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Chemical Residue Testing and the Role of Proficiency Testing Material at the Centre for Veterinary Drug Residues

Submitted by: Canada



**Food Safety Cooperation Forum Partnership
Training Institute Network Proficiency Testing
Workshop
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Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Canadian Food Inspection Agency



Our vision:

To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:

Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Chemical Residue Testing and the Role of Proficiency Testing Material at the Centre for Veterinary Drug Residues

Presenter: Connie Neiser

Canada's National Centre of Expertise for Drug Residue Testing in Foods of Animal Origin

Canada

Introduction:

- CFIA Facts and Figures

CFIA-CVDR Activities:

- Diagnostic Testing
- Method Development
- Proficiency Testing Program

APEC PT review



Canadian Food Inspection Agency

As Canada's largest science-based regulatory enforcement Agency, the CFIA administers and enforces 14 federal Acts and their associated regulations.

CFIA Vision

- To excel as a science-based regulator, trusted and respected by Canadians and the international community.

CFIA Mission

- Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

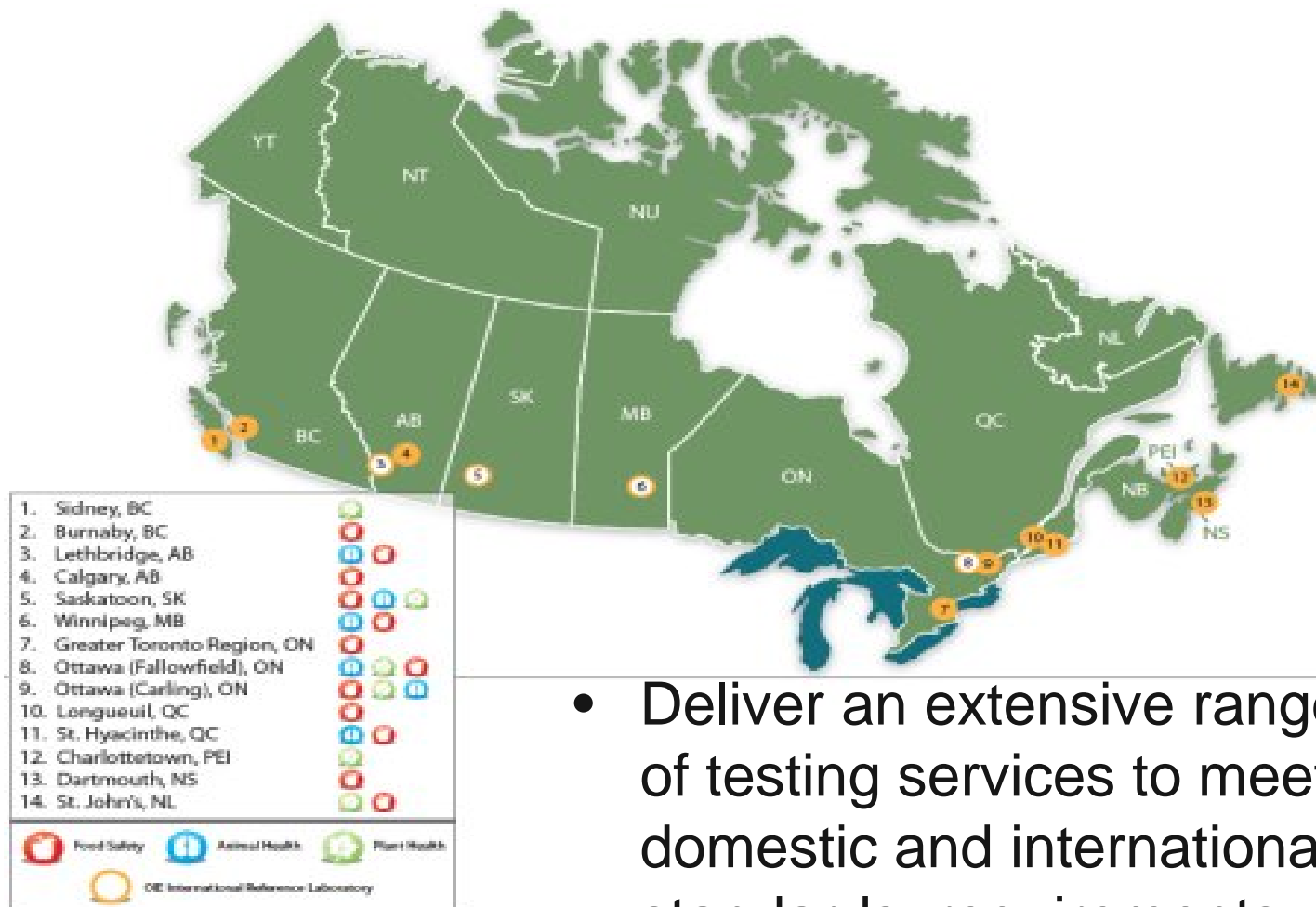


The CFIA includes inspection staff, as well as additional essential positions relating to audit and evaluation, legal, human resources and corporate services.

Inspection staff includes the field inspection staff as well as a variety of other inspection positions such as chemists, risk assessors, supervisors and scientific researchers.

These positions are crucial to the CFIA's inspection and enforcement responsibilities as they conduct tasks such as laboratory testing, food safety investigations and veterinary evaluation.

CFIA Laboratories



- Deliver an extensive range of testing services to meet domestic and international standards, requirements and obligations

CVDR

Centre for Veterinary Drug Residues
Centre de détection de résidus médicamenteux vétérinaires



Method
Development
Non-routine
Testing

Proficiency
Testing



Food Testing





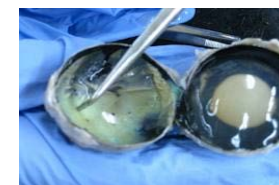
Species

- Cattle
- Swine
- Horse
- Poultry
- “Minor species” (bison, elk, deer, ostrich, etc)



Sample Matrices

- Muscle
- Kidney
- Liver
- Fat
- Retina



Our Scope of Accreditation

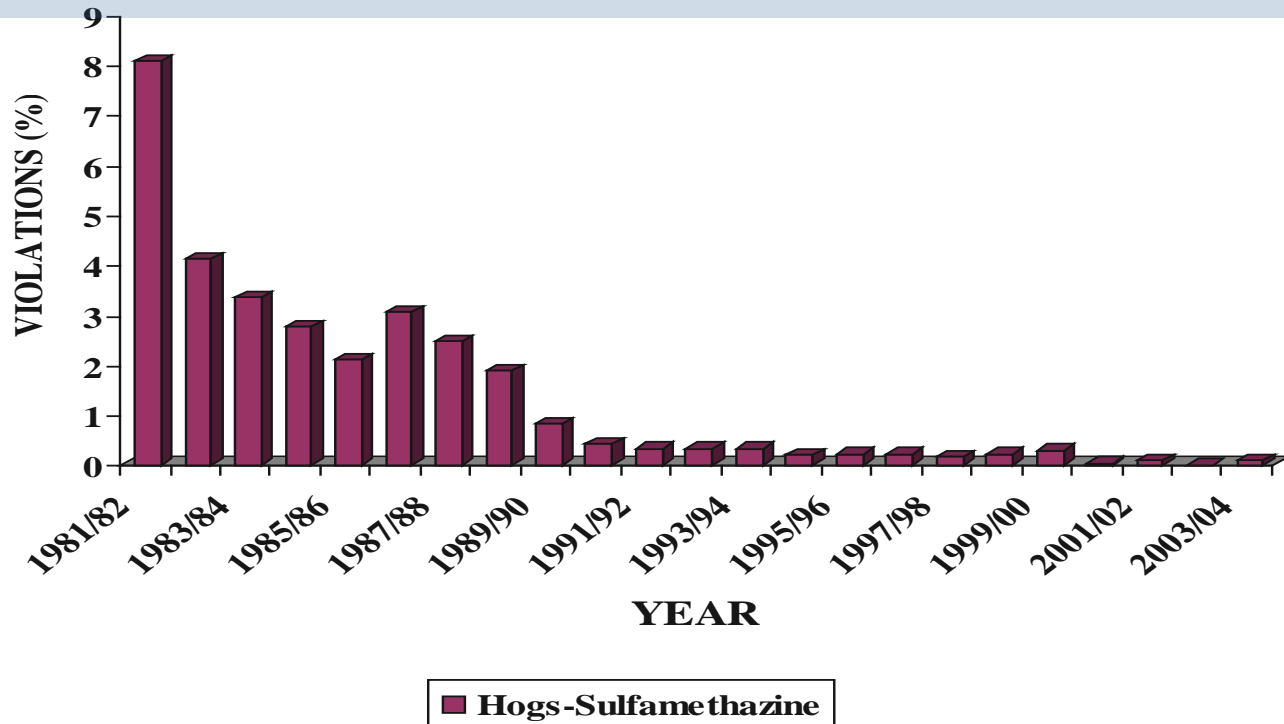
Veterinary drug residues which include the following classes of compounds:

- anthelmintics,
- antibiotics,
- analgesics,
- antimicrobials,
- beta-agonists,
- coccidiostats,
- hormones and hormone-like substances,
- non-steroidal anti-inflammatories,
- tranquilizers and
- pesticides used for veterinary purposes

Food Testing at CVDR supports:

1. Monitoring sampling - probes the food supply for potential contamination.
2. Directed sampling - focuses on identified chemical contamination issues
3. Compliance sampling - seeks removal of food in violation of standards from the marketplace.

Residues - Sulfamethazine



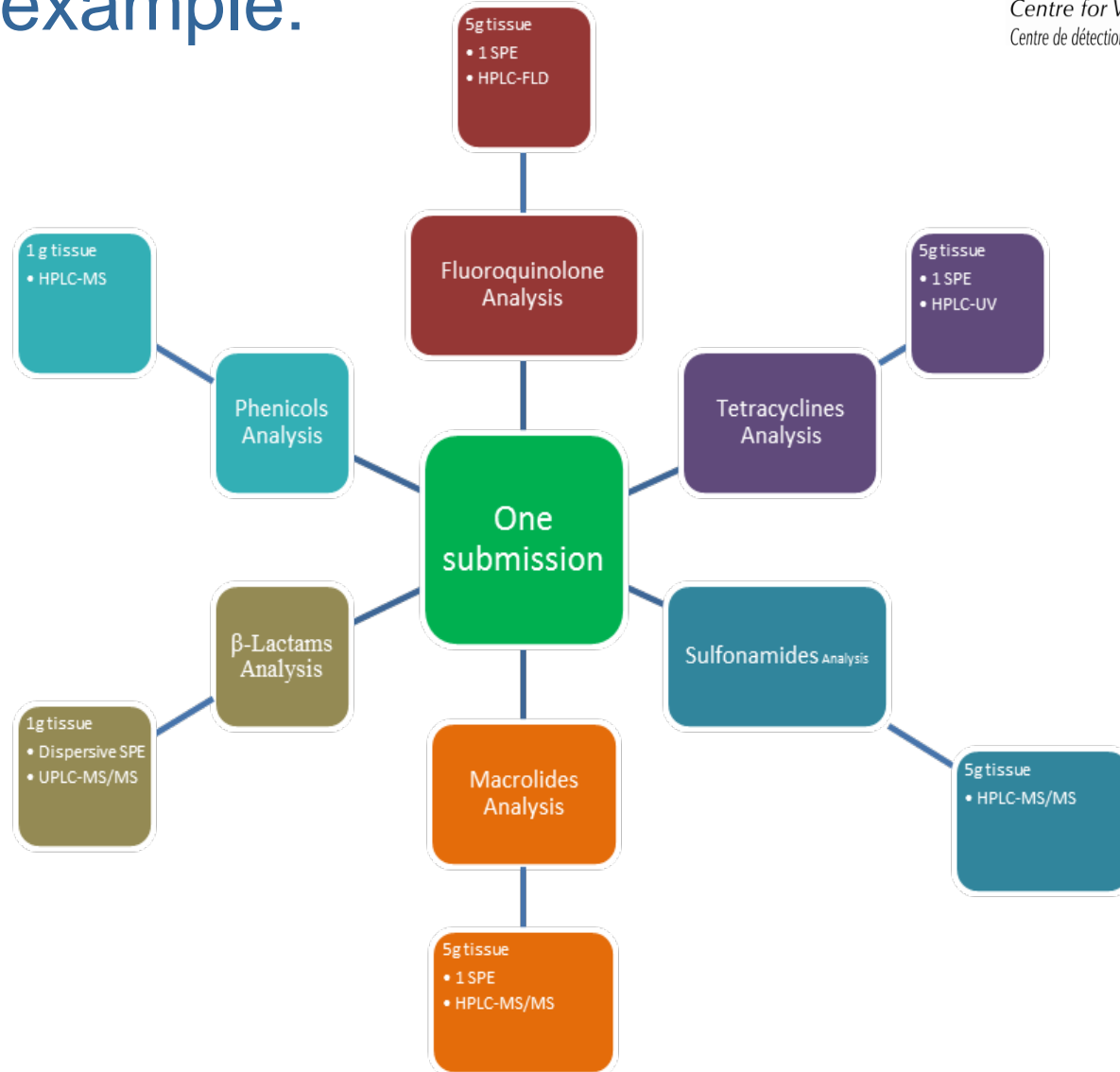
Late 1970 ~ 1980 Trade issue (high violation of Sulfa Drugs on Pork). Directed sampling program has led to better managed sulfa drug use in the porcine industry.

Method Development Activities

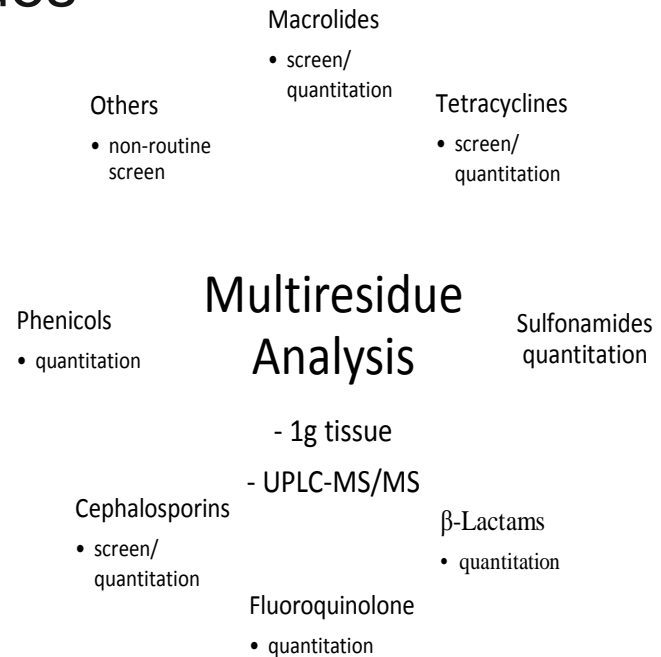
Where a routine test is not available to address an identified need or there is a special request for a non-routine analysis, activities will include:

1. Development and validation of new testing methodology for the screening and determination of veterinary drug residues.
2. Modification, improvement and validation of published or existing test methodology for the screening and determination of veterinary drug residues
3. Development and validation of mass spectral techniques for the confirmation of the identity of veterinary drug residues.

For example:



One method/ One extraction/
One analyst/ Three injections →
60+ veterinary drug residues
screened



Recall Our Scope of Accreditation

Diagnostic Testing in support of Veterinary drug residues which include the following classes of compounds:

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- tranquilizers and
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Available providers of PT material for veterinary drug residues in foods of animal origin

....there isn't many – some options include:

- FAPAS (<http://www.fapas.com/prog.cfm?currsch=fapas>)
- Special Invitations
 - France - Fougères Laboratory; China – CNAS_APEC
- CFIA – Centre for Veterinary Drug Residues, Saskatoon Laboratory

Proficiency Testing Unit

Main Purpose

CVDR's PT Program - To provide PT material and reports for assessing, monitoring and improving the capabilities of laboratories participating in Canada's National Chemical Residue Monitoring Program (NCRMP).

CFIA labs as well as any third party laboratory providing testing in support of CFIA's mandate require accreditation to ISO/IEC 17025, which requires participation in PT schemes to support accredited tests

Proficiency Testing Unit

Structure:

PT Advisory committee

Proficiency Testing Chemist

Technical support

Annually, the PTU sets up a schedule based on NCRMP and Lab capabilities, priorities and regulations.

The schedule is set up such that a subset of residues from within each of 13 veterinary drug classes are assessed 3 times per year.

PT Preparation

Tissue (kidney, liver, muscle) is routinely used and fortified as opposed to incurred sources.

Lyophilized material is preferred for shipment.

Some rounds still use frozen material, as opposed to lyophilized – this is an area for improvement and development.

Results Evaluation

$Z = (x - X) / \sigma$ where

- x = result reported by a participant
- X = the assigned value
- σ = standard deviation

$Z = (x - X) / \sigma$ where

- x = result reported by a participant
- X = the assigned value
- σ = standard deviation

Results Evaluation

Standard deviation

- The value of the standard deviation for proficiency testing is derived from a general model for the reproducibility of the measurement method.
- Horwitz model: $RSD_R (\%) = 2C^{-0.1505}$

where RSD_R = the relative standard deviation among laboratoires

C = analyte concentration in mass/mass units

$$-0.1505 = 0.5 \log_{10} 2$$

<u>Concentration</u>	<u>Pred RSD_R (%)</u>
10 ng/g	33%
1 ng/g	45%

- CFIA/CVDR PT-Program – Range CV% = 22 -33%

Results Evaluation

Z-scores values are interpreted as follows:

$ z < 2$	Satisfactory
$2 < z < 3$	Questionable
$ z > 3$	Unsatisfactory

Summary reports are prepared and distributed by PTU

APEC PT Participation

Summer 2013 - Invitation to participate.

December 2013 - Two freeze-dried chicken muscle samples were received, analysed and reported to APEC PT organizers.

Drugs of Interest in PT samples:

- Nitrofurans
 - 3-amino-2-oxazolidone (AOZ), 5-morpholinomethol-3-amino-2-oxazolidone (AMOZ)
- Fluoroquinolones
 - Ciprofloxacin
- Sulfonamides
 - While the sulfonamides were included in the PT sample, our routine method hadn't been validated for sulfonamides in chicken muscle so those residues were not included in the reported round results.

Methods at Analysis

- Nitrofurans, class-specific method, was used to determine the quantitative levels of AOZ and AMOZ.
- Fluoroquinolones, class specific method, was used to determine the quantitative levels of ciprofloxacin.
- The multi-residue method was used on the PT material in a non-routine manner.
 - The multi-residue method had not validated for chicken muscle at the time – so results were non-routine.
 - The multi-residue screen results were later reviewed relative to the APEC interim PT results summary report for fitness of determinative approach for Ciprofloxacin, Sulfadimidine and Sulfaquinoxaline

APEC PT Participation

Nitrofurans (protein bound) Method Overview:

- Tissue is washed with methanol and ethanol to remove extractable nitrofurans and other material.
- Acid hydrolysis releases the protein bound nitrofurans. Those are derivatized in the presence of hydrochloric acid and 2-nitrobenzaldehyde.
- The derivatized products are extracted from the neutralized extract into ethyl acetate.
- The extract is evaporated, reconstituted in water, washed with hexane, filtered and analysed by LC-MS/MS.

Any residue detected is reported. Limit of Detection (LOD) is nominally set at 0.0005 ppm

Z-scores: AOZ → -1.32 ; -1.77 AMOZ → Z-scores: -1.38 ; -1.27



APEC PT Participation

Fluoroquinolones determinative method overview:

- Acidic ethanol extraction
- Followed with strong cation exchange solid phase extraction column clean up
- The eluted extract is evaporated, reconstituted in 0.1% Formic Acid:Acetonitrile (90:10), filtered and analysed by HPLC with fluorescence detection.

Reporting limit (LOD) = 0.002 ppm

Ciprofloxacin, z-score results: 0.92; -0.45

Post APEC PT – A Review of Multi-Residue capabilities

	Ciprofloxacin		Sulfadimidine		Sulfaquinoxaline	
Results for Samples A and B, ppb	A	B	A	B	A	B
Median ¹ , (APEC report)	78	427	36	123	874	202
Class specific method, reported result (mean) ²	101	406	---	---	---	---
Z-score ²	0.92	-0.45	---	---	---	---
%RD from Median (Class-Specific result)	26%	-5.0%				
Multi-residue, screen, determinative review (mean)	81	424	31	130	687	162
%RD from Median (Multi-residue result)	3.8%	-0.7%	-15%	5.5%	-24%	-22%

Notes:

¹ Estimated from Interim Report; ² As presented in Interim Report

Other Notes:

Sulfamethoxazole – Not included in Multi-residue screening method.

Nitrofurans – Not included in Multi-residue screening method.



PT Participation – A SUCCESS STORY:

1. Successful z-scores for our routine tests.
2. The APEC chicken muscle PT screening results from the multi-class Antimicrobials method has demonstrated fitness of the quantitative approach for the analysis of Ciprofloxacin and the two sulfonamide residues in chicken muscle.

Acknowledgements

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