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

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 12, 2010

MEMORANDUM

Subject:	Name of Pesticide Product:	ZonaStat-H
	EPA File Symbol:	86833-R
	DP Barcode:	D370441
	Decision No.:	420440
	PC Code:	176603 Glycoprotein (Porcine zona pellucida)
	Action Code:	R110

From: Breann Hanson, Biologist BHanson
Alternative Risk Integration and Assessment (ARIA) Team
Risk Integration, Minor Use, Emergency Response Branch (RIMUERB)
Registration Division (RD) (7505P)

Through: John Redden, ARIA Team Leader JR
RIMUERB/RD; 7505P

To: Jennifer Gaines, RM Team 07
Insecticide-Rodenticide Branch
RD; 7505P

Applicant: Humane Society of the United States
2100 L Street NW
Washington, DC 20037

FORMULATION FROM LABEL:

Active Ingredient:
176603 Glycoprotein (Porcine zona pellucida (PZP)) 100 µg*

* The product contains 100 µg of PZP per 0.5 mL dose

ACTION REQUESTED: The Product Manager requests: "Please review the enclosed acute tox study for the new a.i., porcine zona pellucida, to support the new registration of ZonaStat-H, a wild horse contraceptive. I have enclosed the study (MRID 47859803), CSF, cover letter, and label. Please let me know if you require any additional data. Thanks…"

BACKGROUND: The Humane Society of the United States (HSUS) (herein, the "registrant") has applied for registration of ZonaStat-H, EPA File Symbol: 86833-R, for use as a contraceptive in feral and wild horses and burros. The product contains a new active ingredient, porcine zona pellucida (PZP), derived from a naturally-occurring animal glycoprotein, which has not yet been registered with the Agency. The registrant has requested an exemption from the acute toxicity testing requirements. The bases for exempting ZonaStat-H were listed in a submitted toxicology volume (MRID 47859803) and include: a) documented safety and history of use of the components comprising the vaccine; b) the biology and properties of ZonaStat-H, along with the nature and fate of the product's metabolites, do not suggest that the product is toxic or pathogenic; c) the method of delivery (by injection) limits route of exposure for target and non-target animals; and d) extensive field and laboratory data document the safety of the vaccine product.

Composition: ZonaStat-H is an emulsion consisting of two components: a) a naturally occurring, chemically unmodified glycoprotein, PZP, and b) an adjuvant. PZP induces little or no immune response unless administered with an adjuvant (Bhatnager etal., 1989). Adjuvants are associated with side effects including injection site reactions such as granulomas and sterile abscesses, systemic effects such as fever, lethargy, and loss of appetite, and sometimes autoimmune diseases (Hanly etal., 1997).

ZonaStat-H uses 2 adjuvants; Modified Freund's Complete Adjuvant (mFCA) for primer injections, and Freund's Incomplete Adjuvant (FIA) for booster injections. In a variety of studies FCA/FIA vaccines produced antibody levels higher than other adjuvants (please see MRID 47859803).

Although FCA and FIA are not approved by the Federal Drug Administration or U.S. Department of Agriculture for commercial vaccines, the data amassed by the registrant on treated horses do not support the negative results observed which preclude use in human vaccines. The registrant presumes this can be due to several reasons. First, the efficacy and the type and magnitude of side effects elicited vary with species, route of administration, and adjuvant (MRID 47859803). Reports of side effects associated with Freund's adjuvants are derived from studies on laboratory animals, which include mice, rats, hamsters, guinea pigs and rabbits. The side effects noted in the studies submitted by the registrant on horses infer the safety of using FCA/FIA. Secondly, dosages administered in studies that did report side effects were considered extremely high relative to body weight. The dosage for the proposed use on wild horses is minimal compared to doses tested on laboratory animals. Thirdly, the composition of mineral oils has changed significantly over the 40-50 years in which this research has been done (Lindblad, 2000). In more recent preparations, unsaturated and aromatic hydrocarbons have been

removed, leaving less reactive, longer-chain saturated hydrocarbons which are non-carcinogenic in mice via the dermal or inhalation route; non-mutagenic by Ames Test, and non-fetotoxic and non-tetratogenic in rats treated via oral gavage (Stewart-Tull, 1997). Finally, another concern with the use of adjuvants is the risk of aggravating autoimmune diseases associated with antigens that resemble host proteins. PZP, however, does not cross-react with any equine somatic tissues or protein hormones (Kirkpatrick etal. 1996).

Mechanism of Action: ZonaStat-H stimulates a classic humoral response; the antibodies interfere with fertilization by binding to glycoprotein receptors on thee non-cellular membrane that surrounds the egg of the treated animal, causing steric hindrance of the zona sperm receptor (MRID 47859803).

Fate Post-Injection: Following injection, hydrolysis occurs, and the metabolic products are excreted and eliminated from the body in forms that are indistinguishable from other metabolic products (i.e., water, lactic acid, urea) (MRID 47859803). PZP and the adjuvant antigens are not stored in body tissues, thereby eliminating the possibility of continued exposure of the target animal to the vaccine components, or of non-target animals and humans of exposure to the components. Digestion of vaccine components yields end products comprising amino acids and simple carbohydrates, which elicit no immune response and are bioinactive. Therefore, vaccine components are not transferred through food chains (MRID 47859803).

Method of Administration and Exposure Risk: ZonaStat-H is injected intramuscularly, at a volume of 1 mL, either by hand-held syringe, by syringe attached to a "jab-stick", or by syringe dart. The method of delivery ensures that the target animal receives no aerial, oral, ocular, or general dermal irritation; exposure to non-target animals and humans is "nearly zero" (MRID 47859803).

Potential incidental contact with the contents of unrecovered, non-discharged darts is possible; though "approximately 95% of all darts fired are recovered" (Kirkpatrick, 2008); reducing the number of darts remaining in the environment. The proposed label includes language that instructs applicators to attempt to recover all darts, even proposing that lost darts should be noted and marked with an attempt to recover them at a later period. Also, the darts do not discharge spontaneously or with incidental contact. The registrant states that "[s]triking, stepping on, jiggling, biting, or otherwise casually moving or contacting the dart will not discharge or release the contents of the dart" (MRID 47859803). Therefore, the potential for incidental/unintentional contact is low.

Potential oral consumption by a predator or scavenger is another possibility. However; as the product is broken down into amino acids and simple carbohydrates following ingestion, the product becomes physiologically inactive (Takashima etal., 2008). The inerts within the product are known to either pass through the digestive system without absorption or are broken down.

Potential exposure to the applicator via dermal, oral and/or ocular contact with the product is

possible during handling/loading of the product. Therefore, the registrant is requiring training and certification for applicators (the product is a *Restricted Use Pesticide*), as well as requiring protective clothing during the preparation of the product (gloves).

Field and Laboratory Data on the Safety of ZonaStat-H: Adverse reactions to ZonaStat-H may occur at injection sites; including sterile granulomas and draining abscesses. Draining abscesses are rare though seen more often in horses treated via darts than in horses treated by hand injection (MRID 47859803). In one field study, three visible abscesses out of 26 mares receiving 2-3 injections were observed; all draining from 6-9 days after treatment (Kirkpatrick etal., 1990). In another field study, 1841 dartings of 329 wild horses yielded 19 visible abscesses (1% of all dartings); all drained within 30 days after treatment (Kirkpatrick, 2007). In the same study among zoo animals, 16 abscesses were noted out of 1185 treatments (1.35% of all treatments); all drained and healed without incident. In a western wild horse field study, no visible abscesses were observed in 215 mares receiving the product via hand-injection (Turner etal., 1997). Another study of 15 mares hand-injected with the product resulted in only 1 visible abscess (following a booster injection), which drained without incident (Lyda etal., 2005). In that study, the injection sites of 50 mares treated with the product in 4 different formulations yielded a rate of abscesses of 8% over 12 weeks post-treatment; at 10 months 2 still had palpable subcutaneous abscesses. At 7 months post-treatment, muscle tissue disruption at the injection site was noted in 8 of 28 horses examined; 7 were considered "slight".

Based on the above information, injection site reactions are of little concern to the Agency. The reactions noted do not preclude registration based on injection site irritation.

RECOMMENDATIONS: The registrant's request for a waiver of acute oral, acute dermal, acute inhalation, primary eye irritation, primary dermal irritation and dermal sensitization studies has been reviewed and determined by ARIA to be acceptable. Based on the information provided by the registrant, the overall acute toxicity of this product is expected to be low.

Acute Oral Toxicity: Based on the information that once ingested, the product yields end products comprising amino acids and simple carbohydrates which elicit no immune response and are bioinactive, and the low probability of oral contact, the product may be placed into acute oral toxicity category IV.

Acute Dermal, Inhalation, Eye, and Skin Toxicity: Based on the slight chance of exposure to the product and due to the irritation noted in the field and laboratory studies, the product may be placed into toxicity category III for these routes of exposure.

Skin Sensitization: The product does not have to be labelled as a dermal sensitizer.

The acute toxicity profile for ZonaStat-H, EPA File Symbol: 86833-R is:

Acute oral toxicity	IV	Waived

Acute dermal toxicity	III	Waived
Acute inhalation toxicity	III	Waived
Acute eye irritation	III	Waived
Primary skin irritation	III	Waived
Dermal sensitization	Neg.	Waived

Note to RM: The proposed label does not contain any signal word or EPA recommended precautionary and first aid statements. This reviewer concurs with the proposed precautionary statements regarding accidental needle pricks and contact with mFCA, but the additional statements noted below are additionally recommended. This reviewer strongly recommends that the signal word CAUTION appear on the label.

LABELING: Based on the toxicity profile above, the following are the precautionary and first aid statements for this product as obtained from the Label Review System.

PRODUCT ID #: 086833-00001
PRODUCT NAME: ZonaStat-H

PRECAUTIONARY STATEMENTS

SIGNAL WORD: CAUTION

Hazards to Humans and Domestic Animals: Harmful if absorbed through skin or inhaled. Avoid contact with skin, eyes or clothing. Causes moderate eye irritation. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet. Remove and wash contaminated clothing before reuse. Avoid breathing spray mist.

First Aid:

If on skin:
-Take off contaminated clothing.
-Rinse skin immediately with plenty of water for 15-20 minutes.
-Call a poison control center or doctor for treatment advice.

If inhaled:
-Move the person to fresh air.
-If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
-Call a poison control center or doctor for further treatment advice.

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
-Call a poison control center or doctor for treatment advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-xxx-xxxx for emergency medical treatment information.

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