Real-time Monitoring of Contraceptive Pellet Consumption to Achieve Rat/Mouse Rodent Control

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ABSTRACT: Superior rodent management is critical to preserve the diversity of wildlife, and to mitigate environmental and social damage. Pest management strategies can employ 21st century methods to balance the human-animal conflict, especially regarding rodents. Non-toxic fertility control has several advantages over the use of poison when the following parameters are true: 1) rate of removal exceeds rate of population increase; 2) compounds do not bioaccumulate in the rodents or the environment; 3) non-lethal method targets both males and females; 4) animals can be detected at low densities; 5) cost analysis favors fertility control over lethality; 6) method has socio-political acceptance. Our fertility control system meets each of these requirements.

Utilizing plant-root-based extracts from *Tripterygium wilfordii* Hook F, we have formulated a proprietary rodent feed pellet that is efficacious in reducing mice and rat populations in multiple settings. The pellets target rodents with a population reduction capacity of 98% that is sustainable over 12 months. The cost of the pellet solution is less than 90% of other fertility control products, and less than 47% of poison products. The functional key to the success of fertility-control approaches is optimizing feeding protocols to achieve maximum population reduction. We have developed an application-based system to provide sustainable cost-effective management. The monitoring system captures consumption and feeder service data. Point-of-entry data validation is enforced. Compliance with the service program is monitored and configurable alerts are issued for outlying events. These data are ingested into a data analytics engine which generates real-time dashboards for oversight and analysis. Administrative features provide user management, project definition and configuration. In-line instructional documentation and pellet supply order forms provide support for field technicians. As a 501(c)3 non-profit charity we are funded by Open Philanthropy to provide the rigorous data sets presented: a 21st century solution to rodent management.

KEY WORDS: animal feeding, anticoagulant rodenticides, consumption monitoring, house mouse, informatics, *Mus domesticus*, rodent fertility control, rodent population re-education, second-generation rodenticides

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INTRODUCTION

Commensal rodents have been a challenge for humankind for millennia, destroying crops in the field, contaminating stored grains and other foodstuffs, and passing zoonotic diseases. Our attempts to control rats have primarily depended on use of lethal means, from physical trapping to widespread application of poisons. Since WWII the use of first-, then second-generation poisons has steadily expanded. Today the most common poisons used are second-generation anticoagulant rodenticides (SGARs) (Tripathi 2014).

Our organization, Wisdom Good Works, is a nonprofit entity whose goal is to improve conditions for wildlife by reducing the use of poison for wild population management. In this paper we are focusing on rodents, for which the largest amounts of poison are placed into our environment (Quinn and Swift 2018). We are driven by the positive impact of improving conditions for wildlife.

In recent times the growth of integrated pest management (IPM) strategies has yielded additional rodent population management tools that are non-toxic and do not require the use of SGARs. Strategies such as trapping, both lethal and non-lethal, repellents, physical exclusion, and fertility control have been successfully used (Rahelinirina et al. 2021, Hansen et al. 2016, Dyer and Mayer 2014).

The characterization of a successful fertility control strategy has three primary stakeholders: the public, professional pest managers, and the scientific community. The public, as represented by wildlife groups (California AB1788, AB2552), municipalities, (New York City Council Int 376) and governments have turned up the volume on removing poisons from our environment across the U.S. and Canada (State of Connecticut 2022, Commonwealth of Massachusetts 2021, British Columbia, Canada 2021, Rhode Island 2024,). From our communications with residents in these concerned municipalities we have heard: “Fertility control works in the lab, but can it work in the real world; are our rats another story?”; “How long does it take to work?”; “How long will it last?”; and “How much does it cost?” (pers. commun.). In concert with the increasing visibility of secondary poisoning in other wildlife, residents and government managers are desperate for an alternative.

Fertility control as a strategy presents a conundrum for professional pest managers as their existing business model is built on using poison. The pest management industry is considerable in size, capturing $5 billion in annual revenue (Bell Laboratories Inc., State of the Rodent Control Market, Bell Labs). Upsetting this paradigm is not a trivial task. Without doubt, poison kills rats and is inexpensive to use. However, the practice poses potential for poisoning and death of non-target animals, companion animals, and children, and contaminates the environment. Because it is impossible to poison all rats, not for a lack of trying, poison does not solve the self-perpetuating rodent population problem even after decades of use. The economic issues at play are the low cost of poison deployment when compared to other strategies that are more expensive such as labor for trapping, exclusion repair costs, deploying fertility control product, and monitoring.

For the scientific community pest management strategies are focused on efficacy and mechanisms of action of the active ingredients, side effects, non-target animal welfare, palatability, effects on the food chain, environmental dependencies of the treatment, and field-testing results (Massei et al. 2024).

The approach in this report is based on three essential components of a successful solution: 1) Delivery of the active ingredient(s) to the target animal; 2) Safety of the compound; and 3) Population reduction. Assuming all three components are satisfied, the subsequent steps that need to be taken to advance the strategy to a viable solution are: individual animal studies, efficacy in various field environments, regulatory requirements, and a commercial pipeline for distribution.

Throughout the entire research and development process it is critical to have a monitoring and traceability system in place. Our monitoring system used here captures consumption and service-related data in the field. This collection process enforces data validation at time of entry. Compliance monitoring with configurable alerts also supports accurate data collection. Upon successful validation, the data is staged and entered into an analytics portal, where data can be further correlated with other external data sources. Output from the analytics engine includes automated reporting, chart generation and real-time dashboards.

The data reported here provide a compelling case for the inclusion of a solid fertility control product for rodents that is safe for all animals, effective at rodent population reductions, and poses minimum risk to applicators and the environment.

STUDY AREAS

Three studies were conducted, two in enclosed areas and one in a city block. These studies were classified as: **closed** (100% enclosure rodent exclusion), **open** (site with 0% rodent exclusion), and **semi-open** (site with perimeter barriers that are porous).

Initial Arena Field Study: Semi-closed to Migration

The first study began July 13, 2022 and concluded on January 30, 2023 (201 days) and was conducted in a semi-closed and isolated population of *Mus domesticus* at a mouse population greater than one individual per 5 sq ft. The enclosure contained breeding domestic rock pigeons, *Columba livia*, − life cycles described by Giunchi et al. 2020. The 1,912-sq-ft aviary is located in a 4,900-acre private sanctuary in Kanab, Utah and maintained by Best Friends Animal Society (Figure 1 A). The aviary was serviced daily where pigeon eggs were collected from nests to prevent expansion of the bird population (49 males and females). Prior rodent control consisted of live trapping and relocation of mice. Pigeon feed was open and available to both mice and birds. Our facility hosts, Best Friends Sanctuary, required a mouse population management tool that was non-lethal, solid, easy to deploy by maintenance staff, and capable of reducing mouse populations by 90-100%.

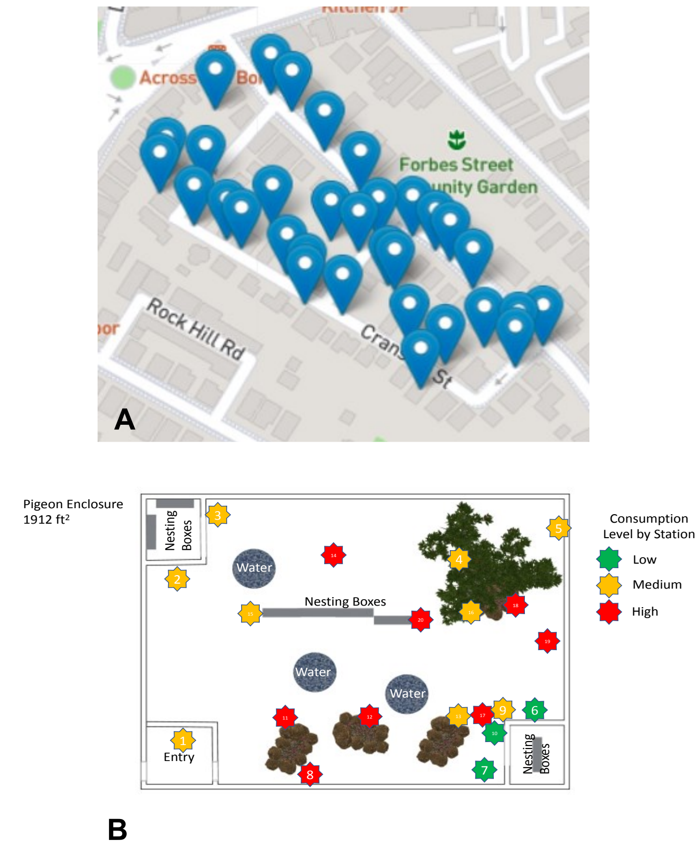

Figure 1.
Panel A is a representative drawing of the pigeon enclosure in the *initial arena study.* Utilizing a camera design application (CamToPlan, Tasmanic Editions) the pellet feeders (shown in three colors) are depicted within the enclosure including interior enrichment elements (rock piles, water basins, nesting boxes, and tree branches).
Panel B is a plot of all feeders deployed in the *open migration residential study.* A map of the residential area (Google Maps), Jamaica Plain in Boston, MA with the feeder location pins positioned by output from the analytics engine of the monitoring system.

The study design for this site involved establishment of a baseline rodent population at the study site compared to final population determination as described in Mayer et al. 2022. Feeding was to be maintained throughout the study once an efficacious active ingredient dose was determined. We utilized a repeated measures strategy comparison of final population estimates as indexed by feed consumption and camera image collections to baseline to determine treatment effect. To determine whether population reduction was a result of treatment vs death, rodent carcasses were collected and compared to life cycle population reduction for the target species. Further, reproductive tissue observations were made throughout the study, including population age ratios, to inform population reduction due to treatment.

Closed Migration Arena Field Study

The study began on August 2, 2023 and was conducted in a 1,200-square-foot building housing 4 separate aviaries and a central enclosed cage workroom for a variety of parrots. It was completely renovated with rodent exclusion modifications and was thus a closed system. An isolated population of *Mus domesticus* was present upon initiation of pellet feeding. The facility is located at the Best Friends Sanctuary described above.

The field experimental design for this site included a full rodent exclusion of the building allowing observation of fertility control-induced rodent population decline to zero. Rodent population was determined by a pellet consumption index, with feeders placed in each individual space within the total building to maintain a feeder density of 1/100 sq ft. Feed consumption was monitored weekly with the data portal as described in materials and methods.

Open Migration Residential Study

The study was conducted in a residential community consisting of 2 full city blocks and 4 adjacent block portions with 32 residential participants, and 36 feeders were placed (Figure 1 B). The community of Jamaica Plain is in Boston, MA.

The design for this study included a baseline assumption utilizing pellet consumption data from feeder acclimatization at peak consumption to subsequent periodic data points throughout the study to determine population decline. Following peak consumption, camera trapping can be employed to determine the percentages of non-target animal feeder visitations. Utilizing camera data. correlated with pellet consumption, estimates of rodent population decline are possible. Weekly site visits provide collection of feeding data, observations of dead rodents and non-target activity. To eliminate the variation of feeder-station servicing, all data collections were performed by Wisdom Good Works staff.

BAIT MATERIALS AND METHODS

Research Fertility Control Pellets

Research batches of rodent fertility control pellets contained 0.2% of the supplement *Tripterygium wilfordii* Hook F (TwHF) and >99% inert ingredients. TwHF is an herbal supplement available commercially, "Thunder God Vine Root Extract - 20:1 Concentration - *Tripterygium wilfordii* - 100 mg Vegan Capsules" (Shining Solace, Sheridan, WY).

The Thunder God Vine, Latin name *Tripterygium wilfordii Hook F* (TwHF), grows in mountainous regions of China. Extracts made from its leaves and roots have been used for centuries in Traditional Chinese Medicine (TCM) primarily to reduce inflammation (Gao et al. 2021). The pharmacologic "side effect" of active ingredients, specifically triptolide, is suppression of mammalian ovarian ovulation (Liu et al. 2011) and testes spermatogenesis (Lue et al. 1998). These contraceptive effects in rat, mouse, non-human primate, women and men are reversible (Chang et al. 2021). Importantly, the contraceptive concentrations of TwHF active ingredients are 10 to 15-fold less than the concentration needed for an effect on rheumatoid arthritis pain and other types of inflammation (Gao et al. 2021). TwHF active ingredients have a rapid onset but because TwHF active ingredients have a short half-life in rat circulation, <15 minutes, they do not accumulate in animal tissues and do not pose a risk of secondary exposure (Liu et al. 2015).

Pellet feed was provided in 100% renewable paper cartons (PlantCarton®, Pactiv Evergreen USA, Lake Forest, IL) enclosed in commercial rodent stations (Protecta EVO Express, Bell Laboratories Inc., Windsor, WI) and was provided *ad libitum.* Initial feeding to determine palatability used control material (Figure 2) without TwHF. An initial dose of 0.1% TwHF (Dose 1, Figure 2) was assessed for palatability and efficacy. Subsequently, a higher dose (Dose 2, Figure 2) of 0.2% TwHF was provided. Feeding stations were replenished as necessary to maintain continuous supply.

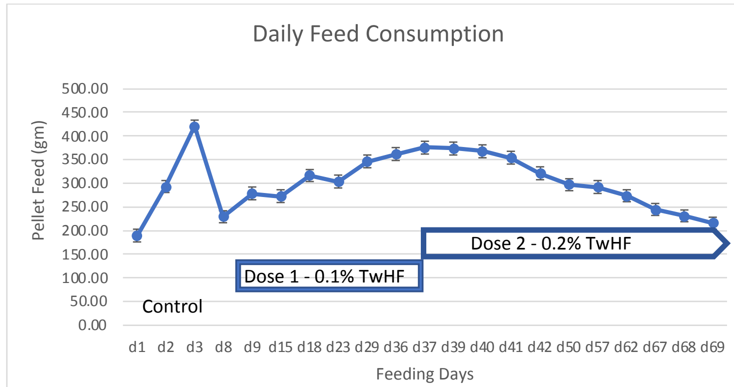

Figure 2. Pellet consumption in pigeon enclosure (Figure 1 B) measured daily (d1-d69) by weight. Initial feeding of control pellets without active ingredient TwHF was administered from d1-d8 (feeding stations n=20). Following initial control treatment, the initial low dose (0.1% TwHF by weight) was administered for 29 days (d8-d37) with no observable adverse effects. On d38, a higher dose (0.2% TwHF) was provided.

Camera data was collected utilizing field cameras (Touard, 20MP 1296P, Toguard Video Inc., Shenzhen, China). Cameras were placed at feeding locations at an optimal height of 61 cm and a distance of 183 cm from feeder entrances. Data image collection began at 18:00 and data were collected daily at 07:00 the following day. Each collection period consisted of 4 days. Each image recorded camera number, date, time of photo and ambient temperature with 3 camera photo bursts set 7 seconds apart to track rodent activity near the feeding stations. This series was followed by a 30-second reset period to avoid duplicate capture of the animal. Collections were performed every 6 weeks throughout the study.

Data Monitoring System

The data monitoring system consists of three modules: pellet consumption and service data collection, data analytics, and an administrative portal. The data monitoring system was used for the closed study (parrot enclosure) and the open study (Jamaica Plain, Boston, MA). Data captured in the collection module includes: the name of the service technician, name of the project, time of data collection, GPS coordinates of the feeder, GPS coordinates of the technician's location when collecting the data, name of the station along with its associated location group, percentage of pellets consumed since last service date, any comments by field technician, images, videos or files relevant to the visit, station location changes if applicable, notation of estimated or exact time, and whether or not a rodent fur sample was collected from this station. The data collection module also provides a summary of previous entries and a request form to order pellets.

The data analytics module enters this consumption data into a data analytics and ad-hoc query system. Features include the ability to: filter, sort and summarize on any data item, consolidate data or drill-down into selected groups, numerous charting options, scheduled PDF reports based on saved queries, real-time view of activities in the field, immediate notification of non-compliance with feeding program, and optional correlation with external data sources via Application Programming Interface (API). The administrative module provides user and security management, project location and feeder configuration, centralized access to all other modules, and oversight tools.

Animal Data Collection: Initial Arena Study

Population levels were determined initially with tracking plates deployed and analyzed as described in Connors et al. (2005) and correlated with consumption (Mayer et al. 2022). Daily feed consumption was estimated to be 3.0 gm/day (Quy et al. 2008). Pellets were weighed prior to deployment for each station and residual station contents weighed to determine the interval consumption amounts. A total of 20 stations were initially deployed in the 1,912 square foot facility (Figure 1 B). Total pellet consumption amounts for each station were used to determine daily average consumption. Stations were assigned an activity rank: Low (17-20 g), Medium (20-25 g) and High (25-33 g) (Figure 1 B).

Determination of population dynamics and reproductive capacity were captured during four trapping events (d41, d42, d43, and d95) using live traps (JT Eaton 420 CL (Twinsburg, OH), and Havahart 1020X-small Woodstream, Lancaster, PA) baited with peanut butter. Trapped animals were placed in a 200-ml glass beaker, weighed, photographed from the bottom, then released back into the enclosure. Testes size was ranked 1-5 using scrotal width adapted from Spears et al. (2013) with 1 indicating sexually immature and 5 sexually mature mice. Lactation was indicated where mammaries were enlarged. Juveniles were distinguished by weight, with juveniles weighing <12 gm and adults weighing >12 gm (Berry and Bronson 1992) (Table 1).

Pigeons were assessed daily for any signs of distress or abnormality. Eggs were collected daily by staff and numbers recorded. Egg laying for the rock pigeon consists of 1-2 eggs per month (OvoControl®, Innolytics LLC, La Quinta, CA). The enclosure consisted of nesting boxes, rock piles, and branches for roosting (Figure 1 B) on a solid cement floor with several inches of dirt cover.

Animal Data Collection: Enclosed Arena Field Study

Pellet consumption data was collected by staff using the web-based data portal as described above. The final TwHF dose used throughout the study was Dose 2, (Figure 3) of 0.2% TwHF. It was determined to be approximately 27 gm per station per day.

Camera trapping was performed as described on the date of initial pellet deployment. Large entry points in the pigeon enclosure were observed by staff; the enclosure was semi-closed with some rodent migration possible. The semi-closed pigeon enclosure provided a comparison between a site with 100% exclusion (parrott enclosure) and a semi-closed site (pigeon enclosure). During the second feeding in the pigeon enclosure feeder stations were reduced from 20 to 5.

Animal Data Collection: Open Migration Residential Study

Pellet consumption data was collected on a weekly basis utilizing the data portal described above. Thirty-three feeder stations were deployed across the site and image collections were made using 6 cameras as described in Materials and Methods. Additional camera trapping was performed on week 12 (October 23, 2023).

Statistics and Animal Welfare

Differences in population numbers were determined by one-way ANOVA with significance set at $p<0.05$. Pearson product moment correlation coefficient was used with time vs consumption.

All procedures followed the Animal Welfare Act guide for care and use of animals, and AVMA guidelines for anesthesia and handling. No animals were euthanized in this study and no animals underwent surgery. This study did not require an Institutional Animal Care and Use Committee approval.

RESULTS

In the initial semi-enclosed arena study, consumption observations of control feed in combination with tracking plate data indicated a palatable product (Figure 2). On the seventh evening of the project a flood of a portion of the facility disrupted feeding. Following restoration of the area, the feeding of "Dose 1", consisting of 0.1% TwHF by weight, began. On d29 of treatment, the dose was increased to "Dose 2" containing 0.2% TwHF by weight. On d40, the health of the mice was assessed during live trapping and no adverse observable effects were noted. By d201 consumption in the enclosure dropped by 99.987% (Figure 3).

Removals of drowned mice from pigeon water bowls were recorded daily by staff. Juvenile mice that enter the bowls are unable to get out and subsequently drown. The staff have used this observation as a measurement of the total mouse population in the enclosure. The average number of drowned mice indicated a positive correlation with consumption measurements. The average daily number of dead mice was recorded monthly: July (d13) 0.23, August (d44) 0.61 and September (d66) 0.18. These data are positively correlated ($r^2 = 0.927$) with population estimates from the consumption index. Additionally, staff collected eggs from the nesting boxes of the pigeons daily and recorded the number collected. The total number of eggs collected over 66 days (86) exceeded the average 75 expected for the rock pigeon during this time (Sauer et al. 2019). Routine veterinary exams over the same periods indicated no adverse health issues on pigeons, corroborating the positive egg collection data.

Table 1. Data for individual animal captures. Treatment day reflects the time (days) from feeding treatment to capture.

Collection Date	Treatment Day	n=	Gender Ratio Female:Male	Avgerage Weight (gm) Female	Average Weight (gm) Males	Juvenile	Adult	Lactating	Testes score
8/21/22	d41	4	(2):(2)	28	32.5	0	4	0	3.0
8/22/22	d42	5	(2):(3)	26.5	22.3	0	5	2	1.5
8/23/22	d43	8	(4):(4)	14	15	4	4	0	1.5
10/15/22	d95	28	(11):(17)	20.63	21.33	8	18	0	0.33
		45	42%:58%	22.28	22.78	27.9%	72%	4.4%	1.6

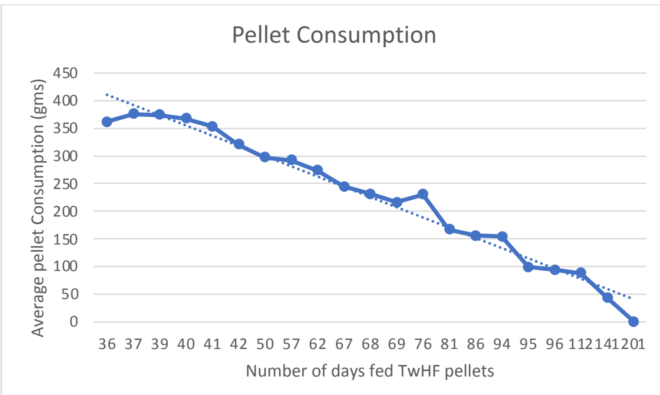

Figure 3. TwHF pellets (0.2% TwHF by weight) were provided continuously in feeders (n=20) for 164 days in the semi-enclosed pigeon arena study. Consumption was measured by weight on the days enumerated on the x axis. The average daily consumption was measured for each collection interval and represented on the y axis. The regression for consumption r^2=0.852.

In addition to good general health observations of trapped mice, we measured body weights, sex ratios, and maturity by sampling (n=45) of the population (Table 1). Average body weight of 21 grams was distributed over a range of 9-33 gm with the average male weight 22.78 gms compared to female weight of 22.28 gms, which are average body weights for wild *Mus domesticus* (Berry and Bronson 1992). The male to female ratio (58:42) further describes an average sex ratio for a wild mouse population (Szenczi et al. 2013). The juvenile population was 27.9% of the total sample, indicating a declining population (Berry and Bronson 1992).

Periodic consumption measurements were made for each station and a daily consumption average was calculated (Figure 3). The regression of consumption over time for d36 to d210 is $r^2 = 0.852$.

Results from this study indicate that the pellet feed is palatable and taken up by both male and female rodents. Population measurements in the form of reduced pellet consumption correlated with camera traps, reduced juveniles in the population, reduced evidence of lactation in females, and testes volume in males support the conclusion that continuous fertility control treatment (0.2% TwHF active ingredient) of rodents can significantly reduce wild rodent populations in an avian facility. Further, the fertility control treatment used as described here had no observable adverse effect on pigeons.

In the second field study (August 2023 - March 2024), data was collected and reported via web-based data portal as described in methods. In two enclosures described in Methods, we measured reduced consumption of the pellets by mice in both enclosures. Data from a continuation of the first study recommencing in August 2023 (eight months following the first study in July 2022) in the semi-open pigeon arena is captured in Figure 4, Panel A. During the eight-month interval between the first pigeon enclosure study and the second, no pellet feeding was done. Using the data portal that analyzed 422 independent data points, we measured a consumption of 73,351 gm of pellets over 7 months, with a peak in month 3 of 13,275 gms. In month 7 we measured 1,200 gms consumed, representing a 91% reduction over 7 months. In September we received an alert from the portal to check with staff on feeding frequency and found a gap in their feeding protocol which was quickly remedied.

We compared the data from the semi-open pigeon enclosure following the feeding gap to that of the closed parrot enclosure with full rodent exclusion during the same time frame. The data represented in Figure 4 B (parrot enclosure) shows a very different pattern of consumption to that observed in Figure 4 A, the pigeon enclosure. In the parrot enclosure we measured a total consumption of 3,300 gms of pellets with a 100% reduction achieved in 4 months. The peak consumption was measured in month 1 at 1,800 grams consumed from 6 feeding stations.

The results from the second study in the closed parrot enclosure indicated that without any immigration of rodents, population decline was more rapid (50% reduction in 30 days) than in a semi-closed pigeon site (50% population reduction in 210 days) and eradication appears to be possible with 100% exclusion.

The third study reported here commenced on August 1, 2023, and is ongoing. It represents a fully open rodent migration site described above (Figure 1 A). Within the residential community of Jamaica Plain in the city of Boston, we treated rodents over two city blocks with margins that involved 31 residential addresses and deployment of 33 feeding stations. The data collected utilizing the data portal is shown in Figure 5. Over the 7 months analyzed,

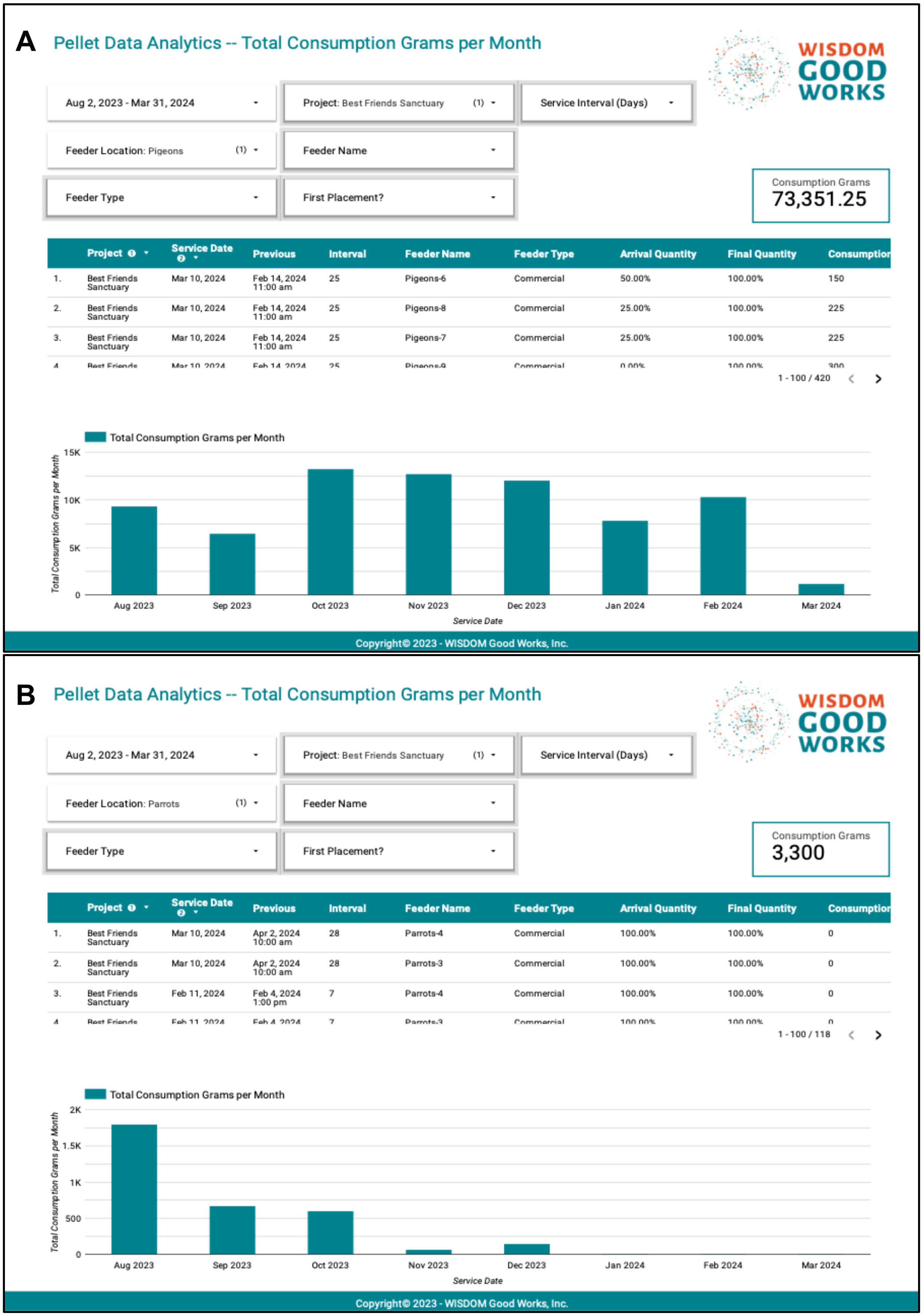

Figure 4. Output from the analytics portal (A) semi-open pigeon arena study and (B) enclosed arena field study. Each panel shows total pellet consumption by month for each study.

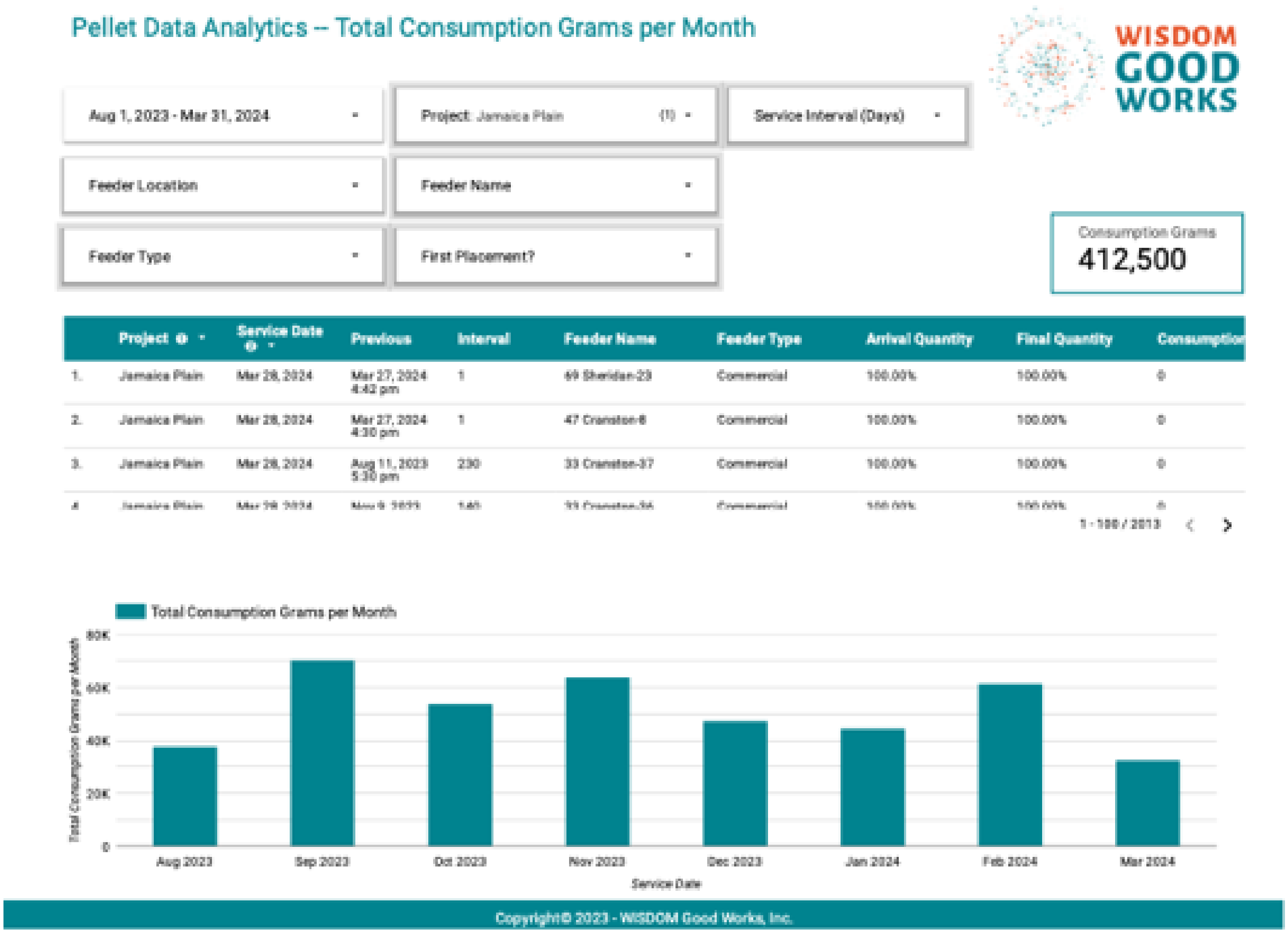

Figure 5. Output from the analytics portal for the *open migration residential study*. Total pellet consumption from 33 feeders is displayed by month (August 2023 - March 2024).

we measured a total consumption of 412,500 gms of pellets deployed in 33 feeding stations. Analyzing 3,984 individual data points we observed a peak consumption of 70,425 gms in the month of September, and a consumption reduction of 53.89% over 8 months. A complication of an open migration site is the presence of other non-target animals. Utilizing camera traps, n=6 over 4 nights, we were able to capture non-target feeding events. In 613 camera captures we measured 40% rats, 2% dogs, 2% birds, 8% squirrels, 33% raccoons, and 15% false triggers.

Results from this study indicated that in a site open to rodent migration such as a residential neighborhood, a rodent population reduction to 50% was achieved in 8 months. The consumption/population reduction data was more variable by month. Further, the data collection was confounded by non-target species feeding from the stations; this required many more camera collections.

DISCUSSION

There is no question among government agencies, wildlife managers, and public opinion that a humane, safe, efficacious, and cost-effective alternative to poison would be a superior approach for rodent management. Rodent fertility control has a high potential to meet this challenge as rodent fecundity is the root cause of continued infestations. The data presented here support further investigation of this potential. Reproduction is the root cause of sustained rodent infestations and targeting the birth rate of mice and rats using a traditional medicine approach, chemical intervention, or gene drive is the obvious pathway. Because gene drive strategies are still years away in research (Clark et al. 2024), and chemicals in use today are potential carcinogens (National Toxicology Program 2011) or toxins (Gadelha et al. 2014), an approach that is available and promising is use of a traditional Chinese medicine that has been available and used for centuries: TwHF.

TwHF mediated contraception occurs at much lower doses than for anti-inflammatory effects, resulting in significantly reduced risk of systemic toxicity. For many women and men who suffer from rheumatoid arthritis, continued use of TwHF for many weeks to several months is needed to gain relief from their symptoms (Zang et al. 2024). Meanwhile they are cautioned that they will likely be infertile, but once they stop using TwHF their fertility will be restored and healthy babies born (Chang et al. 2021). Reproductive "toxicity" is the activity we use to cause infertility in targeted rodent pest populations such as house mice, and brown and black rats.

The criteria for a rodent fertility control tool used in this study are: safe plant-derived solid pellets that encapsulate a contraceptive with a short half-life in the target animals and no adverse observable effects in non-target species; further documented efficacy; low cost; and ease of monitoring. This product could replace a commercially available fertility control that has limitations, such as a product that is liquid with a short environmental shelf life, requires a specifically designed tier 1 bait station delivery, and contains 4-vinylcyclohexene diepoxide, a known carcinogen (National Toxicology Program 2011).

An approach to practical application of a rodent fertility control strategy is to use the animals' natural adaptive characteristics. An oral approach to success is to provide an extremely palatable feed that is satisfying, out-competes common food sources, and is continually available. In the first study reported here, the daily pigeon food was available *ad libitum*. In the presence of the pigeon food, the mice continued to consume the pellets and thereby received sufficient doses of TwHF containing the fertility control active ingredients. We therefore provided continuous feed for the target animals, mice. As the study progressed, pigeon exclusion feeders were developed to prevent them from eating the pellets. Neither pigeon health nor fertility was negatively impacted from eating scattered pellets, with egg tracking for 80 days showing an average daily egg collection of 1.26 (pers. commun., BFAS staff).

Consumption increased commensurate with population estimates. Considering a growing population of mice, it is necessary to understand that with water, food, and habitat shelter, 90-100% of the female mice were pregnant at the start of the project, a normal reproductive rate (Berry and Bronson 1992). Applying this to our observations, and because the gestation period for mice is 18-22 days (Murray et al. 2010), it is reasonable to assume that the peak of the starting population decline would not be before d28-d39 of our study. Our data report the decline of population commenced on d29 of treatment.

Because continuous feeding of the pellets to the target population is essential, practical application monitoring of the consumption was developed for ease of use and tracking. With the data portal system reported above, we were able to visualize the fertility control management potential by comparing a completely closed system to a semi-open or open system. Our data confirmed our hypothesis that fertility control in closed settings would reach peak population consumption within the d28-d39 period (measured as d28), and that a decline in population (r^2 closed = .776 vs r^2 semi-open = .685) would then occur. In the semi-open arena, we reached peak consumption in 80 days. Further, in the closed arena the consumption and population (confirmed by staff) dropped to 0 and remains to the date of this publication. In the semi-open arena, we have reached a 90.9% depletion in 182 days from peak. These data argue for use on islands where rodent in-migration can be controlled.

The most challenging environment, from a rodent migration standpoint, is a completely open setting. We collected data from a residential setting where human food waste, free rodent migration, harborage, and water is freely available. As expected, feed acceptance was immediate and peak consumption was reached in one month. The decline in consumption was slower to progress, with a 53.9% reduction in 213 days. These data show population time-to-reduction in the open rodent migration study to be longer than in the semi-open study; the closed migration study showed the fastest decline in population after commencement of feeding. As with all rodent population reduction strategies, a solid integrated pest management strategy is essential, with exclusion as a major factor. Based on the presence of other non-target animals in this study area, i.e., raccoons, it is necessary to develop more exclusionary feeding stations. There were continued surveys by the residents and our staff with no evidence of observable adverse effects in the non-target animals by staff or visible in our camera traps.

The results presented here provide a strong case for continuation of the study of this pellet formulation. The reduction of rodent population in a balanced manner without the use of poison can improve our environment and safety for our wildlife and companion animals that are at risk of preying upon poisoned rodents. We suggest this single step forward can reduce the human-animal conflict.

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LITERATURE CITED

Bell Laboratories, Inc. 2023. State of the rodent control market. https://giecdn.blob.core.windows.net/fileuploads/document/2023/12/07/2023-rodent-som.pdf

Berry, R., and F. Bronson. 1992. Life history and bioeconomy of the house mouse. Biological Reviews of the Cambridge Philosophical Society 67(4):519-50.

British Columbia, Canada. 2021. SGAR use in British Columbia, https://www2.gov.bc.ca/gov/content/environment/pesticides-pest-management/legislation-consultation/rodenticide-ban#list

California AB1788. 2020. California Ecosystems Protection Act chapter 250, State of California Food and Agriculture Code §12978.7

California AB 2522. 2024. Pending Governor's Signature https://legiscan.com/CA/text/AB2552/id/2987234

Chang, Z., W. Qin, H. Zheng, K. Schegg, L. Han, X. Liu, Y. Wang, Z. Wang, H. McSwiggin, H. Peng, S. Yuan, J. Wu, Y. Wang, S. Zhu, Y. Jiang, H. Nie, Y. Tang, Y. Zhou, M. J. M. Hitchcock, Y. Tang, and W. Yang. 2021. Triptonide is a reversible non-hormonal male contraceptive agent in mice and non-human primates. Nature Communications 23;12(1): 1253.

Clark, A. C., A. Alexander, R. Edison, K. Esvelt, S. Kamau, L. Dutoit, J. Champer, S. E. Champer, P. W. Messer, and N. J. Gemmell. 2024. A framework for identifying fertility gene targets for mammalian pest control. Molecular Ecology Resources https://doi.org/10.1111/1755-0998.13901

Commonwealth of Massachusetts. 2021. House docket, No. 4206.

Connors, M., E. Schauber, A. Forbes, C. Jones, B. Goodwin, and R. Ostfeld. 2005. Use of track plates to quantify predation risk at small spatial scales. Journal of Mammalogy 86(5): 991-996.

Dyer, C. A., and L. P. Mayer. 2014. Sprague Dawley female rat consumption of a liquid bait containing vinylcyclohexene diepoxide and triptolide leads to subfertility. Proceedings of Vertebrate Pest Conference 26:390.

Gadelha, I., N. Fonseca, S. Oloris, M. Melo, and B. Soto-Blanco. 2014. Gossypol toxicity from cottonseed products. Scientific World Journal 2014:231635

Gao, J., Y. Zhang, X. Liu, X. Wu, L. Huang, and W. Gao. 2021. Triptolide: pharmacological spectrum, biosynthesis, chemical synthesis and derivatives. Theranostics 11(15):7199-7221.

Giunchi, D., N. Mucci, D. Bigi, C. Mengoni, and N. E. Baldaccini. 2020. Feral pigeon populations: their gene pool and links with local domestic breeds. Zoology (Jena) 142: 125817.

Hansen, S. C., C. Stolter, C. Imholt, and J. Jacob. 2016. Plant secondary metabolites as rodent repellents: a systematic review. Journal of Chemical Ecology 42(9):970-983.

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

Liu, L, J. Zhang, Z. Wang, D. Xu, Z. Jiang, T. Wang, W. Ju, and L. Zhang. 2015. Gender differences in the toxicokinetics of triptolide after single- and multiple-dose administration in rats. Drug Resistence 65(11):602-606.

Lue, Y, A. Sinha 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(4):479-486.

Massei, G., J. Jacob, and L. Hinds. 2024 Developing fertility control for rodents: a framework for researchers and practitioners. Integrative Zoology 19:87-107.

Mayer, L. P., C. G. Knox, C. A. Dyer, T. K. Ohmar, and T. Barnes. 2022. The economic, social and political impact of the California Ecosystems Protection Act. Proceedings of Vertebrate Pest Conference 30:30.

Murray, S. A., J. L. Morgan, C. Kane, Y. Sharma, C. S. Heffner, J. Lake, and L. R. Donahue. 2010. Mouse gestation length is genetically determined. PLoS One. https://doi.org/10.1371/journal.pone.0012418

National Toxicology Program. 2011. 4-Vinyl-1-cyclohexene diepoxide. Report on Carcinogens 12:436-437.

New York City Council. 2024. Establish a rat contraceptive pilot program, Int 0736-2024 https://legistar.council.nyc.gov/LegislationDetail.aspx?ID=6632239&GUID=F286354E-D685-47A7-B3C4-983AAC4AB6C7

Quinn, N., and C. Swift. 2018. What do we need to know to assess individual and population-level effects on wildlife from anticoagulant rodenticides? Proceedings of Vertebrate Pest Conference 28:235-242.

Quy, R. J., R. W. Watkins, M. S. Lamber, and D. P. Cowan. 2008. Validating census methods to measure changes in house mouse populations. Pest Management Science 65(3): 275-282.

Rahelinirina, S., K. Scobie, B. Ramasindrazana, V. Andrianaivo-arimanana, F. Rasoamalala, L. N. Randriantseheno, J. S. Rakotoniaina, O. Gorgé, X. Lambin, E. Valade, S. Telfer, and M. Rajerison. 2021. Rodent control to fight plague: field assessment of methods based on rat density reduction. Integrative Zoology 16(6):868-895.

Rhode Island. 2024. Senate Bill 2745 https://legiscan.com/RI/bill/S2745/2024

Sauer, J., D. Niven, J. Hines, D. Ziolkowski, K. Pardieck, J. Fallon, and W. Link. 2019. The North American breeding bird survey, results and analysis 1966-2019 Version 2.07 .2019. USGS Patuxent Wildlife Research Center, Laurel, MD.

Spears, J., A. Matthews, and S. Hartwig. 2013. Evaluation of noninvasive methods of predicting testicular weight and development in an inbred and outbred strain of laboratory mouse. Theriogenology 79(3):566-573.

State of Connecticut. General Assembly. 2022. Senate File No. 241 March 28, 2023. Section 22a-50 general statutes.

Szenczi, P., O. Bánszegi, Z. Groó, and V. Altbäcker. 2013. Anogenital distance and condition as predictors of litter sex ratio in two mouse species: a study of the house mouse (*Mus musculus*) and mound-building mouse (*Mus spicilegus*). PLoS One 8(9):e74066

Tripathi, R. 2014. Integrated management of rodent pests. Pages 419-459 *in* P. A. Dharam, editor. Integrated pest management: current concepts and ecological perspective. Academic Press Elsevier, Cambridge, MA.

Zhang X., J. Xia, Y. Jiang, D. S. Pisetsky, J. S. Smolen, R. Mu, S. Dai, M. E. Weinblatt, T. K. Kvien, J. Li, T. Dörner, Y. Zhang, L. Lu, C. Yang, P. Yang, Y. Zhang, C. Xi. Z. Zhao, and P. E. Lipsky. 2024. International consensus guidance for the use of *Tripterygium wilfordii* Hook F in the treatment of active rheumatoid arthritis. Journal of Immunology 142: 103148.