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Reduced fecundity in free-ranging Norway rats after baiting with a liquid fertility control bait

Gary W. Witmer, USDA, APHIS, National Wildlife Research Center (retired), 4101 Laporte Ave., Fort Collins, CO 80521, USA *garywitmer51@gmail.com*
Stefanie Raymond-Whish, SenesTech, Inc., 3140 N. Caden Ct., Flagstaff, AZ 86004, USA

Abstract: Norway rats (*Rattus norvegicus*) cause extensive crop loss, infrastructure damage, and are vectors for zoonotic diseases. Due to reduced efficacy, environmental and animal welfare concerns related to traditional pest management tools, such as rodenticides, it is imperative to find new methods for controlling commensal rodents. Fertility control is emerging as a safe, humane, and effective method of long-term population management. Vinylcyclohexene diepoxide (VCD) and triptolide are 2 compounds that are known to target and inhibit ovarian function. Furthermore, triptolide debilitates spermatogenesis and sperm motility. We prepared liquid bait containing no active ingredients (control) or containing VCD (0.098%) and triptolide (0.0012%, treatment) and offered *ad libitum* for 56 days to male and female Norway rats placed in open, indoor arenas and allowed to breed for 4 rounds (a total of 138 days). The first 3 breeding rounds of treatment-matched matings produced fewer pups in the treatment rats compared to control rats ($P < 0.001$). In the fourth breeding round, control rats were cross-bred with treatment rats. There were no differences in pup numbers between these cross-breeding groups, but the litter sizes in both groups were smaller than those seen in the control female/control male matings. In addition to reduced pup numbers, treatment males and females exhibited decreased reproductive organ weights without any effect on adrenal, kidney, spleen, and liver weights compared to control rats. Use of a liquid contraceptive bait containing VCD and triptolide may be a suitable alternative to the traditional pest management tools used to control wild rat pest populations.

Key words: baiting, commensal rodents, contraceptives, fertility control, Norway rat, population management, *Rattus norvegicus*, rodenticides

Commensal rodents cause significant damage and problems to humans worldwide on a continual basis. Rodent pests consume and damage human foods in the field and in storage, damage property and infrastructure, and harbor and vector numerous diseases that can be passed to humans and livestock (Witmer 2007, Witmer and Singleton 2010, Firth et al. 2014). In the United States, it is estimated that there could be up to 1.4 billion Norway or brown rats (*Rattus norvegicus*; Figure 1) that cause extensive damage in both agricultural and urban/suburban settings at a projected cost of $19 billion USD in economic losses annually (Pimentel et al. 2005).

Globally, the ecological and economic damages caused by rodent pests are incalculable. Acute and anticoagulant rodenticides are most commonly used to manage rodent populations in the United States and around the world (Witmer and Eisemann 2007, Witmer and Pitt 2012, Capizzi et al. 2014). Rodenticides can be effective in the short-term in reducing rat populations, but rats are efficient at reproduction and rat populations can quickly rebound, often in excess of the original population size (Corrigan 2001, Singleton et al. 2007, Hein and Jacob 2015).

Other issues surround the use of rodenticides for controlling rat populations including genetic and behavioral resistance to rodenticides (Quy et al. 1998, Pelz et al. 2005, Haniza et al. 2015), non-target consumption of rodenticides (Eason et al. 2002, Elliott et al. 2014), humaneness of rodenticide mechanism of action (Mason and Littin 2003, Meerburg et al. 2008), and increasing restrictions on the residential and commercial use of current rodenticides on the market (Leggett 2013). Therefore, alternative approaches need to be developed to manage rodent populations and reduce the damage they cause. Many non-lethal methods (e.g., deterrents, barriers, and traps) of rodent damage reduction are only marginally effective or are not cost effective (Witmer 2007, Almeida et al. 2013). However, fertility control may pro-

Figure 1. Norway rat (*Rattus norvegicus*) damaging native vegetation in Hawaii, USA (*photo courtesy of U.S. Department of Agriculture*).

vide an effective, long-term strategy to reduce rodent populations (Gao and Short 1993, Chambers et al. 1999, Humphrys and Lapidge 2008, Jacob et al. 2008, Fagerstone et al. 2010).

The occupational chemical 4-vinylcyclohexene diepoxide (VCD) has been well studied and has been demonstrated to specifically and permanently deplete ovarian primordial follicles by accelerating the natural process of atresia (Springer et al. 1996) leading to premature ovarian failure and the inability to reproduce. Triptolide, a diterpenoid triepoxide extracted from the traditional Chinese medicinal plant (Thunder God vine; *Tripterygium wilfordii* Hook F.) has been demonstrated to alter estrus cyclicity and reduce the number of developing ovarian follicles (Xu and Zhao 2010, Liu et al. 2011). Furthermore, triptolide treatment results in reversible infertility in male rats by reducing epididymal sperm content, completely inhibiting sperm motility, and decreasing epididymal tubule volume (Lue et al. 1998, Huynh et al. 2000). The use of VCD to promote ovarian failure in combination with triptolide to further promote anti-fertility actions in both female and male rats may be a successful strategy to cause immediate infertility in rats and may be a long-term tool in managing wild rat populations.

Contemporary fertility control methods used to reduce wildlife numbers and the damage caused by these species have been short-term approaches because the chemicals being used do not cause permanent sterility (Humphrys and Lapidge 2008, Jacob et al. 2008). The combination of triptolide and VCD in a liquid fertility control bait could cause immediate infertility in male and female rats as a result of triptolide action on spermatogenesis and sperm function and growing/developing ovarian follicles, respectively. Subsequently, permanent infertility can be achieved in female rats as a result of VCD action on primordial follicles of the ovary. Previously, it has been shown that liquid bait containing VCD and triptolide compromises fertility in wild-caught Norway rats (Witmer et al. 2017) and black rats (*R. rattus*; Siers et al. 2017) in a controlled caged setting.

The objective of this study was to provide the liquid contraceptive bait to rats housed in an open room group-housed setting to determine if the reproductive output continued to be lowered, as in the cage study, given the social interactions in rat populations that may limit access to food, water, bait, and mate resources. It is hypothesized that fewer litters and pups will be produced in rats that consumed the liquid contraceptive bait containing VCD and triptolide compared to control rats that did not.

Study area

With permission from landowners, wild Norway rats were live-trapped on privately owned property near Fort Collins, Colorado, USA. We captured 30 sexually mature (based on size) female rats and 12 sexually mature male rats using Tomahawk traps (Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) baited with grain and apple (*Malus* spp.) slices. We doused captured rats with Delta Dust (Bayer CropScience LP, Research Triangle Park, North Carolina, USA) to eliminate ectoparasites before being transported to the U.S. Department of Agriculture's National Wildlife Research Center (NWRC) in Fort Collins. At NWRC, the rats were weighed and then housed individually in polycarbonate, 25.4 by 48-cm rat cages. We provided the rats with commercial rodent chow and water *ad libitum* at all times and they were given either an apple or carrot (*Daucus* spp.) slice daily. Rats were also given a den tube, either burlap pieces or cotton balls for nesting material, and chew sticks for enrichment. The room conditions for the rats were as follows: 22°C, ambient humidity and 12-hour on/off light cycle. We placed the rats under quarantine for 3.5 weeks to allow for any female rats that were pregnant at the time of capture to have their litters. This also assured that all rats were old enough to be sexually mature.

Methods

Bait

Liquid contraceptive bait was obtained from SenesTech, Inc. (Flagstaff, Arizona, USA). The compound 4-vinylcyclohexene diepoxide was obtained from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). Triptolide (>98% pure by high-performance liquid chromatography analysis) was obtained from Stanford Chemicals (Irvine, California, USA). Other proprietary materials and methods were used to increase palatability of the liquid bait formulation. Control bait was formulated in the same way as the treatment bait but did not contain the 2 active ingredients.

Experimental free-range rooms

To test the efficacy of the liquid bait in a free-ranging population of rats, we placed male and female rats in 2 separate 6.7 by 6.7 by 2.7-m-high animal research rooms, one a control room and one a treatment room, and allowed them to roam freely. We spread pine (*Pinus* sp.) shavings around the floor of both rooms to serve as bedding and also to absorb urine and feces from the rats. We randomly placed 12 Protecta bait stations (Bell Labs, Madison, Wisconsin) within each room to provide either liquid bait or water. Six bait stations housed Helland Liquid dispensers (JT Eaton, Twinsburg, Ohio, USA) that contained either control or treatment bait, depending on the room. The remaining 6 bait stations housed Helland Liquid Dispensers that contained only water. This allowed the rats to choose the type of liquid they consumed from familiar containers. Furthermore, each room was outfitted with 4 43 by 28 by 30-cm-high plastic storage bins with 2 holes cut in the sides of the boxes and 6 empty Protecta bait stations to serve as den boxes. The storage bins and empty bait stations were filled with a thin layer of corn cob bedding and 3–4 cotton balls were placed in the containers for nesting material. The rooms also contained 4 food stations, several PVC tube hides, wood chew blocks, and other materials for enrichment.

We placed 15 randomly assigned control female rats and 15 treatment female rats in the rooms for 24 hours prior to 6 males being added to each room. At the time that the females were added to the rooms, the control bait and treatment bait were placed in the respective rooms. No pre-baiting period was conducted. Rats were allowed to freely consume control or treatment bait for 8 weeks, after which all bait was removed. Bait consumption was measured and Helland dispensers were replenished with fresh bait every 3–4 days. As time continued, fighting among the rats occurred in both rooms and some male rats had to be removed from the rooms due to extensive injuries to their bodies and tails. The minimum number of males in each room, at any given time or day, was 2 males.

Control and treatment rats remained in their respectively assigned rooms for 89 days. Assuming a gestation period of 21–25 days and that postpartum female rats will breed during the postpartum estrus, and factoring in potential lactation induced implantation-delay, the 89 days that the rats were in their respective treatment rooms is equivalent to 3 breeding rounds. For the fourth and final breeding round, we captured all control males and placed them in the treatment room, and all treatment males were captured and placed in the control room. The combined control and treatment rats were group-housed together for 23 days, after which all males were trapped out, euthanized, and necropsied. Female rats remained in the rooms for 26 days to allow for delivery of their final litters. This cross-breeding between control and treatment animals allowed for determination of recovery of fertility in treatment male and female rats. Throughout the study, when litters were found, pups were counted, collected, and immediately euthanized by isoflurane exposure. The experiment was carried out according to an NWRC Institutional Animal Care and Use Committee approved study protocol (QA-2151).

Tissue collection

Following the removal of males from the control and treatment group-housed rat rooms, we euthanized males by CO_2 exposure followed by cervical dislocation. We removed organs, testes, and epididymis and cleaned them of fat and connective tissues. Individual wet weights of testes and epididymis were normalized to individual body weights. To determine testis volume, the long and the short axis of the testis was measured, to the nearest 0.1 mm. Testis volume was estimated using the formula for a prolate spheroid (length x width 2 x 0.523).

Twenty-six days after the removal of male rats

Table 1. Weekly bait consumption in free-ranging wild-caught Norway rats (*Rattus norvegicus*) over an 8-week baiting period. Values are expressed in liters. Chi-square test applied to a contingency table to determine differences in bait consumption between rats consuming control bait and rats consuming treatment bait ($\chi^2 = 1435$, df = 7, $P < 0.001$).

	Week								
	1	2	3	4	5	6	7	8	Total
Control	1.625	2.924	2.609	2.578	3.765	4.580	3.333	4.054	25.470
Treatment	1.216	2.273	0.954	0.846	1.232	1.218	0.933	0.943	9.617

Table 2. Norway rat (*Rattus norvegicus*) pup numbers and litters from treatment-paired breeding rounds. Chi-square test applied to contingency table to determine differences in pup numbers between control and treatment breeding pairs ($\chi^2 = 43.82$, df = 2, $P < 0.001$).

	Breeding round					
	1	2	3	Total number of pups	Number of litters	Average litter size
Control	147	82	26	255	30	8.5
Treatment	0	3	9	12	2	6

Table 3. Norway rat (*Rattus norvegicus*) pup numbers and litter sizes from the control and treatment animal cross-breeding round. Expected number of pups determined using the average litter size from the control female and control male breeding rounds 1–3. Chi-square test applied to contingency table to determined differences in observed and expected number of pups from control and treatment rat cross breedings ($\chi^2 = 1.267$, df = 1, $P \approx 0.2604$).

	Observed number of rat pups	Expected number of pups at optimal fertility	Number of litters	Average litter size
Control (n = 14 female rats)	93	119	14	6.64
Treatment (n = 15 female rats)	80	128	12	6.67

from experimental rooms, we euthanized female rats and collected organs. We normalized individual wet weights of the ovaries and uteri to individual body weights. Uterine weights from females determined to be in estrus by vaginal cytology were excluded from uterine weight data.

For both male and female rats, liver, spleen, kidneys, and adrenal glands were removed and trimmed of fat and connective tissue. Wet weights of individual organs from each rat were normalized to individual body weight.

Histological evaluation

We fixed ovaries in 10% neutral phosphate buffered formalin, stained with hematoxylin and eosin, and sectioned (5 μm thick). Oocyte-containing follicles were classified and counted in every tenth section (Mayer et al. 2002). Primordial follicles contained an oocyte surrounded by a complete ring of squamous granulosa cells; primary follicles contained an oocyte surrounded by >1 layer of granulosa cells; primary follicles contained an oocyte surrounded by a complete layer of cuboidal granulosa cells; secondary follicles contained an oocyte surrounded by >1 layer of granulosa cells; antral follicles contained an oocyte surrounded by multilayered granulosa cells and an antral cavity. Follicles were only counted if the oocyte nucleus was visible. Evaluators were blinded to treatment versus control samples.

Statistical analyses

We compared tissue weights and ovarian follicle counts using unpaired, 2-tailed *t*-tests with alpha set at 0.05. Chi-square tests were applied to contingency tables to determine differences between control and treatment bait consumption and pup numbers with alpha set at 0.05. We performed all statistical analyses using GraphPad Prism (Version 6.05 for Windows, GraphPad Software, Inc., La Jolla, California).

Results

We measured bait consumption over the 8 weeks that bait was provided to the free-ranging wild-caught Norway rats. On a weekly basis, a chi-square test showed that treatment rats consumed less ($P < 0.0001$) bait than control rats (Table 1). Likewise, the total bait consumption in treatment rats was lower (9.617 L) than in the control rats (25.470 L). In control rats, the amount of bait consumed during weeks 5–8 was 22.6–43.7% greater than week 4. This increase in bait consumption coincides with the time in which control rats started delivering pups.

During the treatment-paired mating period (89 days), fewer ($P < 0.001$) pups were born in the treatment room compared to the control room (Table 2). During breeding round 1, 147 pups from 16 litters were born in the control room and no pups were born in the treatment room. During breeding round 2, 82 pups from 10 litters were born in the control room and 3 pups from 1 litter were born in the treatment room. During breeding round 3, 26 pups from 4 litters were born in control room and 9 pups from 1 litter were born in the treatment room. In total, for the 3 breeding rounds, 255 pups were born in the control room, and 12 pups were born in the treatment room, resulting in a 95.3% reduction in reproductive capacity after 8 weeks of liquid contraceptive bait consumption.

In the final breeding round, control males were placed in the treatment room with treatment female and treatment males were placed in the control room with control females for 23 days. These cross-treatment breeding pairings resulted in 93 pups from 14 litters being born to control female and treatment male pairings and 80 pups from 12 litters being born to the treatment female and control male pairings (Table 3). Using the average litter size from control rat breeding rounds 1–3 (Table 2), the expected number of pups from successful breeding and implantation was determined. In control females ($n = 14$), 119 pups were expected in 1 breeding round and in treatment females ($n = 15$), 128 pups were expected. A chi-square test was applied to a contingency table with expected and observed pup numbers from control female and treatment male (ConF/TrtM) and treatment female and control male (TrtF/ConM) pairings, and results found no differences in pup numbers ($P > 0.05$), indicating

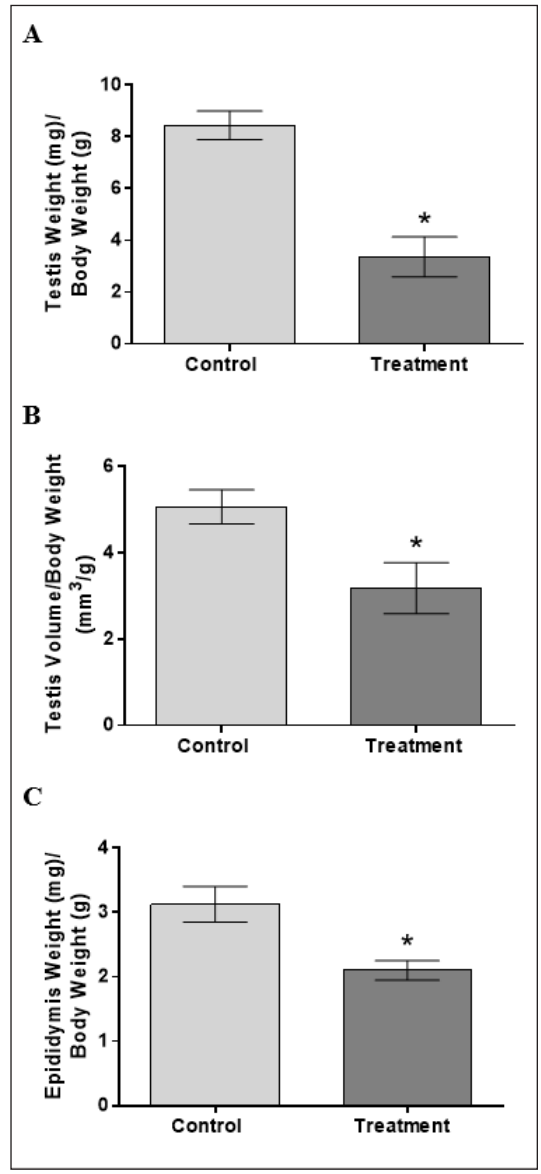

Figure 2. Testes and epididymis weights in males consuming bait containing 4-vinylcyclohexene diepoxide and triptolide. Wild-caught male Norway rats (*Rattus norvegicus*; $n = 6$) consumed either control (light bar) or treatment bait (dark bar) for 56 days in a free-range group-housed setting with female rats. After 4 breeding rounds, testes (A) and epididymis (C) were collected, weighed, and normalized to body weight. Testicular volume (B) was calculated using the formula for a prolate spheroid (length x width x 0.523). Values are represented as mean mg/gm body weight ± SEM with right and left testes combined for each animal. Statistically significant differences in tissue weight or testis volume from control indicated by (*) for $P < 0.05$.

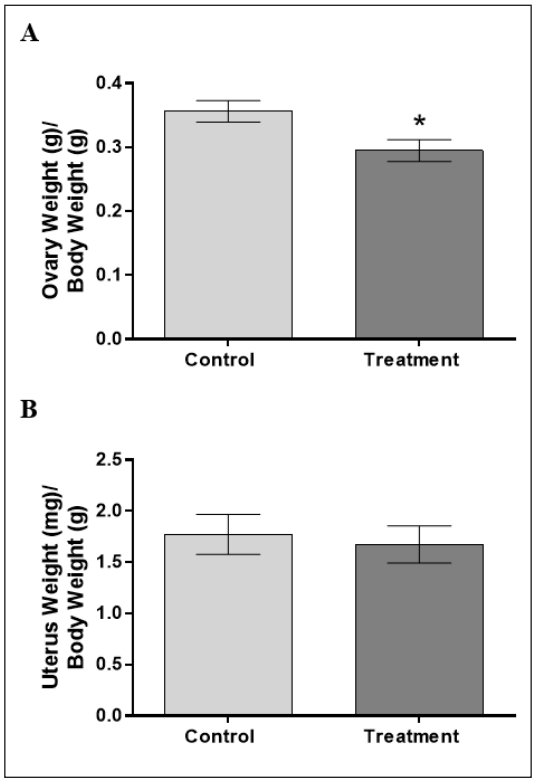

Figure 3. Ovary and uterus weight in female Norway rats (*Rattus norvegicus*) consuming bait containing 4-vinylcyclohexene diepoxide and triptolide. Wild-caught female Norway rats consumed either control (light bar; $n = 14$) or treatment bait (dark bar; $n = 15$) for 56 days in a free-range group-housed setting with male rats. After 4 breeding rounds, ovaries (A) and uteri (B) were collected, weighed, and normalized to body weight. Uterine weights from rats in estrus at the time of necropsy were excluded. Values are represented as mean mg/gm body weight ± SEM with left and right ovary weights combined for each animal. Statistically significant differences in ovarian weight from control indicated by (*) for $P < 0.05$.

recovered fertility in both treatment males and females. Although treatment males and females recovered fertility after consumption of treatment bait, litter sizes were smaller in cross-breeding mating (ConF/TrtM 6.64 ± 0.84 pups; TrtF/ConM 6.66 ± 0.98 pups) compared to control male and female matings (8.50 ± 0.46 pups).

We determined weights of reproductive organs from male and female rats and compared them between treatment groups. Combined (left and right) testis weight was 2.5-fold lower in treatment males ($P < 0.001$) compared to control males (Figure 2A). Additionally, testis volume was reduced ($P < 0.05$) in treatment males compared to control males (Figure 2B). Combined (left and right) epididymal weight was 33% lower in treatment males compared to control males (Figure 2C). Combined (left and right) ovary weights were lower ($P < 0.05$) in treatment females compared to control females (Figure 3A). There were no differences in uterine weights from female rats not in estrus between control and treatment females ($P > 0.05$; Figure 3B).

Testes from male rats were prepared for histological examination. Micrographs of representative testis tissue from control and treatment male rats are shown (Figure 4). Testes of control and treatment male rats have a well-organized progression from spermatogonia lining the basement membrane to spermatids further in the tubules. Spermatocytes and spermatozoa are clearly present in the luminal space of the seminiferous tubules from the control male rat testes (Figure 4A). In contrast, the tubules of treatment male rat testes (Figure 4B) show a reduction in spermatocytes and spermatozoa in the luminal space.

Ovaries from female rats were prepared for histological evaluation. Micrographs of representative ovaries from control and treatment female rats are shown (Figure 5). Relative to controls, there was a reduction ($P < 0.05$) of 53.2% in primary follicles and a 33.2% reduction in secondary follicles (Figures 6B and 6C) in treatment females. Although not statistically significant, there was a 28.8% reduction in primordial follicles in treatment female rats compared to controls (Figure 6A). There were no differences ($P > 0.05$) in antral follicle numbers between control (65.31 ± 13.18 follicles) and treatment (83.88 ± 14.20 follicles) female rats. In both male and female rats, there were no differences ($P > 0.05$) in body weight or in normalized wet weights of adrenal glands, kidneys, spleen, or livers (Table 4).

Discussion

Total bait consumption in the treatment rats was 62% less than that consumed by control rats, which was consistent with previous results in cage studies with lab (Dyer et al. 2013) and wild-caught rats (Witmer et al. 2017). Although bait consumption was reduced in treatment rats, bait was consumed at efficacious amounts to reduce total number of litters and pups born, decrease some ovarian follicle populations, and

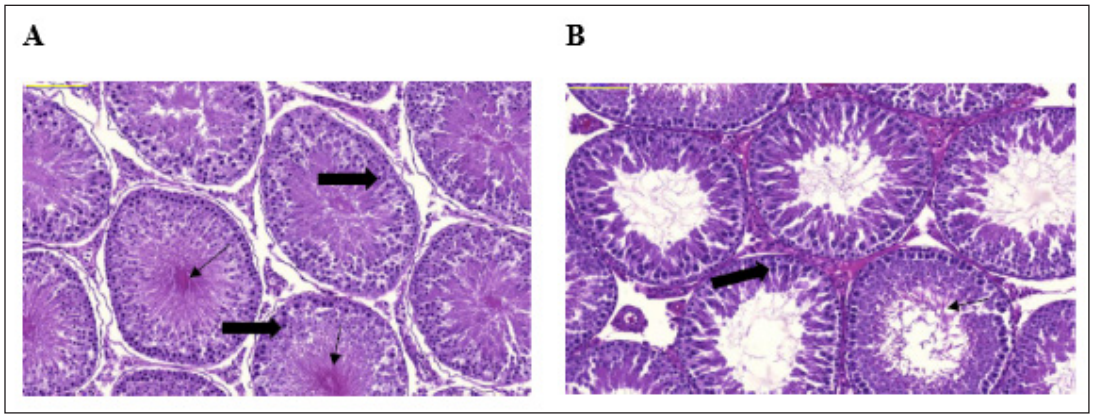

Figure 4. Micrographs of control (A) and treatment (B) Norway rat (*Rattus norvegicus*) testes. Small arrow = spermatozoa; large arrow = spermatocytes. Magnification for images is 20X.

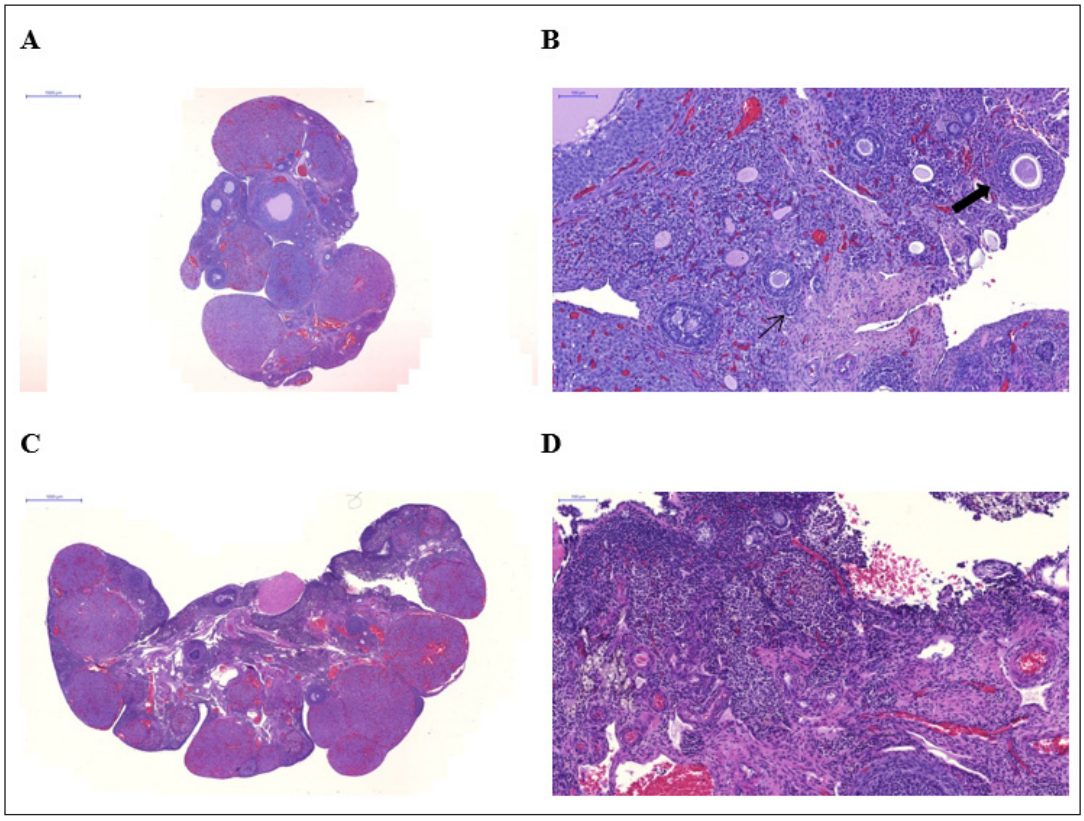

Figure 5. Micrographs of control and treatment Norway rat (*Rattus norvegicus*) ovaries. Wild-caught female Norway rats in a free-range group-housed setting with male rats consumed either control bait (A, B) or treatment bait containing 4-vinylcyclohexene diepoxide and triptolide (C, D) for 56 days. Following 4 breeding rounds, ovaries were collected, fixed, sectioned, and stained with hematoxylin and eosin. A, B = control ovary sections; C, D = treatment ovary sections. Small arrow = healthy primary follicle; closed large arrow = healthy secondary follicle. Magnification for A and C, 1.8X. Magnification for B and D, 13.9X.

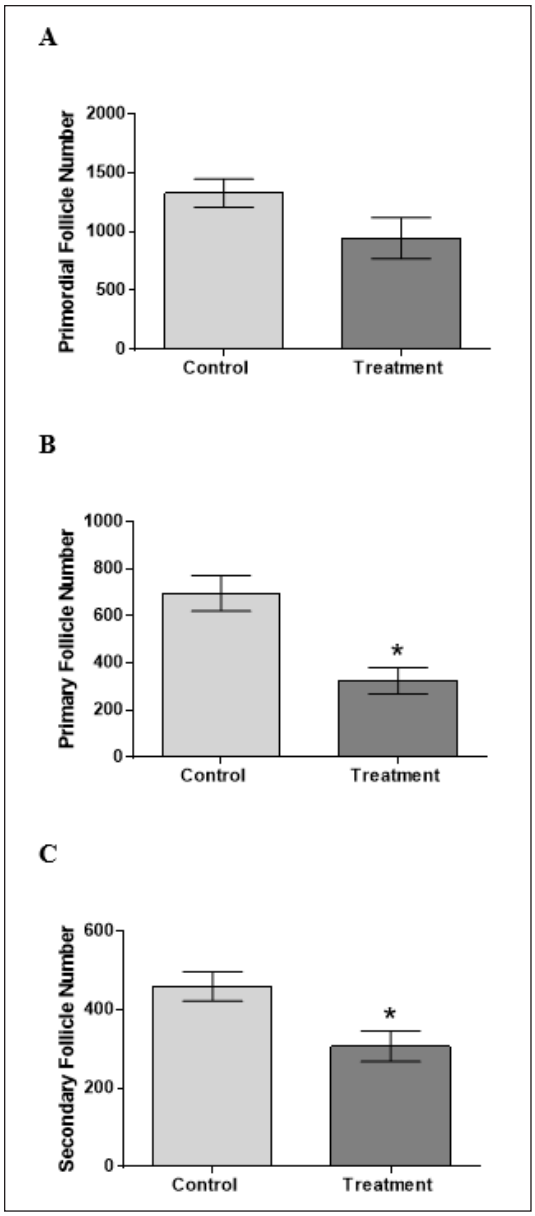

Figure 6. Effect of fertility control bait containing 4-vinylcyclohexene diepoxide and triptolide on ovarian follicle number. Wild-caught female Norway rats (*Rattus norvegicus*) consumed either control (light bar) or treatment bait (dark bar) for 56 days in a free-range group-housed setting with male rats. Eighty-two days after the removal of bait, ovaries were prepared for histological evaluation. Follicles were classified as primordial (A), primary (B), or secondary (C) and counted. Values are represented as mean follicle numbers ± SEM. Differences in follicle numbers from control are indicated by (*) for $P < 0.05$.

reduce testicular spermatozoa resulting in an overall 95% reduced fertility compared to that of control rats.

This reduction in fertility is striking when the time of bait and mate introductions are taken into consideration. Previous studies with the liquid contraceptive bait have been conducted with a 15-day pre-treatment period prior to pairing males and females for breeding (Siers et al. 2017, Witmer et al. 2017). In the current study, no pre-treatment period was included to mimic practical use practices in field settings where isolation of males and females from each other is not feasible. The current study results show immediate action of the contraceptive bait on diminishing male and female rat reproductive capacity as demonstrated by no pups being born in the first breeding round after bait was introduced.

During the first 4 weeks of bait consumption in the control and treatment rats, there were increases in bait intake followed by slight declines in week 3 and week 4. Weeks 3 and 4 coincide with the time that pregnant female rats in the control group were nearing the end of their gestation period. Pregnant rats have been shown to increase their food intake up until right before parturition, when there is a decline, and then increased food intake during lactation (Kristal and Wampler 1973, Morgan and Winick 1981). The pattern of increased bait intake followed by decreased bait intake in control rats is similar to increased food uptake in pregnant rats. The degree of increased bait consumption in treatment rats is not as much as seen in control rats, but the pattern of increased and decreased bait consumption is present. The increases in bait consumption in treatment rats throughout the 8-week baiting period may be associated with pseudopregnancy. Pseudopregnant rats may also demonstrate increases in food intake until their bodies recognize that placental hormones are not present (Bourne and Read 1982). The pattern of increased and decreased bait consumption mimicking food intake in pregnant rats compounded by the possible decreased palatability in treatment bait may explain the drastic differences in total bait consumption between control and treatment rats.

Throughout the 3 breeding cycles of the treatment-paired male and female matings,

Table 4. Body and organ weights from wild-caught Norway rats (*Rattus norvegicus*) after consumption of control or treatment bait. Values are expressed as wet tissue weight (mg) normalized to body weight ± SEM. Left and right adrenal and kidney weights from each animal were combined.

Treatment	Sex	Body weight (g)	Adrenal	Kidney	Spleen	Liver
Control bait	Male	359.9 ± 38.87	0.2765 ± 0.02994	8.811 ± 0.5911	4.148 ± 0.8299	42.35 ± 3.288
	Female	329.0 ± 17.54	0.4414 ± 0.04069	8.093 ± 0.2415	4.516 ± 0.4146	49.86 ± 1.662
Treatment bait	Male	350.8 ± 37.59	0.3486 ± 0.0413	9.466 ± 1.036	3.377 ± 1.049	37.58 ± 4.529
	Female	355.7 ± 13.84	0.419 ± 0.01572	8.32 ± 0.349	4.865 ± 0.3572	49.42 ± 1.120

there was a decline in number of pups born to control rats with each subsequent breeding round. The number of pups born to control rats declined from 147 pups born in the first breeding round to 26 pups born in the third breeding round. This decline may be explained by the removal of control males from the population due to injuries sustained from fighting. Males removed from the colony had extensive injuries to their bodies and tails. It is unknown whether the injuries were caused by a dominate male or dominate female in the colony. The injuries exhibited by male rats were not exclusive to control rats; treatment male rats also experienced injuries that caused them to be removed from the treatment rat colony. At the lowest level, both the control and treatment rat colonies had 2 males present before healed rats could rejoin the colony. With fewer male rats present, this could possibly lead to fewer male and female interactions for breeding, resulting in declines in pup numbers in the control group.

Throughout the treatment-paired mating period, the treatment rats had a 95.3% reduction in number of pups born compared to control rats. In the second and third breeding round of the treatment-paired matings, 1 litter was born in each round resulting in a total of 12 pups born to treatment rats in the first 3 breeding rounds. The first treatment litter of 3 pups was born 77 days after the start of treatment bait delivery in comparison to the first control litter of 10 pups being born 30 days after the start of control bait consumption. The second treatment litter of 9 pups was born 101 days after the start of treatment bait delivery. Therefore, in addition to reducing the total number of pups born to treatment rats, treatment bait consumption also caused a delay in the time in which treatment pups were born compared to control pups. In the fourth breeding cycle, cross-breeding between control females and treatment males, as well as treatment females and control males resulted in pups being born in both groups without there being any difference in the number of pups born to either group. The first litter delivered in breeding round 4 was from a TrtF/ConM pairing, which was 26 days after the control males had been placed with treatment females. These results indicate the effect of triptolide on diminishing fertility had been reversed after 57 days since the removal of treatment bait and treatment males and females were regaining their fertility.

Although fertility was starting to be restored in treatment rats in the latter breeding rounds of the treatment-paired breeding round and in the cross-bred breeding round, some aspects of fertility were not restored to that of controls. Some of the litters born to the cross-bred groups were of the expected size (8–10 pups); however, some litter sizes were as low as 1–2 pups in females from both groups, resulting in average litter sizes in cross-bred groups being smaller (ConF/TrtM, 6.64 pups; TrtF/ConM, 6.67 pups) than litter sizes from control female and control male breedings (8.5 pups). All control females (n = 14) became pregnant by a treatment male in the fourth breeding round and 12 of 15 treatment females became pregnant by control males. Decreased litter sizes in cross-bred pairings and only 80% of treatment females delivering litters indicate that fertility in both treatment males and females is not fully restored to that of control rats. Although infertility achieved in the treatment rats was not permanent, reduced litter sizes and sustained infertility in 20% of the treatment females up to at least 139 days after the start of treatment bait consumption would provide an overall favorable effect on controlling wild rat population numbers.

Treatment male rats experienced decreases in testis and epididymal weight, and likewise also exhibited decreased testis volume compared to control rats. Additionally, treatment male rats showed reductions in spermatozoa within the luminal space of testicular seminiferous tubules compared to controls. These results are similar to male rats treated with triptolide who show decreased tubule volume and reduced epididymal sperm content (Lue et al. 1998, Huynh et al. 2000). With reductions of spermatozoa in the testicular seminiferous tubules, it is possible that this condition would progress to decreases in sperm content within epididymis tubules, as was shown by Lue et al. (1998) in male rats that were treated with triptolide. Although neither testosterone nor gonadotropin levels were measured in this study, reductions in male reproductive tissue weights and declines in spermatogenesis cell types are indicative of when male Norway rats exhibit low testosterone and low luteinizing hormone and gonadotrophic releasing hormone (Wang et al. 2002). Collectively, these results show reduced fertility in treatment males who consumed the liquid contraceptive bait.

Ovary weights were decreased in treatment females compared to controls; however, there were no differences in uterine weight between groups. This reduction in ovary weight is similar to lower ovary weights observed in rats treated with triptolide (Liu et al. 2011) and could be the result of the decrease in primary and secondary follicles observed in treatment rat ovaries. Liu et al. (2011) also observed decreases in uterine weight in rats treated with triptolide, which is likely due to the compound's alterations in estradiol production from granulosa cells (Zhang et al. 2012). The non-significant difference in uterine weight between treatment groups is likely a reflection of the treatment rats' reversal of infertility after the removal of bait and restoration of any inhibition in progesterone and estradiol production that may have been caused by triptolide.

Histological examination of ovaries showed a reduction of primary and secondary follicles in treatment females compared to controls, which are caused by the actions of both VCD and triptolide. This result is different from wild rats given the liquid contraceptive bait in a controlled cage setting, where treatment females had reduced primordial follicles in addition to reductions in primary and secondary follicles (Witmer et al. 2017). The reduction in primordial follicles is a result of VCD action (Kao et al. 1999). Treatment females did exhibit a non-statistically significant reduction in primordial follicles, indicating that VCD did promote primordial follicle number declines, but also that treatment females did not consume enough bait to reach efficacious VCD doses that would elicit significant declines in primordial follicles.

Further evidence that VCD did have some effect on the treatment ovaries were the declines in primary and secondary follicles, suggesting less recruitment of growing follicles from the reduced primordial follicle pool. Additionally, reduced secondary follicle numbers reflect triptolide action as triptolide has been shown to target secondary follicle reduction by apoptosis (Xu and Zhao 2010). Because the rats were housed in a free-range setting, it was not feasible to collect individual bait consumption amounts; thus, we do not know how much bait each individual female consumed and whether the amount consumed was near the effective dose levels for VCD needed to produce significant declines in primordial follicles.

Also unknown at this point is whether rat behaviors and social interactions within the treatment rat colony, such as bait guarding, changed, which might have impacted overall bait consumption for some rats in the colony. Undoubtedly, the bait exposure time in this study to allow for VCD depletion of all primordial follicles was not achieved, and thus permanent sterility in female rats was not attained and these females would continue to go through estrus cyclicity until the primordial follicle pool was exhausted. Further studies are planned to determine the number of days in which wild-caught rats need to consume treatment bait to achieve complete follicle depletion and subsequent sterility.

Normalized adrenal gland, kidney, spleen, and liver weights did not differ between treatment groups. Additionally, there were no differences in those tissue weights between males and females. These results show that there are no gross effects of VCD or triptolide on those vital organs and that these active ingredients in the fertility control bait formulation target the male and female reproductive systems.

Management implications

Even given the behavioral interactions and social hierarches that can develop in a free-ranging Norway rat colony, our study animals willfully consumed a liquid contraceptive bait containing VCD and triptolide. The reproductive capacity of rats consuming treatment bait compromised fertility in males and females in the 3 successive breeding cycles. Therefore, using liquid bait containing VCD and triptolide as a strategy to control rat population numbers may be a suitable alternative to the current toxic rodenticides used to manage rodent pest populations. We recommend that a longer and larger-scale trial with free-ranging rats be conducted.

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Literature cited

Almeida, A., R. Corrigan, and R. Sarno. 2013. The economic impact of commensal rodents on small businesses in Manhattan's Chinatown: trends and possible causes. Suburban Sustainability 1(1):2.

Bourne, A., and R. Read. 1982. Plasma insulin response and food intake in pseudopregnant rats. Comparative Biochemistry and Physiology 73:279–281.

Capizzi, D., S. Bertolino, and A. Mortelliti. 2014. Rating the rat: global patterns and research priorities in impacts and management of rodent pests. Mammal Review 44:148–162.

Chambers, L., M. Lawson, and L. Hinds. 1999. Biological control of rodents—the case for fertility control using immunocontraception. Pages 215–242 *in* G. R. Singleton, L. A. Hinds, H. Leirs, and Z. Zhang, editors. Ecologically-based management of rodent pests. Australian Centre for International Agricultural Research, Canberra, Australia.

Corrigan, R. 2001. Rodent control: a practical guide for pest management professionals. GIE Media, Cleveland, Ohio, USA.

Dyer, C., S. Raymond-Whish, S. Schumuki, T. Fisher, B. Pyzyna, A. Bennett, and L. Mayer. 2013. Accelerated follicle depletion in vitro and in vivo in Sprague-Dawley rats using the combination of 4-vinylcyclohexene diepoxide and triptolide. Journal of Zoo and Wildlife Medicine 44(4S):S9–S17.

Eason, C., E. Murphy, G. Wright, and E. Spurr. 2002. Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. Ecotoxicology 11:35–48.

Elliott, J., S. Hindmarch, C. Albert, J. Emery, P. Mineau, and F. Maisonneuve. 2014. Exposure pathways of anticoagulant rodenticides to non-target wildlife. Environmental Monitoring and Assessment 186:895–906.

Fagerstone, K., L. Miller, G. Killian, and C. Yoder. 2010. Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human–wildlife conflicts in North America. Integrative Zoology 1:15–30.

Firth, C., M. Bhat, M. Firth, S. Williams, M. Frye, P. Simmonds, J. Conte, J. Ng., J. Garcia, N. Bhuva, B. Lee, X. Che, P. Quan, and W. Lipkin. 2014. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal *Rattus norvegicus* in New York City. mBio 5(5): e01933-14.

Gao, Y., and R. Short. 1993. The control or rodent populations. Oxford Reviews of Reproductive Biology 15:265–310.

Haniza, M., S. Adams, E. Jones, A. MacNicoll, E. Mallon, R. Smith, and M. Lambert. 2015. Large-scale structure of brown rat (*Rattus norvegicus*) populations in England: effects on rodenticide resistance. PeerJ 3: e1458.

Hein, S., and J. Jacob. 2015. Recovery of small rodent populations after population collapse. Wildlife Research 42:108–118.

Humphrys, S., and S. Lapidge. 2008. Delivering and registering species-specific oral and anti-fertility products: a review. Wildlife Research

35:578–585.

Huynh, P., A. Hikim, C. Wang, K. Stefonovic, Y. Lue, A. Leung, V. Atienza, S. Baravarian, V. Reutrakul, and R. Swerdloff. 2000. Long-term effects of triptolide on spermatogenesis, epididymal sperm function, and fertility in male rats. Journal of Andrology 21:689–699.

Jacob, J., G. Singleton, and L. Hinds. 2008. Fertility control of rodent pests. Wildlife Research 35:487–493.

Kao, S., I. Sipes, and P. Hoyer. 1999. Timecourse of 4-vinylcyclohexene diepoxide-induced follicle distruction in rats and mice. Reproductive Toxicology 13:67–75.

Kristal, M., and R. Wampler. 1973. Food and water intake prior to parturition in the rat. Physiological Psychology 1:297–300.

Leggett, M. 2013. Assessment of risk of pesticide use under the Federal Insecticide Fungicide and Rodenticide Act and the Endangered Species Act; how much conservatism is too much? Outlooks on Pest Management 24:24–27.

Liu, J., Z. Jiang, L. Liu, Y. Zhang, S. Zhang, J. Xiao, M. Ma, and L. Zhang. 2011. Triptolide induces adverse effects on reproductive parameters of female Sprague-Dawley rats. Drug and Chemical Toxicology 34:1–7.

Lue, Y., A. Hikim, C. Wang, A. Leung, S. Baravarian, V. Reutrakul, R. Sangsawan, S. Chaichana, and R. Swerdloff. 1998. Triptolide: a potential male contraceptive. Journal of Andrology 19:479–486.

Mason, G., and K. Littin. 2003. The humaneness of rodent pest control. Animal Welfare 12:1–37.

Mayer, L., N. Pearsall, P. Christian, P. Devine, C. Payne, M. McCuskey, S. Marion, I. Sipes, and P. Hoyer. 2002. Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene diepoxide. Reproductive Toxicology 16:775–781.

Meerburg, B., F. Brom, and A. Kijlstra. 2008. The ethics of rodent control. Pest Management Science 64:1205–1211.

Morgan, B., and M. Winick. 1981. A possible control of food intake during pregnancy in the rat. British Journal of Nutrition 46:29–37.

Pelz, H., S. Rost, F. Hünerberg, A. Heiberg, K. Baert, A. MacNicoll, C. Prescott, A. Walker, J. Oldenburg, and C. Müller. 2005. The genetic basis of resistance to anticoagulants in rodents. Genetics 170:1839–1847.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52:273–288.

Quy, R., A. MacNicoll, and D. Cowan. 1998. Control of rats resistant to second-generation anticoagulant rodenticides. Proceedings of the Vertebrate Pest Conference 18:262–267.

Siers, S., B. Pyzyna, L. Mayer, C. Dyer, I. Leinbach, R. Sugihara, and G. Witmer. 2017. Laboratory evaluation of the effectiveness of the fertility control bait ContraPest on wild-captured back rats (*Rattus rattus*). Final Report QA-2570. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, Hilo, Hawaii, USA.

Singleton, G., P. Brown, J. Jacob, and K. Aplin. 2007. Unwanted and unintended effects of culling: a case for ecologically-based rodent management. Integrative Zoology 2:247–259.

Springer, L., M. McAsey, J. Flaws, J. Tilly, I. Sipes, and P. Hoyer. 1996. Involvement of apoptosis in 4-vinylcyclohexene diepoxide-induced ovotoxicity in rats. Toxicology and Applied Pharmacology 139:394–401.

Wang, C., A. Hikim, M. Ferrini, J. Bonavera, D. Vernet, A. Leung, Y. Lue, N. Gonzalez-Cadavid, and R. Swerdloff. 2002. Male reproductive ageing: using the brown Norway rat as a model for man. Novartis Foundation Symposium 242:82–97.

Witmer, G. 2007. The ecology of vertebrate pests and integrated pest management. Pages 393–410 *in* M. Kogan and P. Jepson, editors. Perspectives in ecological theory and integrated pest management. Cambridge University Press, Cambridge, United Kingdom.

Witmer, G., and J. Eisemann. 2007. Rodenticide use in the United States: an overview. Proceedings of the Wildlife Damage Management Conference 12:114–118.

Witmer, G., and W. Pitt. 2012. Invasive rodents in the United States: ecology, impacts and management. Pages 47–75 *in* J. Blanco and A. Fernandes, editors. Invasive species: threats, ecological impact and control methods. Nova Science Publishers, Inc., New York, New York, USA.

Witmer, G., S. Raymond-Whish, R. Moulton, B. Pyzyna, E. Calloway, C. Dyer, L. Mayer, and P. Hoyer. 2017. Compromised fertility in free feeding of wild-caught Norway rats (*Rattus norvegicus*) with a liquid bait containing 4-vinylcyclohexene diepoxide and triptolide. Journal of Zoo

and Wildlife Medicine 48:80–90.

Witmer, G., and G. Singleton. 2010. Sustained agriculture: the need to manage rodent damage. Pages 1–38 *in* F. Wager, editor. Agricultural production. Nova Science Publishers, Inc., New York, New York, USA.

Xu, C., and Y. Zhao. 2010. Apoptosis of rat's ovarian follicle cells induced by triptolide in vivo. African Journal of Pharmacy and Pharmacology 4:422–430.

Zhang, J., L. Liu, X. Mu, Z. Jiang, and L. Zhang. 2012. Effect of triptolide on estradiol release from cultured rat granulosa cells. Endocrine Journal 59:473–481.

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Gary W. Witmer, Ph.D., has recently retired from the U.S. Department of Agriculture National Wildlife Research Center. He was a supervisory research wildlife biologist there for many years. His research focused on rodent populations and damage management.

Stefanie Raymond-Whish (photo unavailable), Ph.D., was an employee of SenesTech biotechnology company at the time of this study.