Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Analysis of Dioxins/Furans/PCBs in Feeds at CFIA Calgary Laboratory

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Agenda

- Background & History
- Method Development & Validation
- Analysis of Feeds at the Calgary Lab
- Summary
- Acknowledgements

CFIA Business Lines

- Food Safety - Mitigate risks for food safety and ensures health & safety for Canadians and for export purposes
- Animal Health – Protect and ensures the health of animals in Canada
- Plant Health – Protect and ensures the health of forests, plants and crops in Canada

CFIA Labs

- Food Safety Labs - Calgary, Burnaby, Saskatoon, Greater Toronto Area (GTA), Longueil, Ottawa Lab- Carling, Dartmouth, St. Hyacinthe

- Animal Health Labs -Winnipeg, Lethbridge
- Plant Health Labs - Charlottetown, Sydney, Ottawa Lab - Fallowfield

Calgary Lab Testing

Microbiology

- Analysis of food products and related samples for detection of disease-causing and risk-indicating micro-organisms.
- Test for bacterial toxin genes and for the ability of food to support bacterial growth.
- Accredited for performing all national food testing for *E. coli* O157:H7 and *Campylobacter* bacteria.
- Analysis of cooked and raw meats to confirm species identification

Pesticide residues

- Analysis of fruit, vegetables, fertilizers, nuts & seeds, fish and animal feeds for over 500 pesticide residues.

Environmental contaminants

- Analysis of animal-based foods, milks, fish, fertilizers and animal feeds for various persistent organic pollutants (substances that are toxic and accumulate in the environment and animals). Some of the substances tested for include dioxins, furans, PCBs and PAHs.

Research and development

- Develop and implement new analytical chemistry methods for food safety testing.

Quality management

- All CFIA laboratories have demonstrated conformance to ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*. The Standards Council of Canada (SCC) evaluates our laboratories against this international standard, as a formal verification of the CFIA's capability to produce accurate and reliable results, within our accredited scope of testing.

Reference; https://inspection.canada.ca/science-and-research/our-laboratories/calgary/eng/1549643819070/1549643933428

Investigations & Method Development Projects: Examples of some Investigations at Calgary Lab

CFIA Labs may be involved in local, national and international investigations and incidents, when expertise is needed for testing of contaminants

- Agricultural spills impacting rivers (pesticides in fish, food)
- Medicated feed contamination (antibiotics in eggs)
- Industrial incidents (dioxin in oils)
- Food Production issues (MCPD in soya sauce)
- Food fraud (melamine in baby formulae)
- E.coli O157 in beef
- E.coli O121 in flour
- Dioxins, Furans & PCBs in seal blubber for export purposes (2011)
- Pesticides, Dioxins, Furans & PCBs in seal meat (muscle and liver) for export purposes (2018)
- Dioxins, Furans & PCBs in Cauliflower (2020)

Feed - Dioxins/Furans/PCBs investigation at Calgary Lab

2004 -2005

Tested feed samples and found high levels of PCB 126
The copper sulphate added to the feed was issue
After investigation, it was found that the process to burn wires to recuperate copper was the issue

2007-2008 and 2021 - 2022

Tested milk and feed samples and found high level of octa-dioxin (OCDD)
After investigation, it was found that the treated lumber near the silage was the issue

Method Validation – Nested Experimental Design

Method Performance Parameters

Selectivity (Specificity)

Trueness (only if there is a CRM)

Limit of Detection (LOD)

Limit of Quantification (LOQ)

Decision Limit (CCα) only if MRL exists

Detection capability (CCβ)

Precision (Reproducibility)

Recovery

Uncertainty

Stability

Robustness (optional)

Participation in Proficiency Testing (PT) or Blind Spike

Reference: CL SOP 10 V3: *"Method Validation using Nested Experimental Design"*

- The nested design approach is a series of fortification experiments carried out at various concentration levels in several matrix samples over two days or analysts in triplets.
- Nested Experiment Scenarios

	Scenarios						
	A	B	C	D	E	F	G
fortification levels (excluding blank)	4	4	4	4	4	2	2
matrices	5	4	5	6	6	2	2
repeat batches (on different days or with different analysts)	2	2	2	2	2	2	2
replicate spikes at each level/batch	2	3	3	2	3	2	3
Total number of spikes for uncertainty	80	96	120	96	144	16	24
# of days or batches to complete	10	8	10	12	12	4	4
# of blanks per batch	2	3	3	2	3	2	2

For example in Scenario B, The number of extractions or fortifications required to is:

fortification levels x matrices x (days or analysts) x replicates =

4 X 4 X 2 X 3 = 96

- Data processing is done by using SAS software computerized ANOVA analysis.

Animal Feed Analysis

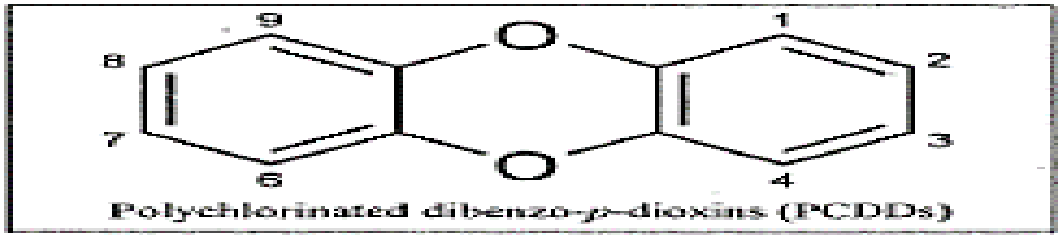
- Feed and Feed Additives extend across a wide and diverse range of livestock feed ingredients such as:
  - fish meal,
  - fish oil,
  - fish feed,
  - minerals,
  - mineral complexes,
  - macropremixes,
  - anti-caking agents,
  - vegetable oils (palm oils, hydrogenated vegetable (palm) oils)
  - By-products of vegetable oils manufacturing (calcium salts of fatty acids, fractionated palm fatty acid distillates, hydrogenated palm fatty acid distillates, Palm palmitic acid).

References: RG-8 Regulatory Guidance: Contaminants in Feed, Section 2
CFIA Calgary Laboratory Analytical Method EC-001-V8.0

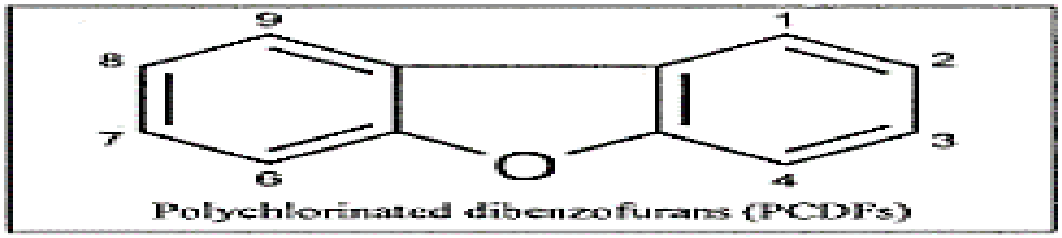
Dioxin, Furan & Dioxin-Like PCB Compounds of Interest

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are referred to simply as “dioxins”.

- Produced unintentionally as unwanted by-products.
- Lipophilic and bio accumulate.
- Concentrations are expressed in toxic equivalents (TEQ) of the most toxic congener (2,3,7,8-TCDD).
- There are 17 congeners that are of toxicological concern.



Polychlorinated dibenzo-*p*-dioxins (PCDDs)

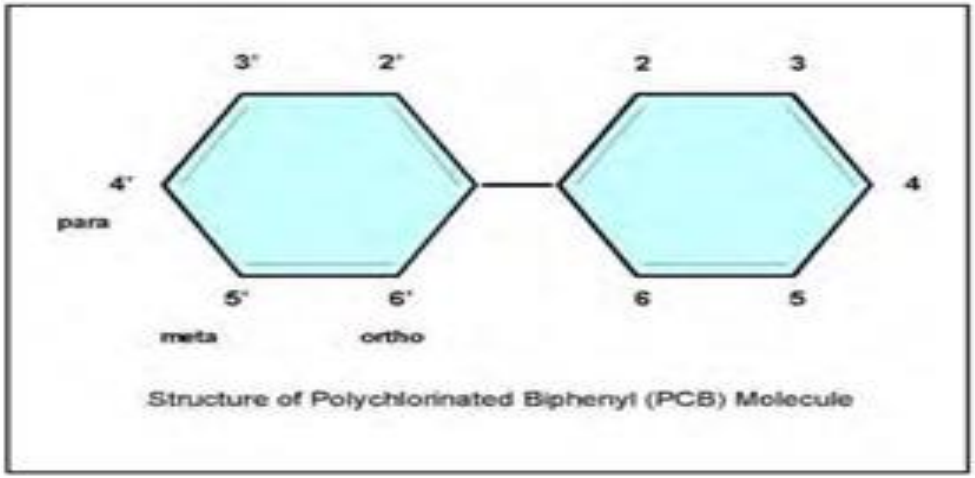


Polychlorinated dibenzofurans (PCDFs)

References: RG-8 Regulatory Guidance: Contaminants in Feed, Section 2
Fact Sheet: Dioxins & Furans, PSCP Canada V1.2.1; https://gost.tpsgc-pwgsc.gc.ca/Contfs.aspx?ID=66&lang=eng
Polychlorinated Biphenyl (PCB) Total Maximum Daily Load (TMDL) Handbook, EPA 841-F-11-006, December 2011

Dioxin, Furan & Dioxin-Like PCB Compounds of Interest

- Some Polychlorinated biphenyls (PCBs) congeners are considered to be “dioxin-like” because of their chemical structure and biological activity.
- Intentionally manufactured for use in industrial applications.
- Lipophilic and bio accumulate.
- The most toxic dioxin-like PCBs can also be expressed in toxic equivalents (TEQ).
- The Calgary Laboratory reports 12 of these toxic congeners, as well as 6 Marker PCBs, which are the most common and are used to assess overall contamination level



Dioxin, Furan & PCB Limits in Feed

Livestock Feed Ingredient	Action Level (ng WHO-TEQ/kg)
Fish Meal	3
Fish Oil	16
Fish Feed	6
Minerals, Mineral Complexes, Macropremixes, and Anti-caking Agents	1.5
Vegetable Oils	0.75 (dioxins and furans only)
By-products of Vegetable Oil Manufacturing	1.5

References: RG-8 Regulatory Guidance: Contaminants in Feed, Section 2
CFIA Calgary Laboratory Analytical Method EC-001-V8.0

Background Contamination Reduction

- All labware and equipment is pre-cleaned twice with each of 3 solvents prior to use to reduce background contamination.
- All reagents and solvents are High Resolution Grade (or equivalent).
- All substrates, glass fibre filters, glass wool, etc are cleaned with solvents before used.
- Only aluminum foil and dust-free wipes are used to cover surfaces and equipment to keep cleaned items free from dust.
- A strict cleaning schedule of the entire laboratory and its equipment helps ensure that background levels stay within acceptable limits.

Reference: CFIA Calgary Laboratory Analytical Method EC-001-V8.0

Sample Extraction: Quality Assurance

Samples are analyzed using Isotope Dilution.

- This is done by adding isotopically-labelled analytes (surrogates) prior to extraction.
- The analytes are quantitated using a ratio of labelled:unlabelled compound.
- This ensures the most accurate results regardless of variation in sample matrix or extraction.

The Calgary Laboratory evaluates each batch based on the following Quality Controls:

- Reagent Blank – to assess background levels using Shewart Control Charts using 95% Confidence Intervals.
- Certified Reference Material (CRM) – Purchased from Wellington Laboratories (ISO 9001:2015, ISO/IEC 17025:2017 and ISO 17034:2016 accredited).
- A typical batch size for Feed Analysis consists of these control samples plus 8 samples for a total batch size of 10 extractions.

Sample Extraction: Method

- Feed & feed additive samples are extracted using soxhlet with a solvent mixture of 70:30 Ethanol: Toluene using Ottawa sand as the support substrate.
- Samples are then solvent exchanged and water is removed with sodium sulfate.
- Samples are then concentrated by rotary evaporation

- Hydrogenated palm oils are dissolved in hexane using heat and sonication. The sample is then passed through 40g of 44% acidified silica to remove large amounts of unwanted lipids. Samples are then solvent exchanged and then concentrated by rotary evaporation
- Liquid Oil samples are diluted in hexane.

Reference: CFIA Calgary Laboratory Analytical Method EC-001-V8.0

Sample extraction:

Sample Clean-up and Final Extract Preparation

Semi-automated sample clean-up is performed using the Econoprep from Fluid Management Systems Inc.

- Removes interferences from sample extracts.
- Separates the analytes of interest into 2 fractions for GC/HRMS analysis.
- Uses a high-capacity acidified silica column to oxidize fat and other compounds.
- An alumina column retains compounds of interest while hexane washes interferences away.
- A carbon/celite column separates the dioxins/furans/coplanar PCBs from the WHO-PCBs/Marker-PCBs. These are eluted into 2 different fractions.

These two fractions are then concentrated and evaporated to dryness.

Each sample is diluted to a final volume of 20µL with Internal Standard Solution in Nonane.

References: CFIA Calgary Laboratory Analytical Method EC-001-V8.0
CFIA Calgary Laboratory ECU SOP 15 V5.0

GC - High Resolution Mass Spectrometry (HRMS) Using the Thermo DFS

Sample Analysis is performed on the Thermo DFS Magnetic Sector High Resolution Mass Spectrometer with dual GCs. The Dioxin/Furan/Coplanar-PCB fraction is injected on one GC and the WHO-PCBs/Marker-PCB fraction is injected on the other GC.

Food import-export control requires compliance with numerous government and analytical methods. The DFS Magnetic Sector GC-HRMS is the only GC-MS compliant with any existing official methods and regulations, including US EPA, EU, and JIS methods

<u>Thermo DFS Performance characteristics</u>

- Sensitivity (EI GC/MS): >200:1 for 20 fg 2378TCDD at m/z 322, R = 10,000, S/N=PtP 4SD.
- Mass accuracy <2 ppm
- Resolution (static) >60,000 (10% valley)
- Scan rates 0.1 to 10,000 seconds/decade (continuously variable)
- Mass range 2 – 6000 Da; 2-1200 Da at full accelerating voltage.

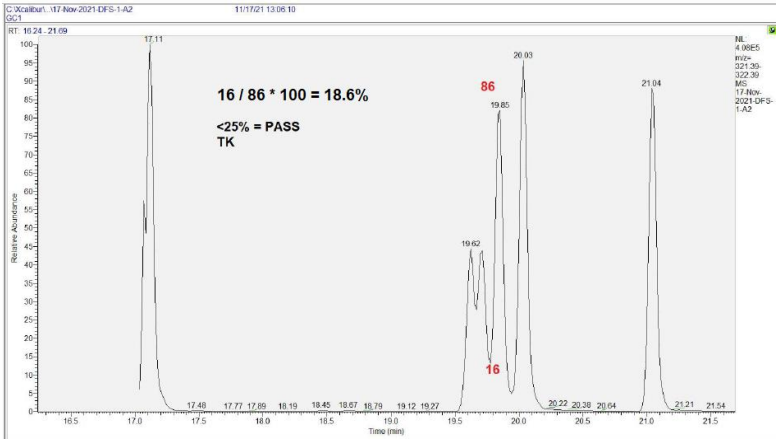
References: CFIA Calgary Laboratory Analytical Method EC-001-V8.0
ThermoFisher Document PS30096-EN 0121

DFS Quality Control Criteria

Mass Resolution – the instrument must maintain a minimum mass resolution of ≥10,000 throughout the run.

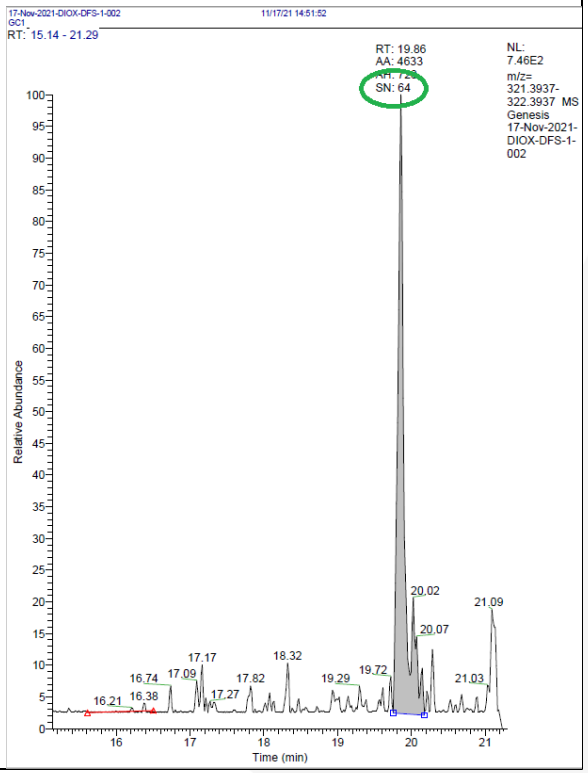
Column Resolution

The GC must resolve the isomers 2,3,7,8-TCDD (analyte of interest) and 1,2,3,8-TCDD (not of interest) at ≤25% height resolution.



Sensitivity

The Signal:Noise ratio of 2,3,7,8-TCDD peak in the 0.05ppb standard must be ≥15.



Data Analysis

The Calgary Laboratory adheres to the GC-HRMS Acceptance Criteria defined in EPA Standard Method 1613B for Dioxins/Furans, and EPA Standard Method 1668C for PCB analysis. This includes the following criteria:

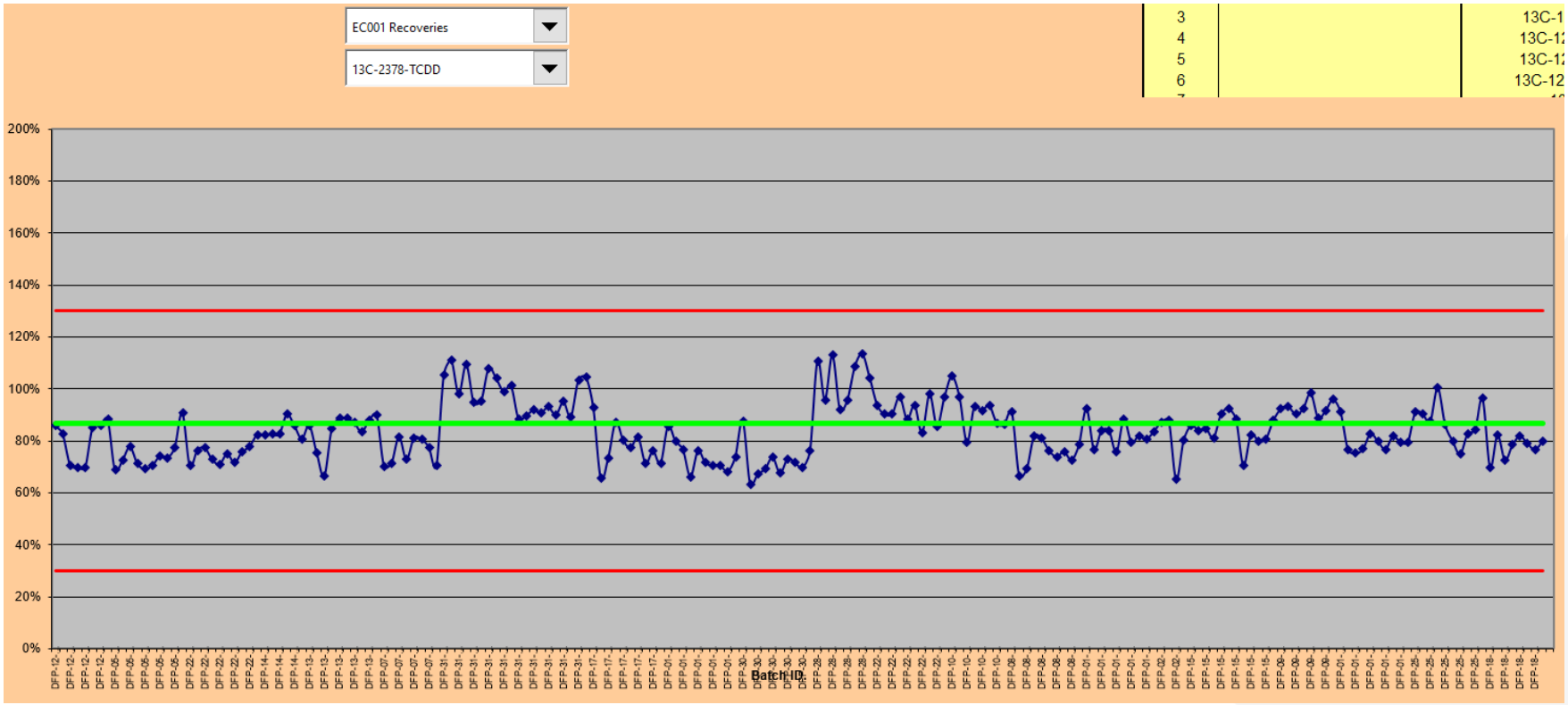
- Continuing Calibration Verification (CCV) standards must meet EPA1613B and 1668C criteria.
- Retention time for each analyte must be within 0.1 min of the retention time relative to their labelled isotopic analogs.
- Ion ratios within 15% of the theoretical value for compound identification.
- Surrogate recoveries between 30-130%.
- Instrument Mass Resolution ≥10,000
- Additional Criteria are:
    - Correlation Coefficients are ≥0.999 for five point calibration curve
    - Certified Reference Material (CRM) - contains 17 certified Dioxin and Furan (DFs) compounds and 12 certified PCBs; results must pass and uncertified PCB compounds are also evaluated and be within the charting limits

References: CFIA Calgary Laboratory Analytical Method EC-001-V8.0
United States Environmental Protection Agency, Method 1613B, October 1994
United States Environmental Protection Agency, Method 1668C, April 2010

^{13}C Surrogate Recoveries

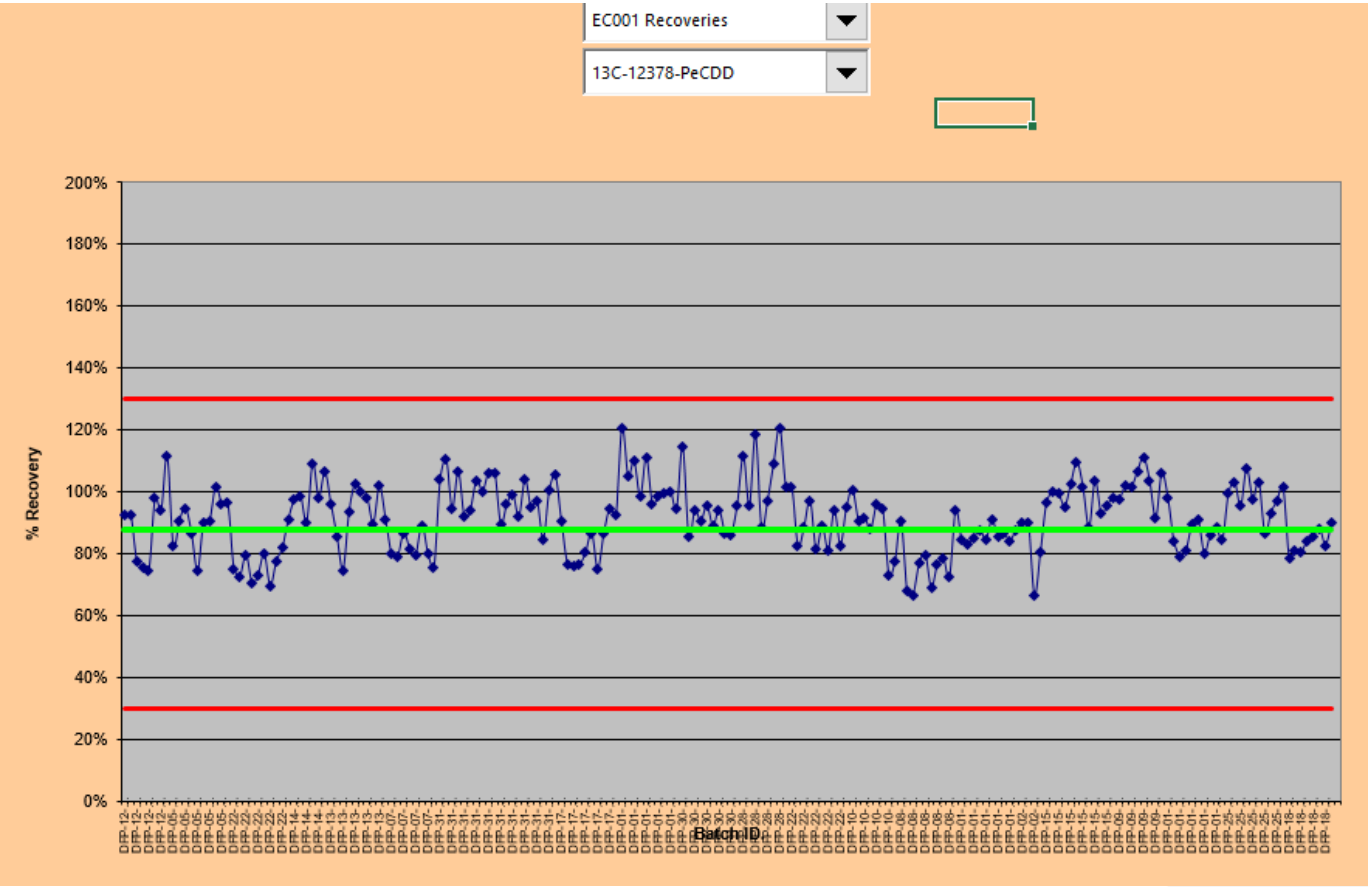
^{13}C Surrogates are added to Reagent Blanks, CRMs and samples. Recoveries are plotted on control charts This data is used to detect trends and to review historical data.

$^{13}C_{12}$-2,3,7,8-TCDD Surrogate Recoveries



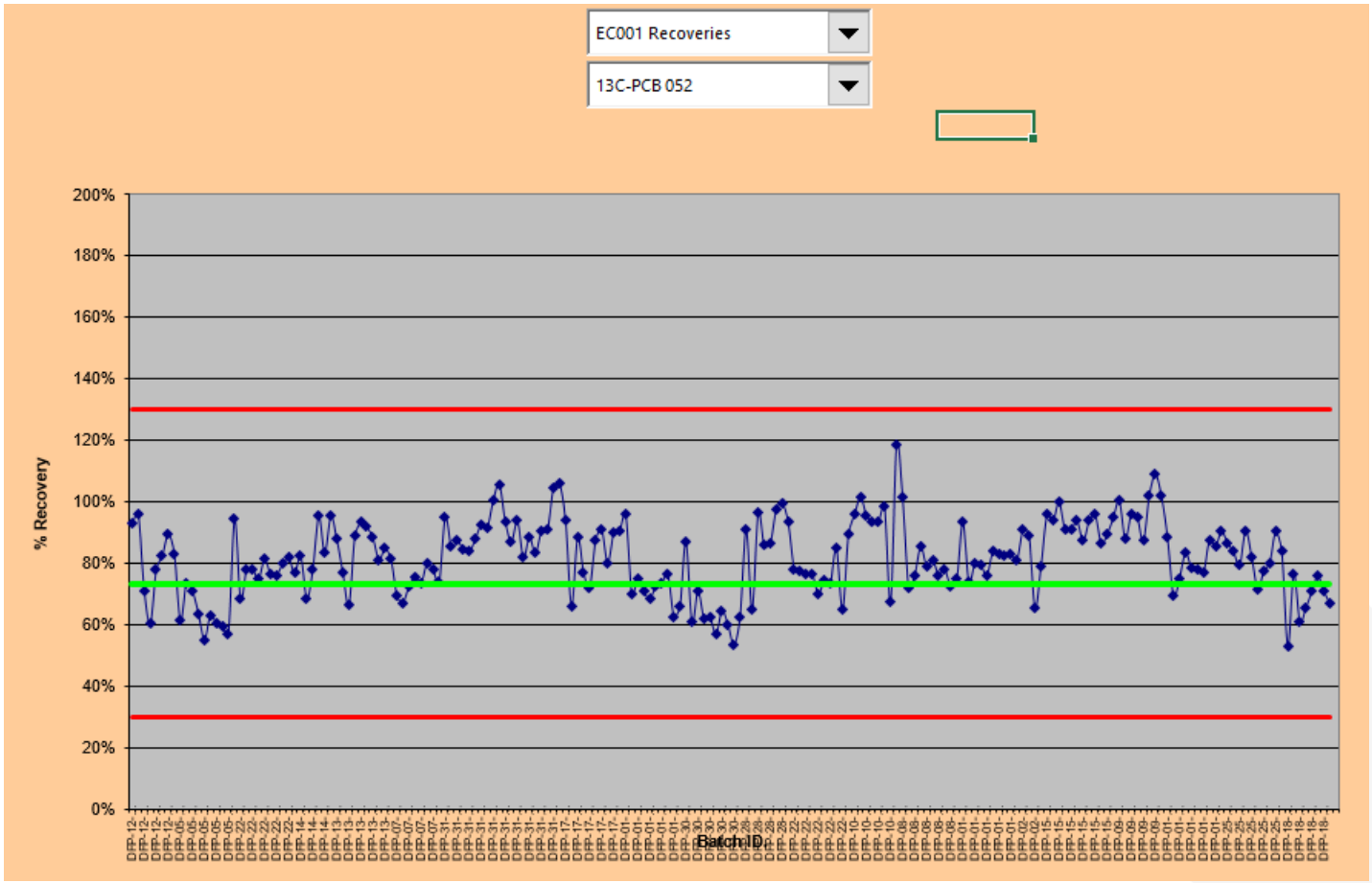
^{13}C Surrogate Recoveries

$^{13}C_{12}$-1,2,3,7,8-PeCDD Surrogate Recoveries



^{13}C Surrogate Recoveries

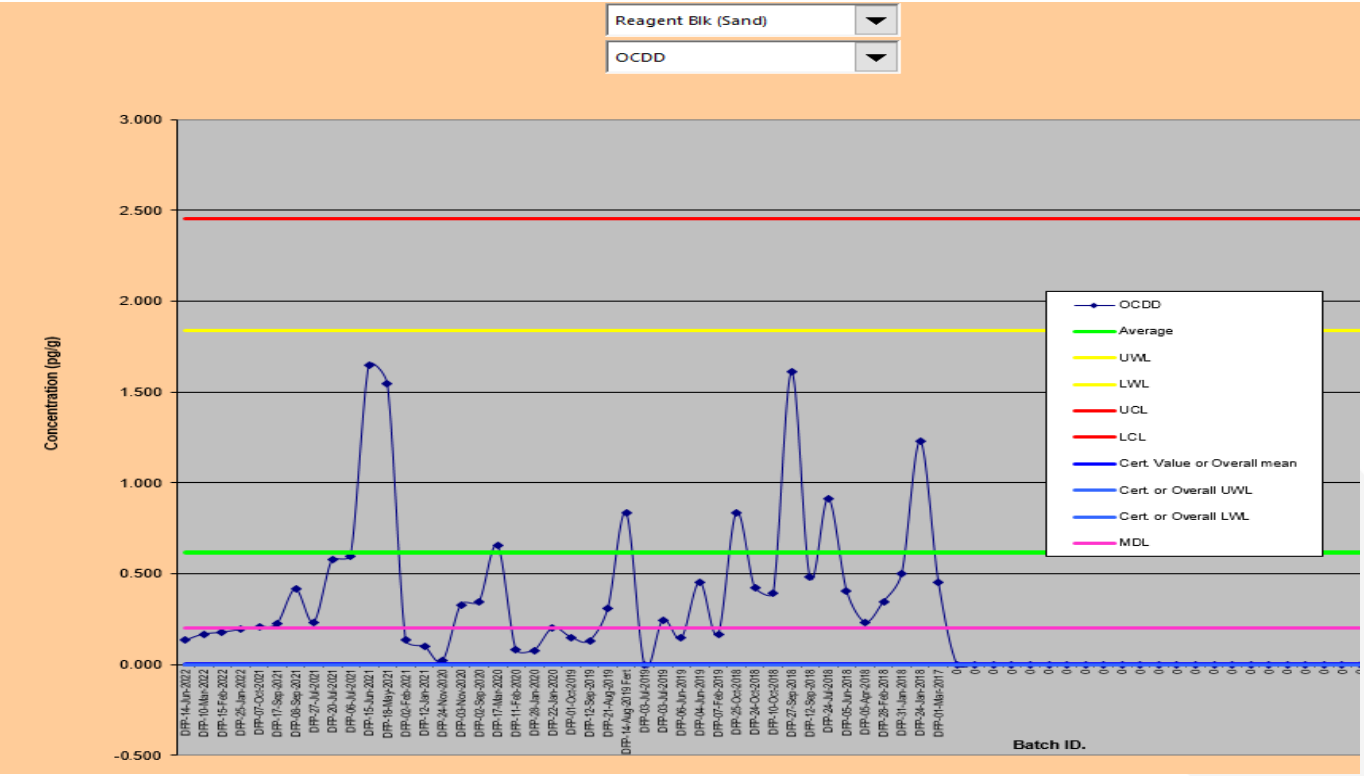
Marker PCB: $^{13}C_{12}$-PCB 52 Surrogate Recoveries



Reagent Blank

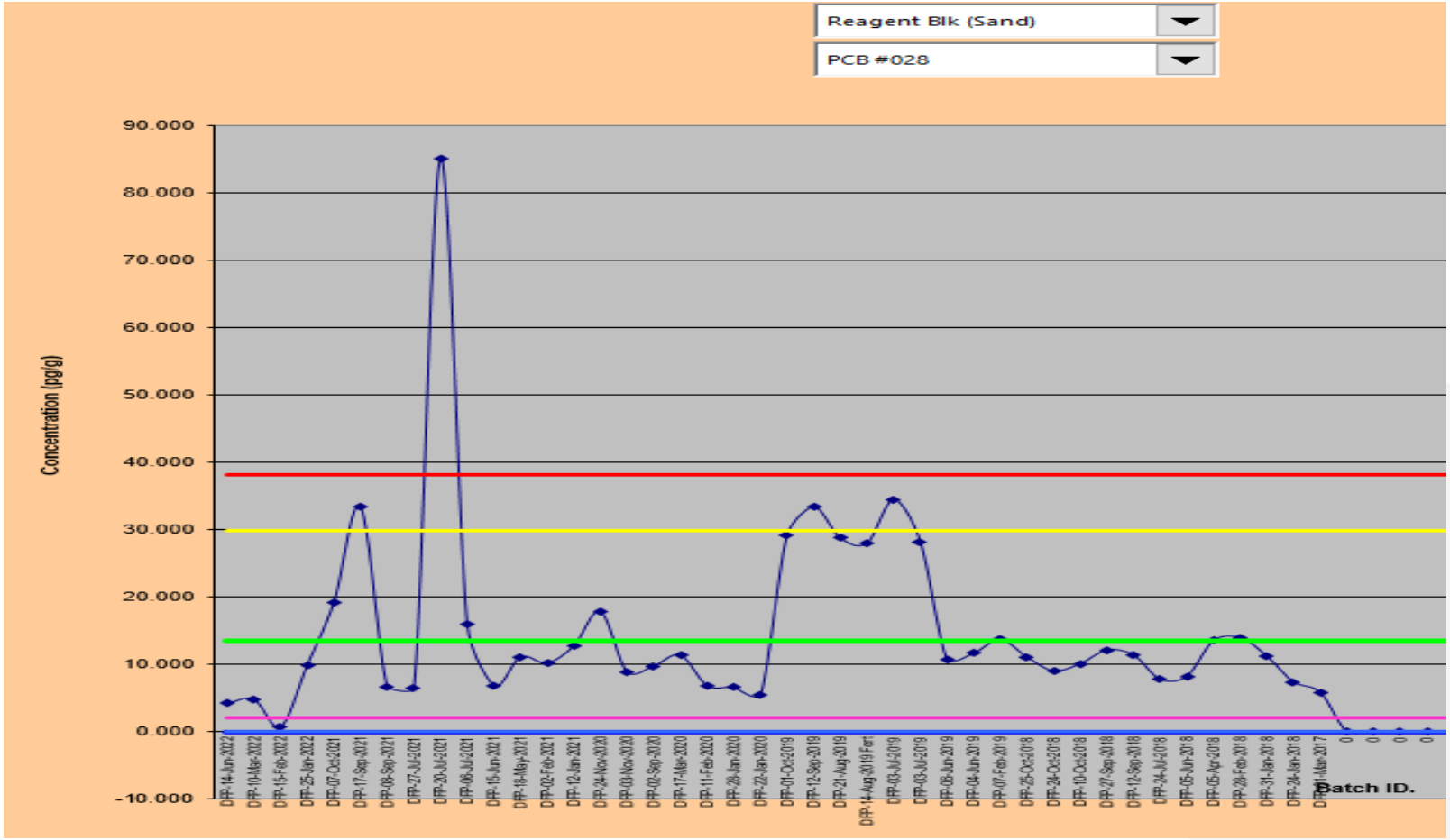
All analytes are plotted on control charts to determine if the Reagent Blank (RB) is acceptable for the batch, based on 95% confidence intervals.

OCDD (in pg/g) in Reagent Blank - Feed Method



Reagent Blank

Marker PCB-028 (in pg/g) in Reagent Blank - Feed Method



Result Reporting

The concentration (in ng/kg) of each Dioxin, Furan and Dioxin-like PCB is multiplied by its respective Toxic Equivalency Factor (TEF), and the resulting concentrations are summed to estimate a 2,3,7,8-TCDD toxic equivalent concentration (as ngTEQ/kg).

Total TEQ (ng/kg) = sum of {[each PCDD] x TEF + [each PCDF] x TEF + [each PCB] x TEF}

Of particular interest to regulators are 3 values which are calculated in different ways:

- TEQ-Lower Bound
- TEQ-Upper Bound
- EMPC-Upper Bound

Table 5a: Dioxins, Furans and PCBs Toxic Equivalency Factors (TEF)

Analyte	2005 WHO TEF
2378-TCDF	0.1
12378-PeCDF	0.03
23478-PeCDF	0.3
123478-HxCDF	0.1
123678-HxCDF	0.1
234678-HxCDF	0.1
123789-HxCDF	0.1
1234678-HpCDF	0.01
1234789-HpCDF	0.01
OCDF	0.0003
2378-TCDD	1
12378-PeCDD	1
123478-HxCDD	0.1
123678-HxCDD	0.1
123789-HxCDD	0.1
1234678-HpCDD	0.01
OCDD	0.0003
PCB 077	0.0001
PCB 081	0.0003
PCB 126	0.1
PCB 169	0.03
PCB 105	0.00003
PCB 114	0.00003
PCB 118	0.00003
PCB 123	0.00003
PCB 156	0.00003
PCB 157	0.00003
PCB 167	0.00003
PCB 189	0.00003

References: Federal Contaminated site risk assessment in Canada: Toxicological Reference Values (TRVs) VERSION 3.0 . Pub.: 200301
CFIA Calgary Laboratory Analytical Method EC-001-V8.0

Summary

- Calgary Lab tests variety of Feed and other matrices for Dioxins, Furans and PCBs for more than 20 years
- We have strict method validation procedures
- We follow QA/QC protocol before reporting
- Participate annually in different PT programs
- Calgary Lab is accredited by SCC under the ISO/IEC 17025:2017

Thank you

Environmental Contaminants Unit:

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Any Questions

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