1	0	1	
Malta	1	0		
the Netherlands	1	3	1	2
Poland	6	0	2	
Portugal	1	0	1	
Slovakia	2	0	1	
Slovenia	2	0	1	
Spain	12	2	3	
Sweden	1	0		
United Kingdom	1	1	1	1
Iceland	1	0		
Norway	1	0		
Switzerland	1	0	1	
TOTAL	83	20	35	9

Response: 42,7 %

Annex 2. Results of the questionnaire

Section 1. General information

1. Is your institute an official control laboratory:
Yes: 35 No: 9

2. Is your laboratory carrying out an active monitoring for botanic undesirable substances:
Yes: 28 No: 16

3. The amount of samples that are declared to be evaluated for botanic undesirable substances, the total amount in the framework of Directive 95/53/EC, and the number of labs stating that no control program is effectuated:

	For undesirable substances	Total for 95/53/EC	No program
A	150-200/yr	3000	
B	6-64/yr	10000	1
CH	40	1600	
CY	0	10	
CZ			1
D	41 / 335 / 70-100 / 0 / 400 / 30 / 150	? / 335 / 1600 / ? / 400 / ? / 800	4
DK	250-600/yr *	8000	
ES	39 / 0	135 / 500	1
EST			1
F	0 / 5	100 / 3000	2
FI	340	5000	
GR			1
HU	20	20	
I	0	50-100	1
IRL	20	1500	
LU	0	300	
LV	0	500-550	
NL	250; 83	1700; 2200	1
P	50	32	1
PT			1
SK	0	0	
SLO	0	1500	
UK	100	20	1
total number of labs	28		16

*: *for composition in general, including undesirable substances*

Section 2. Results from active control programs

A total of 27 laboratories stated that they are carrying out an active monitoring for botanic undesirable substances. Nine of these labs declared to have a number of zero samples evaluated. As a consequence, 18 labs among the 44 respondents have actually tested and eventually found botanic undesirable substances.

4. Indication of the way the evaluation is performed:

Macroscopic (by eye, or by binocular at low magnifications):	18
Microscopic (by compound microscope, at higher magnifications):	15

(One or both answers were possible).

Equipment used: Sieves (four labs), DIC mounting, mounting media: phenolglycerol (once), lugol (once), chemical analysis for isothiocyanaat and vinylthiooxazolidone (once).

5A. Overview of the returned results of the monitoring

Undesirable substance	Number of samples, reporting country	Frequency of occurrence >50%	25-50%	5-25%	<5%	0%	Most common feed ingredient
Castor oil plant — *Ricinus communis*	? D					0	rape seed, maize, feed
	? D					0	
	? NL				<5		
	6* B			17			
Crotalaria spp	? D					0	cattle feed
	? D					0	
	? NL				<5		
	3* B					0	
Thorn apple - *Datura stramonium*	? D	*frequent in some years*					wheat, maize, soybean, linseed, sunflower, rapeseed, feeds
	? D	*sporadically*					
	? NL				<5		
	25 HU			24			
	70 D				<5		
	61* B			10			
	200 FI				<5		
	250-600 DK				<<5		
Rye ergot - *Claviceps purpurea*	? D				<5		wheat, rye, barley, triticale, rapeseed, feeds
	? NL				<5		
	2 A				<5		
	3 P			5-25			
	11 D			5-25			
	20 IRL	*no results yet for 2006*					
	20 D		25-50				
	39 ES	*no results reported*					
	70 D				<5		
	94* B			25			
	83 NL				2.4		
	162** B			22			
	300 D			5-25			
	250-600 DK				<<5		

**: total amount of samples investigated in the years 1995-2005, excluding targeted sampling.*

***: results for the year 2006, excluding targeted sampling.*

5B. Overview of the returned results of the monitoring, second part

Undesirable substance	Number of samples, reporting country	Frequency of occurrence >50% 25-50% 5-25% <5% 0%	Most common feed ingredient
Sareptian mustard — *Brassica juncea* ssp. *juncea*	? D 2-3 F 8* B 250-600 DK	*found occasionally* <5 13 <<5	cattle cake, linseed, rapeseed, feeds
Chinese mustard — *Brassica juncea* ssp. *juncea* var. *lutea*	? D 2-3 F 250-600 DK	*found occasionally* <5 <<5	cattle cake, rapeseed
Indian mustard — *Brassica juncea* ssp. *intregifolia*	? D 2-3 F 250-600 DK	*found occasionally* <5 <<5	cattle cake, rapeseed
Black mustard — *Brassica nigra*	? D 2-3 F	*found occasionally* <5	cattle cake, rapeseed
Ethiopian mustard — *Brassica carinata*	? D 2-3 F	*found occasionally* <5	cattle cake, rapeseed
Purghera — *Jatropha curcas*	? D ? D	0 0	
Croton — *Croton tiglium*	? D ? D	0 <5	
Mowrah, Bassia, Madhuca — *Madhuca longifolia* — *Madhuca indica*	? D	0	
Lolium temulentum	? D 1 P 2* B	0 <5 0	
Lolium remotum	? D 2* B	0 0	
Apricots — *Prunus armeniaca*	? D	0	
Bitter almond — *Prunus dulcis*	? D	0	
Beech mast — *Fagus silvatica*	? D	0	
Camelina — *Camelina sativa*	? D	0	

**: total amount of samples investigated in the years 1995-2006.*

6. Additional remarks

Undesirable substance	**Remark**	
Castor oil plant — *Ricinus communis*	should be kept in the list for toxicity	
Crotalaria spp	very toxic, common weed in tropic/subtropic regions	
Thorn apple - *Datura stramonium*	should be kept in the list for toxicity; in seed lots from N and S America	
Rye ergot - *Claviceps purpurea*	in whole cereal feeds, but low; seems to increase in last years	
Sareptian mustard — *Brassica juncea* ssp. *juncea*		sometimes present in feeds high in rapeseed; no need to mention separately; not extremely toxic, some used for human consumption
Chinese mustard — *Brassica juncea* ssp. *juncea* var. *lutea*		
Indian mustard — *Brassica juncea* ssp. *intregifolia*	spice in human nutrition	
Black mustard — *Brassica nigra*		
Ethiopian mustard — *Brassica carinata*	difficult to get reference samples	
Purghera — *Jatropha curcas*	very toxic, should be kept in the list; no reference samples available	
Croton — *Croton tiglium*	very toxic, should be kept in the list; no reference samples available	
Mowrah, Bassia, Madhuca — *Madhuca longifolia* — *Madhuca indica*	not very toxic, no adverse reactions with up to 20%; no reference samples available	
Lolium temulentum	not very toxic, almost extinct in M Europe (Red List)	
Lolium remotum	not very toxic, almost extinct in M Europe (Red List)	
Apricots — *Prunus armeniaca*	no relevance	
Bitter almond — *Prunus dulcis*	no relevance; no reference samples available	
Beech mast — *Fagus silvatica*	was used as animal feed in historic times, not very toxic	
Camelina — *Camelina sativa*	no adverse reactions with up to 20%, pigs most sensible; produced for human consumption	

Section 3 Proposals for future developments; expertise maintenance

7. Undesirable substances that can be deleted from Directive 2002/32/EC:
- No deletions (six labs);
- Ergot, when alkaloids can be detected chemically (one lab);
- *Camelina sativa* (three labs);
- *Lolium remotum*, beech mast, *Madhuca spec.* (two labs each);
- Mustard species, *Jatropha*, *Croton*, apricots, bitter almond (one lab each);
- Delete all substances which are not found in last 25 years, and without risk according to EFSA (one lab).

8. Undesirable substances that could be considered to be added to Directive 2002/32/EC:
- *Ambrosia spec*. (five labs);
- A final list of seeds containing alkaloids (two labs). Possible species to include: *Xanthium spec*., *Hyoscyamus spec*., *Abutilon spec*., *Atropa belladonna*;
- *Galium aparine* (two labs);
- *Nerium oleander*, *Tilletia spec*., *Polygonum persicaria*, *Colchicum autumnale*, *Acroptilon (Centaurea) repens*, *Agrostemma githago*, *Coronilla varia* (one lab each).

9. The way in which knowledge on the detection and identification of undesirable substances is maintained:

Using text books:	25
Browsing the internet for information:	23
Implementation of dedicated knowledge or expert systems:	10
No activities to increase knowledge:	6
Sufficient knowledge available in the research group:	6

Other, specified as:
- Seed collection for reference (4 labs);
- Meeting with colleagues;
- Reports from other projects;
- Database.

10. The desire to increase knowledge:

By training sessions:	28
By a network of experts:	24
By using text books:	19
By a dedicated knowledge or expert system:	15
No, there is no need to increase:	3

Other, specified as:
- Ring tests (two labs);
- Exchange of samples.