Veterinary Medicine Research & Development
Kalamazoo, Michigan 49007
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zoetis

NADA 141-322, IMPROVEST® (Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate, 0.2 mg/mL) Sterile Solution for Injection

Environmental Impact Technical Section

Supplemental New Animal Drug Application

For the temporary suppression of estrus in gilts intended for slaughter

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APPROVAL

Dated: 17 July 2017 Signed:

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1. PHASE I ASSESSMENT

1.1. Introduction

In accordance with the Code of Federal Regulations (21CFR 25.15(a)), "All applications or petitions requesting agency action require the submission of an EA [environmental assessment] or a claim of Categorical Exclusion [CE]." Under NADA 141-322, Zoetis has presented an EA to address the environmental safety of the use of IMPROVEST® (Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate) Sterile Solution (hereinafter referred to as IMPROVEST) in swine for the temporary immunological castration (suppression of testicular function) and control of boar taint.

The aim of the present EA is to address the environmental safety of the use of IMPROVEST for the temporary suppression of estrus in gilts intended for slaughter.

The following guidance documents were used in preparing this EA: CVM #89 [1] and AHI/CVM Draft 10 [2].

1.2. Description of the Product

IMPROVEST is an immunological product. IMPROVEST stimulates the pig's immune system to produce antibodies that temporarily neutralizes endogenous gonadotropin releasing factor (GnRF), a factor that controls testicular and ovarian function via the gonadotropic hormones LH and FSH.

IMPROVEST is an injectable sterile solution containing an incomplete analog of natural gonadotropin releasing factor (GnRF) conjugated to diphtheria toxoid in an adjuvanted formulation. Immunization with a two dose regimen of IMPROVEST, with a four week interval between doses, stimulates the pig's immune system to produce antibodies which can neutralize its own GnRF. Pigs given an initial dose of IMPROVEST are immunologically primed but do not produce sufficient antibodies to have any physiological effect. Following receipt of the second dose, the pig's immune system responds with a strong antibody response. These antibodies bind to and neutralize circulating GnRF in the bloodstream. Neutralization of GnRF blocks the hypothalamic-pituitary-gonadal endocrine axis, thereby suppressing gonadal function, including both sex hormone production and reproductive capability.

In entire male pigs, this results in the temporary immunological castration (suppression of testicular function) and control of boar taint. Boar taint is principally caused by the accumulation of two lipophilic compounds, androstenone and skatole, in the fat of maturing male pigs. These compounds are relatively volatile and are therefore released during cooking and eating, causing an offensive smell. When administered to gilts, a similar mechanism of action leads to the temporary suppression of estrus. Full suppression of estrus was demonstrated to last from 4 weeks to at least 10 weeks after the second dose.

The immunogen in IMPROVEST is a peptide-protein (synthetic 2-10 peptide analogue of GnRF conjugated to diphtheria toxoid (DT), hereinafter may be referred to as 2-10GnRF-DT) formulated in an aqueous polysaccharide solution as the adjuvant (DEAE-dextran in water) and preserved with chlorocresol. The composition of a 2-mL dose of IMPROVEST is as follows (Table 1):

Table 1. Composition of a 2-mL dose of IMPROVEST

Ingredient	Purpose	Quantity
2-10GnRF-DT	Immunogen	0.4 mg
Diethylaminoethyl-dextran hydrochloride	Adjuvant	300 mg
Urea	Protein solubilizing agent	4 mg
Chlorocresol	Preservative	2 mg
Water for injection	Diluent	to 2 mL

1.2.1. 2-10GnRF

The synthetic 2-10 amino acid GnRF analog (2-10GnRF) is identical to the naturally occurring 1-10 peptide compound except that it lacks one amino acid, 'pyroGlu' on the N-terminal end. The amino acid sequence of the analog is: His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2.

1.2.2. Diphtheria Toxin and Toxoid

Diphtheria toxin is an enzyme naturally produced by non-genetically modified, lysogenized strains of Corynebacterium diphtheriae. The toxin is released extracellularly as a single 535 amino acid polypeptide chain of approximately 66 kDa and is composed of two fragments, 'A' and 'B', both of which are required to confer toxicity [3]. The diphtheria toxin is detoxified by conversion to diphtheria toxoid by chemical modification using formaldehyde. The toxoid cannot be separated into A and B fragments, has no enzymatic activity and does not bind to sensitive cell membranes [3]. DT is one of the major components of the DPT vaccine used to immunize nearly 100% of the children in developed countries.

1.2.3. 2-10GnRF-DT Conjugate

2-10GnRF is covalently conjugated to formaldehyde-detoxified diphtheria toxoid carrier via a stable thioether bond. It is produced using thiol-halide chemistry via a N-succinimidyl S acetyl thioacetate mediated process.

1.3. Posology and Method of Administration

IMPROVEST should be administered via subcutaneous injection into the post auricular region of the neck. A safety injector should be used, preferably one which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. Each intact male pig or gilt should receive two 2-mL doses of IMPROVEST. The first dose should be administered no earlier than 9 weeks of age. The second dose should be administered at least 4 weeks after the first dose. Male pigs should be slaughtered no earlier than 3 weeks and no later than 10 weeks after the second dose. In case of misdosing, the animal should be re-dosed immediately.

1.4. Summary of the EA for Use of IMPROVEST in Male Pigs

The PEC in soil (PEC_{soil}) of the 2-10GnRF-DT conjugate active ingredient and of all other constituents was calculated using assumptions provided in AHI/CVM Environmental Risk Assessment Forum guidance document Draft 10 [2] for pigs weighing 50 kg and 100 kg. These body weights would be typical for the size of pigs that would receive IMPROVEST. It was assumed that the product would be used according to label directions in all male pigs of

the herd (50% of the herd). It was assumed that all ingredients would be excreted unchanged, without any metabolism occurring. The results of these calculations are summarized in Table 2.

Table 2. PEC_{soil} Concentrations of the Components of IMPROVEST Using Draft 10

Item	2-10GnRF-DT Conjugate	DEAE-Dextran	Urea	Chlorocresol
PEC_{soil} - 50 kg pig	0.0406 µg/kg	30.4 µg/kg	0.406 µg/kg	0.203 µg/kg
PEC_{soil} - 100 kg pig	0.0203 µg/kg	15.2 µg/kg	0.203 µg/kg	0.101 µg/kg

These concentrations are all well below the 100 µg/kg level of concern recommended in the Guidance For Industry - Environmental Impact Assessments (EIA's) For Veterinary Medicinal Products (VMP's) - Phase I, VICH GL6 - Final Guidance (# 89) [1].

In addition, as the the 2-10GnRF is the major part of the naturally produced 1-10GnRF, it would be susceptible to the vast array of proteases, peptidases and other enzymes produced by environmental microbes and fungi in manure and soil under various conditions, and in those plants which produce proteases
[4, 5, 6, 7, 8, 9, 10, 11, 12 13, 14, 15, 16, 17, 18, 19]. Likewise, diphtheria toxin has been shown to be degraded by *Escherichia coli* and many species from the ubiquitous Bacillaceae family of bacteria isolated from the environment [20]. Additionally, at least some of the toxin will adsorb to clays in soil, resulting in a reduction of the lethal activity of the toxin [21]. The toxoid, should it reach the environment, will be degraded in much the same way and will not accumulate. The same would be true for the conjugate.

The manufacture of diphtheria toxin and toxoid is conducted in a dedicated GMP facility with dedicated equipment and a closed system designed for containment. The room in which the production equipment is contained is maintained at negative pressure relative to adjacent areas. All discard fluids containing DT are autoclaved or discarded into the process waste system and subsequently decontaminated via heat treatment before disposal. Therefore, there will be no contamination of the environment due to the production of the diphtheria toxin or toxoid.

The EA concluded that based on all available information, no further Phase II assessment was warranted for the use of IMPROVEST in male pigs. A Finding of No Significant Impact (FONSI) was issued.

1.5. Updated PEC_{soil} Calculations – Male and Female Pigs

The assumptions to be used to calculate the PEC_{soil} for the use of IMPROVEST in gilts are in essence the same as those used to perform the calculations for male pigs:

- All gilts in the fattening swine herd will be treated with IMPROVEST (= 50% of the herd).
- Each 2-mL dose of the product contains 0.4 mg of the active ingredient, 2-10GnRF-DT conjugate.
- The product will be administered to sexually maturing/mature gilts in two doses at an interval of at least 4 weeks between doses, with the first dose being administered no earlier than 9 weeks of age.
- PEC calculations are shown for pigs with body weights of 50 kg and 100 kg; these body weights would be typical for the size of gilts that would receive IMPROVEST, given the target slaughter live weight for both barrows and gilts of 128.6 kg (283 lbs) [22].
- The manure production period is 60 d, and a pig produces 4.1 kg manure/d
- For the 100-kg pig, the amount of manure produced per day is adjusted upward in an amount proportional to that of the 50 kg pig as described in Draft 10 [2].
- The concentration of a compound in excreta is: a = (b x c x d)/e, where;

  a = wet weight concentration of drug substance in manure in mg/kg
  b = total dose administered to each animal/d: 0.4 mg
  c = fraction of animals treated: 0.5
  d = number of days animals are treated: 2 d

- The concentration of a compound in soil (PEC_{soil}) is derived from the following:

  Amount of pig manure applied to 1 acre of land: 22700 kg
  Weight of soil in an acre which is 15 cm (6") deep: 910500 kg
  PEC_{soil} = (Conc. in manure x kg of manure/acre)/910500 kg of soil

- 100% of the dose is excreted, with no metabolism.

Given the above, the calculated PEC_{soil} values will be those depicted in Table 2 for male pigs. The worst case scenario would be when a farmer decides to adopt this immunization technology throughout and use IMPROVEST in all of his slaughter pigs, i.e. both in male pigs and in gilts – which is not unrealistic. Therefore, calculations have been performed using the worst-case assumptions but assuming that all male pigs and gilts are treated, meaning 100 % of the herd. Resulting calculations are as follows (Table 3 for 50 kg pigs; Table 4 for 100 kg pigs):

Table 3. PEC_{soil} of the Components of IMPROVEST Using Draft 10: 50 kg Pig

Item	2-10GnRF-DT Conjugate	DEAE-Dextran	Urea	Chlorocresol
a = mg component/kg manure = (b x c x d)/e				
b = total dose/d, mg	0.4	300	4	2
c = fraction treated	1.0	1.0	1.0	1.0
d = # d treated	2	2	2	2
Manure production period, d	60	60	60	60
Daily manure production, kg	4.1	4.1	4.1	4.1
e = kg manure produced	246	246	246	246
a =	0.003252 mg/kg	2.44 mg/kg	0.03252 mg/kg	0.01626 mg/kg
manure/acre, kg	22700	22700	22700	22700
weight of soil, 15 cm	910500	910500	910500	910500
conc. in soil (PECsoil) = (conc. in manure x kg of manure/acre)/910500 kg of soil				
(a x 22700)/910500 kg				
=	$8.1x10^{-5}$ mg/kg	0.061 mg/kg	$0.811*10^{-3}$ mg/kg	$0.405*10^{-3}$ mg/kg
PECsoil =	**0.081 µg/kg**	**61 µg/kg**	**0.811 µg/kg**	**0.405 µg/kg**

Table 4. PEC_{soil} of the Components of IMPROVEST Using Draft 10: 100 kg Pig

Item	2-10GnRF-DT Conjugate	DEAE-Dextran	Urea	Chlorocresol
a = mg component/kg manure = (b x c x d)/e				
b = total dose/d, mg	0.4	300	4	2
c = fraction treated	1.0	1.0	1.0	1.0
d = # d treated	2	2	2	2
Manure production period, d	60	60	60	60
Daily manure production, kg	8.2	8.2	8.2	8.2
e = kg manure produced	492	492	492	492
a =	0.001626 mg/kg	1.21951 mg/kg	0.01626 mg/kg	0.00813 mg/kg
manure/acre, kg	22700	22700	22700	22700
weight of soil, 15 cm	910500	910500	910500	910500
conc. in soil (PECsoil) = (conc. in manure x kg of manure/acre)/910500 kg of soil				
(a x 22700)/910500 kg				
=	$4.05x10^{-5}$ mg/kg	0.0304 mg/kg	$0.405x10^{-3}$ mg/kg	$0.203x10^{-3}$mg/kg
PECsoil =	**0.0405 µg/kg**	**30.4 µg/kg**	**0.405 µg/kg**	**0.203 µg/kg**

Even if the PEC_{soil} is now twice the values calculated for the male pigs alone, all concentrations remain well below the 100 µg/kg level of concern recommended in the Guidance For Industry - Environmental Impact Assessments (EIA's) For Veterinary Medicinal Products (VMP's) - Phase I, VICH GL6 - Final Guidance (# 89) [1].

1.6. Disposal of Unused IMPROVEST or Waste Materials Derived from its Use

Any unused IMPROVEST or waste materials derived from its use should be disposed of in accordance with local requirements.

1.7. Overall Conclusions

The information provided in this Phase I EA is submitted regarding IMPROVEST (Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate) Sterile Solution (active ingredient: Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate) for administration to gilts. The EA was prepared in accordance with the VICH Phase I guidance (CVM Guidance for Industry 89). IMPROVEST should be classified as among those in question 17 of the Phase I Decision Tree, ("*17. Is the environmental concentration of the VMP [Veterinary Medicinal Product] in soil (PECsoil) < 100 µg /kg?...Yes, then STOP"*). IMPROVEST Sterile Solution warrants a finding of no significant environmental impact.

Calculated worst-case PEC_{soil} values for the conjugate were approximately 1,000- to 2,000-fold lower than the 100 µg/kg trigger, even when assuming that all animals on a farm (male and female) would be treated with IMPROVEST. Updated, more recent information confirms that, should the 2-10GnRF-DT conjugate and the precursors individually reach the environment, they should be degraded. Therefore, no significant environmental exposure of 2-10GnRF-DT conjugate or its precursors is expected from the use of IMPROVEST in both male and female pigs.

Taken together, the information provided in this EA supports and confirms the conclusion that this product should have no untoward effects on the environment, and no Phase II assessment is required.

1.8. Description of Any Alternatives to the Proposed Use

The only alternative to the proposed action is the "no action" alternative, which would be the failure to approve the new animal drug application (NADA) for Improvest. However, based on our analysis in this environmental assessment, we do not believe that significant environmental impacts will occur from this action; therefore, the "no action" alternative was eliminated from consideration.

1.9. Agencies and Persons Consulted

During the preparation of this environmental assessment, no external agencies or persons were consulted.

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