

Wildlife Services <b>NWRC</b> National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Chemistry Lab Unit	Invoice #: 17-019 Date: November 6, 2017 Page: 1 of 19
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To: Dr. Gary Witmer  
Research Wildlife Biologist  
NWRC

Subject: Determination of Diphacinone and Brodifacoum in Salamanders, Crickets, Water, and Baits (QA-2688)

Methods: Non-GLP (salamanders, crickets, water); Method 163A (baits)

Analysis Dates: 9/12, 9/13, 9/14, 9/19, 9/25, 9/27, 9/28, 10/13, 10/27, and 10/30/2017

Notebook Reference: AC-161, pp. 86-109

QC Notebook Reference: QC-33, p. 137; AC-162, p. 4

Analyst: Steve Volker

#### Sample Descriptions:

*Ensatina* salamanders (n=8), *Aneides* salamanders (n=14), *Batrachoseps* salamanders (n=36), crickets (n=24 composite samples), water (saturated with ground bait, n=12), and baits (n=4) were received between 6/2/2017 and 9/25/2017 for analysis of diphacinone and brodifacoum. All samples were stored at -20°C until time of analysis.


#### Sample Preparation and Extraction:

##### Homogenization:

Baits and salamanders (whole bodies) were homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenized samples were transferred immediately to vacuum sealable bags while still frozen and stored at -20°C. Cricket samples, consisting of between 11 and 27 individual crickets, were ground into a paste using a glass rod and stored at -20°C.

##### Extraction of salamanders and crickets:

Homogenized sample (70-80 mg) was weighed into a 1.5-mL microcentrifuge tube, 50 µL DI water added, and the sample vortex mixed 4-5 s to form a suspension. Surrogate analytes (20 µL, 16 µg/mL D<sub>4</sub>-diphacinone and 17 µg/mL chlordifacoum in acetonitrile) and 1.180 mL of acetonitrile (ACN) were added and the sample vortex mixed twice for 15-20 s. An excess of NaCl (~120 mg) was added to produce a water:ACN phase separation and the sample vortex mixed twice for 15-20 s. The extract was clarified by centrifugation (12,000 RCF) and 0.900 mL of supernatant transferred to a dispersive solid-phase extraction (dSPE) tube containing MgSO<sub>4</sub> (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and MgSO<sub>4</sub> by vortex mixing for 4-5 s followed by centrifugation at 12,000 RCF for 2-3 s to clarify the supernatant. 0.400 mL of supernatant was then transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 100 µL ACN followed by 400 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC/MS analysis.

 Analyst	11/6/2017 Date	 QC Specialist	11/6/17 Date	 Reviewer	11/6/17 Date
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**Extraction of Water:**

Water samples (10-50 mL) were warmed to room temperature (overnight in a hood), vortex mixed 4-5 s, centrifuged at 1400 RCF for 2 minutes, and then 8-10 mL of supernatant filtered through a 0.7- $\mu$ m glass fiber syringe filter into a 15-mL polypropylene tube. A portion of the filtered sample (1.5 mL) was transferred to a 10-mL glass tube and surrogate analytes (10  $\mu$ L) added. Acetonitrile (2.0 mL), 1M HCl (0.5 mL), and excess NaCl (~1 g) were added and the sample vortex mixed 4-5 s. Chloroform (0.5 mL) was added and the sample vortex mixed 4-5 s, let set for 5-10 minutes, and then vortex mixed again. The sample was then centrifuged at 1400 RCF for 1 minutes and 1.5 mL of the upper ACN/chloroform layer transferred to a 1.5-mL microcentrifuge tube. The solvents were removed in a 45°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 90  $\mu$ L ACN followed by 360  $\mu$ L pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC/MS analysis.

**Baits:**

All baits were assayed by NWRC Method 163A. To assess trace level residues of rodenticides, 0.600 mL of microwave extract from Method 163A procedure was transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 300  $\mu$ L ACN followed by 1200  $\mu$ L pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC/MS analysis.

**Instrument methods:****Salamanders and Crickets:****Agilent 1290 Infinity II HPLC with G6470A QQQ**

Column	Xbridge C18, 2.5- $\mu$ m, 2.1 x 50 mm, Waters P/N 186003085				
Mobile phase A	90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile)				
Mobile phase B	Acetonitrile				
Flow rate	0.800 mL/min	<u>Time (min)</u>	<u>%A</u>	<u>%B</u>	
Column temp.	60°C	0.00	90%	10%	
Injection volume	7.5 $\mu$ L	0.50	90%	10%	
Run time	4.0 min	3.00	20%	80%	
		3.01	0%	100%	
Source	AJS ESI, negative mode	3.50	0%	100%	
Gas temp.	300°C	3.51	90%	10%	
Gas flow	5 L/min				
Nebulizer	45 psi				
Sheath gas	250°C, 7 L/min				
Capillary	-4500 V				
Nozzle	-500 V				
	Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)
	Diphacinone	339.1	<b>167.1</b> 145	100	23 18
	D4-Diphacinone	343.1	167.1	120	23
	Chlordifacoum	477.1	135.1	61	37
			135.0		44
	Brodifacoum	522.9	<b>80.9</b>	165	50

**BOLD** = product ion used for quantitation

Water:

Same conditions as for salamanders and crickets with the following changes:

Flow rate 0.650 mL/min  
Run time 3.5 min

<u>Time (min)</u>	<u>%A</u>	<u>%B</u>
0.00	85%	15%
0.50	85%	15%
2.30	30%	70%
2.31	0%	100%
2.90	0%	100%
2.91	85%	15%

Baits (LCMS):

Same conditions as for water, but 1.5 µL injection volume.

Baits (Method 163A):Agilent 1100 Series HPLC with G1315B Diode Array Detector (DAD) and G1321A Fluorescence Detection (FLD)

Column	Gemini C18, 3-µm, 3 x 150 mm, Phenomenex P/N 00F-4439-Y0			
Mobile phase A	5-mM tetrabutylammonium phosphate (TBAP) in 50%(pH 8.5 6-mM phosphate)/50%(methanol)			
Mobile phase B	5-mM TBAP in methanol			
Flow rate	0.650 mL/min	<u>Time (min)</u>	<u>%A</u>	<u>%B</u>
Column temp.	60°C	0.00	85%	15%
Injection volume	10 µL	1.00	85%	15%
Run time	26 min	17.00	45%	55%
		17.01	0%	100%
Detector	UV (DAD); 325 nm	23.00	0%	100%
		23.01	85%	15%
Detector	Fluorescence (FLD)			
Excitation	310 nm			
Emission	390 nm			

**Detection and Quantitation Limits:**

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of brodifacoum that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL for each analyte. This was performed by comparing the analyte response observed in fortified control matrix with the baseline noise observed at the same retention time in control matrix. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations for diphacinone and brodifacoum in each control matrix.

Detection Limit (DL) and Quantitation Limit (QL)

<u>Control Matrix</u>	<u>Diphacinone</u>		<u>Brodifacoum</u>	
	<u>DL</u>	<u>QL</u>	<u>DL</u>	<u>QL</u>
<i>Ensatina</i> Salamanders (whole body)	5.9 ng/g	19.6 ng/g	6.6 ng/g	21.9 ng/g
<i>Aneides</i> Salamanders (whole body)	7.5 ng/g	25.1 ng/g	8.6 ng/g	28.6 ng/g
<i>Batrachoseps</i> Salamanders (whole body)	8.9 ng/g	29.8 ng/g	8.9 ng/g	29.7 ng/g
Crickets	4.9 ng/g	16.2 ng/g	5.9 ng/g	19.7 ng/g
Water (saturated with ground bait)	0.080 ng/mL	0.267 ng/mL	0.13 ng/mL	0.419 ng/mL
Baits (Method 163A)	2.8 µg/g	9.40 µg/g	0.043 µg/g	0.142 µg/g
Baits (LCMS)	0.0072 µg/g	0.0241 µg/g	0.0081 µg/g	0.0270 µg/g

**Results:**

Triplicate preparations of all samples were prepared, except when sample size was insufficient. Rodenticide residues for salamanders and crickets are reported in units of ng/g, equivalent to parts per billion (ppb). Water results are reported in units of ng/mL, also equivalent to ppb. Rodenticide concentrations in bait formulations are reported in units of µg/g, equivalent to parts per million (ppm).

If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results that are greater than the DL, but less than the QL are identified by an asterisk "\*". Care should be taken when evaluating results below the QL as the variability will be significantly greater than the variability observed in quality control (QC) samples. Results above the QL are reported to three significant figures.

*Desmognathus* salamanders (whole body)

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-13	QP3 (Control)	9/14/2017	ND	ND
S170602-14	QP6 (Control)	9/14/2017	ND	ND
S170602-19-A		9/14/2017	ND	101
S170602-19-B	QP1 (Brodifacoum, Dermal + Cricket)	9/14/2017	ND	95.9
S170602-19-C		9/14/2017	ND	100
S170602-20-A		9/14/2017	ND	86.9
S170602-20-B	QP4 (Brodifacoum, Dermal + Cricket)	9/14/2017	ND	85.7
S170602-20-C		9/14/2017	ND	85.5
S170602-21-A		9/14/2017	ND	50.1
S170602-21-B	QP7 (Brodifacoum, Dermal + Cricket)	9/14/2017	ND	50.7
S170602-21-C		9/14/2017	ND	48.3
S170602-26-A		9/14/2017	ND	ND
S170602-26-B	QP2 (Diphacinone, Dermal + Cricket)	9/14/2017	ND	ND
S170602-26-C		9/14/2017	ND	ND
S170602-27-A		9/14/2017	ND	ND
S170602-27-B	QP5 (Diphacinone, Dermal + Cricket)	9/14/2017	ND	ND
S170602-27-C		9/14/2017	ND	ND
S170602-28-A		9/14/2017	ND	ND
S170602-28-B	QP8 (Diphacinone, Dermal + Cricket)	9/14/2017	ND	ND
S170602-28-C		9/14/2017	ND	ND
DL (ng/g) =			5.9	6.6
QL (ng/g) =			19.6	21.9

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentration less than the Quantitation Limit (QL).

*Aneides* salamanders (whole body)

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-09	QO3 (Control)	9/19/2017	ND	ND
S170602-10	QO6 (Control)	9/19/2017	ND	ND
S170602-11	QO9 (Control)	9/19/2017	ND	ND
S170602-12	QO14 (Control)	9/28/2017	ND	ND
S170711-31-A	QO13 (Control)	9/28/2017	ND	ND
S170711-31-B		9/28/2017	ND	ND
S170711-31-C		9/28/2017	ND	ND
S170711-32-A	QO12 (Control)	9/28/2017	ND	ND
S170711-32-B		9/28/2017	ND	ND
S170711-32-C		9/28/2017	ND	ND
S170602-15-A	QO1 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	108
S170602-15-B		9/28/2017	ND	98.0
S170602-15-C		9/28/2017	ND	103
S170602-16-A	QO4 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	ND
S170602-16-B		9/28/2017	ND	46.6
S170602-16-C		9/28/2017	ND	38.8
S170602-17-A	QO7 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	85.5
S170602-17-B		9/28/2017	ND	97.1
S170602-17-C		9/28/2017	ND	89.3
S170602-18-A	QO10 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	239
S170602-18-B		9/28/2017	ND	214
S170602-18-C		9/28/2017	ND	224
S170602-22-A	QO2 (Diphacinone, Dermal + Cricket)	9/28/2017	182	ND
S170602-22-B		9/28/2017	176	ND
S170602-22-C		9/28/2017	165	ND
S170602-23-A	QO5 (Diphacinone, Dermal + Cricket)	9/28/2017	ND	ND
S170602-23-B		9/28/2017	ND	ND
S170602-23-C		9/28/2017	ND	ND
S170602-24-A	QO8 (Diphacinone, Dermal + Cricket)	9/28/2017	9.0 *	ND
S170602-24-B		9/28/2017	13.7 *	ND
S170602-24-C		9/28/2017	9.8 *	ND
S170602-25-A	QO11 (Diphacinone, Dermal + Cricket)	9/28/2017	ND	ND
S170602-25-B		9/28/2017	ND	ND
S170602-25-C		9/28/2017	ND	ND
		DL (ng/g) =	7.5	8.6
		QL (ng/g) =	25.1	28.6

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

***Batrachoseps* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-30-A	QS22 (control)	9/19/2017	ND	ND
S170602-30-B		9/19/2017	ND	ND
S170602-30-C		9/19/2017	ND	ND
S170711-04-A	QS9 (Control)	9/19/2017	ND	22.0 *
S170711-04-B		9/19/2017	ND	22.6 *
S170711-04-C		9/19/2017	ND	21.2 *
S170711-08-A	QS17 (Control)	9/19/2017	ND	ND
S170711-08-B		9/19/2017	ND	8.8 *
S170711-08-C		9/19/2017	ND	ND
S170711-12-A	QS26 (Control)	9/19/2017	ND	ND
S170711-12-B		9/19/2017	ND	ND
S170711-12-C		9/19/2017	ND	ND
S170711-17-A	QS34 (Control)	9/19/2017	ND	ND
S170711-17-B		9/19/2017	ND	ND
S170711-17-C		9/19/2017	ND	ND
S170602-31-A	QS6 (Brodifacoum, Dermal)	9/19/2017	ND	22.8 *
S170602-31-B		9/19/2017	ND	16.5 *
S170602-31-C		9/19/2017	ND	18.2 *
S170602-32-A	QS11 (Brodifacoum, Dermal)	9/19/2017	ND	82.1
S170602-32-B		9/19/2017	ND	61.9
S170602-32-C		9/19/2017	ND	74.4
S170602-33-A	QS36 (Brodifacoum, Dermal)	9/19/2017	ND	29.8
S170602-33-B		9/19/2017	ND	38.5
S170602-34-A	QS38 (Brodifacoum, Dermal)	9/19/2017	ND	103
S170602-35-A	QS51 (Brodifacoum, Dermal)	9/19/2017	ND	64.4
S170602-35-B		9/19/2017	ND	71.4
S170602-35-C		9/19/2017	ND	71.3
S170711-01-A	QS5 (Brodifacoum, Cricket)	9/19/2017	ND	87.9
S170711-01-B		9/19/2017	ND	72.5
S170711-01-C		9/19/2017	ND	95.1
S170711-02-A	QS7 (Diphacinone, Cricket)	9/19/2017	ND	10.1 *
S170711-02-B		9/19/2017	ND	12.7 *
S170711-02-C		9/19/2017	ND	9.3 *
S170711-03-A	QS8 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-03-B		9/25/2017	ND	ND
		DL (ng/g) =	8.9	8.9
		QL (ng/g) =	29.8	29.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

***Batrachoseps* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-05-A		9/25/2017	ND	54.7
S170711-05-B	QS10 (Brodifacoum, Cricket)	9/25/2017	ND	54.6
S170711-05-C		9/25/2017	ND	60.4
S170711-06-A		9/25/2017	ND	ND
S170711-06-B	QS13 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-06-C		9/25/2017	ND	ND
S170711-07-A		9/25/2017	ND	ND
S170711-07-B	QS14 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-07-C		9/25/2017	ND	ND
S170711-09-A		9/25/2017	ND	48.0
S170711-09-B	QS19 (Brodifacoum, Cricket)	9/25/2017	ND	55.9
S170711-09-C		9/25/2017	ND	49.9
S170711-10-A		9/25/2017	ND	ND
S170711-10-B	QS23 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-10-C		9/25/2017	ND	ND
S170711-11-A		9/25/2017	ND	ND
S170711-11-B	QS24 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-11-C		9/25/2017	ND	ND
S170711-13 <sup>a</sup>	QS27 (Brodifacoum, Cricket)	N/A	N/A	N/A
S170711-14-A		9/25/2017	ND	73.5
S170711-14-B	QS30 (Brodifacoum, Dermal)	9/25/2017	ND	84.4
S170711-14-C		9/25/2017	ND	83.7
S170711-15-A		9/25/2017	ND	ND
S170711-15-B	QS31 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-15-C		9/25/2017	ND	ND
S170711-16-A		9/25/2017	ND	ND
S170711-16-B	QS33 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-16-C		9/25/2017	ND	ND
S170711-18-A		9/25/2017	ND	64.1
S170711-18-B	QS35 (Brodifacoum, Cricket)	9/25/2017	ND	65.6
S170711-18-C		9/25/2017	ND	64.0
S170711-19-A		9/25/2017	ND	ND
S170711-19-B	QS39 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-19-C		9/25/2017	ND	ND
		DL (ng/g) =	8.9	8.9
		QL (ng/g) =	29.8	29.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

<sup>a</sup> No sample available.



***Batrachoseps* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-20-A		9/27/2017	ND	ND
S170711-20-B	QS40 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-20-C		9/27/2017	ND	ND
S170711-21-A		9/27/2017	ND	ND
S170711-21-B	QS42 (Brodifacoum, Cricket)	9/27/2017	ND	ND
S170711-21-C		9/27/2017	ND	ND
S170711-22-A		9/27/2017	ND	33.0
S170711-22-B	QS43 (Brodifacoum, Dermal)	9/27/2017	ND	34.1
S170711-22-C		9/27/2017	ND	34.7
S170711-23-A		9/27/2017	ND	ND
S170711-23-B	QS44 (Diphacinone, Cricket)	9/27/2017	ND	ND
S170711-23-C		9/27/2017	ND	ND
S170711-24-A		9/27/2017	ND	ND
S170711-24-B	QS48 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-24-C		9/27/2017	ND	ND
S170711-25-A		9/27/2017	ND	ND
S170711-25-B	QS52 (Diphacinone, Cricket)	9/27/2017	ND	ND
S170711-25-C		9/27/2017	ND	ND
S170711-26-A		9/27/2017	ND	ND
S170711-26-B	QS53 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-26-C		9/27/2017	ND	ND
S170711-27-A		9/27/2017	ND	ND
S170711-27-B	QS55 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-27-C		9/27/2017	ND	ND
S170711-28-A		9/27/2017	ND	90.8
S170711-28-B	QS56 (Brodifacoum, Cricket)	9/27/2017	ND	91.4
S170711-28-C		9/27/2017	ND	ND
S170711-29-A		9/27/2017	ND	37.3
S170711-29-B	QS57 (Brodifacoum, Dermal)	9/27/2017	ND	35.0
S170711-29-C		9/27/2017	ND	34.2
S170711-30-A		9/27/2017	ND	ND
S170711-30-B	QS58 (Diphacinone, Cricket)	9/27/2017	ND	ND
S170711-30-C		9/27/2017	ND	ND
DL (ng/g) =			8.9	8.9
QL (ng/g) =			29.8	29.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

## Crickets

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-51	Control Tissue 1/2"	9/13/2017	ND	ND
S170711-52	Control Tissue Pinheads	9/12/2017	ND	ND
S170711-45	Placebo Diphacinone + no potato (PDFC1), n=20	9/13/2017	31.5	ND
S170711-45-A		9/12/2017	31.2	ND
S170711-46	Placebo Diphacinone + no potato (PDFC2), n=21	9/13/2017	18.8	ND
S170711-46-A		9/12/2017	15.8 *	ND
S170711-47	Placebo Diphacinone + no potato (PDFC3), n=24	9/13/2017	19.5	ND
S170711-47-A		9/12/2017	14.6 *	ND
S170711-48	Placebo Brodifacoum + no potato (PBFC1), n=22	9/13/2017	ND	ND
S170711-49	Placebo Brodifacoum + no potato (PBFC2), n=23	9/13/2017	ND	ND
S170711-50	Placebo Brodifacoum + no potato (PBFC3), n=21	9/13/2017	ND	ND
S170602-36-A	Brodifacoum + potato (BFC1), n=15	9/13/2017	ND	296
S170602-36-B		9/13/2017	ND	282
S170602-36-C		9/13/2017	ND	309
S170602-37-A	Brodifacoum + potato (BFC2), n=14	9/13/2017	ND	589
S170602-37-B		9/13/2017	ND	687
S170602-38-A	Brodifacoum + potato (BFC3), n=13	9/13/2017	ND	538
S170602-38-B		9/13/2017	ND	672
S170602-38-C		9/13/2017	ND	528
S170602-39-A	Diphacinone + potato (DFC1), n=11	9/13/2017	1490	ND
S170602-39-B		9/13/2017	1600	ND
S170602-40-A	Diphacinone + potato (DFC2), n=15	9/13/2017	3130	ND
S170602-40-B		9/13/2017	3040	ND
S170602-40-C		9/13/2017	2620	ND
S170602-41-A	Diphacinone + potato (DFC3), n=14	9/13/2017	1140	ND
S170602-41-B		9/13/2017	1260	ND
S170711-33-A	Brodifacoum + no potato (BFC4), n=24	9/13/2017	ND	495
S170711-33-B		9/13/2017	ND	519
S170711-33-C		9/13/2017	ND	530
S170711-34-A	Brodifacoum + no potato (BFC5), n=23	9/13/2017	ND	423
S170711-34-B		9/13/2017	ND	420
DL (ng/g) =			4.9	5.9
QL (ng/g) =			16.2	19.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

## Crickets

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-35-A	Brodifacoum + no potato (BFC6), n=23	9/13/2017	ND	560
S170711-35-B		9/13/2017	ND	638
S170711-35-C		9/13/2017	ND	490
S170711-36-A	Diphacinone + no potato (DFC4), n=27	9/12/2017	1060	ND
S170711-36-B		9/12/2017	950	ND
S170711-36-C		9/12/2017	943	ND
S170711-37-A	Diphacinone + no potato (DFC5), n=27	9/12/2017	907	ND
S170711-37-B		9/12/2017	1140	ND
S170711-37-C		9/12/2017	1050	ND
S170711-38-A	Diphacinone + no potato (DFC6), n=21	9/12/2017	2040	ND
S170711-38-B		9/12/2017	2350	ND
S170711-38-C		9/12/2017	1720	ND
S170711-39-A	Diphacinone + dusted (DD1), n=23	9/12/2017	1740	ND
S170711-39-B		9/12/2017	1950	ND
S170711-39-C		9/12/2017	1780	ND
S170711-40-A	Diphacinone + dusted (DD2), n=25	9/12/2017	3090	ND
S170711-40-B		9/12/2017	3490	ND
S170711-40-C		9/12/2017	3410	ND
S170711-41-A	Diphacinone + dusted (DD3), n=18	9/12/2017	4200	ND
S170711-41-B		9/12/2017	4280	ND
S170711-41-C		9/12/2017	3460	ND
S170711-42-A	Brodifacoum + dusted (BD1), n=16	9/12/2017	9.9 *	3320
S170711-42-B		9/12/2017	7.8 *	3080
S170711-42-C		9/12/2017	9.5 *	3260
S170711-43-A	Brodifacoum + dusted (BD2), n=23	9/12/2017	9.7 *	3620
S170711-43-B		9/12/2017	7.1 *	3220
S170711-43-C		9/12/2017	6.2 *	3180
S170711-44-A	Brodifacoum + dusted (BD3), n=18	9/12/2017	7.1 *	2670
S170711-44-B		9/12/2017	7.5 *	3160
S170711-44-C		9/12/2017	6.0 *	2830
DL (ng/g) =			4.9	5.9
QL (ng/g) =			16.2	19.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

## Water (saturated with ground bait)

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-03-A	Water/Diphacinone #1	10/13/2017	6.31	ND
S170602-03-B		10/13/2017	6.44	ND
S170602-03-C		10/13/2017	6.15	ND
S170602-04-A	Water/Diphacinone #2	10/13/2017	9.02	ND
S170602-04-B		10/13/2017	9.63	ND
S170602-04-C		10/13/2017	8.74	ND
S170602-05-A	Water/Diphacinone #3	10/13/2017	17.6	ND
S170602-05-B		10/13/2017	18.0	ND
S170602-05-C		10/13/2017	17.6	ND
S170606-01-A	Water/Diphacinone #4	10/13/2017	3.52	ND
S170606-01-B		10/13/2017	3.34	ND
S170606-01-C		10/13/2017	3.39	ND
S170606-02-A	Water/Diphacinone #5	10/13/2017	4.84	ND
S170606-02-B		10/13/2017	4.89	ND
S170606-02-C		10/13/2017	4.77	ND
S170606-03-A	Water/Diphacinone #6	10/13/2017	3.89	ND
S170606-03-B		10/13/2017	3.57	ND
S170606-03-C		10/13/2017	3.36	ND
S170602-06-A	Water/Brodifacoum #1	10/13/2017	ND	5.78
S170602-06-B		10/13/2017	0.080 *	5.78
S170602-06-C		10/13/2017	ND	5.69
S170602-07-A	Water/Brodifacoum #2	10/13/2017	0.125 *	29.3
S170602-07-B		10/13/2017	0.147 *	29.6
S170602-07-C		10/13/2017	0.133 *	29.5
S170602-08-A	Water/Brodifacoum #3	10/13/2017	0.131 *	29.9
S170602-08-B		10/13/2017	0.110 *	28.6
S170602-08-C		10/13/2017	0.127 *	30.7
S170606-04-A	Water/Brodifacoum #4	10/13/2017	0.134 *	26.5
S170606-04-B		10/13/2017	0.109 *	24.7
S170606-04-C		10/13/2017	0.127 *	25.2
S170606-05-A	Water/Brodifacoum #5	10/13/2017	0.121 *	18.5
S170606-05-B		10/13/2017	0.140 *	19.4
S170606-05-C		10/13/2017	0.123 *	19.5
S170606-06-A	Water/Brodifacoum #6	10/13/2017	0.100 *	18.9
S170606-06-B		10/13/2017	0.171 *	18.8
S170606-06-C		10/13/2017	0.119 *	18.4
DL (ng/mL) =			0.080	0.13
QL (ng/mL) =			0.267	0.419

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

**Baits (Method 163A)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (µg/g)	Observed Brodifacoum Concentration (µg/g)
S170925-01-D		10/27/2017	0.424 <sup>a</sup>	ND
S170925-01-E	Placebo Diphacinone Bait	10/27/2017	0.266 <sup>a</sup>	ND
S170925-01-F		10/27/2017	0.278 <sup>a</sup>	ND
S170925-02	Placebo Brodifacoum Bait	10/27/2017	ND	ND
S170925-03-D		10/27/2017	46.8	ND
S170925-03-E	Diphacinone Conservation 50 (0.0050%) Bait	10/27/2017	46.3	ND
S170925-03-F		10/27/2017	46.1	ND
S170925-04-D		10/27/2017	ND	26.0
S170925-04-E	Brodifacoum Conservation 25 (0.0025%) Bait	10/27/2017	ND	27.2
S170925-04-F		10/27/2017	ND	25.8
		DL (µg/g) =	2.8	0.043
		QL (µg/g) =	9.40	0.142

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

\* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

<sup>a</sup> Method 163A is not sufficiently sensitive to detect diphacinone concentrations less than 2.8 µg/g. To better assess trace level contamination in the baits extracts were also tested by a more sensitive LCMS method with detection limits of 0.0072 µg/g for diphacinone and 0.0081 µg/g for brodifacoum. The placebo diphacinone bait (S170925-01) had diphacinone concentrations of 0.278 – 0.424 µg/g. None of the other baits had detectable contamination.

**QC Results:****QC Recoveries – *Ensatina* Salamander (whole body, S170602-13)**

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-41	9/14/2017	0	ND	N/A	0	ND	N/A
QC-42	9/14/2017	0	ND	N/A	0	ND	N/A
QC-43	9/14/2017	0	ND	N/A	0	ND	N/A
QC-44	9/14/2017	52.9	53.4	101%	52.7	61.3	116%
QC-45	9/14/2017	53.5	54.8	102%	53.3	66.7	125%
QC-46	9/14/2017	52.0	51.1	98.3%	51.8	64.6	125%
QC-47	9/14/2017	427	400	93.7%	425	508	120%
QC-48	9/14/2017	393	364	92.6%	391	472	121%
QC-49	9/14/2017	400	364	91.0%	398	448	113%
QC-50	9/14/2017	4400	4240	96.4%	4380	4750	108%
QC-51	9/14/2017	4360	4250	97.5%	4340	4720	109%
QC-52	9/14/2017	4380	4200	95.9%	4370	4850	111%
		DL (ng/g) =	5.9		DL (ng/g) =	6.6	
		QL (ng/g) =	19.6		QL (ng/g) =	21.9	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – *Aneides* Salamanders (whole body, S170711-31)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-29	9/28/2017	0	ND	N/A	0	ND	N/A
QC-30	9/28/2017	0	ND	N/A	0	ND	N/A
QC-31	9/28/2017	0	ND	N/A	0	ND	N/A
QC-32	9/28/2017	53.3	64.1	120%	53.1	62.1	117%
QC-33	9/28/2017	52.6	48.3	91.8%	52.4	60.1	115%
QC-34	9/28/2017	51.5	49.4	95.9%	51.3	50.3	98.1%
QC-35	9/28/2017	407	389	95.6%	405	428	106%
QC-36	9/28/2017	401	382	95.3%	400	400	100%
QC-37	9/28/2017	409	406	99.3%	407	428	105%
QC-38	9/28/2017	4110	4010	97.6%	4090	4140	101%
QC-39	9/28/2017	4410	4310	97.7%	4390	4570	104%
QC-40	9/28/2017	4340	4330	99.8%	4320	4400	102%
		DL (ng/g) =	7.5		DL (ng/g) =	8.6	
		QL (ng/g) =	25.1		QL (ng/g) =	28.6	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – *Batrachoseps* Salamanders (whole body, S170602-29)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-53	9/19/2017	0	ND	N/A	0	ND	N/A
QC-54	9/19/2017	0	ND	N/A	0	ND	N/A
QC-55	9/19/2017	0	ND	N/A	0	ND	N/A
QC-65	9/25/2017	0	ND	N/A	0	ND	N/A
QC-66	9/25/2017	0	ND	N/A	0	ND	N/A
QC-67	9/25/2017	0	ND	N/A	0	ND	N/A
QC-77	9/27/2017	0	ND	N/A	0	ND	N/A
QC-78	9/27/2017	0	ND	N/A	0	ND	N/A
QC-79	9/27/2017	0	ND	N/A	0	ND	N/A
QC-56	9/19/2017	53.9	47.7	88.5%	53.7	56.3	105%
QC-57	9/19/2017	51.7	46.5	89.9%	51.5	57.0	111%
QC-58	9/19/2017	52.2	52.8	101%	51.9	55.0	106%
QC-68	9/25/2017	53.9	51.9	96.3%	53.7	64.1	119%
QC-69	9/25/2017	52.9	56.3	106%	52.7	57.9	110%
QC-70	9/25/2017	54.8	55.7	102%	54.5	69.5	128%
QC-80	9/27/2017	53.2	56.7	107%	53.0	57.2	108%
QC-81	9/27/2017	52.2	48.4	92.7%	51.9	61.2	118%
QC-82	9/27/2017	52.7	59.3	113%	52.5	63.0	120%
QC-59	9/19/2017	398	371	93.2%	396	346	87.4%
QC-60	9/19/2017	389	384	98.7%	388	363	93.6%
QC-61	9/19/2017	393	376	95.7%	392	381	97.2%
QC-71	9/25/2017	404	412	102%	402	462	115%
QC-72	9/25/2017	412	395	95.9%	410	483	118%
QC-73	9/25/2017	415	423	102%	413	471	114%
QC-83	9/27/2017	472	483	102%	470	527	112%
QC-84	9/27/2017	468	462	98.7%	466	426	91.4%
QC-85	9/27/2017	469	446	95.1%	467	543	116%
QC-62	9/19/2017	4330	4210	97.2%	4320	4040	93.5%
QC-63	9/19/2017	4410	4200	95.2%	4390	3880	88.4%
QC-64	9/19/2017	4210	4110	97.6%	4200	3640	86.7%
QC-74	9/25/2017	4140	4080	98.6%	4120	4190	102%
QC-75	9/25/2017	4250	4240	99.8%	4230	4330	102%
QC-76	9/25/2017	4320	4320	100%	4300	4380	102%
QC-86	9/27/2017	3570	3490	97.8%	3560	3980	112%
QC-87	9/27/2017	3720	3540	95.2%	3700	4150	112%
QC-88	9/27/2017	3670	3540	96.5%	3650	4060	111%
		DL (ng/g) =	8.9		DL (ng/g) =	8.9	
		QL (ng/g) =	29.8		QL (ng/g) =	29.7	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).



## QC Recoveries – Crickets (S170711-52)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-1	9/13/2017	0	ND	N/A	0	ND	N/A
QC-2	9/13/2017	0	ND	N/A	0	ND	N/A
QC-3	9/13/2017	0	ND	N/A	0	ND	N/A
QC-13	9/12/2017	0	ND	N/A	0	ND	N/A
QC-14	9/12/2017	0	ND	N/A	0	ND	N/A
QC-15	9/12/2017	0	ND	N/A	0	ND	N/A
QC-4	9/13/2017	54.3	54.2	99.8%	54.1	61.4	113%
QC-5	9/13/2017	54.3	50.4	92.8%	54.0	63.3	117%
QC-6	9/13/2017	57.7	50.8	88.0%	57.5	60.5	105%
QC-16	9/12/2017	54.8	51.1	93.2%	54.6	65.1	119%
QC-17	9/12/2017	53.3	59.5	112%	53.1	59.0	111%
QC-18	9/12/2017	56.5	53.7	95.0%	56.3	62.1	110%
QC-7	9/13/2017	390	349	89.5%	389	447	115%
QC-8	9/13/2017	426	387	90.8%	425	436	103%
QC-9	9/13/2017	399	376	94.2%	397	452	114%
QC-19	9/12/2017	421	408	96.9%	420	464	110%
QC-20	9/12/2017	430	400	93.0%	428	465	109%
QC-21	9/12/2017	404	382	94.6%	403	450	112%
QC-10	9/13/2017	4620	4390	95.0%	4600	4870	106%
QC-11	9/13/2017	4480	4250	94.9%	4460	4780	107%
QC-12	9/13/2017	4480	4150	92.6%	4470	4620	103%
QC-22	9/12/2017	4560	4420	96.9%	4540	4720	104%
QC-23	9/12/2017	4280	4130	96.5%	4270	4310	101%
QC-24	9/12/2017	4610	4440	96.3%	4590	4660	102%
		DL (ng/g) =	4.9		DL (ng/g) =	5.9	
		QL (ng/g) =	16.2		QL (ng/g) =	19.7	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Recoveries – Water (saturated with ground placebo brodifacoum bait (S170925-02))**

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/mL)	Observed Diphacinone Concentration (ng/mL)	% Recovery	Theoretical Brodifacoum Concentration (ng/mL)	Observed Brodifacoum Concentration (ng/mL)	% Recovery
QC-113	10/13/2017	0	ND	N/A	0	ND	N/A
QC-114	10/13/2017	0	ND	N/A	0	ND	N/A
QC-115	10/13/2017	0	ND	N/A	0	ND	N/A
QC-116	10/13/2017	0.924	1.06	115%	0.920	1.04	113%
QC-117	10/13/2017	0.924	1.12	121%	0.920	1.11	121%
QC-118	10/13/2017	0.924	1.03	111%	0.920	1.01	110%
QC-119	10/13/2017	10.4	11.0	106%	10.3	11.0	107%
QC-120	10/13/2017	10.4	11.1	107%	10.3	11.0	107%
QC-121	10/13/2017	10.4	11.0	106%	10.3	10.8	105%
QC-122	10/13/2017	74.8	79.0	106%	74.5	64.7	86.8%
QC-123	10/13/2017	74.8	79.0	106%	74.5	67.0	89.9%
QC-124	10/13/2017	74.8	78.7	105%	74.5	66.6	89.4%
		DL (ng/mL) =	0.080		DL (ng/mL) =	0.13	
		QL (ng/mL) =	0.267		QL (ng/mL) =	0.419	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

**QC Recoveries – Baits (Method 163A, S170925-02)**

ID	Analysis Date	Theoretical Diphacinone Concentration (µg/g)	Observed Diphacinone Concentration (µg/g)	% Recovery	Theoretical Brodifacoum Concentration (µg/g)	Observed Brodifacoum Concentration (µg/g)	% Recovery
QC-137	10/27/2017	0	ND	N/A	0	ND	N/A
QC-138	10/27/2017	0	ND	N/A	0	ND	N/A
QC-139	10/27/2017	0	ND	N/A	0	ND	N/A
QC-140	10/27/2017	52.5	51.6	98.3%	27.1	25.9	95.6%
QC-141	10/27/2017	51.8	53.4	103%	26.7	26.3	98.5%
QC-142	10/27/2017	52.5	52.2	99.4%	27.1	26.6	98.2%
		DL (µg/g) =	2.8		DL (µg/g) =	0.043	
		QL (µg/g) =	9.40		QL (µg/g) =	0.142	

**QC Recoveries – Baits (LCMS Method, S170925-02)**

ID	Analysis Date	Theoretical Diphacinone Concentration (µg/g)	Observed Diphacinone Concentration (µg/g)	% Recovery	Theoretical Brodifacoum Concentration (µg/g)	Observed Brodifacoum Concentration (µg/g)	% Recovery
QC-137	10/27/2017	0	ND	N/A	0	ND	N/A
QC-138	10/27/2017	0	ND	N/A	0	ND	N/A
QC-139	10/27/2017	0	ND	N/A	0	ND	N/A
QC-140	10/27/2017	52.5	64.7	123%	27.1	18.0	66.4%
QC-141	10/27/2017	51.8	64.6	125%	26.7	17.7	66.3%
QC-142	10/27/2017	52.5	64.7	123%	27.1	17.3	63.8%
		DL (µg/g) =	0.0072		DL (µg/g) =	0.0081	
		QL (µg/g) =	0.0241		QL (µg/g) =	0.0270	