Residue Chemistry Studies for Veterinary Drugs

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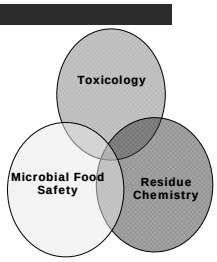
Human Food Safety Assessment

- Risk = Hazard x Exposure
- Toxicology and Microbial Food Safety: identify and characterize the risk of potential adverse health effects
- Residue Chemistry – assess the exposure and mitigate any identified hazard by controlling the exposure with assignment of tolerance and withdrawal period



Presentation Topic

- Improving residue chemistry submissions
  - Focus on tissue residue depletion study for withdrawal period calculation

Tissue Residue Depletion Study - Objective is to establish the withdrawal period

- Conduct a residue depletion study under field conditions
- Use the regulatory method to measure marker residues in edible tissues
- Determine how long it takes the marker residues to deplete to below the tolerance

Before conducting depletion study!

- First establish:
  - ADI
  - marker residue
  - tolerance
  - target tissue
  - validated analytical method (LOQ < tolerance)

Definition of Residue:

- Any compound present in the edible tissues after treatment with the drug.
- Includes parent drug, metabolites, and any substance formed in or on food.
- The definition is broad enough to include resistant bacteria.

Edible Tissues:

- Muscle
- Liver
- Kidney
- Fat/Skin
- Milk
- Eggs

Definitions of Tolerance and Withdrawal Period

- Tolerance – legal safety limit for residues in edible tissues of food animals
- Withdrawal Period – the amount of time between last treatment of the food animal and when residues have depleted to below the tolerance.

Two Parts for Tissue Residue Depletion Study

1. In-Life Portion – Market size animals dosed under field (or field-like) conditions
   - according to proposed label (highest dose, longest duration)
   - sample animals at timepoints after drug is withdrawn
   - GLP-like
2. Analytical Phase – collect and analyze tissues for residue concentrations
   - GLPs

Tissue Residue Depletion Study

Critical! GLP Compliance Statement
- Deviations from GLP

Critical! Quality Assurance Statement
- Dates and Type of Inspection

Critical! Protocol
- Amendments

X. STATEMENT OF INSPECTIONS PREPARED AND SIGNED BY THE QUALITY ASSURANCE UNIT

725417-004
Pilot Study: ^{14}C-Benzocaine Total Residue Depletion

QA Statement

This was a pilot study performed to enhance the design of a subsequent GLP Study. Even though this was considered a pilot study, certain parameters were audited by the Quality Assurance Unit. The analyses were generally carried out in compliance with the GLP regulations. Standard curves and/or QC standards may not have been processed on each day that samples were run. Although, experience with the HPLC method for Benzocaine and its metabolites, in this laboratory, have shown that the standard curve does not drift. Therefore, study personnel were confident that reliable data were obtained.

Dates Audited	Date Reported	Parameter
December 7, 1992	January 23, 1993	HPLC Analysis
December 18, 1992	January 23, 1993	Combustion Analysis
July 9, 12, 13, 1993	July 13, 1993	Final Report

Kathleen Magliere Zajd 7-13-93
Kathleen Magliere Zajd, B.S.
Quality Assurance

In-Life Portion – Animal Information

- Study Animals
  - Numbers
  - Breed or species or strain
  - Males/females
  - Health Records
  - Critical! Records of any prior drug treatments
  - Age
  - Critical! Weight
    - Were animals weighed close to the start of dosing period?
    - Appropriate weight for market size

Table 1. Serial numbers, body weights, and gender of the fish used in the benzocaine pilot studies.

Serial Number	Depuration Time	Weight grams	Gender
BZ-4	0	1153	male[a]
BZ-5	0	1250	male
BZ-6	0	1179	male
BZ-7	20 min	1540	male
BZ-8	20	892	male[a]
BZ-9	20	1120	male
BZ-10	40 min	1482	male
BZ-11	40	1389	male
BZ-12	40	785	male[a]
BZ-13	80 min	1582	male[a]
BZ-14	80	1881	male
BZ-15	80	1706	male
BZ-16	2.67 hr	1488	male
BZ-17	2.67 hr	1502	male[a]
BZ-18	2.67 hr	999	male
BZ-19	0	1975	male; exposed, unused
BZ-20	0	677	male; exposed, unused
BZ-21	8.25 hr	1485	male
BZ-22	8.25	1073	female[a]
BZ-23	8.25	1740	male
BZ-24	0	1069	female
BZ-25	25 hr	1057	female
BZ-26	25	1210	male
BZ-27	25	872	male[a]
BZ-28	42 hr	709	female
BZ-29	42	652	female
BZ-30	135 hr	562	male
BZ-31	135	533	female

[a]Disposition of radioactivity in these fish studied by whole-body autoradiography.

In-Life Portion – Housing/Feed

- Critical! Description of housing – does it mimic commercial conditions?
  (individually or group housed, pen size, light/dark cycle, air flow, etc.)
- Feed – composition and analyzed for contaminants
- Water – analyzed for contaminants
- Environmental conditions
  (daily temperatures, etc)
- Critical! Acclimation Period - enough information to demonstrate that animals aren't stressed and are eating normally prior to test period

TROUT CHOW 3/16 PELLETS SILVER CUP LOT# 12/21/04 3/16

Test Code	Assay / Analyte		Result	Units	
PRKR	Protein, Kjeldahl (N x 6.25)	Protein	45.6	%	
FTAH	Fat, acid hydrolysis	Fat	10.8	%	
FIBR	Fiber, crude	Fiber - Crude	2.63	%	
ORGP	Organophosphate pesticides				Initials:
		Diazinon	< 0.0200	ppm	
		Disulfoton	< 0.0200	ppm	
		Ethion	< 0.0200	ppm	Initials:
		Malathion	0.160	ppm	
		Methyl Parathion	< 0.0200	ppm	
		Parathion	< 0.0200	ppm	
		Thimet	< 0.0200	ppm	
		Thiodan	< 0.0200	ppm	
		Trithion	< 0.0200	ppm	
RSPB	Organochlorine pest.&PCB's				
		Heptachlor Epoxide	< 0.0200	ppm	
		Heptachlor	< 0.0200	ppm	
		DDE	< 0.0200	ppm	
		Lindane	< 0.0200	ppm	
		Endrin	< 0.0200	ppm	

Acclimation period							
Date	Time	Temp	DO	DO	DOchrg	pH	pH
mm/dd/yy	hh:mm:ss	°C	%	mg/L			mV
1/25/2005	9:36:11	17.04	65.8	6.35	31.8	7.44	-46
1/25/2005	10:36:11	17.04	70.2	6.78	32.9	7.42	-45
1/25/2005	11:36:11	17.05	69.7	6.73	32.9	7.44	-46
1/25/2005	12:36:10	17.04	70.9	6.85	32.9	7.45	-47
1/25/2005	13:36:10	17.05	71.8	6.94	32.9	7.46	-47
1/25/2005	14:50:29	17.05	69.7	6.73	32.9	7.51	-51
1/25/2005	15:50:29	17.05	68.7	6.63	32.9	7.51	-51
1/25/2005	16:50:29	17.05	70.6	6.81	32.9	7.51	-51
1/25/2005	17:50:29	17.05	70.1	6.76	31.8	7.51	-50
1/25/2005	18:50:28	17.05	71.8	6.93	32.9	7.52	-51
1/25/2005	19:50:29	17.05	71.8	6.94	32.9	7.52	-51
1/25/2005	20:50:28	17.04	73.2	7.07	32.9	7.55	-53
1/25/2005	21:50:28	17.06	74.2	7.16	32.9	7.54	-52
1/25/2005	22:50:28	17.05	75.7	7.31	32.9	7.57	-54
1/25/2005	23:50:27	17.03	76.6	7.4	32.9	7.57	-54
1/26/2005	0:50:27	17.06	76.6	7.39	32.9	7.58	-55
1/26/2005	1:50:27	17.05	77.3	7.47	32.9	7.58	-55
1/26/2005	2:50:27	17.05	78	7.53	32.9	7.59	-56
			78.2	7.55	32.9	7.6	-56

In-Life Portion – Dosing Information

- Test Material – certificate of analysis
- Route of Administration
  - Medicated feed
  - Drinking water
  - Injectable product
  - Intramammary infusion
  - Added directly to holding tank (fish)

In-Life Portion – Dosing Information

- Critical! How was dose prepared?
  - SOP follow?
  - Description of how medicated feed was mixed, syringes were filled, or medicated water prepared

In-Life Portion – Dosing Information

- Critical! Verify concentration in dosage form before dosing animals
  - For medicated feeds, provide mixing records, demonstration of homogeneity, sampling records
  - Analysis
- Critical! Provide documentation that animals were dosed correctly

Animal ID	TRT Group	B. Weight (kg)	Dose Vol.[1] (ml/50kg)	Calculated Dose (ml)[2]	Total Dose (ml)[3]	Dose rate (mg/kg)
103	1	139	-	-	-	-
309	1	163	-	-	-	-
329	1	129	-	-	-	-
644	1	122	-	-	-	-
316	2	173	1.5	5.190	5.2	0.947
324	2	146	1.5	4.380	4.4	0.949
328	2	127	1.5	3.810	3.9	0.967
468	2	118	1.5	3.540	3.6	0.961
963	2	153	1.5	4.590	4.6	0.947
244	3	137	1.5	4.110	4.2	0.966
307	3	158	1.5	4.740	4.8	0.957
311	3	146	1.5	4.380	4.4	0.949
916	3	113	1.5	3.390	3.4	0.948
964	3	108	1.5	3.240	3.3	0.963

In-Life Portion – Sample Collection

- Slaughter of animals at various timepoints after dosing period – follows protocol
  - Critical! Obtain residue data around the tolerance on linear portion of depletion curve.
  - Critical! Values must be >LOQ to be used for the withdrawal calculation
- Method of slaughter
  - SOPs
  - Critical! Doesn't interfere with analysis of test material
- Tissues collected
  - SOPs
  - Critical! Correct sample size
  - Critical! Document handling of tissue samples from collection through analysis

End of In-Life Phase of Study

Study Day	Date	Treatment of Groups A, B, C, D, E and F	Slaughter
0	04/06/04	X	
3	07/06/04		Group A + 1 animal from Group G
7	11/06/04		Group B
14	18/06/04		Group C+ 1 animal from Group G
21	25/06/04		Group D
28	02/07/04		Group E
35	09/07/04		Group F + 1 animal from Group G

SCHEDULE OF OPERATIONS:

The experimental start date, *i.e.*, Day 0 - first phase, was 20 September 2001. The in-life phase of the study ended on Day 180, 19 March 2002.

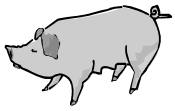
Study Day	Event
Day -7	Acclimation of animals to facilities started Feed and water samples collected Blood for preparation of plasma collected
Day -1	Animals weighed and allocated
Day 0	Animals treated Animals observed approximately hourly for 4 hours after treatment. Collect blood 2 to 6 hours after treatment
Day 62	Two Animals in Group 1 blood sampled and then necropsied and tissues collected
Day 63	Animals in Group 2 blood sampled and then necropsied and tissues collected
Day 77	Animals in Group 3 blood sampled and then necropsied and tissues collected
Day 91	Animals in Group 4 blood sampled and then necropsied and tissues collected
Day 105	Animals in Group 5 blood sampled and then necropsied and tissues collected
Day 119	Animals in Group 6 blood sampled and then necropsied and tissues collected
Day 133	Animals in Group 7 blood sampled and then necropsied and tissues collected
Day 179	Two Animals in Group 1 blood sampled and then necropsied and tissues collected
Day 180	Animals in Group 8 blood sampled and then necropsied and tissues collected

Analytical Phase

- Analytical lab facility
- Analysis of tissues for drug residues - SOPs
- Storage stability of drug in tissues
  - Critical! Demonstrate stability if samples are stored longer than 1 week
  - may be a separate study

Analytical Phase

- Critical! Demonstrate that method is performing successfully prior to analyzing test samples
  - may be a separate study
  - provide validation data
- Critical! Provide method performance data for analysis of test samples



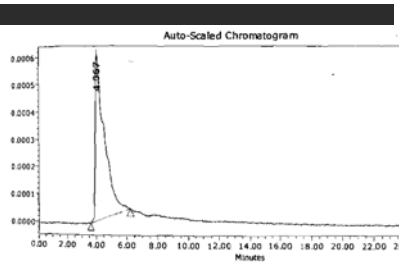
Provide standard curve preparation

These standard solutions are used to prepare the further standards in the range: 32, 20, 16, 8, 4, 2, 1.5, 1 µg/ml in accordance with the following table:

Table 1. Standard Solutions.

REQUIRED OXYTETRACYCLINE CONCN (µg/ml)	VOLUME (ml)	CONC (µg/ml)	USING pH 4.5 BUFFER DILUTE TO (ml)
32	3.2	1000	100
20	20	100	100
16	16	100	100
8	8	100	100
4	4	100	100
2	20	10	100
1.5	15	10	100
1	10	10	100

Provide representative chromatograms



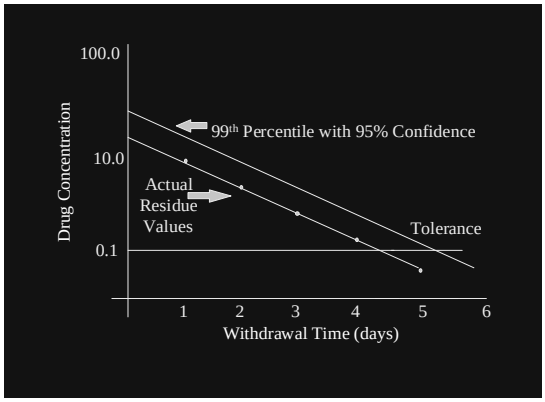
Provide residue data for individual animals, means, and standard deviation

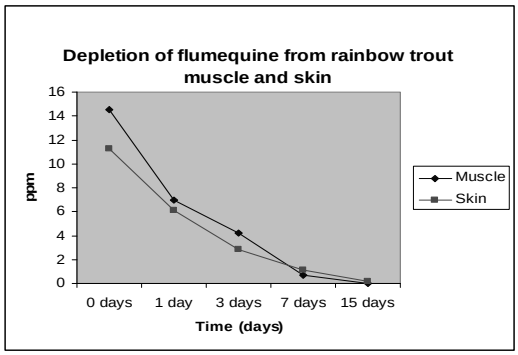
Concentrations in rainbow trout muscle/skin exposed to 17.8 mg/mL for 60 minutes at 17°C.		
Withdrawal Time (hours)	**Concentration (ppm)**	**Mean ± standard deviation**
0	61.4 41.0 47.0 38.6 54.8 50.4	48.9±8.5
0.5	32.4 20.6 28.1 34.7 16.9 26.0	26.5±6.8
1	19.7 11.0 14.9 14.0 11.5 20.8	15.3±4.1

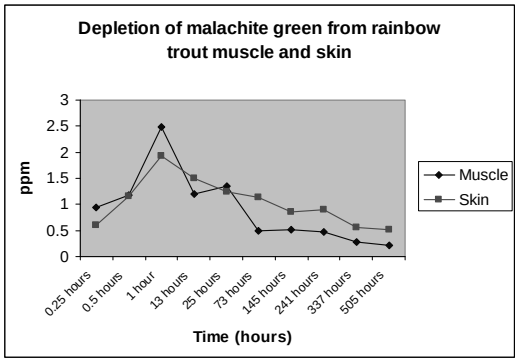
Residue concentrations <LOQ can't be used for withdrawal period calculation

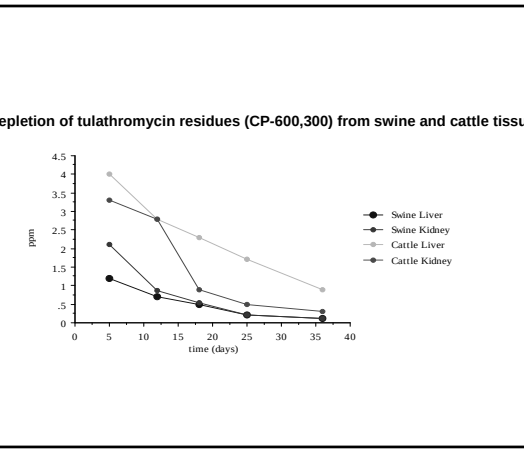
Treatment Group: 5

Animal ID	Day 105 Liver ng/g	Hqtr Mus ng/g	Fat ng/g	Kidney ng/g	InjectSite ng/g
037	BLQ	BLOD	BLQ	BLOD	274
223	5.57	BLOD	BLQ	BLOD	17.8
315	13.0	BLQ	10.2	BLQ	5,380
317	BLQ	BLOD	6.80	8.98	125
915	7.32	120	BLQ	BLOD	198
Average	**5.48**	**BLQ**	**BLQ**	**BLQ**	**230**




Depletion of flumequine from rainbow trout muscle and skin
ppm
16
14
12
10
8
6
4
2
0
0 days
1 day
3 days
7 days
15 days
Time (days)
Muscle
Skin


Depletion of malachite green from rainbow trout muscle and skin
ppm
3
2.5
2
1.5
1
0.5
0
0.25 hours
0.5 hours
1 hour
13 hours
25 hours
73 hours
145 hours
241 hours
337 hours
505 hours
Time (hours)
Muscle
Skin


Depletion of tulathromycin residues (CP-600,300) from swine and cattle tissues
ppm
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
0
5
10
15
20
25
30
35
40
time (days)
Swine Liver
Swine Kidney
Cattle Liver
Cattle Kidney

Key Issues

- Were animals acclimated adequately?
- Dose accountability – Was dosage form prepared properly? Did each animal receive the intended dose?
- Was "chain of custody" documented: collection of tissues, initial preparation of tissues, shipment to lab, storage of tissues until analysis?

Key Issues (continued)

- Was method validated prior to analyzing test samples?
- Were method performance data provided for analysis of test samples?
- Were an adequate number of residue concentrations generated on terminal portion of depletion curve?

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