UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: July 8, 2010

SUBJECT: ZONASTAT-H. Immunocontraceptive Vaccine for Limiting the Population of Wild and Feral Horses and Burros.

PC Code: 176603
Decision No.: 420440
Petition No.: N/A
Risk Assessment Type: Non-food use
TXR No.: N/A
MRID No.: N/A

DP Barcode: D370377
Registration No.: N/A
Regulatory Action: Section 3
Case No.: N/A
CAS No.: N/A
40 CFR:N/A

Ver.Apr. 2010

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HED

1. CONCLUSIONS

HED has no objections to the Section 3 registration of ZonaStat-H. There are no occupational or postapplication concerns for human health risk because of the very limited potential for human exposure. It is recommended that ZonaStat-H be administered by hand injection when possible because of the slightly increased occurrence of abscesses when using a dart rifle.

II. BACKGROUND

The Humane Society of the United States has applied for a Section 3 registration for ZonaStat-H. ZonaStat-H is an injectable immunocontraceptive vaccine and is to be used by certified applicators only. The registrant intends its use on wild horses and burros.

III. DISCUSSION

Ingredients: ZonaStat-H contains porcine zona pellucida antigen (the glycoprotein layer surrounding the oocyte) and an adjuvant. The adjuvant is used to stimulate a more robust immune response because porcine zona pellucida (PZP) is weakly antigenic by itself. Modified Freund's Complete Adjuvant (mFCA) is used for primary vaccination and Freund's Incomplete Adjuvant (FIA) is used for booster vaccinations.

Ovaries are collected from freshly slaughtered pigs at USDA inspected slaughterhouses and frozen. Screening for bacterial pathogens is conducted for each batch. The oocytes are isolated and zona pellucidae are collected, diluted, frozen, and protein concentration is determined by electrophoresis.

Mode of Action: Vaccination causes the production of anti-zona pellucida antibodies, which bind to the zona pellucida of the oocyte and block sperm attachment to zona pellucida receptors.

Treatment of Horses and Burros: The antigen and adjuvant are mixed shortly before injection using two glass syringes connected by a luer lock. Intramuscular injection is made into the hip or gluteus muscles. The registrant proposes delivery by hand-held syringe, jabstick, or by a syringe dart fired by a blow-pipe, CO_2-powered gun, or .22 caliber dart rifle.

The priming dose of PZP with mFCA is followed in 2 – 4 weeks by a booster of PZP with FIA. Annual boosters are of PZP with FIA. Contraceptive efficacy was found to be greatest when the booster is delivered 1 – 3 months before the beginning of the breeding season. A single priming dose is also effective at a reduced level when delivered 1 – 3 months before the breeding season.

Guideline Testing: The registrant submitted waiver requests for the subchronic, developmental, reproductive, genotoxicity, neurotoxicity, and immuntoxicity studies ordinarily required of a terrestrial, non-food use pesticide. There are currently no guideline requirements specific for testing contraceptives in wildlife.

The waiver request are granted based on the lack of toxicity to the target animal; history of safe use of the vaccine; 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.

There are numerous published journal articles on the use of PZP antigen in horses in the package provided by the registrant as well as found in a literature search. These included reports from Assateague Island National Seashore, Maryland; Shackleford Banks, North Carolina; Elko and

Ely Districts of Nevada Bureau of Land Management; Little Book Cliffs Wild Horse Range, Colorado; McCullough Peaks Herd Management Area, Wyoming; Pryor Mountain Wild Horse Range, Wyoming; an un-named Northern California wild horse sanctuary; and Virgin Islands National Park, St. John (burros).

The articles provide an extensive literature on efficacy, safety in target animals, mode of action, and describe management options for use of the vaccine. The articles were generally well written and described the methods and limitations of making observations on wild, free-roaming horses. However, the articles were not intended for regulatory purposes and sometimes different articles emphasized different aspects, such as safety or efficacy, in the same herds over overlapping time periods which sometimes made interpretation difficult for this risk assessment.

As noted earlier, 2 different adjuvants are used with PZP in ZonaStat-H in the proposed registration. The adjuvant used in the primary vaccination is Modified Freund's Complete Adjuvant (**mFCA**) and the adjuvant used in subsequent boosters is Freund's Incomplete Adjuvant (**FIA**).

The early studies used Freund's Complete Adjuvant (**FCA**) in the initial injection instead of mFCA. Beginning in 2002, mFCA was substituted for FCA for the initial injection, as is currently proposed for registration. Because generally similar results were reported regardless of which adjuvant was used for initial vaccination, and because there are so much data for the earlier studies, this risk assessment reports results from studies using both priming adjuvants.

Dietary Exposure: The potential for human dietary exposure through consumption of horse meat is assessed because there used to be horse slaughter plants in this country. It is concluded that there would be little likelihood of human systemic exposure to PZP through dietary exposure because PZP is a glycoprotein which is too large to pass through membranes of the digestive tract intact. Digestion into component amino acids and simple sugars in the stomach and small intestine would occur before absorption. Even if intact PZP were somehow to be absorbed, it is weakly antigenic and requires an adjuvant to stimulate an immune response when injected.

This was confirmed in a study in which rabbits were fed PZP: 4 NZW rabbits per group were treated orally with 400 μg PZP + S-TDCM adjuvant and 4 were treated with adjuvant alone. This compares to 100 μg PZP per dose of ZonaStat-H. ELISA analysis showed that rabbits did not develop circulating anti-PZP IgG antibodies when tested at dilutions of 1:10 to 1:1000. The number of embryos and stage of embryos in treated animals was not affected when compared to controls (Barber and Fayrer-Hosken, 2000).

Occupational Exposure: Applicators could potentially be exposed to ZonaStat-H by dermal or ocular routes while loading a syringe or by accidental self-injection. There are few concerns for dermal or ocular exposure because PZP a weak antigen and is unlikely to be absorbed intact for the same reasons as described in the Dietary section above.

Accidental self-injection could result in the same effects in humans as occur in horses, *i.e.* infertility. A physical injury could also 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 intended for use as a restricted pesticide used only by certified applicators. According to the registrant, ZonaStat-H has been administered to approximately 2,700 horses by dart, hand injection, or jab stick without reported injuries by the applicators (MRID 47859806). A summary of training requirements for applicators is shown in the Appendix.

Postapplication Exposure: There is the possibility of postapplication exposure through contact with a dart which had not discharged. Dart recovery records are available for 3 sites (MRID 47859806). At Assateague Island National Seashore, Maryland, for the years 1994 – 2007, there were 1,185 darts fired of which 1,115 were recovered. At Cape Lookout National Seashore, North Carolina, there were 313 darts fired and 301 recovered for the years 2001 – 2007. At Little Book Cliffs Wild Horse Range, Colorado, there were 146 darts fired and 140 recovered for the years 2003 – 2007.

Individuals using the dart guns reportedly made every effort to retrieve darts whether they struck the target or not. But as reported above, approximately 5% of darts were not recovered when used in different types of terrain: beaches/dunes/forest/marsh in Maryland and North Carolina and canyon/plateau/ forest/grassland in Colorado. It was not reported how many of the darts struck their target and had discharged the vaccine, but it is believed likely that the majority of darts struck their target and had discharged the contents appropriately. Of the darts which missed the horse, some would have discharged the contents upon striking brush or the ground. Degradation of the glycoprotein in the environment would then occur with no concerns for exposure by this scenario.

It is therefore believed that only a small percentage of unrecovered darts would have retained the contents. Human or environmental exposure to vaccine in these darts is unlikely because discharge requires a significant impact with sufficient velocity to set off the charge releasing the contents. According to the registrant, "Striking, stepping on, jiggling, biting, or otherwise casually moving or contacting the dart will not discharge or release the contents of the dart (MRID 47859806).

Safety to Horses: The articles evaluated safety in horses as related to injection site reactions, longevity and body condition, developmental/reproductive effects, and behavioral effects.

Longevity and body condition: Treatment of mares at Assateague Island National Seashore was associated with a greatly increased lifespan. In the study group, there were 42 untreated mares which lived an average of 6 years, 11 mares treated < 3 years lived an average of 10 years, and 19 mares treated for ≥ 3 years lived an average of 19 years (Kirkpatrick and Turner, 2007).

The greatly increased lifespan in treated mares is believed due to the reduced physiological stresses of gestation and lactation. Body condition scores for mares were consistently lower for lactating mares than non-lactating mares (Turner and Kirkpatrick, 2002 and Ransom, et al, 2010).

Injection site reactions: Nodules (~25 mm in diameter) were reported commonly after injection of either PZP/mFCA or PZP/FIA after darting. Abscesses were relatively rare, but were slightly

more common in horses that were darted than in horses that were hand injected. Also reported were swelling and stiffness.

There were opportunities for long-term observations of the horses on Assateague Island, some of which were acclimated to humans. There may have been fewer opportunities for long-term observations of free-roaming horses in the western states, although these horses were sometimes kept in a holding pen after injection for a long enough time for close observation for lesions. The authors of the various studies did a generally good job of describing limitations of the studies and opportunities for observation.

At Assateague Island, there were 3 abscesses after 381 treatments by dart gun or jab stick (0.7%), 2 of which occurred after use of FCA and 1 after use of FIA (Kirkpatrick, et al, 1990 and Lyda, et al, 2005). In a study in Nevada, no abscesses were observed after hand injection of 60 wild mares using PZP/FCA and PZP/FIA (Turner, et al, 1997). Also reported for Nevada mares (Turner, et al, 2001), no abscesses were observed after 155 mares received 2 injections hand injections each (PZP/FCA and PZP/FIA, some also received Carbopol® adjuvant).

Another study in Nevada compared injection site reactions with two adjuvants using hand injection. The initial injection was with PZP/FCA for 7 mares and was PZP/mFCA for 8 mares. The booster for both groups was PZP/FIA. The only injection site reaction was an abscess which followed booster injection with FIA and healed without incident (Lyda, et al, 2005).

One article compared type of injection site reaction with methods of injections (hand injection, CO_2 blowgun, or .22 caliber dart rifle) and adjuvant (FCA, mFCA, FIA). Two herds in Wyoming and one in Colorado were assessed (Roelle and Ransom, 2009). Reactions following hand injection were rare: out of 100 hand injections there was 1 nodule and 2 observations of swelling. In the 2 herds that were darted, 25% of the horses had nodules (both herds), 11% and 33% had swelling, 1% and 12% had stiffness, and 1% and 6% had abscesses. Nodules were the most common reaction and sometimes persisted for a year, but did not cause noticeable change in range of movement or locomotion. Abscesses were too rare for analysis of covariates; and 4 of the 8 observed abscesses occurred in a single mare. There was no relationship between type of adjuvant and injection site reactions, suggesting that reactions are more associated with trauma from dart delivery rather than adjuvant alone.

Behavioral effects: The social behavior of horses treated with PZP was evaluated in several studies. There were only minor effects noted, as described below.

The behavior of 43 mares on Assateague Island National Seashore was observed for 3 months during the 1997 breeding season. Mares were either being currently treated with PZP or had previously been treated with PZP; untreated controls were not available. There were no significant differences between currently treated and previously treated Assateague mares in regard to activity budgets, although there was a trend for currently treated mares to spend more time in social behavior. Treatment did not affect spatial relationships between mares and stallions, social rank, or rates of aggression given or received (Powell, 1999).

The behavior of 30 mares in 13 harem groups on Shackelford Banks, North Carolina was observed during the non-breeding season. Mares were in various treatment statuses or had been untreated. Contracepted mares changed groups more often than untreated mares, visited more groups than untreated mares, and exhibited more reproductive interest. For both contracepted and untreated mares, the number of group changes and number of groups visited decreased with the number of years that mares were pregnant (Nuñez, et al, 2009).

The behavior of PZP-treated and untreated mares in 3 herds in Wyoming and Colorado were observed from April to October each year from 2003 – 2006. Treated mares received more reproductive behavior from stallions than untreated mares. Body condition was the strongest predictor of feeding, resting, maintenance, and social behaviors. Nursing mares had lower body condition than mares without a foal and there was no difference in body condition between treated and untreated mares (Ransom, et al, 2010).

Developmental and reproductive effects: Mares returned to fertility after discontinuation of PZP booster vaccinations, when treated for < 7 years; mares treated for 7 years did not return to fertility. Foals which were *in utero* at the time of treatment, matured, and gave birth to normal foals, as described below.

There are numerous reports detailing the contraceptive efficacy of PZP vaccination over the years. Initial studies used a priming injection of PZP/FCA while more recent studies used mFCA adjuvant as is used in the current registration. A study compared antibody titers from use of PZP/FCA with PZP/mFCA (Lyda, et al, 2005). It was found that PZP/mFCA had higher titers, although not statistically significant, than did PZP/FCA, indicating that PZP/mFCA should be as efficacious as the PZP/FCA adjuvant used in the earlier studies.

Analysis of fecal and urinary hormones has been used to monitor estrous cyclicity. Treatment for a single year did not appear to disrupt ovarian function, and fertility returned after discontinuation of treatment once antibody titers had fallen (Liu, et al, 1989). Ovulation rates and urinary estrogens declined with increasing years of treatment (Kirkpatrick, et al, 1995). For mares treated for 1, 2, or 3 years, the return to fertility was 100%, 100%, and 69%, respectively (n=53). For mares treated for 4 or 5 years (n=5), the return to fertility was 100%. No mares treated for 7 years returned to fertility (n=5). It took a longer time for mares to return to fertility the more years that they had been treated (Kirkpatrick and Turner, 2002).

In another study, fecal hormones from Assateague mares were monitored for 2 years. Mares were either being currently treated with PZP or had previously been treated with PZP; untreated controls were not available. All mares showed some evidence of cyclicity, but there was ovulatory failure (increased total estrogen excretion that was not followed by an increase in luteal protestagen) in both currently treated mares (2/3) and previously treated mares (3/9). The study authors concluded that the anovulatory state was episodic with variable durations (Powell and Monfort, 2001).

The incidence of seasonal births (April, May, and June) was calculated for PZP-treated foals on Assateague Island National Seashore for the years 1990 – 2002 (Kirkpatrick and Turner, 2003). Fecal and urinary hormones were monitored to determine pregnancy status in order to detect

early neonatal loss. The incidence of foals born in season was 76% for untreated mares (69/91) compared to 65% for treated mares (50/77). For mares foaling within 1 year of treatment (ineffective contraception), 69% of foals were born in season (20/29). For mares treated for longer than 2 years and then withdrawn from treatment, 62% foaled in season (30/48). Differences between treated groups and untreated mares were not statistically significant.

Mares which were vaccinated while pregnant have foaled normally and their foals, if untreated, have in turn foaled normally (Kirkpatrick and Turner, 2002). This is probably because there is not significant passage of maternal antibodies through the equine placenta.

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APPENDIX

Training Requirements from MIRD 47859806:

Application of ZonaStat-H is restricted to trained applicators. Applicators will be instructed in specific safety precautions to prevent accidental dermal or ocular exposure or needle stick. Precautions required of applicators include:

1. "One-hand" insertion of needle into adjuvant vial and replacement of plastic safety cover over needle;
2. Proper disposal of used needles and darts in sharps containers;
3. Proper disposal of syringes in clearly marked "Biohazard" bags;
4. Use of high-quality glass syringes to prevent breakage;
5. Wearing of latex or vinyl examination gloves during all operations in which accidental dermal exposure could occur, including washing of mixing syringes; and
6. Washing site of needles stick or cut with soapy water and disinfection of wound with alcohol or other disinfectant or antiseptic.