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Environmental Assessment for IMPROVEST®
(Gonadotropin Releasing Factor Diphtheria Toxoid Conjugate)
Sterile Solution

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APPROVAL

Dated: 4 November 2010 Signed: Richard C Greening

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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]." Therefore, PAH is submitting, under [redacted], an EA to address the environmental safety of the use of IMPROVEST® (Gonadotropin Releasing Factor Diphtheria Toxoid Conjugate) Sterile Solution (hereinafter may be referred to as IMPROVEST) in swine for the temporary immunological castration (suppression of testicular function) and control of boar taint.

The following guidance documents were used in preparing this EA: CVM #89 [1], AHI/CVM Draft 10, [2] and CVMP [3].

1.2. Description and Composition

IMPROVEST is an immunological product for the temporary immunological castration (suppression of testicular function) and control of boar taint in entire male pigs. 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. IMPROVEST stimulates the pig's immune system to produce antibodies that temporarily neutralizes endogenous gonadotropin releasing factor (GnRF), which leads to suppression of testicular function and boar taint.

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:

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 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-NH_2.

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 [4]. 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 [4]. 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. The conjugate, which serves as the immunogen, is also the immunogen in IMPROVAC® and has a Pfizer identification number of PF-04331424-00-0001.

1.3. Target Species and Indication

IMPROVEST is indicated for the temporary immunological castration (suppression of testicular function) and control of boar taint in intact male pigs intended for slaughter.

1.4. Pharmacological Properties

Immunization with IMPROVEST induces an immune response against endogenous GnRF, a factor that controls testicular function via the gonadotropic hormones LH and FSH. The active ingredient in this immunological is a synthetically produced analogue of GnRF, which is conjugated with an immunogenic carrier protein. The conjugate is adjuvanted to increase the level and duration of effect.

The effects of immunization derive from the reduction in testicular function resulting from reduced GnRF activity. This leads to reduced production and concentration of testosterone and other testicular steroids, including androstenone, and another major contributor to boar taint, skatole.

Boars given an initial dose of IMPROVEST are immunologically primed but retain their full testicular function until they receive the second dose, which induces a strong immune response to GnRF and causes temporary immunological suppression of testicular function. This directly controls the production of androstenone and, by removing the inhibitory effect of testicular steroids on hepatic metabolism, indirectly reduces levels of skatole. This effect is apparent within one week of treatment but it may take up to 3 weeks for any existing concentrations of boar taint compounds to be reduced to insignificant levels.

1.5. Posology and Method of Administration

Entire male pigs from 9 weeks of age onwards should be vaccinated with 2 doses of 2 mL of IMPROVEST at least 4 weeks apart, with the second dose given 4 to 8 weeks prior to slaughter. In case of suspected misdosing, the animal should be revaccinated immediately. IMPROVEST is administered by subcutaneous injection in the neck, immediately behind the ear.

1.6. Predicted Environmental Concentration (PEC) Calculations

The PEC in soil (PEC_{soil}) of the 2-10GnRF-DT conjugate active ingredient was calculated using assumptions provided in AHI/CVM Environmental Risk Assessment Forum guidance document Draft 10 [2] and the CVMP technical guidance document [3].

1.6.1. AHI/CVM Draft 10

PEC_{soil} was calculated based on the methods described in AHI/CVM Draft 10 guidance document [2]. The following assumptions are made:

- All males in the 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 male pigs in two doses at an interval of at least 4 weeks between doses, with the second dose being administered approximately 4 - 8 weeks prior to slaughter.
- 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 pigs that would receive IMPROVEST.
- 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 [1].
- The manure production period is 60 d, and a pig produces 4.1 kg manure/d
- 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.

The worst-case PEC_{soil} calculations for the components of IMPROVEST are shown below.

PEC_{soil} Concentrations 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	0.5	0.5	0.5	0.5
d = # d treated	2	2	2	2
e = kg manure prodn' period	246	246	246	246
a =	0.00163 mg	1.220 mg	0.0163 mg	0.00813 mg
manure/acre, kg	22700	22700	22700	22700
weight of soil, 15 cm	910500	910500	910500	910500
conc. in soil (PEC_{soil}) = (conc. in manure x kg of manure/acre)/910500 kg of soil				
=	(a x 22700)/910500 kg			
=	0.0000406 mg/kg	0.0304 mg/kg	0.000406 mg/kg	0.000203 mg/kg
PEC_{soil} =	0.0406 µg/kg	30.4 µg/kg	0.406 µg/kg	0.203 µg/kg

PEC_{soil} Concentrations 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	0.5	0.5	0.5	0.5
d = # d treated	2	2	2	2
e = kg manure prodn' period	492	492	492	492
a =	0.000813 mg	0.610 mg	0.00813 mg	0.00407 mg
manure/acre, kg	22700	22700	22700	22700
weight of soil, 15 cm	910500	910500	910500	910500
conc. in soil (PEC_{soil}) = (conc. in manure x kg of manure/acre)/910500 kg of soil				
=	(a x 22700)/910500 kg			
=	0.0000203 mg/kg	0.00152 mg/kg	0.000203 mg/kg	0.000101 mg/kg
PEC_{soil} =	0.0203 µg/kg	1.52 µg/kg	0.203 µg/kg	0.101 µg/kg

1.6.2. CVMP Guidance

As another means of calculating the PEC_{soil}, the methods outlined in the guidance presented by the Committee for Medicinal Products for Veterinary Use (CVMP) Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38, are used [3]. The following assumptions are made:

- The "fattening pig" category most closely aligns with the indicated body weights (BWs) of the male pigs to be treated. In this instance, the mid-range BW of the given BW range of pigs in this category, 25 – 125 kg, is 75 kg.
- The number of animal cycles, animal/(place • y), is given as '3'.
- The percent of the herd treated is given as 50%.
- The nitrogen production rate of a 75 kg BW pig was adjusted proportionally upward from the default value of 7.5 kg nitrogen/y given for the default BW of 65 kg BW. The proportional value is 8.65 kg nitrogen/y for the 75 kg BW pig.
- One hundred percent (100%) of the dose is excreted, with no metabolism.
- All other default values used in the PEC_{soil} calculations are shown in the following table.

PEC_{soil} Concentrations of the Components of IMPROVEST Using CVMP Methods

Item	Value			
	2-10GnRF-DT Conjugate	**DEAE-Dextran**	**Urea**	**Chlorocresol**
Individual dose (D), mg/kg BW	0.00533	4.00	0.0533	0.0267
Treatments per animal, (Ad)	2			
Average BW, kg	75			
Number of animals/y (P), animal/(place • y)	3			
N application rate to soil (AN), kg/(ha • y)	170			
Fraction of animals treated (Fh)	0.5			
Fraction of active ingredient excreted, Fex	1			
Soil density (R), kg/m^3	1500			
Area of hectare (A), m^2	10000			
Soil penetration depth (Depth), m	0.05			
Nitrogen production rate (N), kg /y	8.65			
Housing factor (H)	1			
Conversion factor (Cf), (μg/mg)	1000			
$PEC_{soil_initial}$ (μg/kg, 5 cm depth) =	[(D • Ad • BW • P • AN • Fh • Fex)/(R • A • Depth • N • H)] • Cf			
=	0.0314	25.58	0.314	0.1574
$PEC_{soil_initial}$ corrected for 15 cm (6") plow depth =	0.0105	8.53	0.105	0.052

The worst-case PEC_{soil} of the active ingredient (2-10GnRF-DT conjugate) using AHI/CVM guidance for the 50 kg pig is 0.0203 µg/kg or 20.3 ng/kg; for the 100 kg pig, the respective values are 0.0102 µg/kg or 10.2 ng/kg. Similarly, the worst-case $PEC_{soil_initial}$ using the CVMP calculation method was 0.0105 μg/kg, or 10.5 ng/kg. These concentrations are approximately 2,500- to 10,000-fold 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].

Additionally, the worst-case PEC_{soil} of all of the other components of IMPROVEST are well below the Phase I trigger limit.

1.7. Degradation of 2-10GnRF, Diphtheria Toxin/Toxoid, and 2-10GnRF-DT in the Environment

1.7.1. 2-10GnRF and analogs

The structure of 2-10GnRF in IMPROVEST is identical to that of the naturally occurring compound, except the pyro-glutamic acid on the N-terminal end is absent. As the 2-10GnRF is the major part of the a naturally produced GnRF, it would be susceptible to the vast array of proteases, peptidases and other enzymes produced by environmental microbes and in those plants which produce proteases [5, 6, 7, 8].

1.7.2. Diphtheria toxin/toxoid

Diphtheria toxin is a naturally produced peptide, so it would be susceptible degradation in environmental matrices via the microflora. 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 [9, 10]. Additionally, at least some of the toxin will adsorb to clays in soil, resulting in a reduction of the lethal activity of the toxin [11]. Although the diphtheria toxoid is a chemically modified form of diphtheria toxin, it is expected that it will be degraded in the environment in much the same way as diphtheria toxin. Therefore, the toxoid, even if it reaches the environment, will be degraded and will not accumulate.

1.7.3. 2-10GnRF-DT

2-10GnRF-DT conjugate, although structurally different from either of the components separately, would very likely be degraded by environmental microflora if it were to reach the environment. Any potential exposure of the conjugate would be of limited duration and the low PEC_{soil} should ensure that there would be no negative effects on environmental organisms. Additionally, it was assumed in the worst case PEC_{soil} calculations that 100% of the 2-10GnRF-DT would be excreted by the treated pig. This is a highly unrealistic assumption which has implications for ecotoxicity, *viz.*, no negative effects on natural environmental species is anticipated.

1.8. Biocontainment of Diphtheria Toxin/Toxoid from the Production Facility

The manufacture of diphtheria toxin and toxoid at the Pfizer Lincoln, NE site is conducted in a facility which has a closed system designed for containment. The equipment which comes into direct contact with the toxin is dedicated to its production, labeled and segregated to clearly designate its use. The room in which the production equipment is contained is maintained at negative pressure relative to adjacent areas. Autoclaving of minor equipment used and all discarded fluids containing the toxin is done by the use of validated procedures and confirmed monitoring. 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 biocontainment documentation for our back-up supplier of diphtheria toxin, Shantha Biotechnics Limited, was not available at the time of this submission. These data, when available, will be submitted post approval in an appropriate supplement to the NADA prior to use of any Shantha diphtheria toxin in the manufacture of IMPROVEST.

1.9. 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.

1.10. Conclusions

The information provided in this Phase I EA is submitted regarding IMPROVEST (Gonadotropin Releasing Factor Diphtheria Toxoid Conjugate) Sterile Solution (active ingredient: Gonadotropin Releasing Factor Diphtheria Toxoid Conjugate) for administration to intact male pigs for the indications listed in Section 1.3. The EA was prepared in accordance with the VICH Phase I guidance (CVM Guidance for Industry 89) and in accordance with the guidance presented by the Committee for Medicinal Products for Veterinary Use (CVMP): Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL6 and GL38. 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 (PEC_{soil}) < 100μg/kg?...Yes,* then *STOP"*). IMPROVEST Sterile Solution warrants a finding of no significant environmental impact.

Experimental data show that the conjugate is not detected in the sera of treated pigs shortly after injection and up to seven days post-injection of IMPROVEST and that worst-case PEC_{soil} values for the conjugate were approximately 2,500- to 10,000-fold lower than the 100 μg/kg trigger. Additionally, 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.

For these reasons, a Phase II assessment is unwarranted. The use of IMPROVEST for the indications stated on the product label should not release 2-10GnRF-DT conjugate into the environment at levels expected to exert negative effects on non-target environmental species.

Finally, the European Medicines Agency's European Public Assessment Report has concluded that there are no concerns with regard to the ecotoxicity of IMPROVAC, a product having the identical active ingredient and formulation as that of IMPROVEST with the exception that the preservative in IMPROVAC is thimerosal rather than chlorocresol [12].

Taken together, the information provided in this EA supports the conclusion that this product should have no untoward effects on the environment.

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