**QA-1434 Final Report: Assessing the potential hazard of anticoagulant rodenticides to non-target reptiles**

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Gary Witmer, Ph.D., Research Project Leader, and Richard Mauldin, Study Director, USDA National Wildlife Research Center, 4101 Laporte Avenue, Fort, Collins, Colorado 80521-2154

Cooperative Agreement with Island Conservation, No. 7485-0766-RA

**Background:** Very little is known about the potential hazards of anticoagulant rodenticides (used for invasive rodent control or eradication) to reptiles. We have wanted to complete a study like this for some time, but were unable to secure funding. A study protocol, QA-1434, was approved on October 14, 2009. Details of the study objective and methods are presented in the protocol. We entered into a cooperative agreement with Island Conservation which is providing the additional funds that were needed. The cooperative agreement was signed by both parties on December 22, 2009. We had hoped to begin the study with a constrictor snake (e.g., *Boa constrictor*) because a ban on their import, keeping, breeding, transport, and sale was being considered by Congress to reduce the number of introductions occurring in parts of the US (notably Florida). However, we were unable to obtain enough snakes, so we began the study with a turtle species because these became available from a reptile distributor.

**Ornate Wood Turtles:** Thirty Central American ornate wood turtles (*Rhinoclemmys pulcherrima*) were transported to NWRC in May 27, 2010. Turtles have been maintained in individual tanks and provided with a den box, water *ad libitum*, and given about 60 g of chopped vegetables and fruit mixed with some wet cat food daily. A heat lamp was placed over the cages. Cedar mulch was used initially as a cage floor covering, but was replaced with heavy brown paper once the study began (i.e., dosing of turtles) to facilitate feces collection and to better allow determination of emesis/regurgitation should that occur. While the turtles were in quarantine, solutions were prepared of brodifacoum and diphacinone using propylene glycol as solvent. Active ingredients were obtained from Bell Laboratories in Wisconsin. The NWRC Analytical Chemistry Unit assured that the concentrations were correct and that the suspensions were uniform. We experimented with some blue dyes in the suspension so that we would be able to detect emesis and to look for evidence of material passage through the GI tract after dosing. We removed all red and blue fruits from the diet so as to not interfere with the detection of blue dye and so that evidence of bleeding would be more readily detected on paper or in feces. Data sheets for behavioral observations were created and the research and animal care staff met regularly to discuss progress, to assure consistency, and to make any necessary changes in procedures. An oral gavage practice session was also held with the Attending Veterinarian present. We also practiced necropsy with a turtle that had to be euthanized because of its condition (impacted intestine was noted).

Turtles were fasted one day before dosing. Turtles were dosed by oral gavage on July 19, 2010, and a second time a week later (July 26). Each of 8 dosed turtle received either 166 µg/kg of diphacinone solution or 84.0 µg/kg of brodifacoum. Eight control turtles were gavaged with the vehicle/solvent only. Turtle behavior has been observed several times daily. Feces was collected whenever observed in a cage, placed in a zip-lock bag, labeled and frozen for later residue and/or blood analysis. Food consumption was also monitored. Food consumption varied by individual and was, in part, related to the size variability of the turtles (0.2-1.0 kg). However, the turtles seemed to eat about 20-30 g of food per day and there did not seem to be much difference across treatments. No turtles died to date after dosing. No bleeding was observed. The blue dye was observed in the feces of a few turtles. We euthanized the treatment turtles on August 2, 2010. We bagged, labeled, and froze samples of liver, kidney, muscle, lung, spleen, and heart tissue for later residue analyses. We also collected histopathology samples and blood samples. On that day, we also administered the high dose (1.7 mg/kg diphacinone or 0.79 mg/kg brodifacoum) to 4 each of the remaining control turtles. Procedures for these turtles thereafter followed the previous trial procedures.

None of the turtles dosed twice with the high dose of diphacinone or the high dose of brodifacoum died during the 2-week exposure period before euthanasia and none exhibited unusual behaviors. Histological preparation and analysis of tissue samples (liver, lung, heart, kidney, skeletal muscle) showed no overt pathological signs. Preliminary diphacinone residue analyses of turtle liver samples were completed. Samples were weighed for analysis at 0.50-0.60g. A total of twelve turtle livers were analyzed, 4 high dose turtle livers (dosed twice 1-week apart at 1.7 mg/kg) and 8 low dose turtle livers (dosed twice 1-week apart at 166 µg/kg). The samples were analyzed using Agilent HPLC instrumentation and a Phenomenex Gemini C18 column. All spiking was performed with Eppendorf Repeater Plus positive displacement pipetter with 1-ml alloquots. 200mg/3mL Phenomenex Strata-X-AW SPE cartridges were used (Zymark RATNH2.SPE method). Samples and standards were diluted to a final volume of 1.0 ml with 5-mM TBAP in 40% (pH 8.5 6-mM phosphate buffer)/60%(MeOH). Final filtration was performed with 0.45-µm PTFE syringe filters directly into HPLC auto-sampler vials. The mean diphacinone concentration for the high dose turtle livers was 1.30µg/g with a range of 1.19µg/g-1.40µg/g. The mean diphacinone concentration for the low dose turtle livers was 0.35µg/g with a range of 0.23µg/g-0.53µg/g. The turtle liver diphacinone levels are shown in the table below:

Turtle Liver No. Sample Concentration (µg/g)

2 High Dose Diphacinone 1.40

4 High Dose Diphacinone 1.31 Mean= 1.30

22 High Dose Diphacinone 1.19 High= 1.40

28 High Dose Diphacinone 1.29 Low= 1.19

3 Low Dose Diphacinone 0.32

7 Low Dose Diphacinone 0.24

10 Low Dose Diphacinone 0.33

13 Low Dose Diphacinone 0.23 Mean= 0.35

14 Low Dose Diphacinone 0.43 High= 0.53

17 Low Dose Diphacinone 0.37 Low= 0.23

18 Low Dose Diphacinone 0.53

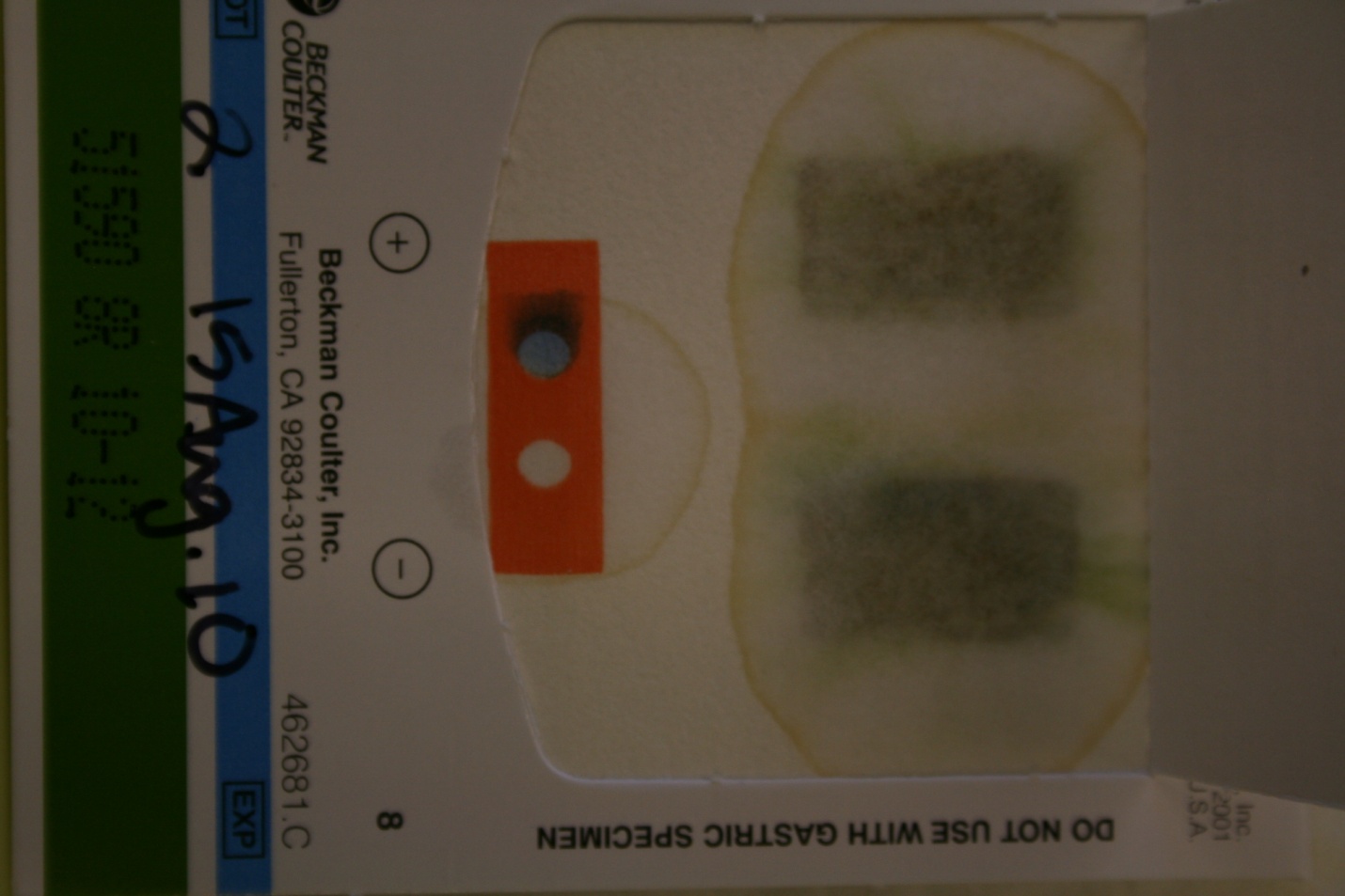
26 Low Dose Diphacinone 0.39

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Difficulties were encountered with the chemical assay method for the brodifacoum samples and those samples were re-analyzed after the analytical method was improved.

A few of the turtle fecal samples were examined for blood using the Hemoccult and Hemoccult Sensa stool guaiac test. In this test, fecal samples are smeared onto absorbent paper which turns blue following the addition of test reagent if blood is present in the stool. A few samples of high dose brodifacoum (# 29) and high dose diphacinone (#2) samples were found to contain blood (see figures below notice blue/ blue-green color on absorbent paper surrounding fecal samples). Although these are very useful data, the diet of test animals can affect results. Therefore, we will compare positive results after dosing to results from the same animals prior to dosing thereby allowing us to determine if the occult blood is a result of the administered anticoagulant.





The mean diphacinone concentration for the high dose turtle muscle was 1.33µg/g with a range of 0.96µg/g-1.91µg/g. The mean diphacinone concentration for the low dose turtle muscle was 0.25µg/g with a range of 0.14µg/g-0.45µg/g. The turtle muscle diphacinone levels are shown in the table below:

Turtle Musc. No. Sample Concentration (µg/g)

2 High Dose Diphacinone 1.24

4 High Dose Diphacinone 1.91 Mean= 1.33

22 High Dose Diphacinone 1.24 High= 1.91

28 High Dose Diphacinone 0.963 Low= 0.963

3 Low Dose Diphacinone 0.150

7 Low Dose Diphacinone 0.249

10 Low Dose Diphacinone 0.204

13 Low Dose Diphacinone 0.142 Mean= 0.247

14 Low Dose Diphacinone 0.454 High= 0.454

17 Low Dose Diphacinone 0.230 Low= 0.142

18 Low Dose Diphacinone 0.303

26 Low Dose Diphacinone 0.241

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The mean brodifacoum concentration for the high dose turtle livers was 1.31µg/g with a range of 0.86µg/g-2.02µg/g. The mean brodifacoum concentration for the low dose turtle livers was 0.32µg/g with a range of 0.17µg/g-0.72µg/g. The turtle liver brodifacoum levels are shown in the table below:

Turtle Liver No. Sample Concentration (µg/g) \_\_\_\_\_

12 High Dose Brodifacoum 1.25

16 High Dose Brodifacoum 1.12 Mean= 1.31

20 High Dose Brodifacoum 0.861 High= 2.02

29 High Dose Brodifacoum 2.02 Low= 0.861

5 Low Dose Brodifacoum 0.432

6 Low Dose Brodifacoum 0.192

8 Low Dose Brodifacoum 0.191

9 Low Dose Brodifacoum 0.334 Mean= 0.320

11 Low Dose Brodifacoum 0.174 High= 0.721

15 Low Dose Brodifacoum 0.342 Low= 0.174

19 Low Dose Brodifacoum 0.721

25 Low Dose Brodifacoum 0.179

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Analytical methods for determination of brodifacoum residue levels varied somewhat from those used for diphacinone. Turtle liver samples were weighed for analysis at 0.50-0.55g. A total of twelve turtle liver tissues were analyzed for residue determination, four high dosed and eight low dosed animals. Samples were analyzed using Agilent HPLC instrumentation and a Phenomenex Gemini C18 column. Also, 200mg/3mL Phenomenex Strata-X-AW SPE cartridges were used with the SPE clean-up done manually on the manifold. Samples and standards were diluted to a final volume of 1.0 mL with 5-mM TBAP in 20% (pH 8.5 6-mM phosphate buffer)/80%(MeOH). Method 139A was modified to include the MARS microwave, eliminate the need for Na2SO4, and improve recoveries.

The mean brodifacoum concentration for the high dose turtle muscle was 0.48µg/g with a range of 0.35µg/g-0.55µg/g. The mean brodifacoum concentration for the low dose turtle muscle was 0.09µg/g with a range of 0.06µg/g-0.14µg/g. The turtle muscle brodifacoum levels are shown in the table below:

Turtle Musc.No. Sample Concentration (µg/g) \_\_\_\_\_

12 High Dose Brodifacoum 0.515

16 High Dose Brodifacoum 0.353 Mean= 0.482

20 High Dose Brodifacoum 0.547 High= 0.547

29 High Dose Brodifacoum 0.511 Low= 0.353

5 Low Dose Brodifacoum 0.0686

6 Low Dose Brodifacoum 0.0947

8 Low Dose Brodifacoum 0.144

9 Low Dose Brodifacoum 0.0798 Mean= 0.0892

11 Low Dose Brodifacoum 0.0642 High= 0.144

15 Low Dose Brodifacoum 0.0569 Low= 0.0569

19 Low Dose Brodifacoum 0.144

25 Low Dose Brodifacoum 0.0612

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Detailed information on the turtles used in the study is presented in the table at the end of the report. Most animals maintained or gained weight over the course of the study. No turtles died during the study and no hemorrhaging was noted.

**Boa constrictors:** Thirty-nine boa constrictor (*Boa constrictor*) snakes arrived at NWRC on November 3, 2010. They were treated for mites, weighed and placed in individual cages. The room was maintained at about 80 oF and a relative humidity of 55%. The floor of the cages was lined with a sheet of brown paper (for ease of feces collection) taped down at the sides of the cage. Each cage contained a water bowl. The snakes were fed 1 dead rat per week. Initial snake weights ranged from 350 g to 5,000 g. Most snakes gained weight over the course of the study. The snakes were dosed by oral gavage on November 15-16 with the same high and low dosages as used with the turtles. A second dose was administered on November 22-23. No snakes died during the course of the study and none showed adverse symptoms. Feces was collected regularly from the snake cages and frozen, but has not been tested for the presence of blood. All snakes were euthanized on November 29-30. Livers were removed and frozen for later residue analysis. The rest of the snake carcass was frozen for later residue analysis.

The mean diphacinone concentration for the high dose boa constrictor livers was 0.89µg/g with a range of 0.54µg/g-1.12µg/g. The mean diphacinone concentration for the low dose boa constrictor livers was 0.49µg/g with a range of 0.35µg/g-0.77µg/g. The boa constrictor liver diphacinone levels are shown in the table below:

Boa Liver No. Sample Concentration (µg/g) \_\_\_

6 Low Dose Diphacinone 0.411

12 Low Dose Diphacinone 0.657

14 Low Dose Diphacinone 0.515

18 Low Dose Diphacinone 0.765 Mean= 0.494

19 Low Dose Diphacinone 0.410 High= 0.765

31 Low Dose Diphacinone 0.457 Low= 0.346

32 Low Dose Diphacinone 0.392

39 Low Dose Diphacinone 0.346

1 High Dose Diphacinone 0.754

9 High Dose Diphacinone 0.909 Mean= 0.886

13 High Dose Diphacinone 1.11 High= 1.12

17 High Dose Diphacinone 0.691 Low= 0.538

20 High Dose Diphacinone 0.930

22 High Dose Diphacinone 1.04

34 High Dose Diphacinone 1.12

37 High Dose Diphacinone 0.538

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The mean diphacinone concentration for the high dose boa constrictor whole body (WB) was 0.32µg/g with a range of 0.07µg/g-0.63µg/g. The mean diphacinone concentration for the low dose boa constrictor whole body was 0.07µg/g with a range of 0.06µg/g-0.12µg/g. The boa whole body diphacinone levels are shown in the table below:

Boa WB No. Sample Concentration (µg/g)

MLOD= 0.061 µg/g

6 Low Dose Diphacinone 0.061 0.048 MLOD: Samples that were

12 Low Dose Diphacinone 0.064 below MLOD were reported

14 Low Dose Diphacinone 0.077 as 0.061 µg/g, actual values

18 Low Dose Diphacinone 0.061 0.053 are reported in red to the right

19 Low Dose Diphacinone 0.061 0.056

31 Low Dose Diphacinone 0.061 0.032 Mean= 0.071 / 0.060

32 Low Dose Diphacinone 0.061 0.035 High= 0.119

39 Low Dose Diphacinone 0.119 Low= 0.061 / 0.032

1 High Dose Diphacinone 0.069

9 High Dose Diphacinone 0.195 Mean= 0.322

13 High Dose Diphacinone 0.415 High= 0.631

17 High Dose Diphacinone 0.314 Low= 0.069

20 High Dose Diphacinone 0.203

22 High Dose Diphacinone 0.631

34 High Dose Diphacinone 0.499

37 High Dose Diphacinone 0.249

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The mean brodifacoum concentration for the high dose boa constrictor livers was 1.30µg/g with a range of 0.82µg/g-2.10µg/g. The mean brodifacoum concentration for the low dose boa constrictor livers was 0.60µg/g with a range of 0.49µg/g-0.85µg/g. The boa liver brodifacoum levels are shown in the table below:

Boa Liver No. Sample Concentration (µg/g) \_\_\_\_

5 Low Dose Brodifacoum 0.703

7 Low Dose Brodifacoum 0.846

8 Low Dose Brodifacoum 0.516

24 Low Dose Brodifacoum 0.496 Mean= 0.599

27 Low Dose Brodifacoum 0.513 High= 0.846

29 Low Dose Brodifacoum 0.485 Low= 0.485

35 Low Dose Brodifacoum 0.702

36 Low Dose Brodifacoum 0.533

2 High Dose Brodifacoum 0.821

3 High Dose Brodifacoum 1.05 Mean= 1.30

10 High Dose Brodifacoum 1.16 High= 2.10

15 High Dose Brodifacoum 2.10 Low= 0.821

26 High Dose Brodifacoum 1.29

28 High Dose Brodifacoum 1.33

30 High Dose Brodifacoum 1.03

33 High Dose Brodifacoum 1.62

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The mean brodifacoum concentration for the high dose boa constrictor whole body (WB) was 0.63µg/g with a range of 0.33µg/g-0.93µg/g. The mean brodifacoum concentration for the low dose boa constrictor whole body was 0.07µg/g with a range of 0.06µg/g-0.09µg/g. The boa whole body brodifacoum levels are shown in the table below:

Boa WB No. Sample Concentration (µg/g) \_\_\_\_\_

5 Low Dose Brodifacoum 0.072

7 Low Dose Brodifacoum 0.071

8 Low Dose Brodifacoum 0.064

24 Low Dose Brodifacoum 0.072 Mean= 0.072

27 Low Dose Brodifacoum 0.062 High= 0.087

29 Low Dose Brodifacoum 0.073 Low= 0.062

35 Low Dose Brodifacoum 0.072

36 Low Dose Brodifacoum 0.087

2 High Dose Brodifacoum 0.741

3 High Dose Brodifacoum 0.796 Mean= 0.632

10 High Dose Brodifacoum 0.628 High= 0.929

15 High Dose Brodifacoum 0.756 Low= 0.331

26 High Dose Brodifacoum 0.376

28 High Dose Brodifacoum 0.331

30 High Dose Brodifacoum 0.929

33 High Dose Brodifacoum 0.502

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Detailed information on the boa constrictors used in the study is presented in the table at the end of the report. Most animals maintained or gained weight over the course of the study. No boas died during the study and no hemorrhaging was noted.

**Ameiva lizards:** Fifty wild-caught *Ameiva ameiva* lizards were received from Guyana, South America, on July 28, 2011. Only 1 lizard arrived dead. Snout-vent lengths (59-178 mm) and weights (3.5-159 g) varied greatly, but most were in good condition. The group contained 22 females and 28 males. A few that were particularly small or appeared in rather poor condition were not used in the trial. Most adjusted well to captivity and began feeding on insects right away. Lizards were randomly assigned divided into 5 groups: control (n=9), 1x diphacinone (n=10), 1x brodifacoum (n=9), 10x diphacinone (n=9), and 10x brodifacoum (n=9). After a quarantine and acclimation period of about 2 weeks, the lizards were dosed (oral gavage) with the anticoagulant solutions or the placebo (control group). The dose concentrations were the same as for the previous turtle and boa snake trials. The first gavages were performed on August 8-11, 2011, and a week later (August 15-16, 2011) the second oral gavage was administered. The blue dye in the gavage solutions appeared in feces within a few days of each gavage. A few lizards regurgitated some of the gavage solution: 7 of the 46 lizards in the first gavage session and 2 of 46 in the second gavage. Euthanasia and necropsy of all lizards still alive (n=42) occurred 1 week (on August 24-25, 2011) after the second gavage. A few lizards appeared lethargic at the trial progressed; the behavioral observation data sheets have yet to be analyzed to see if this might be a treatment effect. A few lizards showed some bleeding during the trial; daily observation records were examined to determine how many and to which treatment group they belonged. Four lizards died during the trial: 3 were from the 1x brodifacoum group and 1 was in the 10x diphacinone group. Because none died in the 10x brodifacoum group, it is probable that the deaths were not anticoagulant poisoning related. Furthermore, the in-depth necropsy of a dead lizard from the 1x brodifacoum group revealed no evidence of internal hemorrhaging. The final weights of lizards euthanized and necropsied at the end of the trial varied from 29.9-144.5 g. Most lizards had gained weight over the course of the trial, but a few lost weight.

Residue analyses for the Ameiva lizards were completed in December, 2011. Ameiva liver samples were weighed for analysis at 0.25-0.30 g. A total of sixteen Ameiva liver tissues were analyzed for diphacinone residue determination, eight low dose and eight high dose. The mean concentration for the low dose Ameiva livers was 0.142 µg/g, the lowest concentration was 0.083 µg/g, and the highest concentration was 0.357 µg/g. The mean concentration for the high dose Ameiva livers was 0.116 µg/g, the lowest concentration was 0.083 µg/g, and the highest concentration was 0.188 µg/g. Liver samples #12 and #14 were lost during sample processing and therefore were not analyzed. The Ameiva liver diphacinone levels are shown in the table below:

Ameiva Liver No. Sample Concentration (µg/g) \_\_\_\_\_\_

8 Low Dose Diphacinone 0.154

12 Low Dose Diphacinone Not Analyzed

13 Low Dose Diphacinone 0.357

14 Low Dose Diphacinone Not Analyzed Mean= 0.142

16 Low Dose Diphacinone 0.110 High= 0.357

23 Low Dose Diphacinone 0.183 Low= 0.060

31 Low Dose Diphacinone 0.083

34 Low Dose Diphacinone 0.083

46 Low Dose Diphacinone 0.083

49 Low Dose Diphacinone 0.084

9 High Dose Diphacinone 0.083

15 High Dose Diphacinone 0.083 Mean= 0.116

22 High Dose Diphacinone 0.083 High= 0.188

26 High Dose Diphacinone 0.101 Low= 0.049

27 High Dose Diphacinone 0.148

40 High Dose Diphacinone 0.188

41 High Dose Diphacinone 0.161 MLOD = 0.083 µg/g,

42 High Dose Diphacinone 0.083 numbers in red fall below

and are reported as 0.083

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Ameiva whole body (WB; minus liver and gall bladder) samples were weighed for analysis at 0.25-0.30 g. A total of eighteen Ameiva whole body tissues were analyzed for diphacinone residue determination, ten low dose and eight high dose. The mean concentration for the low dose Ameiva whole body was 0.022 µg/g, the lowest concentration was 0.018 µg/g, and the highest concentration was 0.035 µg/g. The mean concentration for the high dose Ameiva whole body was 0.032 µg/g, the lowest concentration was 0.018 µg/g, and the highest concentration was 0.076 µg/g. The Ameiva whole body diphacinone levels are shown below:

Ameiva WB No. Sample Concentration (µg/g) \_\_\_\_\_\_

8 Low Dose Diphacinone 0.035

12 Low Dose Diphacinone 0.018

13 Low Dose Diphacinone 0.018

14 Low Dose Diphacinone 0.018 Mean= 0.022

16 Low Dose Diphacinone 0.018 High= 0.035

23 Low Dose Diphacinone 0.029 Low= 0.018

31 Low Dose Diphacinone 0.018

34 Low Dose Diphacinone 0.018

46 Low Dose Diphacinone 0.018

49 Low Dose Diphacinone 0.019

9 High Dose Diphacinone 0.018

15 High Dose Diphacinone 0.018 Mean= 0.032

22 High Dose Diphacinone 0.021 High= 0.076

26 High Dose Diphacinone 0.034 Low= 0.018

27 High Dose Diphacinone 0.042

40 High Dose Diphacinone 0.018

41 High Dose Diphacinone 0.076 MLOD = 0.018 µg/g,

42 High Dose Diphacinone 0.026 numbers in red fall below and

are reported as 0.018

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Ameiva liver (with gall bladder) samples were weighed for analysis at 0.25-0.30 g. A total of fourteen Ameiva liver tissues were analyzed for brodifacoum residue determination, six low dose and eight high dose. The mean concentration for the low dose Ameiva livers was 0.976 µg/g, the lowest concentration was 0.561 µg/g, and the highest concentration was 1.449 µg/g. The mean concentration for the high dose Ameiva livers was 6.377 µg/g, the lowest concentration was 1.983 µg/g, and the highest concentration was 10.542 µg/g. MLOD was 0.009 µg/g. The Ameiva liver brodifacoum levels are shown in the table below:

Ameiva Liver No. Sample Concentration (µg/g) \_\_\_\_

1 Low Dose Brodifacoum 0.805

3 Low Dose Brodifacoum 1.449 Mean= 0.976

7 Low Dose Brodifacoum 0.561 High= 1.449

21 Low Dose Brodifacoum 0.616 Low= 0.561

28 Low Dose Brodifacoum 1.177

33 Low Dose Brodifacoum 1.250

2 High Dose Brodifacoum 10.059

10 High Dose Brodifacoum 8.366 Mean= 6.377

17 High Dose Brodifacoum 4.318 High= 10.542

18 High Dose Brodifacoum 1.983 Low= 1.983

24 High Dose Brodifacoum 5.834

32 High Dose Brodifacoum 3.489

35 High Dose Brodifacoum 10.542 **\*\*MLOD = 0.009 µg/g\*\***

36 High Dose Brodifacoum 6.422

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Ameiva whole body (WB; minus liver and gall bladder) samples were weighed for analysis at 0.49-0.51 g. A total of fourteen Ameiva whole body tissues were analyzed for brodifacoum residue determination, six low dose and eight high dose. The mean concentration for the low dose Ameiva whole bodies was 0.117 µg/g, the lowest concentration was 0.080 µg/g, and the highest concentration was 0.173 µg/g. The mean concentration for the high dose Ameiva whole bodies was 1.161 µg/g, the lowest concentration was 0.267 µg/g, and the highest concentration was 2.153 µg/g. MLOD was 0.012 µg/g. The Ameiva whole body brodifacoum levels are shown in the table below:

Ameiva WB No. Sample Concentration (µg/g)

1 Low Dose Brodifacoum 0.080

3 Low Dose Brodifacoum 0.122 Mean= 0.117

7 Low Dose Brodifacoum 0.098 High= 0.173

21 Low Dose Brodifacoum 0.109 Low= 0.080

28 Low Dose Brodifacoum 0.173

33 Low Dose Brodifacoum 0.121

2 High Dose Brodifacoum 1.067

10 High Dose Brodifacoum 1.349 Mean= 1.161

17 High Dose Brodifacoum 0.559 High= 2.153

18 High Dose Brodifacoum 0.267 Low= 0.267

24 High Dose Brodifacoum 1.182

32 High Dose Brodifacoum 0.818

35 High Dose Brodifacoum 2.153 **\*\*MLOD = 0.012 µg/g\*\***

36 High Dose Brodifacoum 1.892

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Detailed information on the Ameivas used in the study is presented in the table at the end of the report. Most Ameivas lost a small amount of weight over the course of the study. There were one death in the 10x diphacinone-dosed Ameivas; external hemorrhaging was noted in that lizard. There were 4 deaths in the brodifacoum-dosed Ameivas, but none showed signs of hemorrhaging.

**Green Iguanas:** Fifty green iguanas (*Iguana iguana*) were wild-caught in southern Florida and shipped to NWRC on January 11, 2012. All appeared to arrive in good health. The iguanas ranged from about 25-50 cm in total length with snout-vent lengths of 130-230 cm. Weights were from 50-590 g. One iguana was placed in each aquarium. Iguanas were dosed on February 1-2, 2012. They received their second dose on February 8-9, 2012. The diphacinone and brodifacoum doses were the same as per the previous species. All Iguanas were euthanized and necropsied on February 15-16, 2012. Methods used were the same as with the Ameiva lizards, with the exception that the Iguanas were fed a mixture of chopped vegetables and fruit (versus crickets for the Ameivas).

Residue analyses for the Iguana lizards were completed in September, 2012. A total of nineteen Iguana liver tissues were analyzed for diphacinone residue determination, nine low dose and ten high dose. The mean concentration for the low dose Iguana livers was 0.297 µg/g, the lowest concentration was 0.152 µg/g, and the highest concentration was 0.52 µg/g. The mean concentration for the high dose Iguana livers was 0.575 µg/g, the lowest concentration was 0.211 µg/g, and the highest concentration was 1.33 µg/g. All Iguana liver and whole body diphacinone levels are shown in the table below.

Similarly, nineteen whole body diphacinone levels were determined. The mean concentration for the low dose Iguana whole bodies was 0.036 µg/g, the lowest concentration was below the Minimum Level of Detection (MLOD = 0.026 µg/g, and the highest concentration was 0.068 µg/g. The mean concentration for the high dose Iguana whole bodies was 0.328 µg/g, the lowest concentration was 0.032 µg/g, and the highest concentration was 1.28 µg/g. All Iguana liver and whole body diphacinone levels are shown in the table below:

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Diphacinone Conc. (µg/g) | |
| Iguana ID | Treatment | Carcass | Liver |
| 12 | Low (Diphacinone) | 0.0442 | 0.511 |
| 15 | Low (Diphacinone) | < MLOD | 0.152 |
| 19 | Low (Diphacinone) | < MLOD | 0.0919 |
| 23 | Low (Diphacinone) | < MLOD | 0.291 |
| 31 | Low (Diphacinone) | < MLOD | 0.266 |
| 37 | Low (Diphacinone) | < MLOD | 0.233 |
| 41 | Low (Diphacinone) | < MLOD | 0.279 |
| 48 | Low (Diphacinone) | 0.068 | 0.52 |
| 50 | Low (Diphacinone) | 0.0512 | 0.327 |
|  |  |  |  |
| 4 | High (Diphacinone) | 0.374 | 0.551 |
| 14 | High (Diphacinone) | 0.256 | 0.731 |
| 21 | High (Diphacinone) | 0.191 | 0.573 |
| 22 | High (Diphacinone) | 0.669 | 1.33 |
| 25 | High (Diphacinone) | 1.28 | 0.867 |
| 28 | High (Diphacinone) | 0.0713 | 0.369 |
| 33 | High (Diphacinone) | 0.0319 | 0.259 |
| 42 | High (Diphacinone) | 0.0577 | 0.211 |
| 45 | High (Diphacinone) | 0.0478 | 0.308 |
| 47 | High (Diphacinone) | 0.301 | 0.55 |

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A total of nineteen Iguana liver tissues were analyzed for brodifacoum residue determination, nine low dose and ten high dose. The mean concentration for the low dose Iguana livers was 1.220 µg/g, the lowest concentration was 0.811 µg/g, and the highest concentration was 1.880 µg/g. The mean concentration for the high dose Iguana livers was 3.508 µg/g, the lowest concentration was 2.440 µg/g, and the highest concentration was 5.870 µg/g. All Iguana liver and whole body brodifacoum levels are shown in the table below.

Similarly, nineteen whole body brodifacoum levels were determined. The mean concentration for the low dose Iguana whole bodies was 0.177 µg/g, the lowest concentration was below the Minimum Level of Detection (MLOD = 0.010 µg/g, and the highest concentration was 0.365 µg/g. The mean concentration for the high dose Iguana whole bodies was 1.576 µg/g, the lowest concentration was 0.818 µg/g, and the highest concentration was 3.430 µg/g. All Iguana liver and whole body brodifacoum levels are shown in the table below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Brodifacoum Conc. (µg/g) | | |
| Iguana ID | Treatment | | Carcass | Liver |
| 10 | Low (Brodifacoum) | | < MLOD | 1.63 |
| 13 | Low (Brodifacoum) | | 0.365 | 1.88 |
| 18 | Low (Brodifacoum) | | 0.167 | 0.939 |
| 24 | Low (Brodifacoum) | | 0.297 | 1.18 |
| 35 | Low (Brodifacoum) | | 0.175 | 1.28 |
| 36 | Low (Brodifacoum) | | 0.15 | 1.41 |
| 39 | Low (Brodifacoum) | | 0.233 | 1.02 |
| 43 | Low (Brodifacoum) | | 0.0785 | 0.811 |
| 44 | Low (Brodifacoum) | | 0.115 | 0.833 |
|  |  | |  |  |
| 1 | High (Brodifacoum) | | 2.52 | 5.87 |
| 5 | High (Brodifacoum) | | 1.49 | 4.22 |
| 8 | High (Brodifacoum) | | 3.43 | 3.36 |
| 16 | High (Brodifacoum) | | 1.29 | 3.07 |
| 26 | High (Brodifacoum) | | 1.27 | 2.82 |
| 29 | High (Brodifacoum) | | 0.818 | 2.44 |
| 32 | High (Brodifacoum) | | 0.974 | 2.64 |
| 34 | High (Brodifacoum) | | 1.88 | 3.15 |
| 38 | High (Brodifacoum) | | 0.961 | 3.3 |
| 49 | High (Brodifacoum) | | 1.13 | 4.21 |

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Detailed information on the Iguanas used in the study is presented in the table at the end of the report. Most Iguanas maintained or gained a small amount of weight over the course of the study, but some lost a small amount of weight. There were no deaths in the diphacinone-dosed Iguanas, but bleeding was noted in 7 of the 19 dosed animals. There was one death in the low-dose brodifacoum group and that Iguana had some external bleeding and blood in the mouth. There were no deaths in the high-dose brodifacoum group, but one animal had internal hemorrhaging. Three other animals had external bleeding from torn toenails.

**Table of average residue levels across species in QA-1434:**

**Average Diphacinone Residue Levels (µg/g)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Tissue** | **High Dose** | **Low Dose** |
| Turtle | Liver | 1.30 | 0.35 |
| Boa | Liver | 0.87 | 0.49 |
| Ameiva | Liver | 0.12 | 0.14 |
| Iguana | Liver | 0.58 | 0.30 |
| Turtle | Muscle | 1.33 | 0.25 |
| Boa | Whole Body | 0.32 | 0.07 |
| Ameiva | Whole Body | 0.03 | 0.02 |
| Iguana | Whole Body | 0.33 | 0.04 |

**Average Brodifacoum Residue Levels (µg/g)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Tissue** | **High Dose** | **Low Dose** |
| Turtle | Liver | 1.31 | 0.32 |
| Boa | Liver | 1.30 | 0.60 |
| Ameiva | Liver | 6.37 | 0.98 |
| Iguana | Liver | 3.51 | 1.22 |
| Turtle | Muscle | 0.48 | 0.09 |
| Boa | Whole Body | 0.63 | 0.07 |
| Ameiva | Whole Body | 1.16 | 0.12 |
| Iguana | Whole Body | 1.58 | 0.18 |

The table below summarizes the results of the twice oral-gavaged cage trials:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Brodifacoum 1X | Brodifacoum 10X | Diphacinone 1X | Diphacinone 10X |
| Turtles | 8 animals  No deaths  No hemorrhaging | 4 animals  No deaths  No hemorrhaging | 8 animals  No deaths  No hemorrhaging | 4 animals  No deaths  No hemorrhaging |
| Boas | 8 animals  No deaths  No hemorrhaging | 8 animals  No deaths  No hemorrhaging | 8 animals  No deaths  No hemorrhaging | 8 animals  No deaths  No hemorrhaging |
| Ameivas | 9 animals  3 deaths, but  no hemorrhaging | 9 animals  1 death, but  no hemorrhaging | 10 animals  No deaths, but  no hemorrhaging | 9 animals  1 death with  external hemorrhaging |
| Iguanas | 9 animals  1 death with external hemorrhaging | 10 animals  No deaths, 1 with  external hemorrhaging | 9 animals  No deaths, but 2 with external &/or  internal hemorrhaging | 10 animals  No deaths, but 5 with external &/or  internal hemorrhaging |

Gavage dose per gavage:

Brodifacoum 1X = 84 µg/kg

Brodifacoum 10X = 0.79 mg/kg

Diphacinone 1X = 166 µg/kg

Diphacinone 10X = 1.7 mg/kg

The table suggests that turtles and boas may not be as sensitive to anticoagulants as are lizards. However, we note that the deaths of most (4 of 5) Ameivas showed no evidence of hemorrhaging, and hence, death may not have been because of anticoagulant poisoning.

General Comment on the Residue Levels:

With diphacinone dosing, the turtles and boas tended to have higher residue levels. The opposite was true with brodifacoum where lizards had higher residue levels.

**Table of detailed information on the animals used in QA-1434.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **QA-1434 Turtle Weight and Fate Table** | | | | | | | |
| **Species** | **Animal ID** | **Sex** | **Treatment** | **Start  Weight (g)** | **End  Weight (g)** | **Alive/Dead  (A/D)** | **Notes on Adverse Signs** |
| turtle | 3 | F | Diph | 761.0 | 782.0 | A |  |
| turtle | 7 | F | Diph | 687.5 | 678.2 | A |  |
| turtle | 10 | F | Diph | 455.0 | 468.4 | A |  |
| turtle | 13 | F | Diph | 965.0 | 963.7 | A |  |
| turtle | 14 | M | Diph | 331.5 | 325.8 | A |  |
| turtle | 17 | F | Diph | 583.5 | 570.4 | A |  |
| turtle | 18 | M | Diph | 373.5 | 374.1 | A |  |
| turtle | 26 | M | Diph | 193.5 | 192.0 | A |  |
| turtle | 2 | M | Diph. 10X | 421.0 | 421.7 | A |  |
| turtle | 4 | F | Diph. 10X | 637.5 | 675.9 | A |  |
| turtle | 22 | F | Diph. 10X | 778.0 | 785.3 | A | not very responsive from 7/20-7/22 & 7/27-7/28 & 8/2-8/4 & 8/6 |
| turtle | 28 | M | Diph. 10X | 322.0 | 318.4 | A | black spots in feces on 7/30. |
| turtle | 5 | F | Brod | 648.0 | 657.0 | A |  |
| turtle | 6 | M | Brod | 553.5 | 554.1 | A |  |
| turtle | 8 | M | Brod | 294.5 | 298.7 | A |  |
| turtle | 9 | F | Brod | 777.5 | 783.9 | A |  |
| turtle | 11 | M | Brod | 253.5 | 256.5 | A |  |
| turtle | 15 | M | Brod | 493.5 | 591.1 | A |  |
| turtle | 19 | M | Brod | 366.5 | 360.9 | A |  |
| turtle | 25 | F | Brod | 902.5 | 918.6 | A |  |
| turtle | 12 | F | Brod. 10X | 762.0 | 774.9 | A |  |
| turtle | 16 | F | Brod. 10X | 886.0 | 936.7 | A | slow to respond 7/27 & 8/2-8/6 & 8/8 & 8/10 |
| turtle | 20 | M | Brod. 10X | 435.5 | 440.8 | A |  |
| turtle | 29 | M | Brod. 10X | 309.0 | 318.8 | A | slow to respond and not eating on 8/4 & 8/6 |
| turtle | 1 | F | Control | 841.5 | 856.3 | A |  |
| turtle | 23 | M | Control | 234.0 | 335.7 | A |  |
| turtle | 24 | F | Control | 755.5 | 740.2 | A | not normal, spotted liver; worms in feces on 7/30. |
| turtle | 30 | F | Control | 369.0 | 385.2 | A | spotted liver |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **QA-1434 Boa Weight and Fate Table** | | | | | | | |
| **Species** | **Animal ID** | **Sex** | **Treatment** | **Start  Weight (g)** | **End  Weight (g)** | **Alive/Dead  (A/D)** | **Notes on Adverse Signs** |
| Boa | 6 | M | Diph | 3730.0 | 3660.0 | A |  |
| Boa | 12 | F | Diph | 2500.0 | 2560.0 | A |  |
| Boa | 14 | F | Diph | 1200.0 | 2710.0 | A |  |
| Boa | 18 | F | Diph | 1350.0 | 1850.0 | A |  |
| Boa | 19 | M | Diph | 1330.0 | 1500.0 | A |  |
| Boa | 31 | M | Diph | 580.0 | 580.0 | A |  |
| Boa | 32 | F | Diph | 700.0 | 600.0 | A |  |
| Boa | 39 | M | Diph | 470.0 | 550.0 | A |  |
| Boa | 1 | F | Diph. 10X | 4440.0 | 4470.0 | A | extra large liver |
| Boa | 9 | F | Diph. 10X | 1930.0 | 1900.0 | A |  |
| Boa | 13 | F | Diph. 10X | 2720.0 | 3190.0 | A |  |
| Boa | 17 | F | Diph. 10X | 1780.0 | 1760.0 | A |  |
| Boa | 20 | M | Diph. 10X | 1180.0 | 1180.0 | A | kidney more brown; small yellow lessions on kidney;  blood found in feces on 11/20 & 11/22/10 |
| Boa | 22 | M | Diph. 10X | 780.0 | 860.0 | A |  |
| Boa | 34 | F | Diph. 10X | 620.0 | 690.0 | A |  |
| Boa | 37 | M | Diph. 10X | 370.0 | 420.0 | A |  |
| Boa | 5 | F | Brod | 5190.0 | 5180.0 | A | Yellowish mass filling right air sac, 8-10 in. long; spots/bumps on  lining of air sac which were not attached (easily peeled off) |
| Boa | 7 | F | Brod | 3660.0 | 3210.0 | A |  |
| Boa | 8 | M | Brod | 2180.0 | 2240.0 | A |  |
| Boa | 24 | M | Brod | 480.0 | 550.0 | A |  |
| Boa | 27 | F | Brod | 880.0 | 940.0 | A |  |
| Boa | 29 | F | Brod | 2160.0 | 2430.0 | A |  |
| Boa | 35 | F | Brod | 550.0 | 600.0 | A |  |
| Boa | 36 | M | Brod | 710.0 | 590.0 | A |  |
| Boa | 2 | M | Brod. 10X | 4290.0 | 5210.0 | A |  |
| Boa | 3 | M | Brod. 10X | 3670.0 | 3890.0 | A |  |
| Boa | 10 | F | Brod. 10X | 2140.0 | 2900.0 | A |  |
| Boa | 15 | F | Brod. 10X | 1890.0 | 1870.0 | A |  |
| Boa | 26 | F | Brod. 10X | 1220.0 | 1450.0 | A |  |
| Boa | 28 | F | Brod. 10X | 680.0 | 700.0 | A |  |
| Boa | 30 | M | Brod. 10X | 500.0 | 600.0 | A | kidney contains lesions (creamy yello, 1-2 mm, round-oval); kidney  color not mahogany, medium/yellow brown; multifocal, entire length  of kidney, left side is more yellow/brown; liver is also mottled |
| Boa | 33 | F | Brod. 10X | 880.0 | 860.0 | A |  |
| Boa | 4 | M | Control | 3790.0 | 3940.0 | A |  |
| Boa | 11 | F | Control | 2590.0 | 2510.0 | A |  |
| Boa | 16 | F | Control | 1660.0 | 1830.0 | A |  |
| Boa | 21 | F | Control | 1350.0 | 1350.0 | A |  |
| Boa | 23 | M | Control | 710.0 | 850.0 | A |  |
| Boa | 25 | F | Control | 590.0 | 640.0 | A | very shriveled skin so soaked for 2 hrs on 11/18/10 |
| Boa | 38 | M | Control | 400.0 | 400.0 | A |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **QA-1434 Ameiva Weight and Fate Table** | | | | | | | |
| **Species** | **Animal ID** | **Sex** | **Treatment** | **Start  Weight (g)** | **End  Weight (g)** | **Alive/Dead  (A/D)** | **Notes on Adverse Signs** |
| Ameiva | 08 | M | Diph | 118.0 | 119.1 | A | worms in feces found on 8/12/11 |
| Ameiva | 12 | F | Diph | 44.0 | 40.1 | A |  |
| Ameiva | 13 | M | Diph | 88.5 | 78.7 | A | difficulty walking and eyes closed on 8/20/11 |
| Ameiva | 14 | M | Diph | 51.0 | 66.8 | A | gray spot on back left leg seen on 8/10/11 |
| Ameiva | 16 | F | Diph | 61.0 | 60.3 | A |  |
| Ameiva | 23 | M | Diph | 56.0 | 53.0 | A | green/blue bile in stomach |
| Ameiva | 31 | F | Diph | 73.0 | 73.3 | A | fluid in paracartal sack; liver is pale/tan with rounded  edgeand spots that do penetrate liver only on surface;  liver does not look good; 6 eggs; very lethargic on 8/11/11 |
| Ameiva | 34 | F | Diph | 54.5 | 56.2 | A | very dark lungs |
| Ameiva | 46 | M | Diph | 101.5 | 105.0 | A |  |
| Ameiva | 49 | M | Diph | 141.5 | 136.6 | A |  |
| Ameiva | 09 | M | Diph. 10X | 104.5 | 111.7 | A | lession on skin on left side above hind leg |
| Ameiva | 15 | M | Diph. 10X | 42.0 | 37.7 | A | blood on tail on 8/22/11; Liver is very pale and mushy |
| Ameiva | 22 | F | Diph. 10X | 36.5 | 35.5 | A |  |
| Ameiva | 26 | F | Diph. 10X | 51.0 | 49.3 | A |  |
| Ameiva | 27 | M | Diph. 10X | 71.0 | 74.5 | A |  |
| Ameiva | 37 | F | Diph. 10X | 65.5 | 57.0 | D | skin on head crusty and eye swollen on 8/16; blood on head and cage on 8/17; died and had blood on head on 8/18/11 |
| Ameiva | 40 | F | Diph. 10X | 74.0 | 64.9 | A |  |
| Ameiva | 41 | M | Diph. 10X | 102.0 | 100.3 | A | a little bit of blood found on bedding on 8/23/11 |
| Ameiva | 42 | M | Diph. 10X | 90.0 | 93.2 | A |  |
| Ameiva | 01 | M | Brod | 112.0 | 108.1 | A |  |
| Ameiva | 03 | M | Brod | 117.5 | 114.3 | A | mahogany/chestnut liver color; profusely mottled kidneys |
| Ameiva | 07 | M | Brod | 142.0 | 144.5 | A | lessions on liver |
| Ameiva | 21 | F | Brod | 36.0 | 38.2 | A | fatty liver |
| Ameiva | 28 | F | Brod | 62.0 | 59.7 | A |  |
| Ameiva | 30 | F | Brod | 51.0 | 51.5 | D | died on 8/18/11 |
| Ameiva | 33 | M | Brod | 55.5 | 49.9 | A |  |
| Ameiva | 44 | M | Brod | 103.0 | 96.5 | D | died on 8/16/11; possibly not due to anticoagulant poisoning |
| Ameiva | 47 | F | Brod | 70.0 | 69.5 | D | died on 8/11/11 and had blue liquid around mouth |
| Ameiva | 02 | M | Brod. 10X | 137.0 | 132.1 | A | roundworm |
| Ameiva | 10 | M | Brod. 10X | 96.5 | 98.7 | A |  |
| Ameiva | 17 | M | Brod. 10X | 45.0 | 45.8 | A |  |
| Ameiva | 18 | F | Brod. 10X | 47.0 | 45.5 | A | sore on left side above hind leg |
| Ameiva | 24 | F | Brod. 10X | 39.0 | 39.4 | A | swollen joints; diarrhea on 8/24/11 |
| Ameiva | 32 | F | Brod. 10X | 60.0 | 61.4 | A |  |
| Ameiva | 35 | F | Brod. 10X | 76.0 | 30.6 | A |  |
| Ameiva | 36 | M | Brod. 10X | 61.5 | 63.6 | A | swollen arm on 8/16-8/17/11 |
| Ameiva | 43 | M | Brod. 10X | 108.0 | N/A | D | not eating well on 8/7/11; euthanized on 8/20/11 |
| Ameiva | 04 | M | Control | 79.5 | Unknown | A | sore found on hip on 8/18/11 |
| Ameiva | 05 | M | Control | 117.0 | 109.2 | A | lessions on liver |
| Ameiva | 06 | M | Control | 150.0 | 142.4 | A |  |
| Ameiva | 11 | M | Control | 37.5 | 34.6 | A |  |
| Ameiva | 19 | F | Control | 31.5 | 29.9 | A |  |
| Ameiva | 20 | F | Control | 50.0 | 47.4 | A | 3 eggs |
| Ameiva | 25 | F | Control | 79.5 | 75.5 | A | small amount of blood on inside of cage possibly from tail; 5 eggs; a lot of blue/green bile in swollen stomach |
| Ameiva | 29 | F | Control | 58.0 | 62.4 | A | liver fatty |
| Ameiva | 45 | M | Control | 92.0 | 92.2 | A |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **QA-1434 Iguana Weight and Fate Table** | | | | | | | |
| **Species** | **Animal ID** | **Sex** | **Treatment** | **Start  Weight (g)** | **End  Weight (g)** | **Alive/Dead  (A/D)** | **Notes on Adverse Signs** |
| Iguana | 12 | M | Diph | 408.6 | 405.5 | A | blood found on head, chest, and cage 2/10-2/11/12 |
| Iguana | 15 | M | Diph | 292.5 | 300.5 | A |  |
| Iguana | 19 | M | Diph | 236.5 | 248.0 | A |  |
| Iguana | 23 | F | Diph | 258.5 | 249.0 | A |  |
| Iguana | 31 | M | Diph | 72.5 | 73.0 | A | dropped tail during gavaging on 2/2/12 b/c thrashing against wall of  isoflurane chamber |
| Iguana | 37 | M | Diph | 99.0 | 96.0 | A |  |
| Iguana | 41 | M | Diph | 112.0 | 122.0 | A |  |
| Iguana | 48 | F | Diph | 54.0 | 54.0 | A | bleeding in thoracic cavity (more than usual) |
| Iguana | 50 | F | Diph | 106.0 | 103.5 | A |  |
| Iguana | 4 | F | Diph. 10X | 214.5 | 221.0 | A |  |
| Iguana | 14 | M | Diph. 10X | 459.0 | 432.0 | A | blood found in cage 2/15/12; enlarged gall bladder (possibly necrotic  or blood clot) |
| Iguana | 21 | M | Diph. 10X | 292.0 | 282.5 | A | blood coming from mouth and nose before necropsy; bleeding in  small intestine |
| Iguana | 22 | M | Diph. 10X | 235.5 | 236.5 | A |  |
| Iguana | 25 | M | Diph. 10X | 104.0 | 106.5 | A | blood in mouth/nose; toothpick-sized woody piece in gut |
| Iguana | 28 | M | Diph. 10X | 81.0 | 85.0 | A | tore 2 toenails on 2/10/12; blood coming out of nose before  necropsy; nothing looked abnormal upon necropsy |
| Iguana | 33 | M | Diph. 10X | 91.0 | 95.5 | A |  |
| Iguana | 42 | F | Diph. 10X | 72.0 | 73.0 | A |  |
| Iguana | 45 | M | Diph. 10X | 56.5 | 61.5 | A |  |
| Iguana | 47 | F | Diph. 10X | 82.0 | 78.5 | A | blood in mouth before necropsy; red fluid in thoracic cavity |
| Iguana | 10 | M | Brod | 365.0 | 338.5 | D | died 2/14/12; tip of nose had coagulated blood on it and the mouth  had fresh blood in it (started bleeding 2/13) |
| Iguana | 13 | M | Brod | 412.0 | 384.0 | A | bleeding due to ripped toenail 2/12/12 |
| Iguana | 18 | F | Brod | 203.0 | 195.5 | A |  |
| Iguana | 24 | M | Brod | 90.0 | 88.0 | A |  |
| Iguana | 35 | F | Brod | 69.5 | 70.0 | A |  |
| Iguana | 36 | F | Brod | 143.0 | 141.5 | A |  |
| Iguana | 39 | M | Brod | 108.5 | 107.0 | A |  |
| Iguana | 43 | M | Brod | 104.0 | 111.5 | A |  |
| Iguana | 44 | M | Brod | 148.5 | 140.0 | A |  |
| Iguana | 1 | M | Brod. 10X | 460.5 | 454.0 | A | had swollen stifle joint on right leg, upon necropsy yellow mucous  (some parts fibrous; some liquidy) was found. |
| Iguana | 5 | F | Brod. 10X | 491.0 | 493.0 | A | bleeding from torn toenail on 2/5/12; small amount of blood found  in cage on 2/9/12; left lung bloody |
| Iguana | 8 | M | Brod. 10X | 233.5 | 238.0 | A | blood in thoracic cavity |
| Iguana | 16 | M | Brod. 10X | 272.0 | 259.0 | A |  |
| Iguana | 26 | M | Brod. 10X | 85.5 | 88.0 | A |  |
| Iguana | 29 | M | Brod. 10X | 103.5 | 106.0 | A |  |
| Iguana | 32 | M | Brod. 10X | 63.0 | 65.5 | A |  |
| Iguana | 34 | F | Brod. 10X | 79.0 | 80.0 | A |  |
| Iguana | 38 | M | Brod. 10X | 77.0 | 81.5 | A |  |
| Iguana | 49 | M | Brod. 10X | 66.0 | 67.0 | A | dropped tail on 1/30/12; bleeding from a couple of torn toenails  on 2/9/12; small amount of blood found on hide on 2/13 & 2/15/12 |
| Iguana | 7 | M | Control | 289.5 | 292.5 | A |  |
| Iguana | 9 | F | Control | 269.0 | 269.5 | A |  |
| Iguana | 11 | M | Control | 524.5 | 544.5 | A |  |
| Iguana | 17 | M | Control | 218.5 | 214.5 | A |  |
| Iguana | 20 | F | Control | 155.0 | 150.5 | A |  |
| Iguana | 27 | M | Control | 102.5 | 106.5 | A |  |
| Iguana | 30 | M | Control | 71.0 | 74.0 | A |  |
| Iguana | 40 | M | Control | 129.0 | 127.0 | A | bleeding from torn toenail on 2/6/12 |
| Iguana | 46 | M | Control | 69.5 | 68.5 | A |  |