



**Registration of the Contraceptive ZonaStat-H, for  
Population Control of Wild and Feral Horses and Burros**

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# **Registration of the New Active Ingredient Porcine Zona Pellucida Formulated as a Contraceptive, “ZonaStat-H,” to Aid in the Control of Wild and Feral Horse and Burro Populations**

## **Regulatory Rationale**

The U.S. Environmental Protection Agency (hereon referred to as EPA or the Agency) is granting the first registration of a pesticide product containing the active ingredient porcine zona pellucida (PZP).

Wild horses and burros inhabiting private and state-owned lands are the responsibility of state agencies which have to address issues involving these animals when conflicts arise. Federal agencies such as the Department of Interior (DOI), the U.S. Forest Service (USFS), Bureau of Land Management (BLM), and the Department of Defense (DOD) manage lands that support wild horses and burros. The Free-Roaming Wild Horse and Burro Act gives BLM the authority for managing wild horse and burro populations on BLM land. BLM also works with the USFS to manage these populations on national forests land. The National Park Service (NPS) considers wild horses and burros to be an “exotic species” or “feral livestock” and manages herds found on Park lands.

Like other animals (e.g. deer, Canada geese, etc.), horses may be pests in some situations. Expanding populations of feral horses are adversely affecting public and private lands by over-grazing and competing with native populations of animals for food. Wild horse and burro populations that are not managed have the potential to reach densities that damage land and wildlife habitat; conflicts with livestock growers and recreational users may also arise. For example, wild horses on Assateague Island can destabilize and reduce the height of the dunes that protect the island by trampling and grazing intensively on dune grass and salt meadow hay. Additionally, wild horses feeding on western rangelands can overlap with those of cattle, sheep, and elk, and can result in direct competition with these animals for food. Since wild burros inhabit more arid regions, their impacts are seen close to water sources. Wild burros are seen as a threat to natural resources such as the desert tortoise and desert bighorn sheep.

## **I. Chemical Information**

**Chemical Name:** Porcine Zona Pellucida (PZP)

**EPA PC Code:** 176603

**CAS Number:** None

Porcine zona pellucida is a complex of four different acidic glycoproteins, ZP1 (80,000-90,000KD), ZP2 (60,000 – 65,000 KD), ZP3 (55,000 KD), and ZP4 (20,000 – 25,000 KD). PZP consists of approximately 70 – 80% ZP3. To obtain the PZP antigen, porcine ovaries are collected from freshly slaughtered female pigs at USDA-inspected slaughterhouses, and immediately frozen. Oocytes are collected, washed, and then homogenized in a buffered salt

solution. Then the zonae are heat-solubilized in a phosphate buffer solution (PBS) then diluted. The resulting zona pellucida solution is frozen until use. To create the end use product, ZonaStat-H, 100 µg PZP antigen is dissolved in 0.5 mL PBS, which is then emulsified in the field by the applicator in 0.5 mL Modified Freund's Complete Adjuvant (mFCA) or Modified Freund's Incomplete Adjuvant (mFIA).

**Mode of Action:** PZP antigen is the glycoprotein layer that surrounds the oocyte and is weakly antigenic by itself. Therefore, PZP is emulsified with an adjuvant (mFCA for the primary vaccination and mFIA for booster vaccinations) which stimulates a stronger immune response. This results in the creation of anti-zona peullucida antibodies which bind to the zona pellucida of the oocyte, alter their conformation, and block sperm attachment to the zona pellucida receptors.

**Registrant:** Humane Society of the United States (HSUS)

**Proposed Product:** The proposed ZonaStat-H product (EPA File Symbol 86833-R), is an injectable immunocontraceptive vaccine for use only on female feral and wild horses and burros. As a Restricted Use Pesticide (RUP), ZonaStat-H can only be administered by certified applicators, or by persons under the direct supervision of a certified applicator. ZonaStat-H is injected into the target animals by one of the following three methods: hand deliver, jab-stick delivery, and remote (dart) delivery.

## II. Human Health Risk

A summary of the human health effects and risk of PZP as assessed in the Agency document entitled "*ZONASTAT-H. Immunocontraceptive Vaccine for Limiting the Population of Wild and Feral Horses and Burros*" dated July 8, 2010, is provided below.

The HSUS submitted waiver requests for the toxicity studies ordinarily required for a terrestrial, non-food use pesticide. The waiver requests were granted due to lack of toxicity on the target animal; a history of safe use of the vaccine, explained further below; the mode of action and fate of the product's metabolites; the limited opportunity of exposure to non-target animals, applicators, and the public; and lack of immunotoxicity as shown in the published scientific literature.

Under the sponsorship of the National Park Service (NPS), testing of ZonaStat-H began in 1988 on wild horses at Assateague Island National Seashore (ASIS) and it has subsequently been tested on white-tailed deer, zoo animals, African elephants, and other animals. Between 1994 and 2007, 901 female horses were darted with PZP by two applicators without any incident or report of harm to the applicators. Since 2004, ZonaStat-H has been administered to an estimated 1800 western wild horses on 47 herd management areas by researchers and Bureau of Land Management personnel. Also, 136 zoos have administered ZonaStat-H to captive animals with no reports of harm or adverse effects to applicators.

The basic biology and properties of ZonaStat-H, i.e., its mechanism of action - ZonaStat-H contains porcine zona pellucida antigen (the glycoprotein layer surrounding the oocyte) and an



adjuvant resulting in the creation of anti-zona pellucida antibodies which bind to the zona pellucida of the oocyte and block sperm attachment to zona pellucida receptors - and the nature and fate of the product's metabolites, do not suggest that the product has the potential to be toxic or pathogenic. This is further supported by information that shows that once the product is ingested, it is broken down to amino acids and simple carbohydrates which do not cause an immune response and are biologically inactive. Additionally, PZP and the adjuvant antigens are not stored in body tissues.

**a. Toxicological End Points**

No toxicological end points were established.

**b. Dietary Exposure**

The Agency has determined that neither a tolerance nor a tolerance exemption is necessary for this active ingredient at this time. In the past, horse slaughter plants existed in the United States and some horse meat was used for animal feed and human food (though virtually all meat was exported). EPA does not believe there are any horse slaughter plants currently operating in the United States. Because of this and the BLM assertion that no horses or burros will be sold to slaughter houses, the Agency is confident that treated animals will not be used as food or feed.

The Agency has also determined that this use of PZP would not result in residues in treated animals. Once the contraceptive is injected into the animal, both components of the contraceptive are detected by the humoral immune system and are broken down into resulting products that bear no resemblance to the original contraceptive and are excreted and eliminated from the body in forms that cannot be distinguished from other metabolic products, such as CO<sub>2</sub>, water, lactic acid, and urea. Likewise, the antibodies that are produced in response to ZonaStat-H injection are broken down into their component amino acids, and recycled into other body proteins or metabolized and excreted as urea, CO<sub>2</sub>, and water.

**c. Occupational Risk**

Applicators could potentially be exposed to ZonaStat-H by dermal or ocular routes while loading a syringe or by accidental self-injection. There are no occupational concerns as a result of potential dermal or ocular exposure because PZP is a weak antigen and is unlikely to be absorbed intact for the same reason described in the Dietary Exposure section.

Handler Exposure and Risk: Accidental self-injection could result in infertility in females, with no reproductive effect on males. Though public literature indicates that the contraceptive effects of PZP treatment administered annually for up to 5 years to horses are reversible (Kirkpatrick and Turner, 2002), there are no data available for humans exposed to PZP. There is evidence that annual treatment of horses for longer than 7 years results in irreversible infertility, but it is unlikely that accidental self-injection would occur routinely. The ZonaStat-H label includes the statements: "If pregnant, take precaution when preparing, loading and recovering darts to not self-inject. Accidental injection may cause infertility in women."

A physical injury could occur as a result of self-injection, especially if there was tissue trauma from a dart gun. The likelihood of accidental self-injection will be minimized because the product is classified as a Restricted Use Pesticide used only by trained certified applicators or persons under their direct supervision (see Section IV). Applicators are required to wear latex or vinyl examination gloves when handling the product and during all operations in which accidental dermal exposure could occur, including washing of mixing syringes.

Occupational Post-Application Exposure and Risk: There is the possibility of post application exposure through contact with an undischarged dart. Dart recovery data of 329 different horses treated with PZP are available for 3 sites – Assateague Island National Seashore, MD where 1,185 darts were fired in which 1,115 were recovered from 1994-2007; Cape Lookout National Seashore, NC, fired 313 darts and recovered 301 for the years 2001-2007; and 146 darts were fired at Little Book Cliffs Wild Horse Range, CO, with 140 recovered for the years 2003-2007.

While individuals using the dart guns reportedly made every effort to retrieve darts whether they struck the target or not, approximately 5% of the darts were not recovered (as reported above). Some of the darts that missed the horse would have discharged upon striking the ground or surrounding brush resulting in degradation of the glycoprotein into the environment. Therefore, it is believed that only a small amount of unrecovered darts would have retained their contents. Even if an unretrieved dart still retained its contents, exposure to humans or the environment is unlikely because a significant impact is required with enough velocity to result in discharge of the dart contents.

#### **d. Residential Risk**

A residential risk assessment was not conducted because there is no residential use associated with this product.

### **III. Environmental Risk**

A summary of the environmental fate and ecological risks of PZP as assessed in the Agency document titled “*Section 3 Request for ZonaStat, a New Chemical Proposed for Use to Control Wild Horses and Burros*” dated October 4, 2010 is provided below.

Waiver requests were submitted to fulfill the required ecological effects and environmental fate guideline studies. For the reasons listed below, the waivers were granted.

Exposure to non-target organisms is not likely to occur because of the targeted nature of the application. Given the lack of potential exposures, it is unnecessary to generate most of the data generally required for outdoor uses. The Agency determined that these studies are not considered necessary to support the proposed uses of PZP for several reasons:



Due to the application route of injection to the target animal, potential exposure routes for non-target organisms resulting from labeled uses is limited. Exposure to carnivores, exposure to excreted material, and exposure to off-target darts are the potential routes of exposure identified by the Agency and these pathways are unlikely to result in potential risks to non-target organisms at levels of concern to the Agency. Since PZP is deactivated in the digestive tract and absorption from the GI tract is expected to be limited, dietary exposure to ZonaStat is not expected to result in adverse effects at levels of concern to the Agency. Additionally, the short half-life in treated mammals suggests that the potential for secondary exposure to carnivores or scavengers is limited. PZP is not excreted intact from treated animals; it breaks down into amino acids and simple carbohydrates. Off-target, intact darts result in exposure to PZP if the non target contacts the dart with enough impact that the contents are injected. However, it is important to note that PZP has a short shelf life of only 24 hours when removed from frozen storage.

There is very limited potential for water contamination through the use of this product as the product is not expected to be excreted intact from treated animals. The only exposure to aquatic ecosystems would be through darts that missed their target. A dart contains 100 µg of active ingredient and even if the contents of a dart were to enter a small, 20,000,000 liter pond the resulting concentration would be 0.005 ng/L.

Potential exposures to non-target animals are not expected to result in any significant risk concerns to terrestrial or aquatic organisms from the proposed use of PZP.

#### **IV. Regulatory Decision**

Consistent with the requirements of FIFRA section 3(c)(5), EPA is unconditionally registering the product ZonaStat-H, containing porcine zona pellucida to be used as a contraceptive for wild horses and burros. Pursuant to the provisions of section 3(c)(4) of FIFRA, the Agency published a Notice of Receipt (NOR) of the registration application in the *Federal Register* on January 27, 2010 (Docket No. EPA-HQ-OPP-2009-0800). One anonymous comment was submitted in response to the NOR, it did not present any new information or data, but implied that equines on BLM managed lands treated with PZP could end up in the food chain and questioned EPA's jurisdictional regulatory oversight on contraceptive vaccines. The BLM has asserted on their website

[http://www.blm.gov/wo/st/en/prog/wild\\_horse\\_and\\_burro/wh\\_b\\_information\\_center/Fact\\_Sheet.html](http://www.blm.gov/wo/st/en/prog/wild_horse_and_burro/wh_b_information_center/Fact_Sheet.html)

that "The BLM has not been selling any wild horses or burros to slaughterhouses or to "killer buyers." During the comment period on the proposed registration decision for ZonaStat-H, one comment was submitted that expressed support for its registration. The commenter is developing another contraceptive for horses. In addition to supporting the registration, he also offered suggestions and critiques regarding the Training Manual. These comments will be passed on to the HSUS for their consideration.

##### **A. Data Requirements**

No additional data is required to support the proposed registration.

## B. Labeling Requirements

The following requirements have been imposed:

- Restricted-Use Pesticide classification limiting application to Department of Interior, and all its designated agents (i.e., National Park Service, Bureau of Land Management, U.S. Fish & Wildlife Service); State departments of agriculture/livestock and wildlife, and their designated agents; Federally recognized Indian tribes, and their designated agents; Department of Defense and its designated agents; Public and private wild horse sanctuaries and reserves; Humane Society of the United States designated agents; USDA and all its designated agents (i.e., U.S. Forest Service, Animal and Plant Health Inspection Service).
- Use limited to only two animals: Wild and feral horses (*Eqqus caballus*) and feral burros (*Eqqus asinus*).
- Label statement restricting the application of ZonaStat-H to horses or burros that will not be used as food or feed.
- Personal Protective Equipment requirements include: long sleeved shirt and long pants, gloves and shoes plus socks to mitigate occupational exposure.
- Advisory statement for female applicators: “If pregnant, take precaution when preparing, loading and recovering darts to not self-inject. Accidental injection may cause infertility in women.”