



# ZEUTERIN™/ ESTERILSOL™



May 2016

## Product Profile and Position Paper

Zeuterin™/EsterilSol™ is a non-surgical sterilant for male dogs delivered via intratesticular injection. The active ingredient is zinc gluconate neutralized with arginine. The formulation causes permanent sterility in one treatment. The process of neutering with Zeuterin/EsterilSol is also known as “zinc neutering.”

# ZEUTERIN™/ESTERILSOL™

NON-SURGICAL STERILANT FOR MALE DOGS

## INTRODUCTION

This Product Profile and Position Paper was created from a review of both published and unpublished literature; promotional information; and input from experienced users, representatives of relevant organizations, and related experts. ACC&D did not conduct the referenced clinical research; data quoted were obtained from these external sources.

This formulation of the chemical compound zinc gluconate neutralized with arginine was developed to chemically sterilize male dogs. The formulation was initially developed by Pet Healthcare International; it received approval from the U.S. Food & Drug Administration (FDA) in 2003 and was distributed in the U.S. under the name Neutersol® until early 2005. Ark Sciences, Inc., subsequently acquired rights to the technology and registered it under the name EsterilSol™ in Mexico, Colombia, Bolivia, Panama, and Turkey.<sup>1</sup> On February 17, 2014, Ark Sciences launched Zeuterin™ in the U.S.<sup>2</sup> The product is presently approved for male dogs between three and ten months of age in the U.S., and for male dogs over three months of age in other countries. Unfortunately, at the present time EsterilSol is not commercially available in any country. As of this writing and due to company restructuring and distributor and regulatory hurdles, Zeuterin is not available for purchase in the U.S.

Zeuterin and EsterilSol have the same chemical composition and are derived from natural ingredients: zinc, glucose and arginine. The product, delivered by intratesticular injection, causes permanent infertility in a single treatment without removal of the testicles. Light sedation is recommended; anesthesia is not necessary. Zeuterin/EsterilSol can offer savings of time, money, and procedure and recovery space for veterinary clinics, animal shelters, and pet sterilization programs. Additionally, it provides an option for pet owners who seek a less-invasive method of sterilization, who want their dogs' testicles to remain intact, or whose pets cannot safely be anesthetized. It may also be useful in settings in which surgery is difficult to safely perform. As with any medical intervention, safety and effectiveness depend upon proper administration. To help ensure proper administration, veterinary professionals must be trained by Ark Sciences prior to purchasing product.

## MECHANISM OF ACTION

The exact mechanism of action of Zeuterin/EsterilSol is not known. The product is administered as an intratesticular injection into the center of the testicle via the dorsal cranial portion of testicle, parallel to the longitudinal axis. After injection, the compound diffuses in all directions from the center of the testis. In the concentration used, zinc gluconate acts as a spermicide and destroys spermatozoa in all stages of development and maturation. Zinc gluconate is absorbed and metabolized by the body within 72 hours after the injection. As the dog's body increases blood flow and creates inflammation to heal, it results in permanent and irreversible fibrosis in the seminiferous tubules, rete testis, and epididymis. This process results in permanent sterilization, and the endocrine feedback system remains intact.

Within one to three days after injection, sperm production is shut down. However, because sperm produced before the procedure can remain in the vas deferens and epididymis for up to 30 days, Ark Sciences states that a sexually mature zinc neutered dog should be separated from female dogs in heat for up to 60 days following zinc neutering (versus 21 days following vasectomy or castration).

Following injection, the testicles atrophy over a period of time ranging from weeks to months, resulting in a reduction in testicular size and changes in shape or texture. These changes may or may not be symmetrical.

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<sup>1</sup> Ark Sciences currently owns all rights and intellectual property. Neutersol, which was developed by Pet Healthcare International, was introduced in the U.S. in 2003 by Addison Laboratories. In 2005, production and distribution were discontinued after a business divorce between Pet Healthcare International and Addison Laboratories. Pet Healthcare International no longer has any rights to this technology.

<sup>2</sup> For multiple years prior to the February 2014 launch, Zeuterin was used in the U.S. in a post-FDA, pre-commercial launch capacity.

## ZEUTERIN OR “ZINC NEUTERING” IN THE U.S.

Ark Sciences launched Zeuterin commercially in the U.S. in February 2014. The original formulation was submitted to and approved by the US FDA for sterilization of male dogs from three to ten months of age. ACC&D understands that the decision to focus on this age range was based on cost constraints. Ark Sciences reports that it has since submitted paperwork to the FDA to extend registration to male dogs over three months old; however, approval has not yet been granted.<sup>3</sup> Zeuterin has regulatory approval for dogs with testicular widths of 10.0 to 27.0mm. However, ACC&D is familiar with veterinarians who have used the product in dogs with testicular widths of up to 31.0mm, and is not aware of instances where this has compromised the product’s efficacy or safety. Ark Sciences reports that data is available showing that sterility can be achieved in testicles up to 31.0mm, although to ACC&D’s knowledge this has not been publically released. The label allows for use “by or on the order of a licensed veterinarian.”

The initial focus of the US launch was the nonprofit sector, with the goal of high volume sales to neuter shelter dogs; pricing was—and still is—lower for nonprofit entities. In Fall 2015, Ark Sciences promoted Zeuterin with a special discount. For nonprofits, vials were sold for \$60 per 3.0ml vial, but additional free vials available with each purchase lowered the net price to \$25 per vial. For for-profit entities, vials were sold for \$90 per 3.0ml vial; additional free vials with each purchase lowered the net price/vial to as low as \$50 for those who purchased in bulk. Ark Sciences reports that on average, 1.0ml will sterilize one dog (the range, according the dosing scale, is 0.4ml to 2.0ml per dog, depending on his testicle size). With this promotional pricing, the per-dog cost of Zeuterin for a nonprofit could be as low as \$3.33 to \$16.67, depending on testicle size; the per-dog product cost for a for-profit entity could range from \$6.67 to \$33.33. ACC&D asked several U.S. veterinarians who currently use Zeuterin to approximate the cost of additional materials (syringes, needles, sedation, NSAIDs, tattoo supplies, gauze, chlorhexadine for disinfection, and reversal). These veterinarians estimated between \$5 and \$15 per zinc neuter, depending on type of sedation used and weight of the dog.

As noted above, due to company restructuring and distributor and regulatory hurdles, Zeuterin is not presently available for purchase. Future product cost may differ from prices listed above.

## ESTERILSOL INTERNATIONALLY

Ark Sciences introduced EsterilSol in Mexico in 2008 and began selling the product to private practice veterinarians, government programs, and non-governmental organizations. In 2010 EsterilSol received regulatory approval in Bolivia, Panama, and Colombia; the product was subsequently approved in Turkey. In these countries, EsterilSol is approved for use in dogs three months of age and older. In Colombia it is also approved for use in cats. International distribution was halted due to Ark Sciences’ use of a new U.S.-based manufacturer to produce the product (see below for further details), and anticipated international pricing information is not currently available. In some countries in which EsterilSol is not approved by regulatory agencies, it has been used on a limited basis in field research with government approval.

### *ACC&D Field Studies*

In 2009-2010, ACC&D supported several EsterilSol field projects and studies. The grants sought to help organizations extend reach of their population control programs and gather data about product use in the field. More information is available at <http://www.acc-d.org/available-products/sponsored-field-studies>.

## USE AND EFFECTIVENESS

Zeuterin/EsterilSol is administered via an injection to each testicle with either a 28 gauge, 3/4-inch or a 30 gauge, 1/2-inch needle, depending on testicle size. Dosage is determined by measuring each testicle with a caliper provided by Ark Sciences. The correct dose is indicated on the caliper and is based on the maximum width of each testicle. As noted above, Zeuterin/EsterilSol is labeled for use in dogs with an individual testicular width of 10.0 to 27.0mm, although the product has been used in dogs with testicular widths of up to 31.0mm, and ACC&D is unaware of any adverse impacts on safety or efficacy with these larger animals. Some dogs’ testicles will not fall into this range.

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<sup>3</sup> Data submitted for the extension include a field study, which included 53 sexually mature male dogs. In addition, the post-FDA approval study in the U.S. included more than 3,000 dogs, a proportion of which were over 10 months of age; Ark Sciences has collected data on all zinc neuters performed during this post-approval period.

Experienced practitioners report that the process of measuring the testicular width to determine dose, preparing the injections, and administering the injection into each testicle takes two to five minutes. Proper administration is critical to reduce the risk of complications (see below section).

As noted above, Zeuterin is currently produced in 3.0ml vials; an individual dog receives 0.4–2.0ml of product, depending on his testicle size. Zeuterin has no preservatives and must be discarded 10 hours after opening. This combination of factors leads some veterinary professionals to recommend consolidating zinc neuters to maximize cost effectiveness.

Anesthesia is not necessary for the zinc neutering procedure. Ark Sciences recommends light sedation to ensure that the dog holds still during the injection; reversible sedation is commonly used so that dogs are awake and alert in as little as 15-20 minutes after the injection. Most programs with which ACC&D is familiar routinely sedate dogs (to varying degrees, depending on the dog and the situation) prior to injection.

Many people without experience using this technology express concern about its potential to cause pain. In studies reviewed and accepted by the FDA (in which 76% of dogs were not sedated), only 2.5% of dogs showed discomfort by moving or vocalizing. The other 97.5% did not show any reaction to the injection. Scrotal pain was the most common local reaction observed in the FDA-reviewed study, reported in 6.3% of dogs. To prevent discomfort caused by post-injection swelling, Ark Sciences recommends using a non-steroidal anti-inflammatory drug (NSAID).

In the clinical trials presented to the FDA, this formulation was found to cause permanent sterility in 99.6% of treated dogs. Pre-pubescent males never become fertile. Post-pubescent males become infertile within 30 days after treatment.

## TESTOSTERONE, BEHAVIOR, AND HEALTH

To date, two studies have evaluated the effect of zinc neutering on testosterone levels. These studies indicate that, particularly long-term, Zeuterin/EsterilSol does not reduce testosterone to the same extent as surgical castration. When reviewing and interpreting the results of these studies, however, it is imperative to keep in mind that testosterone levels naturally vary significantly between dogs, and even within individual animals, over time.

One study submitted to the FDA measured the effect of Zeuterin on male beagles (aged 6 months at the start of the study) in a clinical setting. At two years post-treatment, mean serum testosterone levels for dogs who received varying quantities of zinc gluconate neutralized with arginine were between 41.0 and 52.0% lower than mean serum testosterone levels of dogs in the control group. At the same time, the majority of dogs' (31 of 40) testosterone levels remained in the same range as the control dogs, the latter of whose natural testosterone levels varied widely.

A second study included dogs of varied breed mixes, sizes, and ages in Chilean Patagonia. Led by Veterinarians Without Borders/Vétérinaires Sans Frontières-Canada (VSB/VSF), it compared testosterone levels of dogs who were surgically castrated, zinc neutered, and intact. Most EsterilSol-treated dogs remained similar to their baseline testosterone levels four and six months post-treatment, but the study also found that four months after injection, 22% of dogs had testosterone levels comparable to the surgically castrated cohort. Six months after injection, 31% of dogs had testosterone levels comparable to the surgically castrated dogs. The study concluded at six months post-treatment. It is important to note that testosterone levels following zinc neutering cannot be equated with fertility; even dogs with higher testosterone were unable to reproduce.

The effect of maintaining higher levels of testosterone on hormone-dependent behaviors and diseases has not been established. Some persons seek behavioral changes commonly associated with surgical castration, and thus view a lesser impact on testosterone production as a drawback. Other pet owners cite health and behavior benefits from retaining some level of circulating testosterone. It is important to remember that while surgical castration has been promoted as a treatment for behavior problems, it has only been shown to be linked to decreases in indoor urine marking, roaming, sexual mounting, and dog-to-dog aggression around females in estrus. Moreover, it does not always decrease these behaviors. Although more is known about the non-reproductive (behavior and health) effects of surgical castration than zinc neutering, further study on both methods is merited.

In conjunction with its study of testosterone, and with support from ACC&D, VSB/VSF collaborated with dog population control, behavior, and veterinary epidemiology experts to objectively evaluate behavior in free-roaming dogs before and after surgical and zinc neutering; findings were compared to dogs left intact. The study evaluated such behaviors

as activity level, aggression, sexual activity, home range, interaction with people, and reactivity. Of the chemically sterilized dogs, no significant changes in courtship behavior, sexual activity or roaming range were noted post-treatment. A statistically significant increase in aggressive behaviors toward other dogs was noted post-treatment, though the authors caution that this was with a small sample size. The authors also note that causes of aggression in dogs are variable and not well understood, and that findings concerning behavior of free-roaming dog populations do not necessarily translate to that of indoor dogs. Additional studies are needed to provide a more complete picture of what behavior changes may be expected with either sterilization method in different populations

With regard to health, surgically castrated dogs have been found to have lower risk for some health problems but higher risk for others. Recent studies have contributed data on health considerations for specific breeds, but these findings should not be extrapolated to all breeds. There is no conclusive evidence that surgical castration makes male dogs healthier overall.

Dogs sterilized with either Zeuterin/EsterilSol or surgical castration may continue to engage in mating behavior. Therefore, neither method of sterilization should be expected to prevent the spread of transmissible venereal tumors (TVT's). (Male dogs surgically castrated prior to puberty display significantly less mating behavior than those castrated as adults. Comparable data on Zeuterin/EsterilSol is not available.)

## IDENTIFYING ZINC NEUTERED DOGS

As with surgically spayed female dogs, visual confirmation of sterility may be difficult in Zeuterin/EsterilSol-treated dogs. Although testicles atrophy over time, the decrease in size is variable. Palpation of testicles may reveal an abnormal contour and/or texture resulting from scar tissue; however, other conditions, such as the zoonotic disease *Brucella canis*, also cause atrophy and scarring of the testes. Given the likely difficulty of identifying a dog who has been zinc neutered, he should be identified as sterilized with Zeuterin/EsterilSol. Ark Sciences provides identification tags and recommends a "Z" tattoo on the abdomen near the inner thigh. Some programs with which ACC&D is familiar have also used tattoos indicating sterilization status on the interior of the ear. Alternately, or in addition, a microchip may be used; microchip companies will add information on neuter status to a dog's record if this information is provided when pet owners register their pet's microchip.

While these methods of identifying dogs as sterilized are likely sufficient for owned animals, ACC&D recognizes that they have limitations for free-roaming populations. The latter cohort may lack a collar or resist the human handling necessary to view a tattoo. This has prompted ACC&D to initiate a project to develop an improved method for identifying non- surgically sterilized dogs and cats.<sup>4</sup>

## COMPLICATIONS

During FDA-required Target Animal Safety work, this formulation of zinc gluconate caused no deaths and was determined to be safe. In the past decade (and on a more limited basis for the past several decades), thousands of male dogs have been successfully neutered with this zinc gluconate formulation with no reports of any long-term detrimental effects.

One of Zeuterin's greatest appeals is that it does not require general anesthesia or a surgical incision, completely removing risk factors (the most serious of which is death) associated with surgery. (One U.S. study of anesthesia-related deaths found the risk for dogs to be 1 in 1,849.) However, any medical intervention presents risks, and environment, individual animal physiology and health status, and/or other factors might also influence the rate of adverse reactions among zinc-neutered animals.

Given the possibility of adverse reactions, ACC&D does not recommend using zinc neutering for dogs without a human caretaker or possibility of post-treatment observation. We emphasize the need for at minimum a community caretaker to observe the animal and report any post-treatment adverse reactions to a veterinarian able to provide appropriate veterinary care. We would recommend the same for surgically sterilized dogs.

Complications reported with Zeuterin/EsterilSol use include—but are not limited to—the following:

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<sup>4</sup> For more information on this initiative, please visit <http://www.acc-d.org/research-innovation/acc-d-flagship-initiatives/flagship-initiative-marking-and-id>

- **Testicular swelling and pain.** Mild to moderate testicular swelling is to be expected and peaks 24-48 hours post-injection. Follow-up veterinary consultation is indicated for dogs displaying severe swelling. Mild to moderate pain is also to be expected and peaks 48-72 hours post-injection. Ark Sciences recommends administering an injectable non-steroidal anti-inflammatory drug (NSAID) at the time of treatment to minimize post-injection discomfort.
- **Vomiting.** In one study, 12 out of 270 dogs (4.4%) vomited one to four times within four hours post-injection. Ark Sciences notes that vomiting is more likely to occur if xylazine is used as a sedative. Fasting for 12 hours before the injection is recommended to decrease the chance of vomiting.
- **Injection site reactions.** In some cases, adverse reactions may occur at the injection site. Reactions range from scaly scrotal skin to ulceration and (rarely) tissue necrosis requiring scrotal ablation (surgical removal of the scrotal tissue and contents). Adverse reactions are believed to be primarily due to imprecise injection technique or failing to follow the recommended administration protocol in other ways (e.g. incorrect needle size, use of product from a vial that was first punctured more than ten hours prior).<sup>5</sup> Serious reactions may occur when a dog chews or licks his scrotum due to irritation caused by a mild reaction. In order to prevent adverse reactions, it is critical that practitioners follow injection technique and all administration protocol precisely.

The rate of injection-site reactions requiring follow-up treatment ranged from 0.7% to 3.9% in early studies. Incidence decreased substantially as better practices (including administering a light sedative to provide chemical restraint during injection and using separate needles for drawing up and injecting the solution) were implemented after the initial introduction. Reports from a large field trial in Mexico showed an average complication rate of 1.2%. In Peru in 2010, 249 dogs were treated and only three adverse reactions (1.2%) were reported. However, in Colombia and the Dominican Republic in 2010, higher rates of injection site reactions were reported. (The number of dogs in these two projects was too small for a percentage of reactions to be statistically reliable.)

It is important that dogs have appropriate after-care with access to a veterinarian if any problems arise. A veterinarian should know how to perform a scrotal ablation, which may be required in a worst-case scenario. Ark Sciences' Client Information Sheet recommends that dogs be prevented from licking or biting the scrotum. (Of 270 dogs that participated in the clinical trial reviewed by the FDA, two developed serious scrotal ulcerations. In both cases these reactions were associated with the dogs chewing the scrotum in the days following their injection. Ark Sciences recommends use of an Elizabethan collar for dogs observed licking or chewing the scrotum.)

The [Client Information Sheet](#) also advises that a dog's exercise be limited to leash walks for several days, and that he not be kept on hard or wet surfaces following the procedure. Dog caregivers are asked to contact the administering veterinary professional if they see "any redness, discharge, or broken skin in the scrotal area" in order to immediately address any complications. After sedation, dogs are less able to regulate their body temperature and should be kept in a comfortable environment (usually inside) for the first night after treatment. Because of the need to control and monitor dogs post-injection, use on dogs that do not have owners or caregivers that can and will comply with guidelines may result in more frequent and/or more severe adverse reactions.

### **What about other species?**

In the past, this formulation was used off-label to sterilize other species, including cats, bears, wolves, goats and other livestock. As noted above, EsterilSol was approved for use in cats in Colombia. Ark Sciences reports that it plans to seek U.S. regulatory approval for use in cats in the future. However, no published or otherwise publically available studies establish dosage, safety, or efficacy in cats (or any species other than dogs).

Zeuterin/EsterilSol presents a less invasive neutering method for cats. Time savings may differ compared to dogs because surgically castrating cats is a simpler and faster procedure than surgically castrating dogs. The impact of Zeuterin/EsterilSol on cat behavior (e.g. urine marking, vocalizing, fighting with other cats) has not been established. Data on what behavior changes can be expected will help guide pet owners, veterinarians, animal welfare organizations, and those managing feral and free-roaming cat populations.

<sup>5</sup>Zeuterin/EsterilSol does not contain any preservatives. Each 3 ml vial holds enough product to treat more than one dog. Ark Sciences recommends that unused product be discarded within ten hours after the first puncture of the vial to prevent potential product contamination, which may lead to adverse reactions.

- **Other complications.** Complications in formal research studies, reports to the FDA, and anecdotal reports have included vocalization (indicating pain), neutrophilia, anorexia, diarrhea, leukocytosis, lethargy, muscle tremor, hypertension, and tachycardia. The package insert should be referenced for details.

(A description and discussion of adverse reactions observed in the FDA-reviewed clinical trial are available through the Freedom of Information Act. The report describing the FDA-reviewed studies is available on ACC&D's website at <http://www.acc-d.org/available-products/zeuterin-esterilol>).

## CONCLUSION

Zeuterin/EsterilSol is a safe, effective method for sterilizing male dogs without surgery. Though sedation is recommended and administration protocol requires precision and care, this method may offer savings in cost, time, and facility requirements, thus helping animal welfare organizations sterilize more animals and/or redirect resources to other lifesaving projects. It also presents an option for pet owners who would prefer to sterilize their dog without surgery, or whose pets cannot safely undergo anesthesia. It is critical that a dog be monitored by an owner/caretaker in the days following treatment and have access to professional veterinary care, if necessary. Zeuterin/EsterilSol has regulatory approval in the US, Mexico, Colombia, Panama, Bolivia, and Turkey; however, as discussed above, sales are suspended due to company restructuring.

## RECOMMENDATIONS AND DISCUSSION

ACC&D believes that Zeuterin/EsterilSol can be a meaningful tool for organizations to reach further in their sterilization programs, and for veterinarians to offer options to clients. It may offer cost savings relative to surgical castration. The shorter procedure and recovery time required for zinc neutering may provide a significant advantage to organizations with space limitations, allowing them to sterilize more animals. Savings in cost, time, and space will vary depending on an organization's infrastructure and efficiencies achieved with surgical sterilization.

ACC&D recognizes that sterilizing males alone may have limited impact on population size unless a very high rate is achieved. Female reproduction must be controlled to achieve population stability or reduction. However, fertility control is part of a comprehensive approach to manage dog populations and improve animal welfare in any community. Population control programs around the world provide sterilization for both females and males as part of their work, and the ability to spend less time and money on male sterilization may free up resources to sterilize more females.

ACC&D realizes that administration protocol and injection technique are critical to reducing the incidence of adverse reactions. Ark Sciences currently requires all practitioners to receive hands-on training, a practice ACC&D supports. We urge all practitioners to follow administration protocol exactly. As with surgical castration, systems should be put in place to help dog owners and caregivers provide after-care for treated dogs and to receive immediate follow-up care for any adverse reactions that occur. In a worst-case scenario, this could require a scrotal ablation. Minor reactions can become severe if prompt care is not sought or received. Dog owners should adhere to all follow-up care instructions carefully and contact their veterinarian with any questions or concerns. Use in dogs without owners or caregivers who can provide the recommended monitoring and care post-treatment (as may be the case for unowned or loosely-owned dogs) may result in a higher rate of and more severe adverse reactions. Organizations that are able to follow up directly with dogs in their home (or, for unowned dogs, their home territory) for several days post-injection may be able to use Zeuterin/EsterilSol on these dogs more safely.

Most pet owners, veterinarians, and animal welfare organizations are accustomed to the risks and benefits of surgical sterilization. While many are enthusiastic about the concept of alternative non-surgical methods of sterilization, specific new approaches can be met with caution and some skepticism. ACC&D feels strongly that any new sterilization method should be introduced with careful thought and adequate resources for education so that potential users can determine the best use of new non-surgical methods. ACC&D encourages veterinarians, animal welfare organizations, and dog caregivers/owners to consider new sterilization methods with an open mind, to explore the characteristics of any method, and to consider how alternate methods might help them achieve their goals.

As with any medical intervention, ACC&D encourages all pet owners, veterinarians, and organizations to consider the associated risks and benefits and to engage in open discussion with other stakeholders (i.e. family members, practice partners, organizational staff/volunteers) about potential use.

When treating un-owned or loosely owned free-roaming dog populations as part of TNR (Trap/Neuter/Return) or ABC (Animal Birth Control) programs, both surgery and Zeuterin/EsterilSol treatment present risks for the individual animal, especially when dogs are released soon after the procedure with limited or no supervision. Organizations need to weigh the risks and benefits for both individual animals and the population as a whole. With Zeuterin/EsterilSol, the potential with for mild adverse injection site reactions to progress to serious reactions in dogs that are not properly supervised should be considered carefully. ACC&D recommends use be limited to dogs with owners/caregivers that can provide appropriate care.

Additional information is available at <http://www.acc-d.org/available-products/zeuterin-esterilsol>. Questions and comments can be directed to [info@acc-d.org](mailto:info@acc-d.org). Ark Sciences' web address is [www.arksciences.com](http://www.arksciences.com).



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