Title:

Dermal toxicity of sodium bicarbonate to control Coqui frogs, *Eleutherodactylus coqui*, in Hawaii

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Executive Summary

1. We evaluated the dermal toxicity of two sodium bicarbonate applications (a powder and a slurry solution) under laboratory conditions.

2. We evaluated four grades of sodium bicarbonate: USP #1 powder, USP #2 fine granular, USP #5 course granular, and industrial powder.

3. Efficacy varied across grades of sodium bicarbonate and between the slurry and powder applications.

4. USP #1, the finest grade of the four sodium bicarbonate powders was found to be the most effective and one of the least expensive powders, sold at $24.30 per 100 lbs.

5. A powder application rate of USP #1 at 400 lbs per acre was the lowest application rate resulting in >80% mortality within 24 hours after initial exposure.

6. Slurry solution of 25% concentration USP #1 was the lowest application resulting in >80% mortality within 24 hours after initial exposure.

7. A powder application of sodium bicarbonate (USP #1) is cheaper and potentially easier to apply than a slurry solution.

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INTRODUCTION

Coqui frogs, *Eleutherodactylus coqui* native to the Puerto Rico are widespread in the Hawaiian Islands and have affected Hawaii's floriculture and agriculture industries, as well as real estate, private industry, and human health (Kraus and Campbell 2002, Beard and Pitt 2005, Beard and Pitt 2006, Beard 2007). The discoveries of frogs in certified nurseries make them a quarantine issue that will greatly impact the exportation of disease and pest-free nursery products to the mainland and destinations abroad. The threat of frogs invading fragile habitats of Hawaii's rare and endangered plants and animals is also a concern. Coqui frog densities have been estimated to reach greater than 89,000 frogs per hectare, at which they are capable of consuming approximately 675,000 arthropod prey items per hectare in a single night (Woolbright et al 2006). Once established in native habitats the frogs could compete directly with native birds for limited food resources, as well as prey on endemic arthropods (Beard and Pitt 2005).

During the past seven years, the National Wildlife Research Center's Hilo Hawaii Field Station has conducted multiple studies to investigate chemical agents to manage coqui frogs throughout the Hawaiian Islands (Campbell 2002a, Pitt and Sin 2004a and 2004b, Pitt and Doratt 2006a, 2006b, and 2006c). We identified four chemical agents (caffeine, citric acid, hydrated lime, endosulfan) as effective frog toxicants that could be utilized efficiently under certain conditions.

Although these four chemicals were found to be effective, each one displayed drawbacks. For example registration restrictions prevented the wide spread use of caffeine. Citric acid had some phytotoxic effects to sensitive plants and there is reluctance in the public sector to use this chemical due to its high cost (Pitt et al. 2008).

Thionex, a restricted use insecticide (active ingredient endosulfan) was highly effective in laboratory and field trails, but its use is restricted to certified applicators. In April 2008, the quarantine exemption permit for use of hydrated lime as a control agent expired thus, making citric acid the only chemical registered for frog control by the general public. Therefore, additional research is needed to find other effective chemical tools.

The purpose of this study was to investigate acute dermal toxicity of sodium bicarbonate to coqui frogs, determine the lowest application at which the frogs would have >80% mortality after 24 hours, and determine an application rate and a solution concentration rate for possible registration purposes to help control this invasive frog.

METHODS

Collection site

Sub-adult and adult male and female coqui frogs were collected from February 2008 through July 2008 in Lava Tree State Monument, west of Pahoa, Hawaii.

Housing of animals

Before each trial 70 frogs were hand captured and placed inside a plastic terrarium containing moist (de-chlorinated water) paper towels and taken to an animal holding facility at the National Wildlife Field Station, Hilo, Hawaii. There, the frogs were maintained for less than 16 hours and the temperature in the room ranged from 20 to 28°C.

Afterwards, all of the frogs were sexed and their snout vent length (SVL) and body mass recorded. The gender of each frog was determined by following the guidelines described by Woolbright (1989) (adult male will have a more elastic skin underneath their chin [vocal sac] in contrast the adult females will have a much tighter vocal sac. If the female is gravid a cluster of eggs should be visible underneath her abdomen area or on the sides of her torso). The frogs' body mass was recorded after voiding the bladder then blotting dry its body surface moisture with sheet of Kimwipe® tissue and weighing it to 0.01g on a Mettler PE 3600 electronic balance.

After each of the frog was measured, they were placed inside individually labeled 42 oz plastic container (10.5-14.4 x 10.5-14.4 x 8.8 cm) containing 6 ml of distilled water and a folded tissue (11.4 x 21.3 cm) sheet. After 24 hours, the frogs were transferred into individual mesh cages (4.5 x 4.5 x 5 cm) (Teflon, Chemware PTFE laboratory matting, Saint-Gobain Performance Plastic, Seattle, WA) for treatment.

Randomization

Prior to the exposure trials the frogs were randomly assigned to one of the treatment groups using a randomization computer program (random-number generator) called RANDSEL (Sugihara 1997). RANSDEL was programmed to assign the frogs uniformly by their body mass to minimize body mass variation as a possible covariate in the influence of the frog's health status when exposed to the chemical.

Chemicals

Four product grades of sodium bicarbonate powder were tested in this study: USP#1 powder, USP #2 fine granular, USP #5 course granular, and Industrial powder. Distilled water was used as a negative control group and citric acid and hydrated lime were used as positive control groups to compare frog mortality (Table 1). For sodium bicarbonate a total of seven powders and four solutions of varying concentrations were tested (Table 2.)

Chemical application

Once the frogs were placed inside their individual mesh cages, the correct amount of powder or solution was applied directly onto the frog's dorsal surface. Application rates for the powders were converted from lbs per acre to grams per cm^2 (Table 3). Before and after treatment exposure the mesh cages containing the frogs were kept inside a plastic container (3.45 x 41.5 x 1.8 cm) containing 100 ml of distilled water and 5 folded paper towels (24.5 x 13 cm) sheet to maintain a moist environment.

Powders

A pistol grip blow gun (Model HDA50400AV; Husky Co., Pacific, MO) connected to an air compressor (Model D55168; DeWalt Industrial Tool C., Baltimore, MD) was used to administer the chemical powder onto the frogs. The tip of the blow gun was connected to a piece of tygon vacuum tubing which was also connected to a polypropylene check valve. A 2.5 cm diameter filter washer (chemical holding tray) was then connected onto the check valve before blowing the chemical onto the frogs (Figure 1). Before administering the chemical powder onto the frogs, the correct amount of powder was placed inside the filter washer and weighed (0.0001g) using a Sartorius CP 124S electronic balance. A glass globe (surface area 78.54 cm^2) was used to shield the area surrounding the mesh cage as the powder was applied onto the frog (Figure 2). Quality assurance trials showed that this application technique evenly distributed the powder onto the bottom of the glass globe surface.

Solutions

For solutions, 1.0 ml of the appropriate concentration was administered on the dorsal surface of the frogs using a single channel pipette (Cat no. 4600100; Finnpipette, Thermo Electron Corp. Vantaa, Finland). The solution was applied through the top of the mesh cage, which was then tapped to remove excess liquid. Once the treatment had been applied to all the frogs in the treatment group, the mesh cages with the frogs were placed back inside their plastic observation container.

All solution concentrations were calculated and expressed on a weight to volume basis. Thus, a 20 % solution would have 20 g of solute, mixed with water to produce a final solution of 100 ml. In cases where all of the solution was not fully dissolved, the concentration of the slurry was calculated in a similar manner.

Post-treatment observation

During the powder trials the frog health status was recorded 15, 30, 60 minutes and 24 hours after initial exposure. Mortality was defined by the frog's losing its ability to right itself. Any survivors were euthanized after 24 hours except for trials with extended observation periods.

We conducted a longer post-treatment observation period of four days for the most effective slurry solution. After the first 24 hours the frogs were taken out of their treatment mesh cages and placed in a clean observation container. Everyday each container received approximately 3-4 pin head sized crickets per frog and feeding observations were recorded. The frogs were also transferred to another clean observation container after two days.

Data Analysis

Descriptive statistics of application rate, concentration solution, frog health status, time of death, and percent mortality were performed using SAS 9.1® (SAS Institute 2003).

RESULTS

Sodium Bicarbonate Powders

A total of 470 individual coqui frogs were exposed to one of the four grades of sodium bicarbonate powders. Of those four, two powders were highly effective on the frogs 24 hours after initial exposure: USP #1 and USP #2. Both grades had 95% mortality after 24 hours at an application rate of 400 lbs per acre (Table 4). USP #1, the finest grade of the four powders, showed a slightly higher percent mortality within the different observation periods 15, 30 and 60 minutes compared to USP # 2 (Table 5). In comparison, there were no mortalities in the control group while 16% citric acid solution had an average of 97% mortality and at 200 lbs per acre hydrated lime powder had 95% mortality.

Sodium Bicarbonate Solutions

A total of 157 individual coqui frogs were used for testing the solution forms of sodium bicarbonate. Of the four sodium bicarbonate grades a 25% concentration slurry solution of USP #1 was the lowest concentration resulting in 89% mortality both 24 hours and 4 days after initial exposure (Table 6).

No mortalities occurred in the control group, 16% citric acid solution had an average of 98% mortality, and 3% hydrated lime solution had 8% mortality after 24 hours. Hydrated lime was the only treatment that showed an increase in percent mortality after 48 hours of initial exposure. Percent mortality increases from 20% after 24 hours to 80% after 48 hours (Table 6).

In the longer post-treatment observation trial all the frogs that were transferred to a new observation container fed and behaved similar to the frogs that were in the control group except for the hydrated lime group as stated above. No signs of irritation or sluggishness were observed throughout the four days.

Discussion

When comparing the powder application rates and solution concentrations of all four sodium bicarbonate product grades, USP #1, the finest of all the powders and the least expensive powder ($24.30 per 100 lbs) had the highest percent mortality of coqui frogs in our laboratory trials (Table 7). A powder application rate of 400 lbs per acre and a 25% concentration solution were the lowest tested levels to show a mortality rate of >80% 24 hours after initial exposure.

When the frogs were initially treated with either application method (powder or solution), only the frogs that were treated with the powder displayed a slight irritation reaction (rubbing of eyes and jumping). This irritation behavior was seen in frogs treated with application rates between 250 and 500 lbs per acre.

To better assess how well sodium bicarbonate USP #1 would work in the field we recommend a field assessment of the effectiveness of sodium bicarbonate as a coqui control agent both as a dust application of 400 pounds per acre and a spray application of 25% concentration solution, as well as an evaluation of non-target impacts.

We also advise that a dust application would be more advantageous than a slurry solution spray because of its lower cost and less extensive labor required. The approximate cost of treating one acre with USP #1 powder applied at 400 lbs per acre would be $97, compared to a 25% slurry solution of the same powder, which would cost $500 (if 1000 gallons per acre were used). The cost of treating one acre with 16% citric acid solution at the same rate would cost approximately $1180.

LITERATURE CITED

Beard, K. H. 2007. Diet of the invasive frog, *Eleutherodactylus coqui*, in Hawaii. *Copeia* 2007, 281-291.

Beard, K. H., and W. C. Pitt. 2005. Potential consequences of the coqui frog invasion in Hawaii. Diversity and Distributions. 11:427-433.

Beard, K. H., and W. C. Pitt. 2006. Potential predators of an invasive frog *Eleutherodactylus coqui* in Hawaii rain forests. Journal of Tropical Ecology. 22(4):345-347.

Campbell, E. W. 2002a. Dermal toxicity of selected agricultural pesticides, Pharmaceutical products, and household chemicals to introduced *Eleutherodactylus* frogs in Hawaii. Rep. No. QA-693. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Campbell, E. W. 2002b. Field efficacy trials of the directed spray application of caffeine solutions for controlling introduced *Eleutherodactylus* frogs in floriculture and nursery crops in Hawaii. Rep. No. QA-846. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Kraus, F., and Campbell, E. W. 2002. Human-mediated escalation of a formerly eradicable problem: the invasion of Caribbean frogs in the Hawaiian Islands. Biological Invasion, 4: 327-332

Pitt, W. C. and Doratt, R. 2006a. Efficacy of hydrated lime on *Eleutherodactylus coqui* and an operational field-application assessment on the effects on non-target invertebrate organisms Rep. No. QA-1208 and 1264. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Pitt, W. C. and Doratt, R. 2006b. Screening for the evaluation of selected chemicals and pesticides to control Eleutherodactylus frogs in Hawaii. Rep. No. QA-1208. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Pitt, W. C. and Doratt, R. 2006c. Field efficacy trials of spray application of Thionex 3EC solution for controlling the introduced frog *Eleutherodactylus coqui* in floriculture and nursery crops in Hawaii. QA-1208. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Pitt, W. C., Higashi, M., Swift, R., and Doratt, R. 2008. Phytotoxicity of 16% citric acid solution on native Hawaiian plants under greenhouse conditions QA-1331. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Pitt, W. C. and Sin, H. 2004a. Dermal toxicity of citric acid based pesticides to introduced *Eleutherodactylus* frogs in Hawaii. Rep. No. QA-992. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Pitt, W. C. and Sin, H. 2004b. Field efficacy and invertebrate nontarget hazard assessment of citric acid spray application for control of introduced *Eleutherodactylus* frogs in Hawaii. Rep. No. QA-1048. USDA/APHIS/WS/NWRC, Hilo, Hawaii.

Ruibal, R. 1962. The adaptive value of bladder water in the toad, *Bufo cognatus*. Physiological Zoology, 35: 218-223.

SAS, 9.1, 2004, SAS Institute, Inc., Cary, North Carolina

Sugihara, T., S. 1997. RANDSEL: Randomly selecting and assigning animals to treatment groups. Wildlife Society Bulletin, **25**: 183-184

Woolbright, L. 1989. Sexual dimorphism in *Eleutherodactylus coqui*: selection pressure and growth rates. Herpetologica. 45: 68-74.

Woolbright, L., Hara, A. H., Jacobsen, C. M., Mautz, W. J., Benevides, F. J. 2006. Population Densities of the Coqui, *Eleutherodactylus coqui* (Anura: Leptodactlylidae) in Newly Invaded Hawaii and in Native Puerto Rico. Journal of Herpetology. 40: 122-126.

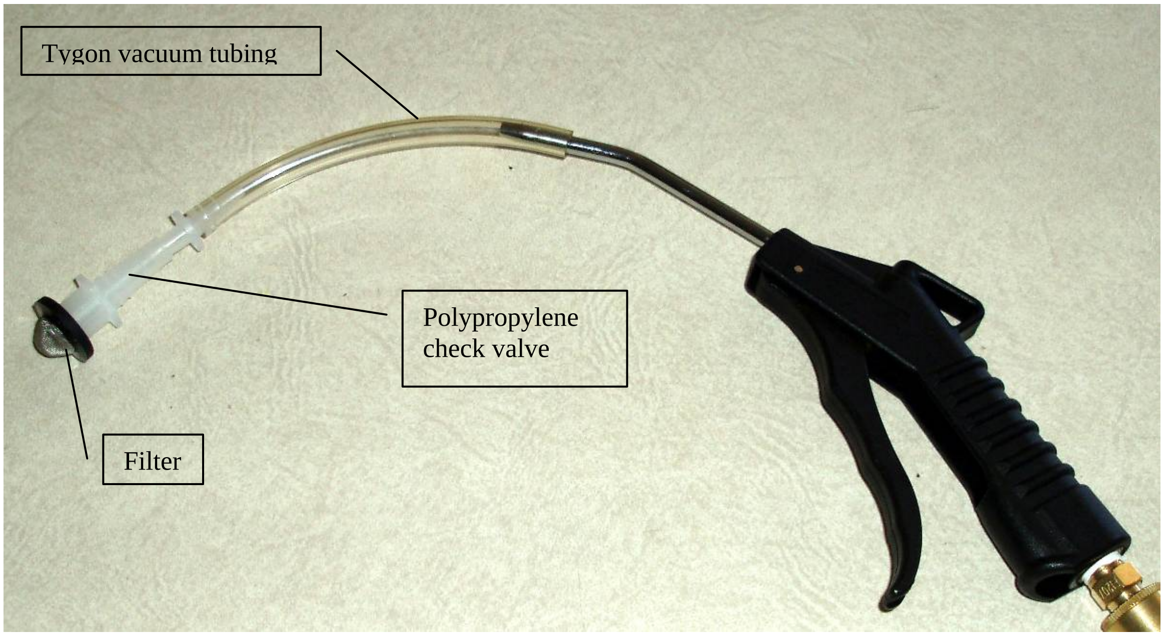

Figure 1. Chemical powder applicator pistol grip blow gun attached to tygon vacuum tubing, polypropylene check valve and filter washer.

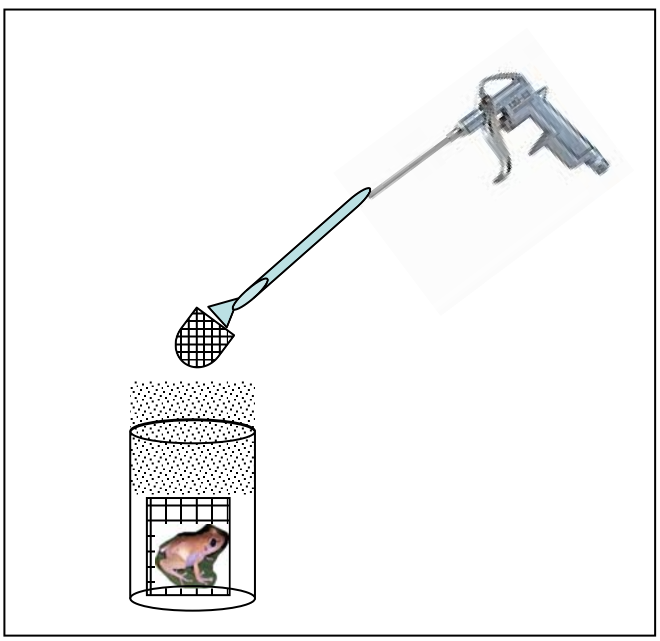

Figure 2. Drawing of the powder application process. Blow gun, treatment mesh cage with frog, and glass globe. Surface area of glass globe 78.54 cm^2.

Table 1. Chemical reference of products tested.

Chemical	CAS	Batch No.	Manufacture	Grade
Sodium Bicarbonate USP No. 1	144-55-8	0000822151	FMC Corporation, Philadelphia, PA	Powdered
Sodium Bicarbonate USP No. 2	144-55-8	0000775731	FMC Corporation, Philadelphia, PA	Fine Granular
Sodium Bicarbonate USP No. 5	144-55-8	0000784698	FMC Corporation, Philadelphia, PA	Course Granular
USP Powdered No. 5	144-55-8	0000786290	FMC Corporation, Philadelphia, PA	Industrial
Citric acid anhydrous	77-92-9	A7129	BEI, Hilo , HI	Granular
Calcium Hydroxide (hydrated lime)	1305-62-0	na	Ash Grove, Portland, OR	Powder
Distilled Water	na	na	Longs Drug Store, Hilo, HI	na

Notes: *EPA Reg No.

Table 2. Sodium bicarbonate powder and concentration solution formulations.

Formulation	Application rate(lbs/acre)	Formulation	Percent concentration application (1 ml)
Powder	100	Liquid	8
Powder	150	Liquid	12
Powder	200	Liquid	20
Powder	250	Liquid	25
Powder	300		
Powder	400		
Powder	500		

Table 3. Sodium bicarbonate powder application rate conversions from pounds per acre to grams per cm^2.

Application rate (lbs/acre)	Application rate (g/cm^2)*
100	0.0880
150	0.1319
200	0.1759
250	0.2199
300	0.2647
400	0.3526
500	0.4406

Notes: *Surface area treated (bottom of glass globe) 78.54 cm^2

Table 4. Sodium Bicarbonate powder application (lbs per acre) and percent mortality after 24 hours exposure.

Application rate (lbs per acre)	100		150		200		250		300		400		500	
Treatment	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**
Distilled water (1 ml)	10	0%	10	0%	19	0%	20	0%	10	0%	10	0%	10	0%
16% Citric Acid solution (1 ml)	*	*	10	90%	20	100%	10	100%	9	100%	*	*	10	100%
Calcium hydroxide (Hydrated lime) powder	10	30%	10	60%	20	95%	10	100%	10	100%	*	*	10	100%
Sodium Bicarbonate, USP #1 powder	9	33%	9	44%	18	83%	18	78%	27	70%	20	95%	10	100%
Sodium Bicarbonate, USP #2 fine granular	10	40%	10	70%	19	84%	20	70%	28	75%	20	95%	10	100%
Sodium Bicarbonate, Industrial powder	10	10%	10	50%	18	67%	20	80%	8	50%	10	100%	10	90%
Sodium Bicarbonate, USP #5 course granular	10	20%	10	20%	20	70%	18	72%	8	50%	10	100%	10	90%

Notes: *No frogs were tested.

Table 5. Sodium Bicarbonate USP #1 and USP# 2 powder application (lbs per acre) and percent mortality after 15, 30, and 60 minutes exposure.

Application rate (lbs per acre)		100		150		200		250		300		400	
Treatment	**Time**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**	**n**	**Percent Mortality**
Sodium Bicarbonate, USP #1 powder	15 minutes	9	0%	9	0%	18	0%	18	11%	27	0%	20	15%
Sodium Bicarbonate, USP #2 fine granular		10	0%	10	0%	19	0%	20	0%	28	*	20	15%
Sodium Bicarbonate, USP #1 powder	30 minutes	9	22%	9	0%	18	33%	18	17%	27	26%	20	30%
Sodium Bicarbonate, USP #2 fine granular		10	0%	10	0%	19	16%	20	10%	28	25%	20	50%
Sodium Bicarbonate, USP #1 powder	60 minutes	9	22%	9	0%	18	56%	18	33%	27	63%	20	90%
Sodium Bicarbonate, USP #2 fine granular		10	0%	10	40%	19	58%	20	40%	28	46%	20	75%

Notes: *Frogs were not observed.

Table 6. Sodium Bicarbonate percent concentration solutions, time of death, and percent mortality.

Treatment groups	15 minutes after treatment	30 minutes after treatment	1 hour after treatment	24 hour after treatment
Distilled water (1 ml)	0 of 37 (0%)	0 of 37 (0%)	0 of 37 (0%)	0 of 37 (0%)
16% Citric acid (1 ml)	24 of 40 (60%)	35 of 40 (88%)	38 of 40 (85%)	39 of 40 (98%)
3% Hydrated lime (1 ml)	0 of 40 (0%)	0 of 40 (0%)	0 of 40 (0%)	3 of 40 (8%)*
8% Sodium Bicarbonate USP #1 powder (1 ml)	0 of 10 (0%)	0 of 10 (0%)	0 of 10 (0%)	2 of 10 (20%)
8% Sodium Bicarbonate USP #2 fine granular (1 ml)	0 of 10 (0%)	0 of 10 (0%)	0 of 10 (0%)	3 of 10 (30%)
8% Sodium Bicarbonate USP #5 course granular (1 ml)	0 of 10 (0%)	0 of 10 (0%)	0 of 10 (0%)	3 of 10 (30%)
8% Sodium Bicarbonate Industrial powder (1 ml)	0 of 10 (0%)	0 of 10 (0%)	0 of 10 (0%)	2 of 10 (20%)
12% Sodium Bicarbonate Industrial powder (1 ml)	0 of 19 (0%)	0 of 19 (0%)	5 of 19 (26%)	11 of 19 (58%)**
20% Sodium Bicarbonate USP #1 powder (1 ml)	0 of 19 (0%)	4 of 19 (21%)	13 of 19 (68%)	15 of 19 (79%)
20% Sodium Bicarbonate USP #2 fine granular (1 ml)	0 of 20 (0%)	1 of 20 (5%)	11 of 20 (55%)	15 of 20 (75%)
20% Sodium Bicarbonate USP #5 course granular (1 ml)	0 of 20 (0%)	7 of 20 (35%)	13 of 20 (65%)	16 of 20 (80%)
20% Sodium Bicarbonate Industrial powder (1 ml)	0 of 20 (0%)	2 of 20 (10%)	8 of 20 (40%)	14 of 20 (70%)
25% Sodium Bicarbonate Industrial powder (1 ml)	0 of 19 (0%)	9 of 19 (47%)	12 of 19 (63%)	17 of 19 (89%)**

Note: *3% Hydrated lime percent mortality increases from 20% after 24 hours to 80% after 48 hours
**No change in mortality after 4 days after initial application.

Table 7. Chemical cost comparisons.

Product	Cost
Sodium Bicarbonate USP #1 powder	$24.30 per 100 lbs
Sodium Bicarbonate USP #2 fine granular	$24.85 per 100 lbs
Sodium Bicarbonate USP #5 course granular	$34.30 per 100 lbs
Sodium Bicarbonate, Industrial powder	$22.75 per 100 lbs
Calcium hydroxide powder	$9.05 per 35 lbs
16% Citric acid solution	$44.20 per 50 lbs

Notes: Cost of sodium bicarbonate quoted by FMC chemical products 2/26/08.
Cost of Citric acid, hydrated lime, & sodium bicarbonate quoted by BEI, Hawaii 2/26/08