United States
Environmental Protection Agency
Office of Chemical Safety and Pollution Prevention
(7505P)

Pesticide Fact Sheet

Name of Chemical:	**Porcine Zona Pellucida (PZP)**
Reason for Issuance:	**New Chemical Nonfood Use**
Date Issued:	**January 2012**

1. Description of Chemical

Glycoprotein Complex:	ZP1 (80,000-90,000 KD), ZP2 (60,000-65,000 KD), ZP3 (55,000 KD), and ZP4 (20,000 – 25,000 KD)
Common Name:	Porcine Zona Pellucida (PZP)
EPA PC Code:	176603
Chemical Class:	Contraceptive
Registration Status:	New Chemical, nonfood use
Pesticide Type:	Mammalian Contraceptive
U.S. Technical Registrant:	Humane Society of the United States 2100 L St. NW Washington, DC 20037

2. Use Patterns and Formulations

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 pellucida antibodies which bind to the zona pellucida of the oocyte, alter their conformation, and block sperm attachment to the zona pellucida receptors.

Application Sites: ZonaStat-H will be used to control female wild and feral horse and burros privately or publicly owned, in areas where they have become a nuisance and are capable of doing environmental damage.

Methods of Application: The vaccine will be injected intramuscularly in hip or gluteus muscles by hand-held syringe, syringe mounted on a jabstick, or by syringe dart fired from a CO_2 or cartridge-powered projection system.

Application Rate: The application rate is 1.0 cc of PZP + adjuvant (modified Freund's complete adjuvant for the initial application, then modified Freund's incomplete adjuvant for follow-up applications). A second administration is given 2 to 4 weeks after the initial priming dose, then annually thereafter.

3. Science Findings

Available product chemistry data supporting the use of ZonaStat-H including product chemistry, toxicology, efficacy, and ecological effects and environmental fate are summarized below in Tables 1 and 1.1.

Table 1. Product Chemistry Summary

Common name	Porcine Zona Pellucida (PZP)
Color	Clear
Physical State	Active: Aqueous solution or powder EU: Thick, white aqueous emulsion
Odor	Odorless
Oxidation/Reduction Action	Denatured by acid or base, no incompatibility
pH	7.0 – 7.04
Flammability	Nonflammable (protein)
Explodability	Not explosive (protein)
Storage stability	Frozen liquid (or powder in desiccant) is viable for 2 years.
Corrosion Characteristics	No corrosive activity.

TOXICOLOGY SUMMARY

The Registrant submitted waiver requests for the acute oral, acute dermal, acute inhalation, primary eye irritation, primary dermal irritation, and dermal sensitization studies. The waiver requests were reviewed and determined to be acceptable.

Table 2. Acute Toxicity Data

GUIDELINE NO.	STUDY TYPE	MRID NO.	RESULTS	TOXICITY CATEGORY
870.1100	Acute Oral Toxicity	47859803	Waived	IV
870.1200	Acute Dermal Toxicity	47859803	Waived	III
870.1300	Acute Inhalation Toxicity	47859803	Waived	III
870.2400	Acute eye irritation	47859803	Waived	III
870.2500	Primary skin irritation	47859803	Waived	III
870.2600	Dermal sensitization	47859803	Waived	Negative

- Toxicity Category III = Precautionary Statements Required

Chronic toxicity data were not submitted. There is no human exposure from use of ZonaStat-H, therefore no toxicity endpoints were selected because of the very limited potential worker and dietary exposure.

ECOLOGICAL EFFECTS

Waivers were submitted to fulfill required ecological effects and environmental fate guideline studies for the registration of ZonaStat-H because of the limited potential for environmental releases. Since the product is labeled only for injection to target animals by hand or dart, is expected to be deactivated in the digestive tract, and has a short half-life in treated mammals, the limited potential risks to non-target organisms resulting from the proposed registration of ZonaStat-H are not expected to exceed the Agency's concern levels.

Risk to Endangered Species

The following table summarizes the conclusions of potential concerns for direct and indirect effects to federally-listed threatened and endangered species (listed species). Because the proposed uses cannot be geographically limited, all federally listed species are assumed to be potentially indirectly affected. The available data suggest that potential exposures to non-target animals is not expected to result in any significant risk concerns to terrestrial or aquatic organisms from the proposed use. However, indirect effects (potentially beneficial or negative) to Listed species could not be precluded.

Table 3. Potential Effects to Federally Listed Taxa

Listed Taxa	Direct Effects	Indirect Effects
Terrestrial and semi-aquatic plants – monocots and dicots	No	Yes
Terrestrial invertebrates	No	Yes
Birds (surrogate for terrestrial-phase amphibians and reptiles)	No	Yes
Mammals	No	Yes
Aquatic vascular plants	No	Yes
Aquatic non-vascular plants	No	Yes
Freshwater fish (surrogate for aquatic-phase amphibians)	No	Yes
Freshwater invertebrates	No	Yes
Freshwater benthic invertebrates	No	Yes
Estuarine/Marine fish	No	Yes
Estuarine/Marine crustaceans	No	Yes
Estuarine/Marine mollusks	No	Yes

EFFICACY

As ZonaStat-H does not bear claims to control pests that may pose a threat to human health, pursuant to OPPTS 810.1000(b)(2), the requirement for demonstration of efficacy is waived. In lieu of efficacy studies, the registrant provided various peer-reviewed published articles demonstrating ZonaStat's efficacy as a contraceptive for wild horses and burros.

The principle of efficacy of PZP in horses was first demonstrated by Liu et al. (1989) by inhibiting fertility in 12 of 14 captive fertile domestic and wild mares (*Eqqus caballus*), which persisted for 7 months. The researchers inoculated the mares with 4 hand injections of PZP with aluminum hydroxide gel. As the aluminum hydroxide gel was found to be only moderately effective in most of the horses, it was therefore substituted by FCA and FIA at 2-4 week intervals. A fifth booster injection was administered 6-9 months after the fourth injection. This study also demonstrated that anti-PZP antibody titers of 64% or greater were associated with effective contraception, and that a decline in contraceptive effect correlated with a decline in antibody titers.

Kirkpatrick et al. (1990) demonstrated PZP effectiveness in a study conducted at Assateague Island National Seashore (ASIS), MD in which 26 mares were remotely injected with a priming dose of 65-100 µg PZP in FCA and either one or two boosters of PZP in FIA at three-week intervals based on the determination by Liu et al. (1989) that at least two inoculations are required in horses so antibody titers are raised high enough for a minimum of 6 months. Upon the first inoculation, antigen recognition is initiated which increases antibody titers temporarily. Then, the second inoculation causes increased titers that last for several months, with each follow-up inoculation prolonging the duration of high titers (Kirkpatrick, et al. 1990).

During this study, 14 of the 26 treated mares were already pregnant upon inoculation and gave birth to healthy foals approximately 1 – 3 months after the last inoculation. By October 1998, there was only one pregnancy out of the 26 treated mares, as indicated by analysis of urinary steroids, with zero pregnancies among the 18 receiving 3 inoculations, and one pregnancy out of the 8 receiving two inoculations. The following spring, August 1989, only one of the 26 treated mares produced foals (Kirkpatrick, et al. 1990). Of the 26 treated mares, 14 were boosted again a year later with a single remotely delivered dart containing PZP in FIA. Only 1 of the 14 boosted mares was pregnant and produce a foal the following year, compared to 10 of 22 "sham-treated and untreated mares (45.5%) (Kirkpatrick, et al. 1991). Additional studies were carried out during the next 6 years which demonstrated foaling rates of 3.8% (4 foals in 105 mare-years) among PZP-treated mares compared to 46.2% in untreated mares (Kirkpatrick, et al. 1991). Zero population growth was achieved in 2 years, with an initial decline in the population becoming apparent in 8 years of inoculations and by year 11, the population declined from 175 to 135 horses, a decrease of 22.8% (Kirkpatrick and Turner 2008).

Turner et al. (1996) conducted a study at Virgin Islands National Park, St. Johns, VI (VINP) on free-roaming feral burros (*Eqqus asinus*) to assess the effectiveness of PZP as a contraceptive with results comparable to those seen in the Assateague Island studies. In this study, 16 female burros were treated with PZP contraceptive. Burros were given an initial one- or two-injection PZP treatment and, after 10 – 12 months, were given a one-injection PZP booster treatment. Initial treatment consisted of: (1) two separate injections (3 weeks apart) of a 1.0 mL emulsion, containing 65 µg PZP plus FCA (first injection) followed by a booster of FIA (n = 13); or (2) a single injection containing 130 µg PZP emulsified in FCA (n = 3). The single injection was a time-released method with release rates projected to be continuous across 4 weeks, with greatest release in weeks 1 and 4 followed by a booster shot at the end of the 4 weeks (Turner et al., 1996).

Zero of 13 females darted with a priming dose of 65 – 100 µg in FCA and a booster of 65 – 100 µg PZP in FIA produced foals in the period 12 – 24 months after treatment, while 1 of the 3 females receiving the single dose produced foals. Furthermore, 6 of 11 control females gave birth in that time period. Unlike wild and feral horses, feral burros are not seasonal breeders, and some of the burros were pregnant at the time of treatment.

The results of this study indicate the two-injection protocol was more effective than the single-injection in preventing pregnancies.

The effectiveness of the adjuvant used is an important factor in how efficacious the PZP epitope is as an immunocontraceptive (Lyda, et al. 2005). Since 1998, PZP has been used in captive free-ranging wild horses with a high degree of efficacy, utilizing Freund's Complete Adjuvant (FCA) as the adjuvant of choice for the initial inoculation and Freund's Incomplete Adjuvant (FIA) for booster inoculations. The use of FCA has resulted in 90% or greater efficacy, however two side effects can occur from its use: 1) Injection site reactions, including open abscesses and 2) false-positive tuberculosis (TB) tests in treated animals. The primary ingredient in the FCA is *Mycobacterium tuberculosis* which can cause antibodies against the TB organism. As a result of these side effects, the United States Department of Agriculture (USDA) has voiced opposition to the use of FCA.

Therefore, modified Freund's Complete Adjuvant (mFCA) has been substituted for FCA in titer trials of captive mares. These trials demonstrated no significant difference between mares hand-injected with 65-100 µg PZP in mFCA followed by a booster shot of 65-100 µg in FIA and mares treated with 65-100 µg PZP in FCA followed by a booster of 65-100 µg in FIA. Lyda et al. (2005) reported that 7 of 8 (87.5%) of mares treated with PZP and mFCA remained above the contraceptive titer threshold 10 months after treatment. The effectiveness of mFCA as an adjuvant was verified with these studies.

4. Summary of Regulatory Position and Rationale

Available data provide adequate information to support the unconditional registration of ZonaStat-H as a tool for management of nuisance feral and wild horses, and burros.

Like other animals (e.g. deer, Canada geese, etc.), horses may be pests in some situations. As a result of Federal protection, lack of natural predators, and fecundity (herd sizes can double in about four years), wild horse and burro herd populations have significantly increased, exceeding the BLM appropriate population levels of 27,200 in BLM managed lands. To help control these populations, BLM removes wild horses and burros and transfers them to private ownership or maintains them in BLM holding facilities.

With high population levels and the inability to sell or adopt out all captured wild horses and burros, the BLM has expressed that there is an explicit need to manage wild horse and burro populations because uncontrolled populations may lead to adverse environmental effects such as degradation of wildlife and native vegetation habitat. Additionally, these populations may lead to conflicts with other rangeland uses such as cattle grazing and recreation.

With these factors in mind, EPA is proposing to register ZonaStat-H and PZP for use to control wild and feral horse and burro populations. The Agency feels that ZonaStat-H will provide BLM a much needed alternative control method for wild horse and burro populations. The Agency believes that ZonaStat-H and PZP meet the standard for unconditional registration in FIFRA § 3(c)(5) including that it will not cause any unreasonable adverse effects on the environment. Therefore, the Agency proposes to grant this registration with the labeling requirements below.

5. Labeling Restrictions

To mitigate any risks, 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.

- A warning that pregnant women must not be involved in handling or injecting ZonaStat-H and that all women should be aware that accidental self-injection may cause infertility.

6. Data Requirements

The registrant has fulfilled all data requirements, resulting in an unconditional registration of ZonaStat-H.

7. CONTACT PERSON AT EPA

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DISCLAIMER: The information presented in this Pesticide Fact Sheet is for informational purposes only may not be used to fulfill data requirements for pesticide registration and reregistration. The information is believed to be accurate as of the date on the document.

APPENDIX I

GLOSSARY OF TERMS AND ABBREVIATIONS

ADNT	Acute delayed neurotoxicity
a.i.	Active Ingredient
aPAD	Acute Population Adjusted Dose
ARI	Aggregate Risk Index
BCF	Bioconcentration Factor
CAS	Chemical Abstracts Service
ChE	Cholinesterase
ChEI	Cholinesterase inhibition
cPAD	Chronic Population Adjusted Dose
%CT	Percent crop treated
DAT	Days after treatment
DEEM-FCID	Dietary Exposure Evaluation Model - Food Consumption Intake Database
DNA	Deoxyribonucleic acid
DNT	Developmental neurotoxicity
DIT	Developmental immunotoxicity
DWLOC	Drinking Water Level of Comparison.
EC	Emulsifiable Concentrate Formulation
EEC	Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem.
EPA	U.S. Environmental Protection Agency
FQPA	Food Quality Protection Act
GLC	Gas Liquid Chromatography
GLN	Guideline Number
LC_{50}	Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water, air or feed, e.g., mg/l, mg/kg or ppm.
LD_{50}	Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, inhalation). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.
LOAEL	Lowest Observed Adverse Effect Level
LOAEC	Lowest Observed Adverse Effect Concentration
LOC	Level of Concern
LOD	Limit of Detection
LOQ	Limit of Quantitation
mg/kg/day	Milligram Per Kilogram Per Day
mg/L	Milligrams Per Liter
MOE	Margin of Exposure
MRID	Master Record Identification (number), EPA's system of recording and tracking studies submitted
MTD	Maximum tolerated dose
NA	Not Applicable
NOEC	No Observable Effect Concentration
NOEL	No Observed Effect Level

NOAEL	No Observed Adverse Effect Level
NOAEC	No Observed Adverse Effect Concentration
NPDES	National Pollutant Discharge Elimination System
OP	Organophosphate
OPP	EPA Office of Pesticide Programs
OPPTS	EPA Office of Prevention, Pesticides and Toxic Substances
PAD	Population Adjusted Dose
PAG	Pesticide Assessment Guideline
PAM	Pesticide Analytical Method
PHED	Pesticide Handler's Exposure Data
PHI	Preharvest Interval
ppb	Parts Per Billion
PPE	Personal Protective Equipment
ppm	Parts Per Million
PRZM/EXAMS	Tier II Surface Water Computer Model
RAC	Raw Agriculture Commodity
RBC	Red Blood Cell
RED	Reregistration Eligibility Decision
REI	Restricted Entry Interval
RfD	Reference Dose
SCI-GROW	Tier I Ground Water Computer Model
SF	Safety Factor
TGAI	Technical Grade Active Ingredient
UF	Uncertainty Factor
µg	micrograms
µg/L	Micrograms Per Liter
µL/g	Microliter per gram
USDA	United States Department of Agriculture
WPS	Worker Protection Standard

APPENDIX II

Citations Considered to be Part of the Data Base Supporting the Registration of Porcine Zona Pellucida.

MRID	Citation	Receipt Date
47859801	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Product Efficacy: (Wild Horses and Burros). Unpublished study prepared by The Humane Society of the United States.	September 17, 2009
	Liu, I.K.M., M. Bernoco, and M. Feldman. 1989. Contraception in mares heteroimmunized with pig zonae pellucidae. Journal of Reproduction and Fertility. 85:19-29.	September 17, 2009
	Kirkpatrick, J.F., I.K.M. Liu, and J.W. Turner, Jr. 1990. Remotely delivered immunocontraception in feral horses. Wildlife Society Bulletin. 18:326-330.	September 17, 2009
	Kirkpatrick, J.F., I.K.M. Liu, T.W. Turner, and M. Bernoco. 1991. Antigen recognition in feral mares previously immunized with porcine zonae pellucidae. Journal of Reproduction and Fertility Supplement. 44:321-325.	September 17, 2009
	Kirkpatrick, J.F. and A. Turner. 2008. Achieving population goals in a long-lived wildlife species (*Eqqus caballus*) with contraception. Wildlife Research. 35:513-519.	September 17, 2009
	Turner, J.W., I.K.M. Liu, and J.F. Kirkpatrick. 1996. Remotely delivered immunocontraception in free roaming feral burros (*Eqqus asinus*). Journal of Reproduction and Fertility. 107:31-35.	September 17, 2009
	Lyda, R.O., J.R. Hall, and J.F. Kirkpatrick. 2005. Comparison of Freund's complete and Freund's modified adjuvants used with a contraceptive vaccine in wild horses (*Eqqus caballus*). Journal of Zoo and Wildlife Medicine. 36:610-616.	September 17, 2009
47859802	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Product Identity and Composition. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859803	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Toxicology – Acute. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859804	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Human Exposure. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859805	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Ecological Effects. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859806	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Human Exposure. Unpublished study prepared by Humane Society of the United States.	September 17, 2009
47859807	Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Environmental Fate. Unpublished study prepared by Humane Society of the United States.	September 17, 2009