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RIKILT Report 2012.012

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Report 2012.012 **October 2012**

Project number:	120.72.250.01
BAS-code:	WOT-02-004-025
Project title:	Statistical foundation of a risk-based National Plan for feedingstuffs (incl. analysis of trends)
Project leader:	M. de Nijs

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This research was funded by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.

Distribution list:

- NVWA, Rik Herbes

Executive summary

Trend analysis on monitoring data could be a suitable method to identify feed commodities with higher dioxin or DL-PCB concentrations, thus contributing to risk-based monitoring. The aim of the present study was to obtain insight into background levels of dioxins and dioxin-like PCBs (DL-PCBs) in feed and potential trends therein. For this, monitoring data collected in the Netherlands from 2001-2011 were reviewed (around 4900 feed samples).

Most samples were first screened with the DR CALUX® bioassay and, if suspected, the samples were analysed with GC-HRMS (N±800). However, besides these samples, from 2003-2010, yearly on average 120 samples were picked at random and analysed directly with GC-HRMS. The results from the screening were used to determine the extent of non-compliant samples. The fraction of samples exceeding maximum levels (MLs) for either dioxins or the sum of dioxins and DL-PCBs was below 1%, except for fish meal, clay minerals and vegetable oils. Non-compliance with action levels was 2-3 times higher than for MLs and just above 1% in animal fat, pre-mixtures and feed materials of plant origin.

Samples randomly analysed with GC-HRMS were used for trend analysis. In none of the feed materials a significant trend was observed in the levels, which is primarily linked to the fact that most background levels were below or around the limit of quantification (LOQ) of the GC-HRMS method. A further reduction of the LOQs might be required to improve this situation and to allow an earlier detection of potential incidents. In general, lower dioxin and DL-PCB levels were present in the samples collected in the Netherlands vs. the data presented recently by EFSA, especially when focussing on the upper end of the distribution curve (e.g. 95th percentile) which was used to set the MLs. In fact, based on the results collected in the Netherlands, for many feed materials, the ML for the sum of dioxins and DL-PCBs could be set at the ML for dioxins only, thus eliminating the need for two MLs without allowing higher levels of dioxins.

A representative amount of samples needs to be collected each year to obtain statistically significant results and to track down incidents. Based on this study, the following recommendations can be given.

The following feed materials should stay a key part of the NCP:

- Vegetable oils
- Animal fat
- Fish meal
- Complete feed

The amount of samples collected for the following feed materials is assumed to be sufficient:

- Feed materials of plant origin, including vegetable oils
- Animal fat
- Fish meal and fish oil
- Clay minerals
- Feed materials of mineral origin
- Complementary cattle feed

The amount of samples taken of the following feed group should increase:

- Complete feed, especially pig feed

The amount of samples taken of the following feed materials may decrease:

- Choline chloride
- Premixtures

To attain at a more risk-based approach, more focus should also be laid on monitoring exporting countries as 88% of all feed materials used in the Netherlands are imported. Hereby, precisely reporting the country of origin is important.

This report shows that it is very useful to analyse historical data to look for potential trends in both the levels of contaminants in animal feed and the extent of non-compliance. On the basis of these results monitoring strategies can be re-evaluated and optimized into risk-based monitoring.

Technical summary

Exposure to dioxins and dioxin-like PCBs (DL-PCBs) can cause a wide range of toxicological effects on human and animal health. Around 90% of human exposure results from the consumption of food of animal origin (Fürst *et al*., 1992) and animals are exposed mainly through feed. Monitoring and taking measures to prevent the contamination of feed is, therefore, an important step to reduce the uptake of these contaminants by food-producing animals and thus by humans. In this report historical monitoring data from the period 2001-2011 were used to give insight into background levels and potential trends of dioxin and DL-PCBs in animal feed in the Netherlands.

The monitoring data used were collected from the databank of the Program for the Quality of Agricultural Products (KAP). The KAP databank is previously managed by RIKILT and since 2010 by RIVM; the around 4900 feed samples analysed for this report have been submitted to the databank and are results from the Dutch National Control Plan for animal feed, relating to monitoring only (i.e. no data from follow-up studies in the case of incidents). Most samples were first screened with the DR CALUX® bioassay and, if suspected, the samples were analysed with GC-HRMS (N±800). However, besides these samples, from 2003 till 2010, on average 120 samples were picked at random and analysed directly with GC-HRMS based on Commission Recommendation 2004/704/EC (hereafter called EU monitoring samples). The results from the screening were used to determine the fraction of samples exceeding the action and maximum levels and the EU monitoring samples were used in the statistical analysis.

A limited amount of animal feed data was chosen to investigate in detail levels and trends, based upon the usage of the material as animal feed (quantity) and on potential risk materials. By choosing the feed materials wheat, maize and oil seeds/fruits approx. 66% of the total volume of animal feed in the Netherlands is represented. Incidents have been found in the past in artificially dried feed materials (e.g. bakery waste, grass/alfalfa), naturally or industrially contaminated feed materials (e.g. clay minerals, choline chloride) and fats/oils (esp. fish oil). The planning was to look into levels and trends of these feed materials individually, but due to the low amount of samples present, we could only focus on the main feed categories. In the annex of this report the usage in the Netherlands is described in more detail. As 88% of all feed materials are imported, it would have been important to also relate the results found to the main exporting countries being responsible for around 98% of all feed imports into the Netherlands. The countries are Argentina (25%), Brazil (23%), Germany (20%), Belgium (7.5%), Malaysia (7%), USA (7%), France (4.5%) and Indonesia (4%). However, the number of samples measured with GC-HRMS for these countries was too low to be used for further analysis and also in 35% of all samples collected the country of origin was unknown.

The strategy used for the trend analysis is to first combine data at a high level, by, for example, combining all feed materials to major feed groups. An analysis was then carried out on feed subgroups and in some cases for specific feed materials (e.g. clay minerals and fish meal). Samples which exceeded the regulatory limit were linked further to the specific countries.
A descriptive analysis was carried out and results are shown as averages, median, 95th and 99th percentile values. Histograms are used to display the data, with year of sampling on one axis and contaminant content on the other. Using simple regression analysis, trend lines through the averages were calculated and displayed. In addition the statistical test Mann-Kendall was used to

examine the significance of the trends observed. Background concentrations were compared to the results from the database evaluation by EFSA (2010), the latter being used for reassessment of the limits. The results presented in this report will give suitable information for discussing the current monitoring strategies in the Netherlands including the potential use of lower decision limits for certain feed categories. Recommendations on sampling can be used to adjust the Dutch National Control Plan for animal feed (NCP).

The main conclusions of the study are:

- Dioxin and DL-PCB concentrations are in most feed materials low, being at or around the LOQ. Also the fraction of samples exceeding maximum levels was below 1% in most feed materials, except for fish meal, clay minerals and vegetable oils.
- The background concentrations found in feed materials of plant origin (excl. vegetable oils) stay far below the regulatory limits and the monitoring can be continued as it is.
- In vegetable oils and animal fats the number of samples collected with GC-HRMS was too low to investigate on trends. As more or less often incidents were observed in this study as well as by RASFF, we advise to keep monitoring these feed materials on a regular basis.
- Fish meal samples repeatedly exceeded regulatory limits and even though no trend could be found, sampling fish meal should be kept part of the NCP. The number of samples taken for fish oil is too low to draw conclusions, although in general higher dioxin and DL-PCB concentrations were observed compared to all other feed materials. However, as this feed material only forms a small proportion in animal diets and is used in low quantities in the Netherlands, the number of samples collected does not need to be increased.
- Almost every year, clay mineral samples exceeded the limit. This underlines the necessity to keep clay minerals included as part of the annual monitoring program, but as the use in animal diets concerns low quantities the amount sampled per year can remain as it is. In choline chloride, none of the samples exceeded the regulatory limits and the number of samples taken per year can be limited.
- Looking into dioxin and DL-PCB concentrations of compound feedingstuffs may also be a better approach instead of checking feed additives, minerals and pre-mixtures individually. In this scenario still the individual feed materials have to be monitored from time to time and it might than be necessary to also lower the action or decision levels for compound feedingstuffs.
- Monitoring complementary cattle feed remains important, but the number of samples taken per year is sufficient. In contrast, the amount of samples collected for e.g. complete pig feed is recommended to be increased. In general, only a low amount of complete feed samples were taken in 2010 and 2011, which should be increased. The dioxin and DL-PCB concentrations in poultry feed are generally low and if high dioxin and DL-PCB levels are found in eggs, this is most likely linked to a contamination of the soil. Therefore, poultry feed should still be measured on a basic level, but monitoring poultry end products is important as well.
- In most feed materials lower background concentrations for dioxins and DL-PCBs were observed compared to the calculations made by EFSA based on the data sent in by the Member States (2010). Difference can particularly be found in the average values, the 95th and 99th percentile and are much smaller in median values. In general, similar 95th

percentile dioxin and DL-PCB concentrations were only found in feed additives, pre-mixtures and fish oil if compared to the EFSA dataset.

- In general no significant change of dioxin and DL-PCB concentrations in feed was found. Often levels remained within a certain range and were generally low.
  - The slight decrease of dioxin levels observed in the feed subgroup forages and roughage, clay minerals as well as in the main feed group of compound feedingstuffs is probably biased, as it is linked to a reduction of the LOQs in 2007. In fish meal no significant change of the average dioxin levels can be found and in the main feed group of plant origin (excl. vegetable oils) and in feed additives dioxin background levels seem to stay around the same level with no significant change.
  - In fish meal and feed for fur animals, pets and fish no significant change of the average DL-PCB levels can be observed and in feed of plant origin (excl. vegetable oils), in feed additives and compound feed (excl. fur animals, pets and fish) DL-PCB background levels seem to stay around the same level with no significant change.
  - Although in the feed subgroup forages and roughage and clay minerals as well as compound feed (excl. fur animals, pets and fish) average dioxin and DL-PCB levels tend to decrease significantly, this reduction can again be linked to lowering the LOQs of dioxins in 2007 and the trend is probably fake. Also in feed for fur animals, pets and fish total dioxin and DL-PCB concentrations tend to decrease significantly, but due to the low number of samples available having relatively low concentrations and the short time frame (2004-2008), the outcome of the trend analysis may not be representative. In fish meal no change can be found and in the main feed group of plant origin (excl. vegetable oils) and in feed additives the average dioxin and DL-PCB concentrations vary per year, but no significant trend can be seen.
  - The outcome of the trend analysis has to be treated with caution when looking into trends in values which are below or close to the reporting limit or LOQ.

The main recommendations of the study are:

- It is important to continue taking a representative amount of samples of the different feed groups, i.e. enough samples to obtain statistically significant results and to track down incidents.
- Lower decision limits might be used for certain feed materials, allowing an earlier detection of potential incidents. This applies e.g. for dioxins in feed materials of plant origin, animal fat and other land animal products, fish oil and feed materials of mineral origin. For vegetable oils, and compound feedingstuffs the decision limits for both dioxins and DL-PCBs may be reduced. The decision limits for DL-PCBs could be lowered in fish meal and clay minerals. In practice this could be done by lowering the so-called decision limit for the GC-HRMS method and maybe also the DR CALUX® bioassay. This may include a reconsideration of using separate action levels for dioxins and DL-PCBs.
- There appears to be a clear discrepancy between the datasets of EFSA and the Netherlands. It is of interest to further investigate the possible reasons for this discrepancy. The fact that especially the higher end of the distribution is clearly different, by having higher concentrations, has serious implications with respect to the limits.

- In general the fraction of samples exceeding the maximum levels is rather low. Most of the time these cases were first discovered with the bioassay and not in the EU-monitoring samples. The approach to use a combination of a screening and confirmatory method allows large numbers of samples to be tested and as such clearly increases the chance to detect non-compliant samples.
- The results obtained with the DR CALUX® bioassay are expressed in a qualitative way (negative/suspected) and not yet used to its full potential. It should be investigated whether the semi-quantitative results obtained with the bioassay can somehow be included in trend analysis on background levels.
- Regularly also negative DR CALUX® samples are analysed using the GC-HRMS method (check on false-negatives), but these values are not kept in the laboratory system LIMS after verification and are, therefore, also not stored in the databank KAP. It is recommended to keep and gather these values to help to investigate trends in future more precisely also for low dioxin and DL-PCB concentrations.
- By using the Mann-Kendall test and/or Sen's slope estimator, trends do not need to be adjusted for outliers and using these statistical tests in future trend analysis may be of advantage.

  Looking into trends found in background levels for NDL-PCBs may be of advantage and help to develop sampling strategies.
- More focus should be laid in future on monitoring exporting countries as 88% of all feed materials used in the Netherlands are imported. Furthermore, verifying and reporting the country of origin correctly is important. In samples analysed often the country of origin is unknown (in 35% of the samples collected) or seems to be notified incorrectly (e.g. palm kernel oil originating from the Netherlands).

This report shows that it is very useful to analyse historical data to look for potential trends in both the levels of contaminants in animal feed and the extent of non-compliance. On the basis of these results monitoring strategies can be re-evaluated and optimized into risk-based monitoring.

List of abbreviations

AL	(regulatory) Action level
Avg.	Average
DR CALUX®	Dioxin Responsive Chemical-Activated LUciferase gene eXpression
EFSA	European Food Safety Authority
GC-HRMS	Gas Chromatography - High Resolution Mass Spectrometry
KAP	Program for the Quality of Agricultural Products (KAP)
[lb]	Lower bound
LOQ	Limit of Quantification
ML	(regulatory) Maximum level
Mt	Million tons
NCP	Dutch National Control Plan for animal feed
NVWA	Netherlands Food and Consumer Product Safety Authority
DL-PCBs	Dioxin-like Polychlorinated Biphenyls
PCDDs	PolyChlorinated Dibenzo-p-Dioxins
PCDFs	PolyChlorinated DibenzoFurans
PDV	Dutch Product Board Animal Feed (“Productschap Diervoeder’)
RASFF	Rapid Alert System for Feed and Food
StDev	Standard deviation
TEF	Toxic Equivalence Factor
TEQ	Toxic Equivalents
TCDD	2,3,7,8-TetraChloroDibenzo-p-Dioxin
TSE	Transmissible spongiform encephalopathies
[ub]	Upper bound
WHO-TEF	World Health Organization-Toxic Equivalent Factor

Abbreviations of Member States and other countries

AR	Argentina
AT	Austria
BE	Belgium
BR	Brazil
CN	China
DE	Germany
DK	Denmark
ES	Spain
EU	European Union consisting of 27 Member States
FR	France
GB	United Kingdom
ID	Indonesia
IT	Italy
MY	Malaysia
NL	the Netherlands
NO	Norway
PE	Peru
PH	Philippines
SB	Solomon Islands
UN	Unknown country of origin
US	United States of America

Contents

1 Introduction

Dioxins are a group of persistent chemicals formed by natural or industrial processes, such as combustion (burning) processes. Polychlorinated biphenyls (PCBs) have been used in transformers, building materials, lubricants, coatings, plasticizers and inks, although their production and use is not allowed anymore. Some of the PCBs have toxicological properties that are similar to dioxins and are therefore called “dioxin-like” PCBs (DL-PCBs). The relevant dioxins and DL-PCBs are highly resistant to breakdown processes, and consequently persist in the environment. Due to their physical (lipophilic nature) and chemical (stability) properties, these toxic compounds can also accumulate in human and animal tissue. Exposure to dioxins and DL-PCBs can result in a wide range of toxicological effects on human and animal health, including cancer and disturbance of the reproductive and immune system (SANCO, 2001). To protect human and animal health, dioxin and DL PCB concentrations in food and feed must be limited and potential sources need to be identified and eliminated.

It is estimated that up to 90% of human exposure to dioxins and DL-PCBs results from the consumption of food, mainly linked to foodstuff of animal origin (Fürst *et al*., 1992). Another route of exposure is the inhalation and ingestion of particles from air, contributing to less than 10% of daily intake. The route of exposure in animals is through feed, contaminated soil or air particles.

Dioxins and DL-PCBs can enter the human food chain via different pathways. One of them includes the consumption of contaminated feed by animals used for food (European Commission, 2000). Several dioxin feed incidents found in the past emphasise the special concern and impact of this contamination route on the occurrence of dioxins and DL-PCBs in food of animal origin (Table 1.1).

Contamination may occur as a result of industrial processes applied to animal feed (European Commission, 2000); e.g. the inadequate drying process used for bakery waste in 2003 and 2008, which led to high dioxin levels in feed. Inadequate drying also seems to underlie the contamination of organic corn in 2010, cacao fatty acid distillates in 2011 and of sugar beet pulp in 2011. Another example is the use of contaminated materials during the production process, like lime from a PVC-production plant used in the production of citrus pulp in 1998, pineapple sawdust treated with pentachlorophenol mixed with choline chloride in 2002 and contaminated HCl used for gelatine production in 2006. In other cases there is a clear case of fraud like the use of oil not intended for human or animal consumption in feed in 2010. Contamination may also occur by using naturally contaminated feed materials, like certain clay minerals (kaolinic clay, ball clay). There are also a number of cases with contaminated zinc and other minerals, some due to recycling of materials. Still a mystery is one of the largest incidents in Belgium in 1998, where 200 litres of PCB-oil ended up in vegetable fat used for animal feed.

Monitoring and taking measures on feed and its contamination routes are important steps to reduce the dioxin uptake by animals and humans. These measures should form a crucial part of the management strategy of food safety authorities, such as the NVWA (Netherlands Food and Consumer Product Safety Authority). By focusing on feed ingredients, a dioxin contamination can be identified in an early stage and often before it enters the food supply chain via animal products. Finding out which feed materials may bear a higher risk of being contaminated with dioxins and DL-PCBs is hereby crucial.

Table 1.1. Several examples of dioxin feed incidents.

Year	Country	Incident – source of contamination	References
1998	Germany Brazil	Contaminated lime as neutralization for citrus pulp used as animal feed	(Malisch, 2001)
1999	Belgium	Animal feed had been prepared with fat contaminated with PCB oil	(Bernard *et al*., 1999)
1999	Austria Germany the Netherlands	Use of contaminated kaolinic clay for mixing vitamins and minerals into feed	(Jobst et al., 2000)
2000	Germany Belgium Spain	PCB-contaminated sawdust used as carrier for a choline chloride premix used as animal feed component	(Llerena *et al*., 2003)
2003	Germany the Netherlands	Due to an inappropriate drying process high dioxin levels were formed in dried bakery waste used as animal feed	(Hoogenboom *et al*., 2004)
2004	the Netherlands	Potato peelings used as feed, which were contaminated due to the use of kaolinitic clay for sorting potatoes in a production process of French fries	(Hoogenboom *et al*., 2010)
2006	the Netherlands Belgium	Use of fat from a gelatine production site using contaminated hydrochloric acid	(Hoogenboom et al., 2007)
2008	Ireland the Netherlands	High levels of dioxin in pork as a result of contaminated feed produced from bakery waste which was artificially dried using PCB-containing oil	(Bradley, 2008)
2010	the Netherlands Germany	Contaminated organic corn, possibly due to inappropriate drying	RASFF
2010	Germany	A batch of fatty acids, meant to be used for technical purposes, got mixed with fat for the production of feed	(European Commission, 2010)
2011	the Netherlands Brazil	Fat of Brazilian cocoa beans used as feed contained to high dioxin levels after being inappropriately dried	(NVWA, 2011a)
2011	Germany the Netherlands	Contaminated sugar beet pulp; probably due to inappropriate drying	RASFF

Dioxins and dioxin-like PCBs have not been routinely monitored in feed commodities in the Netherlands until 2001. The discovery of elevated levels of dioxins in citrus pulp pellets imported from Brazil in 1998 (Malisch, 2001), and the Belgian dioxin incident in 1999 (Bernard *et al*., 1999) formed the starting point for systematic national monitoring. In 2002 regulatory limits on dioxins permitted in feed were set for the first time (Directive 2002/32/EC). Initially, standards have been set for dioxins only, but in November 2006 DL-PBCs have been added (Commission Directive 2006/13/EC). The official limits are stated in more detail in chapter 2 of this report. In addition, the European Commission encourages a proactive approach to reduce the concentration of dioxins and DL-PCBs, present in food and feed, by setting so-called action levels (ALs, Commission Recommendation 2006/88/EC). If the AL is found to be exceeded, the food product can still be consumed, but further action needs to be taken to disclose the source. Once identified, measures should be taken to prevent or reduce the contamination. The scheme below illustrates the relation between the different intervention levels (see Figure 1.1). Although, it appears that currently no target levels will be established, which were a part of the original EU-strategy on dioxins and DL-PCBs.

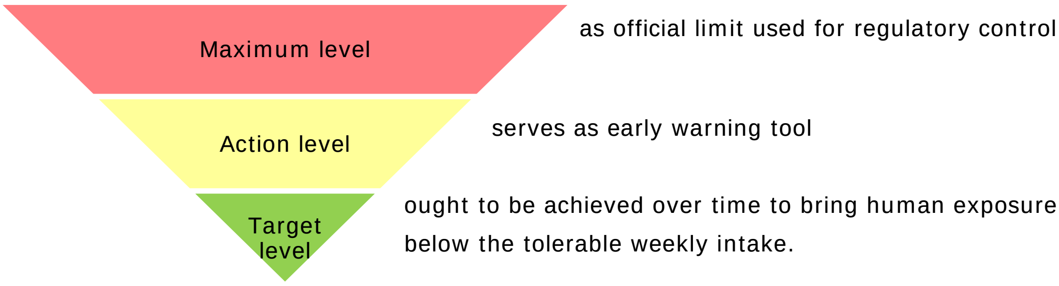

Figure 1.1. Overview intervention levels of undesirable substances in animal feed.

In order to monitor if these measures have an effect, the EC has asked all Member States to measure background levels of dioxins and DL-PCBs in defined feed categories for a certain number of years on a yearly basis and to present these data to the EFSA (Commission Recommendation 2004/704/EC). In the Netherlands around 120 feed samples were examined with GC-HRMS annually from 2003 until 2009.

Recently, the EFSA (European Food Safety Authority) has evaluated the available data, covering monitoring samples collected from 1999 to 2008. EFSA came to the conclusion that hardly any reduction of the background levels of dioxins and DL-PCBs can be observed over this period. This means that the regulatory limits introduced in 2001 and 2006 cannot be further reduced, since otherwise an unreasonably high amount of samples would have to be rejected.

In the Netherlands most sampling strategies of the National Control Plan are based on incidents found in the past, recommendations of the European Commission or other research and own experience gained over the years. To a certain extent, unpredictability persists but NVWA attempts to permanently improve the monitoring and surveillance system to an even more risk-based approach. In this context a trend analysis on the amount of dioxins and DL-PCBs in feed was carried out by RIKILT in 2007 (Adamse *et al.*, 2007) using monitoring data. Within this study difficulties were found in forecasting trends as the dataset used contained only the most recent laboratory results starting from 2002 till 2005. In general, a low number of measurements was available, making it difficult to give advice on certain feed materials. By now, more results have been collected and a more representative trend analysis can be achieved. The objective of this report is to obtain insight into background levels of dioxins and DL-PCBs in feed materials on the basis of Dutch monitoring data collected from 2001 till 2011. The outcome can be used to identify commodities and countries of origin with higher risks. The results can help to support risk management strategies of the NVWA and to reach a more risk-based sampling in the national feed monitoring program. Furthermore, informing the public about dioxin levels that have actually been found in food and feed is recommended to increase consumers' confidence and trust in food and feed control.

2 Materials and methods

In this chapter the steps taken to execute the trend analysis are described. The main steps of the trend analysis are:

1. Description of the methods of analysis;
2. Data collection and pre-analysis, including a differentiation between DR CALUX® and GC-HRMS samples;
3. Classification of main feed groups and subgroups;
4. Identification of critical feed materials and/or subgroups via (1) incidents (RASFF); (2) gathering information on accessible commodities used in the feed sector in the Netherlands; including a differentiation between domestic and imported feed;
5. Description and selection of statistical analysis; including points of attention when analysing the results.

2.1 Methods of analysis

CALUX method

Most collected samples were first screened with the Dioxin Responsive Chemical-Activated LUciferase gene eXpression (DR CALUX®) cell-based assay, using a so-called qualitative approach. This method is used to eliminate negative samples, in order to reduce the costs of analysis, and thus allow a larger number of samples to be analysed. The response of the test samples is compared with that of a reference sample having a certain limit. This decision limit is a level set by RIKILT and is different for the various types of feed materials due to the different MLs and ALs. In the EU legislation every feed (sub)group has two MLs, one for dioxins and one for the sum of dioxins and DL-PCBs (Table 2.2, section 2.3). In addition, there are two ALs, one for dioxins, one for DL-PCBs. In principle the decision limit used by RIKILT is based on the lowest AL for each feed (sub)group. Since dioxins and DL-PCBs are not separated during clean-up, it must be assumed that the response is completely caused by one group of compounds or the other. In practice the level of the reference sample used for comparison is at the lowest AL, e.g. 0.57 ng TEQ/kg in the case of compound feed and non-fat feed ingredients (e.g. including feed additives, minerals and pre-mixtures), 0.4 pg TEQ/g for plant-derived fat, 1.0 pg TEQ/g for animal derived fat, 3.1 pg TEQ/g for fish oil, 0.76 ng TEQ/kg for fish meal and 1.84 ng TEQ/kg for fish food. Samples showing a lower response than the reference sample are registered as negative. If the results obtained are higher the sample is declared suspected, and the so-called gas chromatography - high resolution mass spectrometry (GC-HRMS) technique is used to determine the level (quantitative approach). Overall, this is a rather conservative approach when trying to detect samples exceeding the ML but it aims at reducing the chance on a false-negative result to a minimum.

The main disadvantage of this approach is that the true value for samples which are found to be negative with the DR CALUX® assay is not known. A level might be estimated but the true level must be determined by GC-HRMS. This is due to the fact that the CALUX response can be caused by other Ah-receptor agonists that survive the clean-up procedure but are not necessarily dioxin-like in terms of toxicity and persistence. In addition, there are some differences between the official TEF-values and the relative response of the different dioxins and DL-PCBs in the test. Thus, if the sample is declared negative, the precise dioxins and/or DL-PCB value is not known but could be in the range of 0 to the decision limit.

GC-HRMS method

Applying the GC-HRMS confirmatory method, concentrations of relevant congeners of dioxins and DL-PCBs are measured and recalculated to Toxic Equivalence (TEQ) values through multiplication by its Toxicity Equivalent Factor (TEF) (Ahlborg *et al.*, 1992). In this report so-called upper bound [ub] levels are reported, which means that if the concentration of a specific congener is below its LOQ, the level of that congener is assumed to be the LOQ.

During the past years there has not been a change in the measurement accuracy of the GC-HRMS method at RIKILT: the extended measurement uncertainty is 10% . However, the limit of quantification (LOQ) per congener and also the reporting limits have decreased in the middle of 2007. This influences the outcome of the upper bound [ub] levels. Lowering the LOQ will thus result in lowering the [ub]-levels. Therefore, trends found in lower concentrations can be subject to detection capabilities ([ub] vs. [lb]) and checking for trends below a certain dioxin and DL-PCB concentration is difficult as the LOQ/reporting limits were adjusted (from 0.29 to 0.17 ng TEQ/kg for dioxins [ub] and thus from 0.31 to 0.19 ng TEQ/kg for the sum of dioxins and DL-PCBs[ub]; the LOQs for the DL-PCBs remained unchanged, viz. 0.02 ng TEQ/kg). In this way a fictive decreasing trend might be seen between the years 2001-2006 and 2007-2011 if most concentrations found are at or below the reporting limit.

2.2 Data pre-analysis

Data on dioxin and DL-PCB levels in feed was gathered using the databank of the Program for the Quality of Agricultural Products (KAP)[1]. The KAP databank has been filled and managed by RIKILT and since 2010 by RIVM. During the past years (2001-2011) around 4900 feed samples were taken in the framework of monitoring programs (random selection) and will be used within this report (see Table 2.1). All samples are analysed by RIKILT[2] and were collected at random in the framework of the Dutch National Control Plan for animal feed (NCP).

Sample selection

As mentioned before, from 2003 until 2009 a certain amount of samples was collected on a yearly basis for the European monitoring program (based on Commission Recommendation 2004/704/EC), hereafter named EU monitoring samples. These samples (about 120 per year) were measured directly with GC-HRMS and are ideal for trend analysis since they were sampled randomly. Also feed materials, which are known to regularly give a "false-positive" DR CALUX® response (e.g. alfalfa, fish oil), are in general measured directly with GC-HRMS and will be used as being a part of the EU monitoring samples (see column 6, Table 2.1). For determining the background levels, GC-HRMS samples which were analysed as a follow-up of a suspected DR CALUX® result (column 2), were not included in the analysis. This approach was chosen since inclusion of only suspected DR CALUX® samples would cause a bias on the levels. Also all samples tested negative with DR CALUX® and which were not tested with GC-HRMS were excluded from

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1 KAP is a collaboration between agricultural businesses and the Dutch government. The KAP databank is designed to process the results of monitoring programs and contains levels of contaminants and residues measured in agricultural products.

2 RIKILT, as National Reference Laboratory of the Netherlands, has measured all samples in accordance with the European legislation on foodstuffs and animal feed.

the analysis as the true value is not known. However, negative DR CALUX® samples, measured with GC-HRMS for quality assurance of the bioassay (see column 4, Table 2.1), were included[3]. These samples were actually first measured with GC-HRMS and not till then with the DR CALUX®.

Table 2.1 gives an overview of the total amount of feed samples collected for the analysis on dioxin and DL-PCBs over the past 11 years.

Table 2.1. Overview of the number of samples tested on the content of dioxins and DL-PCBs, 2001-2011.

Year	DR CALUX®			GC-HRMS only		Total
	Suspected	Negative		EU monitoring	Other	
			*Verification**			
2001	30	386	*9***		4**	420
2002	25	354			1	380
2003	69	314	*11*	122		505
2004	95	306		109		510
2005	109	467	*8*	128		704
2006	66	223	*10*	111		400
2007	84	141		111		336
2008	77	168	*1*	109		354
2009	87	244	*2*		138	469
2010	87	217	*3*		97	401
2011	67	373			19	459
Total	**796*** **	**3193**	***44***	**690**	**259**	**4938**

* *A certain amount of samples tested negative with DR CALUX® have also been measured with GC-HRMS. This is for verification purposes to regularly check on the reliability of the DR CALUX® bioassay. These samples are not included in the total sum as they are already counted via the column of the negative samples. N.B. Negative samples confirmed via GC-HRMS are in general not registered in LIMS and KAP, thus likely these samples have been reported by accident. However, there are chosen to be included in the analysis.*

** *In seven samples only the concentration of dioxins, but not DL-PCBs is available. These samples are therefore excluded from the calculation of the background levels and trend analysis.*

*** *Five suspected samples were not measured with GC-HRMS and are therefore excluded from the dataset.*

So in 2007 e.g., a total of 336 samples were analysed of which 225 were tested with the DR CALUX® assay and 111 were analysed directly with GC-HRMS for the EU-database. Of the 225 samples tested with DR CALUX®, 84 samples showed a suspected response and were also analysed by GC-HRMS. In this year no information is available on negative DR CALUX® samples, tested for quality control purposes with GC-HRMS. For the EU monitoring program 111 samples were measured directly with GC-HRMS.

[3] N.B. For checking the reliability of the DR CALUX® method, yearly a certain percentage of negative samples are tested at random with the GC-HRMS method. According to Commission Regulation 152/2009/EC on methods of sampling and analysis for the official control of feed, 2 to 10% of all negative samples should be confirmed with a quantitative method such as GC-HRMS.

Many trend analysis approaches require the removal of samples collected too frequently in time, as they typically consist of highly correlated, redundant information that might be inappropriate for use in trend analyses. The dataset was therefore also examined on double measurements of the same feed material within one company on the same day. Samples taken from different feed materials within one company on the same day are seen as being independent from each other and do not need to be considered. Overall, samples have been taken from different batches and/or are linked to a different producer respectively composition of the feed materials. Therefore, samples were not excluded based on these criteria, as the characteristics differed and it cannot be seen as a repetition of one measurement. However, selective samples, which have not been chosen at random, were excluded. They may influence background levels and the trend analysis, especially when linked to incidents.

Unless stated otherwise, the upper bound limit (as described in Annex II) is used for all samples tested with GC-HRMS. This approach can be seen as worst case scenario as it might overestimate the true dioxin concentration. On the other hand, maximum levels and action levels as laid down in the regulation are based on upper bound limits as well. A comparison of the results found using either upper or lower bound limits are also given within this report and individual results of dioxins and DL-PCB levels are illustrated. In Annex II an explanation is given of the calculation of the TEQ values.

A total of around 4900 samples have been collected from 2001 till 2011, with an average amount of 450 samples per year (StDev: 102). Of these, around 960 samples (20%) were analysed at random with GC-HRMS (EU monitoring data) and used for further analysis. In 2001, 2002 and 2011, a low number of samples has been measured with GC-HRMS, which might influence the outcome of the trend analysis. From 2003 till 2010 on average 120 EU monitoring samples per year (StDev: 15, including quantified negative DR CALUX® samples) have been quantified with GC-HRMS and only this period was used for trend analysis. However, background levels were calculated over the whole time period (2001-2011). In Annex III an overview is given of the amount of samples gathered per feed category.

Summary - Data pre-analysis

- From 2001-2011 around 4900 feed samples were collected in the Netherlands in the framework of monitoring programs (NCP). These samples were used to determine the extent of non-compliance.
- Around 960 feed samples of EU monitoring data and other samples randomly analyzed with GC-HRMS from the period 2003-2010 were used for the trend analysis; on average 120 samples per year (StDev: 15). The samples were collected in the framework of the Dutch monitoring program (NCP) and have been analysed by RIKILT. Background levels were calculated over the whole time period (2001-2011) using the EU monitoring data and including samples measured directly with GC-HRMS in the other years.

2.3 Classification of feed groups

From a practical point of view, all feed materials were grouped into four main categories. Each category is further divided into a limited number of subgroups. This classification is based on the Commission Regulation 575/2011/EU (catalogue of feed materials) and the categorization of the regulatory limits (ALs and MLs). The division was chosen as the results are otherwise too extensive for evaluation purposes and/or the amount of samples per group is too low for trend analysis. The categorization of feed materials as carried out during a previous trend analysis (Adamse *et al*., 2007), merging similar feed materials to one feed subgroup, has been adopted. The AL for dioxins and DL-PCBs as well as the ML for dioxins and the sum of dioxins and DL-PCBs are included in Table 2.2; according to Commission Directive 2006/13/EC.

Table 2.2. Grouping of feed materials into main feed categories and feed (sub)groups, including regulatory limits as specified in Commission Directive 2006/13/EC (expressed in ng TEQ/kg with 12% moisture, except for fat/oil, ng TEQ/kg fat) and based on WHO-TEFs 1998) ***("old" TEFs and limits)****.*

Feed categories		**AL** (dioxins)	**AL** (DL-PCBs)	**ML** (dioxins)	**ML** (dioxins + DL-PCBs)
1. Feed materials of plant origin					
1.1. Feed materials of plant origin, excl. vegetable oils					
Cereal grains and their by-products	Barley, brewers' grains, maize, millet, oat, rice, rye, spelt, triticale, wheat	0.5	0.35	0.75	1.25
Forages and roughage	Alfalfa, clover, duckweed, grass, herbs	0.5	0.35	0.75	1.25
Oil seeds/fruits and their by-products	Cocoa, copra (coconut), cole-/rapeseed, groundnut, linseed, palm kernel, sesame seed, soya (bean), sunflower seed	0.5	0.35	0.75	1.25
Other feed materials of plant origin	1. *Legume seeds and their by-products of:* lentils, peas 2. *Tubers, roots and their by-products of:* carrot, manioc (tapioca), potatoes, sugar beet 3. *Other seeds/fruits and their by-products of:* citrus pulp 4. *Other plants, algae and their by-products of:* algae/seaweed, (sugar) cane molasses, sugar candy waste	0.5	0.35	0.75	1.25
1.2. Vegetable oils and by-products					
Vegetable oil/fat		0.5	0.5	0.75	1.5
Fatty acids [A]	incl. glycerine/glycerol	0.5	0.5	0.75	1.5
2. Feed materials of animal origin					
2.1. Land animal (by-)products					
Animal fat		1	0.75	2	3
Other land animal products	milk/eggs and their by-products	0.5	0.35	0.75	1.25

Feed categories		AL (dioxins)	AL (DL-PCBs)	ML (dioxins)	ML (dioxins + DL-PCBs)
2.2. Fish, other aquatic animals and their by-products					
Fish meal		1	2.5	1.25	4.5
Fish oil		5	14	6	24
Fish protein, hydrolysed		1.75	7	2.25	11
3. Feed additives, minerals and pre-mixtures					
Feed additives [B]	see Table 2.4 *Binders and anti-caking agents*	0.5 0.5	0.35 0.5	1 0.75	1.5 1.5
Feed materials of mineral origin	see Table 2.4	0.5	0.35	1	1.5
Pre-mixtures [C]		0.5	0.35	1	1.5
4. Compound feedingstuffs [D]					
4.1. Compound feed, excl. fur animals, pets and fish					
Ruminant feed	cattle feed, goat feed, sheep feed	0.5	0.5	0.75	1.5
Pig feed		0.5	0.5	0.75	1.5
Poultry feed		0.5	0.5	0.75	1.5
Miscellaneous feed materials	bakery waste [E], catering reflux (incl. cooking oil), mixed fat, yeast	0.5	0.5	0.75	1.5
4.2. Feed for fur animals, pets and fish					
Fish food		1.75	3.5	2.25	7
Pet food	bird food, horse feed, pet food, rabbit food	1.75	3.5	2.25	7

[A] *If the content of mixed fat and fatty acids is unknown, then this feed material is seen as compound feedingstuffs. Otherwise a ratio is used for the different feed materials (e.g. vegetable oil, animal fat and fish oil). In this context the AL and ML of compound feedingstuffs is used.*

[B] *A feed additive is used to favourably affect the characteristics and nutritional value of feed (material) by e.g. enhancing the flavour, colour or nutritional quality of the feed and/or optimize performance of the livestock. The definition is laid down in Article 2 (2) and 5 (3) of Council Regulation 1831/2003/EC.*

[C] *Pre-mixtures are defined as “mixtures of feed additives or one or more feed additives with feed materials or water used as carriers, not intended for direct feeding to animals” (Article 2 (2) Council Regulation 1831/2003/EC). The function is to optimize mixing of feed additives in feedingstuffs.*

[D] *Compound feedingstuffs is defined as “mixtures of feed materials, whether or not containing additives, for oral animal feeding in the form of complete or complementary feedingstuffs” (Council Directive 96/24/EC).*

[E] *Bakery waste includes biscuit meal, breadcrumb, bread meal and dough mixture.*

Starting from 18th of April 2012, the European Commission has changed the ALs and MLs for feed materials, based on the WHO-TEF 2005 instead of WHO-TEF 1998 values. Table 2.3 shows the "new" ALs/MLs based on Commission Regulation 2012/277/EU.

Table 2.3. New regulatory limits per feed category and (sub)group as specified in Commission Regulation 2012/277/EU (expressed in ng TEQ/kg product with 12% moisture, expect for fat/oil, ng TEQ/kg fat, and based on WHO-TEFs 2005) ***("new" TEFs and limits)***.

Feed categories	**AL** (dioxins)	**AL** (DL-PCBs)	**ML** (dioxins)	**ML** (dioxins + DL-PCBs)
1. Feed materials of plant origin				
1.1. Feed materials of plant origin, excl. vegetable oils	0.5	0.35	0.75	1.25
1.2. Vegetable oils and by-products	0.5	0.5	0.75	1.5
2. Feed materials of animal origin				
2.1. Land animal (by-)products				
Animal fat, excl. fish oil	0.75	0.75	1.5	2
Other land animal products	0.5	0.35	0.75	1.25
2.2. Fish, other aquatic animals and their by-products				
Fish meal	0.75	2	1.25	4
Fish oil	4	11	5	20
Fish protein, hydrolysed	1.25	5	1.75	9
3. Feed additives, minerals and pre-mixtures				
Feed additives Binders and anti-caking agents	0.5 0.5	0.35 0.5	1 0.75	1.5 1.5
Feed materials of mineral origin	0.5	0.35	0.75	1.0
Pre-mixtures	0.5	0.35	1	1.5
4. Compound feedingstuffs	0.5	0.5	0.75	1.5
Compound feed, excl. fur animals, pets and fish	0.5	0.5	0.75	1.5
Feed for fur animals, pets and fish	1.25	2.5	1.75	5.5

If not stated differently, the "old" regulatory limits (as shown in Table 2.2) are mentioned and shown in this report.

Feed additives may be further classified into the categories as stated in more detail in Annex IV. Following a comprehensive overview is given of this classification by stating the actual components sampled within this research. Hereby, feed materials of mineral origin are stated as well.

Table 2.4. Complete overview of feed additives and feed materials of mineral origin included within the study as specified in the European Union Register of Feed Additives (pursuant to Council Regulation1831/2003/EC) and the Catalogue of feed materials (Commission Regulation 575/2011/EU), 2001-2011.

Technological feed additives	
Preservatives	calcium formiate (E238), calcium propionate (E282), citric acid (E330), fumaric acid (E297), potassium sorbate (E202), sodium benzoate (E700), sorbic acid (E200)
Antioxidants	ascorbic acid (E300), propyl gallate (E310)
Emulsifying and stabilizing agents, thickeners and gelling agents	lecithin (E322)
Binders, anti-caking agents and coagulants	clay minerals (e.g. bentonite E558, kaolinitic clays E559, kieselgur - diatomaceous earth E551c, sepiolite E562/3, lignosulphonates E565, zeolite such as clinoptilolite E567/8)
Acidity regulators	ammonium chloride (E510)
Sensory feed additives	
Colorants	canthaxanthin (E161g)
Flavouring substances	sodium saccharin (E954)
Nutritional feed additives	
Vitamins and pro-vitamins	ascorbic acid (vitamin C, E300), bentaine HCl, choline chloride (vitamin B4), vitamin mix
Trace elements (Micro minerals)	copper chelate (E4), copper sulphate (E4), iron chelate (E1), iron oxide (E1), iron sulphate (E1), manganese chloride (E5), manganese oxide (E5), manganese sulphate (E5), potassium iodide (E2), zinc acetate (E6), zinc chelate (E6), zinc oxide (E6), zinc sulphate (E6)
Amino acids	
Feed materials of mineral origin	
	calcium carbonate (E170), dicalcium phosphate (E341), magnesium acetate, magnesium phosphate (E343), magnesium oxide (E530), mono-ammonium phosphate (E342), mono-calcium phosphate (E341), potassium chloride (E508), sodium butyrate, sodium bicarbonate (E500), table salt (sodium chloride)

Below, a graphical overview is given of the feed categories and (sub)groups used within this report. Figure 2.1 pictures the distribution of the untargeted samples analysed with GC-HRMS per year for each feed (sub)group. The actual amount shown is based on the selection which is made of the samples used for the analysis.

The amount of samples taken each year within a subgroup varies, as well as the total amount taken comparing all subgroups. This has to be considered when looking at individual feed groups as it can limit the reliability of the trend analysis; e.g. a low number of samples of fish oil as well as vegetable oil were analysed.

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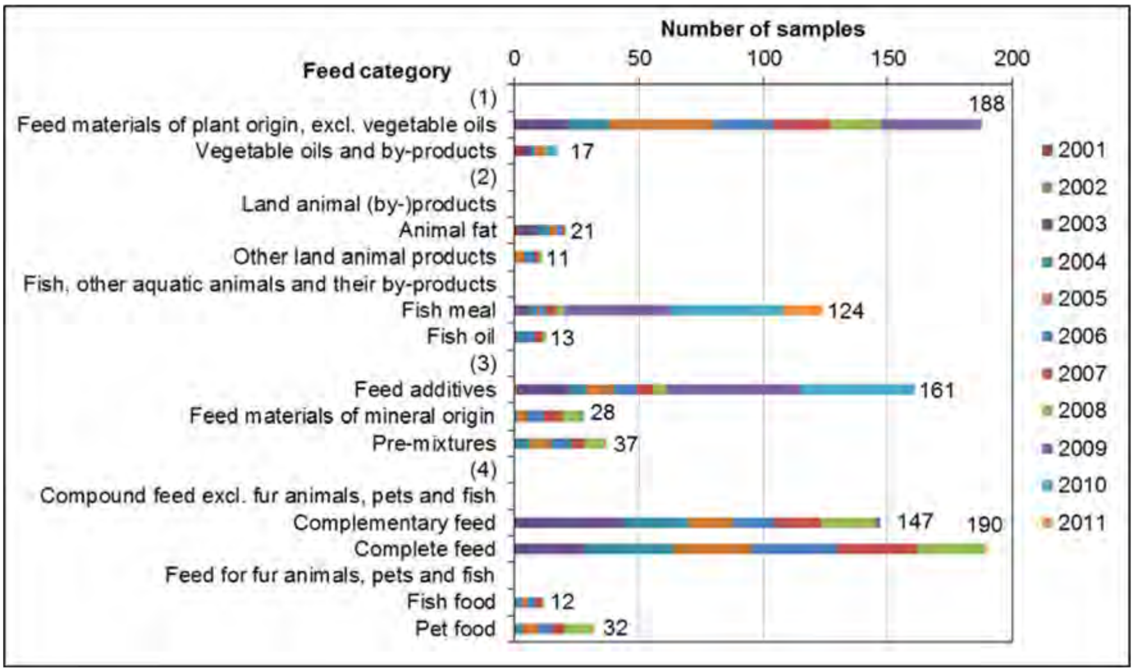

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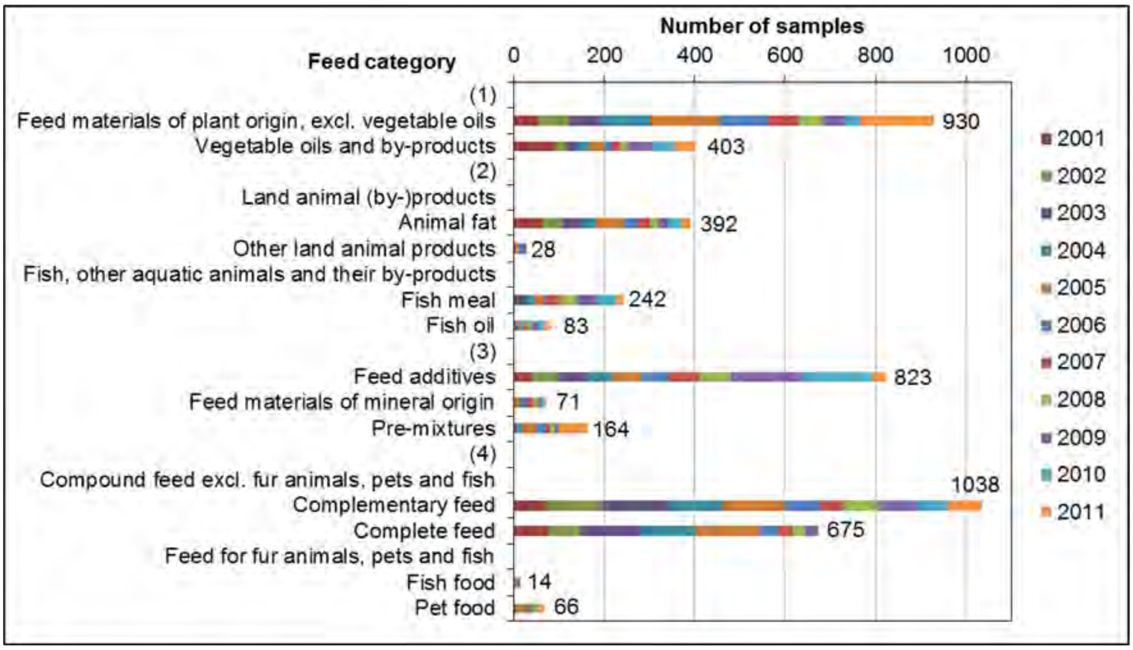

(1) Feed materials of plant origin, (2) Feed materials of animal origin, (3) Feed additives, minerals and pre-mixtures, (4) Compound feedingstuffs

Figure 2.1. Distribution of samples across feed (sub)groups for (A) the EU monitoring samples and (B) all samples collected.

Summary - Classification of feed groups

- All samples used within this report were grouped into the following four main feed categories: (1) feed materials of plant origin, (2) feed materials of animal origin, (3) feed additives, minerals and pre-mixtures and (4) compound feedingstuffs.
- Per category, feed subgroups were classified and an overview is given of the individual feed materials sampled.
- The amount of samples taken is not distributed equally per feed category over years, which may affect the outcome of the trend analysis.

2.4 Identification of critical feed materials

The Rapid Alert System for Feed and Food (RASFF) was used to look into the amount of incidents found in feed in the past in which a dioxin level above the regulatory limit was notified. From 2002 till 2010 102 incidents have been reported. Incidents notified via RASFF cannot be used for trend analysis, as on one hand the number of data is limited and on the other hand it has to be considered that only results exceeding the official limits have been reported. The measurements carried out by the competent authority which are below the limit do not need to be notified; therefore, in general higher total values are expected. For this reason, the RASFF dataset has been used to identify potential “at risk” feed groups in relation to the country of origin. In general the highest total amount of notifications is for feed originating from Germany (N=23 out of 102, 23%) and it is noticeable that 14 incidents can be linked to green forage, mainly notified in 2002 and 2003. A reason may be a higher dioxin contamination rate of the soil. Furthermore, it is prominent that a total amount of 38 incidents (38%) are notified in the category of feed additives and the highest amount can be found in Poland over the years (9 incidents mainly linked to dried sea shells). In general, the average amount of notifications and dioxin and DL-PCB levels found decreased and is in 2006 till 2010 lower compared to the years before. However, the data collected unfortunately did not reveal a clear picture of bottlenecks: feed groups or country of origin to focus on. Looking into more detail at the import statistics of the Netherlands might help to distinguish feed materials of importance.

Annex V gives an overview of the average amount of feed material used in the Netherlands each year and the percentage imported. On average the highest amount of feed materials used in the Netherlands can be found in the main feed groups of cereal grains and their by-products (47% of total feed consumption; especially wheat 23%, maize 13% and barley 4%) and oil seeds/fruits and their by-products in the form of oil cakes (30% of total feed consumption; especially soya (bean) 15%, cole-/rapeseed 6%, palm kernel 5% and sunflower 3%). Within the trend analysis, therefore, attention will be paid in more detail to wheat, maize, oil seeds/fruits and vegetable oils. However, as the number of samples collected and measured with GC-HRMS was too low for the individual feed materials, the trend analysis can only be carried out for the feed subgroup forages and roughage. However, it was also looked into background levels for the other feed subgroups, such as cereal grains and their by-products, oil seeds/fruits and their by-products as well as vegetable oils and by-products.

In the past, high dioxin levels were detected in a number of incidents (as stated in Table 1.1), such as citrus pulp, choline chloride, bakery waste, clay minerals (e.g. kaolinite, sepiolite) and recently again in fats and oils. Dried products, such as grass and alfalfa, are also at risk, due to the potential formation of dioxins in case an inappropriate drying process is applied. All the above mentioned feed materials have been included in the Dutch National Control Plan for animal feed (NCP) (see Annex I), linked to a risk-based monitoring approach. Therefore, a focus will be put on these feed materials as well; excluding citrus pulp. The latter was a problem in the late nineties, but since then the Brazilian government has to provide certificates (proof of the absence of dioxins) with every shipment entering the Netherlands and until now no incident has been notified again, according to the NVWA (NCP). Concluding, it is not necessary to include citrus pulp in the list of critical materials. According to an EFSA opinion (2010) the highest dioxin levels found can be linked to fish oil, so this feed material also deserves special attention. However, in the Netherlands vegetable and animal fat/oil is generally used in feed in low quantities (2% of total feed consumption, 0.3 Mt) and thus lead to a small contribution of the total amount used. This has to be kept in mind when an advice on this feed material is given based on the results of the analysis. Also for the above mentioned feed materials the number of samples collected and measured with GC-HRMS was too low per individual feed materials. The trend analysis can therefore only be carried out for the feed material clay minerals belonging to the feed subgroup of binders, anti-caking agents and coagulants (feed additives).

A high amount of the feed materials used is imported (88% respectively 12.7 Mt). A differentiation is made between direct and indirect import. The latter means that foreign feed materials are further processed in the Netherlands before they are used as feed. In this case not only the origin could contribute to the presence of dioxins, but also the production process. In 68 % (8.6 Mt) feed materials are directly imported and in 32% (4.1 Mt) the feed is further processed in the Netherlands. Due to the high amount of foreign feed materials, it seems important to also look in more detail into the main countries exporting feed materials to the Netherlands. Eurostat was used to look into the percentage of feed entering the Netherlands coming from other EU-27 Member States and/or from countries outside the EU. Based on the average volume (in tons) per year and on the data available between 2001 till 2010, the main countries where imported feed in the Netherlands originates from are Argentina (25%), Brazil (23%), Germany (20%), Belgium (7.5%), Malaysia (7%), USA (7%), France (4.5%) and Indonesia (4%). Next to the above mentioned countries, a focus will be laid on countries in which a repeated exceedance of maximum limits of dioxin was found during the analysis. Table 2.5 gives an overview of the main countries involved per feed category. A detailed table can be found in Annex VI.

Table 2.5. Countries of origin per feed category and (sub)group, based on the average volume exported to the Netherlands, 2001-2010. *Source: Eurostat (DS_018995).*

Feed category	**% total** [A]	**Country of origin (%)** [B]
1. Feed products predominantly imported from countries outside the EU-27		
Other feed materials of plant origin		
- vegetables and their by-products	85 ± 7	BR: 46, AR: 25, US: 13
Oil seeds/fruits and their by-products		
- soya (bean)	96 ± 1	AR: 48, BR: 47
- palm nut or kernel	95 ± 2	MY: 64, ID: 31
- sunflower seed	86 ± 8	AR: 83
- copra (coconut)	90 ± 11	ID: 61, PH: 28
2. Feed products predominantly imported from other EU-27 Member States		
Cereal grains and their by-products		
- wheat	100	DE: 56, BE: 30, FR: 13
- maize	100	DE*: 56, FR*: 38, BE*: 5
- rice	94 ± 48	NB. Since 2007 not imported anymore.
Forages and roughage	100	FR: 73, DE: 14, BE: 13 (for alfalfa)
Oil seeds/fruits and their by-products		
- rapeseed	100 ± 2	DE: 82, BE: 12
- linseed	75 ± 23	NB. High StDev as from 2001-2005 coming from US and AR, but since 2006 coming from DE and BE.
- groundnut	96 ± 30	NB. High StDev as in 2001 high import from SN, but since 2002 DE is the main contributor with 92%.
Fish meal	88 ± 15	DE: 69, DK: 11, FR: 5, N.B. High StDev as fish meal comes also from countries outside EU-27 such as PE: 12.
Miscellaneous feed materials		
- residues of starch and similar manufacture	99 ± 1	BE: 39, FR: 34, DE: 25
- brewing or distilling waste	87 ± 10	DE: 56, BE: 26, US: 12
Feed for fur animals, pets and fish		
- dog/cat food - retail sale	94 ± 4	DE: 33, FR: 29, BE: 11, GB: 8

Feed category	% total [A]	Country of origin (%) [B]
3. Feed products imported from both outside or inside EU-27		
Oil seeds/fruits and their by-products		
- cotton seed		Until 2007 mainly from outside EU-27. Starting from 2007 coming from DE*, but the amount in tons imported decreased over the years.
- other oil seeds		DE*: 44, AR: 27, ES: 17, BR: 5
Compound feed, excl. fur animals, pets and fish		US: 46, DE: 35, BE: 10, FR: 6

Abbreviations used: AR = Argentina, AT = Austria, BE = Belgium, BR = Brazil, DE = Germany, ES = Spain, FR = France, GB = United Kingdom, ID = Indonesia, IT = Italy, MY = Malaysia, PE = Peru, PH = Philippines, SN = Senegal, US = United States of America

[A] *The first percentage stated is based on the average amount in tons in the specified feed (sub)group making a division between (1) outside and (2) inside EU-27 divided by the total average amount in tons per the same feed (sub)group. This means that in (1) the % stated relates to feed exported from countries outside the EU and in (2) the % stated relates to feed exported from other EU-27 Member States to the Netherlands. The average amount in tons is based on the Dutch import statistics from 2001 till 2010.The standard deviation (StDev) was calculated on basis of the percentage per year. The percentage per year was calculated by the amount in tons per specified feed (sub)group making again a division between (1) outside and (2) inside EU-27 divided by the total amount in tons per the same feed (sub)group in each year from 2001 till 2010.*

[B] *The percentage stated per country of origin is based on the average amount in tons per specified feed (sub)group per country of origin from 2001 till 2010 divided by the average of the total amount in tons per feed (sub)group.*

* *Likely cotton seed and other oil seeds are not cultivated but instead further processed in Germany. Also maize is probably imported from the US and after further processing, by-products are exported by Germany, Belgium and France.*

The feed consumption provided by the PDV (Annex V) is considered to give a good overview of the actual amount of feed materials used in the Netherlands as the quantity of feed exported is extracted from the feed produced (own resources) and/or imported. However, a point of attention is the fact that via trade statistics it is reported that e.g. Germany exports soya (beans) and sunflower seeds as feed to the Netherlands, whereas it hardly cultivates these plant species. In fact, these feed materials have probably been exported from Argentina and Brazil to Germany, where they are processed e.g. to oil and the by-products are exported as feed to the Netherlands. The real country of origin, therefore, cannot be specified, but within this report it will be assumed that these feed materials originate from the countries as stated in the database, not at least because they also have the responsibility by law to monitor the material that has been imported. Wheat, maize, barley and oats are in general grown and processed in the European countries as indicated.

To be able to analyse the dioxin and DL-PCB concentrations in different countries of origin, a sufficient amount of samples needs to be present per year. Figure 2.2 gives an overview of the number of samples taken per country of origin for (A) the untargeted samples analysed with GC-HRMS (EU monitoring) and (B) all samples collected including samples measured with DR CALUX® only. As can be seen in Figure 2.2 A, the number of samples collected and measured with GC-HRMS is low per country (except: the Netherlands and in case the origin is unknown). Therefore no trend analysis was carried out per country. In order to use samples for trend analysis related to the country of origin in future, it is important to register originating countries correctly. Also in 33% of the cases (N=1616 of N_{total}=4938) the country of origin was unknown.

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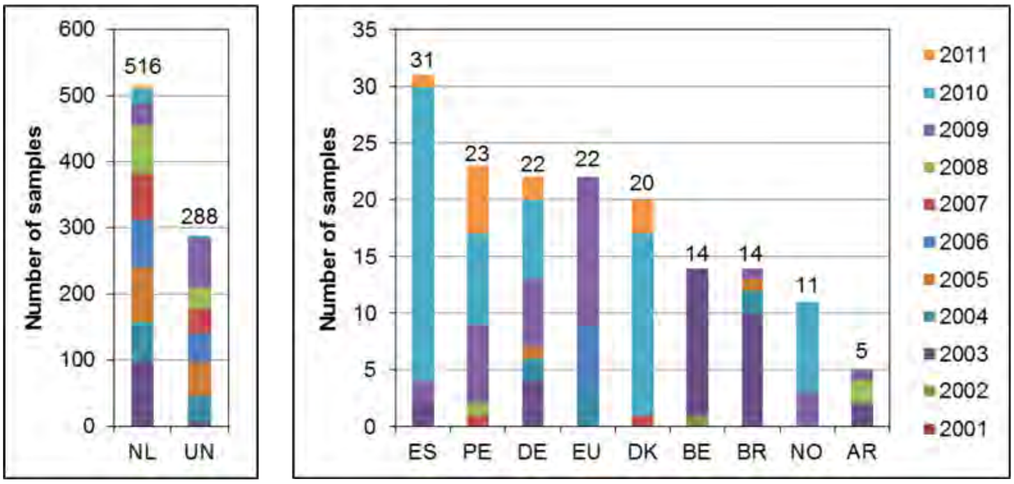

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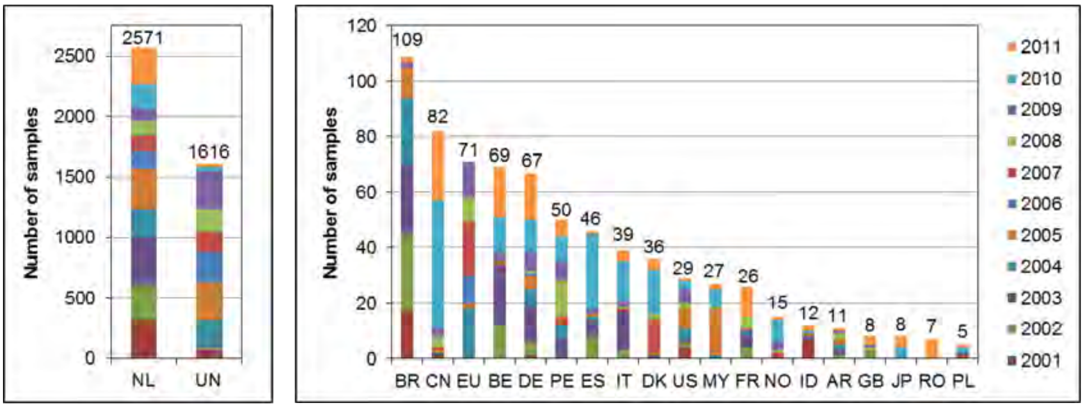


Abbreviations used: AR = Argentina, BE = Belgium, BR = Brazil, CN = China, DE = Germany, DK = Denmark, ES = Spain, EU = European Union consisting of 27 Member States excluding those mentioned separately, FR = France, GB = United Kingdom, ID = Indonesia, IT = Italy, MY = Malaysia, NL = the Netherlands, NO = Norway, PE = Peru, UN = origin unknown, US = United States of America.

Figure 2.2. Distribution of samples across country of origin for (A) the EU monitoring samples, (B) all samples collected.

Summary - Identification of critical feed materials

- Based on incidents found in the past and import volume, critical feed materials chosen to focus on during the trend analysis are: wheat, maize, grass/alfalfa, oil seeds/fruits, choline chloride, clay minerals, fats/oils (esp. fish oil), fish meal and bakery waste. However, the number of samples collected and measured with GC-HRMS was too low per individual feed material and therefore a focus is laid on the feed (sub)groups: cereal grains and their by-products, forages and roughage, oil seeds/fruits and their by-products, vegetable oils and by-products, animal fat, fish oil, fish meal and feed additives.
- Based on import volume, countries of origin chosen to focus on in more detail for the trend analysis are: Argentina, Brazil, Germany, Belgium, Malaysia, USA, France, Indonesia and all countries found repeatedly exceeding maximum dioxin levels in the past. However, the number of samples collected and measured with GC-HRMS was too low for these countries to be used for further analysis.

2.5 Statistics used for trend analysis on monitoring data

Results are first visualized with the help of histograms and a descriptive analysis is carried out. The sample year is displayed on the X axis and the dioxin concentration (in ng TEQ/kg) on the left Y axis. The number of samples tested per year (stated as N) is further visible on a second Y axis on the right side of the graph. Results of dioxin concentrations are shown as averages, median and 90^{th} percentile (90^{th} perc); with median and 90^{th} percentile absent when less than 5 and 10 samples, respectively, have been analysed. In addition, limits (legal maximum limit, ML) will be indicated in the graph using a grey background. It is important to always check the values stated on the left Y-axis, as in some cases the trend values calculated might be far below the regulatory limit. For a better visualization the limit might not be visible in the graph itself; therefore the limit is also stated below each graph to avoid misconception. MLs were first set in 2006 for all feed categories (Commission Directive 2006/13/EC), but will be used in the graph for all years (2001 till 2010).

The average (avg), defined as arithmetic mean within this report, is the central tendency of all sample outcomes. The larger the sample size (amount of samples tested per year), the more reliable the average, as this value is greatly influenced by outliers (e.g. incidents). For comparison also the median is shown in the graph. The median is a value which separates the higher half of a sample outcome (dioxin concentrations) from the lower half, being the middle value. Compared to the average value, it is a good indicator if a distribution of measurements is skewed, as in this way outliers become less important. The 90^{th} percentile (90^{th} perc), also shown in the graph, indicates that 90% of the sample outcomes is below the given concentration respectively 10% is above the value. This statistic value may be useful as it gives a picture of the bandwidth of dioxin concentrations.

In order to visualize trends in the graph, regression analysis was used by adding a linear trend line to the graph. The trend line is based on the average dioxin concentrations per year. Regression is used to predict future trends on the basis of tendencies found in the past and to look at the variability across time periods. Hereby the equation $y = mx + b$ describes a straight line for a set of data where x is the independent variable, y is the dependent variable, m represents the slope of the line (also known as the regression coefficient) and *b* represents the y-intercept (that is, the point where the line crosses the left y-axis). Furthermore, R^2 is the correlation factor which indicates how well the trend line fits the observations. A R^2 value of zero indicates no relationship (absolute randomness) between the x variable (year) and y variable (average dioxin concentration), whereas a R^2 value of 1 indicates perfect correlation meaning that the values of y all lie on the trend line. If R^2 is below 0.3, no correlation can be found and a trend cannot be analysed as there is no connection between changing dioxin concentrations over the years.

In order to estimate background levels, which can be compared with the EFSA data, the average, median (Q(.50)), 95th and 99th percentile (Q(.95), Q(.99)) were calculated for the complete time period and are illustrated in a table before each section. The Q(.95) is an important value as the MLs are set on the basis that 5% exceed the background levels, thus using the Q(.95).

Furthermore, a more in depth statistical analysis is carried out. Dioxin data are assumed to be not normally distributed as dioxins and DL-PCBs are usually present in low concentrations and an asymmetric distribution is expected. Furthermore, in respect to the dataset, not always a constant number of monitoring samples is taken per given period of time (year) and the feed materials are not always linked to the same production site and/or country of origin. In this case a non-parametric statistical test might be used, such as the Mann-Kendall test.

The Mann-Kendall test measures the presence of monotonic increasing or decreasing trends, by comparing the relative magnitudes of sample data rather than the data values themselves (Gilbert, 1987). The advantage of this test is that the data need not to conform to any particular distribution and missing values are allowed. The template MAKESENS (Salmi *et al*., 2002) was used to assess trends with the Mann-Kendall test. This template provides a significance value for the annual average dioxin and DL-PCB concentrations per feed (sub)group per time period ($N \leq 8$ years, from 2003 till 2010). It, therefore, indicates whether the trend found with the descriptive analysis (graphs) really changed significantly over the period of investigation. The significance values of the Mann-Kendall test are described in Table 2.6.

Table 2.6. Significance in Mann-Kendall Test.

Symbol	**Level of significance [A]**
***	If trend at 0.001 level of significance
**	If trend at 0.01 level of significance
*	If trend at 0.05 level of significance
+	If trend at 0.1 level of significance
blank	If trend at >0.1 level of significance

[A] *The significance level 0.001 means that there is a 0.1% probability that the values are from a random distribution and that there is no trend. Thus the existence of a monotonic trend is very probable. Respectively the significance level 0.1 means that there is a 10% probability that no trend can be found, thus it is still very likely that an increasing or decreasing trend exists.*

A trend found with a significance level of e.g. p=0.05 (significance: *) means that there is a 5% probability that the values are from a random distribution and that there is no trend. Thus the existence of a monotonic trend is very likely (95%). The S value indicates an upward (positive value) or downward (negative value) trend. The higher the value the more increasing or decreasing trend can be found. The Mann-Kendall test requires at least 4 average values in a time series, thus a maximum of 4 years is allowed in which no samples have been collected.

A disadvantage is the fact that both the Mann-Kendall test as well as the descriptive analysis do not take the amount of samples tested per year into account as the estimations are based on the average values per year. Before using the statistical tests it was checked that a minimum amount of samples (e.g. N=5) is present per year.

Some general issues should be considered before analysing and interpreting the collected dataset and starting with the trend analysis, such as:

- <u>Number of years</u>:
  Trends have been investigated over a time period of 8 years (2003-2010). The shorter the time period, the greater is the potential for error. Eight years can been seen as a reasonable time scale to be able to analyse trends within the framework of this report. Nevertheless, dioxin and DL-PCB concentrations are expected to change slowly over time as being quite persistent chemicals with a long half-life. On the other hand, this is exactly the period where various measures were taken to reduce the levels, including the establishments of limits and increased monitoring, both by governments and companies.
- <u>Presence of outliers</u>:
  It is important to determine whether outliers are due to random variability or if they reflect a real change in the general trend. Annex VIII gives an overview of potential outliers within the dataset.
- <u>Number of samples</u>:
  To get reliable results on trends a minimum number of samples should be collected each year per feed (sub)group. In addition, if N fluctuates a lot during the years, the outcome on trends may not be representative as average dioxin and DL-PCB concentrations might be influenced. In some years more samples of a certain feed group have been taken than in other years, if e.g. an incident was found related to a company, a country, a specific feed material or a production process applied. Furthermore, in 64% of the samples (N=3149 of N_{total}=4938) the precise dioxin concentration is not known; only that it is below the decision limit of the DR CALUX® bioassay. This is due to the fact that in these samples a negative result was found with CALUX and the samples were not quantified further with GC-HRMS. A lot of samples could therefore not be included in the trend analysis.
- <u>Exogenous factors which can influence the trend</u>:
  Changes over time in factors related to the indicator of interest (dioxin concentration within a feed (sub)group during 8 years) must also be considered. Examples are:
  - A fictive decreasing trend might be seen between the years 2001-2006 and 2007-2011 if most concentrations found are at or below the reporting limit/LOQ (see section 2.1 for further explanation).
  - Starting from 2005, dioxin concentrations were calculated on the basis of the product containing 12% moisture. The dioxin concentration in feed samples containing less/more moisture was adjusted. This approach was chosen as all regulatory limits set by

Commission Directive 2006/13/EC are based on a product containing 12% moisture. Prior to 2005 concentrations were not adjusted. Since the moisture content of many feeds is often close to 12% the results from before and after 2005 can still be compared. However, caution of interpreting trends found between 2001-2004 and 2005-2011 is in place for feed materials high or low in water content, e.g. liquid feeds and minerals, respectively. Products measured on the fat/oil basis are excluded from this conversion and are until now reported on the dioxin concentration measured without adjustment.

- o Changes in legislation. End of 2006, regulatory limits for dioxins and dioxin-like PBCs were regulated for the first time for all feed categories. Initially only a maximum dioxin level in citrus pulp has been established by law starting from 2002. Feed producers might not have immediately taken action on higher dioxin or DL-PCB levels present in certain feed materials. Concluding, trends might not be visible yet.

In conclusion, the issues mentioned could influence the trend analysis and to determine the real cause of a trend is of utmost importance.

Summary - Statistics used for trend analysis on monitoring data

- Results are visualized with the help of histograms and a descriptive analysis (average, StDev, median, 95th and 99th percentile). Regression analysis was used by adding a linear trend line to the graph.
- As dioxins and DL PCBs are usually present in low concentrations and an asymmetric distribution is expected, in addition to the regression analysis also the non-parametric statistical test Mann Kendall was used to predict trends.
- Points of attention:
  - o A minimum number of samples has to be present per year per feed (sub)group to get reliable results on trends.
  - o Strong fluctuations in the amount of samples taken per year per feed (sub)group may result in misinterpretation of trends.
  - o Trends found in low dioxin and DL-PCB concentrations should be taken with caution as in the middle of 2007 the LOQ/reporting limits were adjusted.
  - o Starting from 2005, dioxin concentrations were calculated on the basis of product containing 12% moisture. Prior to 2005 concentrations were not adjusted, therefore changes found between 2001-2004 to 2005-2010 should be treated with caution, especially in feed materials with a higher or lower water content than most feeds.
  - o The late establishment of regulatory limits for DL-PCBs in 2007 might have influenced the compliance of feed producers at a later time period compared to dioxins.

3 Results

This chapter starts with an overview of all samples tested in the monitoring program and the samples found to exceed the regulatory limits. Subsequently, upper bound levels of the random samples analysed by GC-HRMS (EU monitoring samples) are compared to lower bound dioxin and DL-PCB levels in order to establish the range of uncertainty in the outcome of the results. In the end, trends of dioxins, dioxin-like PCBs (DL-PCBs) and the sum of dioxins and DL-PCBs are investigated. First, contamination levels are characterized in terms of average, median (Q(.50)), 95^{th} and 99^{th} percentile (Q(.95) and Q(.99)). Levels of the EU monitoring data are compared to the data analysis of European data by EFSA (EFSA, 2010), which formed the basis for the "new" regulatory limits. Values were established for both sets of TEF-values resulting in TEQ_{WHO98} and TEQ_{WHO05}. However, for a better illustration graphs and tables are only shown using the TEQ_{WHO98}. The descriptive analysis is visualized using frequency distribution curves and trends during the years are shown using regression analysis as well as the Mann-Kendall test (statistical analysis). A summary of the results of the statistical test can be found in Annex X. The results of the trend analysis are shown per feed subgroup as specified in section 2.3. In case dioxin levels exceed the action level (AL) or maximum level (ML) the origin of the commodity (country of origin) has been identified. At the beginning of each section a table also provides an overview of the total amount of the selected feed materials used in the Netherlands. In case the feed is imported, the country of origin is provided as well.

3.1 Non-compliance with regulatory limits

In Table 3.1 an overview of all samples found to exceed the regulatory limits, namely the action level (AL) and maximum level (ML) is given. The AL serves as early warning tool and in case dioxin concentrations are found to exceed the AL, actions should be taken to disclose the source of dioxins and DL-PCBs. The ML is the official limit used for regulatory control and if this limit is exceeded the food product in question may not be used for human consumption anymore. In this overview also all suspected or negative samples found with the DR CALUX® are included (N_{total}). It is important to acknowledge that all samples included are taken at random and are not the result of targeted sampling. Table 3.1 gives an overview of the percentage of samples exceeding the AL for dioxins (PCDD/Fs) and DL-PCBs and the ML for dioxins and for the sum of dioxins and DL-PCBs per feed category. Hereby also a 10% margin relating to the measurement uncertainty is included by decreasing the concentration of each sample measured with 10% prior to comparison with the limits. This margin has only been applied in this section and thus not for the trend analysis or calculation of background levels.

In fish meal and clay minerals (binders, anti-caking agents and coagulants) the AL for dioxins was exceeded to a high extent with 4.1% and 9.8%, respectively. Hereby, the ML for dioxins was exceeded as well in 3.7% and 3.4% of the cases. In addition, DL-PCBs play a role in fish meal and the AL was exceeded with 2.5%, whereas for clay minerals DL-PCBs do not seem to be an issue. For both feed materials also the ML for the sum of dioxins and DL-PCBs was exceeded with 1.7% and 1.5%, respectively.

Another feed material of concern could be vegetable oil, in which 3% of the samples exceeded the AL and 1.7% the ML of dioxins. In feed materials of plant origin (excl. vegetable oil) only in 1.1% the AL of dioxins was exceeded. In animal fat, DL-PCBs seem to be of more concern and in 1.3% the AL of DL-PCBs was exceeded.

In all other feed materials no or only a low (<1%) non-compliance with the regulatory limits was found.

When comparing these findings to the results found by using the "new" TEF_{WHO05} values instead of the TEF_{WHO98} and the new ALs and MLs (see Table 3.2), the non-compliance found in most feed categories is comparable. However, the number of samples exceeding the ML of dioxins in fish meal decreased with 2.5%. Also in clay minerals the AL of dioxins is now with 8.9% in less cases non-compliant, which is a decrease of around 1%. For all other feed materials around the same number of non-compliant samples was found.

Concluding, using the "new" TEF_{WHO05} values may have an impact on the number of fish meal samples rejected in future and the concentrations found may differ from background levels in the past.

Table 3.3 gives an overview of the total number of samples which exceed the AL and ML. This gives a better picture on the amount of samples involved, as some individual samples may exceed both ALs and/or MLs. In this overview, pre-mixtures now seem to exceed the ALs with 1.2%, which was below 1% before looking at the individual ALs. In the case of fish meal, Table 3.3 indicates that the fraction of samples exceeding the MLs markedly decreased from 4.1 to 1.2%. This can be explained by the reduction of the TEFs for the mono-ortho PCBs in 1 sample and the reduction of the TEFs for two specific PCDFs (e.g. 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF) in the other 6 samples. Due to a relatively high concentration of these compounds and the fact that all 7 samples were close to the ML, less non-compliant samples were found when using the new TEFs (WHO_{2005}).

Table 3.4 shows that in the majority of samples exceeding the AL the country of origin was either unknown or the Netherlands. In the case of the ML, most samples originated from the Netherlands. Of course this is primarily due to the much larger sample numbers from these origins. It should be mentioned that the AL for DL-PCBs and ML for the sum of dioxins and DL-PCBs only applies since the end of 2006.

Summary - Non-compliance with regulatory limits

- The fraction of samples exceeding the maximum levels was below 1% for most feed materials, except for fish meal, clay minerals and vegetable oils. In addition, in animal fat, pre-mixtures and feed materials of plant origin (except vegetable oils) just above 1% of the samples were non-compliant with the ALs.
- Slight differences can be seen when comparing TEQ_{WHO98} vs. TEQ_{WHO05} values. When using the TEF_{WHO05} instead of TEF_{WHO98}, in clay minerals the amount of samples exceeding the AL of dioxins decreases. In fish meal a decrease can be seen as well if looking into the amount of non-compliant samples for the ML of dioxins. Using the "new" TEF_{WHO05} values may thus have an impact on the number of fish meal samples rejected.

Table 3.1. Number (N) and percentage (%) of samples exceeding the action levels (AL) and maximum levels (ML) according to Commission Directive 2006/13/EC (expressed on the basis of 12% moisture in ng TEQ/kg, expect for fat/oil, ng TEQ/kg fat) using WHO-TEFs 1998 ***("old" TEFs and limits).***

Feed category	$\mathbf{N}_{total}$	**AL (PCDD/Fs)**			**AL (DL-PCBs)**			**ML (PCDD/Fs)**			**ML (sum PCDD/Fs + DL-PCBs)**		
		AL	Samples > AL		AL	Samples > AL		ML	Samples > ML		ML	Samples > ML	
			N	**%**		**N**	**%**		**N**	**%**		**N**	**%**
Feed materials of plant origin	**1333**	**0.5**	**22**	**1.7**	**n.a.**	**2**	**0.2**	**0.75**	**9**	**0.7**	**n.a.**	**4**	**0.3**
Feed materials of plant origin, excl. vegetable oils	930	0.5	10	1.1	0.35	1	0.1	0.75	2	0.2	1.25	3	0.3
Vegetable oils and by-products	403	0.5	12	3.0	0.5	1	0.2	0.75	7	1.7	1.5	1	0.2
Feed materials of animal origin	**754**	**n.a.**	**13**	**1.7**	**n.a.**	**11**	**1.5**	**n.a.**	**11**	**1.5**	**n.a.**	**7**	**0.9**
Land animal (by-)products	420	n.a.	3	0.7	n.a.	5	1.2	n.a.	2	0.5	n.a.	3	0.7
- *Animal fat*	*392*	*1*	*3*	*0.8*	*0.75*	*5*	*1.3*	*2*	*2*	*0.5*	*3*	*3*	*0.8*
- *Other land animal products*	*28*	*0.5*	*0*	*0*	*0.35*	*0*	*0*	*0.75*	*0*	*0*	*1.3*	*0*	*0*
Fish, other aquatic animals and their by-products	334	n.a.	10	3.0	n.a.	6	1.8	n.a.	9	2.7	n.a.	4	1.2
- *Fish meal*	*242*	*1*	*10*	*4.1*	*2.5*	*6*	*2.5*	*1.25*	*9*	*3.7*	*4.5*	*4*	*1.7*
- *Fish oil*	*83*	*5*	*0*	*0*	*14*	*0*	*0*	*6*	*0*	*0*	*24*	*0*	*0*
- *Fish protein, hydrolysed*	*9*	*1.75*	*0*	*0*	*7*	*0*	*0*	*2.25*	*0*	*0*	*11*	*0*	*0*
Feed additives, minerals and pre-mixtures	**1058**	**0.5**	**34**	**3.2**	**n.a.**	**2**	**0.2**	**n.a.**	**12**	**1.1**	**1.5**	**6**	**0.6**
Feed additives	823	0.5	33	4.0	0.5	1	0.1	1	12	1.5	1.5	6	0.7
- *Binders, anti-caking agents and coagulants*	*325*	*0.5*	*32*	*9.8*	*0.5*	*0*	*0*	*0.75*	*11*	*3.4*	*1.5*	*5*	*1.5*
Feed materials of mineral origin	71	0.5	0	0	0.35	0	0	1	0	0	1.5	0	0
Pre-mixtures	164	0.5	1	0.6	0.35	1	0.6	1	0	0	1.5	0	0
Compound feedingstuffs	**1793**	**n.a.**	**6**	**0.3**	**n.a.**	**0**	**0**	**n.a.**	**5**	**0.3**	**n.a.**	**3**	**0.2**
Compound feed, excl. fur animal, pets and fish	1713	0.5	6	0.4	0.5	0	0	0.75	5	0.3	1.5	3	0.2
- *Complementary feed*	*1038*	*0.5*	*5*	*0.5*	*0.5*	*0*	*0*	*0.75*	*5*	*0.5*	*1.5*	*3*	*0.3*
- *Complete feed*	*675*	*0.5*	*1*	*0.1*	*0.5*	*0*	*0*	*0.75*	*0*	*0*	*1.5*	*0*	*0*
Feed for fur animals, pets and fish	80	1.75	0	0	3.5	0	0	2.25	0	0	7	0	0
- *Fish food*	*14*	*1.75*	*0*	*0*	*3.5*	*0*	*0*	*2.25*	*0*	*0*	*7*	*0*	*0*
- *Pet food*	*66*	*1.75*	*0*	*0*	*3.5*	*0*	*0*	*2.25*	*0*	*0*	*7*	*0*	*0*

Table 3.2. Number (N) and percentage (%) of samples exceeding the action levels (AL) and maximum levels (ML) according to Commission Regulation 2012/277/EU (expressed on the basis of 12% moisture in ng TEQ/kg, expect for fat/oil, ng TEQ/kg fat) and using WHO-TEFs 2005 ***("new" TEFs and limits)***.

Feed category	N_{total}	**AL (PCDD/Fs)**			**AL (DL-PCBs)**			**ML (PCDD/Fs)**			**ML (sum PCDD/Fs + DL-PCBs)**		
		AL	Samples > AL		AL	Samples > AL		ML	Samples > ML		ML	Samples > ML	
			N	**%**		**N**	**%**		**N**	**%**		**N**	**%**
Feed materials of plant origin	**1333**	**0.5**	**22**	**1.7**	**n.a.**	**2**	**0.2**	**0.75**	**10**	**0.8**	**n.a.**	**3**	**0.2**
Feed materials of plant origin, excl. vegetable oils	930	0.5	9	1.0	0.35	1	0.1	0.75	2	0.2	1.25	2	0.2
Vegetable oils and by-products	403	0.5	13	3.2	0.5	1	0.2	0.75	8	2.0	1.5	1	0.2
Feed materials of animal origin	**754**	**n.a.**	**14**	**1.9**	**n.a.**	**9**	**1.2**	**n.a.**	**5**	**0.7**	**n.a.**	**7**	**0.9**
Land animal (by-)products	420	n.a.	3	0.7	n.a.	3	0.7	n.a.	2	0.5	n.a.	4	1.0
- *Animal fat*	*392*	*0.75*	*3*	*0.8*	*0.75*	*3*	*0.8*	*1.5*	*2*	*0.5*	*2*	*4*	*1.0*
- *Other land animal products*	*28*	*0.5*	*0*	*0*	*0.35*	*0*	*0*	*0.75*	*0*	*0*	*1.25*	*0*	*0*
Fish, other aquatic animals and their by-products	334	n.a.	11	3.3	n.a.	6	1.8	n.a.	3	0.9	n.a.	3	0.9
- *Fish meal*	*242*	*0.75*	*11*	*4.5*	*2*	*6*	*2.5*	*1.25*	*3*	*1.2*	*4*	*3*	*1.2*
- *Fish oil*	*83*	*4*	*0*	*0*	*11*	*0*	*0*	*5*	*0*	*0*	*20*	*0*	*0*
- *Fish protein, hydrolysed*	*9*	*1.25*	*0*	*0*	*5*	*0*	*0*	*1.75*	*0*	*0*	*9*	*0*	*0*
Feed additives, minerals and pre-mixtures	**1058**	**0.5**	**31**	**2.9**	**n.a.**	**2**	**0.2**	**n.a.**	**10**	**0.9**	**1.5**	**5**	**0.5**
Feed additives	823	0.5	30	3.6	0.5	1	0.1	1	10	1.2	1.5	5	0.6
- *Binders, anti-caking agents and coagulants*	*325*	*0.5*	*29*	*8.9*	*0.5*	*0*	*0*	*0.75*	*10*	*3.1*	*1.5*	*4*	*1.2*
Feed materials of mineral origin	71	0.5	0	0	0.35	0	0	0.75	0	0	1	0	0
Pre-mixtures	164	0.5	1	0.6	0.35	1	0.6	1	0	0	1.5	0	0
Compound feedingstuffs	**1793**	**n.a.**	**6**	**0.3**	**n.a.**	**0**	**0**	**n.a.**	**5**	**0.3**	**n.a.**	**2**	**0.1**
Compound feed, excl. fur animal, pets and fish	1713	0.5	6	0.4	0.5	0	0	0.75	5	0.3	1.5	2	0.1
- *Complementary feed*	*1038*	*0.5*	*5*	*0.5*	*0.5*	*0*	*0*	*0.75*	*5*	*0.5*	*1.5*	*2*	*0.2*
- *Complete feed*	*675*	*0.5*	*1*	*0.1*	*0.5*	*0*	*0*	*0.75*	*0*	*0*	*1.5*	*0*	*0*
Feed for fur animals, pets and fish	80	1.75	0	0	3.5	0	0	2.25	0	0	7	0	0
- *Fish food*	*14*	*1.25*	*0*	*0*	*2.5*	*0*	*0*	*1.75*	*0*	*0*	*5.5*	*0*	*0*
- *Pet food*	*66*	*1.25*	*0*	*0*	*2.5*	*0*	*0*	*1.75*	*0*	*0*	*5.5*	*0*	*0*

Table 3.3. Total number (N) and percentage (%) of samples exceeding the action levels (AL) and maximum levels (ML) per feed category using TEF_{WHO98} and TEF_{WHO05}.

Feed category	N_{total}	> AL				> ML			
		TEF_{WHO98}		TEF_{WHO05}		TEF_{WHO98}		TEF_{WHO05}	
		N	%	N	%	N	%	N	%
Feed materials of plant origin	**1333**	**22**	**1.7**	**22**	**1.7**	**10**	**0.8**	**11**	**0.8**
Feed materials of plant origin, excl. vegetable oils	930	10	1.1	9	1.0	3	0.3	3	0.3
Vegetable oils and by-products	403	12	3.0	13	3.2	7	1.7	8	2.0
Feed materials of animal origin	**754**	**18**	**2.4**	**16**	**2.1**	**13**	**1.7**	**7**	**0.9**
Land animal (by-)products	420	6	1.4	4	1.0	3	0.7	4	1.0
- *Animal fat*	*392*	*6*	*1.5*	*4*	*1.0*	*3*	*0.8*	*4*	*1.0*
- *Other land animal products*	*28*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Fish, other aquatic animals and their by-products	334	12	3.6	12	3.6	10	3.0	3	0.9
- *Fish meal*	*242*	*12*	*5.0*	*12*	*5.0*	*10*	*4.1*	*3*	*1.2*
- *Fish oil*	*83*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
- *Fish protein, hydrolysed*	*9*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Feed additives, minerals and pre-mixtures	**1058**	**35**	**3.3**	**32**	**3.0**	**12**	**1.1**	**11**	**1.0**
Feed additives	823	33	4.0	30	3.6	12	1.5	11	1.3
- *Binders, anti-caking agents and coagulants*	*325*	*32*	*9.8*	*29*	*8.9*	*11*	*3.4*	*10*	*3.1*
Feed materials of mineral origin	71	0	0	0	0	0	0	0	0
Pre-mixtures	164	2	1.2	2	1.2	0	0	0	0
Compound feedingstuffs	**1793**	**6**	**0.3**	**6**	**0.3**	**5**	**0.3**	**5**	**0.3**
Compound feed, excl. fur animal, pets and fish	1713	6	0.4	6	0.4	5	0.3	5	0.3
- *Complementary feed*	*1038*	*5*	*0.5*	*5*	*0.5*	*5*	*0.5*	*5*	*0.5*
- *Complete feed*	*675*	*1*	*0.1*	*1*	*0.1*	*0*	*0*	*0*	*0*
Feed for fur animals, pets and fish	80	0	0	0	0	0	0	0	0
- *Fish food*	*14*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
- *Pet food*	*66*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*

Table 3.4. Number (N) and percentage (%) of samples exceeding the action levels (AL) and maximum levels (ML) per country of origin, according to Commission Directive 2006/13/EC using WHO-TEFs 1998.

Origin	**N_{total}**	**AL (PCDD/Fs)** Samples > AL		**AL (DL-PCBs)** Samples > AL		**ML (PCDD/Fs)** Samples > ML		**ML (sum PCDD/Fs + DL-PCBs)** Samples > ML		**Feed materials exceeding 1 or both MLs**
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
NL	2571	24	0.9%	2	0.1%	12	0.5%	9	0.4%	clay minerals* (N=4), bakery waste (N=2), mineral mix cattle (N=2), grass (N=1), alfalfa (N=1), fish meal (N=1), animal fat (N=1), fatty acids (N=1)
UN	1616	37	2.3%	10	0.6%	17	1.1%	8	0.5%	coconut oil (N=6), clay minerals (N=5), fish meal (N=5), animal fat (N=1), soya bean (N=1), copper sulphate (N=1)
EU	71	2	2.8%	0	0.0%	1	1.4%	0	0.0%	fish meal (N=1)
BE	69	5	7.2%	1	1.4%	2	2.9%	1	1.4%	clay minerals* (N=1), animal fat (N=1)
DE	67	1	1.5%	0	0.0%	1	1.5%	1	1.5%	bakery waste (N=1)
ES	46	3	6.5%	0	0.0%	1	2.2%	1	2.2%	clay minerals* (N=1)
DK	36	1	2.8%	0	0.0%	1	2.8%	0	0.0%	fish meal (N=1)
US	29	1	3.4%	0	0.0%	1	3.4%	0	0.0%	fish meal (N=1)
FR	26	1	3.8%	2	7.7%	1	3.8%	0	0.0%	fish meal (N=1)
Total	**4948**	**75**	**1.5%**	**15**	**0.3%**	**37**	**0.7%**	**20**	**0.4%**	

Abbreviations used: AL = action level, BE = Belgium, DE = Germany, DK = Denmark, ES = Spain, EU = European Union consisting of 27 Member States, FR = France, ML = maximum level resp. regulatory limit, NL = the Netherlands, UN = Unknown country of origin, US = United States of America.

* *Clay minerals are most likely only imported by the Netherlands or Belgium and are not mined within these countries.*

3.2 Upper bound vs. lower bound concentrations

According to an article of the EFSA, in general no correlation can be found between the concentrations of dioxins and DL-PCBs due to the different sources of contamination and different origins of the feed commodities (EFSA, 2005). When looking into the contribution of dioxins and DL-PCBs to the TEQ-concentrations using the Dutch monitoring data, in general there appears to be a higher contribution of dioxins in most feed groups and only in the feed categories forages and roughage, minerals, fish meal, fish oil and fish food a relatively higher amount of DL-PCBs were present. However, it should be realized that the upper bound principle may cause a bias on dioxins since the limit of quantification (LOQ) is higher for dioxins than for DL-PCBs. Levels of dioxins and DL-PCBs are normally expressed as upper bound levels, where the levels of non-detected congeners are assumed to be present at the LOQ before being multiplied with the TEF value and forming the TEQ-concentrations. The higher LOQ and the on average higher TEF-values for dioxins could cause the on average higher contribution of dioxins to the total dioxins and DL-PCB concentration.

In addition, at low background levels there may be a large difference of upper bound vs. lower bound levels. When using lower bound, levels of non-detected congeners are assumed to be zero. In this chapter upper bound levels [ub] are compared to lower bound levels [lb] for dioxins, DL-PCBs and the sum of dioxins and DL-PBCs. The graphical overview is used to look into the range of uncertainty which may be present in the results of the trend analysis. Figures are shown for the eight feed categories: (1) feed materials of plant origin, excl. vegetable oils (2) forages and roughage (3) fish meal, (4) feed additives, (5) compound feed, excl. fur animals, pets and fish, (6) feed for fur animals, pets and fish and (7) complementary and (8) complete feed. The selection is made on the basis of the feed groups in which a sufficient number of samples was available to carry out a trend analysis. These groups are analysed on trends later on in this chapter (section 3.3 till section 3.6).

3.2.1 Dioxins

Figure 3.1 gives an overview of [ub] and [lb] dioxin concentrations per feed group of the EU monitoring data found between 2001 and 2011. It can be observed that in general the higher the dioxin concentrations found, the less difference there is between the normal distribution curves (see e.g. fish meal (Figure 3.1 C)). The lower the dioxin levels, the more difference exists between the two graphs of [ub] and [lb] (see e.g. feed materials of plant origin, excl. vegetable oils (Figure 3.1 A), forages and roughage (Figure 3.1 B), compound feed excl. fur animals, pets and fish (Figure 3.1 E), complementary feed (Figure 3.1 G) and complete feed (Figure 3.1 H)). In the category feed additives (Figure 3.1 D) and feed for fur animals, pets and fish (Figure 3.1 F), dioxin levels of [ub] and [lb] seem to align at above 0.6 ng TEQ/kg. The difference in outcome for the Q(.99) is low for this two feed materials.

These findings seem to be understandable as at higher dioxin concentrations for most individual congeners the concentration is known or is high enough to make the assumed LOQ-levels of non-detected congeners insignificant. In conclusion, when interpreting the results on trends found for low dioxin concentrations, caution should be taken.

3.2.2 Dioxin-like PCBs

Figure 3.2 gives an overview of [ub] and [lb] DL-PCB concentrations per food product of the EU monitoring data found between 2001 and 2011. It can be observed that in general in all products the DL-PCB concentrations are around the same level for [ub] and [lb] and almost no differences can be seen. These findings are not influenced by low or high concentrations which is linked to the fact that for non-ortho DL-PCBs for most congeners the values are known, being above the LOQ. In a ddition, for most DL-PCBs TEF values are 100 to 1000-fold lower compared to the TEF values used for dioxins. This means that the individual DL-PCB concentrations per non-detected congener have less impact on the final TEQ value. In conclusion, the analysis carried out on trends found for DL-PCBs in feed is not influenced by [ub] vs. [lb] levels.

3.2.3 Sum of dioxins and dioxin-like PCBs

Figure 3.3 gives an overview of [ub] and [lb] concentrations of the sum of dioxins and DL-PCBs per feed group of the EU monitoring data found between 2001 and 2011. It can be observed that in all feed materials with overall low dioxin and DL-PCB levels, variation can be seen between the normal distribution curves (see feed materials of plant origin, excl. vegetable oils (Figure 3.3 A), forages and roughage (Figure 3.3 B), compound feed excl. fur animals, pets and fish (Figure 3.3 E), complementary feed (Figure 3.3 G) and complete feed (Figure 3.3 H)). For all other feed materials, the difference found between [ub] and [lb] levels is rather small. In conclusion, when interpreting the results for the above mentioned feed materials on trends, caution should be taken, as due to the low levels of dioxins and DL-PCBs present in these feed materials, a fictive trend might be found. The graphics suggest that in these feeds often the concentration for certain congeners could not be quantified and the LOQ was used, leading to the differences found between [lb] and [ub] levels. As in the middle of 2007 LOQs were lowered, a fictive decreasing trend might be seen between the years 2001-2006 and 2007-2010.

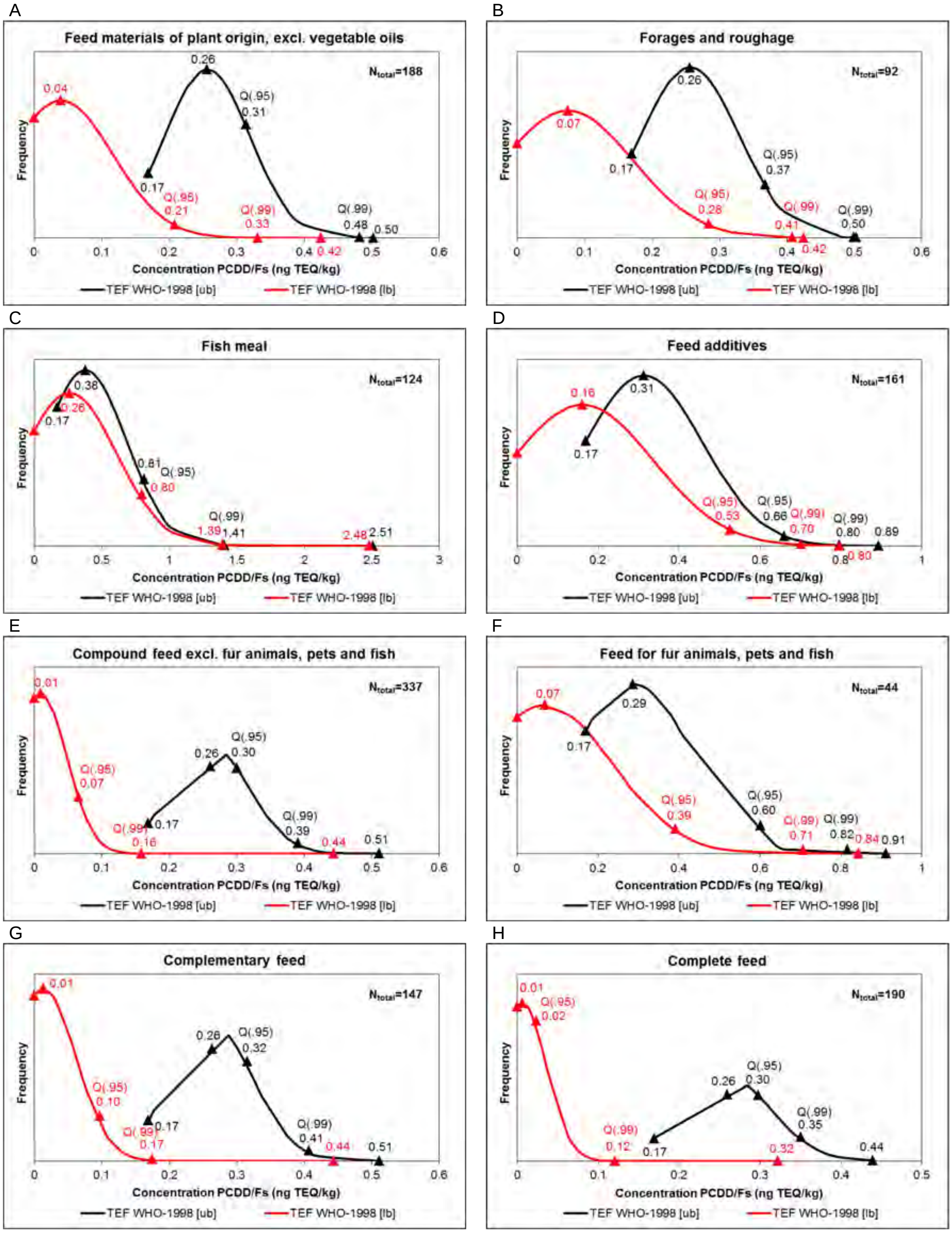


Figure 3.1. Frequency distribution curve of upper bound [ub] and lower bound [lb] dioxin concentrations in (A) feed materials of plant origin, excl. vegetable oils, (B) forages and roughage, (D) fish meal, (D) feed additives, (E) compound feed excl. fur animals, pets and fish, (F) feed for fur animals, pets and fish, (G) complementary feed and (H) complete feed.

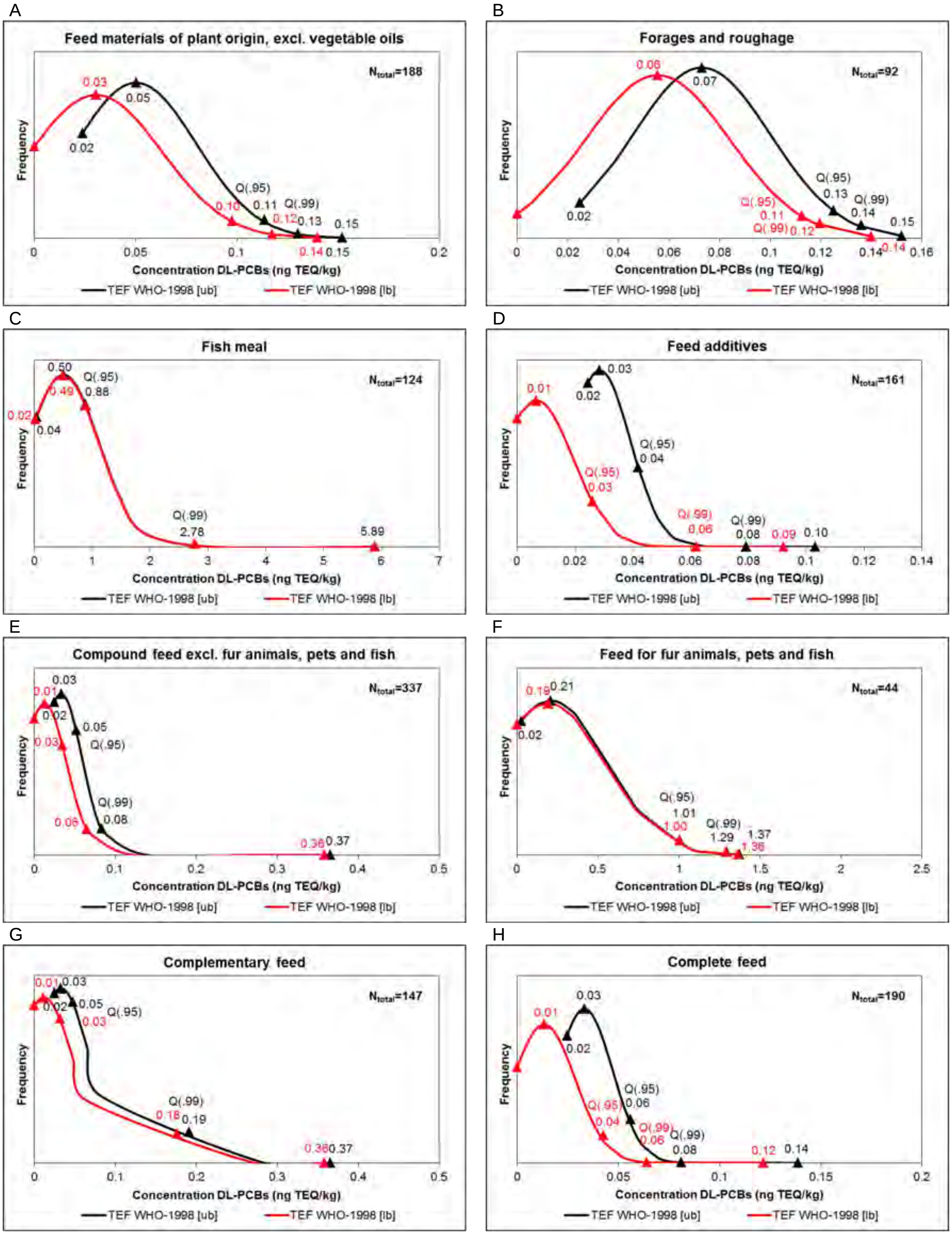


Figure 3.2. Frequency distribution curve of upper bound [ub] and lower bound [lb] DL-PCB concentrations in (A) feed materials of plant origin, excl. vegetable oils, (B) forages and roughage, (D) fish meal, (D) feed additives, (E) compound feed excl. fur animals, pets and fish, (F) feed for fur animals, pets and fish, (G) complementary feed and (H) complete feed.

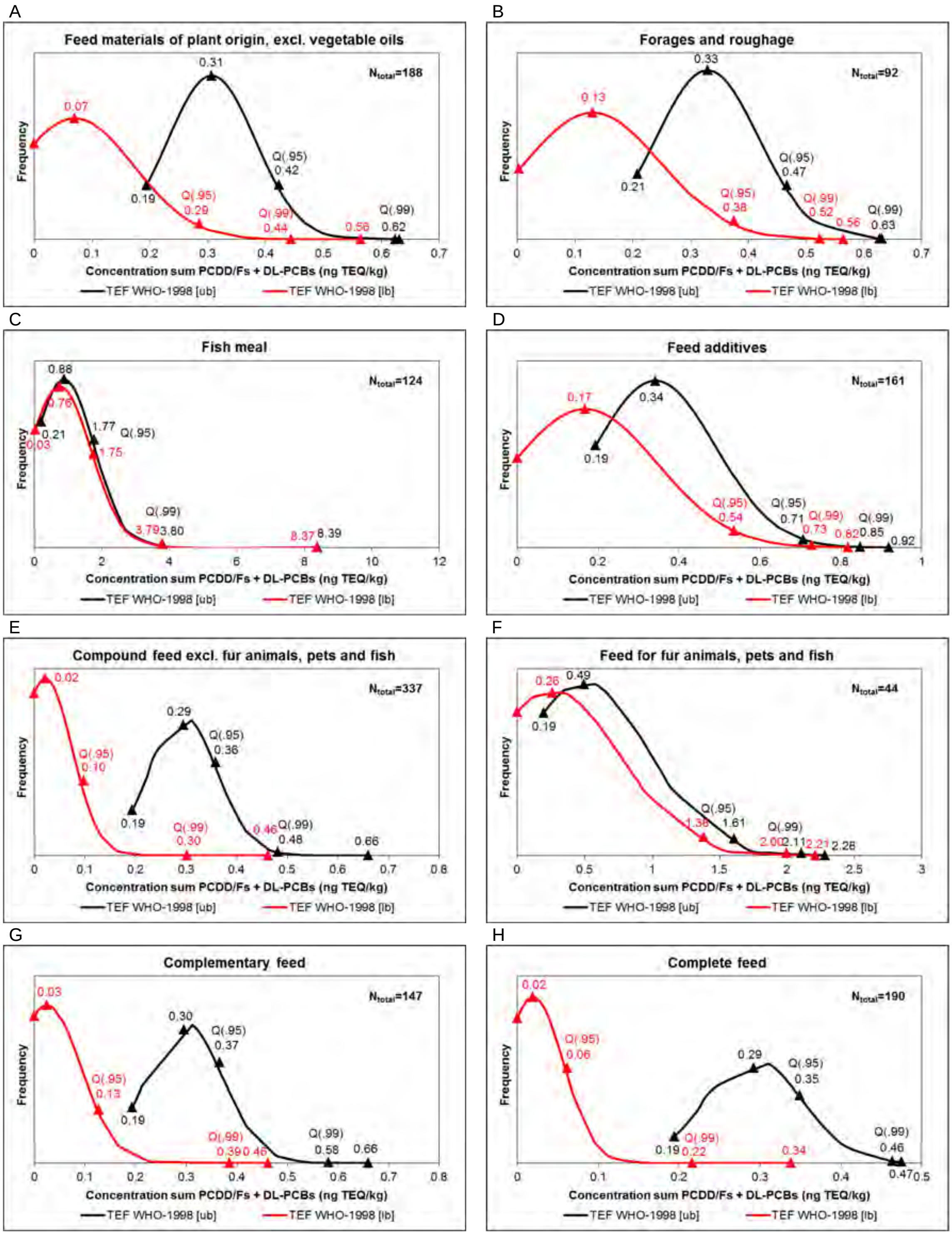


Figure 3.3. Frequency distribution curve of upper bound [ub] and lower bound [lb] dioxin and DL-PCB concentrations in (A) feed materials of plant origin, excl. vegetable oils, (B) forages and roughage, (D) fish meal, (D) feed additives, (E) compound feed excl. fur animals, pets and fish, (F) feed for fur animals, pets and fish, (G) complementary feed and (H) complete feed.

Summary - Upper bound vs. lower bound concentrations

- Dioxins: The higher the dioxin concentrations found, the less variation can be seen between [ub] and [lb] levels. At around 0.6 ng TEQ/kg dioxin levels seem to align and below this concentration differences become rather large, which should be taken into account when interpreting results found for low dioxin concentrations.
- DL-PCBs: In general, the DL-PCB concentrations are around the same level for [ub] and [lb]. The use of upper vs. lower bound levels therefore does not influence the outcome of the analysis on trends.
- Sum dioxins and DL-PCBs: Only in feed materials with overall low dioxin and DL-PCB levels (e.g. feed materials of plant origin, excl. vegetable oils and compound feed excl. for fur animals, pets and fish) still large differences can be seen between [ub] and [lb] concentrations. This should be taken into account when interpreting the results on trends for these feed materials.

3.3 Levels and trends in feed materials of plant origin

Feed materials of plant origin form, with 52-65 % for cereal grains and legumes and 22-40% for (by-) products of plant origin, the major dietary source for food producing animals, with the exception of fish (see Annex IX). Too high dioxin levels in this feed category might therefore significantly contribute to the dioxin burden in the animal and eventually in the food end product.

Table 3.5 gives an overview of the main feed materials of plant origin used in the Netherlands. Staple feed (e.g. cereal grains, potatoes, forages and roughage) is mainly produced in the Netherlands or imported from other European Member States. Contrarily, the majority of vegetable oils and oilseeds/fruits are imported and the highest proportion originates from countries outside the EU.

Table 3.5. Overview of the Dutch feed consumption for specified feed materials of plant origin. Only feed groups for which data was available are shown.

Feed material	**Dutch feed consumption** [A]		**Domestic feed** [A]		**Import** [A]			**Country of origin** [B]
					direct	**indirect**		
	x1000 tons	%	x1000 tons	%	x1000 tons	x1000 tons	%	%
1.1. Feed materials of plant origin, excl. vegetable oils								
Cereal grains and their by-products								
total	6858	47	1298	19	5560	1067	81	-
wheat (by-products)	3302	23	911	28	2391	634	72	EU: 100 (DE: 56, BE: 30, FR: 13)
maize (by-products)	1864	13	120	6	1744	356	94	EU: 100 (DE: 56, FR: 38, BE: 5)
barley	613	4	153	25	460	-	75	n.a.
Forages and roughage *								
total	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-
fodder, dried	122	1	109	90	13	-	10	EU: 100 (FR: 73, DE: 14, BE: 13)
Oil seeds/fruits and their by-products								
total	4432	30	-	-	4431	2812	100	-
soya (bean)	2238	15	-	-	2238	2042	100	EU: 4, AR: 48, BR: 47
cole-/rapeseed	934	6	-	-	934	169	100	EU: 100 (DE: 82, BE: 12)
palm kernel	787	5	-	-	787	-	100	EU: 5 (DE: 5), MY: 64, ID: 31
sunflower seed	397	3	-	-	397	234	100	EU: 14 (DE: 11), AR: 83
copra	20	0	-	-	20	-	100	EU: 10 (DE: 9), ID: 61, PH: 28
linseed	18	0	-	-	18	-	100	EU: 75 (DE: 48, BE: 26), US: 20, AR: 5
other oilseeds	15	0	-	-	15	-	100	EU: 62 (DE: 44, ES: 17), AR: 27, BR: 5
Other feed materials of plant origin								
total	1280	9	52	0	1228	-	96	-
Legume seeds and their by-products								
- feed legume	110	1	-	-	110	-	100	n.a.
- lupins	25	0	-	-	25	-	100	n.a.
Tubers, roots and their by-products								
- sugar beet pulp	284	2	38	14	246	-	86	n.a.
- manioc	146	1	-	-	146	19	100	n.a.
Other seeds/fruits and their by-products								
- citrus pulp	340	2	-	-	340	-	100	n.a.
Other plants, algae and their by-products								
- molasses	263	2	10	4	253	-	96	n.a.
- vinasses	111	1	-	-	111	-	100	n.a.

Feed material	Dutch feed consumption [A]		Domestic feed [A]		Import [A]			Country of origin [B]
					direct	indirect		
	x1000 tons	%	x1000 tons	%	x1000 tons	x1000 tons	%	%
1.2. Vegetable oils and their by-products								
Vegetable oil/fat								
total	179	1	-	-	179	-	100	-
palm oil	99	1	-	-	99	-	100	EU: 5 (DE: 5), MY: 64, ID: 31
soybean oil	36	0	-	-	36	-	100	EU: 4, AR: 48, BR: 47
coconut oil	26	0	-	-	26	-	100	EU: 10 (DE: 9), ID: 61, PH: 28
palm kernel oil	11	0	-	-	11	-	100	EU: 5 (DE: 5), MY: 64, ID: 31
rapeseed oil	5	0	-	-	4	-	80	EU: 100 (DE: 82, BE: 12)
sunflower oil	2	0	-	-	2	-	100	EU: 14 (DE: 11), AR: 83
other	1	0	-	-	1	-	100	EU: 62 (DE: 44, ES: 17), AR: 27, BR: 5
By-products								
fatty acids **	27	0	-	-	27	-	100	n.a.

Abbreviations used: AR = Argentina, BE = Belgium, BR = Brazil, DE = Germany, ES = Spain, EU = European Union consisting of 27 Member States, FR = France, ID = Indonesia, MY = Malaysia, n.a. = not available, NL = the Netherlands, PH = Philippines, US = United States of America.

a *average 2004/2005 till 2008/2009, Source: PDV (see Annex V).*
b *average 2001 till 2010, Source: Eurostat (see Annex VI).*
* *Only the amount of dried fodder is known, which is mainly used for horses. However, ruminants also graze outside and/or use other sources such as silage. The complete amount of forages and roughage used is therefore unknown.*
** *Most fatty acids are linked to processed palm oil and therefore involves feed materials of foreign origin. Fatty acids are however an end product of production processes carried out in the Netherlands or neighbour European countries.*

For vegetable oils different limits (ALs and MLs) are set by law compared to all other feed materials of plant origin, therefore, the analysis is carried out for both feed groups separately.

3.3.1 Level and trends in feed materials of plant origin, excl. vegetable oils

In this section the levels and trends will be described for the main feed category feed materials of plant origin and in more detail for the subgroup forages and roughage. All results are also compared to the study by EFSA (2010) consisting of data from various EU member states.

First, an overview is given of the descriptive analysis (see Table 3.6). When comparing all feed subgroups, the highest concentrations can be observed in forages and roughage, especially when looking at the Q(.95) and Q(.99). The concentrations found in the feed subgroups cereal grains, oil seeds/fruits and other feed materials of plant origin are around the same level and no differences can be observed. Due to the low amount of samples available per feed subgroup and year, a trend analysis was only carried out in the group of forages and roughage.

Table 3.6. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in feed materials of plant origin, excl. vegetable oils from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
AL[1]	0.5	0.35	
ML[1]	0.75		1.25
Feed materials of plant origin, excl. vegetable oils (N=188)			
avg	0.26	0.05	0.31
Q(.50)	0.29	0.04	0.31
Q(.95)	0.31	0.11	0.42
Q(.99)	0.48	0.13	0.62
Cereal grains and their by-products (N=32)			
avg	0.24	0.03	0.27
Q(.50)	0.29	0.02	0.31
Q(.95)	0.29	0.04	0.33
Q(.99)	0.29	0.04	0.33
Forages and roughage (N=92)			
avg	0.26	0.07	0.33
Q(.50)	0.27	0.07	0.33
Q(.95)	0.37	0.13	0.47
Q(.99)	0.50	0.14	0.63
Oil seeds/fruits and their by-products (N=33)			
avg	0.24	0.03	0.28
Q(.50)	0.29	0.02	0.31
Q(.95)	0.29	0.04	0.33
Q(.99)	0.30	0.08	0.37
Other feed materials of plant origin (N=31)			
avg	0.27	0.03	0.30
Q(.50)	0.29	0.03	0.32
Q(.95)	0.29	0.04	0.33
Q(.99)	0.30	0.05	0.33
EFSA[2] - feed materials of plant origin excl. vegetable oils (N=378)			
avg	0.21	0.08	0.29
Q(.50)	0.12	0.03	0.19
Q(.95)	0.48	0.25	0.73
Q(.99)	1.15	0.82	1.41

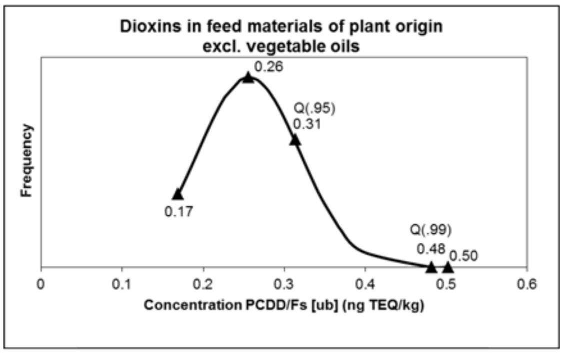
1 *Source: Commission Directive 2006/13/EC.*
2 *Source: (EFSA, 2010).*

Dioxins

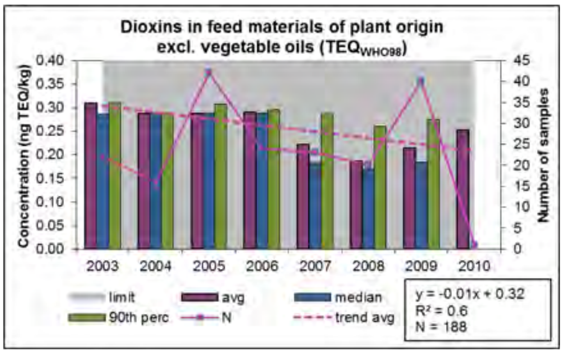
As shown in Table 3.6 and Figure 3.4 A, the average dioxin concentrations found in feed materials of plant origin are around the same level as the concentration found by EFSA. However, in the EFSA data the bandwidth of the dioxin concentrations is higher by having 40-50% higher Q(.95) and Q(.99) values.

Figure 3.4 B shows that no significant change of the average dioxin levels can be found from 2003 till 2010, although the concentrations seem to be slightly lower starting from 2007. This lower levels are linked to the fact that the reporting limits were reduced in 2007, which means that levels of individual congeners were often below the LOQ. No conclusions on trends can be drawn and dioxin concentrations seem to stay around the same level during the past years. In 2010 a low number of samples were measured directly with GC-HRMS as the EU monitoring program stopped in 2009.

A

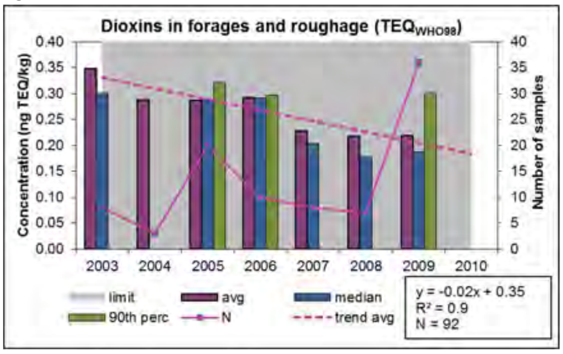


B



S=-14, Signific.: none
Limit (ML) = 0.75 ng TEQ/kg

C



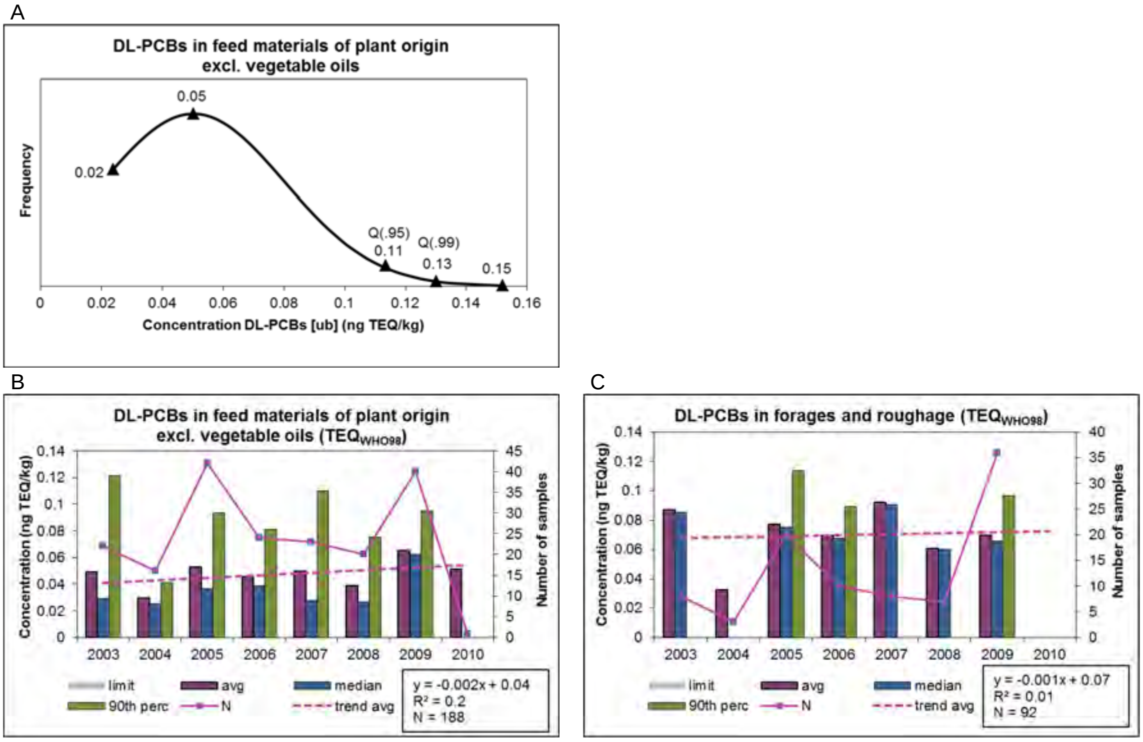
S=-15, Signific.: p=0.05
Limit (ML) = 0.75 ng TEQ/kg

Figure 3.4. Dioxins [ub] in feed materials of plant origin, excl. vegetable oils.
(A) frequency distribution curve using TEFWHO98 values.
(B) trends in the average dioxin concentrations per year.
(C) trends in the feed subgroup forages and roughage.

Dioxin-like PCBs

The average DL-PCB levels found in feed materials of plant origin are low being around 0.05 ng TEQ/kg (see Table 3.6 and Figure 3.5 A). This is comparable with the levels found by EFSA. Again, the bandwidth of the DL-PCB concentrations is higher in the EFSA data by having 4 to 6 times higher Q(.95) and Q(.99) values.

Figure 3.5 B shows that no significant change of the average DL-PCB levels in feed materials of plant origin can be found from 2003 till 2010. Average DL-PCB concentrations seem to stay around the same level and the 90th percentile fluctuates during the years. Also in the feed group forages and roughage (Figure 3.5 C) no significant trend can be seen.



S=8, Signific.: none
Limit (ML) = n.a.

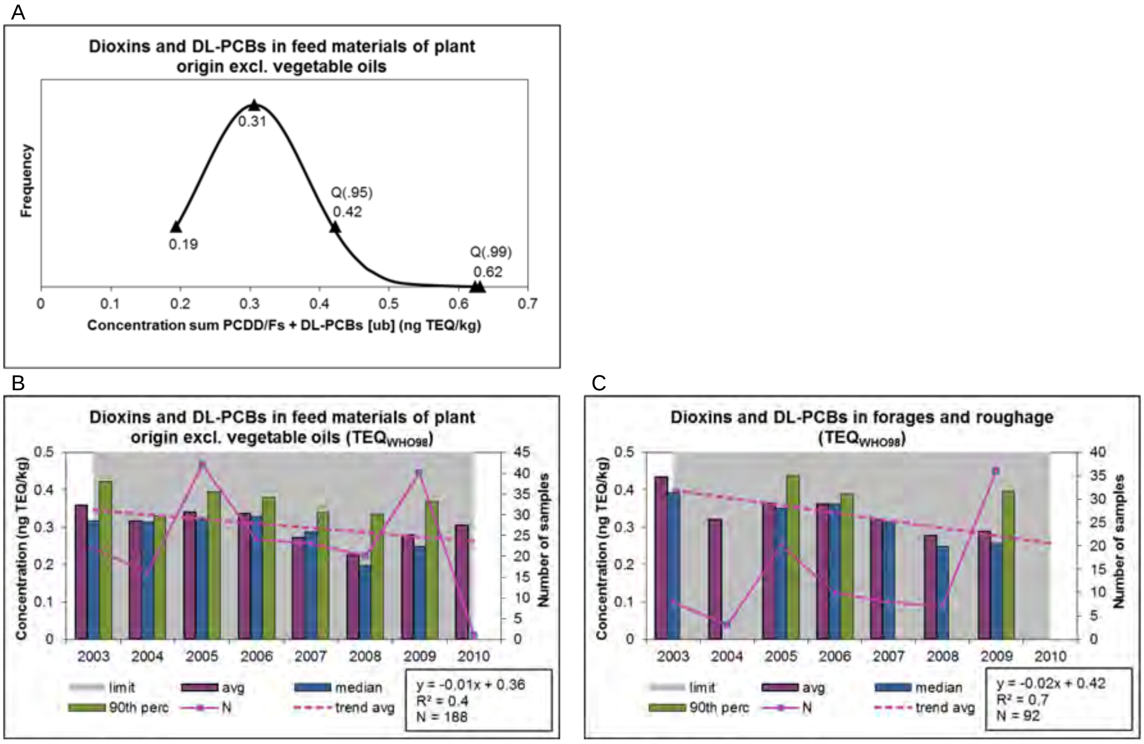
S=-1, Signific.: none
Limit (ML) = n.a.

Figure 3.5. DL-PCBs [ub] in feed materials of plant origin, excl. vegetable oils.
(A) frequency distribution curve using TEFWHO98 values.
(B) trends in the average DL-PCB concentrations per year.
(C) trends in the feed subgroup forages and roughage.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in feed materials of plant origin is around the same level as the EFSA concentrations (see Table 3.6 and Figure 3.6 A), only Q(.95) levels are 2,5 times higher in the EFSA dataset.

Figure 3.6 B shows that average dioxin and DL-PCB levels in feed materials of plant origin did not change significantly during the last decade. The concentrations vary per year, but no trend can be seen. When looking into the feed subgroup forages and roughage (Figure 3.6 C), at first dioxin and DL-PCB concentrations tend to decrease significantly (p=0.01, Mann-Kendall). However, the slight decrease (5%, slope m=-0.02) is again linked to the fact that the reporting limits were reduced in 2007 and the concentrations were at/below the LOQ. No conclusions on trends can be drawn and concentrations seem to stay around the same level during the past years.



S=-14, Signific.: none
Limit (ML) = 0.75 ng TEQ/kg

S=-13, Signific.: p=0.1
Limit (ML) = 1.25 ng TEQ/kg

Figure 3.6. Dioxins and DL-PCBs [ub] in feed materials of plant origin, excl. vegetable oils.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average dioxin and DL-PCB concentrations per year.
(C) trends in the feed subgroup forages and roughage.

Table 3.7 gives an overview of the amount of samples exceeding the regulatory limits (ML) and/or action levels (AL) in the feed group of forages and roughage. In 2002 and 2003, dioxin levels were exceeded once. In all other years no samples were detected which were non-compliant with the regulatory limits. As this feed materials are seldom imported, it can be assumed that if high dioxin concentrations are found, that they originate from grass and alfalfa grown and processed in the Netherlands and likely linked to certain geographical areas.

Being a substantial part of the daily diet for some animals (e.g. cattle), this feed materials are advised to be monitored on a regular basis by staying a part of the NCP. Another reason is also the fact that grass and alfalfa seem to have a higher bandwidth (Q(.95) and Q(.99)) of dioxin concentrations compared to the other feed materials of plant origin. In general, they form a potential risk of containing higher dioxin levels as in some cases an industrial drying process is applied, which may lead to an additional formation of dioxins, on top of contamination already present through the soil and deposition from the air. In 2010 no samples were collected in the feed group of forages and roughage and in 2011 only a low number of samples were taken. The reason is unknown.

The results found are assumed to not influence the trend analysis for the main feed group of feed materials of plant origin as no significant change of the dioxin and/or DL-PCB concentrations has been found. In addition, no outliers could be distinguished as stated in Annex VIII. The sample with the high concentration found in 2003 (see Table 3.7, feed group forages and roughage) cannot influence the trend analysis as this sample was first screened with the DR CALUX®. Because it was found to be suspected, the sample was not included in the calculation of the background levels and the trend analysis.

Table 3.7. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group forages and roughage.

		Forages and roughage										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	26	34	36	34	58	48	37	37	36	-	7
Above ML (PCDD/Fs + DL-PCBs) of 1.25 ng TEQ/kg	N		1	1								
	Conc.		1.9	1.4								
	Origin		NL	NL								
Above ML (PCDD/Fs) of 0.75 ng TEQ/kg	N		1	1								
	Conc.		1.7	1.2								
	Origin		NL	NL								
Above AL (PCDD/Fs) of 0.5 ng TEQ/kg	N		2	2	3		1		1			
	Conc.		0.6 - 1.7	0.7 – 1.2	0.6 – 0.7		0.7		0.6			
Above AL (DL-PCBs) of 0.35 ng TEQ/kg	N											
	Conc.											

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), ML = maximum level (regulatory limit), NL = the Netherlands.

* N_{total} *refers to the total amount of samples measured of the feed material in that year.*

Oil seeds/fruits and their by-products form one third of the total feed used in the Netherlands, with soya being the main feed material within this feed group. Table 3.8 gives an overview of all non-compliant samples during the past 11 years. In 2007, the dioxin and DL-PCB levels were exceeded linked to the same soya sample. In all other years, the dioxin concentrations measured remain well below the limit and none exceeded either the ML (1.25 ng TEQ/kg for the sum of dioxins and DL-PCBs) or the AL (0.5 ng TEQ/kg for dioxins and 0.35 ng TEQ/kg for DL-PCBs). However, the number of samples collected per year is rather low and it is not clear if this amount is sufficient to monitor the risks. Non-compliance with regulatory limits of vegetable oil samples is demonstrated in section 3.1.

All feed materials belonging to this feed subgroup are imported to the Netherlands, and soya predominantly originates from Argentina and Brazil. A total of 17 samples (of N_{total} = 47) originated from these two countries (Argentina N=7, Brazil N=10) and for 25 samples the country of origin was unknown. When collecting samples, more attention should be paid naming the country of origin and sampling strategies should focus on countries with a high import quantity compared to the domestic production.

Table 3.8. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group oils seeds/fruits and their by-products.

		Oil seeds / fruits and their by-products										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	7	1	6	21	39	12	7	8	4	5	23
Above ML (PCDD/Fs + DL-PCBs) of 1.25 ng TEQ/kg	N							1				
	Conc.							1.9				
	Origin							UN				
Above ML (PCDD/Fs) of 0.75 ng TEQ/kg	N											
	Conc.											
	Origin											
Above AL (PCDD/Fs) of 0.5 ng TEQ/kg	N							1				
	Conc.							0.6				
Above AL (DL-PCBs) of 0.35 ng TEQ/kg	N							1				
	Conc.							1.3				

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), ML = maximum level (regulatory limit), UN = unknown.

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

In the other two feed groups (cereal grains and their by-products, other feed materials of plant origin) no ALs and MLs were exceeded.

Around 75% of wheat and 94% of maize is imported, mainly from Germany, Belgium or France. However, only 19 samples (of N_{total} = 143) originated from these countries. This is not a representative sample unit for risk based monitoring of feed materials linked to the country of origin. The amount of samples taken per country of origin should form a part of the NCP and it is advised to base it on the quantity imported compared to domestic production. The origin of some samples is also not mentioned (wheat N=25, maize N=22). For processed materials this approach might be less feasible since batches could be mixed. Furthermore, contamination may also occur during the processing step.

In general, the dioxin and DL PCB concentrations in feed materials of plant origin stay far below the regulatory limit, which is exceeded only seldom. Concluding, monitoring these feed materials can be continued as it is.

3.3.2 Levels and trends in vegetable oils and by-products

In the feed group vegetable oils and by-products a too low number of samples were measured with GC-HRMS to be able to look into trends, therefore only background levels were investigated. By-products of vegetable oils are e.g. fatty acids mainly originating from palm oil.

Dioxins

As shown in Table 3.9 and Figure 3.7 A, the average dioxin concentrations found in vegetable oils and by-products are generally around the same level when comparing the Dutch data vs. EFSA, staying between 0.26 and 0.28 ng TEQ/kg. However, in the EFSA data the bandwidth of the dioxin concentrations is higher by having almost two times higher Q(.95) and Q(.99) values.

Table 3.9. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in vegetable oils and their by-products from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
AL[1]	0.5	0.5	
ML[1]	0.75		1.5
Vegetable oils and by-products (N=17)			
avg	0.28	0.07	0.35
Q(.50)	0.29	0.04	0.32
Q(.95)	0.35	0.18	0.49
Q(.99)	0.41	0.24	0.55
EFSA[2] - vegetable oils and their by-products (N=68)			
avg	0.26	0.15	0.41
Q(.50)	0.23	0.11	0.37
Q(.95)	0.57	0.44	0.88
Q(.99)	0.86	0.87	1.15

1 *Source: Commission Directive 2006/13/EC.*
2 *Source: (EFSA, 2010).*

Dioxin-like PCBs

DL-PCB concentrations found by EFSA are slightly higher, especially when looking at the Q(.95) and Q(.99).

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs is comparable with the levels found by EFSA, being around 0.35 ng TEQ/kg (see Table 3.9 and Figure 3.7 C). Again, the bandwidth of the dioxin and DL-PCB concentrations is higher in the EFSA data by having 40-50% higher Q(.95) and Q(.99) values.

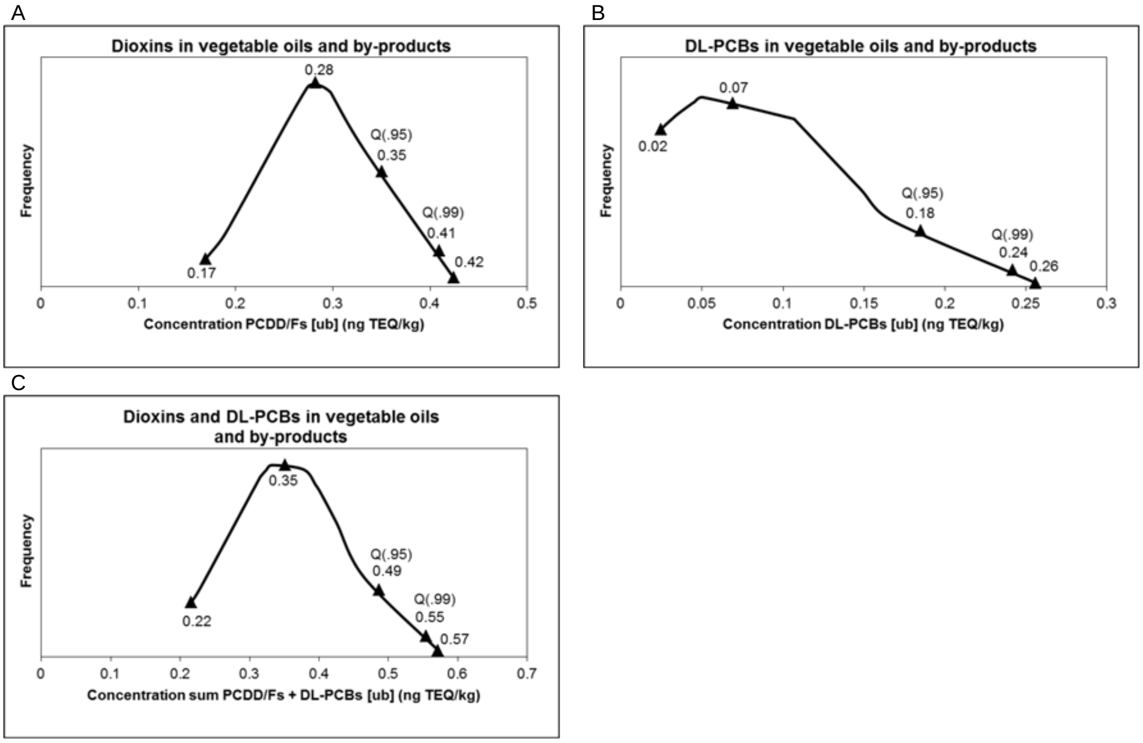


Figure 3.7. Frequency distribution curve of the feed group vegetable oils and by-products for (A) dioxins, (B) DL-PCBs and (C) the sum of dioxins and DL-PCBs; using TEF_{WHO98} [ub] values

Table 3.10 gives an overview of the amount of samples exceeding the regulatory limits (ML) and/or action levels (AL) in the feed group of vegetable oil/fat. In 2011, six coconut oil samples were found to be above the ML for dioxins (0.75 ng TEQ/kg) and just below the ML for the sum of dioxins and DL-PCBs (1.5 ng TEQ/kg). In general, vegetable oils should be monitored regularly as from time to time incidents seem to occur.

The majority of oils is imported, e.g. soybean oil originating from Argentina and Brazil, sunflower oil originating from Argentina and Germany and palm kernel oil originating from Malaysia and Indonesia. However, none or only a small amount of samples is collected or registered as originating from these countries. In the majority of the oil samples the country is unknown (69%, N=247 of N_{total}=359) or it is stated that the oils originate from the Netherlands (N=100). Oil seeds are not cultivated within the Netherlands, but might be processed here and it needs to be differentiated if the dioxin contamination comes from the raw materials or via the production process (e.g. oil extrusion). Stating the country of origin of the raw materials as well as the production site is, therefore, crucial and would help to predict risks related to certain countries.

Fatty acids are a by-product of e.g. the palm oil production. The number of samples taken is rather low as most of the times samples are collected from the vegetable oil and not the by-products. Since 2002, no sample were found to be above the regulatory limits (ML) again.

Table 3.10. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group vegetable oil/fat.

		Vegetable oil / fat										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	88	21	16	26	32	14	17	17	45	43	40
Above ML (PCDD / Fs + DL-PCBs) of 1.5 ng TEQ/kg	N											
	Conc.											
	Origin											
Above ML (PCDD / Fs) of 0.75 ng TEQ/kg	N											6
	Conc.											1.3 - 1.5
	Origin											UN
Above AL (PCDD / Fs) of 0.5 ng TEQ/kg	N				1			1				6
	Conc.				0.6			0.7				1.3 - 1.5
Above AL (DL-PCBs) of 0.5 ng TEQ/kg	N											
	Conc.											

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), ML = maximum level (regulatory limit), UN = unknown.

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

Table 3.11. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group fatty acids.

		Fatty acids										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total} *	6	3	6	1	2	1	2	3	4	7	9
Above ML (PCDD / Fs + DL-PCBs) of 1.5 ng TEQ/kg	N	1										
	Conc.	4.3										
	Origin	NL										
Above ML (PCDD / Fs) of 0.75 ng TEQ/kg	N	1										
	Conc.	2.3										
	Origin	NL										
Above AL (PCDD / Fs) of 0.5 ng TEQ/kg	N	1		2				1				
	Conc.	2.3		0.7 – 0.8				0.6				
Above AL (DL-PCBs) of 0.5 ng TEQ/kg	N	1										
	Conc.	1.9										

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), ML = maximum level (regulatory limit), NL = the Netherlands.

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

Summary - Levels and trends in feed materials of plant origin

- **Feed materials of plant origin, excl. vegetable oils**
  - Dioxin and DL-PCB concentrations are generally low and stay far below the regulatory limit. Monitoring these feed materials can be continued as it is.
  - Dioxins: average dioxin concentrations in feed materials of plant origin (excl. vegetable oil) are within the same range as the EFSA data. The dioxin concentrations seem to stay on a constant level over time with no significant change.
  - DL-PCBs: average DL-PCB concentrations are within the same range as the EFSA data. No significant trend can be seen and the 90th percentile fluctuates during the years.
  - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are around the same level compared to the Dutch data. In feed materials of plant origin no significant change of the average dioxin and DL-PCB levels was found.
- **Vegetable oils and their by-products**
  - Dioxin and DL-PCB concentrations stay far below the regulatory limit, but as more or less frequently incidents occur, this feed group should be monitored regularly.
  - Dioxins: average dioxin concentrations in feed materials of plant origin (excl. vegetable oil) are within the same range as the EFSA data.
  - DL-PCBs: average DL-PCB concentrations are within the same range as the EFSA data.
  - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are around the same level compared to the Dutch data.
- Too high dioxin and DL-PCB levels in feed of plant origin may significantly contribute to the dioxin burden in the animal and thus eventually in the food end product, since it is forming the major dietary source of most farm animals.
- Reporting the country of origin is important. We recommend to implement the amount of samples taken per country of origin into the NCP, based on the quantity imported compared to the domestic production. For processed materials this might be less feasible since batches may be mixed and the contamination may also occur during the

3.4 Levels and trends in feed materials of animal origin

Table 3.12 gives an overview of the main feed materials of animal origin used in the Netherlands. Not in all cases the quantity imported or domestically produced is known. However, in contrast to vegetable oils, the production and trade of animal fats can be considered as a regional activity and no or only a low quantity is imported (exception: fish oil and fish meal). The feed material of animal origin used most are land animal products (including animal fat), followed by fish meal. The contribution of fish meal, to the dietary intake of animals such as ruminants, pigs and poultry (0-4%) is relatively low, whereas fish meal and fish oil forms with 50% and 25%, respectively, a major dietary source for carnivorous fish species, e.g. farmed eel or salmon (see Annex IX and III). These two feed materials are, therefore, critical in the farmed fish production as they may significantly contribute to the dioxin burden in fish as feed or food end product.

Table 3.12. Overview of the Dutch feed consumption for specified feed materials of animal origin. Only feed groups for which data was available are shown.

Feed material	Dutch feed consumption [A]		Domestic feed [A]		Import [A]			Country of origin [B]
					direct	indirect		
	x1000 tons	%	x1000 tons	%	x1000 tons	x1000 tons	%	%
2. Feed materials of animal origin								
2.1. Land animal (by-)products								
Animal fat, excl. fish oil *								
total	129	1	129	100	-	-	-	-
bovine fat (tallow)	25	0	25	100	-	-	-	-
porcine fat (lard)	48	0	48	100	-	-	-	-
other animal fat	56	0	56	100	-	-	-	-
Other land animal products								
total	326	2	56	17	270	-	83	-
whey powder	195	1	33	17	162	-	83	n.a.
milk powder, skimmed	131	1	23	18	108	-	82	n.a.
2.2. Fish, other aquatic animals and their by-products								
Fish meal								
total	n.a.	n.a.	n.a.	n.a.	52 [B]	n.a.	n.a.	EU: 88 (DE: 69, DK: 11, FR: 5), PE: 12
Fish oil								
total	2	0	-	-	2	-	100	n.a.

Abbreviations used: DE = Germany, DK = Denmark, EU = European Union consisting of 27 Member States, FR = France, n.a. = not available, PE = Peru.

[A] *average 2004/2005 till 2008/2009, Source: PDV (see Annex V).*
[B] *average 2001 till 2010, Source: Eurostat (see Annex VI).*
* *In the overview of the PDV no clear differentiation is made if the animal fat originates from domestic or foreign origin. After consulting an expert the assumption is made that animal fat used in feed mainly originates from the Netherlands.*

3.4.1 Levels and trends in land animal (by-)products

The feed group of land animal (by-)products is further divided into animal fat and other land animal products (e.g. milk/eggs and their by-products). As the number of samples collected and measured with GC-HRMS per group was too low, it was not possible to look into trends and only background levels have been investigated for animal fat. All results are also compared to the study by EFSA (2010) consisting of data from various EU member states.

Dioxins

As shown in Table 3.13 and Figure 3.8 A, the average dioxin concentrations found in animal fat are approximately within the same range as the concentration found by EFSA. However, in the EFSA data the bandwidth of the dioxin concentrations is higher by having more than 3fold higher Q(.95) and Q(.99) values. Also the levels found in all other land animal products is comparable with the concentrations found by EFSA.

Table 3.13. Dioxin and DL-PCB concentrations (in pg TEQ/g) found in animal fat and other land animal products from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
Animal fat			
AL[1]	1	0.75	
ML[1]	2		3
NCP (N=21)			
avg	0.38	0.40	0.78
Q(.50)	0.36	0.46	0.83
Q(.95)	0.53	0.71	1.25
Q(.99)	0.54	0.84	1.33
EFSA[2] - animal fat incl. milk fat and egg fat (N=37)			
avg	0.44	0.36	0.79
Q(.50)	0.31	0.29	0.72
Q(.95)	1.72	0.78	2.10
Q(.99)	1.81	0.86	2.17
Other land animal products			
AL[1]	0.5	0.35	
ML[1]	0.75		1.25
NCP (N=11)			
avg	0.26	0.05	0.31
Q(.50)	0.29	0.02	0.31
Q(.95)	0.30	0.17	0.48
Q(.99)	0.32	0.27	0.58
EFSA[2] - other land animal products (N=31)			
avg	0.19	0.08	0.27
Q(.50)	0.12	0.06	0.21
Q(.95)	0.82	0.23	0.92
Q(.99)	0.91	0.46	0.95

1 Source: Commission Directive 2006/13/EC.
2 Source: (EFSA, 2010).

Dioxin-like PCBs

The average DL-PCB levels found in animal fat are around the same level when comparing the Dutch vs. the EFSA data, staying between 0.36 and 0.40 pg TEQ/g (see Table 3.13 and Figure 3.8 B). However, the DL-PCB concentrations found by EFSA are slightly lower, when looking at the Q(.95) and Q(.99). In the feed group other land animal products the levels found by EFSA are comparable with the Dutch background concentrations.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in feed materials of plant origin is around the same level when comparing the Dutch data vs. EFSA, being around 0.78 pg TEQ/g (see Table 3.13 and Figure 3.8 C). Again, the bandwidth of the dioxin and DL-PCB concentrations is higher in the EFSA data by having 30-40% higher Q(.95) and Q(.99) values. Also the levels found in all other land animal products is comparable with the concentrations found by EFSA, but the amount of samples available is too low to draw final conclusions.

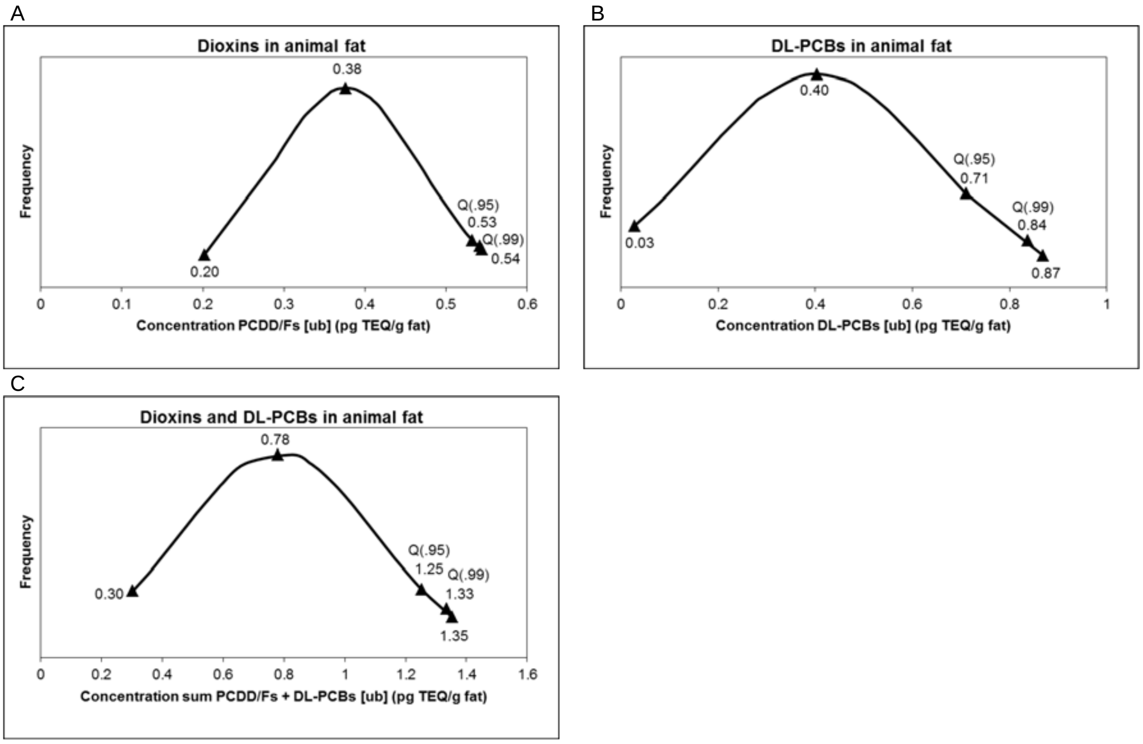


Figure 3.8. Frequency distribution curve of the feed group animal fat for (A) dioxins, (B) DL-PCBs and (C) the sum of dioxins and DL-PCBs; using TEF_{WHO98} [ub] values.

Animal fat seems to be a critical feed material as an extremely high dioxin concentration has been found in one sample in 2005 (50 pg TEQ/g, see Table 3.14). It is worth mentioning that later on this finding turned out to be linked to an incident. The animal fat, imported from Belgium, derived from the production of gelatine and the contamination was caused by the use of contaminated hydrochloric acid for treatment of the pig bones (Hoogenboom et al., 2007). The values found are only linked to dioxins and the concentration of DL-PCBs stays below the AL (0.75 pg TEQ/g). As this sample has first been screened suspected with the DR CALUX® and can further be linked to an incident, it was not included in the calculation of the background levels and the trend analysis.

Table 3.14. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group animal fat.

		Animal fat										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	64	43	39	37	61	32	25	17	23	30	21
Above ML (PCDD/Fs + DL-PCBs) of 3 pg TEQ/g	N			1		1					1	
	Conc.			3.9		50.0					7.7	
	Origin			NL		BE					BE	
Above ML (PCDD/Fs) of 2 pg TEQ/g	N					1					1	
	Conc.					50.0					2.3	
	Origin					BE					BE	
Above AL (PCDD/Fs) of 1 pg TEQ/g	N			1		1					1	
	Conc.			1.2		50.0					2.3	
Above AL (DL-PCBs) of 0.75 pg TEQ/g	N			1	3						1	
	Conc.			2.8	0.9 – 2.1						5.5	

Abbreviations used: AL = action level, BE = Belgium, Conc. = concentration (in ng TEQ/kg), ML = maximum level (regulatory limit), NL = the Netherlands, UN = unknown.

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

Recently (end 2010), in Germany an incident was reported in which fat, intended to be used for technical purposes, has intentionally been mixed with fat for feed production (see Table 1.1). Also here the problem is that in case dioxin concentrations are found to exceed the ML, most of the times the exceedance of the regulatory limit is high, forming a substantial risk that also the animal derived food may exceed the limit. Furthermore, it indicates that a previous or still on-going incident has been overlooked causing the contamination of animals that were already slaughtered, as shown in the Irish incident (Heres et al., 2010). This feed material is therefore advised to be monitored on a regular basis, which has already been recommended in the previous trend analysis (Adamse *et al.*, 2007).

In the feed group other land animal products no ALs and MLs were exceeded, but the total number of samples collected is too low to draw conclusions (N_{total}=28, $N_{GC\text{-}HRMS}$=11).

3.4.2 Levels and trends in fish, other aquatic animals and their by-products

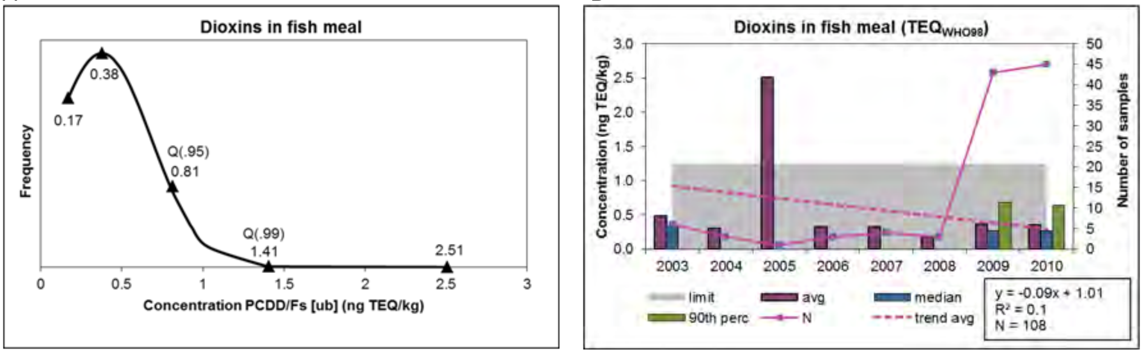
The feed group of fish, other aquatic animals and their by-products is further divided into fish meal and fish oil. Although the number of samples increased for fish oil from 2001 till 2011, as advised in the previous trend analysis (Adamse *et al.*, 2007), the amount taken was still too low to carry out a trend analysis. Therefore, only in fish meal it was looked into trends. But for both feed groups, background levels were investigated. All results are also compared to the study by EFSA (2010) consisting of data from various EU member states.

Dioxins

As shown in Table 3.15 and Figure 3.9 A, the average dioxin concentrations found in fish meal are within the same range as the levels found by EFSA. Fish oil concentrations are slightly lower compared to EFSA. However, the number of fish oil samples collected is too low to give a representative picture on the general background levels and no conclusions can be drawn.

Figure 3.9 B shows that no significant change of the average dioxin levels can be found from 2003 till 2010. It can be observed that until 2008 a low amount of samples was measured (N≤6) and not till 2009 the number increased rapidly. The concentrations found in 2009 and 2010 are, however, comparable to the years before and the average dioxin background levels seem to stay around the same level during the years. In 2005, only one sample was measured, which was further established as outlier within this feed group (see Annex VIII), having a dioxin concentration of 2.51 ng TEQ/kg. After excluding this sample, still no trend can be seen.

A B



S=-4, Signific.: none
Limit (ML) = 1.25 ng TEQ/kg

Figure 3.9. Dioxins [ub] in fish meal.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average dioxin concentrations per year.

Table 3.15. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in fish meal from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

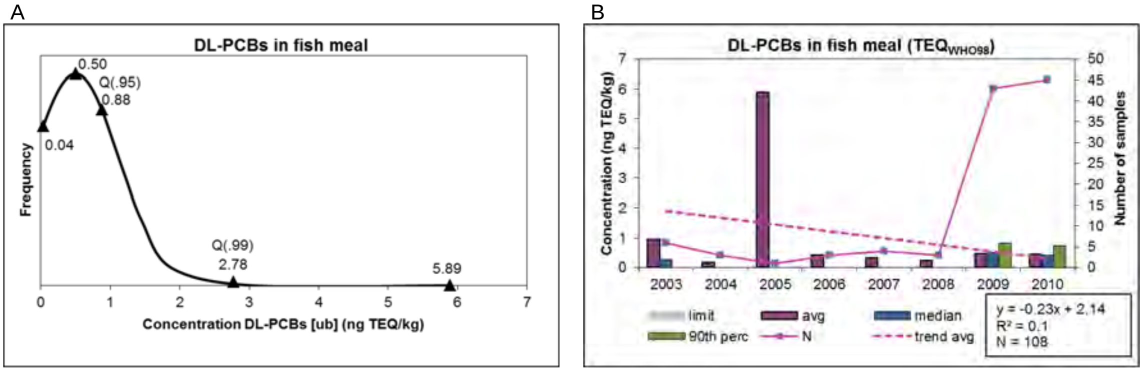
	PCDD/Fs	**DL-PCBs**	**sum PCDDF/s + DL-PCBs**
Fish meal			
AL[1]	1	2.5	
ML[1]	1.25		4.5
NCP (N=124)			
avg	0.38	0.50	0.88
Q(.50)	0.28	0.35	0.63
Q(.95)	0.81	0.88	1.77
Q(.99)	1.41	2.78	3.80
EFSA[2] - aquatic products excl. fish oil and protein (N=128)			
avg	0.41	0.86	1.27
Q(.50)	0.35	0.67	1.04
Q(.95)	0.97	2.53	3.43
Q(.99)	1.61	3.96	4.77
Fish oil			
AL[1]	5	14	
ML[1]	6		24
NCP (N=13)			
avg	2.18	7.51	9.69
Q(.50)	2.16	7.47	9.45
Q(.95)	3.58	10.92	14.37
Q(.99)	3.93	11.38	15.29
EFSA[2] - fish oil (N=89)			
avg	2.83	7.14	9.97
Q(.50)	2.52	6.38	8.64
Q(.95)	5.53	16.17	22.08
Q(.99)	7.08	26.31	32.89

1 Source: Commission Directive 2006/13/EC.
2 Source: (EFSA, 2010).

Dioxin-like PCBs

The average DL-PCB levels found in fish meal are comparable with the levels found by EFSA and only the Q(.95) is slightly lower in the Dutch data, being almost 50% lower. The levels found in fish oil are slightly higher compared to the concentrations found by EFSA.

Figure 3.10 B shows that no significant change of the average DL-PCB levels in fish meal can be found from 2003 till 2010. Again, average DL-PCB concentrations seem to stay around the same level, even when excluding the one outlier sample found in 2005 with a concentration of 5.89 ng TEQ/kg. This is the same sample as described before (section dioxins).



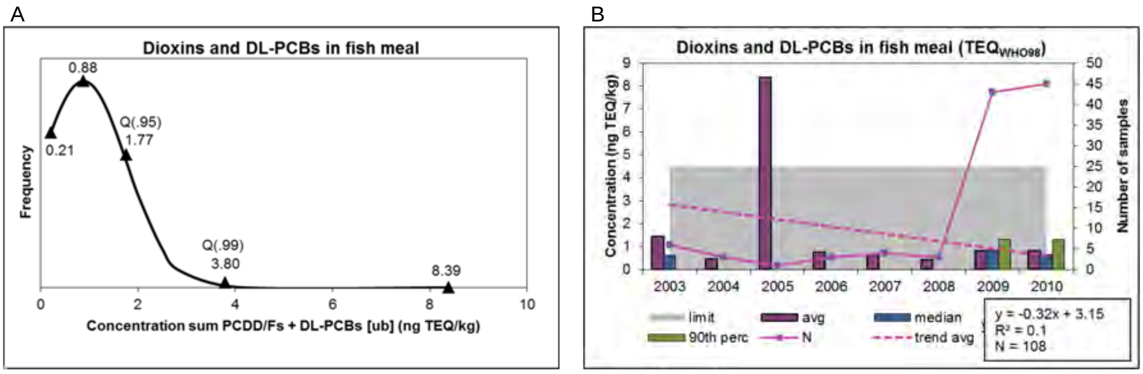
S=-2, Signific.: none
Limit (ML) = n.a.

Figure 3.10. DL-PCBs [ub] in fish meal.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average DL-PCB concentrations per year.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in fish meal is with 0.88 ng TEQ/kg slightly lower than the concentrations found by EFSA (see Table 3.15 and Figure 3.11 A). Especially the Q(.95) is higher in the EFSA dataset, having 40-50% higher values. The levels found in fish oil are slightly higher compared to the concentrations found by EFSA, but the amount of samples available is too low to draw final conclusions.

Figure 3.11 B reveals no trend and the average dioxin and DL-PCB levels in fish meal seem to stay constant from 2003 till 2010. Also, after excluding the outlier found in 2005, which is actually also the only sample measured in that year, no significant trend can be found.



S=-4, Signific.: none
Limit (ML) = 4.5 ng TEQ/kg

Figure 3.11. Dioxins and DL-PCBs [ub] in fish meal.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average dioxin and DL-PCB concentrations per year.

Fish meal seems to be critical feed material, due to repeatedly exceeding the limits (see Table 3.16) and being used in most animal diets. It is advised that fish meal remains a key part of the annual monitoring program (NCP). Over the past years the amount of samples taken has increased indicating that fish meal is already considered to be important, however, the amount collected in 2011 is rather low compared to the years before.

Table 3.16. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed material fish meal.

		Fish meal										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	-	-	24	20	21	5	32	36	43	45	16
Above ML (PCDD/Fs + DL-PCBs) of 4.5 ng TEQ/kg	N				1	2			1			
	Conc.				7.9	8.0 – 8.4			5.2			
	Origin				UN	UN			UN			
Above ML (PCDD/Fs) of 1.25 ng TEQ/kg	N				1	3		3			1	1
	Conc.				2.1	1.5 – 2.5		1.4 - 1.6			1.4	1.4
	Origin				UN	UN		FR, EU, DK			NL	US
Above AL (PCDD/Fs) of 1 ng TEQ/kg	N				1	4		3			1	1
	Conc.				2.1	1.1 – 2.5		1.4 - 1.6			1.4	1.4
Above AL (DL-PCBs) of 2.5 ng TEQ/kg	N			1	1	2		1	1			
	Conc.			3.0	5.7	5.8 – 5.9		3.5	4.9			

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), DK = Denmark, EU = European Union consisting of 27 Member States, FR = France, ML = maximum level (regulatory limit), NL = the Netherlands, UN = unknown.

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

For fish oil, neither the regulatory limit (24 pg TEQ/g for the sum of dioxins and DL-PCBs) nor the action level (5 pg TEQ/g for dioxins and 14 pg TEQ/g for DL-PCBs) were found to be exceeded during the 11 years. According to the GMP+ BA4 Feed Safety Assurance scheme fish oil needs to be refined before it is allowed to be used as feed in order to lower the levels in this feed material. This could be a reason that concentrations stay within the regulatory limits, although average dioxin and DL-PCB levels are still in a higher range then all other feed materials.

However, the amount of fish oil used as feed in the Netherlands is rather low (<0.01%, 2 of a total of ≈ 14550 thousand tons of all feed materials per year). It only forms a small proportion of animal diets as fish oil is predominantly used for carnivorous fish (see Annex IX). Even if, according to EFSA (2010), the highest dioxin levels found can be linked to fish oil, it is questionable if a non-compliance would lead to a feed or food safety risk. In contrary, fish meal is used more often (see Table 3.12 in the beginning of this section) and some samples exceeded the regulatory limit of 4.5 ng TEQ/kg (see Table 3.16). In most cases the country of origin is unknown, which seem to be a repeating point of attention.

Summary - Levels and trends in feed materials of animal origin

Land animal (by-)products

- **Animal fat**
    - In case dioxin concentrations are found to exceed the ML in animal fats, most of the time the violation of the limit is high, forming a substantial risk. Although the background levels are in general low, more or less frequently incidents occur, which could be observed in this study and also RASFF. It is advised to monitor animal fat on a regular basis.
    - Dioxins: average dioxin concentrations in animal fat are within the same range as the EFSA data.
    - DL-PCBs: average DL-PCB concentrations are slightly higher compared to the EFSA data.
    - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are within the same range compared to the Dutch data.
- **Other land animal products**
    - The amount of samples available is too low to draw conclusions.

Fish, other aquatic animals and their by-products

- **Fish meal**
    - Fish meal is used in all animal diets and as samples repeatedly exceed regulatory limits, it is advised that fish meal remains a key part of the NCP.
    - Dioxins: average dioxin concentrations in fish meal are within the same range as the EFSA data. The dioxin concentrations seem to stay on a constant level over time with no significant change.
    - DL-PCBs: average DL-PCB concentrations are within the same range as the EFSA data. No significant change of the DL-PCB levels can be seen.
    - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are slightly higher compared to the Dutch data, especially the Q(.95). In fish meal no significant change of the average dioxin and DL-PCB levels could be found.
- **Fish oil**
    - The amount of samples available is too low to draw conclusions. In general higher dioxin and DL-PCB concentrations were observed compared to all other feed materials. However, as this feed material only forms a small proportion in animal diets and is used in low quantities in the Netherlands, the number of samples collected does not need to be increased.

3.5 Levels and trends in feed additives, minerals and pre-mixtures

Feed additives, minerals and pre-mixtures contribute 2-4% to the diets of food producing animals (see Annex IX). The purpose is to favourably affect the characteristics and nutritional value of feed. No data are available of the domestic use in the Netherlands or the amount imported.

3.5.1 Levels and trends in feed additives

Incidents were reported in the past for clay minerals and choline chloride, therefore, it was decided to look into more detail in finding trends in these feed materials. Only for clay minerals a sufficient amount of samples (N=123) was available to carry out a trend analysis and to look into background levels. All samples in the group of binders, anti-caking agents and coagulants can be linked to clay minerals, but within this report the complete feed group is stated, rather than the individual feed material. For choline chloride a total number of 150 samples were taken of which 72 were measured with GC-HRMS. Of the 72 samples only 2 were measures directly with GC-HRMS, the other 70 samples were first found to be suspected with the DR CALUX® and therefore could not be included in the analysis. For this reason no data on background levels is available for choline chloride.

Due to the low amount of samples available per feed subgroup and year, a trend analysis was only carried out in the main feed group of feed additives and the feed subgroup of binders, anti-caking agents and coagulants. In addition, background levels were calculated for trace elements. In the study of EFSA (2010), consisting of data from various EU member states, dioxin and DL-PCB concentrations were only gathered for trace elements.

First, an overview is given of the descriptive analysis (see Table 3.17). When comparing the two feed subgroups, the highest concentrations can be observed in binders, anti-caking agents and coagulants, but still there is almost no difference compared to the levels found in trace elements or the main group of feed additives. However, the average and median dioxin values found by EFSA seem to be slightly lower.

Table 3.17. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in feed additives from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

	PCDD/Fs	**DL-PCBs**	**sum PCDDF/s + DL-PCBs**
AL[1]	0.5	0.35	
ML[1]	1		1.5
Feed additives (N=161)			
avg	0.31	0.03	0.34
Q(.50)	0.29	0.02	0.31
Q(.95)	0.66	0.04	0.71
Q(.99)	0.80	0.08	0.85
Binders, anti-caking agents and coagulants (N=123)			
avg	0.33	0.03	0.36
Q(.50)	0.29	0.02	0.31
Q(.95)	0.68	0.04	0.73
Q(.99)	0.82	0.09	0.85
Trace elements (N=30)			
avg	0.26	0.03	0.29
Q(.50)	0.29	0.02	0.31
Q(.95)	0.35	0.04	0.38
Q(.99)	0.37	0.06	0.41
EFSA[2] - additives compounds of trace elements (N=79)			
avg	0.19	0.06	0.25
Q(.50)	0.12	0.03	0.19
Q(.95)	0.71	0.19	0.80
Q(.99)	0.83	1.22	1.34

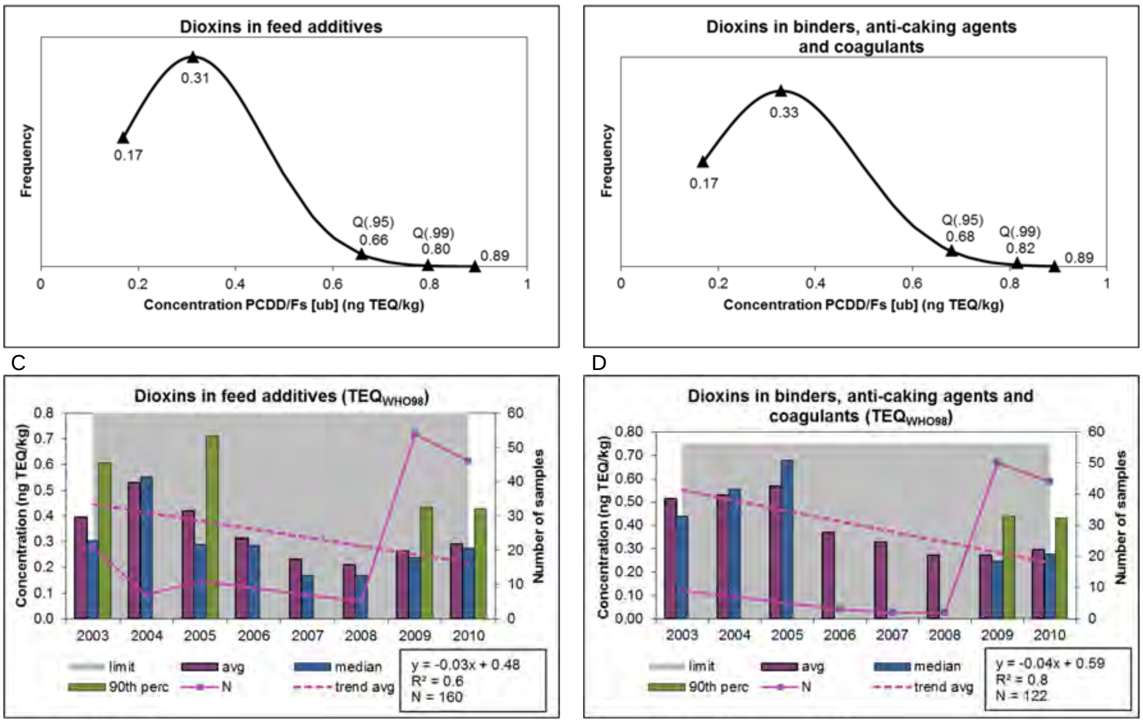
[1] Source: Commission Directive 2006/13/EC.
[2] Source: (EFSA, 2010).

Dioxins

As shown in Table 3.17 and Figure 3.12 A and B, the average dioxin concentrations found in feed additives and in more detail in binders, anti-caking agents and coagulants are around 0.32 ng TEQ/kg. The average and median dioxin levels found in the feed subgroup trace elements are slightly higher compared to EFSA (see Table 3.17). However, in the EFSA data the bandwidth of the dioxin concentrations is higher by having more than twice as high Q(.95) and Q(.99) values.

Figure 3.12 C shows that no significant change of the average dioxin levels can be found from 2003 till 2010 in the main group of feed additives. Even after the number of samples collected was increased rapidly in 2009 and 2010, the same average concentrations can still be observed and there is almost no difference between the average and median values. In the feed subgroup binders, anti-caking agents and coagulants (clay minerals) a slight decrease (7%, slope m=-0.04) can be seen (Figure 3.12 D) with a significance of p=0.05. Due to the low average dioxin concentrations present (<0.6 ng TEQ/kg), the decrease is most likely linked to the reduction of the reporting limits in 2007 and the trend found seems to be fake.

A B



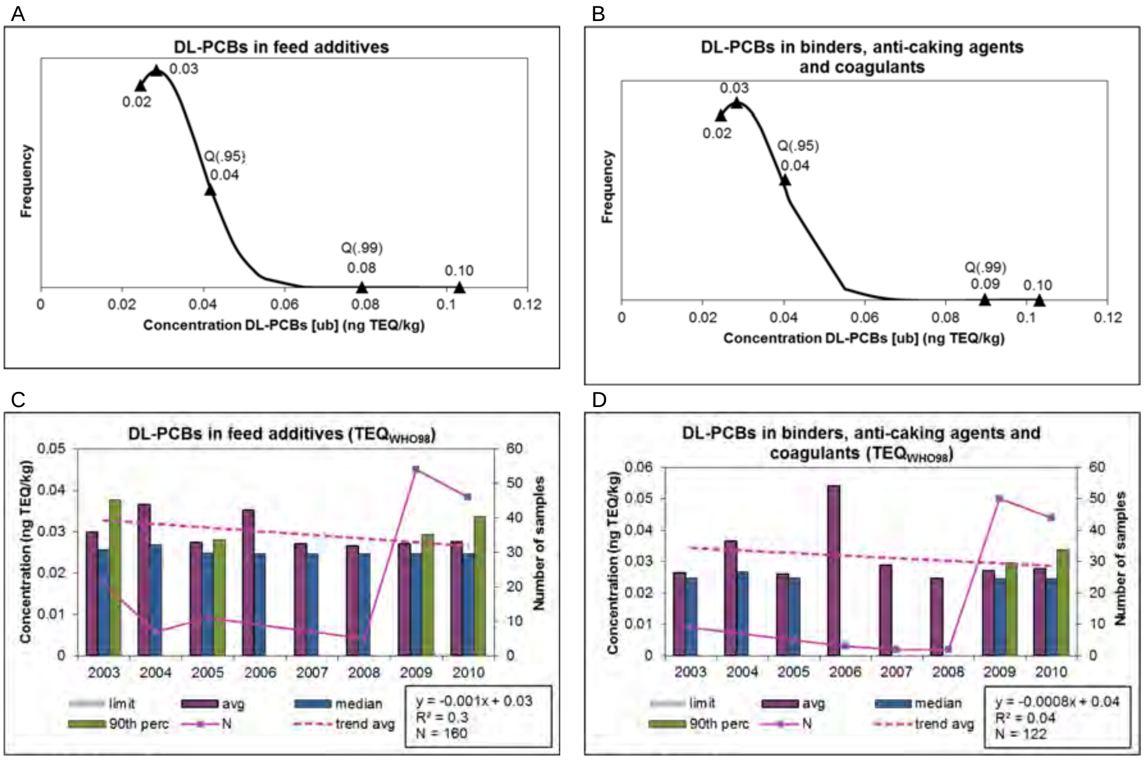
S=-14, Signific.: none | *S=-18, Signific.: p=0.05*
Limit (ML) = 1 ng TEQ/kg | *Limit (ML) = 0.75 ng TEQ/kg*

Figure 3.12. Dioxins [ub] in feed additives and specified in binders, anti-caking agents and coagulants.
(A) + (B) frequency distribution curve using TEF_{WHO98} values.
(C) + (D) trends in the average dioxin concentrations per year.

Dioxin-like PCBs

The average DL-PCB levels found in feed additives and the two subgroups (binders, anti-caking agents and coagulants, trace elements) are around the same level, being 0.04 ng TEQ/kg (see Table 3.17 and Figure 3.13 A and B). This is comparable with the levels found by EFSA. Again, the bandwidth of the DL-PCB concentrations is higher in the EFSA data by having 4 to 10 times higher Q(.95) and Q(.99) values.

Figure 3.13 C shows that no significant change of the average DL-PCB levels in feed additives can be found from 2003 till 2010. Average DL-PCB concentrations seem to stay around the same level as well as median and 90th percentile values. Also in the feed group of clay minerals (binders, anti-caking agents and coagulants, Figure 3.13 D) no significant trend can be seen.



S=-10, Signific.: none
Limit (ML) = n.a.

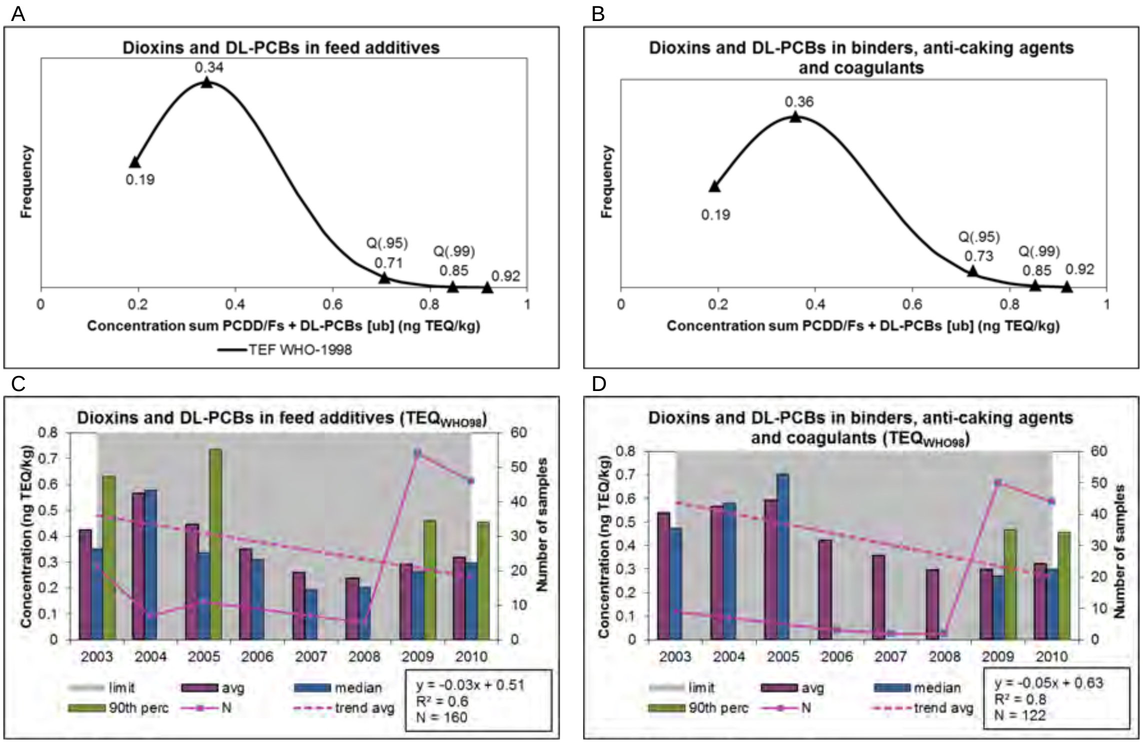
S=-2, Signific.: none
Limit (ML) = n.a.

Figure 3.13. DL-PCBs [ub] in feed additives and specified in binders, anti-caking agents and coagulants.
(A) + (B) frequency distribution curve using TEF_{WHO98} values.
(C) + (D) trends in the average DL-PCB concentrations per year.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in feed additives and the two subgroups (binders, anti-caking agents and coagulants, trace elements) are around the same level, staying between 0.29 and 0.36 ng TEQ/kg (see Table 3.17 and Figure 3.14 A and B). Hereby, the levels found in trace elements are overall lower. The EFSA levels are approximately the same level as the Dutch data and only Q(.99) levels are slightly higher in the EFSA dataset.

Figure 3.14 C shows that average dioxin and DL-PCB levels in feed additives did not change significantly during the last decade. The concentrations vary per year, but no trend can be seen. When looking into the feed subgroup binders, anti-caking agents and coagulants (Figure 3.14 D), at first dioxin and DL-PCB concentrations tend to decrease significantly (p=0.01, Mann-Kendall). However, the slight decrease (8%, slope m=-0.05) is again linked to the fact that the reporting limits were reduced in 2007 and the concentrations were at/below the LOQ. No conclusions on trends can be drawn and also the clay mineral concentrations seem to stay around the same level during the past years.



S=-14, Signific.: none
Limit (ML) = 1.5 ng TEQ/kg

S=-16, Signific.: p=0.1
Limit (ML) = 1.5 ng TEQ/kg

Figure 3.14. Dioxins and DL-PCBs [ub] in feed additives and specified in binders, anti-caking agents and coagulants.
(A) + (B) frequency distribution curve using TEF_{WHO98} values.
(C) + (D) trends in the average dioxin and DL-PCB concentrations per year.

Table 3.18 gives an overview of the amount of samples exceeding the regulatory limits (ML) and/or action levels (AL) in the feed group of feed additives. All samples are linked to clay minerals (binders, anti-caking agents and coagulants), except one sample, which exceeded the ML for dioxins in 2009 and belongs to the group of trace elements (copper sulphate).

Clay minerals belong to the feed subgroup of binders, anti-caking agents and coagulants and may be used for various reasons in feed, e.g. as pelletizing aid in the production of animal feed pellets or as flow ability aid (anti-caking agent and coagulant) for unconsolidated feed ingredients such as soy meal or as binder of mycotoxins. Almost every year, one or more clay mineral samples were found to exceed the limit of 1.5 ng TEQ/kg for the sum of dioxins and DL-PCBs (see Table 3.18). The elevated levels are caused by dioxins only, not by DL-PCBs. These feed materials are presumed to be contaminated with dioxins in ancient times and dioxin contamination can hardly be avoided. A better selection of clay mineral sources/regions clean of dioxins and DL-PCBs may help to reduce levels on a long term.

The frequent non-compliance with the regulatory limits underlines the necessity to keep clay minerals included as part of the annual monitoring program, although the amount sampled per year does not need to be increased. Clay minerals are expected to be tested also on a regular basis by feed producing companies.

Table 3.18. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group feed additives.

		Feed additives										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	40	55	69	53	66	59	67	71	157	156	30
Above ML (PCDD/Fs + DL-PCBs) of 1.5 ng TEQ/kg **	N	1	1			1				1		
	Conc.	4.5	5.7	1.7 - 2.1		2.8				1.7		
	Origin	NL	NL	NL, ES		UN				UN		
Above ML (PCDD/Fs) of 0.75 ng TEQ/kg **	N	1	1	4	1	3	1			1		
	Conc.	4.2	5.4	0.9 – 2.0	0.9	0.9 – 2.7	0.8			1.1		
	Origin	NL	NL	BE, NL, ES	UN	UN	UN			UN		
Above AL (PCDD/Fs) of 0.5 ng TEQ/kg **	N	1	1	8	7	7	2	1	1	4	1	
	Conc.	4.2	5.4	0.6 – 2.0	0.6 – 0.9	0.6 – 2.7	0.6 – 0.8	0.7	0.7	0.6 – 1.1	0.6	
Above AL (DL-PCBs) of 0.5 ng TEQ/kg **	N									1		
	Conc.									0.6		

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), BE = Belgium, ES = Spain, ML = maximum level (regulatory limit), NL = the Netherlands, UN = unknown.

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

** *The limits refer to the ones set for binders and anti-caking agents as in the majority of the samples these limits were exceeded and only one sample was non-compliant with the regulatory limits set for all other feed additives.*

Choline chloride, also known as a member of the vitamin B family, is used as nutritional feed additive for animals in cases where the natural choline content of the feed may not fully cover the minimal requirements. In the past, high dioxin levels were found in this feed additive due to the mixing with contaminated saw dust. The monitoring data used in this study did not reveal either an exceedance of the regulatory limit (1.5 ng TEQ/kg for the sum of dioxins and DL-PCBs) or the action levels (0.5 ng TEQ/kg for dioxins and 0.35 ng TEQ/kg for DL-PCBs). Considering the data available, choline chloride does not seem to form a high risk to feed and food safety as the total quantity used in the animal diet is low (up to 1%) and the average dioxin and DL-PCB concentrations measured were all below the AL and ML. A major reason to monitor this ingredient is the elevated response observed with the DR CALUX® assay which could be linked to the contamination with brominated dioxins. This points to a cross-contamination with brominated compounds during production. In conclusion, the amount of samples currently collected is sufficient or can even be lowered.

3.5.2 Levels and trends in feed materials of mineral origin

For feed materials of mineral origin, a too low number of samples (N=28) was available to look into trends, therefore, only background levels were investigated.

Dioxins

As shown in Table 3.19, the average dioxin concentrations found in feed materials of mineral origin are around 0.24 ng TEQ/kg. The average and median dioxin levels found by EFSA are 50% lower and only the Q(.95) and Q(.99) is around the same level compared to the Dutch data.

Table 3.19. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in feed materials of mineral origin from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
AL[1]	0.5	0.35	
ML[1]	1		1.5
Feed materials of mineral origin (N=28)			
avg	0.24	0.03	0.27
Q(.50)	0.29	0.02	0.31
Q(.95)	0.29	0.03	0.32
Q(.99)	0.31	0.06	0.37
EFSA[2] - feed materials of mineral origin (N=114)			
avg	0.13	0.10	0.23
Q(.50)	0.10	0.03	0.21
Q(.95)	0.29	0.37	0.67
Q(.99)	0.39	0.66	0.98

1 Source: Commission Directive 2006/13/EC.
2 Source: (EFSA, 2010).

Dioxin-like PCBs

The average DL-PCB levels found in feed materials of mineral origin are slightly lower compared to the concentrations found by EFSA, especially when looking into the Q(.95) and Q(.99) values, which are 8 to 12 times higher in the EFSA dataset (see Table 3.19).

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in feed materials of mineral origin is around the same level when comparing the Dutch data vs. EFSA, staying between 0.23 and 0.27 ng TEQ/kg (see Table 3.19). Again, the bandwidth of the dioxin and DL-PCB concentrations is higher in the EFSA data by having twice as high Q(.95) and Q(.99) values.

For these feed materials, no ALs and MLs were exceeded and the dioxin and DL-PCB concentrations found were in general low. Looking into dioxin and DL-PCB concentrations of compound feedingstuffs instead of checking minerals individually could, therefore, be a better approach. In case elevated dioxin and DL-PCB concentrations are found in compound feedingsstuffs, it should be investigated if this is caused by these feed materials. However, in this scenario still feed materials of mineral origin have to be monitored from time to time and it might than be necessary to also lower the action or decision levels for compound feedingstuffs.

3.5.3 Levels and trends in pre-mixtures

For the feed group pre-mixtures, a too low number of samples (N=37) was available to look into trends, therefore, only background levels were investigated.

Dioxins

As shown in Table 3.20, the average dioxin concentrations found in pre-mixtures are around 0.26 ng TEQ/kg. The average dioxin levels found by EFSA are 50% lower and only the Q(.95) is around the same level compared to the Dutch data.

Table 3.20. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in pre-mixtures from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
AL[1]	0.5	0.35	
ML[1]	1		1.5
Pre-mixtures (N=37)			
avg	0.26	0.03	0.29
Q(.50)	0.29	0.02	0.31
Q(.95)	0.32	0.03	0.35
Q(.99)	0.37	0.05	0.40
EFSA[2] - pre-mixtures (N=91)			
avg	0.12	0.03	0.16
Q(.50)	0.07	0.03	0.09
Q(.95)	0.30	0.11	0.34
Q(.99)	0.97	0.27	1.02

1 *Source: Commission Directive 2006/13/EC.*
2 *Source: (EFSA, 2010).*

Dioxin-like PCBs

The average DL-PCB levels found in pre-mixtures are around the same level when comparing the Dutch data vs. EFSA, being around 0.03 ng TEQ/kg (see Table 3.20). However, the bandwidth of the DL-PCB concentrations is higher in the EFSA data by having 3 to 5 times higher Q(.95) and Q(.99) values.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in pre-mixtures is around 0.29 ng TEQ/kg (see Table 3.20). Again, the average dioxin and DL-PCB levels found by EFSA are almost 50% lower and only the Q(.95) is around the same level compared to the Dutch data. Still the bandwidth of concentrations is higher in the EFSA data by having a twice as high Q(.99) value.

For these feed materials, no MLs and only twice ALs were exceeded (2005 and 2006, see Table 3.21). In general, the dioxin and DL-PCB concentrations found were low. Also for pre-mixtures, it therefore seems sufficient to be measured infrequently from time to time and to focus more on elevated dioxin and DL-PCB concentrations found in compound feedingsstuffs. However, there have been incidents with zinc oxide (trace elements) in Italy and Chile and sequestered minerals in the USA and some attention is justified.

Table 3.21 Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group pre-mixtures

		Pre-mixtures										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	3	-	-	14	32	22	8	12	7	2	64
Above ML (PCDD/Fs + DL-PCBs) of 1.5 ng TEQ/kg **	N											
	Conc.											
	Origin											
Above ML (PCDD/Fs) of 1 ng TEQ/kg **	N											
	Conc.											
	Origin											
Above AL (PCDD/Fs) of 0.5 ng TEQ/kg **	N					1						
	Conc.					1.0						
Above AL (DL-PCBs) of 0.35 ng TEQ/kg **	N						1					
	Conc.						0.6					

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), ML = maximum level (regulatory limit).

* *N_{total} refers to the total amount of samples measured of the feed material in that year.*

Summary - Levels and trends in feed additives, minerals and pre-mixtures

- **Feed additives**
  - Dioxins: average dioxin concentrations in feed additives are slightly higher compared to the EFSA data. The dioxin concentrations seem to stay on a constant level over time with no significant change, although in the feed group clay minerals a slight decrease of 7% can be noticed.
  - DL-PCBs: average DL-PCB concentrations are within the same range as the EFSA data. No significant trend can be seen and also median and 90th percentile values stay around the same level.
  - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are around the same level compared to the Dutch data. Overall, levels found in trace elements are slightly lower. In main group of feed additives no significant change of the average dioxin and DL-PCB levels was found. Average dioxin and DL-PCB levels of the feed group clay minerals tend to decrease significantly with around 8%, linked to a reduction of the dioxin levels. However, due to the low levels found the trend analysis has to be treated with caution.
  - Within the feed subgroup of feed additives, almost every year one or two clay mineral samples exceeded the limit, mainly caused by too high dioxin concentrations only and not by DL-PCBs. In general, dioxin and DL-PCB concentrations stay far below the regulatory limit, but as more or less frequently incidents occur, this feed group should be monitored regularly, although the amount sampled per year does not need to be increased.
  - For the feed additive choline chloride the monitoring data did not reveal a non-compliance with the regulatory limit and action levels. Less attention needs to be paid to this feed material in future, although the contamination with brominated compounds deserves attention.
- **Feed materials of mineral origin**
  - Dioxins: average dioxin concentrations in feed materials of mineral origin are two times higher compared to the EFSA data. However, the Q(.95) and Q(.99) values are around the same level.
  - DL-PCBs: average DL-PCB concentrations are slightly lower compared to the EFSA data.
  - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are around the same level compared to the Dutch data.
- **Pre-mixtures**
  - Dioxins: average dioxin concentrations in pre-mixtures are two times higher compared to the EFSA data. However, the Q(.95) values are around the same level.
  - DL-PCBs: average DL-PCB concentrations are within the same range as the EFSA data.
  - Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are around 50% lower compared to the Dutch data, but the Q(.95) values are around the same level.
- All feed materials within this group are used in low quantities in all animal diets, meaning that only high levels will noticeably increase the levels in the feed. Another approach could be to investigate elevated dioxin and DL-PCB concentrations found in e.g. compound feedingstuffs and investigate than if this is caused by these feed materials. However, in this scenario still individual feed materials have to be monitored from time to time and it might be necessary to lower the action/decision limits for compound feedingstuffs.

3.6 Levels and trends in compound feedingstuffs

Compound feedingstuffs is comprised of complete and complementary feed. Complete feed consists of (a combination of) all feed materials as described in the sections before (feed materials of plant and animal origin, feed additives, minerals and pre-mixtures and to some extent maybe also feed from food waste streams). In Annex IX an overview is given of the different percentages used per feed group to form complete feed. If high dioxin and DL-PCB concentrations are found in complete feed, a higher risk for the feed and food safety exists as the animal diet is almost entirely based on that product. Instead, complementary feed is used additionally, mostly in low quantities, as part of the diet, thus forming a lower risk.

Table 3.22 gives an overview of the data available for compound feed produced in the Netherlands, which is used for domestic production or export. The majority of feed is produced for pigs (41%), and in more detail for pigs used for meat production (27%). This is followed by feed produced for dairy cattle (22%) and poultry used either for egg (14%) or meat (12%) production. In general, compound feed as "ready-to-feed" animal food seems to form the primary basis in animal diets used in the Netherlands, leading to a higher risk if dioxin and DL-PCB limits are exceeded.

Table 3.22. Overview of the Dutch feed production for specified compound feedingstuffs.

Feed material	**Feed production** [A]		**Import** [B]			**Country of origin** [B]
			direct	**indirect**		
	x1000 tons	%	x1000 tons	x1000 tons	%	%
5. Compound feedingstuffs						
5.1. Compound feed, excl. fur animals, pets and fish						
total	13721	100	1268	n.a.	n.a.	EU: 53 (DE: 35, BE: 10, FR: 6), US: 46
Pigs						
total	5624	41	n.a.	n.a.	n.a.	n.a.
pigs for fattening	3690	27	n.a.	n.a.	n.a.	n.a.
breeding pigs	1231	9	n.a.	n.a.	n.a.	n.a.
piglets	754	5	n.a.	n.a.	n.a.	n.a.
Poultry						
total	3444	25	n.a.	n.a.	n.a.	n.a.
chicks & layers	1880	14	n.a.	n.a.	n.a.	n.a.
broilers	1600	12	n.a.	n.a.	n.a.	n.a.
Bovine animals						
total	3360	24	n.a.	n.a.	n.a.	n.a.
dairy cow	2992	22	n.a.	n.a.	n.a.	n.a.
for fattening	321	2	n.a.	n.a.	n.a.	n.a.
calves (excl. milk replacers)	72	1	n.a.	n.a.	n.a.	n.a.
other	59	0	n.a.	n.a.	n.a.	n.a.

Feed material	Feed production [A]		Import [B]			Country of origin [B]
			direct	indirect		
	x1000 tons	%	x1000 tons	x1000 tons	%	%
Milk replacers						
total	713	5	n.a.	n.a.	n.a.	n.a.
Other						
total	560	4	n.a.	n.a.	n.a.	n.a.
5.2. Feed for fur animals, pets and fish						
Pet food						
dog/cat food - retail sale	57	0	149	n.a.	n.a.	EU: 94 (DE: 33, FR: 29, BE: 11, GB: 8)

Abbreviations used: BE = Belgium, DE = Germany, EU = European Union consisting of 27 Member States, FR = France, GB = United Kingdom, n.a. = not available, NL = the Netherlands, US = United States of America.

[A] *average 2001 till 2011, Source: Fefac (see Annex VII).*
[B] *average 2001 till 2010, Source: Eurostat (see Annex VI).*

Feed materials which could not be categorized in the other three feed groups, were included in the category of compound feedingstuffs. Miscellaneous feed materials often consist of a mixture of feed materials of plant and animal origin or other feed materials. The feed originates mainly from the remains of the food production industry or food waste (see Table 3.23). In general, these feed materials are used as complementary feed and it is not known in what quantity they form a part of animal diets. The amount of remains of food production used as feed seems to form a substantial part of compound feed if compared to other single feed materials. Food for human consumption is, however, also subject to monitoring of dioxins and DL-PCBs with in some cases even stricter limits. Likely only additional industrial processes applied to the food waste streams may increase the feed safety risk; e.g. the incident found for bakery waste in which an additional heating process was applied. However, it should be kept in mind that mostly food waste streams are transported to companies within the same area as the food processing plant and if an incident occurs high levels might be found in animals in the region.

Table 3.23. Overview of the Dutch feed consumption for miscellaneous feed materials, which could not be categorized in the other feed groups of single feed materials.

Feed material	Dutch feed consumption A		Domestic feed A		Import B direct	Import B indirect	Import B	Country of origin B
	x1000 tons	%	x1000 tons	%	x1000 tons	x1000 tons	%	%
5. Compound feedingstuffs								
Miscellaneous feed materials								
total	1220	8	171	14	1049	208	86	-
residues of starch and similar manufacture	n.a.	n.a.	n.a.	n.a.	574 B	n.a.	n.a.	EU: 99 (BE: 39, FR: 34, DE: 25)
brewing or distilling waste	n.a.	n.a.	n.a.	n.a.	207 B	n.a.	n.a.	EU: 87 (DE: 56, BE: 26), US: 12

Abbreviations used: BE = Belgium, DE = Germany, EU = European Union consisting of 27 Member States, FR = France, GB = United Kingdom, n.a. = not available, NL = the Netherlands, US = United States of America.

[A] *average 2004/2005 till 2008/2009, Source: PDV (see Annex V).*
[B] *average 2001 till 2010, Source: Eurostat (see Annex VI).*

For compound feedingstuffs different limits (ALs and MLs) are set by law for fur animals, pets and fish compared to all other compound feed, therefore, the analysis is carried out for both feed groups separately.

3.6.1 Levels and trends in compound feed, excl. fur animals, pets and fish

In this section the levels and trends will be described for compound feed, excluding fur animals, pets and fish and in more detail for the subgroups complementary cattle feed as well as complete pig and poultry feed. Due to the low amount of samples tested with GC-HRMS for all other feed subgroups, the descriptive analysis as well as trend analysis could only been carried out for these three groups. All results are also compared to the study by EFSA (2010) consisting of data from various EU member states.

First, an overview is given of the descriptive analysis (see Table 3.24). When comparing the dioxin and DL-PCB levels of complementary and complete feed and the three feed subgroups, the concentrations found are overall within the same range. No differences can be observed. We first looked into levels and trends found in the main feed group of compound feedingstuffs, followed by a division into complementary and complete feed.

Table 3.24. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in compound feed, excl. fur animals, pets and fish from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

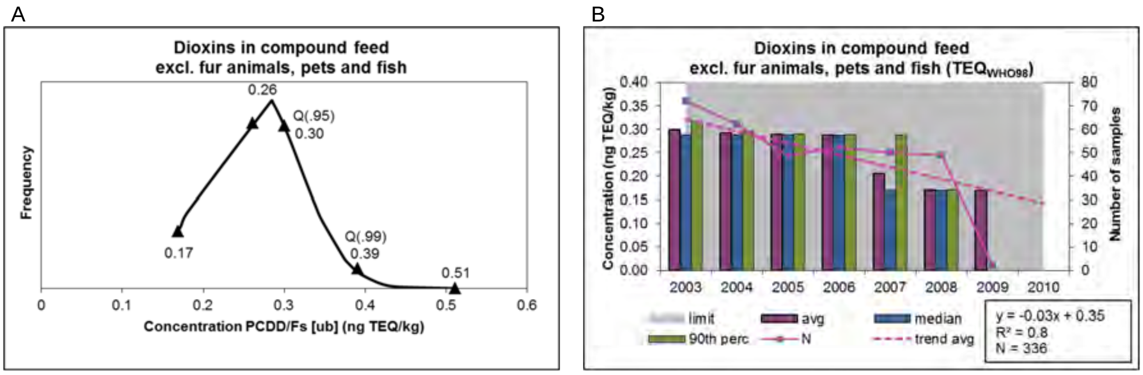
	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
AL[1]	0.5	0.5	
ML[1]	0.75		1.5
Compound feed excl. fur animals, pets and fish (N=337)			
avg	0.26	0.03	0.29
Q(.50)	0.29	0.03	0.31
Q(.95)	0.30	0.05	0.36
Q(.99)	0.39	0.08	0.48
Complementary feed (N=147)			
avg	0.26	0.03	0.30
Q(.50)	0.29	0.02	0.31
Q(.95)	0.32	0.05	0.37
Q(.99)	0.41	0.19	0.58
Complementary cattle feed (N=99)			
avg	0.27	0.03	0.29
Q(.50)	0.29	0.02	0.31
Q(.95)	0.34	0.04	0.37
Q(.99)	0.42	0.07	0.49
Complete feed (N=190)			
avg	0.26	0.03	0.29
Q(.50)	0.29	0.03	0.31
Q(.95)	0.30	0.06	0.35
Q(.99)	0.35	0.08	0.46
Complete pig feed (N=115)			
avg	0.26	0.03	0.29
Q(.50)	0.29	0.03	0.32
Q(.95)	0.30	0.06	0.36
Q(.99)	0.38	0.08	0.46
Complete poultry feed (N=67)			
avg	0.26	0.03	0.29
Q(.50)	0.29	0.03	0.31
Q(.95)	0.29	0.05	0.33
Q(.99)	0.30	0.08	0.35
EFSA[2] - compound feed excl. fur animals, pets and fish (N=482)			
avg	0.22	0.26	0.47
Q(.50)	0.17	0.03	0.22
Q(.95)	0.69	1.63	2.16
Q(.99)	1.14	2.67	3.81

[1] *Source: Commission Directive 2006/13/EC.*
[2] *Source: (EFSA, 2010).*

Dioxins

As shown in Table 3.24 and Figure 3.15 A, the average dioxin concentrations found in compound feed (excl. fur animals, pets and fish) are generally within the same range when comparing the Dutch data vs. EFSA, staying between 0.22 and 0.27 ng TEQ/kg. In the EFSA data the bandwidth of the dioxin concentrations is higher by having 2 to 3fold higher Q(.95) and Q(.99) values.

Figure 3.15 B shows a slight decrease (≈9%, slope m=-0.03) of the average dioxin levels with a high significance (p=0.001, Mann Kendall). The concentrations seem to be slightly lower starting from 2007. This lower levels are linked to the fact that the reporting limits were reduced in 2007, which means that levels of individual congeners were often below the LOQ. This is supported by the fact that the lowest upper bound dioxin levels are before 2007 0.29 and from 2007 on 0.17 ng TEQ/kg, meaning that no concentration can be found below this values. These lowest [ub] concentrations are actually visible in the graph. In addition, the median and 90th percentile values are around the same level as the average, which indicates that there is a low bandwidth of the concentrations found. Obviously, the trend found is fake and dioxin concentrations stayed around the same level from 2003 till 2010.



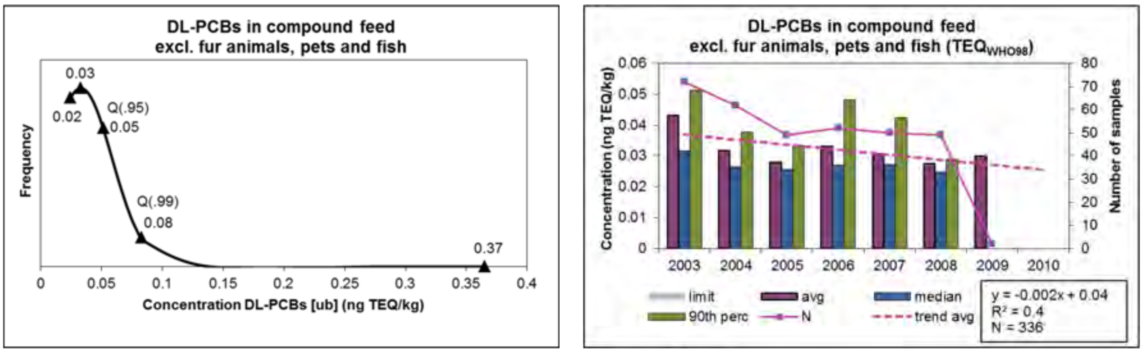
S=-21, Signific.: p=0.001
Limit (ML) = 0.75 ng TEQ/kg

Figure 3.15. Dioxins [ub] in compound feed, excl. fur animals, pets and fish.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average dioxin concentrations per year.

Dioxin-like PCBs

The average DL-PCB levels found in compound feed are around 0.04 ng TEQ/kg (see Table 3.24 and Figure 3.16 A). This is 5 to 8fold lower compared to the levels found by EFSA. Also the Q(.95) and Q(.99) values are a lot higher compared to the Dutch data.

A B



S=-11, Signific.: none
Limit (ML) = n.a.

Figure 3.16. DL-PCBs [ub] in compound feed, excl. fur animals, pets and fish.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average DL-PCB concentrations per year.

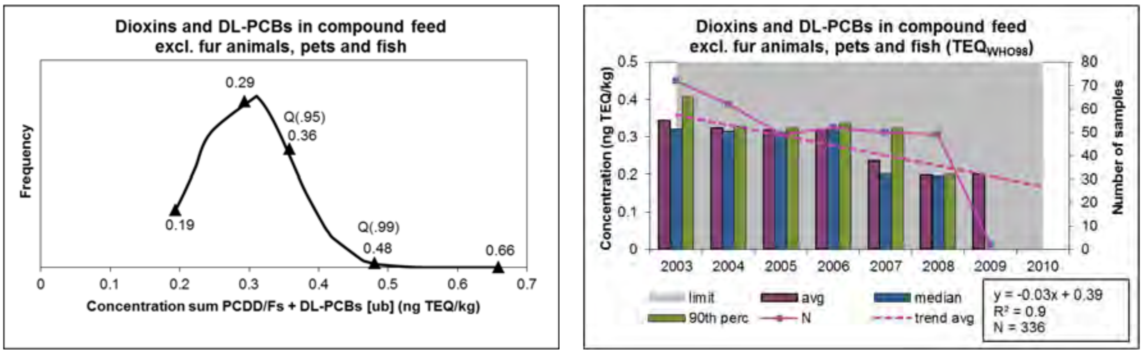
Figure 3.16 B shows that no significant change of the average DL-PCB levels can be found from 2003 till 2010. Average DL-PCB concentrations seem to stay around the same level as well as median and 90th percentile values.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs in compound feed is 0.29 ng TEQ/kg (see Table 3.24 and Figure 3.17 A). The EFSA levels are almost two times higher as the Dutch data and only median values are comparable with the one's of the Netherlands. Again, the bandwidth of the dioxin and DL-PCB concentrations is higher in the EFSA data by having 5 to 6 times higher Q(.95) and Q(.99) values.

Figure 3.17 B shows that average dioxin and DL-PCB levels in compound feed changed significantly during the last decade, with lower concentrations since 2007 (p=0.05, Mann Kendall). This decrease of around 8% is linked to the reduction of dioxin levels only and seems to be biased. Due to the change of the reporting limits in 2007 as described before (section dioxins), more likely concentrations stayed constant during the years and the trend found is fake.

A B



S=-17, Signific.: p=0.05
Limit (ML) = 1.5 ng TEQ/kg

Figure 3.17. Dioxins and DL-PCBs [ub] in compound feed, excl. fur animals, pets and fish.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average dioxin and DL-PCB concentrations per year.

Almost all samples, which exceeded the ALs and MLs belong to complementary feed (see Table 3.25). The three samples, which were found to exceed both MLs in 2003 are related to bread meal originating from Germany and the Netherlands. These samples are likely linked to the bakery waste incident end of 2002/beginning of 2003 (PDV, 2003). The risk of future incidents with bread meal should not be underestimated as the heating/drying process is still a risk factor which can, under certain circumstances, lead to the formation of dioxins. The two non-compliant samples found in 2001 and 2002 were taken from mineral mix for bovine animals. The sample, which exceeded the AL of dioxins in 2004, is the only sample which can be linked to complete (poultry) feed.

Table 3.25. Overview of non-compliance with regulatory limits (ML) and action levels (AL) in the feed group compound feed, excl. fur animals, pets and fish.

		Compound feed, excl. fur animals, pets and fish										
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
	N_{total}*	156	188	279	237	285	116	83	106	115	67	81
Above ML (PCDD/Fs + DL-PCBs) of 1.5 ng TEQ/kg **	N			3								
	Conc.			2.0 – 8.1								
	Origin			NL, DE								
Above ML (PCDD/Fs) of 0.75 ng TEQ/kg **	N	1	1	3								
	Conc.	1.0	1.2	2.0 – 8.1								
	Origin	NL	NL	NL, DE								
Above AL (PCDD/Fs) of 0.5 ng TEQ/kg **	N	1	1	3	1							
	Conc.	1.0	1.2	2.0 – 8.1	0.7							
Above AL (DL-PCBs) of 0.5 ng TEQ/kg **	N											
	Conc.											

Abbreviations used: AL = action level, Conc. = concentration (in ng TEQ/kg), DE = Germany, ML = maximum level (regulatory limit), NL = the Netherlands.

Almost all samples taken for this feed category originate from the Netherlands, which is reasonable as compound feedingsstuffs used in the Netherlands originate mainly from domestic producers. The feed materials used within the feed originate of course also from other countries as described in the sections before (3.3 till 3.5).

Within the group of compound feedingstuffs, cattle feed is actually almost only fed complementary as a substantial part of the diet consists of forages and roughage; excluding milk replacers used for calves. For the other animal species (e.g. poultry and pigs) it is more common to use complete feed, therefore, a too low amount of samples was collected on complementary feed to be able to use for further analysis. A sufficient number of samples was available for complementary cattle feed and complete pig and poultry feed, ergo this feed materials will be investigated in more detail on levels and trends.

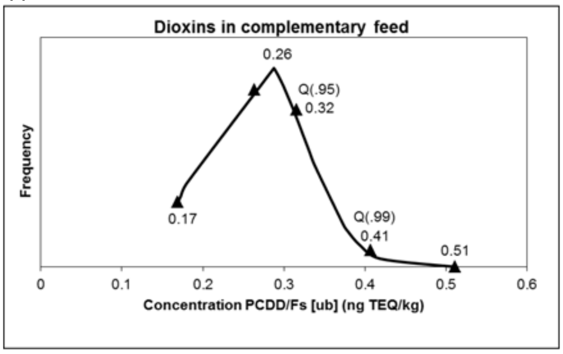
Levels and trends in complementary feed

Dioxins

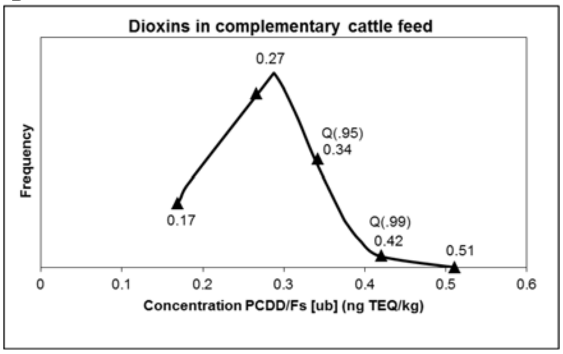
As shown in Table 3.24 and Figure 3.18 A and B, the average dioxin concentrations found in complementary feed and in more detail complementary cattle feed are generally within the same range when comparing the Dutch data vs. EFSA, staying between 0.22 and 0.27 ng TEQ/kg. In the EFSA data the bandwidth of the dioxin concentrations is higher by having 2 to 3fold higher Q(.95) and Q(.99) values.

Figure 3.18 C shows a slight decrease of the average dioxin levels in complementary feed, with a high significance (p=0.001, Mann Kendall). This change of around 8% can be also seen in complementary cattle feed with a significance of p=0.01 (see Figure 3.18 D). This similarity can be explained as cattle feed forms the major part of samples collected within the group of complementary feed (N=99 out of N_{total}=147). Again the concentrations seem to be slightly lower starting from 2007, which is presumably linked to the reduction of the reporting limits. Likely the trend found is fake and dioxin concentrations stayed around the same level from 2003 till 2010.

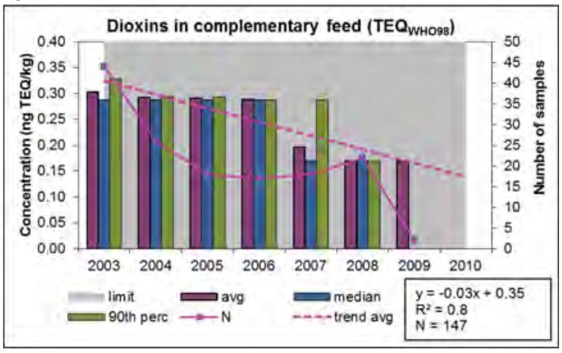
A



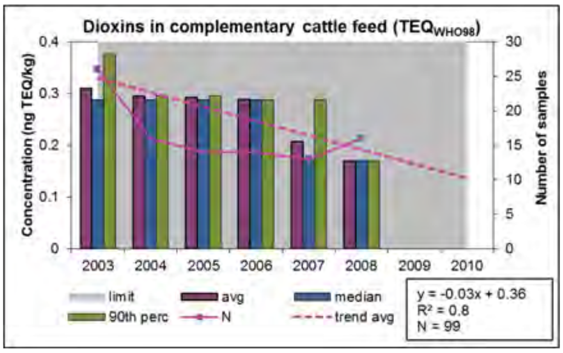
B



C



D



S=-21, Signific.: p=0.001
Limit (ML) = 0.75 ng TEQ/kg

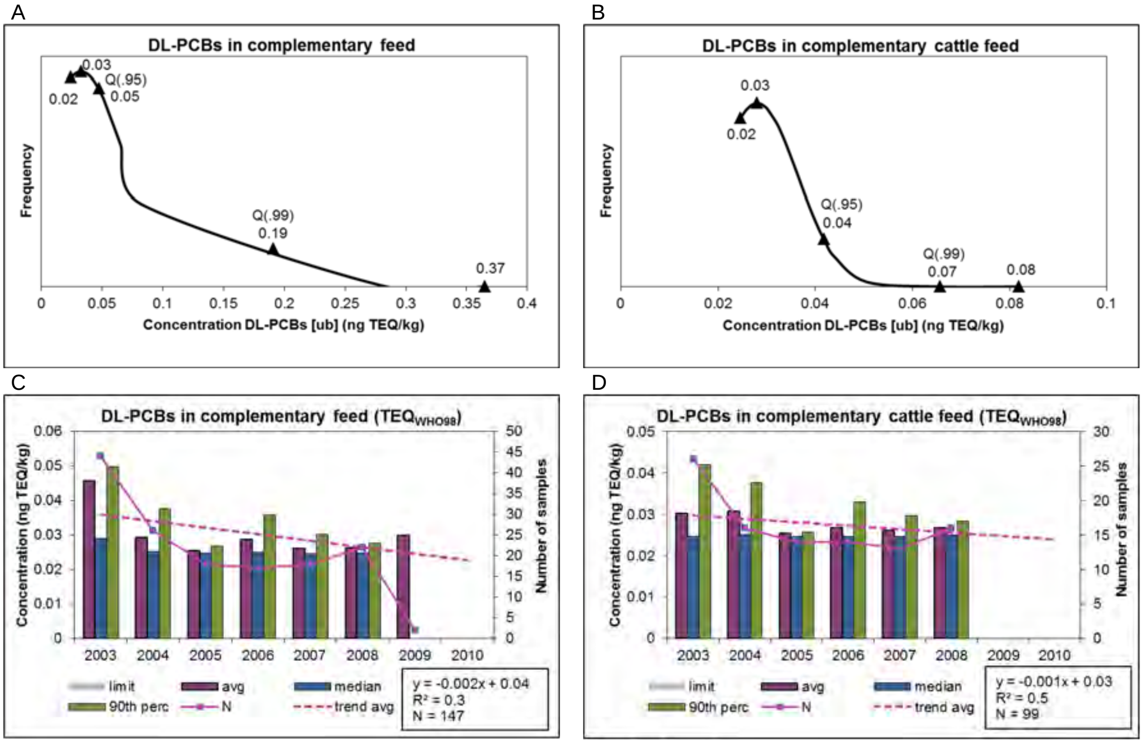
S=-15, Signific.: p=0.01
Limit (ML) = 0.75 ng TEQ/kg

Figure 3.18. Dioxins [ub] in complementary feed and specified in complementary cattle feed.
(A) + (B) frequency distribution curve using TEF_{WHO98} values.
(C) + (D) trends in the average dioxin concentrations per year.

Dioxin-like PCBs

The average DL-PCB levels found in complementary feed and in more detail complementary cattle feed are around 0.03 ng TEQ/kg (see Table 3.24 and Figure 3.19 A and B). This is 5 to 8fold lower compared to the levels found by EFSA. Also the Q(.95) and Q(.99) values are a lot higher compared to the Dutch data.

Figure 3.19 C and D show that no significant change of the average DL-PCB levels can be found from 2003 till 2010. Average DL-PCB concentrations seem to stay around the same level as well as median and 90th percentile values.



S=-3, Signific.: none
Limit (ML) = n.a.

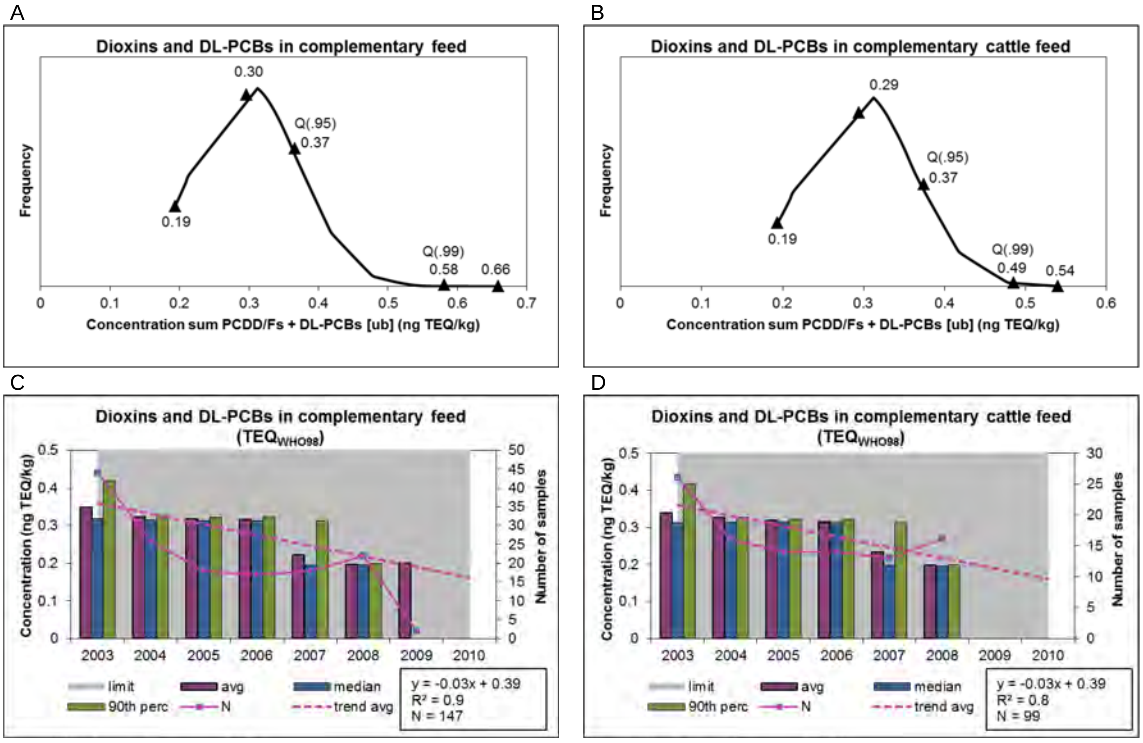
S=-5, Signific.: none
Limit (ML) = n.a.

Figure 3.19. DL-PCBs [ub] in complementary feed and specified in complementary cattle feed.
(A) + (B) frequency distribution curve using TEF_{WHO98} values.
(C) + (D) trends in the average DL-PCB concentrations per year.

Sum of dioxins and DL-PCBs

The average content of the sum of dioxins and DL-PCBs found in complementary feed and in more detail complementary cattle feed is around 0.29 ng TEQ/kg (see Table 3.24 and Figure 3.20 A and B). The EFSA levels are almost two times higher as the Dutch data and only median values are comparable with the one's of the Netherlands. Again, the bandwidth of the dioxin and DL-PCB concentrations is higher in the EFSA data by having 5 to 6 times higher Q(.95) and Q(.99) values.

Figure 3.20 C and D shows that average dioxin and DL-PCB levels in complementary (cattle) feed changed significantly during the last decade, with lower concentrations since 2007 (p=0.01, Mann Kendall). This decrease of around 8% is linked to the reduction of dioxin levels only and has to be treated with caution, due to the change of the reporting limits in 2007 as described before (section dioxins). Likely levels stayed constant during the years and the trend found is fake. Further no or only a low number of samples were measured with GC-HRMS in 2009 and 2010, making it difficult to draw conclusions on recent levels.



S=-19, Signific.: p=0.01
Limit (ML) = 1.5 ng TEQ/kg

S=-15, Signific.: p=0.01
Limit (ML) = 1.5 ng TEQ/kg

Figure 3.20. Dioxins and DL-PCBs [ub] in complementary feed and specified in complementary cattle feed.
(A) + (B) frequency distribution curve using TEF_{WHO98} values.
(C) + (D) trends in the average dioxin and DL-PCB concentrations per year.

Monitoring of cattle feed stays important as dioxin or DL-PCBs may migrate into dairy products or beef. However, the current amount sampled seems to be sufficient.

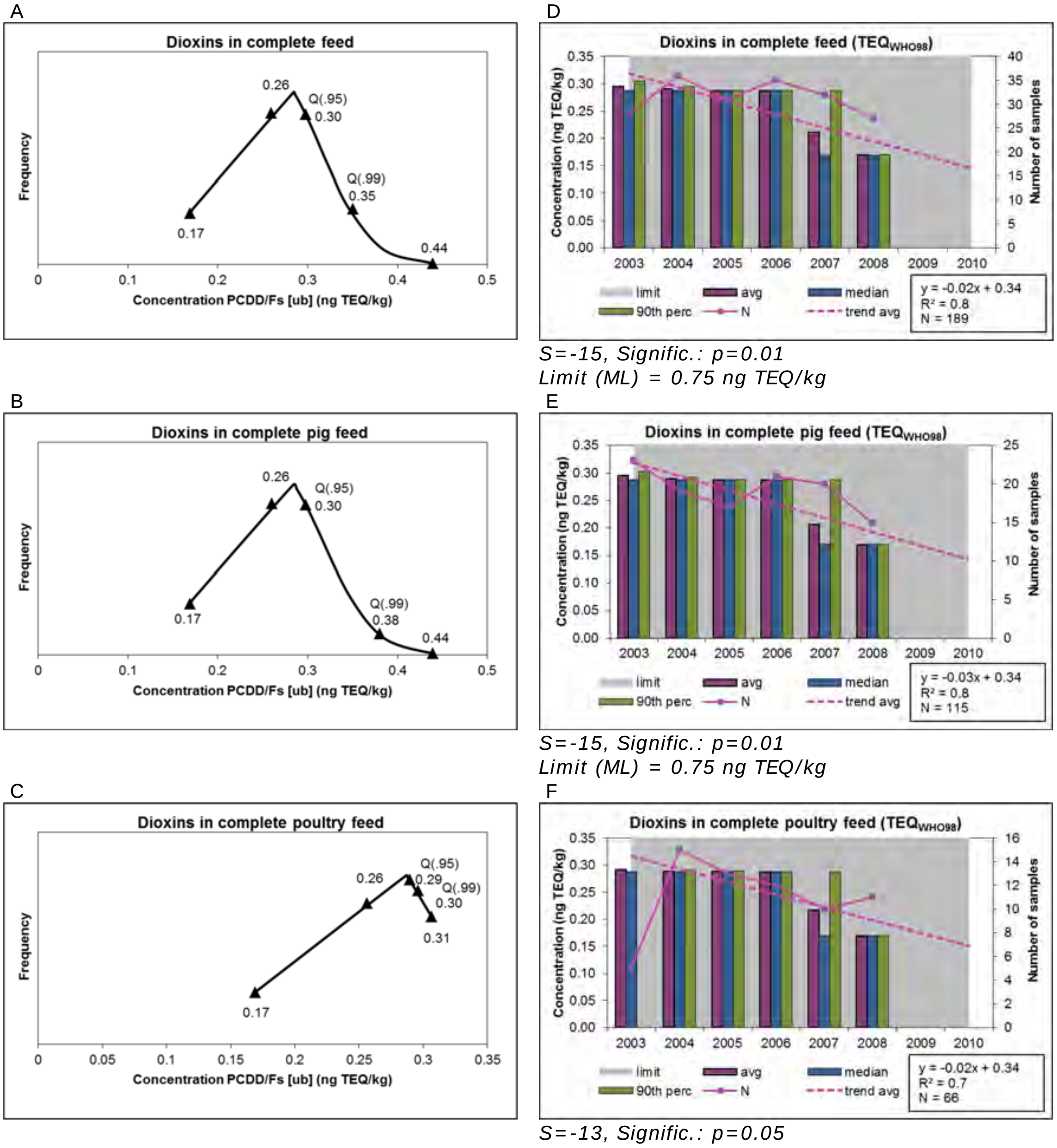
Levels and trends in complete feed

Dioxins

The graphs of complete feed (N=190) and in more detail complete pig feed (N=115) and poultry feed (N=67) are similar (see Figure 3.21 A till F).

As shown in Table 3.24 and Figure 3.21 A till C, the average dioxin concentrations found in complete feed and in more detail complete pig and poultry feed are generally within the same range when comparing the Dutch data vs. EFSA, staying between 0.22 and 0.26 ng TEQ/kg. In the EFSA data the bandwidth of the dioxin concentrations is higher by having 2 to 3fold higher Q(.95) and Q(.99) values.

Figure 3.21 D shows a slight decrease of the average dioxin levels in complete feed, with a significance of p=0.01 (Mann Kendall). This change of 6-9% can be also seen in complete pig and poultry feed with a significance of p=0.01 and p=0.05, respectively (see Figure 3.21 E and F). This similarity can be explained as pig and poultry feed form the major part of samples collected within the group of complete feed and the background levels seem to be within the same range in both groups. Again, the concentrations appears to be slightly lower starting from 2007 and the lower levels found are likely linked to the reduction of the reporting limits. Presumably, the trend found is fake and dioxin concentrations stayed around the same level from 2003 till 2010.



S=-13, Signific.: p=0.05
Limit (ML) = 0.75 ng TEQ/kg

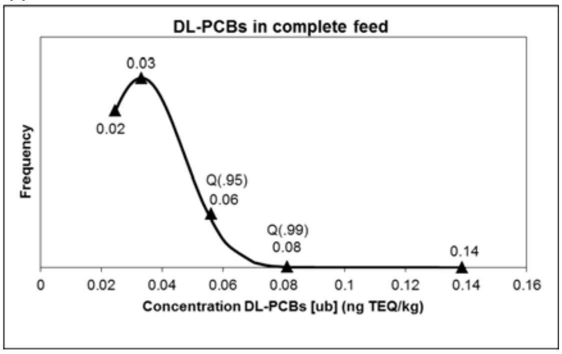
Figure 3.21. Dioxins [ub] in complete feed and specified in complete pig and poultry feed.
(A) + (B) + (C) frequency distribution curve using TEF_{WHO98} values.
(D) + (E) + (F) trends in the average dioxin concentrations per year.

Dioxin-like PCBs

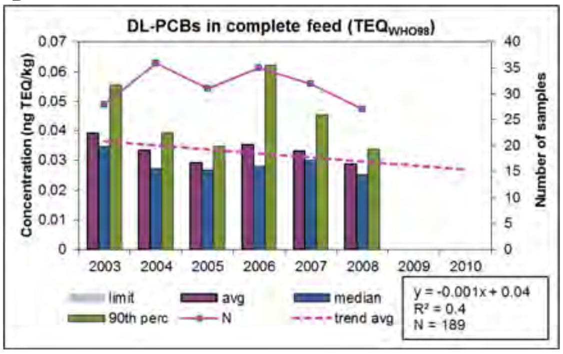
The average DL-PCB levels found in complete feed and in more detail complete pig and poultry feed are around the same level, being around 0.04 ng TEQ/kg (see Table 3.24 and Figure 3.22 A till C). This is 5 to 8fold lower compared to the levels found by EFSA. Also the Q(.95) and Q(.99) values are a lot higher compared to the Dutch data.

Figure 3.22 D till F show that no significant change of the average DL-PCB levels can be found from 2003 till 2010. Average DL-PCB concentrations seem to stay around the same level as well as median values and only and the 90th percentile values fluctuate to a low extend during the years.

A

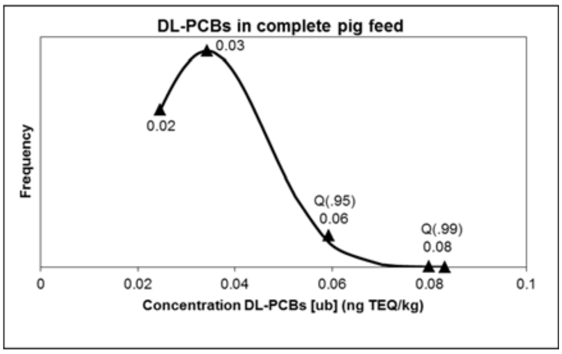


D

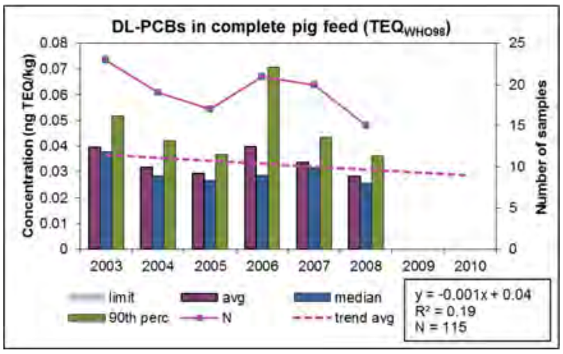


S=-9, Signific.: none
Limit (ML) = n.a.

B

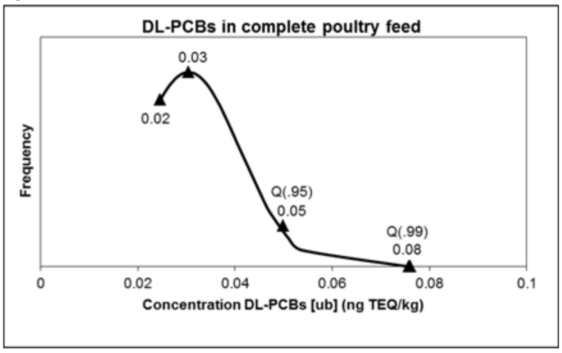


E

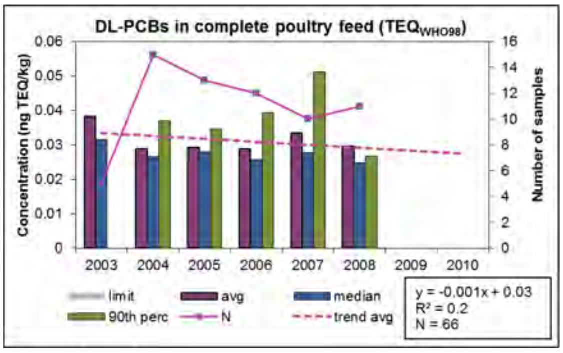


S=-5, Signific.: none
Limit (ML) = n.a.

C



F



S=-1, Signific.: none
Limit (ML) = n.a.

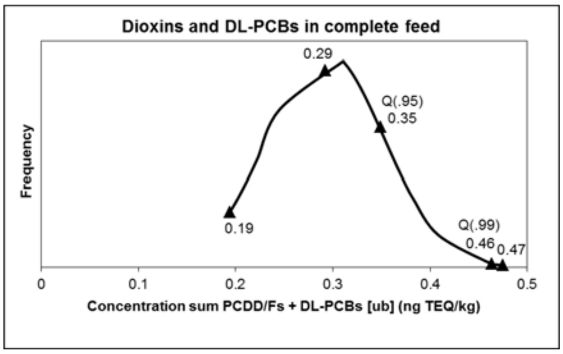
Figure 3.22. DL-PCBs [ub] in complete feed and specified in complete pig and poultry feed.
(A) + (B) + (C) frequency distribution curve using TEF_{WHO98} values.
(D) + (E) + (F) trends in the average DL-PCB concentrations per year.

Sum of dioxins and DL-PCBs

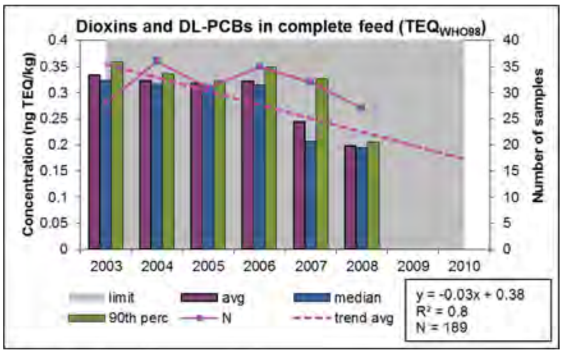
The average content of the sum of dioxins and DL-PCBs found in complete feed and in more detail complete pig and poultry feed is around 0.28 ng TEQ/kg (see Table 3.24 and Figure 3.23 A till C). The EFSA levels are almost two times higher as the Dutch data and only median values are comparable with the one's of the Netherlands. Again, the bandwidth of the dioxin and DL-PCB concentrations is higher in the EFSA data by having 7 to 11 times higher Q(.95) and Q(.99) values.

Figure 3.23 D till F shows that average dioxin and DL-PCB levels in complete (pig/poultry) feed changed significantly during the last decade, with lower concentrations since 2007 ($p=0.05$, Mann Kendall). This decrease of 5-8% is linked to the reduction of dioxin levels only and has to be treated with caution, due to the change of the reporting limits in 2007 as described before (section dioxins). Likely levels stayed constant during the years and the trend found is fake. Further no samples were measured with GC-HRMS in 2009 and 2010, making it difficult to draw conclusions on recent levels.

A

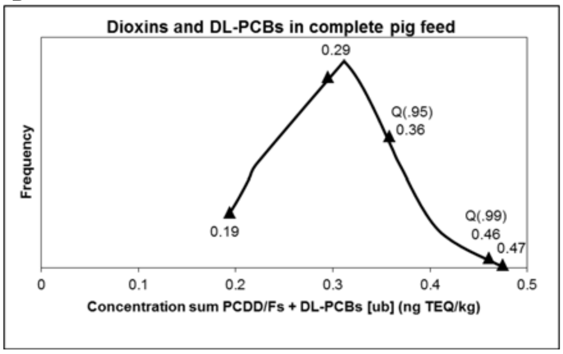


D

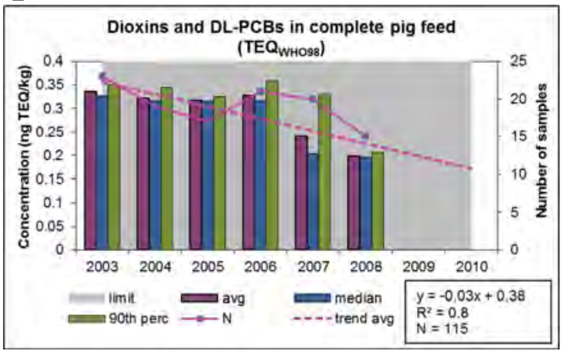


S=-13, Signific.: p=0.05
Limit (ML) = 1.5 ng TEQ/kg

B

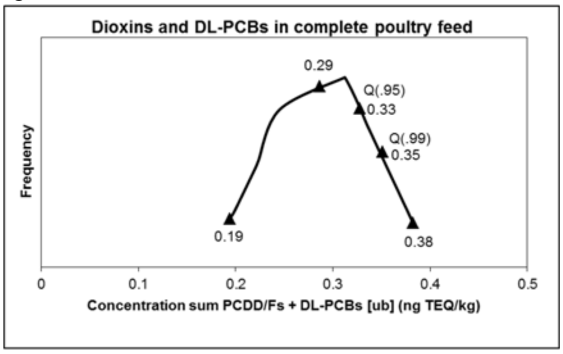


E

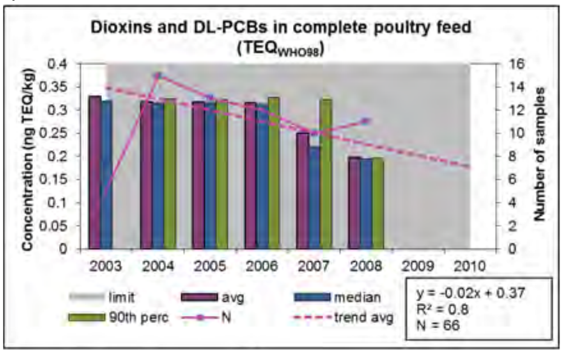


S=-11, Signific.: p=0.05
Limit (ML) = 1.5 ng TEQ/kg

C



F



S=-13, Signific.: p=0.05
Limit (ML) = 1.5 ng TEQ/kg

Figure 3.23. Dioxins and DL-PCBs [ub] in complete feed and specified in complete pig and poultry feed.
(A) + (B) + (C) frequency distribution curve using TEF_{WHO98} values.
(D) + (E) + (F) trends in the average dioxin and DL-PCB concentrations per year.

No or only a low amount of complete feed samples were collected in 2009 and 2010 (N=4, including DR CALUX® measurements). This is alarming, because if high dioxin and DL-PCB concentrations are found in complete feed, levels in the animal (end)product are as well easily above the regulatory limits. In general, a higher risk for the feed and food safety exists in complete feed compared to complementary feed as the animal diet is almost entirely based on that product. Complete feed is therefore advised to be monitored on a regular basis as part of the NCP.

Although, no exceedance of the regulatory limits was found for pig feed, this feed material might form a potential risk. One example is the gelatine incident found in the past with contaminated animal fat used in pig feed. The animal fat, derived from the production of gelatine, was contaminated by the use of dioxin-containing HCl for treatment of the pig bones (Hoogenboom et al., 2007). In 2010 and 2011 no samples were taken from complete pig feed. Such as with complementary cattle feed, it is advised to also collect a sufficient amount of samples from complete pig feed per year.

In general, dioxins and DL-PCB concentrations in poultry feed were found far below the regulatory limit (1.5 ng TEQ/kg). This means that if high dioxin levels are found in eggs and/or chicken meat, this is most likely linked to a contamination of the soil (esp. in free ranging chicken) and the amount of soil ingested by the chicken. In a study of Van Eijkeren *et al*. (2006), chicken are described to absorb 40 to 60 of dioxins and DL-PCBs from soil as compared to around 90 from feed. Therefore, it is important to also monitor poultry end products on a regular basis.

3.6.2 Levels and trends in feed for fur animals, pets and fish

Feed for fur animals, pet and fish food are considered to be complete feed only; therefore, no differentiation is made in various constituents. The majority of samples within this group are collected as pet food (N=32) and additionally only a low amount was measured as fish food (N=12). Generally, the amount of samples taken was low, but as the period of time was shorter (2004-2008 instead of 2003- 2010), meaning that the number of samples collected per year was sufficient, a trend analysis could be carried out. All results are also compared to the study by EFSA (2010) consisting of data from various EU member states.

Dioxins

As shown in Table 3.26 and Figure 3.24 A, the average dioxin concentrations found in feed for fur animals, pets and fish are around 0.29 ng TEQ/kg. The EFSA levels are approximately two times higher as the Dutch data and also the bandwidth of the dioxin concentrations is higher in the EFSA data by having more than twice as high Q(.95) and Q(.99) values.

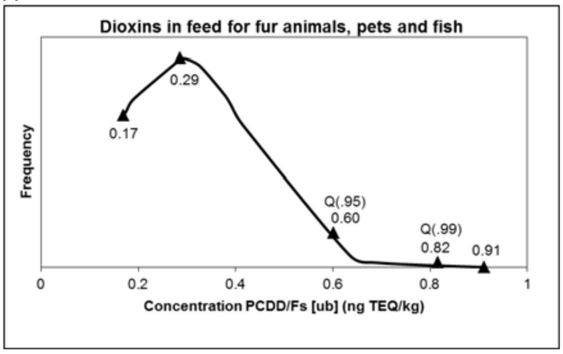
Figure 3.24 B shows that average dioxin levels changed significantly from 2004 till 2008 ($p=0.05$, Mann Kendall). However, this decrease of around 10% should be treated with caution, as it might be linked to the reduction of the reporting limits in 2007. Further, no or only a low number of samples were measured with GC-HRMS in 2009 till 2011, making it difficult to draw conclusions on recent levels.

Table 3.26. Dioxin and DL-PCB concentrations (in ng TEQ/kg) found in feed for fur animals, pets and fish from 2001 till 2011 in terms of average (avg), median (Q(.50)), 95th percentile (Q(.95)) and 99th percentile (Q(.99)); using TEQ_{WHO98} [ub].

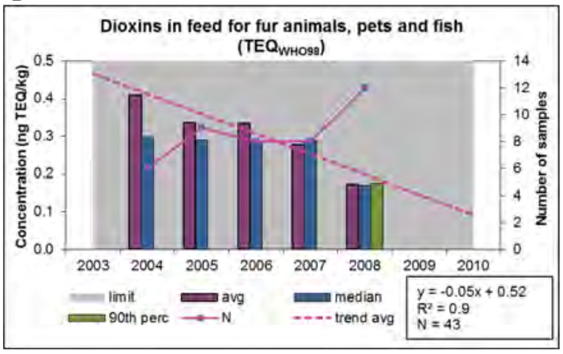
	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
AL[1]	1.75	3.5	
ML[1]	2.25		7
Feed for fur animals, pets and fish (N=44)			
avg	0.29	0.21	0.49
Q(.50)	0.29	0.04	0.32
Q(.95)	0.60	1.01	1.61
Q(.99)	0.82	1.29	2.11
EFSA[2] - feed for fur animals, pets and fish (N=143)			
avg	0.65	1.46	2.11
Q(.50)	0.53	1.34	1.95
Q(.95)	1.57	2.93	4.31
Q(.99)	1.94	4.75	6.89

1 Source: Commission Directive 2006/13/EC.
2 Source: (EFSA, 2010).

A



B



S=-10, Signific.: p=0.05
Limit (ML) = 0.75 ng TEQ/kg

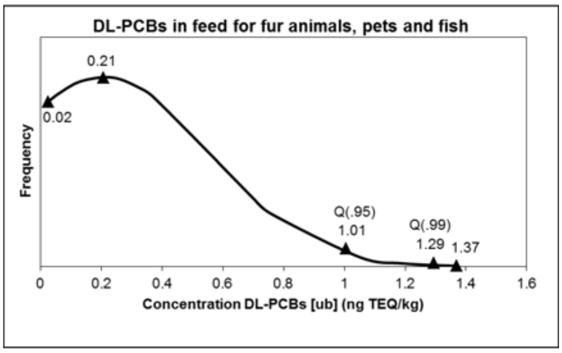
Figure 3.24. Dioxins [ub] in feed for fur animals, pets and fish.
(A) frequency distribution curve using TEF_{WHO98} values.
(B) trends in the average dioxin concentrations per year.

Dioxin-like PCBs

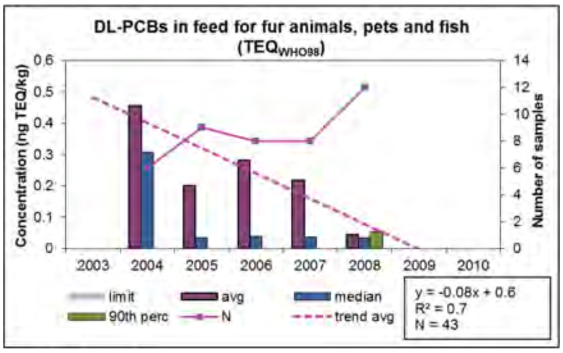
The average DL-PCB levels found in feed for fur animals, pets and fish are around 0.21 ng TEQ/kg (see Table 3.26 and Figure 3.25 A). This is around 80% lower than the levels found by EFSA. Also the bandwidth of the DL-PCB concentrations is higher in the EFSA data by having 2 to 3 times higher Q(.95) and Q(.99) values.

Figure 3.25 B shows that no significant change of the average DL-PCB levels can be found from 2004 till 2008, although the levels seem to be a lot lower in 2008 compared to 2004. Also the median levels are lower starting from 2005. In general, the time period is too short to draw conclusions on the overall tendency and no information is available on recent levels.

A



B



S=-6, Signific.: none
Limit (ML) = n.a.

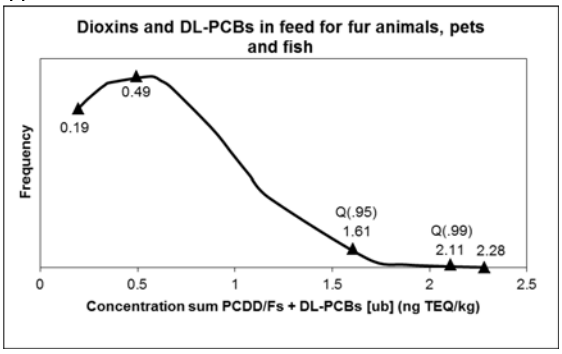
Figure 3.25. DL-PCBs [ub] in feed for fur animals, pets and fish.
(A) frequency distribution curve using TEF$_{WHO98}$ values.
(B) trends in the average DL-PCB concentrations per year.

Sum of dioxins and DL-PCBs

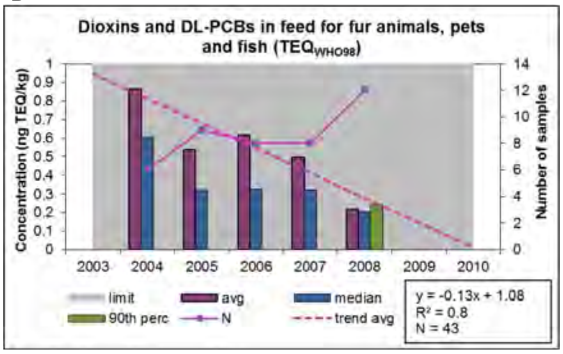
The average content of the sum of dioxins and DL-PCBs in feed for fur animals, pets and fish is around 0.49 ng TEQ/kg (see Table 3.26 and Figure 3.26 A). The EFSA levels are again around 70% higher compared to the Dutch data and also the median values as well as Q(.95) and Q(.99) are at least 2fold higher in the EFSA dataset.

Figure 3.26 B shows that average dioxin and DL-PCB levels changed significantly from 2004 till 2008 (p=0.1, Mann Kendall). This decrease of around 12% (slope m=-0.13) is likely linked to a reduction of both dioxin and DL-PCB levels, but the outcome of the trend should be treated with caution, as it might be linked to the reduction of the reporting limits in 2007 and no information is available on recent levels. Further, the time period is quite short to draw conclusions on the overall tendency.

A



B



S=-8, Signific.: p=0.1
Limit (ML) = 1.5 ng TEQ/kg

Figure 3.26. Dioxins and DL-PCBs [ub] in feed for fur animals, pets and fish.
(A) frequency distribution curve using TEF$_{WHO98}$ values.
(B) trends in the average dioxin and DL-PCB concentrations per year.

The difference found between the EFSA and the Dutch data could be related to a difference in the feed samples tested. If more fish food samples were included in the EFSA dataset, than presumably higher values are found, leading to higher average background levels and a higher bandwidth. In feed for fur animals, pets and fish no ALs and MLs were exceeded and the concentrations found are in general low. Looking into levels and trends in pet food forms also more an issue for animal health and welfare than for feed and food safety. The amount of samples collected recently (2011) is therefore considered to be sufficient. This is also true for fish food.

Summary - Levels and trends in compound feedingstuffs (1)

Compound feed , excl. fur animals, pets and fish

- Compound feed can be divided into complete and complementary feed. Complete feed needs more attention as non-compliant levels found in these feed product form a higher food safety risk for the animal (end)product. This feed product is therefore advised to be monitored on a regular basis as part of the NCP.
- When comparing the dioxin and DL-PCB levels of complementary and complete feed and the three feed subgroups complementary cattle feed and complete pig and poultry feed, the concentrations found are overall within the same range. No major differences can be observed.
- **Complementary feed**
  - <u>Dioxins:</u> average dioxin concentrations in complementary (cattle) feed are within the same range as the EFSA data. In complementary feed no significant change of the average dioxin and DL-PCB levels was found.
  - <u>DL-PCBs:</u> average DL-PCB concentrations are to a quite high extend lower compared to the EFSA data (5-8fold). No significant trend can be seen and also median and 90th percentile values stay around the same level.
  - <u>Sum dioxins and DL-PCBs:</u> The average dioxin and DL-PCB concentrations found by EFSA are almost twice as high compared to the Dutch data and the levels found in the Q(.95) and Q(.99) are even higher. Only the median values stay within the same range compared to the Netherlands. No significant change of the average dioxin and DL-PCB levels was found.
  - Monitoring of cattle feed remains important, but the amount of samples taken per year is sufficient.
- **Complete feed**
  - <u>Dioxins:</u> average dioxin concentrations in complete (pig/poultry) feed are within the same range as the EFSA data. In complete feed no significant change of the average dioxin and DL-PCB levels was found.
  - <u>DL-PCBs:</u> average DL-PCB concentrations are to a quite high extend lower compared to the EFSA data (5-8fold). No significant trend can be seen and the 90th percentile fluctuates during the years.
  - <u>Sum dioxins and DL-PCBs:</u> The average dioxin and DL-PCB concentrations found by EFSA are almost twice as high compared to the Dutch data and the levels found in the Q(.95) and Q(.99) are even higher. Only the median values stay within the same range compared to the Netherlands. No significant change of the average dioxin and DL-PCB levels was found.
  - In pig feed the number of samples tested decreased over the years. It is advised to collect a sufficient amount of samples per year, comparable with the number of e.g. complementary cattle feed.
  - The dioxin and DL-PCB concentration in poultry feed is low. If high dioxin levels are found in eggs and/or chicken meat, this is most likely linked to a contamination of the soil and maybe the wood chips used for bedding. Monitoring of end products is therefore important as well.

Summary - Levels and trends in compound feedingstuffs (2)

- Only a low number of samples were collected and measured with GC-HRMS in 2009 till 2011, making it difficult to draw conclusions on recent levels.
- Feed from residual food streams is already monitored in food processing plants according to EU specifications and only forms a risk when further processed for feed use.

Feed for fur animals, pets and fish

- Dioxins: average dioxin concentrations in feed for fur animals, pets and fish are 50% of the levels found by EFSA and also the Q(.95) and Q(.99) is more than twice as low. A decreasing trend of around 10% is visible, but due to the low number of samples available and the short time frame (2004-2008) the outcome of the trend analysis has to be treated with caution.
- DL-PCBs: average DL-PCB concentrations are to a quite high extend lower compared to the EFSA data (80%). No significant trend can be seen.
- Sum dioxins and DL-PCBs: The average dioxin and DL-PCB concentrations found by EFSA are around 70% higher compared to the Dutch data and also the median values as well as Q(.95) and Q(.99) are at least twice as high. Average dioxin and DL-PCB levels tend to decrease significantly with around 12%, linked to a reduction of both dioxin and DL-PCB levels. However, due to the low number of samples available having relatively low concentrations and the short time frame (2004-2008) the outcome of the trend analysis has to be treated with caution.

4 Discussion and conclusions

Levels and comparison with the legal limits

A major conclusion is that in most feed materials investigated in the Netherlands similar or lower average background concentrations for dioxins and DL-PCBs can be seen compared to the data found by EFSA (2010), which used the data from different Member States. Lower levels can especially be found for the 95th percentile (Q(.95)) as shown in Table 4.1 which compares the Q(.95) found by EFSA with those calculated in the present study based on the EU-monitoring samples and using TEF_{WHO05} values. The regulatory limits (MLs), which were established end of 2011 and came into force beginning of 2012, are based on this EFSA study. Regulatory limits for dioxins and the sum of dioxins and DL-PCBs are generally set by using the 95th percentile (Q(.95)) as ML. The ALs are set at about 2/3 of the ML. The differences between EFSA and our data imply that often the MLs and as a consequence also the ALs appear to be very conservative in relation to the feed materials of the Dutch feed supply chain. In fact, for most materials the ML for the sum of dioxins and DL-PCBs could be set at the ML for dioxins only, thus eliminating the need for two MLs per feed material without allowing higher levels of dioxins. An exception is fish meal and fish oil.

*Table 4.1. Dioxin and DL-PCB concentrations (in ng TEQ/kg) of all feed materials in terms of **95th percentile Q(.95)** for TEQ_{WHO05} [ub], calculated on the basis of EU monitoring (EU) data from 2001 till 2011.*

	N	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
Feed materials of plant origin				
Feed materials of plant origin, excl. vegetable oils				
AL[1]		0.5	0.35	
ML[1]		0.75		1.25
EFSA[2]	378	0.46	0.27	0.69
Total NL	188	0.29	0.13	0.41
Cereal grains and their by-products	32	0.27	0.05	0.31
Forages and roughage	92	0.33	0.15	0.45
Oil seeds/fruits and their by-products	33	0.27	0.06	0.32
Other feed materials of plant origin	31	0.27	0.05	0.31
Vegetable oils and their by-products				
AL[1]		0.5	0.5	
ML[1]		0.75		1.5
EFSA[2]	68	0.56	0.40	0.85
Total NL	17	0.33	0.25	0.52
Feed materials of animal origin				
Animal fat				
AL[1]		0.75	0.75	
ML[1]		1.5		2
EFSA[2]	37	1.57	0.71	1.94
Total NL	21	0.47	0.88	1.33

	N	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
Other land animal products				
AL[1]		0.5	0.35	
ML[1]		0.75		1.25
EFSA[2]	31	0.78	0.27	0.87
Total NL	11	0.28	0.22	0.50
Fish meal				
AL[1]		0.75	2	
ML[1]		1.25		4
EFSA[2]	128	0.80	2.22	2.97
Total NL	124	0.65	1.23	1.89
Fish oil				
AL[1]		4	11	
ML[1]		5		20
EFSA[2]	89	5.03	13.69	19.10
Total NL	13	3.07	15.03	17.87
Feed additives, minerals and pre-mixtures				
Feed additives				
AL[1]		0.5	0.35	
ML[1]		1		1.5
EFSA[2] - additives compounds of trace elements	79	0.69	0.22	0.76
Total NL - feed additives	161	0.64	0.05	0.70
Binders, anti-caking agents and coagulants	123	0.66	0.05	0.71
Feed materials of mineral origin				
AL[1]		0.5	0.35	
ML[1]		1		1.5
EFSA[2]	114	0.27	0.34	0.58
Total NL	28	0.27	0.04	0.31
Pre-mixtures				
AL[1]		0.5	0.35	
ML[1]		1		1.5
EFSA[2]	91	0.28	0.12	0.31
Total NL	37	0.31	0.04	0.34

	N	PCDD/Fs	DL-PCBs	sum PCDDF/s + DL-PCBs
Compound feedingstuffs				
Compound feed excl. fur animals, pets and fish				
AL[1]		0.5	0.5	
ML[1]		0.75		1.5
EFSA[2]	482	0.58	1.50	1.95
Total NL	337	0.28	0.07	0.35
Complementary feed	147	0.29	0.06	0.35
Complete feed	190	0.28	0.07	0.34
Feed for fur animals, pets and fish				
AL[1]		1.25	2.5	
ML[1]		1.75		5.5
EFSA[2]	143	1.31	2.48	3.72
Total NL	44	0.52	1.36	1.87

[1] *Source: Commission Regulation 2012/277/EU.*
[2] *Source: (EFSA, 2010).*

EFSA states in their report that a general caution is in place as some of the results reported and used for the estimation of the average background levels might have originated from specific incidents. This might explain the differences found between the Dutch and EFSA average dioxin and DL-PCB concentrations and is strengthened by the fact that differences in the median (Q.(50)) seem much smaller. Differences occur in particular in the average and 95th or 99th percentile. In some feed materials, there are even lower Q(.50) EFSA values compared to the Dutch data, i.e. the Q(.50) of the sum of dioxins and DL-PCBs in feed materials of plant origin (excl. vegetable oil) calculated by EFSA is around 2-fold lower in comparison to the Dutch values of all feed subgroups within this category. Other examples are compound feed (excl. fur animals, pets and fish) and feed materials of mineral origin. This also points to the inclusion of higher contaminated samples that may be related to incidents.

The Q(.95) of the dioxin and DL-PCB concentrations found in vegetable oils, fish meal, animal fat and other land animal products are around 40% lower than the levels found in the data of EFSA (see Table 4.1). In feed materials of plant origin, excluding vegetable oils and forages and roughage as well as feed materials of mineral origin and feed for fur animals, pets and fish there is even a higher discrepancy and levels are more than 50% lower. The highest difference, with around 80%, can be seen in compound feed (excl. fur animals, pets and fish). Only in feed additives, pre-mixtures and fish oil the Q(.95) is similar in the Dutch data. The question arises if decision limits lower than the action levels should be used for early detection of potential incidents.

In the feed subgroup of forages and roughage slightly higher dioxin and DL-PCB concentrations can be seen compared to the other feed materials of plant origin, which are however still lower compared to the EFSA dataset.

Can we apply lower decision limits and for which feed materials?

The advantages of applying decision limits lower than the AL for certain feed materials is to detect dioxin/DL-PCB incidents quicker and at an early stage by following-up samples in which the levels are clearly different from the normal Dutch background levels. In this way, the impact of incidents can be reduced and also the exposure of the Dutch population to dioxins and DL-PCBs. When such decision limits are exceeded relatively frequent, potential sources can be identified. This may also be of advantage for companies as they can react quicker in case of an incident and or the occurrence of incidents might be prevented or reduced.

By using a combination of the DR CALUX® as screening method and the GC-HRMS as quantification in case of suspected CALUX samples, a higher quantity of samples can be analysed on the presence of dioxins and DL-PCBs. In this way, the chance of detecting samples exceeding the regulatory limits increases and, therefore, also the chance to tackle new or old contamination sources. Hereby, the so-called decision limits used for the DR CALUX® should not be set too high in order to exclude false-negatives, but also to identify samples different from the general Dutch background levels. In practice this could be done by using decision limits, which are more based on the Dutch data as investigated in this study. In practice the decision limits used with DR CALUX® are already rather conservative and it might be worthwhile to focus on the so-called false-positive results first.

Lower decision limits might be used for dioxins in feed materials of plant origin, animal fat and other land animal products, fish oil and feed materials of mineral origin. For vegetable oils, and compound feedingstuffs the decision limits for both dioxins and DL-PCBs may be reduced. The decision limits for DL-PCBs could be lowered in fish meal and clay minerals. Table 4.2 gives an overview of "new" decision limits which could be applied based on the Q(.95) and the decision limits of the screening assay. In those cases where the existing AL is lower than the Q(.95) the proposed DL is equal to the AL. Furthermore a lowest level of 0.35 pg TEQ/g is used based on detection capabilities of both GC-HRMS and bioassays.

Screening assays like CALUX allow a much higher sample throughput due to lower costs and as such increase the chance of detecting non-compliant samples. However, this requires a simple clean-up and normally dioxins and DL-PCBs are not separated. In addition to decision limits for dioxins and DL-PCBs, a DL for the sum of dioxins and DL-PCBs is therefore suggested. This DL is in some cases based on the lowest AL as required by EU-legislation. In other cases an even lower DL might be applied, also based on the new proposed DLs. It is important that performance criteria for testing apply to MLs rather than ALs or alternative DLs.

Table 4.2. Overview of "new" decision limits (DL) for dioxins and DL-PCBs, based on the Q(.95) levels using TEF_{WHO05} [ub] and the detection limits.

Feed	PCDD/Fs		DL-PCBs		sum PCDD/Fs + DL-PCBs	Current DL
	AL[1]	DL	AL[1]	DL	DL CALUX	
Feed materials of plant origin						
Feed materials of plant origin, excl. vegetable oils	0.5	0.35	0.35	0.35	0.35	0.48
Vegetable oils and their by-products	0.5	0.35	0.5	0.35	0.35	0.35
Feed materials of animal origin						
Land animal (by-)products						
- *Animal fat*	0.75	0.5	0.75	0.75	0.63	0.63
- *Other land animal products*	0.5	0.35	0.35	0.35	0.35	0.48
Fish, other aquatic animals and their by-products						
- *Fish meal*	0.75	0.75	2	1.5	0.70	0.70
- *Fish oil*	4	3	11	11	3.10	3.10
Feed additives, minerals and pre-mixtures						
Feed additives	0.5	0.5	0.35	0.35	0.35	0.48
- *Binders, anti-caking agents and coagulants*	0.5	0.5	0.5	0.35	0.35	0.48
Feed materials of mineral origin	0.5	0.35	0.35	0.35	0.35	0.48
Pre-mixtures	0.5	0.35	0.35	0.35	0.35	0.48
Compound feedingstuffs						
Compound feed excl. fur animals, pets and fish	0.5	0.35	0.5	0.35	0.35	0.48
Feed for fur animals, pets and fish	1.25	0.75	2.5	1.5	0.70	0.70

[1] *AL = action levels as laid down in Commission Regulation 2012/277/EU.*

In most feed materials the non-compliance with the regulatory limits (MLs) was below 1% during the whole time period (2001 till 2011) based on the NCP data used in this study. An exception form the feed groups fish meal, clay minerals and vegetable oils. In addition, in animal fat, pre-mixtures and feed materials of plant origin (except vegetable oils) just above 1% of the samples were non-compliant with the ALs. This may support the findings that the decision limit for dioxins and DL-PCBs may be lowered in some feed materials to detect samples different from the average background levels.

Trends

In general, no or only a slight decreasing trend of dioxin and DL-PCB concentrations in feed was observed, with more or less significance. In certain feed materials a trend could not be investigated due to the low number of samples available, e.g. vegetable oils, animal fat and other land animal products, fish oil, feed materials of mineral origin and pre-mixtures.

Dioxins The slight decrease of dioxin levels observed in the feed subgroup forages and roughage, clay minerals as well as in the main feed group of compound feedingstuffs is probably biased, as it is linked to a reduction of the LOQs in 2007. In fish meal no significant change of the average dioxin levels can be found and in the main feed group of plant origin (excl. vegetable oils) and in feed additives dioxin background levels seem to stay around the same level with no significant change.

DL-PCBs In fish meal and feed for fur animals, pets and fish no significant change of the average DL-PCB levels can be observed and in feed of plant origin (excl. vegetable oils), in feed additives and compound feed (excl. fur animals, pets and fish) DL-PCB background levels seem to stay around the same level with no significant change.

Sum of dioxins and DL-PCBs Although in the feed subgroup forages and roughage and clay minerals as well as compound feed (excl. fur animals, pets and fish) average dioxin and DL-PCB levels seem to decrease significantly, this reduction can again be linked to lowering the LOQs of dioxins in 2007 and the trend is probably fake. Also in feed for fur animals, pets and fish total dioxin and DL-PCB concentrations seem to decrease significantly, but due to the low number of samples available having relatively low concentrations and the short time frame (2004-2008), the outcome of the trend analysis may not be representative. In fish meal no change can be found and in the main feed group of plant origin (excl. vegetable oils) and in feed additives the average dioxin and DL-PCB concentrations vary per year, but no significant trend can be seen.

Positive effects of the measures taken in the past might not be seen immediately but, because of the persistent nature of dioxins and DL-PCBs in the environment, only after several years. Maybe 8 years (2003-2010) is still too short to see significant changes in the levels. An important question is whether such measures would result in a decrease in the background levels measured in a small set of random samples or merely in the fraction of non-compliant samples. The latter clearly requires the testing of a larger amount of samples.

Uncertainties in trend analyses are described in detail in this report. One example is that looking into trends in values of low concentrations remains difficult and results found should be treated with caution. The limits of quantification (LOQ) per congener have decreased in the middle of 2007, which may result in a lowering of the upper bound level [ub]. In this way a fictive trend might be seen between the years 2001-2006 and 2007-2010 if most concentrations found are at or below the reporting limit. In some years also more or less samples of certain feed categories were collected, which may bias the result of the trend analysis. In addition, the Mann Kendall test cannot be used to predict no trend/change of the concentrations, thus if levels stay on a constant level. This means, that in some cases where no significance was found, still a significant "zero" trend line could be present.

During the previous trend analysis (Adamse *et al.*, 2007) the advice was given to use a fixed frequency for analysing certain feed subgroups or materials. The reason for not following this advice is unknown and it may have been related to the fact that the amount of feed samples monitored by the NVWA yearly via the NCP is limited. The conclusion of this trend analysis is that it is more important to focus on certain "at risk" feed materials and main importing countries as part of the NCP. However, the risk should not be linked to the import volume only, but also to incidents and elevated dioxin/DL-PCB concentrations found in the past.

The advice of the previous trend analysis (Adamse *et al.*, 2007) to estimate and report a level with DR CALUX® has not been followed up, probably because it is time consuming and, therefore, not cost-effective. The data are still available to allow such an estimation, e.g. in a special project. On the other hand it is also questionable if these values can be used at all for looking into background levels and/or trends as the DR CALUX® is a more qualitative approach and generally dioxin and DL-PCB concentrations are overestimated, especially for low levels. In addition, this screening method does not respond exclusively to the 17 dioxin and 12 DL-PCB congeners. One example is the high false positive rate found for choline chloride, which could be linked to brominated dioxins. These compounds do not have an assigned TEF-value and give a dioxin-like response in the assay, leading to the false positive result for dioxins and DL-PCBs.

Completeness of datasets

With often incomplete data provided by the NVWA on parameters such as country of origin, it is difficult to analyse on certain risk factors. Investigating risks related to certain regions and/or countries of origin is important for setting an efficient monitoring strategy. Only if we have sufficient knowledge on all factors involved in a higher contamination, samples can be taken targeted and more efficiently.

Even though a high amount of feed materials is imported (88% resp. 12.9 Mt. of a total amount of 14.6 Mt), a large number of samples tested (50.7, N=2277 of N_{total}=4490) have been reported to originate from the Netherlands. This is questionable as in some cases also feed materials, such as palm kernels, have been reported as being of Dutch origin, while these plant species are not cultivated in this region. These oil seeds are imported from other countries, but may be processed in the Netherlands. It needs to be differentiated whether the dioxin contamination comes from the raw materials or via the production processes. Basically, all production processes who form a risk should be known. Examples are the artificial drying of grass/alfalfa or mixing/extrusion of fat/oils. Stating the country of origin of the raw materials as well as the production site is, therefore, crucial and would help predicting risks related to certain origins.

Reporting the country of origin needs to remain a point of attention, also because in 35% of the samples collected (N=1589 of N_{total}=4490) the origin was not specified at all and in a lot of samples which exceeded the regulatory limit the country of origin was not stored in the database. In order to allow an assessment of potential countries with a higher risk, more focus should be put in future on reporting the country of origin correctly. This was already recommended by the previous trend analysis (Adamse *et al.*, 2007). Until now improvements can first be seen in 2010, where only nine sample origins were unknown. Before 2010, on average, 75 of 207 samples per year did not have a country of origin reported. Implementing the number of samples taken per country into the NCP is recommended, based on the quantity imported and compared to the domestic production.

The amount of samples collected per country was actually too low to investigate trends within the different countries of origin. This is again influenced by the fact that in 35% of the cases the country of origin is unknown and/or in some cases the Netherlands was stated as origin even if this was not likely.

Main conclusions

Within the feed group of feed materials of plant origin (excluding vegetable oils), dioxin and DL-PCB concentrations stay far below the regulatory limit and monitoring these feed materials can be continued as it is.

In vegetable oils and animal fats, incidents do occur on a regular basis (also again more recently in 2011) and it is advised to monitor this feed material regularly. The EU recently announced that all fats used for feed production should be analysed.

The number of samples taken for fish oil increased, but was still too low to look into trends. In principle, a downward trend might be expected due to the cleaning of fish oil prior to use. Overall, higher absolute dioxin and DL-PCB levels are found in this feed material, compared to all other commodities. However, the effects of non-compliance with dioxin or DL-PCB levels in fish oil seems questionable with respect to forming a feed or food safety risk. Fish oil only forms a small proportion of animal diets and compared to other feed materials, the amount used in the Netherlands is rather low.

Fish meal is used more often and forms a more substantial risk as samples taken repeatedly exceeded regulatory limits. Fish meal should stay a key part of the NCP.

Almost every year several samples of clay minerals exceeded the limit, linked to a contamination with dioxins only and not DL-PCBs. As also high levels were found in certain types of clay in the past, this feed material is advised to be measured on a regular basis, although the current number of samples taken does not necessarily need to be increased. Oppositely, less attention can be paid to choline chloride for which a lot of samples were taken during the past years with none exceeding the regulatory limit. The proportion used in feed is also rather low (up to 1%).

To investigate compound feedingstuffs instead of checking feed additives, minerals and pre-mixtures individually might be another approach, serving as a warning tool. If certain limits are exceeded, the cause of the high concentration should than be investigated into more detail. In this scenario, it has to be kept in mind that the batch/lot of compound feed will have been composed of feed materials originating from more than one company and/or country of origin. In addition, the current action and maximum limits might be too high, also regarding the fact that these regulatory limits may not guarantee that the animal derived food does not exceed the limit. The decision to focus on compound feed rather than ingredients also depends on the analytical capabilities of the methods used for screening and confirmation.

The number of samples collected for complete feed is recommended to be increased as no or only a low amount was taken in 2010 and 2011. Complete feed needs more attention than complementary feed as non-compliant levels found in these feed product form a higher food safety risk for the animal (end)product. When comparing the dioxin and DL-PCB background levels of complementary and complete feed no major differences can be observed and the concentrations found are overall within the same range.

Monitoring complementary cattle feed remains important, but the amount of samples taken per year is sufficient. However, the number of complete pig feed samples decreased over the years and it is advised to adjust the amount of samples taken to a comparable level as collected for e.g. complementary cattle feed. The dioxin and DL-PCB concentration in complete poultry feed is generally low and if high dioxin levels are found in eggs, this is most likely linked to a contamination of the soil, especially as in the past and also more recently too high dioxin levels were found in organic eggs, especially coming from free ranging chicken feeding in soil regularly. Monitoring end products seems to be more reasonable in the case of eggs, but not necessarily for chicken meat.

Feed from residual food streams is already monitored in food processing plants and may only form a risk if further processed for feed.

To arrive at a risk-based monitoring programme solely on the levels detected thus far can be tricky, as incidents can be rather unpredictable as shown e.g. by the incident with sugar beet pulp by the end of 2011. In these feed materials dioxin levels have not been exceeded for a long time and are in general very low. Detailed knowledge about production processes and sources of dioxins and DL-PCBs seems at least of equal importance.

As stated in the introduction, the outcome of this study has been used to identify commodities and countries of origin with higher risks. The results can help the NVWA to reach a more risk-based sampling in the National feed monitoring program. Several feed groups have been described in detail and it has been stated whether they should be sampled to a higher, the same or lesser extent than has been done in the previous years. In the next chapter some specific recommendations have been formulated.

5 Recommendations

5.1 Sampling strategy

5.1.1 General

For future research, but also for traceability reasons, it is recommended to always notify the country of origin, esp. if regulatory limits are exceeded. This could be also done as feedback at a later stage (e.g. after the sample has already been analysed and reported to the NVWA).

Enough samples should be collected per feed (sub)group per year and per country of origin.

Processes forming a risk have to be known well when deciding at which stage a sample has to be collected (e.g. during import or after processing).

5.1.2 Specific

For feed materials of plant origin (excl. vegetable oils), other land animal products, feed additives, minerals and pre-mixtures and compound feed (excl. fur animals, pets and fish), lower decision limits may be used for early detection of potential incidents. The decision limit used in the CALUX-assay is a level set by RIKILT, which is in principle based on the lowest AL for either dioxins or DL-PCBs in each food item. This is a rather conservative decision limit resulting in a relatively high fraction of false-positives. Decision limits used by GC-HRMS are based on the ALs and include a measurement uncertainty of about 10%. Lower decision limits may be used for GC-HRMS. For all the above mentioned feed materials decision limits could be lowered to e.g. 0.35 ng TEQ/kg, which is actually lower than the recommended AL for dioxins and/or DL-PCBs. In this way samples, which are different from the general background levels can be tackled quicker and at an earlier stage.

A representative amount of samples needs to be collected each year to obtain statistically significant results and to track down incidents. Based on this study, the following recommendations can be given.

The feed materials mentioned below should stay a key part of the NCP:

- vegetable oils
- animal fat
- fish meal
- complete feed

The amount of samples collected for the following feed materials is assumed to be sufficient:

- feed materials of plant origin, including vegetable oils
- animal fat
- fish meal and fish oil
- clay minerals
- feed materials of mineral origin
- complementary cattle feed

The amount of samples taken of the following feed group should increase:

- complete feed, especially pig feed

The amount of samples taken of the following feed materials may decrease:

- choline chloride
- premixtures

5.2 Trend analysis

Currently, the results of the DR CALUX® are only stored as suspected (V) or negative (N). Looking into the concentrations measured and how these values could be used or interpreted in future research might be of interest as a lot of samples were measured with the DR CALUX® only and were not quantified with the GC-HRMS. Another approach of looking into trends could be that if less suspected DR CALUX® are found over time, this could be a sign for a decrease in background levels.

Regularly also negative DR CALUX® samples are quantified using the GC-HRMS method. This is done for reliability reasons, but the data are not kept after verification in the laboratory system LIMS and are, therefore, not stored in the KAP databank. By actually storing the exact GC-HRMS value, e.g. in KAP, trends can be investigated more precisely also for low dioxin and DL-PCB concentrations. However, trends found in lower concentrations have to be taken with caution as the trend can be subject to measurement capabilities (upper bound [ub] vs. lower bound [lb] limit). At low concentrations certain congeners may not have been quantified and the reporting limit was used. As in the middle of 2007 reporting limits were lowered, a fictive decreasing trend might be seen between the years 2001-2006 and 2007-2010.

Instead of looking into outliers individually, a future approach could be to use the statistical tests Mann-Kendall and Sen's slope estimator. Trends do not need to be adjusted for outliers by using one or both of these tests when enough years are included in the dataset, 4 and 10 years, respectively. Other statistical analysis could also be used in future to estimate the approximate number of samples needed for a risk-based sampling approach.

5.3 New ideas

Starting from 2012 also new regulatory limits for non-dioxin like PCBs (NDL-PCBs) came into force. Looking into trends found on background levels for NDL-PCBs may be of advantage and help developing sampling strategies. Hereby, it has to be taken into account that the DR CALUX® bioassay cannot be used to screen for NDL-PCBs. However, at this stage these PCBs are determined in samples analysed already by GC-HRMS, so in principle DR CALUX® suspects or EU monitoring samples. It may be argued that samples with high NDL-PCB levels will also contain DL-PCBs and even dioxins, meaning that the bioassay will also detect such samples. This hypothesis may be tested using the database.

With specific statistical tests it is possible to get more insight into the minimum number of samples needed for certain feed groups. These amounts are related to the amount of feed available and the range and distribution of the dioxin concentrations found. It is recommended to perform this test in a future project to improve the effectiveness of the risk-based sampling.

There is a possibility that adulteration takes place with vegetable oils as the regulatory limits are higher for animal fat and fish oil. A research project looking into the authenticity of fat/oils may help to investigate if this is an issue. Next to declaring an incorrect feed compound, feed materials exceeding the ML may also simply be diluted via the use of compound feed, although this is against the European food law. These adulteration issues may need to be discussed in future and could be investigated via other research projects.

A future approach could be also to epidemically measure the dioxin content in blood samples of humans for trend analysis. It is, however, difficult to link the results found to food and/or feed materials and only the general risk and exposure of humans to dioxins and DL PCBs can then be investigated.

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Regulations

Council Regulation 999/2001/EC of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies; latest amended by Commission Regulation 189/2011/EU of 25 February 2011

Council Directive 2002/32/EC of 7 May 2002 on undesirable substances in animal feed

Council Regulation 1831/2003/EC of 22 September 2003 on additives for use in animal nutrition

Commission Regulation 892/2010/EU of 8 October 2009 on the status of certain products with regard to feed additives within the scope of Council Regulation 1831/2003/EC

Council Regulation 767/2009/EC of 13 July 2009 on the placing on the market and use of feed

Commission Directive 2006/13/EC of 3 February 2006 on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs, amending Annexes I and II to Council Directive 2002/32/EC

Commission Recommendation 2006/88/EC of 6 February 2006 on the reduction of the presence of dioxins
Commission Regulation 152/2009/EC of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

Annex I
Overview sampling strategy on dioxins and dioxin-like PCBs of NVWA, according to the Dutch National Control Plan for animal feed, 2006-2011

Year	Feed category	Location	Samples (N)
2011	Pre-mixtures	Pre-mixture & compound feed companies	100
	Feed additives & minerals	Pre-mixture & compound feed companies Import	100
	Fats/oils of both, animal and non-animal origin	Compound feed companies Fat processing companies	100
	Dried feed materials	Food companies	200
	Project Asia	Various	50
			550
2010	**Clay minerals**	Pre-mixture & compound feed companies	50
	Choline chloride	Pre-mixture & compound feed companies Harbour	25 25
	Trace elements (esp. manganese, copper or zinc oxide and copper chelate)	Pre-mixture companies Import	50
	Feed additives and minerals	Pre-mixture & compound feed companies	100
	Fish meal & oil	Compound feed companies Fat processing companies	50
	Fats/oils of both, animal and non-animal origin	Compound feed companies Fat processing companies	100
	Potato waste products	Food companies	25
	Project Asia	Various	50
			475
2009	Commission Recommendation 2004/704/EC	Various, according to recommendation	96
	Clay minerals	Pre-mixture & compound feed companies	50
	Choline chloride	Pre-mixture & compound feed companies Harbour	25 25
	Trace elements (esp. manganese, copper or zinc oxide and copper chelate)	Pre-mixture companies Import	50
	Feed additives and minerals	Pre-mixture & compound feed companies	100
	Fish meal & oil	Where possible	50

Year	Feed category	Location	Samples (N)
2009	**Fats/oils of both, animal and non-animal origin**	Compound feed companies Fat processing companies	100
	Potato waste products	Food companies	25
	Compound feed	Compound feed companies	25
2008	Commission Recommendation 2004/704/EC	Various, according to recommendation	111
	Clay minerals	Pre-mixture & compound feed companies	50
	Minerals, trace elements & choline chloride	Pre-mixture & compound feed companies	40
	Choline chloride	Harbour	10
	Fish meal & oil	Where possible	50
	Fats/oils of both, animal and non-animal origin	Compound feed companies Fat processing companies	50
	Project Residual streams	According to project description	50
	Project Environment: grass (silage)	According to project description	25
			386
2007	Commission Recommendation 2004/704/EC	Various, according to recommendation	111
	Clay minerals	Pre-mixture & compound feed companies	50
	Minerals, trace elements & choline chloride	Pre-mixture & compound feed companies	50
	Fish meal & oil	Where possible	40
	Dried products: grass, alfalfa, bread crumbs	Compound feed companies Drying companies	25
	Project Residual streams	According to project description	50
	ALOM project **animal fat**	According to project description	50
			376
2006	Commission Recommendation 2004/704/EC	Various, according to recommendation	111
	Clay minerals	Pre-mixture & compound feed companies	50
	Minerals, trace elements & choline chloride	Pre-mixture & compound feed companies	50
	Dried products: grass, alfalfa, bread crumbs	Compound feed companies Drying companies	50
	Project Residual streams	According to project description	100
	ALOM project **animal fat**	According to project description	50
			411

Bold: *yearly recurrent feed categories.*
Red: *deviant feed categories, which are not monitored on a regular basis and have been included in the list due to the incidents found in the past (as stated in Table 1.1).*

Annex II
TEQ concept

In general dioxins and dioxin-like PCBs contain mixtures of different PCDD (polychlorinated dibenzo-p-dioxin), PCDF (polychlorinated dibenzofuran) and PCB (polychlorinated biphenyl) congeners. For the purpose of risk assessment and to facilitate regulatory controls, the concept of toxic equivalency (TEQ) was developed to describe the cumulative toxicity of complex mixtures of these compounds (Ahlborg *et al.*, 1992). The relative toxicity of PCDD, PCDF and PCB congeners is compared to the most toxic substance 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), which is considered the reference congener; and a toxic equivalency factors (TEFs) is assigned. A TEQ is calculated by multiplying the actual grams weight of each dioxin and dioxin-like compound by its corresponding TEF and then summing up the results. The overview of TEF values as established by the WHO in 1998 are laid down in Directive 2006/13/EC and is shown below. The new proposed regulatory limits are based on the TEF values as established by the WHO in 2005. Differences in the TEF values are highlighted in bold.

Table 6.1. Overview WHO TEFs 1998 and 2005.

Dioxins (PCDD/Fs)	TEF WHO 1998	TEF WHO 2005	**Dioxin-like PCBs (DL-PCBs)**	TEF WHO 1998	TEF WHO 2005
chlorinated dibenzo-p-dioxins (PCDDs)			**non-ortho substituted PCBs (NO PCBs)**		
2,3,7,8-TCDD	1	1	PCB 77	0.0001	0.0001
1,2,3,7,8-PeCDD	1	1	PCB 81	0.0001	**0.0003**
1,2,3,4,7,8-HxCDD	0.1	0.1	PCB 126	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1	PCB 169	0.01	**0.03**
1,2,3,7,8,9-HxCDD	0.1	0.1			
1,2,3,4,6,7,8-HpCDD	0.01	0.01			
OCDD	0.0001	**0.0003**			
chlorinated dibenzofurans (PCDFs)			**mono-ortho substituted PCBs (MO PCBs)**		
2,3,7,8-TCDF	0.1	0.1	PCB 105	0.0001	**0.00003**
1,2,3,7,8-PeCDF	0.05	**0.03**	PCB 114	0.0005	**0.00003**
2,3,4,7,8-PeCDF	0.5	**0.3**	PCB 118	0.0001	**0.00003**
1,2,3,4,7,8-HxCDF	0.1	0.1	PCB 123	0.0001	**0.00003**
1,2,3,6,7,8-HxCDF	0.1	0.1	PCB 156	0.0005	**0.00003**
1,2,3,7,8,9-HxCDF	0.1	0.1	PCB 157	0.0005	**0.00003**
2,3,4,6,7,8-HxCDF	0.1	0.1	PCB 167	0.00001	**0.00003**
1,2,3,4,6,7,8-HpCDF	0.01	0.01	PCB 189	0.0001	**0.00003**
1,2,3,4,7,8,9-HpCDF	0.01	0.01			
OCDF	0.0001	**0.0003**			

Abbreviations used: “T” = tetra; “Pe” = penta; “Hx” = hexa; “Hp” = hepta; “O” = octa; “CDD” = chlorodibenzodioxin; “CDF” = chlorodibenzofuran; “CB” = chlorobiphenyl.

Source: (Van den Berg et al., 1998) (van den Berg et al., 2006).

Results of the average concentrations of chemicals in each feed group are expressed as lower bound and upper bound means. In case the concentration of one or more congeners is not measured and/or below the limit of quantification, there are two ways in which this data can be treated. One is to assume the concentration for the congener is zero (lower bound limit). The other option is to assume that all values of the different congeners (not known) are equal to the limit of quantification (upper bound limit).

Annex III
Overview number of samples (N) tested on PCDD/Fs and DL-PCBs, per feed category, 2001-2011

Feed category	**DR CALUX®**			**EU monitoring**	**Total**
	Suspected	**Negative**			
			*Verification**		
Feed materials of plant origin	**315**	**831**	***19***	**187**	**1333**
Feed materials of plant origin, excl. vegetable oils	233	520	*11*	177	930
- Cereal grains and their by-products	*15*	*159*		*32*	*206*
- Forages and roughage	*166*	*104*	*9*	*83*	*353*
- Other feed materials of plant origin	*46*	*162*	*1*	*30*	*238*
- Oil seeds/fruits and their by-products	*6*	*95*	*1*	*32*	*133*
Vegetable oils and by-products	82	311	*8*	10	403
- Vegetable oil/fat	*65*	*285*	*6*	*9*	*359*
- Fatty acids	*17*	*26*	*2*	*1*	*44*
Feed materials of animal origin	**157**	**429**	***6***	**168**	**754**
Land animal (by-)products	37	357	*6*	26	420
- Animal fat	*36*	*339*	*4*	*17*	*392*
- Other land animal products	*1*	*18*	*2*	*9*	*28*
Fish, other aquatic animals and their by-products	120	72		142	334
- Fish meal	*57*	*61*		*124*	*242*
- Fish oil	*61*	*9*		*13*	*83*
- Fish protein, hydrolysed	*2*	*2*		*5*	*9*
Feed additives, minerals and pre-mixtures	**203**	**632**	***3***	**223**	**1058**
Feed additives	181	484	*3*	158	823
- Binders, anti-caking agents and coagulants	*85*	*117*		*123*	*325*
Feed materials of mineral origin	1	42		28	71
Pre-mixtures	21	106		37	164
Compound feedingstuffs	**121**	**1301**	***16***	**371**	**1793**
Compound feed excl. fur animals, pets and fish	**118**	**1267**	**15**	**328**	**1713**
Complementary feed	99	799	*11*	140	1038
- Compound feed - ruminants	*18*	*81*		*5*	*104*
- Compound feed - cattle	*55*	*403*	*2*	*101*	*559*
- Compound feed - goat	*1*	*7*		*2*	*10*
- Compound feed - sheep	*2*	*20*		*3*	*25*

Feed category	DR CALUX®			EU monitoring	Total
	Suspected	Negative			
			*Verification**		
- Compound feed - pig	*6*	*50*	*1*	*8*	*64*
- Compound feed - poultry	*5*	*30*	*1*	*11*	*46*
- Compound feed - other	*3*	*62*		*4*	*69*
- Miscellaneous feed materials	*9*	*146*	*7*	*6*	*161*
Complete feed	19	468	*4*	188	675
- Compound feed - cattle	*3*	*28*	*1*	*8*	*39*
- Compound feed - goat		*1*			*1*
- Compound feed - sheep		*6*			*6*
- Compound feed - pig	*11*	*263*	*2*	*113*	*387*
- Compound feed - poultry	*5*	*163*	*1*	*67*	*235*
- Compound feed - other		*7*			*7*
Feed for fur animals, pets and fish	**3**	**34**	**1**	**43**	**80**
Fish food	1	1		12	14
Pet food	2	33	*1*	31	66
Total	**796**	**3193**	***44***	**949**	**4938**

Annex IV
Categories of feed additives as defined in Annex I of Council Regulation 1831/2003/EC

1. Technological additives		**2. Sensory additives**		
(a)	preservatives	(a)	colorants	
(b)	antioxidants		(i)	substances that add/restore colour to feedstuffs
(c)	emulsifiers		(ii)	substances that colours food of animal origin
(d)	stabilisers		(iii)	substances that colour ornamental birds/fishes
(e)	thickeners	(b)	flavouring substances	
(f)	gelling agents	**3. Nutritional additives**		
(g)	binders	(a)	vitamins/pro-vitamins and similar	
(h)	substances for control of radio nucleoid contamination	(b)	compounds of trace elements	
(i)	anticaking agents	(c)	amino acids, their salts and analogues	
(j)	acidity regulators	(d)	urea and derivatives	
(k)	silage additives	**4. Zoo technical additives**		
(l)	denaturants	(a)	digestibility enhancers	
(m)	mycotoxin binders	(b)	gut flora stabilisers	
		(c)	substances which favourably affect the environment	
		(d)	others	

Annex V
Overview total amount of single feed materials used in the Netherlands (x1000 ton), per feed group, 2004/05-2008/09

Source: PDV "Diervoedergrondstoffen in Nederland"

Feed material	2004/2005			2005/2006			2006/2007			2007/2008			2008/2009		
	Import		Total	Import		Total	Import		Total	Import		Total	Import		Total
	direct	indirect		direct	indirect		direct	indirect		direct	indirect		direct	indirect	
1. Feed materials of plant origin															
1.1. Feed materials of plant origin, excl. vegetable oils															
Cereal grains and their by-products	2994	1103	5515	3222	1133	5467	4932	1070	7212	5527	1063	7764	5786	968	8330
wheat and their by-products	943	685	2615	1340	656	2711	2308	612	3864	1715	641	3167	2480	575	4154
maize and their by-products	147	462	609	152	371	523	1598	351	2073	2804	314	3352	2240	282	2764
barley	251		447	226		401	863		967	315		410	645		840
Forages and roughage *	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
fodder, dried	14		127	29		123	20		82			139			138
Oil seeds/fruits and their by-products	1226	2903	4129	1439	2640	4079	1509	2806	4316	1950	2922	4872	1974	2788	4762
soya (bean) and their by-products	106	1984	2090	115	1725	1840	79	2152	2231	185	2340	2525	495	2009	2504
cole-/rapeseed and their by-products	660	22	682	824	32	856	907	106	1014	1060		1060	544	514	1058
palm kernel by-products	992		992	879		879	605		605	833		833	625		625
sunflower seed and their by-products	19	296	315	194	228	422	130	262	392	236	122	358	239	261	500
copra	27		27	34		34	13		13	22		22	5		5
linseed	15		15	14		14	21		21	19		19	21		21
other oilseeds	2		2	17		17	15		15	20		20	23		23

Feed material	2004/2005			2005/2006			2006/2007			2007/2008			2008/2009		
	Import		Total	Import		Total	Import		Total	Import		Total	Import		Total
	direct	indirect		direct	indirect		direct	indirect		direct	indirect		direct	indirect	
Other feed materials of plant origin	1293		1348	1060		1129	1362		1384	1574		1584	849		954
Legume seeds and their by-products															
feed legume	208		208	178		178	124		124	41		41			0
lupins	48		48	48		48	26		26	4		4			0
Tubers, roots and their by-products															
sugar beet pulp, dried	199		244	289		348	246		258	321		321	173		249
manioc	132		132	17		17	259		259	304		304		19	19
Other seeds/fruits and their by-products															
citrus pulp	344		344	255		255	293		293	449		449	359		359
Other plants, algae and their by-products															
molasses	245		255	164		174	275		285	336		346	247		257
vinasses	117		117	109		109	139		139	119		119	70		70
1.2. Vegetable oils and their by-products															
Vegetable oil/fat	199		199	187		187	162		162	177		177	168		168
palm oil	120		120	104		104	79		79	92		92	99		99
soybean oil	37		37	33		33	46		46	41		41	21		21
coconut oil	29		29	25		25	23		23	25		25	27		27
palm kernel oil	9		9	9		9	12		12	14		14	12		12
rapeseed oil				8		8	1		1	4		4	7		7
sunflower oil	1		1	7		7	1		1	1		1	1		1
other vegetable oil/fat	2		2	1		1	1		1	1		1	2		2
Fatty acids **	23		23	29		29	31		31	24		24	27		27
2. Feed materials of animal origin															

Feed material	2004/2005			2005/2006			2006/2007			2007/2008			2008/2009		
	Import		Total	Import		Total	Import		Total	Import		Total	Import		Total
	direct	indirect		direct	indirect		direct	indirect		direct	indirect		direct	indirect	
2.1. Land animal (by-)products															
Animal fat, excl. fish oil ***			135			102			108			147			152
bovine fat (tallow)			22			17			19			32			35
porcine fat (lard)			58			27			30			63			60
other animal fat			55			58			59			52			57
Other land animal products	161		256	163		253	191		286	439		439	397		397
whey powder	45		98	52		103	73		134	341		341	301		301
milk powder, skimmed	116		158	111		150	118		152	98		98	96		96
2.2. Fish, other aquatic animals and their by-products															
Fish meal	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Fish oil	1		1	2		2	3		3	2		2	3		3
3. Feed additives, minerals and pre-mixtures	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4. Compound feedingstuffs	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Miscellaneous feed materials (not mentioned in the other groups)	632	212	1009	774	208	1145	973	192	1338	1040	193	1409	783	237	1200
Grand total	**6521**	**4218**	**12720**	**6876**	**3981**	**12487**	**9151**	**4068**	**14890**	**10709**	**4178**	**16533**	**9960**	**3993**	**16105**

* *Only the amount of dried fodder is known, which is mainly used for horses. However, ruminants also graze outside and/or use other sources such as silage. The complete amount of forages and roughage used is therefore unknown.*

** *Most fatty acids are linked to processed palm oil and therefore involves feed materials of foreign origin. Fatty acids are however an end product of production processes carried out in the Netherlands or neighbour European countries.*

*** *In the overview of the PDV no clear differentiation is made if the animal fat originates from domestic or foreign origin. After consulting an expert the assumption is made that animal fat used in feed mainly originates from the Netherlands.*

Indirect import = the feed materials are of foreign origin, but are processed in the Netherlands

Total = total amount of feed used in the Netherlands (feed consumption). This amount therefore includes feed cultivated/produced in the Netherlands (domestic feed) plus imports minus exports.

n.a. = not available

Annex VI
Overview total amount of feed imported into the Netherlands, per feed group, 2001-2010

Source: Eurostat (DS_018995)

Feed group	Number Eurostat	Outside EU-27 Avg. [A]		Outside EU-27 StDev [B]		Inside EU-27 Avg. [A]		Inside EU-27 StDev [B]		Total import	Country of origin [C]
		x1000 tons	%	x1000 tons	%	x1000 tons	%	x1000 tons	%	x1000 tons	%
1. Feed materials of plant origin											
1.1. Feed materials of plant origin, excl. vegetable oils											
Cereal grains and their by-products											
wheat	8126	0	0	0	0	430	100	55	0	430 *	DE: 56, BE: 30, FR: 13
maize (corn)	8124	0	0	0	0	52	100	18	0	52 *	DE: 56, FR: 38, BE: 5
cereal straw and husks	8111	0	0	0	0	98	100	23	0	98 *	DE: 66, FR: 25, BE: 5
rice	8125	0.4	6	0.4	4	6	94	5	48	6	BE: 51, DE: 28, FR: 15 (N.B. Since 2007 not imported anymore.)
other cereals	8129	0.2	1	0.4	2	18	99	9	2	18	BE: 49, DE: 47
Forages and roughage											
lucerne (alfalfa)	8112	0	0	0	0	17	100	3	0	17	FR: 73, DE: 14, BE: 13
Oil seeds/fruits and their by-products											
soya beans	8131	4204	96	924	1	193	4	54	1	4396	AR: 48, BR: 47
rape or colza seeds	8136	3	0	8	2	801	100	222	2	804	DE: 82, BE: 12
palm nuts or kernels	8138	1194	95	105	2	68	5	25	2	1262	MY: 64, ID: 31, DE: 5
sunflower seeds	8135	388	86	73	8	66	14	37	8	453	AR: 83, DE: 11
coconut or copra	8137	99	90	116	11	11	10	9	11	110	ID: 61, PH: 28, DE: 9
linseed	8134	5	25	7	23	16	75	5	23	22	DE: 48, BE: 26, US: 20, AR: 5
groundnuts	8132	0.2	4	1	30	5	96	3	30	5	DE: 92
cotton seeds	8133	3	99	7	45	0	1	0	39	3	BR: 98 (N.B. Since 2007 mainly coming from DE, but amount decreased.)
other oil seeds, oleaginous fruits and germs of cereals	8139	5	38	8	27	9	62	10	27	14	DE: 44, AR: 27, ES: 17, BR: 5
Other feed materials of plant origin											
leguminous plants	8123	0	0	0	0	13	100	9	0	13	DE: 78, GB: 15, BE: 5
vegetables and their by-products	8119	836	85	89	7	150	15	73	7	986	BR: 46, AR: 25, US: 13, DE: 8, BE: 5

Feed group	Number Eurostat	Outside EU-27 Avg. [A]		Outside EU-27 StDev [B]		Inside EU-27 Avg. [A]		Inside EU-27 StDev [B]		Total import	Country of origin [C]
		x1000 tons	%	x1000 tons	%	x1000 tons	%	x1000 tons	%	x1000 tons	%
swedes, mangolds, fodder roots, hay, clover, sainfoin, forage kale, lupines, vetches and similar forage products	8113	1	2	1	2	26	98	6	2	26	DE: 54, FR: 27, AT: 8, BE: 8
beet pulp, bagasse and other waste of sugar manufacture	8152	2	1	3	2	140	99	32	2	142	DE: 55, BE: 26, FR: 10, AT: 6
1.2. Vegetable oils and their by-products											
2. Feed materials of animal origin											
2.1. Land animal (by-)products											
Other land animal products											
flours, meals and pellets, of meat or meat offal	8141	7	7	5	4	87	93	32	4	94 *	DE: 33, IT: 18, GB: 14, FR: 12, BE: 9
2.2. Fish, other aquatic animals and their by-products											
Fish meal											
flours, meals and pellets, of fish or crustaceans, mollusc or other aquatic invertebrates	8142	6	12	15	15	46	88	22	15	52	DE: 69, PE: 12, DK: 11, FR: 5
3. Feed additives, minerals and pre-mixtures											
4. Compound feedingstuffs											
4.1. Compound feed, excl. fur animals, pets and fish											
preparations of a kind used for animal food	8199	598	47	470	26	670	53	208	26	1268	US: 46, DE: 35, BE: 10, FR: 6
Miscellaneous feed materials											
residues of starch manufacture and similar residues	8151	4	1	5	1	571	99	110	1	574	BE: 39, FR: 34, DE: 25
brewing or distilling dregs and waste	8153	28	13	28	10	179	87	47	10	207	DE: 56, BE: 26, US: 12
wine lees; argol	8194	0	0	0	0	0.2	100	0.4	53	0.2	DE: 96 (N.B. Since 2008 no import anymore.)
4.2. Feed for fur animals, pets and fish											
dog or cat food, put up for retail sale	8195	8	6	5	4	141	94	19	4	149	DE: 33, FR: 29, BE: 11, GB: 8

Abbreviations used: AR = Argentina, AT = Austria, BE = Belgium, BR = Brazil, DE = Germany, DK = Denmark, ES = Spain, FR = France, GB = United Kingdom, ID = Indonesia, IT = Italy, MY = Malaysia, PE = Peru, PH = Phillipines, US = United States of America.

Intra- and Extra EU-27 imports | mainly import from third countries | mainly import from EU-27 MS

[A] *The average amount in tons is based on import data from 2001 till 2010. The average percentage is based on the average amount in tons in the specified feed (sub)group making a division between inside/outside EU-27 divided by the total average amount in tons per the same feed (sub)group.*

[B] *The standard deviation (StDev) was calculated on basis of the percentage per year. The percentage per year was calculated by the amount in tons per specified feed (sub)group making a division between inside/outside EU-27 divided by the total amount in tons per the same feed (sub)group in each year from 2001 till 2010.*

[C] *The percentage country of origin is based on the average amount in tons per specified feed (sub)group per country of origin from 2000 till 2010 divided by the average of the total amount in tons per feed (sub)group. Only percentages of ≥5 are stated in the row "Country of origin".*

* *The data of the import volume provided by Eurostat is lower compared to the PDV. This might relate to the fact that not all data was available for Eurostat due to the free movement of goods between European countries which do not necessarily need to be registered. The data provided by the PDV is supposed to give a better picture about the quantities used and/or imported in the Netherlands.*

Annex VII
Overview total amount of compound feed used in the Netherlands (x1000 ton), per animal species, 2001-2011

Source: Fefac - Compound feed production (http://www.fefac.eu/publications.aspx?CategoryID=2061&EntryID=10802).

	2001	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**
Pigs											
total	5762	5309	5227	5120	5378	5672	5755	6107	5936	5863	5734
pigs for fattening	3693	3353	3404	n.a.	3545	3698	3779	4032	3884	3844	3668
breeding pigs	1254	1222	1129	n.a.	1144	1216	1206	1278	1257	1266	1333
piglets	815	734	694	n.a.	689	758	770	797	795	753	733
Poultry											
total	3466	3296	2745	3080	3426	3533	3407	3710	3623	3793	3801
chicks & layers	1626	1700	1411	n.a.	1868	2007	1877	2047	2083	2135	2042
broilers	1840	1596	1334	n.a.	1558	1526	1530	1663	1540	1658	1759
Bovine animals											
total	3449	3306	3388	3320	3178	3140	3375	3560	3364	3433	3451
dairy cow	3013	2998	3067	n.a.	2916	2881	3012	3186	2923	2982	2943
for fattening	308	266	276	n.a.	262	259	347	343	338	380	432
calves (excl. milk replacers)	128	42	45	n.a.	n.a.	n.a.	0	0	0	0	0
other	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	16	31	103	71	76
Milk replacers	**710**	**666**	**690**	**728**	**728**	**724**	**761**	**759**	**719**	**703**	**652**
Other	**864**	**772**	**796**	**830**	**269**	**422**	**432**	**441**	**437**	**457**	**435**
Pet food	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**66**	**29**	**70**	**61**
Grand total	**14251**	**13349**	**12846**	**13078**	**12979**	**13491**	**13730**	**14643**	**14108**	**14319**	**14134**

Annex VIII
Calculation of outliers

An outlier is an observation that is numerically distant from the rest of the data, being unusually small or unusually large in the dataset (Barnett *et al.*, 1994). Present within the dataset they may influence the outcome of the trend analysis. For this reason outliers have been determined and are related to the results found in *section 3.3 till 3.6*. Based on the assumption that the data is normally distributed, two different methods have been reviewed which are commonly used for pinpointing outliers: z-score method and boxplot method.

In the z-score method, the mean and standard deviation is used to observe the dataset, using the following formula:

$$Z_{ij} = \frac{(x_{ij} - \overline{X}_j)}{StDev_j}$$

x_{ij} = *concentration of the sample i within the feed (sub)group j*
$\overline{X}_j$ = *average/mean of all values of the specific feed (sub)group j*
$StDev_j$ = *standard deviation of all values of the specific feed (sub)group j*

A z-score greater than three is assumed to be an outlier. Since both the mean and standard deviation are affected by the outliers, the outcome of this method might be unreliable. The boxplot method seems to be a better alternative as it depends on the median and not the mean of the data; which makes it also suitable for a not normally distributed dataset. The original boxplot according to Tukey (1977) is graphically constructed by

- putting a line at the height of the sample median Q_2,
- drawing a box from the first quartile Q_1 (25^{th} percentile) to the third quartile Q_3 (75^{th} percentile),
- classifying all points outside the interval (the fence): $[Q_1 - 1.5 * IQR ; Q_3 + 1.5 * IQR]$ as outlier (where the interquartile range $IQR = Q_3 - Q_1$),
- drawing the whiskers (the lines that go from the ends of the box to the most remote points that are no outliers) (Hubert et al., 2006).

In addition, the method of adjusted boxplot especially takes the skewness of the data into account. Instead of using the fence $[Q_1 - 1.5 * IQR ; Q_3 + 1.5 * IQR]$; the boundaries of the interval used were defined, as proposed by Hubert and Vandervieren (2006):

$$[Q_1 - h_{lower}(MC) * IQR ; Q_3 + h_{upper}(MC) * IQR]$$

MC = medcouple (measure of the skewness of a continuous unimodal distribution)

Hereby the dataset fits best to the linear model, corresponding to the following equation:

$$[h_{lower}(MC) = 1.5 + a\ MC ; h_{upper}(MC) = 1.5 + b\ MC] \text{ and}$$

$$a\ MC \approx \frac{Q_1 - Q_\alpha}{IQR} - 1.5 \qquad b\ MC \approx \frac{Q_\beta - Q_3}{IQR} - 1.5$$

Qp denotes the pth quartile of the distribution with $\alpha = 0.0035$ and $\beta = 0.9965$.

In total 1 sample was found to fall outside the interval for dioxins as well as DL-PCBs and the sum of dioxins and DL-PCBs. The adjusted boxplot method was used for each feed subgroup as this is considered to lead to more reliable results as dioxin and DL-PCB concentrations differ within feed categories, especially if linked to different limits. Table 6.2 gives an overview of the calculated (adjusted) boxplot values per feed subgroup. It was not only relied on the latter method, but also looked into detail if certain values are close to the established boxplot interval and therefore should/should not be defined as outlier.

Table 6.2. Overview calculation of (adjusted) boxplot values per feed category.

Dioxins

					Original boxplot		Adjusted boxplot					
Feed category	Q_1	Q_2	Q_3	IQR	Lower fence	Upper fence	Lower fence	aMC	Q_α	Upper fence	bMC	Q
1. Feed materials of plant origin												
1.1. Feed materials of plant origin, excl. vegetable oils												
Cereal grains and their by-products	0.17	0.29	0.29	0.12	-0.01	0.46	0.17	-1.50	0.17	0.30	-1.42	0.30
Forages and roughage	0.18	0.27	0.29	0.11	0.02	0.45	0.17	-1.36	0.17	0.50	0.46	0.50
Other feed materials of plant origin	0.29	0.29	0.29	0.00	0.29	0.29	0.17	513.90	0.17	0.30	48.72	0.30
1.2. Vegetable oils and their by-products												
Oil seeds/fruits and their by-products	0.17	0.29	0.29	0.12	0.00	0.46	0.17	-1.48	0.17	0.30	-1.39	0.30
Vegetable oil/fat	0.29	0.29	0.29	0.01	0.28	0.30	0.17	15.43	0.17	0.42	16.44	0.42
2. Feed materials of animal origin												
2.1. Land animal (by-)products												
Animal fat	0.30	0.36	0.42	0.12	0.13	0.59	0.21	-0.66	0.21	0.54	-0.43	0.54
Other land animal products	0.23	0.29	0.29	0.06	0.14	0.38	0.17	-0.51	0.17	0.32	-1.01	0.32
2.2. Fish, other aquatic animals and their by-products												
Fish meal	0.19	0.28	0.45	0.26	-0.20	0.84	0.17	-1.43	0.17	2.03	4.58	2.03
Fish oil	1.78	2.16	2.58	0.80	0.59	3.78	0.35	0.30	0.35	3.99	0.26	3.99
3. Feed additives, minerals and pre-mixtures												
Feed additives	0.22	0.29	0.35	0.13	0.03	0.54	0.17	-1.08	0.17	0.86	2.50	0.86
Feed materials of mineral origin	0.17	0.29	0.29	0.12	-0.01	0.47	0.17	-1.50	0.17	0.32	-1.25	0.32
Pre-mixtures	0.17	0.29	0.29	0.12	0.00	0.46	0.17	-1.47	0.17	0.38	-0.69	0.38
4. Compound feedingstuffs												
4.1. Compound feed excl. fur animals, pets and fish												
Complementary feed	0.18	0.29	0.29	0.11	0.02	0.45	0.17	-1.39	0.17	0.46	0.14	0.46
- cattle feed	0.23	0.29	0.29	0.05	0.15	0.37	0.17	-0.32	0.17	0.48	2.01	0.48
Complete feed	0.17	0.29	0.29	0.12	0.00	0.46	0.17	-1.48	0.17	0.40	-0.51	0.40
- pig feed	0.17	0.29	0.29	0.12	0.00	0.46	0.17	-1.48	0.17	0.42	-0.38	0.42
- poultry feed	0.17	0.29	0.29	0.12	-0.01	0.47	0.17	-1.50	0.17	0.30	-1.37	0.30
4.2. Feed for fur animals, pets and fish												
Fish food	0.30	0.35	0.47	0.17	0.05	0.72	0.18	-0.77	0.18	0.90	1.09	0.90
Pet food	0.17	0.29	0.29	0.12	-0.01	0.46	0.17	-1.49	0.17	0.30	-1.42	0.30

DL-PCBs

Feed category	Q_1	Q_2	Q_3	IQR	Original boxplot		Adjusted boxplot					
					Lower fence	Upper fence	Lower fence	aMC	Q_α	Upper fence	bMC	Q
1. Feed materials of plant origin												
1.1. Feed materials of plant origin, excl. vegetable oils												
Cereal grains and their by-products	0.02	0.02	0.03	0.00	0.02	0.03	0.02	-1.49	0.02	0.04	2.38	0.04
Forages and roughage	0.05	0.07	0.09	0.04	-0.02	0.15	0.00	-0.38	0.00	0.15	-0.06	0.15
Other feed materials of plant origin	0.03	0.03	0.03	0.00	0.02	0.04	0.02	-1.29	0.02	0.05	2.48	0.05
1.2. Vegetable oils and their by-products												
Oil seeds/fruits and their by-products	0.02	0.02	0.02	0.00	0.02	0.02	0.02	27.32	0.02	0.09	2478.61	0.09
Vegetable oil/fat	0.03	0.03	0.11	0.08	-0.10	0.23	0.02	-1.49	0.02	0.25	0.27	0.25
2. Feed materials of animal origin												
2.1. Land animal (by-)products												
Animal fat	0.20	0.46	0.56	0.35	-0.33	1.09	0.03	-1.02	0.03	0.86	-0.65	0.86
Other land animal products	0.02	0.02	0.04	0.02	0.00	0.06	0.02	-1.50	0.02	0.28	14.04	0.28
2.2. Fish, other aquatic animals and their by-products												
Fish meal	0.18	0.35	0.65	0.47	-0.51	1.35	0.05	-1.20	0.05	4.66	7.11	4.66
Fish oil	5.84	7.47	10.32	4.48	-0.89	17.04	0.34	-0.27	0.34	11.46	-1.25	11.46
3. Feed additives, minerals and pre-mixtures												
Feed additives	0.02	0.02	0.03	0.00	0.02	0.03	0.02	-1.48	0.02	0.10	21.99	0.10
Feed materials of mineral origin	0.02	0.02	0.02	0.00	0.02	0.02	0.02	-0.87	0.02	0.07	3389.09	0.07
Pre-mixtures	0.02	0.02	0.03	0.00	0.02	0.03	0.02	-1.48	0.02	0.05	26.47	0.05
4. Compound feedingstuffs												
4.1. Compound feed excl. fur animals, pets and fish												
Complementary feed	0.02	0.02	0.03	0.01	0.02	0.04	0.02	-1.49	0.02	0.32	53.43	0.32
- cattle feed	0.02	0.02	0.03	0.00	0.02	0.03	0.02	-1.49	0.02	0.08	20.58	0.08
Complete feed	0.03	0.03	0.04	0.01	0.01	0.05	0.02	-1.40	0.02	0.10	5.22	0.10
- pig feed	0.03	0.03	0.04	0.01	0.01	0.06	0.02	-1.40	0.02	0.08	1.90	0.08
- poultry feed	0.02	0.03	0.03	0.01	0.01	0.04	0.02	-1.50	0.02	0.08	4.97	0.08
4.2. Feed for fur animals, pets and fish												
Fish food	0.36	0.64	0.83	0.47	-0.34	1.53	0.14	-1.01	0.14	1.36	-0.36	1.36
Pet food	0.03	0.03	0.04	0.01	0.01	0.05	0.02	-1.09	0.02	0.06	0.40	0.06

Sum dioxins and DL-PCBs

Feed category	Q_1	Q_2	Q_3	IQR	Original boxplot		Adjusted boxplot					
					Lower fence	Upper fence	Lower fence	aMC	Q_α	Upper fence	bMC	Q
1. Feed materials of plant origin												
1.1. Feed materials of plant origin, excl. vegetable oils												
Cereal grains and their by-products	0.20	0.31	0.31	0.12	0.02	0.49	0.19	-1.47	0.19	0.33	-1.35	0.33
Forages and roughage	0.26	0.33	0.37	0.11	0.10	0.53	0.21	-1.01	0.21	0.63	0.96	0.63
Other feed materials of plant origin	0.31	0.32	0.32	0.01	0.30	0.33	0.19	18.98	0.19	0.33	0.25	0.33
1.2. Vegetable oils and their by-products												
Oil seeds/fruits and their by-products	0.20	0.31	0.31	0.12	0.02	0.49	0.19	-1.48	0.19	0.38	-0.95	0.38
Vegetable oil/fat	0.32	0.32	0.40	0.08	0.19	0.53	0.23	-0.48	0.23	0.57	0.50	0.57
2. Feed materials of animal origin												
2.1. Land animal (by-)products												
Animal fat	0.51	0.83	1.02	0.51	-0.25	1.78	0.31	-1.10	0.31	1.35	-0.85	1.35
Other land animal products	0.25	0.31	0.33	0.08	0.14	0.44	0.19	-0.72	0.19	0.60	2.06	0.60
2.2. Fish, other aquatic animals and their by-products												
Fish meal	0.36	0.63	1.13	0.77	-0.79	2.28	0.22	-1.31	0.22	6.54	5.55	6.54
Fish oil	8.05	9.45	11.89	3.84	2.29	17.65	0.72	0.41	0.72	15.44	-0.58	15.44
3. Feed additives, minerals and pre-mixtures												
Feed additives	0.25	0.31	0.37	0.12	0.07	0.56	0.19	-1.04	0.19	0.88	2.65	0.88
Feed materials of mineral origin	0.19	0.31	0.31	0.12	0.02	0.49	0.19	-1.50	0.19	0.39	-0.88	0.39
Pre-mixtures	0.20	0.31	0.32	0.12	0.02	0.49	0.19	-1.46	0.19	0.41	-0.71	0.41
4. Compound feedingstuffs												
4.1. Compound feed excl. fur animals, pets and fish												
Complementary feed	0.21	0.31	0.32	0.10	0.06	0.48	0.19	-1.30	0.19	0.64	1.55	0.64
- cattle feed	0.26	0.31	0.31	0.05	0.19	0.39	0.19	-0.15	0.19	0.52	2.51	0.52
Complete feed	0.22	0.31	0.32	0.10	0.07	0.47	0.19	-1.23	0.19	0.47	-0.02	0.47
- pig feed	0.22	0.32	0.33	0.11	0.06	0.49	0.19	-1.28	0.19	0.47	-0.15	0.47
- poultry feed	0.23	0.31	0.32	0.08	0.11	0.44	0.19	-1.02	0.19	0.37	-0.85	0.37
4.2. Feed for fur animals, pets and fish												
Fish food	0.65	0.99	1.30	0.65	-0.31	2.27	0.32	-0.98	0.32	2.27	0.00	2.27
Pet food	0.21	0.31	0.32	0.11	0.03	0.49	0.19	-1.40	0.19	0.35	-1.27	0.35

In Table 6.3 an overview is given of the one sample with extreme values, which are seen as potential outliers within this report.

Table 6.3. Extreme values per feed subgroup.

Feed material	Sample ID	Year	Origin	Concentration		
				dioxins	DL-PCBs	sum dioxins + DL-PCBs
Fish meal	200159200	2005	UN	**2.51**	**5.89**	**8.39**

Bold *= The concentrations which were identified as outlier are stated in bold.*

Annex IX
Diet composition of food producing animals (in percentage)

Source: Scientific Committee on Animal Nutrition (European Commission, 2000).

Feed material	**Ruminants**		**Pigs**				**Poultry**				**Farmed Fish**	
			Piglets		Fattening pigs		used for meat production		layer		carnivorous species ***	omnivorous species ***
	avg (%)	StDev (%)	avg (%)	StDev (%)	avg (%)	StDev (%)	avg (%)	StDev (%)	avg (%)	StDev (%)	(%)	(%)
cereals and legumes	52	18	58	3	65	16	58	8	63	6	11	30
(by-)products of plant origin	40	28	31	6	29	14	31	9	22	6	12	56
fish meal	3	3	4	2			1	2	1	2	50	10
fat *	2	2	3	4	3	2	5	3	1	1	25	2
pre-mixture **	4	0	4	1	3	1	4	1	2	0	2	2

* *fat can include fat of animal origin and/or vegetable origin; exclusion: in the fish diet it refers to fish oil.*
** *includes feed additives and minerals.*
*** *carnivorous species (e.g. salmon, sea bass, tuna, eel) rely under natural circumstances entirely upon the flesh or meat of a living organism, whereas omnivorous species can survive on flesh as well as plants (e.g. catfish, tilapia).*

Annex X
Results Mann-Kendall

Feed group	N_{years}	PCDD/Fs		DL-PCBs		Sum PCDD/Fs + DL-PCBs	
		Significance	S value	Significance	S value	Significance	S value
Feed materials of plant origin							
Feed materials of plant origin, excl. vegetable oils	8		-14		8		-14
- Forages and roughage	*7*	*	*-15*		*-1*	+	*-13*
Vegetable oils and their by-products	8	*	-18		-8	**	-24
Feed materials of animal origin							
Fish meal	8		-4		-2		-4
Feed additives, minerals and pre-mixtures							
Feed additives	8		-14		-10		-14
- Binders, anti-caking agents and coagulants	*8*	*	*-18*		*-2*	+	*-16*
Compound feedingstuffs							
Compound feed excl. fur animals, pets and fish	**8**	*******	**-21**		**-11**	*****	**-17**
Complementary feed	8	***	-21		-3	**	-19
- Complementary cattle feed	*6*	**	*-15*		*-5*	**	*-15*
Complete feed	6	**	-15		-9	*	-13
- Complete pig feed	*6*	**	*-15*		*-5*	*	*-11*
- Complete poultry feed	*6*	*	*-13*		*-1*	*	*-13*
Feed for fur animals, pets and fish	**5**	*****	**-10**		**-6**	**+**	**-8**

Mann-Kendall test: The significance levels tested are 0.001 (***), 0.01 (**), 0.05 (*) and 0.1 (+).
A positive (negative) S value indicates an upward (downward) trend.

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

RIKILT advises national and international governments on establishing standards and methods of analysis. RIKILT is available 24 hours a day and seven days a week in cases of incidents and food crises.

The research institute in Wageningen is the National Reference Laboratory (NRL) for milk, genetically modified organisms, and nearly all chemical substances, and is also the European Union Reference Laboratory (EU-RL) for substances with hormonal effects.

RIKILT is a member of various national and international expertise centres and networks. Most of our work is commissioned by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the new Dutch Food and Consumer Product Safety Authority. Other parties commissioning our work include the European Union, the European Food Safety Authority (EFSA), foreign governments, social organisations, and businesses.

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