Study Title:
The effects of cooking on diphacinone residues in feral pig tissue

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Executive Summary

1. We examined diphacinone levels in feral pigs to determine the potential risk to people who consume pig meat after the pigs were exposed to rodenticide baits.

2. We examined diphacinone residue levels in cooked and uncooked tissue of feral pigs exposed to sub-lethal quantities of diphacinone rodenticide.

3. Pigs were provided large amounts of baits to consume and than euthanized prior to the onset of symptoms that would indicate rodenticide poisoning or sickness. We exposed feral pigs to two dosages of diphacione: 12.5 mg/kg and 6.25 mg/kg.

4. None of the pigs displayed obvious signs of toxicity during the study period.

5. The highest concentrations of diphacinone were found in liver tissue.

6. Cooking had little effect on residual diphacinone concentrations.

7. There is very little risk to humans and pets from meat harvested from pigs contaminated with diphacinone.

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INTRODUCTION

Rodent control is a high priority for many land management, native species conservation, and ecosystem restoration projects around the Pacific. In Hawaii, cooperative efforts by state and federal conservation agencies and non-governmental land management or native conservation organizations have resulted in the registration of aerial broadcast application of rodent bait containing diphacinone as a method to control rat and mouse depredation in native conservation areas (Swift 1998, Dunlevy et al. 2000, Spurr et al. 2003, Pitt et al. 2006). Diphacinone is an anticoagulant which works by inhibiting the vitamin K cycle, reducing the ability to form essential blood clotting factors and eventually leads to lethal hemorrhaging (Fisher 2006). It is part of the "first generation" of anticoagulants, including chlorophacinone and warfarin, which require multiple feedings to be toxic.

In many of the habitats where diphacinone could potentially be applied, feral pigs (*Sus scrofa*) range freely and are hunted as sources of food and recreation (Giffin 1973, Anderson and Stone 1993, USDA 1999, 2000). A major concern with the use of aerial broadcast rodenticide application is the effect diphacinone will have on the health of wild pigs accidentally exposed to bait pellets and subsequently, the potential risk to hunters, their families, and household animals from consuming tissues from exposed pigs (Eisemann and Swift 2006). Diphacinone rodenticide pellets can be very attractive to feral pigs as shown in a recent evaluation of a diphacinone bait broadcast operation (Pitt et al. 2006). Varying levels of diphacinone residues were found in liver and muscle tissue samples collected from pigs foraging in the treatment area.

Prior laboratory studies have examined acute dietary toxicity and diphacinone residues in domestic pigs. Fletcher (2002) offered Ramik Green® pellets, a commercial rodenticide formulation containing 0.005% diphacinone, to domestic pigs at rates of 0.133 and 0.333 mg/kg body wt/day for 7 consecutive days. Fisher (2006) evaluated persistence of diphacinone after exposing domestic pigs to single doses of 2.5 or 12.5 mg diphacinone/kg body mass. In the sole study using feral pigs, Keith et al. (1990) fed pigs rations of whole corn dosed with 0.007 mg/kg body mass/day for 2 days.

Past studies examined diphacinone concentrations in raw tissue and none looked at the effects of cooking on diphacinone residues. This study examines diphacinone residues levels in raw and cooked tissue of feral pigs exposed to sub-lethal quantities of bait pellets to assess the potential toxicological risks to humans and domestic animals consuming contaminated pig tissue.

METHODS

Test Subjects and Housing

Twelve feral pigs were captured between March 19, 2008 and April 29, 2008 in Puna and South Hilo districts of Hawaii Island. The pigs were caught using leg snares or cage traps and transported to the National Wildlife Research Center (NWRC) Hawaii field station. Each pig was sexed, weighed, assigned a unique identification number, and marked with an AVID (American Veterinary Identification Devices, Norco, CA) microchip (Table 1). The pigs were housed in two at a time in a large roofed chain-link pen and held in individual cages approximately 1.1m x 1.2m (>12 ft^2 of floor space for each animal). Prior to the feeding trials,

pigs were fed commercial hog feed (16% protein) and were acclimated to entering a temporary holding pen while the housing cages were cleaned and water and food replenished. Fresh water was available ad libitum during the entire acclimation period and feeding trials.

Feeding Trials

The bait used for this study was commercially available flavorized ½ inch Ramik Green® CAS #82-66-6 (0.005% (50ppm) active ingredient diphacinone) pellets manufactured by Hacco Inc., Madison, WI. A bioassay provided by Hacco Inc. verified the diphacinone concentration. The baits were kept in ambient temperatures and a representative sample was collected upon receipt and later verified by the NWRC Analytical Chemistry section. We used the average bait pellet mass of 1.2 ± 0.01g (n=31) to estimate spillage that occurred during the feeding trials.

Feeding trials were conducted using one male and one female of similar weight at a time. An overnight fasting period was implemented the day before each trial was set to begin. All food was removed but water remained available ad libitum. We offered the pigs Ramik Green® based on two doses of active ingredient diphacinone, 12.5 and 6.25 mg/kg initial body mass. A control group was offered commercial hog feed at same weight basis as the 12.5 mg/kg diphacinone treatment.

On the morning of day one of treatment, we offered the two test pigs the full dosage of Ramik Green® as a no-choice food. On the morning of day two any bait remaining in the feed trays was weighed and spilled pellets counted and removed. The number of spilled pellets was used to estimate spillage based on the average pellet mass. On day three the pigs were euthanized by a certified operator using a 0.22 caliber rifle and their end weights were recorded. We weighed any bait remaining in the feed trays and counted the spilled pellets.

Diphacinone Residue Analysis

Raw liver, fat, and muscle tissue samples from each animal were collected for diphacinone residue analysis immediately after being euthanized. Muscle and fat are the tissues most likely to be used to human consumption, and since the liver functions to remove toxins from the blood, it is most likely to have the highest concentration of diphacinone. Muscle tissue was taken from the rump/hindquarters while fat was taken from the abdominal area, under the throat, and from the hind quarters.

One third of the liver, fat, and muscle tissue samples were kept raw and the remaining thirds were cooked in a fashion as normally done by hunters consuming feral pigs. The two cooking methods were by boiling and roasting-baking. For boiling, water was heated over a propane burner until it reached a rolling boil and then samples were cooked for approximately 15 minutes, or until the internal temperature reached 160°F as measured with a meat thermometer. For the roasting-baking method, tissue samples were cooked at 350°F for approximately 40 minutes in a conventional convection oven, or until the internal temperature reached 160°F.

All tissue samples were weighed before cooking and then frozen and placed in individually vacuum sealed bags until being sent to NWRC Analytical Chemistry department in Fort Collins, Colorado. At Analytical Chemistry, approximately 20 to 25 grams of each sample was homogenized in a SPEX liquid nitrogen freezer mill and placed in a bag, vacuum sealed and

frozen (-30° C) until analysis. The extracts were cleaned up with solid phase extraction (SPE) columns using acetonitrile as the extraction solution and analyzed by reverse-phase ion-pairing chromatography.

Statistical analyses on diphacinone concentrations were done using SAS Version 9.1 (SAS Institute 2004). We tested the diphacinone residue concentrations for normality using the Anderson-Darling test and compared the three cooking methods using two-way analysis of variance ANOVA (PROC GLM; SAS Institute 2004) followed by Tukey's multiple comparisons test.

RESULTS

Chemical Analysis of Bait

Two samples of the Ramik Green® bait were assayed by NWRC Analytical Chemistry and had a mean concentration of 0.0052% while quality assurance samples indicated a 100-101% recovery. The bait was deemed well within the acceptable range of concentration (Appendix 3).

Feeding Trials

Only eight out of the twelve captured pigs were used for the study due to handling and safety concerns. We planned on conducting one replicate of each trial but conducted a second 12.5 mg/kg diphacinone trial because the first trial had a longer pre-treatment holding period than the rest of the pigs (15 days vs. average of 6 days). Table 1 shows the amount of Ramik Green® or commercial hog feed offered on the first day of treatment, based on the pig's initial body mass.

All pigs were observed consuming the Ramik Green® the first day it was offered. No obvious signs of toxicity (external bleeding, lethargy, decreased food intake) were observed. During tissue sample collection, the blood of all pigs clotted immediately. We visually inspected the gastrointestinal tracts and found the contents of the large and small intestines of all pigs fed Ramik Green® were clearly green in color, indicated the bait had indeed been consumed.

Table 1. Feeding trial design

Dosage Level	Pig ID	Sex	Initial Body Mass (kg)	Ramik Green® Offered (g)
12.5 mg/kg	5	F	11.8	2951.0
12.5 mg/kg	6	M	6.8	1702.5
12.5 mg/kg	11	M	14.1	3518.5
12.5 mg/kg	12	F	13.2	3518.5
6.25 mg/kg	7	F	12.2	1532.3
6.25 mg/kg	8	M	14.1	1759.3
				Hog Feed Offered (g)
Control	9	M	16.8	4199.5
Control	10	F	14.1	3518.5

Diphacinone Consumption

Actual consumption of the active ingredient diphacinone was calculated using amount of Ramik Green® consumed and the pig end body mass. Daily consumption of diphacinone ranged from 0.4-4.2 mg/kg/day, with an average of 3.7 and 1.7 mg/kg/day for the 12.5 and 6.25 mg/kg treatment levels respectively (See Table 2). The average total amount of diphacinone consumed over the two day study period was 7.3 mg/kg for the higher dosage and 3.5 mg/kg for the lower dosage, with the highest amount being 7.7 mg/kg (Pig 12).

Table 2. Consumption in pigs offered Ramik Green®

Dosage Level	Pig ID	End Body Mass (kg)	Total Ramik Green® Consumption (g)	Diphacinone Consumption (mg/kg body mass)		
				Day 2	Day 3	Total
12.5 mg/kg	5	14.2	2001.3	4.2	2.8	7.0
	6	9.8	1442.4	3.7	3.6	7.3
	11	13.6	1992.1	4.0	3.3	7.3
	12	14.1	2173.3	3.9	3.8	7.7
6.25 mg/kg	7	12.8	1430.3	3.5	2.1	5.6
	8	12.3	331.8	0.4	0.9	1.3

Tissue Collection and Analysis

Because all the pigs used for the trials were subadults, it was difficult to collect enough fat tissue to satisfy the minimum 50g sample size needed for bioassays. We were able to extract ample fat for raw analysis for all eight pigs but only enough fat to be roasted for pigs 5 and 6; there was not enough fat from any pig to be boiled and assayed. Approximately 20 to 25 grams of each sample were assayed and analyzed in duplicate by Analytical Chemistry. The mean method limit of detection (MLOD) for diphacinone was 0.027 ppm (see Appendix 1).

Preliminary results indicated that diphacinone concentrations in tissue samples increased after both roasting and boiling (See Appendix 1). These higher concentrations were attributed to water loss that occurred during cooking. To account for water loss, tissue samples were analyzed for water content and the results used to standardize the diphacinone residue levels. One gram samples of tissue were heated to constant weight in aluminum weigh boats at 102°C though two heating cycles of four to eight hours each. The percent difference in water content between raw and cooked samples was used to correct the initial diphacinone residue results (See Appendix 2); all reported diphacinone residue concentrations have been corrected for water loss unless noted otherwise.

Diphacinone Residue Levels

Diphacinone concentrations in all control group tissue samples were less than the minimum level of detection (MLOD). Diphacinone concentrations in all treatment group tissues regardless of dosage or cooking method ranged from 0.205-0.708 ppm for fat, 0.444-3.212 ppm for liver, and 0.032-0.476 for muscle (See Appendix 2). Diphacinone residue levels increased with the actual amount of diphacinone consumed (See Figures 1 and 2). Pigs in the 6.25 mg/kg trial had mean concentrations of 0.223 ppm in fat, 0.826 ppm in liver, and 0.085 ppm in muscle (See Appendix 2). Pigs in the 12.5 mg/kg trial had mean corrected concentrations of 0.508 ppm in fat, 1.856 pp in liver, and 0.254 ppm in muscle.

Overall mean concentrations were 0.198 ppm in muscle, 0.437 ppm in fat, and 1.513 ppm in liver. The highest residual concentration of diphacinone in this study was taken from a roasted liver sampled (3.212 ppm) while the highest detected in a raw sample was taken from a liver (2.690 ppm).

Mean residue levels among the different cooking types can be seen in Table 3. A two-way ANOVA indicated there was no significant difference among cooking methods ($F=0.22$, $df=2$, $P=0.8029$) and the interaction of cooking method and tissue type ($F=0.23$, $df=3$, $P=0.8781$). Across the three tissue types, there was a significant difference in the residue level ($F=33.23$, $df=2$, $P<0.0001$); liver had higher diphacinone concentrations than fat and muscle.

Table 3. Mean ± SE standardized diphacinone residue levels in treatment group tissue samples.

Tissue Type	Cooking Method					
	Raw		Roasted		Boiled	
	n	ppm	n	ppm	n	ppm
Fat	6	0.449 ± 0.08	2	0.401 ± 0.06	--	--
Liver	6	1.375 ± 0.31	6	1.697 ± 0.35	6	1.466 ± 0.25
Muscle	6	0.155 ± 0.05	6	0.243 ± 0.06	6	0.195 ± 0.05

Figure 1. Corrected diphacinone concentrations in all treatment group raw, roasted, and boiled samples.

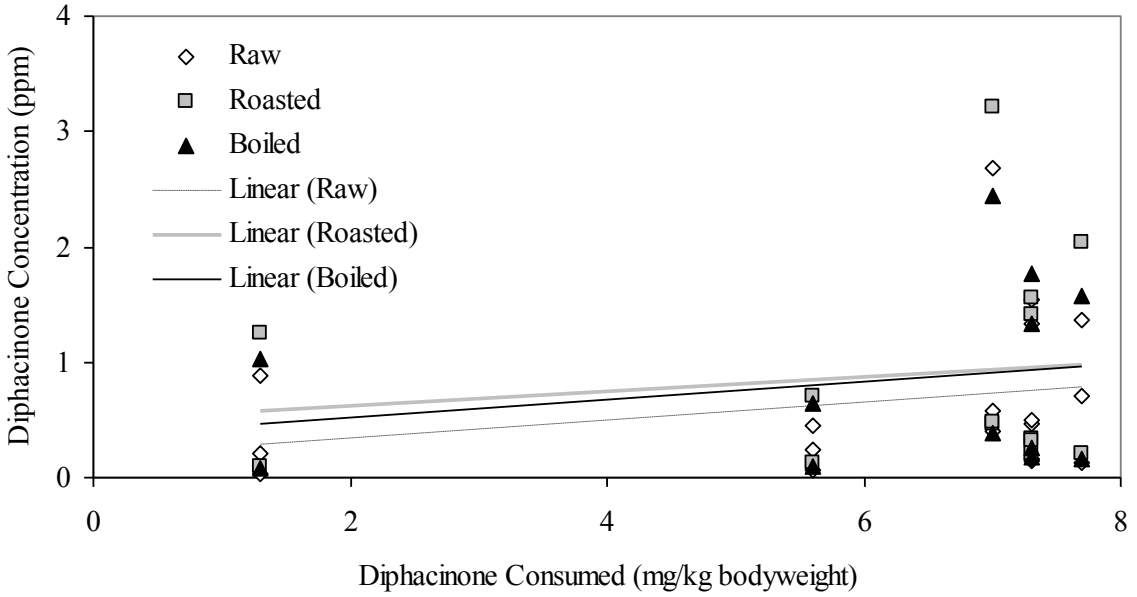
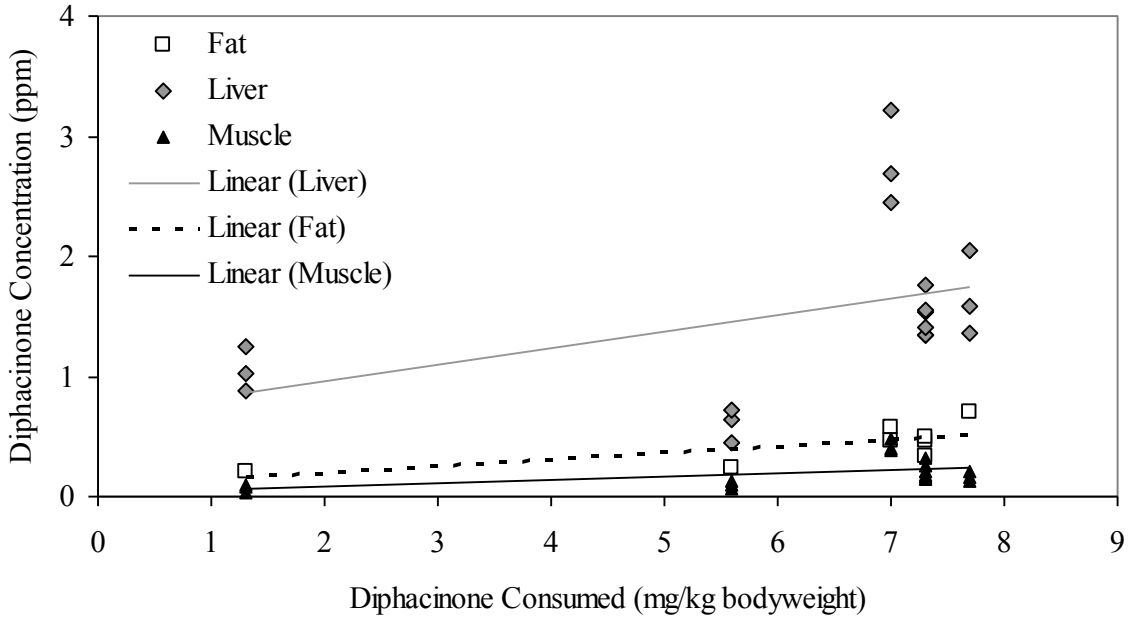


Figure 2. Corrected diphacinone concentrations in all treatment group fat, liver, and muscle samples.



DISCUSSION

Although the daily diphacinone consumption rates for our pigs are higher than the No Observed Effect Level (NOEL) and Lowest Observed Effect Level (LOEL) for rats (0.040 and 0.085 mg/kg/day) even the highest single day consumption rate (4.2 mg/kg) was well below the oral LD50 of 150 mg/kg in pigs (Eisemann and Swift 2006). Shortly after ingestion, pigs can attain high levels of diphacinone without showing signs of poisoning. Fletcher's (2002) 0.333 mg/kg/day dose produced obvious signs of poisoning (external bleeding, lethargy, and decreased food intake), followed by recovery within the 21 day study period and two of the twelve pigs in Fisher's (2006) study exhibited severe lameness when dosed at 0.5 mg/kg/day at days two and six of the 14-day study. Despite our daily diphacinone consumptions (average 1.7 and 3.7 mg/kg/day) being considerably higher than either study, as expected, none of our pigs showed obvious signs of toxicity and all appeared to be in healthy condition prior to being euthanized. However, had our study period been extended, it is likely that we would have observed signs of poisoning.

Among recent studies, pigs in our 12.5 mg/kg dosage group had the highest mean concentrations of diphacinone residue in fat, liver, and muscle tissue. Keith et al. (1990) dosed pigs with 0.007 mg/kg/day for 2 days and 0.018 mg/kg/day for five days and found mean diphacinone concentrations of 0.42 ppm in liver and <MLOD in muscle. When Fletcher (2002) exposed his pigs to 0.333 mg/kg/day for seven days, the pigs had a mean concentration 0.040 ppm in liver and <MLOD in muscle. Fisher (2006) exposed pigs to a single 12.5 mg/kg dose and found diphacinone residues of 0.11 ppm in fat, 0.69 ppm in liver, and 0.05 ppm in muscle. Pitt et al. (2005) found the mean concentration of diphacinone in radio-collared pigs with detectable residue levels to be 1.063 ppm for liver and 0.073 ppm for muscle. In comparison, pigs in our higher dosage treatment group consumed 3.7 mg/kg/day and had mean concentrations of 0.51 ppm in fat, 1.86 ppm in liver, and 0.25 ppm in muscle.

Cooking had little effect on residual diphacinone concentrations in feral pig tissue. Although cooked samples had slightly higher diphacinone concentrations than raw samples even after being corrected for water content, it is likely the decrease in mass can be attributed to other mechanisms besides water loss. Other volatiles such as fat are readily vaporized or otherwise lost during the roasting and boiling processes.

The levels of diphacinone in pig tissue observed in this study were not high enough to be of risk to humans. A comprehensive assessment of hazards from aerial broadcast applications of 0.005% diphacinone baits has already concluded that the risk to humans consuming pigs harvested in treated areas is very low (Eisemann and Swift 2006). Based on the maximum concentrations detected by Pitt et al. (2005) of 3.07 ppm for liver and 0.251 ppm for muscle, Eisemann and Swift (2006) calculated that a 55 kg person would need to consume either 2.33 kg of liver or 28.49 kg of muscle in a single day, or 0.72 kg of liver or 8.77 kg of muscle over multiple consecutive days to receive a dose shown to cause detectable changes in blood clotting in rats. Therefore there is very low risk of exposure to levels high enough to cause harmful effects to humans. Individuals who may be more susceptible to effects of diphacinone such as pregnant women, those with vitamin K deficiency, or liver disorders, should exercise caution if

consuming meat taken from pigs who may have been exposed to the bait and refrain from consuming the liver.

There have also been concerns about potential risk to hunting or pet dogs from hunters feeding contaminated pig carcasses to their dogs (R. Sugihara, personal communication 2008). The amount of diphacinone that is lethal to one half (50%) of experimental animals is known as the lethal dose fifty (LD50). Three separate reports have listed the diphacinone LD50 for dogs as being 0.88 mg/kg, 3.0-7.5 mg/kg, and 5-15 mg/kg. (Erickson and Urban 2004). Based on this study's average diphacinone concentration in liver (1.513 ppm), even at the lowest reported LD50, a 20-kg dog would have to consume 11.6 kg of liver to receive a lethal dose. Therefore the risk of diphacinone exposure is very low to dogs and any accidental poisoning can be easily treated with vitamin K (Eisemann and Swift 2006).

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Appendix 1: Chemical Analyses of Diphacinone in Pig Tissue Samples

Table 1: Uncorrected diphacinone concentrations in tissues

Pig ID	Tissue Type	Cooking Method	Dipahcinone Concentration (ppm)
5	Fat	Raw	0.582
6	Fat	Raw	0.490
7	Fat	Raw	0.241
8	Fat	Raw	0.205
9	Fat	Raw	<MLOD
10	Fat	Raw	<MLOD
11	Fat	Raw	0.468
12	Fat	Raw	0.708
5	Fat	Roast	0.748
6	Fat	Roast	0.547
5	Liver	Boil	2.660
6	Liver	Boil	1.920
7	Liver	Boil	0.699
8	Liver	Boil	1.120
9	Liver	Boil	<MLOD
10	Liver	Boil	<MLOD
11	Liver	Boil	1.460
12	Liver	Boil	1.720
5	Liver	Raw	2.690
6	Liver	Raw	1.540
7	Liver	Raw	0.444
8	Liver	Raw	0.876
9	Liver	Raw	<MLOD
10	Liver	Raw	<MLOD
11	Liver	Raw	1.340
12	Liver	Raw	1.360
5	Liver	Roast	3.650
6	Liver	Roast	1.770
7	Liver	Roast	0.812
8	Liver	Roast	1.420
9	Liver	Roast	<MLOD
10	Liver	Roast	<MLOD
11	Liver	Roast	1.600
12	Liver	Roast	2.320
5	Muscle	Boil	0.461
6	Muscle	Boil	0.310
7	Muscle	Boil	0.115

8	Muscle	Boil	0.098
9	Muscle	Boil	<MLOD
10	Muscle	Boil	<MLOD
11	Muscle	Boil	0.214
12	Muscle	Boil	0.198
5	Muscle	Raw	0.396
6	Muscle	Raw	0.166
7	Muscle	Raw	0.064
8	Muscle	Raw	0.032
9	Muscle	Raw	<MLOD
10	Muscle	Raw	<MLOD
11	Muscle	Raw	0.148
12	Muscle	Raw	0.124
5	Muscle	Roast	0.541
6	Muscle	Roast	0.372
7	Muscle	Roast	0.148
8	Muscle	Roast	0.116
9	Muscle	Roast	<MLOD
10	Muscle	Roast	<MLOD
11	Muscle	Roast	0.242
12	Muscle	Roast	0.238

Table 2: Quality control recovery for diphacinone

ID	Tissue Type	Cooking Method	Fortification Level (ppm)	Percent Recovery
QC 1	Muscle	Raw	Blank	na
QC 2	Muscle	Raw	Blank	na
QC 3	Muscle	Raw	0.212	97.5
QC 4	Muscle	Raw	0.206	112
QC 5	Muscle	Raw	1.04	99.7
QC 6	Muscle	Raw	1.03	92.7
QC 7	Fat	Raw	Blank	na
QC 8	Fat	Raw	Blank	na
QC 9	Fat	Raw	0.206	101
QC 10	Fat	Raw	0.195	102
QC 11	Fat	Raw	1	100
QC 12	Fat	Raw	1.01	98
QC 13	Liver	Raw	Blank	na
QC 14	Liver	Raw	Blank	na
QC 15	Liver	Raw	0.412	89.5
QC 16	Liver	Raw	0.412	92.1

QC 17	Liver	Raw	2.12	86.6
QC 18	Liver	Raw	2.06	80.8
QC 19	Muscle	Roast	Blank	na
QC 20	Muscle	Roast	Blank	na
QC 21	Muscle	Roast	0.204	117
QC 22	Muscle	Roast	0.214	173*
QC 23	Muscle	Roast	1.05	105
QC 24	Muscle	Roast	0.993	101
QC 25	Liver	Roast	Blank	na
QC 26	Liver	Roast	Blank	na
QC 27	Liver	Roast	0.408	109
QC 28	Liver	Roast	0.401	111
QC 29	Liver	Roast	2.08	87.5
QC 30	Liver	Roast	2.1	95.1
QC 31	Liver	Boil	Blank	na
QC 32	Liver	Boil	Blank	na
QC 33	Liver	Boil	0.405	104
QC 34	Liver	Boil	0.412	94.1
QC 35	Liver	Boil	2.12	93.6
QC 36	Liver	Boil	2.1	93.8
QC 37	Muscle	Boil	Blank	na
QC 38	Muscle	Boil	Blank	na
QC 39	Muscle	Boil	0.206	102
QC 40	Muscle	Boil	0.206	96.5
QC 41	Muscle	Boil	1.04	96.0
QC 42	Muscle	Boil	1.05	95.8

*Suspected contamination

Table 3: Method limit of detection (MLOD)

Tissue	**Cooking Method**	**MLOD (ppm)**
Muscle	Raw	0.006
Fat	Raw	0.006
Liver	Raw	0.073
Muscle	Roast	0.041
Liver	Roast	0.015
Liver	Boil	0.027
Muscle	Boil	0.020
	Muscle Mean	0.022
	Liver Mean	0.038
	Overall Mean	0.027

Appendix 2: Corrected Diphacinone Concentrations in Pig Tissue

Table 1. Water content differences between cooked and raw tissue

Tissue	Cooking Method	Water Content	Difference in Water Content
Fat	Raw	39%	
Fat	Roast	24%	38%
Liver	Raw	73%	
Liver	Roast	64%	12%
Liver	Boil	67%	8%
Muscle	Raw	74%	
Muscle	Roast	65%	12%
Muscle	Boil	62%	16%

Table 2. Corrected diphacinone concentrations in pig tissues based on differences in water content between raw and cooked tissue

Pig ID	Tissue Type	Cooking Method	Corrected Diphacinone Concentration (ppm)
5	Fat	Raw	0.582
6	Fat	Raw	0.490
7	Fat	Raw	0.241
8	Fat	Raw	0.205
9	Fat	Raw	<MLOD
10	Fat	Raw	<MLOD
11	Fat	Raw	0.468
12	Fat	Raw	0.708
5	Fat	Roast	0.464
6	Fat	Roast	0.339
5	Liver	Boil	2.442
6	Liver	Boil	1.763
7	Liver	Boil	0.642
8	Liver	Boil	1.028
9	Liver	Boil	<MLOD
10	Liver	Boil	<MLOD
11	Liver	Boil	1.340
12	Liver	Boil	1.579
5	Liver	Raw	2.690
6	Liver	Raw	1.540
7	Liver	Raw	0.444
8	Liver	Raw	0.876
9	Liver	Raw	<MLOD

10	Liver	Raw	<MLOD
11	Liver	Raw	1.340
12	Liver	Raw	1.360
5	Liver	Roast	3.212
6	Liver	Roast	1.558
7	Liver	Roast	0.715
8	Liver	Roast	1.250
9	Liver	Roast	<MLOD
10	Liver	Roast	<MLOD
11	Liver	Roast	1.408
12	Liver	Roast	2.042
5	Muscle	Boil	0.387
6	Muscle	Boil	0.260
7	Muscle	Boil	0.097
8	Muscle	Boil	0.082
9	Muscle	Boil	<MLOD
10	Muscle	Boil	<MLOD
11	Muscle	Boil	0.180
12	Muscle	Boil	0.166
5	Muscle	Raw	0.396
6	Muscle	Raw	0.166
7	Muscle	Raw	0.064
8	Muscle	Raw	0.032
9	Muscle	Raw	<MLOD
10	Muscle	Raw	<MLOD
11	Muscle	Raw	0.148
12	Muscle	Raw	0.124
5	Muscle	Roast	0.476
6	Muscle	Roast	0.327
7	Muscle	Roast	0.130
8	Muscle	Roast	0.102
9	Muscle	Roast	<MLOD
10	Muscle	Roast	<MLOD
11	Muscle	Roast	0.213
12	Muscle	Roast	0.209

Appendix 3: Chemical Analyses of Ramik Green® Bait Samples

Table 1. Diphacinone concentrations in Ramik Green® samples

ID	Concentration (ppm)
1	51.7
2	52.2
Average	52.0

Table 2: Quality control recovery for Ramik Green® Bait

ID	Fortification Level	Percent Recovery
QC 1	53.1	100
QC 2	53.1	101

Table 1. Sex and body mass of captured feral pigs (*Sus scrofa*)

Pig ID	Sex	Initial Body Mass (kg)
1	Male	31.8
2	Female	28.1
3	Male	21.8
4	Female	29.0
5	Female	11.8
6	Male	6.8
7	Female	12.2
8	Male	14.1
9	Male	16.8
10	Female	14.1
11	Male	14.1
12	Female	13.2