ANALYTICAL OPTIONS FOR LOWERING LEGAL LIMITS FOR DIOXINS AND PCB IN FEED AND FOOD AS CONSEQUENCE OF THE EFSA REVISION OF THE TWI

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Introduction

On 20 November 2018, the European Food Safety Authority (EFSA) published the Authority's first comprehensive review of the risks to human and animal health from dioxins and PCBs in food and feed [1]. The panel set a new tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs in food of 2 pg/kg bw/week. This new TWI is seven-times lower than the previous EU tolerable intake set by the Scientific Committee on Food (SCF) in 2001 [2]. Furthermore it was concluded that the toxicity of the most harmful dioxin-like PCB, PCB 126, may be overestimated.

Subsequently the EU Commission informed the EU Reference Laboratory (EURL) that as follow-up of this EFSA opinion maximum levels for dioxins (WHO-PCDD/PCDF-TEQ) and the sum of dioxins and dioxin-like PCBs (WHO-PCDD/PCDF-PCB-TEQ) are expected to be reviewed (lowered) and consequently also the action levels for dioxins and dioxin-like PCBs. However maximum/action levels need to be enforced and therefore it would be necessary, in case in the future lower maximum/action levels are established, that these controls can be performed on a routine, reliable and inexpensive way also enabling the current screening techniques still to be used in official control strategy (including an auto-control programme). Therefore the EURL was asked to prepare a position paper which should address two important aspects:

- Part 1 should highlight the limits of lowering maximum levels (and corresponding action levels) for the different feed and food commodities (i.e. to which extent levels can be lowered purely from an analytical point of view) thereby ensuring a compliance with the analytical requirements of Regulation (EU) 2017/644 (Food) [3] and 2017/771 (feed) [4] but still enabling a routine, reliable and inexpensive analysis and this by most National Reference Laboratories (NRLs) and official control laboratories (OFLs) currently performing analysis of dioxins and PCBs. Also the possibilities of using currently used screening techniques have to be ensured.
- Part 2 should explore how the analytical requirements of Regulation (EU) 2017/644 and 2017/771 could be modified, whilst still ensuring that the analytical results obtained are reliable. This would enable to set possible lower maximum levels which would not be possible with respect to the current legal analytical requirements (and this for most of the NRLs and current official control laboratories).

Materials and Methods: Information and inclusion of National Reference Laboratories in the evaluation

The National Reference Laboratories (NRLs) were informed about the publication of the EFSA opinion and the request of the Commission. It was considered as an important pillar to have a feed-back from the NRL's point of view on both aspects.

- For compilation of an overview as answer to the first task, the EURL developed a questionnaire (as excel file) for a quick and easy response. The excel file had two spreadsheets (one for food, one for feed) listing the existing maximum and action levels and asking to fill in the achievable lower ends of the working range for WHO-PCDD/PCDF-TEQ and WHO-PCB-TEQ, furthermore providing the most basic information on the kind of the detection method. In case laboratories apply bioanalytical screening methods, the achievable lowest cut-off level was of interest.
- With regard to the second aspect, the NRLs could give comments.

Results and discussion

1. Task 1: Options and limits for lowering maximum levels and corresponding action levels purely from an analytical point of view

1.1 Evaluation of proficiency tests (PTs)

As one pillar to address this request from the Commission, the evaluation of certain proficiency tests (PT) results could be seen demonstrating the state of play and options from the EURL's point of view: In the period 2007 – 2018, the EURL has performed 25 PTs with 41 samples. However, the PTs were performed mostly in ranges around present maximum and/or action levels and are therefore only of limited value with regard to check the possibilities in very low concentration ranges.

1.2 Evaluation of questionnaires sent to NRLs

17 NRLs from 17 countries and 6 OFLs sent back questionnaires. 21 participants reported information for confirmatory methods (using GC-HRMS and/or GC-MS/MS) and 4 participants reported information for bioanalytical screening methods. The reported LOQs / achievable lower ends of working range of confirmatory methods were summarized in comparison to maximum and action levels for food and feed as follows:

- WHO-PCDD/PCDF-TEQ for food matrices with legal limits on fat basis
- WHO-PCDD/PCDF-TEQ for food matrices with legal limits on wet weight basis
- WHO-PCB-TEQ for food matrices with legal limits on fat basis
- WHO-PCB-TEQ for food matrices with legal limits on wet weight basis
- WHO-PCDD/PCDF-TEQ for feed matrices with possibly higher lipid content
- WHO-PCDD/PCDF-TEQ for feed matrices with low lipid content
- WHO-PCB-TEQ for feed matrices with possibly higher lipid content
- WHO-PCB-TEQ for feed matrices with low lipid content

1.3 Criteria for limit of quantification as basis for conclusions

An important requirement of analytical criteria is the limit of quantification (LOQ) which for a confirmatory method shall be about one fifth of the maximum level (Commission Regulation (EU) 2017/644 for food, Commission Regulation (EU) 2017/771 for feed). The achievement of this requirement is closely linked with the most important criteria for reliable results from confirmatory methods (trueness and intermediate precision, see chapter 2). A lower LOQ in comparison to the maximum level would be better, however a higher LOQ could be an indication of analytical problems which could have an effect on the reliability of results.

1.4 Limits for lowering of maximum and action levels

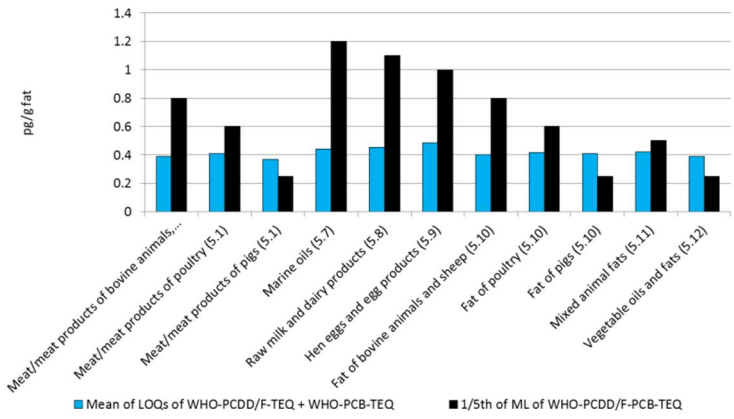

Figure1: WHO-PCDD/PCDF-PCB-TEQ for food matrices with legal limits on fat basis

As example for the resulting evaluation based on the reported data from all 21 laboratories, figure 1 shows the ratio between the mean of all reported LOQs/lower ends of the working range, calculated as sum of WHO-PCDD/PCDF-TEQ and WHO-PCB-TEQ, for food matrices with legal limits on fat basis in comparison with one fifth of the respective maximum level. (The WHO-PCDD/PCDF-PCB-TEQ calculation of LOQs is based on an equal contribution of WHO-PCDD/PCDF-TEQ and WHO-PCB-TEQ to the sum.) For fulfilment of the criterion "LOQ shall be about one fifth of the maximum level", the mean of the reported LOQs should be below one fifth

of the maximum level. For action levels an additional provisional criterion for these evaluations was applied: "LOQ should be about one third of the action level".

Figure 2 shows the same evaluation for food matrices with legal limits on wet weight basis (fish, liver).

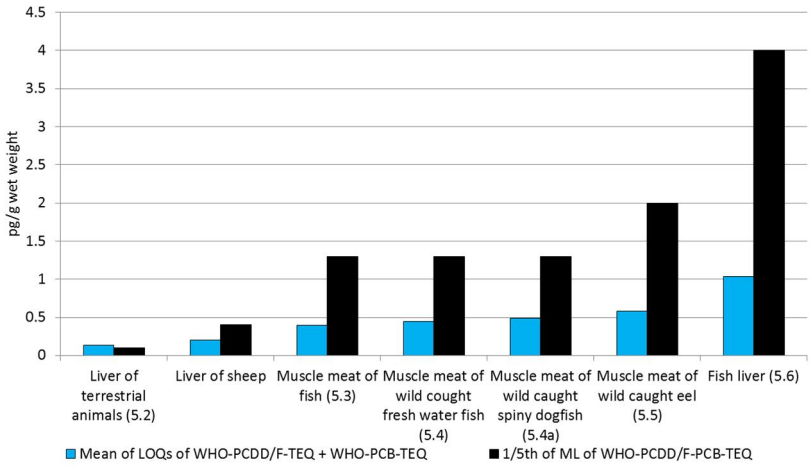

Figure 2: WHO-PCDD/PCDF-PCB-TEQ for food matrices with legal limits on wet weight basis (fish, liver)

Bioanalytical screening methods

In case laboratories apply bioanalytical screening methods, the achievable lowest cut-off level is of interest. Two laboratories reported cut-offs for PCDD/PCDFs and PCBs separately, two laboratories only for the sum of WHO-PCDD/PCDF-TEQ and WHO-PCB-TEQ.

The two laboratories reporting cut-offs for PCDD/PCDFs and dioxin-like PCBs (DL-PCBs) showed that the lowest cut-offs for the relevant food matrices could be on average a factor of 8 (range 1.5 to 40) below the respective maximum levels for PCDD/PCDF with the lowest margin of about 2 for vegetable oils and fat/meat of pigs. The other two laboratories establishing a cut-off only for the sum of PCDD/PCDFs and DL-PCBs reported that the lowest cut-offs are on average a factor of 2 below the maximum levels for the sum of PCDD/PCDFs and DL-PCBs and in the range of the maximum levels for PCDD/PCDFs only.

For feed matrices the results are comparable. In this case only one laboratory reported cut-off values for the sum of PCDD/PCDFs and PCBs. These were on average a factor of 1.5 below the respective maximum levels. For the two laboratories reporting cut-off levels for PCDD/PCDFs the values were on average a factor of 5 (range 2.5 to 1) below the respective maximum levels with the lowest margin again for vegetable oils.

1.5 Conclusions and outlook

The currently applied analytical methods show for several different food and feed matrices a potential for lowering maximum and action levels regarding the possible LOQs/lower ends of working range.

For **food** with maximum levels on fat basis this is the case for meat/fat of bovine animals and sheep, meat/fat of poultry, marine oils, raw milk and dairy products and hen eggs and egg products, whereas maximum levels for meat/fat of pigs and vegetable oils are quite close to the analytical limitations. Additionally legal limits for fish/fish liver and liver of sheep (with maximum levels based on wet weight) could also be lowered.

For infant food, on average the currently applied methods by some laboratories cannot meet the criteria for the maximum levels for WHO-PCDD/PCDF-TEQ and WHO-PCDD/PCDF-PCB-TEQ. Further improvement of the analytical methods and lowering of LOQs is necessary for these matrices. This is also in line with the conclusions of the "Scientific Opinion on the presence of dioxins (PCDD/Fs) and dioxin-like PCBs (DL-PCBs) in commercially available foods for infants and young children" [5], which demands that "sensitivity of the analytical methods should be improved, if lower MLs were to be considered in the future". Additionally regarding the analysis of food for infants and young children it is referred to the document "Enforceability of the proposed maximum and action levels for PCDD/Fs and dioxin-like PCBs in foods for infants and young children (CRL for Dioxins and PCBs in Feed and Food, 1 December 2008)".

In the framework of the adaption of maximum and action levels also the importance of contribution to the certain food matrices should be considered. Important sources for intake of PCDD/PCDFs and/or PCBs from food are amongst others fish, beef and free range eggs. These matrices could be priorities for adjustments.

For **feed** only some matrices currently show options for a possible considerable lowering of maximum and action levels. This includes fish oil and fish protein (>20% fat). In most other cases maximum and action levels are already quite close to the analytical limitations (taking into account the criterion "LOQ shall be about one fifth of the maximum level"). This relatively low margin between LOQs and legal limits could be due to the requirements for use of 10 g of sample for determination of PCDD/PCDFs and PCBs in the standard EN 16215 [6]. Therefore, further improvement of LOQs and lowering of the working range could be achieved by increasing the sample intake.

2. Task 2: Possible amendments of analytical requirements

The most important criteria for reliable results from confirmatory or screening methods are false-compliant rate and repeatability for screening methods and trueness for confirmatory methods, furthermore intermediate precision for both screening and confirmatory methods, set as follows:

	Screening with bioanalytical or physico-chemical methods	Confirmatory methods
False-compliant rate	< 5 %	
Trueness		- 20 % to + 20 %
Repeatability (RSD_r)	< 20 %	
Intermediate precision (RSD_R)	< 25 %	< 15 %

All other parameters are mainly "supporting" parameters which should help to make sure that these decisive parameters are met. Therefore, a modification e.g. of acceptable blank contributions or wider acceptable ranges between lower and upper bound parameters or any other detailed parameters would increase the risk that results are not reliable. Furthermore, they are important parameters in particular for beginners which did not have the opportunity to demonstrate the reliability of their analyses in numerous proficiency tests. Therefore, no significant options are seen to modify the analytical requirements without risking the reliability of the results.

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