CAPE Technologies

Technical Note TN-008

Technical Considerations for TEQ Analysis of Foods and Related Samples

Overview of the immunoassay method for analysis of dioxin/furan/dioxin-like PCB TEQ

Immunoassay analysis of any sample for dioxin/furan/dioxin-like PCB TEQ is comprised of 3 stages which can be considered separately. These are: 1) sample extraction, 2) extract cleanup, and 3) immunoassay analysis of the cleaned and concentrated extract. All samples analyzed using the CAPE Technologies Dioxin/Furan and Dioxin-like PCB Immunoassay Kits are processed to the same sample preparation endpoint for introduction to the immunoassay. The difference between sample types is in the sample preparation procedure, not the immunoassay procedure. This is summarized graphically in the flow chart shown in Figure 1.

The sample preparation procedure varies significantly among sample types at the beginning (the extraction). The next step, the oxidative portion of the extract cleanup, is similar for all sample types, varying mostly by degree of pretreatment and volume of solvent required to process the sample through the cleanup. The final cleanup step, the carbon column adsorption, is nearly identical for all samples, varying only in the amount and possibly the composition of the solvent mixture passed through the column. Because dioxin and related compounds bind very tightly to the carbon column in the presence of the solvents used for column loading, the exact volumes of these solvents do not matter and a variety of samples can be prepared using the same column design and protocol. Thus, when a sample is eluted from the carbon column, the last step before immunoassay analysis, all samples are treated identically (except for possibly using different dilution levels).

Because dioxin-like PCBs are contained in the fraction eluted from the carbon column just before the dioxin/furan fraction, it is simple to prepare any type of sample for both immunoassays using one portion of sample. This allows determination of the separate TEQ contributions from dioxin/furan and from dioxin-like PCBs, as well as the total TEQ by addition of those two results. Technical Note TN-005 provides more detail on the carbon column fractionation procedure, including GC-MS based congener profiles of the fractions.

Application of the immunoassay method to TEQ analysis of foods and food related samples

Application Note AN-008 describes soil and sediment sample preparation, which is standardized for all soil and sediments. In contrast, the variation among foods and food related samples is significant and requires several different approaches for proper processing of samples. Individual application notes are under development for a variety of foods and food related samples, but this Technical Note will provide an overview of those varied sample preparation methods.

Food and food related samples are extracted in whatever way is appropriate for that sample type. Figure 1 shows 4 different boxes (A-D) in the extraction portion of the flow chart. This group of methods is not intended to be all-inclusive, but does apply to the majority of samples commonly analyzed for dioxin/furan/dl-PCB TEQ.

Box A applies to grains, feeds, minerals used as feed additives, and similar samples with low levels of lipid (or possibly industrial waste oil) that can be removed easily from the sample matrix without acid digestion. Extraction is typically performed with hexane or a mixture of hexane and dichloromethane (DCM).

Box B applies to meat, fish, shellfish, and similar samples requiring total tissue disruption for complete analyte recovery. The hydrochloric acid hydrolysate of the tissue sample is extracted with hexane or hexane-DCM. The recovered lipid represents a common starting point for the oxidative portion of the extract cleanup.

Box C applies to milk and liquid milk products, which are a special case. Any extraction procedure can be used which provides complete recovery of the total lipid fraction. As for tissue samples (B above), the extracted lipid represents a common starting point for the oxidative portion of the extract cleanup.

Box D applies to edible oil samples which are essentially extracted lipid already and therefore do not require extraction during the sample preparation. These samples can be diluted with hexane and DCM and taken directly into the oxidative portion of the cleanup procedure.

All the sample extracts or diluted samples from procedures A through D then enter the oxidative pretreatment portion of the cleanup. The primary differences here are in the volume and composition of solvent required to perform the pretreatment and wash the resulting acid silica. Sufficient oxidative pretreatment is performed so that the solvent supernatant is clear and colorless. At this point the pretreated extract is applied to the coupled column system and pressurized to maintain a flow rate of 1 to 2 mL/min. The acid silica from the pretreatment is washed with two successive aliquots of solvent, which are added sequentially to the coupled column system. If the acid silica column shows unused oxidative capacity at the end of sample processing, then the oxidative part of the cleanup is deemed to be complete, pending acceptable results for quality assurance samples.

Practical implications for TEQ analysis of foods and food related samples

Several important implications of Figure 1 are listed below. Product codes are indicated for items available from CAPE Technologies. Consult the Application Note or CAPE Technologies for more specific recommendations.

1) The same immunoassay kits and standards are used for all sample types.

2) The same pipettors and immunoassay readers are used for all sample types (per Equipment List EL-001).

3) Analysis of only dioxin/furan TEQ or only dl-PCB TEQ requires the same amount of sample preparation work; adding the second immunoassay for complete D/F/dl-PCB TEQ analysis incurs only the additional cost of the second immunoassay kit. This applies to all sample types.

4) All food and food related samples use the same disposable materials for the extract cleanup. This includes:
   A) FAS-250 bulk fine acid silica for extract pretreatment (250 g bottle)
   B) SP5 Sample Preparation Kit (25 mm diameter acid silica columns plus carbon columns)

5) All food and food related samples use the same non-disposable hardware for both pretreatment and column portions of the cleanup. This includes (all available from CAPE Technologies):
   A) SF-FAS-1000 polyethylene separatory funnel (1000 mL) modified for acid silica addition to extracts
   B) SP2-RK rack for holding acid silica/carbon column sets
   C) SP3-ST Sample Preparation Starter Kit (pressure manifold system)
   D) PC25 pressure caps for 25 mm diameter acid silica columns (not included in SP3-ST)
   E) NF25 needle funnels for addition of sample or solvent when using pressure manifold

6) Disposables for sample extraction and oxidative pretreatment may vary, but the following list includes the three containers used most commonly at CAPE Technologies (two containers per sample, one for each step). All are borosilicate glass and have teflon lined caps to minimize adsorptive loss of dioxin during sample processing. Non-teflon lined caps can cause significant reductions in recovery of dioxins and should be avoided. This list is provided for customer reference in the expectation that customers outside North America may wish to purchase these items domestically to avoid the high cost of transoceanic shipping of glassware. Customers may also request a quote from CAPE Technologies on these items. Reference prices below are list prices from Fisher Scientific via their web site (fishersci.com) and do not include shipping, duties, or taxes.

container	Fishersci.com part number	case quantity	approx. list cost per bottle/vial (USD on 010409)
250 mL clear nm bottles	05-719-165	cs of 12	$2.10
125 mL clear nm bottles	05-719-161	cs of 12	$2.10
40 mL vials	03-391-7G	cs of 100	$0.90

7) The same equipment as shown in Equipment List EL-002 will be adequate for nearly all sample types. Individual application notes, when available, will provide more detailed information.

Special note on quality assurance (QA) in TEQ analysis of foods and food related samples

Quality assurance (QA) samples for monitoring the entire analytical procedure should match the type of sample being analyzed. Because of the low detection limits usually required for food analysis, CAPE Technologies in house QA generally includes analysis of each sample as a spiked/unspiked pair, with the spike level being at most 2x the target level (for example 1 pg/g spike for grain screening at 0.5 pg/g). Occasional additional spikes at 3x the original spike level are also used to verify an acceptable dose-response relationship. This approach is highly recommended for customers performing the analysis in their own labs. Spiking solutions for this purpose, including both dioxin/furan and dl-PCB, are available from CAPE Technologies.

Figure 1

Analysis of Dioxin/Furan and Dioxin-like PCB TEQ by Immunoassay

Overview of Sample Preparation Procedure and Interface with Immunoassay Procedure

Sample sizes for soils and sediments are typically 5 g, providing detection limits of roughly 5 to 10 pg/g. Sample sizes for feeds and feed related samples can range to 50 g, providing detection limits as low as 0.5 pg/g. Both methods are based on extraction of 25 to 100 pg of analyte for immunoassay analysis.