Date of Approval: March 22, 2011

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-322

IMPROVEST

Gonadotropin Releasing Factor - Diphtheria Toxoid conjugate
Sterile Solution for Injection
Swine, intact males

For the temporary immunological castration (suppression of testicular function) and reduction of boar taint in intact male pigs intended for slaughter.

Sponsored by:

Pfizer, Inc.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-322
B. Sponsor:	Pfizer, Inc. 235 East 42d St. New York, NY 10017 Drug Labeler Code: 000069
C. Proprietary Name:	IMPROVEST
D. Established Name:	Gonadotropin Releasing Factor – Diphtheria Toxoid conjugate (GnRF-DT conjugate)
E. Pharmacological Category:	Immunotherapeutic
F. Dosage Form:	Sterile Solution for Injection
G. Amount of Active Ingredient:	0.2 mg/mL
H. How Supplied:	20, 100, 250, and 500 mL vials
I. How Dispensed:	Rx
J. Dosage:	2, 2 mL (0.4 mg GnRF-DT conjugate) injections
K. Route of Administration:	Injection, subcutaneous
L. Species/Class:	Swine, intact males
M. Indication:	For the temporary immunological castration (suppression of testicular function) and reduction of boar taint in intact male pigs intended for slaughter.

II. EFFECTIVENESS:

A. Mechanism of Action:

Treatment with a 2 dose regimen of IMPROVEST (with an interval between doses of at least 4 weeks) stimulates the pig's immune system to produce antibodies which can neutralize its own gonadotrophin releasing factor (GnRF). These antibodies bind to circulating GnRF in the bloodstream which leads to an inhibition of the hypothalamic-pituitary-gonadal endocrine axis. The ultimate physiological effect is suppression of testicular function including both steroidogenesis and reproductive function.

Boars given an initial dose of IMPROVEST (GnRF-Diphtheria Toxoid (DT) conjugate) are immunologically primed but retain their full testicular function until they receive the 2nd dose which induces a strong immune response and causes temporary immunological suppression of testicular function (immunological castration). IMPROVEST reduces the production of gonadal steroids, including testosterone and, by removing the inhibitory effect of testicular steroids on hepatic metabolism, may indirectly reduce levels of skatole, which is an amine compound derived from the amino acid tryptophan.

B. Dosage Characterization:

1. Type of Study: Clinical Field study

   a. Title: Study 3322E-60-04-305 Dose Justification of GnRF conjugate Product for Injection in Male Pigs

   b. Study Investigator and Study Location:

      Lindy F. Miller, PhD, PAS
      Terre Haute, IN

      LFM Quality Labs, Inc.
      Terre Haute, IN

   c. Study Design:

      1) Objective: The primary objective of this study was to evaluate the effectiveness of GnRF-DT conjugate, administered as 2 subcutaneous 2 mL (400 µg) injections at 18 and 22 weeks of age, to immunologically castrate intact male pigs and reduce boar taint at slaughter to a level comparable to that of physically castrated male pigs (barrows).

      2) Study Animals: One hundred sixty crossbred surgically castrated males (barrows; T01 and T02), 160 intact males (T03 and T04), and 16 sentinel boars (NTX), approximately 3 weeks of age (Day 0), were randomly allotted within castration status to either a 'low' lysine diet or to a 'high' lysine diet (Table 1). There were 10 pens for each of the 4 treatments with 8 pigs per pen.

The 16 sentinel boars were also assigned at random to a sentinel non-treated group (2 pens, 8 pigs per pen).

Table 1. Treatment and dosage structure used to determine dose justification of GnRF-DT conjugate product for injection in male pigs.

Treatment	Dosage	Regimen (age weeks)	Route of Administration	Animals per Treatment
T01 Surgically castrated fed 0.95 – 0.60% lysine diets	2.0 mL saline	2xSID [2] (18 and 22 weeks)	SC [3]	80 (10 pens of 8)
T02 Surgically castrated fed 1.15 – 0.78% lysine diets	2.0 mL saline	2xSID [2] (18 and 22 weeks)	SC [3]	80 (10 pens of 8)
T03 Immunocastrated fed 0.95 – 0.60% lysine diets	2.0 mL GnRF-DT conjugate [1]	2xSID [2] (18 and 22 weeks)	SC [3]	80 (10 pens of 8)
T04 Immunocastrated fed 1.15 – 0.78% lysine diets	2.0 mL GnRF-DT conjugate [1]	2xSID [2] (18 and 22 weeks)	SC [3]	80 (10 pens of 8)
NTX Entire males fed 1.15 – 0.78% lysine diets	2.0 mL saline	2xSID [2] (18 and 22 weeks)	SC [3]	16 (2 pens of 8)

[1] GnRF-DT conjugate contained ± 0.012% thimerosal as preservative.

[2] Single injection.

[3] Subcutaneous.

3) Test Article Administration: Animals received subcutaneous injections (2 mL of the GnRF-DT conjugate to boars and 2 mL saline to barrows) at 18 and 22 weeks of age (Day 105 and 133, respectively). Doses were delivered with an 18 gauge (5/8 inch) needle from a single dose syringe; a new needle was used for each pig. The formulation of GnRF-DT conjugate in this study contained nominal 400 µg GnRF-DT per 2 mL (API), 300 mg DEAE-Dextran in aqueous suspension per 2 mL (adjuvant), and ± 0.012 percent thimerosal (preservative). Physiological saline (sterile 0.9 percent sodium chloride), was used in the injection given to the barrows.

4) Measurement and Observations: In-life phase monitoring included daily general health observations, injection site observations, adverse event observations, body and feed weights, 10^{th} rib ultrasound scanning, collection of blood samples, and behavior assessment. At slaughter, testes and

bulbourethral weights were collected for measurements, as well as fat samples (last rib posterior) for olfactory (trained panel) and chemical (androstenone and skatole) analyses. The trained panel used a Visual Analog Scale (VAS) from 0 (nil odor) to 150 mm (strong odor).

5) Statistical Analysis: The primary effectiveness variables were boar taint (olfactory score, skatole and androstenone levels) and immunological castration (testosterone levels and anti-GnRF titer levels). The 5percent level of significance ($P \leq 0.05$) was used for all hypothesis tests (two-tailed).

d. Results: GnRF-DT conjugate treated boars had higher anti-GnRF antibody titers than barrows after the 2nd injection ($P < 0.0001$, Table 2). Treated boars and barrows did not differ in serum testosterone at 3 and 4 weeks after the 2nd injection (Table 3). Two cryptorchids were identified at slaughter: a treated boar that demonstrated low olfactory scores and a barrow that demonstrated high olfactory scores. Mean olfactory scores tended not to differ between treated boars (immunocastrates) and barrows ($P = 0.07$), unless the cryptorchid barrow was removed ($P = 0.0135$, Table 4).

Table 2. Geometric mean titer of anti-GnRF antibodies (U) in immunocastrates, barrows, and boars.

	Days of Age [a]							
Treatment	91	105	119	133	147	161	175	182
Immunocastrates	10.7 (n=40)	10.5 (n=40)	11.6 (n=40)	13.1 (n=40)	31.9 (n=40)	924.8 [b] (n=40)	645.6 [b] (n=40)	437.0 [b] (n=40)
Barrows	11.2 (n=40)	11.2 (n=40)	11.3 (n=40)	15.0 (n=40)	23.9 (n=39)	17.4 (n=39)	10.7 (n=39)	10.8 (n=49)
Boars	10.5 (n=4)	10.5 (n=4)	10.5 (n=4)	20.6 (n=4)	31.0 (n=4)	18.8 (n=4)	10.5 (n=4)	10.5 (n=4)

[a] The 1st and 2nd GnRF-DT conjugate injections were administered at 126 and 154 days of age, respectively.

[b] Immunocastrates differ ($P < 0.0001$) from barrows at time points indicated. Boars were not included in the statistical analysis.

Table 3. Serum testosterone (ng/mL) in immunocastrates, barrows, and boars.

Treatment	Days of Age [a]							
	91	105	119	133	147	161	175	182
Immunocastrates	1.116 [b] (n=40)	0.599 [b] (n=39)	2.079 [b] (n=40)	1.159 [b] (n=40)	1.745 [b] (n=40)	0.390 [b] (n=40)	0.097 (n=40)	0.144 (n=40)
Barrows	0.050 (n=40)	0.050 (n=40)	0.050 (n=39)	0.050 (n=40)	0.050 (n=39)	0.050 (n=39)	0.050 (n=39)	0.050 (n=49)
Boars	1.106 (n=4)	1.802 (n=4)	1.211 (n=4)	0.738 (n=4)	1.051 (n=4)	0.841 (n=4)	2.410 (n=4)	3.520 (n=4)

[a] The 1st and 2nd GnRF-DT conjugate injections were administered at 126 and 154 days of age, respectively.

[b] Immunocastrates differ ($P \leq 0.05$) from barrows at time points indicated. Boars were not included in the statistical analysis.

Table 4. Least squares means for olfactory scores (VAS, 0-150 mm) in immunocastrates, barrows, and boars.

Variable	Immunocastrates	Barrows (with / without Cryptorchid)	P value (with / without Cryptorchid)	Boars [1]
Olfactory, mm [2]	5.2	3.2 / 2.7	0.07 / 0.0135	45

[1] Arithmetic mean for boars.

[2] Cryptorchid immunocastrate had a mean olfactory score = 4.0 mm; cryptorchid barrow had a mean olfactory score = 82.8 mm.

e. Conclusions: This study supports the target dose of GnRF-DT conjugate of 400 µg per 2 mL dose volume in a thimerosal perservative formulation and the dosing regime of 2 doses administered subcutaneously at 18 and 22 weeks of age with slaughter at 26 weeks of age.

The above study was used to support a 2 mL dose of IMPROVEST, where the 1st dose was administered at 18 weeks of age and the 2nd dose was administered at 22 weeks of age. These animals were then slaughtered 4 weeks (26 weeks of age) after the 2nd injection. Current United States (U.S.) swine management programs provide for a variation in the timing of when pigs may be sent to slaughter (all in/all out vs. selection by body weight). Therefore, information was provided to demonstrate that administering the 2nd injection of IMPROVEST up to 8 weeks prior to slaughter is effective for the temporary immunological castration of intact male pigs (see Section II.C.1) and in reducing boar taint (as measured by aroma and flavor) in pork from treated intact males (see Section II.C.2.c).

Additionally, several international studies were used to support the "temporary immunological castration" portion of the indication. In these studies, thimerosal was used as a preservative in the formulation of the GnRF-DT conjugate. Thimerosal is an

organomercury compound that can have negative effects on the human central nervous system and kidneys. As a precautionary measure, several health organizations (including the FDA) have urged vaccine manufacturers to reduce or eliminate thimerosal in vaccines as soon as possible. [1] Therefore, the sponsor was asked to re-formulate their GnRF-DT conjugate with an alternative preservative. Based on data presented in Section II.C.1.1.d. and in Tables 9, 10, and 11, boars administered 2 doses of GnRF-DT conjugate preserved with 0.1 percent chlorocresol had similar anti-GnRF titers, circulating serum testosterone concentrations, and testes volume compared to boars treated with GnRF-DT conjugate preserved with 0.01 percent thimerosal. Based on the similarity in outcomes between the 2 formulations, the 0.1 percent chlorocresol preserved formulation of GnRF-DT conjugate was administered in the U.S. studies.

C. Substantial Evidence:

Substantial evidence was evaluated in 2 parts: 1) evidence to support the use of IMPROVEST to temporarily immunologically castrate (suppression of testicular function) intact males and 2) evidence to support the use of IMPROVEST to reduce boar taint in intact males intended for slaughter.

1. Temporary Immunological Castration (Suppression of testicular function)

The evaluation of an indication for the GnRF-DT conjugate related to temporary immunological castration (suppression of testicular function) was based on both foreign and U.S. studies, many of which were literature reviewed. These studies took into account its effects on immune and endocrine responses, testis size, animal behavior, and sperm production. Consideration of effects of the GnRF-DT conjugate also needed to take into account the duration of effect on these endpoints for greater than 8 weeks after the 2nd of 2 doses of the GnRF-DT conjugate, as the product labeling stated that *"Pigs should be slaughtered no earlier than 4 weeks and no later than 8 weeks after the 2nd dose"* (of the GnRF-DT conjugate). Specific endpoints evaluated were immunological, i.e., anti-GnRF antibody titers; hormonal, i.e., testosterone, luteinizing hormone (LH); testis size, i.e., weight or volume; and functional, i.e, sexual behavior and spermatogenesis. The hormonal and immunological endpoints represented biological markers for this indication and were viewed with their relationship to functional endpoints of immunological castration, i.e., sexual behavior and spermatogenesis.

[1] http://www.fda.gov/biologicsbloodvaccines/safetyavailability/vaccinesafety/ucm096228.htm

Discussion of the studies used to evaluate these endpoints to support temporary immunological castration are organized in the following manner:

1. Study Identification and Description
2. Immunological - Anti-GnRF antibody titers
3. Endocrine (hormonal; testis steroid production, pituitary LH)
4. Testis size
5. Behavior (sexual, aggressive, and social behaviors)
6. Spermatogenesis

Some of the studies described in this section included barrows (castrated boars), control boars (untreated or treated saline), and boars treated with the GnRF-DT conjugate. For the purposes of temporary immunological castration, the following discussion focused on comparisons between control boars and GnRF-DT conjugate-treated boars only. In addition, all international studies and some U.S. studies were conducted with the GnRF-DT conjugate containing 0.01 percent thimerosal as preservative, while some of the U.S. studies were conducted with the current GnRF-DT conjugate containing 0.1 percent chlorocresol. Study 3920E-60-06-382 (described below) compared the 2 formulations and found no differences in testis size, and immune and endocrine responses.

1. Study Identification and Description:

U.S. Studies

A list of studies the sponsor conducted in the United States appears in Table 5 below. A brief description of each study follows the table.

Table 5. List of U.S. study reports and endpoints evaluated in support of an indication for temporary immunological castration (suppression of testicular function).

	Endpoints Evaluated				
Report Identification:	Anti-GnRF Antibody Titers	Testosterone Concentrations	Testis Size	Sexual behavior	Spermato-genesis
Study 3322E-60-04-305 -Dose Justification of GnRF conjugate Product for Injection in Male Pigs.	x	x	x	x	
Study 3322C-60-04-326 - Multi-Location Clinical Study to Evaluate the Effectiveness of a GnRF conjugate for Injection product to Control Boar Taint in Intact Male Pigs at Slaughter.	x	x	x		
Study 3822C-60-09-760 - Immunization of Intact Male Pigs with IMPROVEST to Control Boar Taint in Market Aged Pigs.	x	x	x	x	
Study 3920E-60-06-382 - Clinical Evaluation of Different Formulations of GnRF conjugate for Injection Containing Alternative Preservatives to Thimerosol.	x	x	x		
Study 3920C-60-05-344 - Impact of Flexible Timing of First and Second Dose of a GnRF conjugate for Injection and Interval to Slaughter on Boar Taint in Male Market Pigs.	x	x	x		
Study 3920W-60-06-466 - Preliminary Evaluation of the Duration of Effect of GnRF conjugate for Injection in Intact Male Pigs.	x	x	x		

a. Study 3322E-60-04-305:

1. Investigator and Study Location:

Lindy F. Miller, PhD, Terre Haute, IN

2. Methods:

Boars were treated with 2 subcutaneous injections of saline or GnRF-DT conjugate (400 µg) at 18 and 22 weeks of age. There were 20 pens of 8 boars in the GnRF-DT conjugate treatment group, and 2 pens of 8 boars in the saline treatment group. Two pigs from each pen were randomly selected for blood collection (anti-GnRF titers and testosterone concentrations). Animals were slaughtered at 26 weeks of age, and weights of testes and bulbourethral glands recorded. Two pens, at random from each treatment, were evaluated for mounting and aggressive behavior (4 minutes per pen in the afternoon, weekly from 16-26 weeks of age).

b. Study 3322C-60-04-326:

1. Investigator and Study Location: This study was conducted at 9 locations in the United States (Table 6):

Table 6. Names of investigators and their locations.

Investigator	Study Location
"In-Life" phase	
J. Jeffrey Chewning, PhD	Summers, AR
Jeffrey Harker, DVM	Frankfort, IN
Lyle Kesl, DVM, PhD	Ames, IA
Kelly Lechtenberg, DVM, PhD	Oakland, NE
Dale Mechler, DVM	Algona, IA
Lindy Miller, PhD	Terre Haute, IN
John Waddell, DVM, MBA	Sutton, NE
Nathan Winkelman, DVM	Rice, MN
Michael Cox, DVM	Sutton, NE

Table 6. Names of investigators and their locations.	
Investigator	Study Location
Sample processing	
John Ymker	Sioux Center, IA
Ken Prusa, PhD	Ames, IA
Consumer panel evaluation	
Ziad Matta	Chicago, IL

2. Methods:

Boars were placed into 3 different treatment groups: 1) saline (17 and 21 weeks of age); 2) 400 µg GnRF-DT conjugate (9 and 21 weeks of age); and 3) 400 µg GnRF-DT conjugate (17 and 21 weeks of age). Animals were slaughtered at 25-27 weeks of age, or 4-6 weeks after the 2nd dose of the conjugate. Blood samples were collected from all pigs (anti-GnRF titers and testosterone concentrations). Testes volume was determined at the time of slaughter. A fourth group of surgically castrated (barrows, treated with saline at 17 and 21 weeks of age) was also included to determine consumer acceptability of pork against GnRF-DT conjugate treated males.

At the time of slaughter, barrow and immunocastrate carcasses were processed to remove the loins for further processing into boneless loin chops. Pork chops were shipped to 1 of 4 geographically separated consumer testing sites to be evaluated for aroma and flavor based on a 9 point descriptive scale. Pork was considered acceptable if at least 2 of the 3 consumers found the samples acceptable for both aroma and flavor.

c. Study 3822C-60-09-760:

1. Investigator and Study Location:

Paul Runnels, DVM, PhD, Richland, MI

2. Methods:

Boars were treated with saline or GnRF-DT conjugate (400 µg) at 18 and 22 weeks of age, and were slaughtered at approximately 26 weeks of age. Blood samples were collected from all pigs (anti-GnRF titers and testosterone concentrations). Scrotal width was measured as an estimate of testis size. Pens of pigs were observed for boar behavior (mounting, aggression, and other) twice per

week (mornings, 5 minutes per 2 adjacent pens) from the time of the 1st dose of the GnRF-DT conjugate through the end of the study.

d. Study 3920E-60-06-382:

1. Investigator and Study Location:

Lindy F. Miller, PhD, Terre Haute, IN

2. Methods:

The purpose of this study was to determine a suitable preservative for the GnRF-DT conjugate to replace 0.01 percent thimerosal used in an earlier formulation. Alternatives evaluated were:

-non-preserved
-3 mg/ml m-cresol + 1 percent benzyl alcohol
-2 percnet benzyl alcohol
-0.1 percent chlorocresol

Boars were treated with saline, GnRF-DT conjugate (400 µg; chlorocresol-preserved), or GnRF-DT conjugate (400 µg; thimerosal-preserved) at 18 and 22 weeks of age, and euthanized at 26 weeks of age. Blood samples were collected from all pigs (anti-GnRF titers and testosterone concentrations). Testes volume was determined at the time of euthanasia.

Based on the results of this study, the sponsor chose to pursue the GnRF-DT conjugate preserved with 0.1 percent chlorocresol (IMPROVEST). The sponsor found that anti-GnRF titers, testosterone concentrations, and testes volume was similar between boars treated with the GnRF-DT conjugate preserved with thimerosal and chlorocresol. Results presented below only included 3 treatment groups: 1) saline-treated boars; 2) GnRF-DT conjugate-treated boars (chlorocresol-preserved); and 3) GnRF-DT conjugate-treated boars (thimerosal-preserved).

e. Study 3920C-60-05-344:

1. Investigator and Study Location:

Kelly Lechtenberg, DVM, PhD, Oakland, NE

2. Methods:

This study was initiated to examine different timings for the 1st and 2nd doses of the GnRF-DT conjugate. The particular GnRF-DT conjugate treatment group described below and for which results will subsequently be discussed represent

the treatment group that complied with labeling directions: 1) administer the 1st dose no earlier than 9 weeks of age; 2) administer the 2nd dose at least 4 weeks after the 1st dose; and 3) slaughter pigs no earlier than 4 weeks and no later than 8 weeks after the 2nd dose.

Boars were treated with saline or 2 doses of the GnRF-DT conjugate (400 µg; 9 and 20 weeks of age), and were slaughtered at 26 weeks of age. Blood samples were collected from all pigs (anti-GnRF titers and testosterone concentrations). Testes volumes were determined at the time of slaughter.

f. Study 3920W-60-06-466:

1. Investigator and Study Location:

Michael J. Prough, Richland, MI

2. Methods:

The purpose of this study was to examine the effect of the dose of GnRF-DT conjugate on anti-GnRF titers, testosterone, and scrotal width. The following treatment groups were used:

-saline
-20 µg GnRF-DT conjugate
-100 µg GnRF-DT conjugate
-300 µg GnRF-DT conjugate
-500 µg GnRF-DT conjugate

Treatments were administered when boars were 12 and 16 weeks of age. Scrotal width was measured and blood samples were collected (anti-GnRF titers and testosterone concentrations) were collected at 4 week intervals from 7, 11, 15, 19, 23, 28, and 32 weeks after the 2nd dose of GnRF-DT conjugate.

International Study Reports

A list of international study reports are listed in Table 7 below. These reports were with the use of the GnRF-DT conjugate preserved with thimerosal. A brief description of each study report follows the table.

Table 7. List of international study reports, and endpoints evaluated in support of an indication for temporary immunological castration (suppression of testicular function).

	Endpoints Evaluated				
Report Identification:	Anti-GnRF Antibody Titers	Testosterone Concentrations	Testis Size	Sexual behavior	Spermato-genesis
Study 9320C-03-07-271 - Efficacy of Boar Taint Control Ten Weeks After the Second of Two Doses of Minimum Potency Improvac™ Given Four Weeks Apart. (U.K.)	x	x			
Study 9322C-07-05-204 - To Demonstrate the Safety and Efficacy of Improvac® in Controlling Boar Taint in Heavy Male Pigs Under Commercial Field Conditions in Italy. (Italy)	x	x			
IRTA Study Report # 31203 (Agrifood Research and Technology Institute, Spain). Performance and qualitative effects of applying the vaccine IMPROVAC to grow-finish pigs. A study carried out under both experimental and commercial conditions. (Spain)			x	x	

a. Study 9320C-03-07-271:

1. Study Location:

Hertfordshire, U.K.

2. Methods:

Boars were treated with saline or 2 doses of GnRF-DT conjugate at approximately 11 and 15 weeks of age. Blood samples (anti-GnRF titers and testosterone concentrations) were collected from study animals at the time of the 1st dose of the GnRF-DT conjugate, and at 4 and 10 weeks after the 2nd dose of the conjugate. Animals were slaughtered at 10 weeks after the 2nd dose.

b. Study 9322C-07-05-204:

1. Study Location:

Cremona, Italy

2. Methods:

There were 3 treatment groups in this study:

-surgically castrated at birth
-2 doses of GnRF-DT conjugate at 10-11 and 26-27 weeks of age
-3 doses of GnRF-DT conjugate at 10-11, 26-27, and 36-37 weeks of age

Blood samples (anti-GnRF titers and testosterone concentrations) were collected from study animals at the time of each dose of the GnRF-DT conjugate, 2 weeks after the 3rd dose (in animals receiving a 3rd dose), and at slaughter (40-41 weeks of age).

c. Agrifood Research and Technology Institute (IRTA Report 31203):

1. Study Location:

Monells (Girona), Spain

2. Method:

Boars were treated with 2 mL of the GnRF-DT conjugate at approximately 11 and 21 weeks of age, and were slaughtered 4 weeks following the 2nd dose of the conjugate. Paired testes weights were determined at slaughter. Animal behaviors were videotaped for 2 hours on given dates during the study; observations were transcribed from the video recordings. The most notable observational periods were at 3 times following the 2nd dose of the GnRF-DT conjugate: 1) 2-3 days

after the 2nd dose; 2) 2 weeks after the 2nd dose; and 2 days before slaughter (2-4 weeks after the 2nd dose). During these observational periods, behavior was categorized as 1) active (standing, drinking, or eating) vs. inactive (sitting or laying down); 2) aggression (at or away from the feeder), and 3) sexual (mounting behavior).

Published Manuscripts of International Studies

A list of manuscripts of international studies is provided in Table 8 below. The study designs generally followed that of the designs employed with the reports listed in Tables 5 and 7 above. Two doses of GnRF-DT conjugate were given at 4-8 week intervals, with variable observation periods after the 2nd dose to characterize immune and endocrine responses, testis size, sexual behavior, and sperm production. These published reports generally corroborated the U.S. and international study reports on the effects of the GnRF-DT conjugate on anti-GnRF titers, circulating concentrations of testosterone, and testis size. The published manuscripts also provided the primary information with respect to the duration of effect of the GnRF-DT conjugate (> 8 weeks after the 2nd dose of the conjugate), sexual behavior, and spermatogenesis. For those manuscripts that provided the primary information on sexual behavior and spermatogenesis, a more detailed description of the study designs was provided when discussing study results.

All published manuscripts reported the use of the GnRF-DT conjugate preserved with thimerosal.

Table 8. List of international reports in the scientific literature, and endpoints evaluated in support of an indication for temporary immunological castration (suppression of testicular function).

	Endpoints Evaluated				
Manuscript Identification:	Anti-GnRF Antibody Titers	Testosterone Concentrations	Testis Size	Sexual behavior	Spermato-genesis
Claus K, Lacorn M, Danowski K, Pearce MC, Bauer A. 2007. Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. Vaccine 25:4689-4696. (Germany)	x	x			
Claus R, Rottner S, Ruckert C: 2008. Individual return to Leydig cell function after GnRH-immunization of boars. Vaccine 26:4571-4578. (Germany)	x	x			
Cronin G, Dunshea F, Butler K, McCauley I, Barnett J and Hemsworth P. 2003. The effects of immuno- and surgical castration on behaviour and consequently growth of group-housed male finisher pigs. Appl Anim Behav Sci. (2003) 81(2):111-126. (Australia)				x	
Einarsson S, Andersson K, Wallgren M, Lundstrom L, Rodriguez-Martinez H. 2009. Short- and long-term effects of immunization against gonadotropin-releasing hormone, using Improvac, on sexual maturity, reproductive organs and sperm morphology in male pigs. Theriogenology 71:302-310. (Sweden)					x

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	Endpoints Evaluated				
Manuscript Identification:	Anti-GnRF Antibody Titers	Testosterone Concentrations	Testis Size	Sexual behavior	Spermato-genesis
Fuchs T, Thun, R, Parvizi N, Nathues H, Koehrmann A, Andrews S, Brock F, Klein G, Sudhaus N, grosse Beilage E. 2009. Effect of a gonadotropin-releasing factor vaccine on follicle stimulating hormone and luteinising hormone concentrations and on the development of testicles and the expression of boar taint in male pigs. Theriogenology 72: 672-680. (Germany)			x		
Hilbe, M., Jaros, P., Ehrensperger, F., Zlinszky, K., Janett, F., Hässig, M., Thun, R. 2006. Histomorphological and immunohistochemical findings in testes, bulbourethral glands and brain of immunologically castrated male piglets. Schweiz. Arch. Tierheilk. 148, 599-608. (Switzerland)			x		x
Rottner, S., R. Claus. 2009. Return of testicular function after vaccination of boars against GnRH. Animal 3, 1279-1286. (Germany)			x		x
Rydhmer L, Lundström K, Andersson K. 2010. Immunocastration reduces aggressive and sexual behaviour in male pigs. Animal pp.1-8. doi:10.1017/S175173111000011x. (Sweden)				x	

Table 8. List of international reports in the scientific literature, and endpoints evaluated in support of an indication for temporary immunological castration (suppression of testicular function).

	Endpoints Evaluated				
Manuscript Identification:	Anti-GnRF Antibody Titers	Testosterone Concentrations	Testis Size	Sexual behavior	Spermato-genesis
Wagner A and Claus R. 2004. Involvement of glucocorticoids in testicular involution after active immunization of boars against GnRH. Reproduction 127:275-283.		x	x		x
Zamaratskaia G, Andersson HK, Chen G, Andersson K, Madej A, Lundström K. 2008a. Effect of a gonadotropin-releasing hormone vaccine (Improvac®) on steroid hormones, boar taint compounds and performance in entire male pigs. Reproduction in Domestic Animals 43(3):351-359. (Sweden)	x	x	x		
Zamaratskaia G, Rydhmer L, Andersson HK, Chen G, Lowangie S, Andersson K, Lundström. 2008b. Long-term effect of vaccination against gonadotropin-releasing hormone, using Improvac™, on hormonal profile and behaviour of male pigs. Animal Reproduction Science 108:37-48. (Sweden)	x	x	x	x	

Results

1. Anti-GnRF Antibody Titers

Results from the U.S. studies for anti-GnRF antibody titers are presented in Table 9. These data demonstrated that antibody titers increased 4 weeks after the 2nd dose of the GnRF-DT conjugate, and for those studies that followed titers for longer periods of time, remained increased for 6-11 weeks after the 2nd dose of the conjugate. In addition, international reports showed that anti-GnRF antibodies remained elevated through at least 12 weeks following the 2nd dose of the GnRF-DT conjugate (Study # 9322C-07-05-204, Study # 9320C-03-07-271, Claus et al., 2007 and 2008, Zamaratskaia et al., 2008a and 2008b; data not shown).

Therefore, the anti-GnRF antibody titers remain elevated for at least 12 weeks following the 2nd dose of the GnRF-DT conjugate, thereby supporting a duration of effect beyond the 8 week period after the 2nd dose of the conjugate specified on product labeling.

2. Endocrinology

Results from the U.S. studies for circulating testosterone concentrations are presented in Table 10. Testosterone concentrations were dramatically reduced in boars given the GnRF-DT conjugate vs. saline-treated boars at 4 weeks after the 2nd dose of the conjugate, and at 6-7 weeks following the 2nd dose in those studies that observed boars beyond 4 weeks. Results from international studies corroborated these results, and demonstrated that testosterone remained low in GnRF-DT conjugate-treated boars for up to 16 weeks following the 2nd dose of the conjugate (Study # 9320C-03-07-271; Study # 9322C-07-05-204; Claus et al., 2007 and 2008; Wagner and Claus, 2004; Zamaratskaia et al., 2008a and 2008b; data not shown). In addition, 1 U.S. study (Study # 3920W-60-06-466) and 2 international studies (Wagner and Claus, 2004; Claus et al., 2008) showed that similar to the reduction in testosterone concentrations, luteinizing hormone (LH) was reduced in GnRF-DT conjugate vs. saline-treated boars (data not shown).

Taken together, the results of the U.S. and international studies showed a reduction in testosterone secretion in GnRF-DT conjugate-treated boars through 16 weeks after the 2nd dose of the conjugate, supporting a duration of effect beyond the 8 week period after the 2nd dose of the conjugate specified on product labeling.

3. Testis size

Results from the U.S. studies for testis size are presented in Table 11. These data demonstrate that treatment of boars with the GnRF-DT conjugate resulted in reduced testis size through 4-6 weeks after the 2nd dose of the conjugate when

compared to saline-treated boars. This was consistent with results from international studies, which showed reduced testis size for 4-22 weeks after the 2nd dose of the GnRF-DT conjugate (IRTA Study Report # 31203; Fuchs et al., 2009; Hilbe et al., 2006; Rottner and Claus, 2009; Wagner and Claus, 2004; Zamaratskaia et al., 2008a and 2008b).

Taken together, results of the U.S. and international studies demonstrated that use of the GnRF-DT conjugate reduced testis size through 22 weeks after the 2nd dose of the conjugate, supporting a duration of effect beyond the 8 week period after the 2nd dose of the conjugate specified on product labeling.

4. Consumer acceptability of pork

Consumer acceptability data was analyzed as a non-inferiority test based on a 0.05 significance level (one-sided), with a non-inferiority margin of 10 percentage points on the original scale. A frequency of 8.4 percent of barrows, 10.6 percent of male pigs dosed with IMPROVEST at 9 and 21 weeks of age and 8.0 percent of male pigs dosed with IMPROVEST at 17 and 21 weeks of age were scored as not acceptable by the consumers (Table 12). Consequently, pork from both GnRF-DT conjugate treated groups was considered to be non-inferior to pork from surgically castrated males.

This study demonstrates that consumers, representative of the U.S. population, found pork from IMPROVEST treated boars to be similarly acceptable as pork from barrows.

Table 9. Anti-GnRF antibody titers in boars treated with Saline or the GnRF-DT conjugate (Conjugate) at specified times relative to the 2nd of 2 doses of the Conjugate in studies conducted in the United States.

			Anti-GnRF Antibody Titers (U)			
Study ID	(N)	Treatment Group	At 2nd Dose	4 weeks After 2nd Dose	At End of Observation Period (weeks after 2nd dose)	Probability
3322E-60-04-305 [1]	4	Saline	31.0	10.5	(4 weeks)	
	40	Conjugate (400 µg)	31.9	437.0	"	
3322C-60-04-326 [2]	208	Saline	17.9	14.1	(4-6 weeks)	
	430	Conjugate 1 (400 µg) [3]	22.1	117.6	"	
	479	Conjugate 2 (400 µg) [3]	42.0	338.5	"	
3822C-60-09-760 [2]	31	Saline	10.3 (1st dose)	9.5	(4 weeks)	P < 0.05
	31	Conjugate (400 µg)	10.7	337.0		
3920E-60-06-382 [1,2]	20	Saline	9.0	9.0	(4 weeks)	P < 0.05
	20	Conjugate (400 µg) [1]	10.5	496.2		
	20	Conjugate (400 µg) [2]	9.0	491.3		
3920C-60-05-344 [1]	40	Saline	9.0	9.0	9.0 (6 weeks)	P < 0.05
	40	Conjugate (400 µg)	9.2	122.4	75.4	
3920W-60-06-466 [1]	5	Untreated			15.83 (11 weeks) [4]	
	7	Conjugate (20 µg)			45.08	
	6	Conjugate (100 µg)			47.47	
	7	Conjugate (300 µg)			73.05	
	6	Conjugate (500 µg)			55.73	

[1] GnRF-DT conjugate contained 0.01% thimerosal as preservative (IMPROVAC).

[2] GnRF-DT conjugate contained 0.1% chlorocresol as preservative (IMPROVEST).

[3] In Study 3322C-60-04-326, boars were 2 doses of conjugate at 9 and 21 weeks of age (Conjugate 1) or 17 and 21 weeks of age (Conjugate 2).

[4] In Study 3920W-60-06-466, antibody titers remained elevated through 11 weeks after 2nd dose on conjugate treated boars, and returned to baseline by 15 weeks.

Table 10. Circulating testosterone concentrations (ng/mL) in boars treated with Saline or the GnRF-DT conjugate (Conjugate) at specified times relative to the 2nd of 2 doses of the Conjugate in studies conducted in the United States.

			Circulating Testosterone (ng/mL)			
Study ID	(N)	Treatment Group	At 2nd Dose	4 weeks After 2nd Dose	At End of Observation Period (weeks after 2nd dose)	Probability (last observation)
3322E-60-04-305 [1]	4	Saline	1.05	3.52	(4 weeks)	
	40	Conjugate (400 µg)	1.75	0.14	"	
3322C-60-04-326 [2]	208	Saline	4.03	7.36	(4-6 weeks)	
	429	Conjugate 1 (400 µg) [3]	3.53	0.12	"	
	478	Conjugate 2 (400 µg) [3]	3.85	0.18	"	
3822-C-60-09-760 [2]	31	Saline	1.90 (1st dose)	3.59	(4 weeks)	P < 0.05
	31	Conjugate (400 µg)	1.98 (1st dose)	0.27		
3920E-60-06-382 [1,2]	20	Saline	5.29	6.88	(4 weeks)	P < 0.05
	20	Conjugate (400 µg) [1]	5.95	0.05		
	20	Conjugate (400 µg) [2]	6.11	0.19		
3920C-60-05-344 [1]	40	Saline	0.78	1.11	2.46 (6 weeks)	P < 0.05
	40	Conjugate (400 µg)	0.80	0.19	0.309	
3920W-60-06-466 [1]	5	Saline			0.90 (7 weeks) [4]	
	7	Conjugate (20 µg)			1.41	
	6	Conjugate (100 µg)			1.68	
	7	Conjugate (300 µg)			0.49	
	6	Conjugate (500 µg)			0.48	

[1] GnRF-DT conjugate contained 0.01% thimerosal as preservative (IMPROVAC).

[2] GnRF-DT conjugate contained 0.1% chlorocresol as preservative (IMPROVEST).

[3] In Study 3322C-60-04-326, boars were 2 doses of conjugate at 9 and 21 weeks of age (Conjugate 1) or 17 and 21 weeks of age (Conjugate 2).

[4] In Study 3920W-60-06-466, antibody titers remained elevated through 7 weeks after 2nd dose on conjugate treated boars, after which testosterone was comparable across treatment groups.

Table 11. Testis size in boars treated with Saline or the GnRF-DT conjugate (Conjugate) at specified times relative to the 2nd of 2 doses of the Conjugate in studies conducted in the United States.

Study ID	(N)	Treatment Group	Testis size	Time of measurement after 2nd dose	Probability
3322E-60-04-305 [1]	4	Saline	700.9 [5]	4 weeks	
	40	Conjugate (400 µg)	320.2	"	
3322C-60-04-326 [2]	184	Saline	528.9 [6]	4-6 weeks	
	404	Conjugate 1 (400 µg) [3]	215.8	"	
	444	Conjugate 2 (400 µg) [3]	212.9	"	
3822C-60-09-760 [2]	31	Saline	5.855 [7]	4 weeks	P < 0.05
	31	Conjugate (400 µg)	4.505	"	
3920E-60-06-382 [1,2]	20	Saline	258.6 [6]	4 weeks	P < 0.05
	20	Conjugate (400 µg) [1]	125.6		
	20	Conjugate (400 µg) [2]	123.9		
3920C-60-05-344 [1]	40	Saline	310.1 [6]	6 weeks	P < 0.05
	40	Conjugate (400 µg)	137.6	"	
3920W-60-06-466 [1]	5	Saline	5.36 [7, 8]		
	7	Conjugate (20 µg)	5.40		
	6	Conjugate (100 µg)	4.68		
	7	Conjugate (300 µg)	2.38		
	6	Conjugate (500 µg)	2.99		

[1] GnRF-DT conjugate contained 0.01% thimerosal as preservative (IMPROVAC).

[2] GnRF-DT conjugate contained 0.1% chlorocresol as preservative (IMPROVEST).

[3] In Study 3322C-60-04-326, boars were 2 doses of conjugate at 9 and 21 weeks of age (Conjugate 1) or 17 and 21 weeks of age (Conjugate 2).

[4] In Study 3920W-60-06-466, antibody titers remained elevated through 7 weeks after 2nd dose on conjugate treated boars, after which testosterone was comparable across treatment groups.

[5] Testis size reported as weight (g).

[6] Testis size reported as volume (cm^3).

[7] Testis size reported as scrotal width (inches).

[8] In Study 3920W-60-06-466, scrotal width was decreased in boars given the 300 and 500 µg doses of conjugate vs. saline-treated boars through 11 weeks after 2nd dose; scrotal width was similar among groups by 15 weeks.

Table 12. Consumer acceptability of pork from barrows (surgical castrates) and IMPROVEST treated male pigs (T02 and T03).

Description	Treatment	Animal Acceptable				Total Obs.
		YES		NO		
		Number	%	Number	%	
Barrows	T01	438	91.6	40	8.4	478
IMPROVEST 9 wk / 21 wk	T02	381	89.4	45	10.6	426 [1]
IMPROVEST 17 wk / 21 wk	T03	439	92.0	38	8.0	477

[1] Fewer total observations due to T02 animals being removed from Site B due to misdosing.

5. Behavior

a. Study # 3222E-60-04-305:

In this study, randomly-selected pens from each treatment group were evaluated for mounting and aggressive behavior (4 minutes per pen in the afternoon, weekly from 16-26 weeks of age; n = 16 for saline-treated boars, n = 32 for GnRF-DT conjugate-treated boars). Treatments were applied when boars were 18 and 22 weeks of age.

Mounting and aggressive behaviors were minimal prior to 22 weeks of age, and were similar between treatment groups. At 23-26 weeks of age (1-4 weeks after the 2nd dose of the GnRF-DT conjugate), mounting and aggressive behavior was greater in saline vs. conjugate treated boars.

b. Study # 3822C-60-09-760:

Boars from saline (n = 31) and GnRF-DT conjugate (n = 31) were observed for mounting, aggressive, and other sexual behavior (i.e., attempted mount, erection, flanking, sniffing prepuce) twice per week (mornings, 5 minutes per 2 adjacent pens) from the time of the 1st dose of the conjugate through the end of the study. Treatments were applied when boars were 18 and 22 weeks of age.

Overall, there were minimal differences in mounting, aggressive, and other behaviors between the 2 treatment groups.

c. IRTA Study Report # 31203:

Behaviors were compared between control (n = 24) and GnRF-DT conjugate-treated (n = 24) boars given the conjugate at a 10 week interval, with slaughter occurring 4 weeks after the 2nd dose. Observations were transcribed from

video recordings collected at 2 hour intervals during a given date at 6 time points in the study:

-at transfer of animals to monitoring barn
-2 and 3 days after the 1st dose of the GnRF-DT conjugate
-2 days prior to the 2nd dose of the GnRF-DT conjugate
-2 and 3 days after the 2nd dose of the GnRF-DT conjugate
-2 weeks after the 2nd dose of the GnRF-DT conjugate
-2 days before slaughter (~ 4 weeks after the 2nd dose of the GnRF-DT conjugate)

Behavior was categorized as 1) active (standing, drinking, or eating) vs. inactive (sitting or laying down); 2) aggression (at or away from the feeder), and 3) sexual (mounting behavior).

More control boars vs. boars given the GnRF-DT conjugate were categorized as "active" at 2 weeks after the 2nd dose of the conjugate and 2 days prior to slaughter. Similar, aggressive behaviors were higher in control boars vs. boars given the GnRF-DT conjugate, but only at 2 days prior to slaughter. At all time points after the 2nd dose of the GnRF-DT conjugate, control boars demonstrated increased sexual activity (mounting) compared to boars given the conjugate.

d. Cronin et al., 2003:

This manuscript described the collection and evaluation of behavior (feeding, social, aggressive, sexual) of control (n = 60) and GnRF-DT conjugate-treated (n = 60) boars given the conjugate at 14 and 18 weeks of age. Behaviors were first documented in a 24 hour time-lapse video recording for each pen of pigs. Recording was at 17 and 21 weeks of age (3 weeks after the 1st and 2nd doses of the GnRF-DT conjugate). Observations were transcribed from the video and reported as a percent of the pigs' time per 24 hours.

At 17 weeks of age (prior to 2nd dose of the GnRF-DT conjugate), there were no differences between control boars and boars given the conjugate with respect to feeding, social, aggressive, and sexual behaviors. At 21 weeks of age (3 weeks after the 2nd dose), social, aggressive, and sexual behaviors were reduced in boars given the GnRF-DT conjugate vs. control boars; feeding behavior did not differ.

e. Rydhmer et al., 2010:

This manuscript described the collection and evaluation of sexual and social behavior in control (n = 64) and GnRF-DT conjugate-treated (n = 48) boars given the conjugate (2 doses at 8 and 4 weeks prior to slaughter). Visual behavioral observations were recorded at 1 week before and 1 week after the

1st dose of the GnRF-DT conjugate, and 1 and 3 weeks after the 2nd dose of the conjugate. Observations were performed between 1000 and 1530 h, with 9 observation rounds per pen in consecutive pen order, with each pen observed for 10 minutes per session in each round.

At 1 and 3 weeks after the 2nd dose of the GnRF-DT conjugate, control boars displayed more sexual/mounting, aggressive, and social behaviors than those boars given the conjugate. Given the short duration after the 2nd dose of the GnRF-DT conjugate in which observations were made (1-3 weeks after the 2nd dose), results in this report only provide information with respect to a reduction in sexual and aggressive behaviors shortly after treatment (i.e., ~ 1 week after the 2nd dose).

f. Zamaratskaia et al., 2008b:

This manuscript described the collection and evaluation of sexual and social behavior in control and GnRF-DT treated boars (n = 12 per group; 2 doses 4-6 weeks apart). Behavior was videotaped from approximately 0800-1700 h during weeks 4, 6, 8, 10, 13, 15, 19, and 21 after the 2nd dose of the GnRF-DT conjugate. Study animals were managed in 2 groups such that both groups were not necessarily represented for all weeks (e.g., only the 2nd group of animals was observed at 19 and 21 weeks). Behavior of all animals in the monitored pens (2-3 pigs) was recorded each minute to obtain the percentage of time spent on the following activities:

-sleeping (lying down, resting)
-walking (sitting, standing, rooting in bedding, walking, running)
-eating (head in trough or waiting for food beside trough or drinking)
-social (several pigs interacting in a non-aggressive way)
-manipulating (nibbling/pushing another pig; has another pig's tail/ear in mouth)
-aggressive (giving or receiving head-knocks or bites)
-mounting (mounting or being mounted)

In addition, expression of sexual behavior was examined in a 7 minute mating test performed 9 and 15 weeks after the 2nd dose for both groups, and 21 weeks for the 2nd group. An observed male was left alone in a pen followed by the entrance of a female in estrus (standing reflex 10 hours prior to mating test). The observation period started when the male first recognized the female's presence in the pen, and ended as soon as the male mounted the female. To prevent fertilization, the pigs were separated at the time of mounting, if mounting occurred. The test was also stopped if the pigs began fighting.

The average percent of day time spent exhibiting social, manipulating, aggressive, and mounting behaviors over the entire study period (up to

22 weeks after the 2nd dose) was greater in control boars vs. boars given the GnRF-DT conjugate. There were no differences in sleeping, walking, and eating behaviors.

Results for the mating study were reproduced in Table 13 below.

Table 13. Sexual behavior of control and GnRF-DT conjugate-treated in a 7-minute mating test performed at 9, 15, and 21 [a] weeks after the 2nd dose of the conjugate.

Behavior	Control Boars		GnRF-DT conjugate Boars	
	Number	Median (min)	Number	Median (min)
Week 9	8		10	
Nose genital	5	1	6	3
Nose/lift side	6	1	5	3
Mount attempt	1	6	0	
Mounting	6	2	0	
End of test [b]	1	4	1	1
Week 15	10		12	
Nose genital	5	1	6	2
Nose/lift side	5	1	5	2
Mount attempt	0		2	3
Mounting	10	2	0	
End of test [b]	0		2	3
Week 21 [a]	6		6	
Nose genital	1	1	4	2
Nose/lift side	1	1	3	3
Mount attempt	0		0	
Mounting	6	1	0	
End of test [b]	0		0	

[a] Numbers of animals tested at 21 weeks included only those from the 2nd group of pigs.
[b] Tests were stopped due to severe fighting.

Results of the mating tests showed that boars given the GnRF-DT conjugate demonstrated no mounting activity at any of the 3 test periods at 9, 15, and 21 weeks after the 2nd dose of the conjugate (2 attempts to mount at 15 weeks). For control boars, 6 of 8 mounted estrous females at week 9, while all tested boars mounted estrous females at 15 and 21 weeks. These results showed that mounting activity was greatly reduced in boars given the GnRF-DT conjugate vs. the control boars.

The videotaped results of the time spent in various social, aggressive, and sexual behaviors, along with the results of the mating tests, demonstrate sexual behavior was dramatically reduced through 21-22 weeks after the 2nd dose of the GnRF-DT conjugate, and covered the timeframe specified for temporary immunological castration on labeling (8 weeks after the 2nd dose).

Conclusions on Animal Behavior

Results from the studies described in this section indicated that treatment of boars with the GnRF-DT conjugate interrupted sexual behavior for a period of up to 22 weeks after administration of the 2nd of 2 doses of the conjugate.

6. Spermatogenesis

Reports on the effects of the GnRF-DT conjugate on spermatogenesis utilized histological evaluation of preserved testicular tissue to evaluate presence or absence of spermatogenic cells at various stages of maturity. Evaluated were the presence and quantity of spermatogenic cell types from the least mature to the most mature: type A and B spermatogonia, primary and secondary spermatocytes, round and elongated spermatids, and spermatozoa. One report also examined the morphological difference in spermatozoa collected from the epididymis as an index of sexual maturity.

a. Einarsson et al., 2009:

This manuscript presented results of 2 experiments evaluating the effect of 2 doses (6 to 10 weeks apart) of the GnRF-DT conjugate on spermatogenesis based on histological sections of testicular samples, and the morphology of spermatozoa collected from the epididymis (assessment of sexual maturity). In both experiments, there were control boars and boars given 2 doses of the GnRF-DT conjugate. The 1st dose was given at 12-15 weeks of age, and the 2nd dose was given at 18-22 weeks of age. Animals were slaughtered at 4 weeks after the 2nd dose of the GnRF-DT conjugate in the 1st experiment (n = 24 control, n = 31 conjugate), and 16 (n= 5 control, n = 6 conjugate) or 22 (n= 5 control, n = 6 conjugate) weeks after the 2nd dose of the conjugate in the 2nd experiment.

With respect to spermatogenesis, the authors noted that spermatogenesis was fully-developed (presence and numbers of all spermatogenic cell types) in control boars slaughtered at 16 and 22 weeks after the 2nd dose of the GnRF-DT conjugate. While at 4 weeks after the 2nd dose, control boars possessed more variation in spermatogenesis than at the later ages, they were considered to fall within the normal range for spermatogenesis. For boars given the GnRF-DT conjugate, there was mild to severe disruption of spermatogenesis, with some considered to be mild (spermatocyte loss with decreased number of layers of germ cells) to severe loss (i.e., complete loss) of germ cells.

Disruption of spermatogenesis based on histological evaluation did not differ among GnRF-DT conjugate-treated boars at 4, 16, and 22 weeks after the 2nd dose of the conjugate.

These authors also collected spermatozoa from the epididymis of study boars to evaluate potential differences in morphology as a basis to assess sexual maturity. After spermatogenesis proper, when spermatozoa are released into the lumen of seminiferous tubules and transported to and through the epididymis, further morphological changes must occur in order for spermatozoa to attain fertilizing capacity. One such change related to sexual maturity is the loss of proximal (to the sperm head) cytoplasmic droplets. The authors previously correlated sexual maturity with the loss of proximal cytoplasmic droplets, and determined that boars with a > 10 percnet of spermatozoa with proximal cytoplasmic droplets and > 10 percent of spermatozoa with abnormal heads represent sexual immaturity. [2] In the current studies, 50 percent of control (non-treated) boars processed at 4 weeks after the 2nd dose of the GnRF-DT conjugate, and 100 percent of saline-treated boars processed at 16 and 22 weeks after the 2nd dose, were deemed sexually mature. In contrast, no GnRF-DT conjugate-treated boars were deemed sexually mature at 4, 16, and 22 weeks after the 2nd dose of the conjugate. The percentage of spermatozoa with proximal cytoplasmic droplets was 12.2, 1.3, and 1.2, and with abnormal heads was 14.5, 4.8, and 6.4, for control boars at 4, 16, and 22 weeks after the 2nd dose. The percentage of spermatozoa with proximal droplets was 66.1, 79.2, and 44.5, and with abnormal heads was 25.2, 28.8, and 44.3, for GnRF-DT conjugate-treated boars at 4, 16, and 22 weeks after the 2nd dose.

This report indicated that spermatogenesis and sexual maturity (based on morphology of epididymal spermatozoa) was disrupted with 2 doses of the GnRF-DT conjugate, confirmed at 4, 16, and 22 weeks after the 2nd dose.

b. Hilbe et al., 2006:

Sixteen boars were given the GnRF-DT conjugate twice at 10 to 16 of age, with the 2nd dose given 4 to 5 weeks after the 1st dose. Fourteen boars were slaughtered over 1 to 16 weeks after the 2nd dose, while 2 boars were given 3 additional doses of the GnRF-DT conjugate and slaughtered at 50 or 60 weeks after the last dose. Four control boars served as negative controls, and were slaughtered at either 6 or 12 months of age.

The authors found that, in control boars, spermatogenesis was fully-developed from spermatogonia up to mature spermatozoa. In all GnRF-DT conjugate-treated boars, the majority of spermatogenic cells visible within the

[2] Einarsson, S., M. Holtman, K. Larsson, I. Settergren, and A. Bane. 1979. The effect of two different feed levels on the development of the reproductive organs in boars. Acta Vet. Scand. 20:1-9.

seminiferous tubules were spermatogonia, with few or no spermatocytes; differential classification of primary and secondary spermatocytes was not possible. For animals slaughtered later in the process (i.e., 12-16 weeks after the 2nd dose), very few spermatocytes and spermatids were discernible. There were no or very few spermatozoa in the head and tail of the epididymis of GnRF-DT conjugate-treated boars; there were plentiful spermatozoa in the epididymis of control boars. This report provided evidence that spermatogenesis was dramatically interrupted through 16 weeks after the 2nd dose. The 14 boars given 2 doses of the GnRF-DT conjugate and slaughtered at 1 to 16 weeks after the 2nd dose had no restoration of normal spermatogenesis, and 13 of 14 boars had paired testis weights that were markedly reduced at the time of slaughter vs. control boars. If it is assumed that testis androgen production was restored at 16 weeks after the 2nd dose of the GnRF-DT conjugate (no endocrine data included in this manuscript), there likely would need to be an additional 5 to 6 weeks (spermatogenesis from type B spermatogonia forward) to observe mature spermatozoa ready for ejaculation in the tail of the epididymis, or approximately 21-22 weeks after the 2nd dose of the conjugate.

This report provided evidence that there was an interruption in spermatogenesis in boars given 2 doses of the GnRF-DT conjugate. The period of time covered was 1-16 weeks after the 2nd dose of the GnRF-DT conjugate. Given where the interruption in spermatogenesis occurred (after type A/B spermatogonia), an additional 5-6 weeks (beyond 16 or more weeks) would be necessary in order to restore full reproductive capacity of GnRF-DT conjugate-treated boars.

c. Rotnner and Claus, 2009:

Boars served as negative controls (n = 4) or were treated with GnRF-DT conjugate (n = 6) at 19 and 26 weeks of age. Boars were slaughtered at 26 weeks after the 2nd dose of the GnRF-DT conjugate. Testes were collected at slaughter and fixed for later histological analyses. A comparison between control and GnRF-DT conjugate-treated boars with respect to the stages of spermatogenesis were examined in histological sections.

While other measures of testis function (i.e., testis size, diameter/area of seminiferous tubules, height/area of seminiferous epithelium, and mitotic activity) were less in GnRF-DT conjugate-treated vs. control boars at 26 weeks after the 2nd dose of the conjugate, there were no differences in frequencies of the contribution of germ cells to the various stages of spermatogenesis (i.e., pre-meiotic, meiotic, post-meiotic). Therefore, while testis function appeared not to have fully recovered at 26 weeks after the 2nd dose of the GnRF-DT conjugate, characteristics of the germ cells in histological sections suggested that spermatogenesis had begun to resume by 26 weeks after the 2nd dose.

d. Wagner and Claus, 2004:

In this study, histological sections were prepared from testes of control boars (n = 5) and boars given the GnRF-DT conjugate (n = 5). Testes were collected at slaughter when boars were 32 weeks of age (3 doses of GnRF-DT conjugate given at 20, 24, and 28 weeks of age).

Histological evaluation showed a dramatic reduction in area of seminiferous tubules and size of Leydig cell cytoplasm/nucleus in GnRF-DT conjugate-treated vs. control boars. The Leydig cell results correlated well with the reduction in testosterone and LH concentrations seen with GnRF-DT conjugate treatment. In addition, evaluation of the presence of spermatogenic cell types in histological sections showed no reduction in the number of type A spermatogonia, but there was ~ 60 percent reduction in GnRF-DT conjugate-treated vs. control boars for type B spermatogonia, Pachytene (primary) spermatocytes, and round/elongated spermatids. Of note was that while the number of type A spermatogonia was not reduced with use of the GnRF-DT conjugate, there was a ~50 percent reduction in mitotic activity. A reduction in mitotic activity did not appear to have an impact on the number of type A spermatogonia for the 4 and 8 weeks after the 2nd and 3rd doses of the GnRF-DT conjugate, though there may be longer term effects on spermatogonia that could have long-term consequences on spermatogenesis.

Conclusions on Spermatogenesis:

Given the results reported in the manuscripts discussed above, there is sufficient scientific information that use of the GnRF-DT conjugate interrupted spermatogenesis for a period of at least 22 weeks following the 2nd dose of the conjugate and supported the duration of 8 weeks stipulated on product labeling.

Overall Conclusions on Temporary Immunological Castration

Results from the U.S. and international studies confirmed that treatment of boars with the GnRF-DT conjugate increased anti-GnRF antibody titers and reduced circulating testosterone concentrations. The effects on these biological markers were for approximately 12-16 weeks following administration of the 2nd of 2 doses of the GnRF-DT conjugate. With respect to the effects on testis size, use of the GnRF-DT conjugate reduced testis size through approximately 22 weeks following the 2nd of 2 doses of the conjugate. For functional indicators of boar reproductive function, use of the GnRF-DT conjugate caused an interruption in sexual behavior and spermatogenesis through approximately 21-22 weeks after the 2nd of tw2 doses of the conjugate. Therefore, the results of the U.S. and international studies support the label indication for temporary immunological castration with a duration of at least 8 weeks following administration of the GnRF-DT conjugate. GnRF-DT conjugate treated males are immunologically castrated and incapable of being used for breeding,

as demonstrated by a reduction in testosterone, smaller testicular size, and disrupted reproductive behavior and spermatogenesis.

2. Reduction of Boar Taint in Intact Males Intended for Slaughter

a. Type of Study: Clinical Field study, sensitive consumer panel sensory evaluation

1. Title: Study 3920C-60-09-771 Consumer Panel Sensory Evaluation of Pork Samples Generated from Clinical IMPROVEST Study 3822C-60-09-760

2. Study Investigator and Location:

Ziad Matta
Peryam & Kroll Research Corporation
Chicago, IL

Peryam & Kroll Research Corporation
Chicago, IL

3. Study Design:

a) Objective: The objective of this study was to conduct sensitive consumer panel sensory evaluations (aroma and flavor) of pork samples generated from saline treated intact boars and boars that had received the 2nd dose of IMPROVEST at 4 weeks prior to slaughter (Study 3822C-60-09-760).

b) Study Animals (Study 3822C-60-09-760): Sixty four intact crossbred male pigs were assigned to 16 pens, with 4 animals per pen of the same treatment group. Treatments were assigned to pens according to a randomized block design.

c) Test Article Administration (Study 3822C-60-09-760): Treatments were sterile saline control (T01, 32 pigs) and IMPROVEST (T02, 32 pigs) (Table 14). Pigs were injected (16 gauge, ½ inch needle) with either 2 mL of saline (T01) or IMPROVEST (T02) subcutaneously in the neck at approximately 18 weeks of age (Day 0) and at approximately 22 weeks of age (Day 28). The sterile saline product consisted of 0.9 percent w/v sodium chloride. The GnRF-DT conjugate formulation consisted of 200 µg GnRF-DT conjugate per mL; 0.1 percent chlorocresol was added as a preservative.

Table 14. Treatment and dosing regimen. [1]						
		Regimen				
Treatment	Dosing	Approx. Weeks of Age	Day of Study	Route of Administration [2]	Number of Animals per Pen	Number of Animals per Treatment Group
T01	2 mL CP [3]	18, 22	0, 28	SC	4	32 (8 pens)
T02	2 mL IVP [4]	18, 22	0, 28	SC	4	32 (8 pens)
[1] Intact males were used in this study.						
[2] Subcutaneously.						
[3] Control product = sterile saline.						
[4] Investigational veterinary product = IMPROVEST.						

d) Measurements and Observations (Study 3822C-60-09-760): In-life phase monitoring included daily general health observations, collection of blood samples, body weights, feed weights, behavior observations, scrotal width measurements, adverse event observations, and injection site reaction observations. At the end of the study, carcass evaluation measures were taken, as well as backfat collected for chemical analysis of androstenone and skatole, and boneless loin chops collected for evaluation by a sensitive consumer sensory panel.

e) Study Samples: Boneless loin chops from 30 saline treated intact male pigs (T01, control) and 30 intact male pigs treated with 2 IMPROVEST doses (T02) were evaluated for aroma and flavor by a panel of 3 sensitive consumers per pair of samples. Individual consumers who were invited to participate were representative of the U.S. population and were comprised of approximately equal numbers of adult (≥18 years old) males and females who like fresh pork and consumed it regularly (at least once per month). Consumers were screened (both aroma and flavor) for sensitivity to boar taint (Tables 15 and 16).

Table 15. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter.		
Description	Number	%
Total days session run	5	----
Total sessions run	33	----
Total consumers invited to participate	840	----
Total consumers screened	648	77.1
Total consumers passed aroma screen	210	32.4
Total consumers passed flavor screen and evaluated study samples	90	13.9

Table 16. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter: Consumer demographics.			
	Recruitment Targets [1]	Sensitive Consumers	
Ethnicity	%	Number	%
Male	50	40	44
Female	50	50	56
Caucasian		61	68
Africa American	13	16	18
Hispanic / Latino	15	12	13
Asian American	5	1	1
Other / Don't know	1	0	0
[1] Respondents who were invited to participate in the study were recruited based on ethnicity quotas to be close to the U.S. Census in 2008.			

Boar samples (T01) were prepared and cooked in a different kitchen than samples from the IMPROVEST treated pigs (T02). Animals were randomly assigned within each block to pairs so each pair contained one T01 and one T02 animal. Pairs were randomly assigned to a serving order such that half would have T01 evaluated first and half would have T02 evaluated first.

f) Measurements: The consumer panelist evaluated the 1st sample as unpleasant ('yes' or 'no'), 1st for aroma and 2nd for flavor. When done, the consumer was presented with the 2nd sample, which was similarly evaluated. Pork from each pig was evaluated by a panel of 3 sensitive consumers (Table 17). Two of 3 consumer panelists had to score a sample as 'unpleasant' for the pork to be considered not acceptable. Conversely,

2 of 3 had to score a sample as 'not unpleasant' for the pork to be acceptable.

Table 17. The number of sensitive consumers used to evaluate pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter.

Treatment	Gender	Dose	Number of Animals Selected for Study	Number of Consumers per Sample	Total Number of Sensitive Consumers
T01	Intact males	2 mL CP [1]	30	3	90 [3]
T02	Intact males	2 mL IVP [2]	30	3	

[1] Control product = saline.

[2] Investigational veterinary product = IMPROVEST.

[3] Each sensitive consumer evaluated 1 sample from T01 and 1 sample from T02. Each sample was evaluated by 3 consumers.

g) Statistical Analysis: Frequency of "unacceptable/acceptable" was analyzed using a generalized linear mixed model with a logit link function. The model included the fixed treatment effect and the random effects block and panel nested within block. The treatment effect was tested at significance level $\alpha = 0.05$. Back-transformed means, in percent form, and their standard errors were calculated for each treatment group. Both aroma and flavor were analyzed in this manner.

4. Results: A total of 648 consumers were screened to identify the 90 consumers who evaluated study samples (Table 15). The frequency of 'not acceptable' aroma scores was significantly less ($P = 0.0075$) for pork samples from the IMPROVEST treated group (T02) than for the saline-treated, intact boar group (T01) (Table 18). The frequency of 'not acceptable' flavor scores was also significantly less ($P = 0.0277$) for pork samples from the IMPROVEST treated group (T02) than for the saline-treated, intact boar group (T01) (Table 19).

Table 18. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter: Aroma Scores "Not Acceptable".

	"Not Acceptable" Aroma Scores (%)	
Treatment	Back-transformed Mean	Standard Error
T01 – Saline	80.0	7.4
T02 – IMPROVEST	43.3	9.2

Table 18. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter: Aroma Scores "Not Acceptable".		
	"Not Acceptable" Aroma Scores (%)	
Treatment	Back-transformed Mean	Standard Error
	P = 0.0075	

Table 19. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter: Flavor Scores "Not Acceptable".		
	"Not Acceptable" Flavor Scores (%)	
Treatment	Back-transformed Mean	Standard Error
T01 – Saline	60.0	9.1
T02 – IMPROVEST	30.0	8.5
	P = 0.0277	

5. Conclusions: IMPROVEST treated animals in this study represented a 4 week duration of effect window (time between 2nd dose and slaughter date). Consumers who were screened for their ability to differentiate between tainted and untainted samples identified, by aroma and flavor, significantly fewer "unacceptable" samples from IMPROVEST treated pigs than from intact boars.

b. Type of Study: Clinical Field study, sensitive consumer panel sensory evaluation

1. Title: Study 3920Z-60-09-798 Consumer Panel Sensory Evaluation of Pork Samples Generated from Clinical IMPROVEST™ Study 3322C-60-09-706 that Represent a 6 Week Duration of Effect Window

2. Study Investigator and Location:

Ziad Matta
Peryam & Kroll Research Corporation
Chicago, IL

Peryam & Kroll Research Corporation
Chicago, IL

3. Study Design:

a) Study objective: The objective of this study was to conduct sensitive consumer panel sensory evaluations (aroma and flavor) of boneless pork loin chop samples generated from non-treated intact boars and boars that had received the 2nd dose of IMPROVEST at 6 weeks prior to slaughter.

b) Study animals (3322C-60-09-706): One thousand intact boars and 200 surgical castrates (barrows) approximately 18 days of age were used. This study consisted of 6 treatment groups (Table 20) with 8 pens per treatment and 25 pigs per pen. Treatments were assigned to pens according to a randomized complete block design.

Table 20. Study design.

Treatment	Gender	Test Article [1]	Lysine Level	Number of Pens	Number of Pigs per Pen
T01	Surgical castrates	None	Low	8	25
T02	Intact males	IMPROVEST 2 mL on Study Day 94 and 122	Low	8	25
T03	Intact males	IMPROVEST 2 mL on Study Day 94 and 122	Medium Low	8	25
T04	Intact males	IMPROVEST 2 mL on Study Day 94 and 122	Medium High	8	25
T05	Intact males	IMPROVEST 2 mL on Study Day 94 and 122	High	8	25
T06	Intact males	None	High	8	25
Total				48	1200

[1] The 1st IMPROVEST injection was administered post-auricular on the left side of the neck and the 2nd injection was administered post-auricular on the right side of the neck.

c) Test article administrations (3322C-60-09-706): Animals in treatment group T01 (barrows) and T06 (intact boars) were non-treated controls. Pigs assigned to treatment groups T02 through T05 were injected with 2 mL per dose of GnRF-DT conjugate. The GnRF-DT conjugate formulation consisted of 200 µg GnRF-DT conjugate per mL; 0.1 percent chlorocresol was added as a preservative. The 1st and 2nd doses were administered on Days 94 and 122, respectively.

d) Measurements and Observations (3322C-60-09-706): "In-life" phase monitoring consisted of daily general health and injection site observations, adverse event observations, animal body weights, and consumption. Monitoring at the time of slaughter included: carcass evaluation measures, primal cuts removed for pork quality assessment, and calculation of fat-free lean.

e) Study Samples: Boneless loin chops collected from animals in treatment group T05 were used in the sensory panel evaluation as this group was fed a high lysine level diet, which corresponded to the high lysine level diet fed to the control (T06) group; thus avoiding confounding dietary lysine level with treatment. Loin chops from 62 pigs treated with 2 doses of IMPROVEST (T05) and 62 non-treated, intact male pigs (T06) were evaluated for aroma and flavor by sensitive consumers. Individual consumers who were invited to participate were representative of the U.S. population and were comprised of approximately equal numbers of adult (≥18 years old) males and females who like fresh pork and consumed it regularly (at least once per month). Consumers were screened (both aroma and flavor) for sensitivity to boar taint (Tables 21 and 22).

Table 21. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 6 weeks prior to slaughter.

Description	Number	%
Total days session run	11	----
Total sessions run	60	----
Total consumer invited to participate	2047	----
Total consumers screened	1556	76.0
Total consumers passed aroma screen	468	30.1
Total consumers passed flavor screen and evaluated study samples	186	12.0
Total repeat consumers from previous studies [1]	1	0.54

[1] Consumers that evaluated study samples from the previous sensitive consumer panel sensory evaluation were invited to participate. Out of the 90 sensitive consumers from the study where samples were generated from boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter, only 1 consumer evaluated study samples in this study.

Table 22. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 6 weeks prior to slaughter: Consumer demographics.

	Recruitment Targets [1]	Sensitive Consumers	
Ethnicity	%	Number	%
Male	50	83	45
Female	50	103	55
Caucasian	66	139	75
Africa American	13	24	13
Hispanic / Latino	15	19	10
Asian American	5	3	2
Other / Don't know	1	1	1

[1] Respondents who were invited to participate in the study were recruited based on ethnicity quotas to be close to the U.S. Census in 2008.

Samples from IMPROVEST treated pigs (T05) were prepared and cooked in a different kitchen than boar samples (T06). Within each block, animals were randomly assigned to pairs so each pair contained one T05 and one T06 animal. Two T06 animals were excluded from this study because they did not meet the inclusion criteria. Therefore, two T05 animals were randomly selected for exclusion to obtain an equal number of animals per treatment. Pairs were randomly assigned to a serving order such that half would have T05 evaluated first and half would have T06 evaluated first.

f) Measurements: The consumer panelist evaluated the 1st sample as unpleasant ('yes' or 'no'), 1st for aroma and 2nd for flavor. When done, the consumer was presented with the 2nd sample that was similarly evaluated. Pork from each pig was evaluated by a panel of 3 sensitive consumers (Table 23). Two of 3 consumer panelists had to score a sample as unpleasant for the pork to be considered not acceptable. Conversely, 2 of 3 had to score a sample as not unpleasant for the pork to be acceptable.

Table 23. The number of sensitive consumers used to evaluate pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 6 weeks prior to slaughter.

Treatment	Gender	Dose	Number of Animals Selected for Study	Number of Consumers per Sample	Total Number of Sensitive Consumers
T05	Intact males	2 mL IVP [1]	62	3	186 [2]
T06	Intact males	None	62	3	

[1] Investigational veterinary product = IMPROVEST.

[2] Each sensitive consumer evaluated 1 sample from T05 and 1 sample from T06. Each sample was evaluated by 3 consumers. Because two T06 animals were removed prior to testing, 2 randomly identified T05 males were not tested in order to balance the presentation of samples to sensitive consumers.

g) Statistical Analysis: Frequency of "unacceptable/ acceptable" was analyzed using a generalized linear mixed model with a logit link function. The model included the fixed treatment effect and the random effects block and panel nested within block. The treatment effect was tested at significance level $\alpha = 0.05$. Back-transformed means, in percent form, and their standard errors were calculated for each treatment group. Both aroma and flavor were analyzed in this manner.

4. Results: A total of 1,556 consumers were screened to identify the 186 consumers who evaluated the study samples (12 percent). The number of pork samples from IMPROVEST treated animals (T05) that were considered "not acceptable" for aroma was significantly less ($P = 0.0180$) than for non-treated control animals (T06) (Table 24). The number of pork samples from IMPROVEST treated animals (T05) that were considered "not acceptable" for flavor was lower than for non-treated control animals (T06), but was not significantly different ($P = 0.1085$) (Table 25).

Table 24. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 6 weeks prior to slaughter: Aroma Scores "Not Acceptable".		
	"Not Acceptable" Aroma Scores (%)	
Treatment [1]	Back-transformed Mean	Standard Error
T05 – IMPROVEST	47.5	7.6
T06 – Control	67.9	6.9
	P = 0.0180	
[1] T06 = non-treated control boars.		

Table 25. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 6 weeks prior to slaughter: Flavor Scores "Not Acceptable".		
	"Not Acceptable" Flavor Scores (%)	
Treatment [1]	Back-transformed Mean	Standard Error
T05 – IMPROVEST	18.8	5.8
T06 – Control	29.3	7.6
	P = 0.1085	
[1] T06 = non-treated control boars.		

5. Conclusions: IMPROVEST treated animals in this study represented a 6 week duration of effect window (time between 2nd dose and slaughter date). Consumers who were screened for their ability to differentiate between tainted and untainted samples identified, by aroma, significantly fewer "unacceptable" samples from IMPROVEST treated pigs than from intact boars. The same consumers identified, by flavor, fewer "unacceptable" samples from IMPROVEST treated pigs than from intact boars.

c. Type of Study: Clinical Field study, sensitive consumer panel evaluation

1. Title: Study 3920Z-60-09-816 Consumer Panel Sensory Evaluation of Pork Samples Generated from Clinical IMPROVEST Study 3322C-60-09-706 that Represent an 8 Week Duration of Effect Window

2. Study Investigator and Location:

Ziad Matta
Peryam & Kroll Research Corporation
Chicago, IL

Peryam & Kroll Research Corporation
Chicago, IL

3. Study Design:

a) Study objective: The objective of this study was to conduct sensitive consumer panel sensory evaluations (aroma and flavor) of pork samples generated from non-treated intact boars and boars that had received the 2nd dose of IMPROVEST at 8 weeks prior to slaughter.

b) Study animals (3322C-60-09-706): One thousand intact boars and 200 surgical castrates (barrows) approximately 18 days of age were used. This study consisted of 6 treatment groups (see Table 20 above) with 8 pens per treatment and 25 pigs per pen. Treatments were assigned to pens according to a randomized complete block design.

c) Test article administrations (3322C-60-09-706): Animals in treatment group T01 (barrows) and T06 (intact boars) were non-treated controls. Pigs assigned to treatment groups T02 through T05 were injected with 2 mL per dose of GnRF-DT conjugate. The GnRF-DT conjugate formulation consisted of 200 µg GnRF-DT conjugate per mL; 0.1 percent chlorocresol was added as a preservative. The 1st and 2nd doses were administered on Days 94 and 122, respectively.

d) Measurements and Observations (3322C-60-09-706): "In-life" phase monitoring consisted of daily general health and injection site observations, adverse event observations, animal body weights, and consumption. Monitoring at the time of slaughter included: carcass evaluation measures, primal cuts removed for pork quality assessment, and calculation of fat-free lean.

e) Study Samples: Boneless loin chops collected from animals in treatment group T05 were used in the sensory panel evaluation as this group was fed a high lysine level diet, which corresponded to the high lysine level diet fed to the control (T06) group; thus avoiding confounding dietary lysine level with treatment. Loin chops from 63 pigs treated with 2 doses of IMPROVEST (T05) and 63 non-treated, intact male pigs (T06) were evaluated for aroma and flavor by sensitive consumers. Individual consumers who were invited to participate were representative of the U.S. population and were comprised of approximately equal numbers of adult (≥18 years old) males and females who like fresh pork and consumed it

regularly (at least once per month). Consumers were screened (both aroma and flavor) for sensitivity to boar taint (Tables 26 and 27).

Table 26. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 8 weeks prior to slaughter.

Description	Number	%
Total days session run	10	----
Total sessions run	59	----
Total consumer invited to participate	2209	----
Total consumers screened	1733	78.5
Total consumers passed aroma screen	520	30.0
Total consumers passed flavor screen and evaluated study samples	189	10.9
Total repeat consumers from previous studies [1]	13	6.9

[1] Sensitive consumers that evaluated study samples from the study where samples were generated from boars receiving the 2nd dose of IMPROVEST at 4 weeks prior to slaughter (90 consumers) or those who successfully passed the aroma screen in the study where samples were generated from boars receiving the 2nd dose of IMPROVEST at 6 weeks prior to slaughter (468 consumers) were invited to participate. Out of this pool of repeat consumers (558 total), only 13 consumers evaluated study samples in this study.

Table 27. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 8 weeks prior to slaughter: Consumer demographics.

	Recruitment Targets [1]	Sensitive Consumers	
Ethnicity	%	Number	%
Male	50	90	48
Female	50	99	52
Caucasian	66	131	69
Africa American	13	23	12
Hispanic / Latino	15	26	14
Asian American	5	4	2
Other / Don't know	1	5	3

[1] Respondents who were invited to participate in the study were recruited based on ethnicity quotas to be close to the U.S. Census in 2008.

Samples from IMPROVEST treated pigs (T05) were prepared and cooked in a different kitchen than boar samples (T06). Samples were randomized for consumer evaluation. Within each block, animals were randomly assigned to pairs so each pair contained one T05 and one T06 animal.

One block had one too few T05 animals, and another block had one too few T06 animals. Therefore, a T06 animal from the former block was paired with a T05 animal from the latter block. Pairs were randomly assigned to a serving order such that half would have T05 evaluated first and half would have T06 evaluated first.

f) Measurements: The consumer panelist evaluated the 1st sample as unpleasant ('yes' or 'no'), 1st for aroma and 2nd for flavor. When done, the consumer was presented with the 2nd sample that was similarly evaluated. Pork from each pig was evaluated by a panel of 3 sensitive consumers (Table 28). One hundred eighty nine sensitive consumers were necessary to complete the testing of 126 samples. Two of 3 consumer panelists had to score a sample as unpleasant for the pork to be considered not acceptable. Conversely, 2 of 3 had to score a sample as not unpleasant for the pork to be acceptable.

Table 28. The number of sensitive consumers used to evaluate pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 8 weeks prior to slaughter.

Treatment	Gender	Dose	Number of Animals Selected for Study	Number of Consumers per Sample	Total Number of Sensitive Consumers
T05	Intact males	2 mL IVP [1]	63	3	189[2]
T06	Intact males	None	63	3	

[1] Investigational veterinary product = IMPROVEST.

[2] Each sensitive consumer evaluated 1 sample from T05 and 1 sample from T06. Each sample was evaluated by 3 consumers.

g) Statistical Analysis: Frequency of unacceptable/acceptable was analyzed with a generalized linear mixed model with a logit link function. The model included the fixed treatment effect and random effects block and panel nested within block. The treatment effect was tested at significance level $\alpha = 0.05$. Back-transformed means, in percent form, and their standard errors were calculated for each treatment group. Both aroma and flavor were analyzed in this manner.

4. Results: A total of 1,733 consumers were screened to identify the 189 who evaluated the study samples (10.9 percent). Consumers who evaluated study samples in study 3920C-60-09-771 (90 consumers) or those who successfully passed the aroma screen in study 3920Z-60-09-798 (468 consumers) were among those invited to participate in this study. Out of this pool of potential repeat consumers, only 13 (6.9 percent of the 189 consumers) evaluated study samples in this study.

The number of pork samples from IMPROVEST treated animals (T05) that were considered not acceptable for aroma was significantly less ($P = 0.0048$) than for non-treated control animals (T06) (Table 29). The number of pork

samples from IMPROVEST treated animals (T05) that were considered not acceptable for flavor was significantly lower (P = 0.0005) than for non-treated control animals (T06) (Table 30).

Table 29. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 8 weeks prior to slaughter: Aroma Scores "Not Acceptable".

	"Not Acceptable" Aroma Scores (%)	
Treatment [1]	Back-transformed Mean	Standard Error
T05 – IMPROVEST	41.2	6.7
T06 – Control	66.8	6.4
	P = 0.0048	

[1] T06 = non-treated control boars.

Table 30. Consumer panel sensory evaluation of pork samples generated from intact boars receiving the 2nd dose of IMPROVEST at 8 weeks prior to slaughter: Flavor Scores "Not Acceptable".

	"Not Acceptable" Flavor Scores (%)	
Treatment [1]	Back-transformed Mean	Standard Error
T05 – IMPROVEST	17.9	6.2
T06 – Control	49.4	9.3
	P = 0.0005	

[1] T06 = non-treated control boars.

5. Conclusions: IMPROVEST treated animals in this study represent an 8 week duration of effect window (time between 2nd dose and slaughter date). Consumers who were screened for their ability to differentiate between tainted and untainted samples identified, by both aroma and flavor, significantly fewer "unacceptable" samples from IMPROVEST treated pigs than from intact boars.

Overall Conclusion of the Reduction of Boar Taint

Sensitive consumers detected less unacceptability of pork due to boar taint (based on aroma and flavor) from IMPROVEST treated pigs when compared to intact male pigs of a similar age at 4 weeks, 6 weeks, and 8 weeks after the 2nd dose of IMPROVEST.

Collectively, the temporary immunological castration and reduction of the boar taint data support that IMPROVEST Sterile Solution is effective for the temporary immunological castration (suppression of testicular function) and reduction of boar taint in intact male pigs intended for slaughter.

III. TARGET ANIMAL SAFETY:

A. Margin of Safety Study

1. Title: **"**Target Animal Safety of GnRF conjugate for Injection in Boars**" Pfizer Study Report No. 3420N-60-07-557.**

   The study was performed in accordance with Good Laboratory Practice (GLP) regulations.

2. Study Director:

   T. Schieber, DVM
   Midwest Veterinary Services, Inc.
   Oakland, NE

3. Study Design:

   a) Objective: The objective of this study was to evaluate the safety of 2 doses (2 mL each) of GnRF-diphtheria toxoid conjugate (GnRF-DT) for injection (commercial formulation) in intact male swine, administered subcutaneously into the same location 14 days apart, with pathology evaluation 14 days following the 2nd injection.

   b) Study Animals: Thirty healthy, mixed-breed intact boars were used, aged 9 weeks at the time of the 1st injection. Boars were acclimatized for 28 days and then each was assigned to 1 of 3 treatments: Control, GnRF-DT at the intended 2 mL dose (1X), or GnRF-DT at 6 times the intended dose (6X). The left and right post-auricular injection sites were marked with an indelible marker.

   c) Test Article Administration: GnRF-DT solution (200 µg/mL) was used as the test article (Lot number L64308), and 0.9 percent sterile saline for injection was used as the control article. Each dose of test or control article was drawn

into a new, sterile syringe fitted with a sterile 16-ga x 0.5 inch needle. Each boar in the control group received 2, 6 mL injections of control article, 1 into each of the left and right injection sites, and the 2 injections were re-administered into the same marked left and right injection sites 14 days later. Each boar in the 1X group was administered 2 mL test article into the left injection site and 2 mL control article into the right injection site, and the 2 injections were re-administered into the same marked left and right injection sites 14 days later. Boars in the 6X group were administered test article exactly as control article was administered to boars in the control group.

d) Measurements and Observations: In-life phase monitoring included daily clinical evaluations, observation, and measurement of injection sites, body weight, quantitative feed consumption, hematology, and clinical chemistry analyses. At postmortem observations 14 days after the 2nd injection, the abdominal cavity was opened, a urine specimen was collected by cystocentesis, and a urinalysis was conducted. A complete necropsy was conducted, select organs were removed and weighed, gross lesions were described, injection sites entirely excised, measured, and described, and a complete set of tissue samples was preserved. Histologic tissue sections were evaluated microscopically by a pathologist.

e) Statistical Analysis: Continuous response variables were analyzed by mixed model analysis of covariance. Repeated measures mixed model analysis of covariance was used for the in-life continuous responses measured at multiple time points. Categorical and incidence data were tabulated.

4. Results:

a) In-Life Observations: Treatment-related clinically observable changes at the intended dosage were limited to transient pruritus of the neck in most animals, lasting up to 2 days in 60 percent of the pigs. Injection sites showed mainly firmness, which lasted no longer than 3 days after the 1st injection but for up to 2 weeks after the 2nd injection in 20 percent of the pigs. Temporary local sensitivity, heat, and erythema also were observed.

Clinical signs in 6X boars were depression and stiffness of the neck lasting up to 5 days. One pig showed a temporary reduction in feed intake, multiple swollen joints, and associated lameness. Other treatment-related changes were lower body weights; increased white blood cell counts (eosinophilia and neutrophilia); slight increases in total serum protein and globulin (neither of which were clinically significant); and slight decreases in serum amylase, albumin, and alkaline phosphatase (none of which were clinically significant). Injection sites showed clinically detectable firmness persisting in all animals for 14 days after the 2nd injection. Pain and sensitivity persisted for up to 5 days, and erythema and heat were more prominent than at the intended 2 mL dose.

b) Postmortem Observations: Injection sites in 1X boars were firm and discolored, with estimated volumes ranging from 1 cm^3 to 25 cm^3. Microscopically, injection sites showed mild to moderate chronic inflammation that did not extend to the deep musculature of the neck. Bulbourethral glands, seminal vesicles, and testes were lower in weight than in the control group. Treatment-related microscopic changes in these tissues consisted of interstitial cell or generalized hypoplasia of testes, decrease in secretions in seminal vesicles, and acinar hypoplasia of the prostate.

Changes at the injection sites in 6X animals were similar to those observed in the 1X animals but were more marked; injection site volumes ranged from 16 cm^3 to 276 cm^3. In addition to decreased weights of bulbourethral glands, seminal vesicles, and testes volume, thymic weights in 6X boars were decreased relative to controls. Microscopic changes were similar to those observed at the intended 2 mL dose, but injection site inflammation was more severe.

5. Conclusions: GnRF-DT administered subcutaneously to intact male swine 2 weeks apart resulted in mild transient injection site reactions at the 1X dose and caused clinical signs of systemic inflammation at a 6-fold multiple of the intended dose. Atrophy of testes, prostate, and bulbourethral glands were expected consequences associated with the intended effect. Additionally, mild to moderate chronic inflammation and discoloration in the subcutaneous tissues at the injection site may result in loss of edible tissues if boars are slaughtered 14 days after injection.

B. Injection Site Tolerance Study

1. Title: **"**Injection Site Tolerance of GnRF conjugate Administered Subcutaneously in Boars" **Report No. 3420N-60-04-327.**

   The study was performed in accordance with Good Laboratory Practice (GLP) regulations.

2. Study Director:

   T. Schieber, DVM,
   Midwest Veterinary Services, Inc.
   Oakland, NE

3. Study Design:

   a) Objective: The objective of this study was to evaluate the injection site toleration of 2 doses (2 mL each) of GnRF-diphtheria toxoid conjugate (GnRF-DT) in intact male swine, administered subcutaneously into the same

location 28 days apart, with evaluation of pathology 2 days following the 2nd injection.

b) Study Animals: Sixteen healthy, mixed-breed intact boars were used, aged 17 weeks at the time of the 1st injection. Animals were acclimatized for 28 days and randomized to treatments with either 0.9 percent saline or GnRF-DT. The left post-auricular injection sites were marked with an indelible marker.

c) Test Article Administration: GnRF-DT solution (200 µg/mL) was used as the test article, and 0.9 percent sterile saline for injection was used as the control article. Each dose of test or control article was drawn into a new, sterile syringe fitted with a sterile 16-ga x 0.5 inch needle. Pigs received 2, 2 mL injections 28 days apart, administered at a right angle to the skin, into the same pre-marked post-auricular location on the left side of the neck. The saline control article was administered by using the same type of equipment, volume, and technique.

d) Measurements and Observations: In-life phase monitoring included daily clinical evaluations and observation and measurement of injection sites. Necropsy was performed 2 days after the 2nd injection. The entire injection site was excised, dimensions measured, and lesions were described. Tissue samples from injection sites were preserved, processed, and evaluated microscopically.

4. Results:

a) *In-Life Observations*: In-Life Observations: Clinical signs in GnRF-DT treated boars were limited to the injection site reactions. Easily observable injection site swelling resolved within 24 hours, and pain on palpation resolved by 48 hours post-injection. Firmness persisted for up to 11 days after the 1st injection in 10 percent of pigs. Dimensional measurements showed that lesions measured a maximum of 28.9 cm^2 in surface area and 18.4 cm^2 in volume approximately 2 to 3 days after the 1st injection.

b) *Gross Injection Site Observations:* Gross injection site alterations in GnRF-DT treated animals consisted of subcutaneous edema with tan or red discoloration. The mean postmortem injection site volume in GnRF-DT treated animals was 132 cm^3 (range 31 cm^3 to 288 cm^3). Microscopic treatment-related injection site alterations included chronic eosinophilic inflammation, edema and hemorrhage in the subcutaneous fat; edema, degeneration and mineralization of the panniculus muscle; and minimal chronic eosinophilic inflammation of the dermis.

5 Conclusions: Two 2 mL injections of GnRF-DT, administered 28 days apart into the same location through a 16-ga x 0.5 inch needle resulted in transient injections

site reactions following each injection. Postmortem evaluation approximately 50 hours after the 2nd injection revealed tissue swelling and discoloration in the subcutaneous tissues at the injection site and may result in loss of edible tissues if boars were slaughtered 48 hours after injection.

IV. HUMAN FOOD SAFETY:

A. Toxicology:

All pivotal testing was conducted in full compliance with the Good Laboratory Practice (GLP) Regulations (21 CFR part 58).

1. Summary of Toxicology Studies

Subchronic Toxicity and Immunogenicity Study

1. Title of the Study: A 45-Day Toxicity Study of Gonadotropin Releasing Factor-Diphtheria Toxoid conjugate for Injection Administered by Oral Gavage or Subcutaneous Injection to Rats

2. Study No: CRL Study No. LIA00414; PAH Study No. 3471N-60-07-230

3. Report Date: February 26, 2009

4. Study Director: Kimberly L. Bonnette, M.S., LATG

5. Study Location (in life): Charles River Laboratories, Preclinical Services, 640 North Elizabeth Street, Spencerville, Ohio 45887, USA.

6. Purpose: The study consisted of 2 phases (a toxicity phase and a toxicokinetic (TK) satellite phase) conducted concurrently in the same facility. The toxicity phase of the study was designed to address any potential toxicological, reproductive, and endocrine effects following chronic oral exposure to IMPROVEST Sterile Solution. The satellite phase of the study was designed to address any potential immunological effect(s) following chronic oral exposure to IMPROVEST Sterile Solution.

7. Experimental Design: In the toxicology phase, Sprague Dawley rats (10/sex/group) were orally gavaged with IMPROVEST Sterile Solution at 6.6, 33, and 66 µg GnRF-DT conjugate/kg body weight (bw)/day, or saline solution (as a negative control). All the male animals were dosed once daily for 45 consecutive days. All the female animals were dosed daily though 45-49 days until diestrus was confirmed. In addition, a group of rats (10/sex), served as a positive control, was subcutaneously injected with IMPROVEST Sterile Solution at 40 µg GnRF-DT

conjugate/kg bw/dose on Days 1 and 29. Mortality and moribundity check, daily cage-side observations, detailed clinical observations, and ophthalmoscopic examinations were performed. Body weight and food consumption were recorded. Estrous cycle was monitored for all females by daily vaginal smear beginning on Day 25 and continuing until the day of necropsy. Urine samples for urinalysis were collected on Day 46. At necropsy, blood samples were collected from fasted males on Day 46 and females in the diestrus phase from Days 46 to 49 and any remaining ones on Day 50. Blood samples were evaluated for clinical pathology (hematology, coagulation, and clinical chemistry). Serum samples were analyzed for IgG and IgM antibodies to GnRF. Serum concentrations of luteinizing hormone (LH), testosterone for males, and progesterone for females were analyzed. At the treatment termination, all surviving animals were sacrificed and subject to a complete gross necropsy. Weights of organ including reproductive organs were obtained. A standard set of tissues were collected from all animals and examined histopathologically.

In the satellite TK phase, animals (6/sex/group) were dosed identically to the animals in the toxicity phase. Mortality and moribundity were checked. Cageside observations were performed and body weight was recorded. Blood samples were collected periodically, and serum concentrations of IgG and IgM antibodies were analyzed. A gross necropsy was performed only on unscheduled deaths, and all surviving animals were euthanized and discarded.

8. Results and Conclusion: There were no treatment-related effects for the orally dosed groups. In contrast, multiple effects on food consumption, clinical pathology, gross pathology and histopathology, organ weights, estrus cycle, and hormone levels were noted in animals subcutaneously injected with 40 µg/kg bw/dose. It was concluded that oral administration did not result in toxicological, reproductive and endocrine effects, or the development of anti-GnRF antibodies at the tested doses. A no-observed-effect-level (NOEL) of 66 µg/kg bw/day was established based on the lack of effects at the highest dose tested.

Acute Toxicity and Immunogenicity Pivotal Study

1. Title of the Study: An 80-Day Toxicity Study of Gonadotropin Releasing Factor-Diphtheria Toxoid conjugate for Injection Administered by Oral Gavage or Subcutaneous Injection to Rats

2. Study No: CRL Study No. LIA00415; PAH Study No. 3471N-60-07-231

3. Report Date: February 27, 2009

4. Study Director: Kimberly L. Bonnette, M.S., LATG

5. Study Location (in life): Charles River Laboratories, Preclinical Services, 640 North Elizabeth Street, Spencerville, Ohio 45887, USA.

6. Purpose: The study was designed to address any potential toxicological and immunological effects of acute oral exposure to IMPROVEST Sterile Solution. The study consisted of 2 phases (a toxicity phase and a satellite phase).

7. Experimental Design: In the toxicity phase, Sprague Dawley rats (10/sex/group) were orally gavaged with IMPROVEST Sterile Solution at 33 and 66 µg GnRF-DT conjugate/kg bw/dose or saline solution (as a negative control) on Days 1, 29, and 57. In addition, a group of animals (10/sex), served as positive control, was subcutaneously injected with the test article (40 µg/kg bw/dose) on Days 1, 29, and 57. Clinical observations, body weight, and food consumption were recorded. Blood samples were collected from fasted animals on Day 81 for hematology, coagulation, clinical chemistry, and hormone analyses, and on Days -4, 64, and 81 for antibody analyses. At terminal sacrifice on Day 81, all surviving animals were sacrificed and subject to a complete gross necropsy examination. Seminal vesicles and coagulating gland, spleen, and thymus were weighed. A series of tissues were collected from the control and 66 µg/kg bw/dose groups, but only the reproductive organs and organs related to immunological functions were processed and examined microscopically.

   In the satellite phase, animals (6/sex/group) were dosed identically to the animals in the toxicity phase. Cage-side observations and body weight were recorded. Blood serum samples were collected from all animals at 0 (prior to treatment), 4, 6, 8, 12, and 24 hours after the 1st dose administration on Day 1 for analyses of plasma LH, progesterone (females) and testosterone (males). Blood serum samples were also collected periodically for anti-GnRF antibody titer determination. The ovaries were collected, processed and examined microscopically for the ovarian corpora lutea count. On Day 82, all surviving animals were euthanized.

8. Results and conclusions: Oral administration of IMPROVEST Sterile Solution did not cause systemic antibody production, and a NOEL of 66 µg/kg bw for GnRF-DT was established based on a lack of acute toxicity and immunological effects at the highest dose tested. The hormonal data was not adequate to be used for the establishment of an acute hormonal NOEL for hormone effects.

A Single Dose Acute Toxicity in Female Rats

1. Title of the Study: Study of the Effects of IMPROVEST Sterile Solution on Luteinizing Hormone, Progesterone and Corpora Lutea in Female Rats

2. Study No: PGRD Study No. 10GR003; PAH Study No. 3491N-60-10-004

3. Report Date: July 9, 2010

4. Study Director: Gregg D. Cappon, Ph.D.

5. Study Location (in life): Developmental and Reproductive Toxicology, Pfizer Drug Safety Research and Development, Groton, Connecticut 06340, USA.

6. Purpose: This study was performed to evaluate the potential for IMPROVEST sterile solution to alter LH and progesterone levels, and corpora lutea number.

7. Experimental Design: Female synchronized Sprague Dawley rats (30/sex/group) between 13-14 weeks of age at dosing were administered a single dose of either saline by oral gavage, IMPROVEST sterile solution by subcutaneous injection at 40 µg GnRF-DT conjugate/kg bw, IMPROVEST sterile solution by oral administration at 33 and 66 µg GnRF-DT conjugate/kg bw, or Triptorelin at 100 µg/kg bw by subcutaneous injection. Animals were observed for moribundity, mortality, and clinical signs. Body weight and food consumption were recorded. Blood samples were collected from all females at 0 (prior to dosing on the day of dose administration), 4, 6, 8, 12, and 24 hours post-dosing. Serum was assayed for concentration of LH and progesterone. Animals were euthanized on Day 15. The ovaries were collected, examined grossly and microscopically, and the number of corpora lutea in each ovary was counted.

8. Results and Conclusion: IMPROVEST Sterile Solution administered orally to female synchronized rats at 33 and 66 µg GnRF-DT conjugate/kg bw had no hormonal effect on LH and progesterone levels evaluated at 0, 4, 6, 8, 12, and 24 hours post-administration; no effects were seen in the number of corpora lutea and estrus cyclicity. In contrast, the GnRF analog agonist Triptorelin caused significant increases in LH and progesterone levels and disrupted estrus cyclicity as expected. A NOEL of 66 µg/kg bw for GnRF-DT was established based on the lack of effects in the highest oral dose tested.

A Single Dose Acute Toxicity Study in Male Rats

1. Title of the Study: Study of the Effects of IMPROVEST Sterile Solution on Luteinizing Hormone and Testosterone in Male Rats

2. Study No: PGRD Study No. 10GR002, PAH Study No. 3491N-60-10-005

3. Report Date: July 15, 2010

4. Study Director: Gregg D. Cappon, Ph.D.

5. Study Location (in life): Developmental and Reproductive Toxicology, Pfizer Drug Safety Research & Development, Groton, Connecticut 06340, USA.

6. Purpose: The study was performed to evaluate the potential of IMPROVEST Sterile Solution to alter LH and testosterone levels in male rats.

7. Experimental Design: Sprague Dawley rats (30/sex/group) were administered either a single dose of either saline (oral gavage), IMPROVEST sterile solution 0.04 mg/kg (subcutaneous injection), IMPROVEST sterile solution 0.033 and 0.066 mg/kg (oral gavage), or Triptorelin 0.10 mg/kg (subcutaneous injection). Blood samples were collected from all males at 0 (prior to dosing on the day of dose administration), 4, 6, 8, 12, and 24 hours post-dosing, and serum was assayed for concentration of LH and testosterone. Males were euthanized 3 days following dose administration and the accessory sex glands (prostate, seminal vesicles and coagulating glands), testes, and epididymides were weighed.

8. Results and Conclusion: Administration of IMPROVEST sterile solution to male rats at 40 µg/kg bw subcutaneously or at 33 and 66 µg/kg bw orally had no effect on LH or testosterone levels. In contrast, Triptorelin caused increased LH and testosterone levels as expected. Male reproductive organ weights were not altered following oral administration of IMPROVEST sterile solution or subcutaneous administration of Triptorelin. However, the weight of the accessory sex glands measured was reduced in animals receiving 40 µg/kg bw IMPROVEST sterile solution via subcutaneous injection. A NOEL of 66 µg/kg bw for GnRF-DT is established from this study based on the lack of effects in the highest oral dose tested.

2. Determination of No Observed Effect Level (NOEL) for chronic exposure and the NOEL for acute exposure (if applicable).

For chronic exposure, a NOEL of 66 µg/kg bw/day can be established based on the lack of any effects via oral route from the 45-day subchronic study in rats. For acute exposure, a NOEL of 66 µg/kg bw can be established based on the lack of effects via oral route from the single dose acute toxicity studies in male and female rats.

3. Acceptable Daily Intake (ADI) and Acceptable Single-Dose Intake (ASDI)*)*

The toxicological acceptable daily intake (ADI) is calculated using the following formulation based on the NOEL of 66 µg/kg bw/day from the 45-day subchronic study in rats and a safety factor of 50:

$$Toxicological\ Acceptable\ Daily\ Intake\ (ADI) = \frac{Lowest\ NOEL}{Safety\ Factor}$$

$$= \frac{66\ \mu g/kg\ bw/day}{50} = 1.32\ \mu g/kg\ bw/day$$

(Equation 1: Toxicological Acceptable Daily Intake (ADI) equals Lowest NOEL divided by Safety Factor, which equals 66 µg/kg bw/day divided by 50, which equals 1.32 µg/kg bw/day.)

Because there is no microbiological ADI, the toxicological ADI (1.32 µg/kg bw/day) is the final ADI for GnRF-DT conjugate.

For GnRF-DT conjugate residues at an injection site, an acceptable single-dose intake (ASDI) of 6.6 µg/kg bw is calculated based on a NOEL of 66 µg/kg bw from the acute oral toxicity study in rats and a safety factor of 10, as follows:

$$Acceptable\ Single\ Dose\ Intake\ (ASDI) = \frac{Acute\ NOEL}{Safe\ Factor} = \frac{66\ \mu g/kg\ bw}{10}$$

$$= 6.6\ \mu g/kg\ bw$$

(Equation 2: Acceptable Single Dose Intake (ASDI) equals Acute NOEL divided by Safe Factor, which equals 66 µg/kg bw divided by 10, which equals 6.6 µg/kg bw.)

4. Safe Concentrations for Total Residues (edible tissues and injection sites)

The calculation of the tissue safe concentrations is based on the *General Principles for Evaluating the Safety of Compounds used in Food-Producing*

Animals (FDA/CVM, revised July 2006). The safe concentration as total GnRF-DT residues (ppm) in each edible tissue of swine is calculated using the following formulation:

$$Safe\ \ Concentration\ \ (SC) = \frac{Acceptable\ \ Daily\ \ Intake\ \ (ADI) \times Human\ Weight}{Consumption\ Value}$$

(Equation 3: Safe Concentration (SC) equals Acceptable Daily Intake (ADI) times Human Weight divided by Consumption Value.)

The average human weight is approximated at 60 kg. The daily consumption values of edible tissues of swine are approximated as 300 g for muscle, 100 g for liver, 50 g for kidney, and 50 g for fat.

Therefore, the safe concentrations for the edible tissues are calculated as (summarized in Table 31):

$$SC\ (muscle) = \frac{1.32\ \mu g / kg\ bw / day \times 60\ kg}{300\ g / day} = 0.264\ \mu g / g = 0.264\ ppm$$

(Equation 4: SC (muscle) equals 1.32 µg/kg bw/day times 60 kg divided by 300 g/day, which equals 0.264 µg/g or 0.264 ppm.)

$$SC(liver) = \frac{1.32\ \mu g / kg\ bw / day \times 60\ kg}{100\ g / day} = 0.792\ \mu g / g = 0.792\ ppm$$

(Equation 5: SC (liver) equals 1.32 µg/kg bw/day times 60 kg divided by 100 g/day, which equals 0.792 µg/g or 0.792 ppm.)

$$SC(kidney) = \frac{1.32\ \mu g / kg\ bw / day \times 60\ kg}{50\ g / day} = 1.584\ \mu g / g = 1.584\ ppm$$

(Equation 6: SC (kidney) equals 1.32 µg/kg bw/day times 60 kg divided by 50 g/day, which equals 1.584 µg/g or 1.584 ppm.)

$$SC(\ fat) = \frac{1.32\ \mu g / kg\ bw / day \times 60\ kg}{50\ g / day} = 1.584\ \mu g / g = 1.584\ ppm$$

(Equation 7: SC (fat) equals 1.32 µg/kg bw/day times 60 kg divided by 50 g/day, which equals 1.584 µg/g or 1.584 ppm.)

Assuming a 60 kg average human body weight and a food consumption factor of 300 g for muscle, a safe concentration for total residues of GnRF-DT at injection site tissues resulting from acute exposure is calculated as:

$$SC(injectionsite) = \frac{ASDI \times Human\ \ Body\ \ Weight}{Food\ \ Consumption\ \ Factor}$$

$$= \frac{6.6\ \ \mu g / kg\ \ bw \times 60\ \ kg\ \ bw}{300\ \ g}$$

$$= 1.320\ \mu g/g = 1.320\ ppm$$

(Equation 8: SC (injection site) equals ASDI times Human Body Weight divided by Food Consumption Factor, which equals 6.6 µg/kg bw times 60 kg bw divided by 300 g, which equals 1.320 µg/g or 1.320 ppm.)

Table 31. Safe Concentrations (SCs) for Total GnRF-DT Residues in Edible Tissues of Swine Using the Food Consumption Factors.

Edible Tissue	Amount Consumed/Day	Safe Concentration (SC)
Muscle	300 g	0.264 ppm
Liver	100 g	0.792 ppm
Kidney	50 g	1.584 ppm
Fat	50 g	1.584 ppm
Injection site	300 g	1.320 ppm

B. Residue Chemistry:

1. Summary of Residue Chemistry Studies

Traditional residue chemistry studies were not conducted for this approval. Due to the instability of GnRF-DT, a peptide protein conjugate, in swine, the Agency considered traditional residue chemistry studies inappropriate for the determination of incurred drug residue concentrations or metabolic profiles in swine. The Agency conducted a worst-case residue exposure assessment for total drug residues in the edible tissues, including the injection site tissue, of swine treated with IMPROVEST Sterile Solution. The Agency's decision criterion is that if the estimated total residue exposure from an edible tissue in the worst-case assessment is lower than the residue safe concentration for that tissue, then the residues in the edible tissue are found to raise no human food safety concerns.

The assessment assumes that at the nominal zero withdrawal (8-12 hours post treatment), the entire administered dose is distributed and then retained in each of the edible tissues including remote muscle, liver, kidney and fat, and in 500 grams of the injection site muscle. In reality, drug absorption, distribution, metabolism,

and elimination would take place after the administration, resulting in reduction of the drug residues to the concentrations less than those predicted in the assessment.

By doing a worst-case assessment, the Agency ensures that conservative human food safety decisions are made in the absence of information obtained through traditional residue chemistry studies.

A Worst-Case Assessment for Total Residues of GnRF-DT in Liver, Kidney, Muscle and Fat

The assessment assumes that an average market-size pig weighs 107 kg, and the liver, kidney, muscle and fat of the pig make up 1.3, 0.6, 54.5 and 35 percent, respectively, of the body weight. The assessment also assumes that the entire 400 µg GnRF-DT dose is distributed to each of the 4 edible tissues and no drug residues are eliminated from the tissues (representing zero hours post treatment). The resulting total residue concentrations in the tissues are shown in Table 32 below:

Table 32. Results of a Worst-Case Assessment for Total GnRF-DT Residues in Muscle, Liver, Kidney, and Fat.

1	2	3	4	5
Edible Tissues	Tissue Weight as of Live Market Size Pig of 107 kg	Tissue Weight Estimated (kg)	Total Tissue Residue Concentration from 400 µg GnRF-DT[1] (µg/g)	Calculated Human Exposure to Residues[2] (µg/person/day)
Muscle	54.5%	58.3	0.0069	2.1
Liver	1.3%	1.4	0.286	28.6
Kidney	0.6%	0.64	0.625	31.3
Fat	35.0%	37.5	0.0107	0.5

[1] calculated by dividing 400 µg of GnRF-DT by the respective tissue weight in Column 3.

[2] calculated by multiplying total tissue residue concentration in Column 4 with the respective food consumption factor (muscle, 300 g; liver, 100 g; kidney, 50 g; fat, 50 g).

Conclusions: The total residues of GnRF-DT in muscle, liver, kidney, and fat are below the respective safe concentrations for the edible tissues and do not raise residue safety concerns at zero withdrawal.

A Worst-Case Assessment for Total Residues of GnRF-DT at the Injection Site

A worst-case scenario for total residues of GnRF-DT conjugate at the injection site is calculated based on the treatment dose of 400 µg:

400 µg/500 g = 0.8 µg/g = 800 ng/g

(Equation 9: 400 µg divided by 500 g, which equals 0.8 µg/g or 800 ng/g.)

Where: 500 g is the standard amount of injection site muscle collected for measuring residue concentrations at the injection site.

Conclusions: At zero withdrawal, the total residues at the injection site are below the safe concentration for the injection site and do not raise residue safety concerns at a zero withdrawal.

2. Target Tissue and Marker Residue Assignment

It is not necessary to assign a target tissue or marker residue for GnRF-DT residues.

3. Tolerance Assignments

It is not necessary to establish a tolerance for GnRF-DT residues in swine.

4. Withdrawal Period

Based on the assessment summarized under section IV. B.1 above, the use of IMPROVEST in swine qualifies for a zero withdrawal.

C. Microbial Food Safety:

Antimicrobial Resistance

GnRF-DT conjugate (IMPROVEST) is neither thought, nor has been reported to impact antimicrobial resistance among bacteria of public health concern in or on treated boars. The Agency has determined that an assessment of the microbial food safety for the use of GnRF-DT conjugate (IMPROVEST) in intact boars is not necessary at this time.

Impact on Human Intestinal Flora

Residues and metabolites of GnRF-DT conjugate (IMPROVEST) in or on the edible tissues of treated boars are neither thought, nor have been reported to impact intestinal flora in human consumers. The Agency has determined that an assessment of the impact of GnRF-DT conjugate (IMPROVEST) on human intestinal flora and establishment of a microbiological acceptable daily intake is not necessary at this time.

D. Analytical Method for Residues:

A regulatory analytical method for residues of GnRF-DT is not required.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to IMPROVEST:

CONTRAINDICATIONS:
Not approved for use in female pigs and barrows.

Do not use IMPROVEST in intact male pigs intended for breeding because of the disruption of reproductive function.

WARNINGS:

WITHDRAWAL PERIODS:
No withdrawal period is required when used according to labeling.

Not for Human Use. Keep Out of Reach of Children.

USER SAFETY WARNINGS:

Warning for person administering IMPROVEST: Accidental self injection could affect reproductive physiology of both men and women and may adversely affect pregnancy. **Pregnant women should not administer this product. Women of childbearing age should exercise extreme caution when handling this product.** Special care should be taken to avoid accidental self injection and needle stick injury when administering the product. Protective clothing including, but not limited to, safety glasses and gloves should be worn. Use a safety injector, preferably one which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. In case of eye contact, rinse immediately with copious amounts of water. In case of skin contact, wash immediately with soap and water. The product should be stored safely out of the reach of children. As a reminder, it is the prescribing veterinarian's responsibility to inform drug administrators of the user safety warnings associated with IMPROVEST.

Advice to the user in the event of accidental self injection: In the event of accidental self injection, wash the injury thoroughly with clean running water. Seek prompt medical attention and take the package leaflet with you. Do not administer the product, and/or any other product with a similar action, in the future.

Advice to the physician: Accidental self injection could affect reproductive physiology of both men and women and may adversely affect pregnancy. If self injection with IMPROVEST is suspected, reproductive physiology should be monitored by assay of testosterone or estrogen levels (as appropriate). The risk of a physiological effect is greater after a 2nd or subsequent accidental injection than after a 1st injection. The patient should be advised not to administer IMPROVEST, and/or any other product with a similar action, in the future.

For customer service, to report suspected adverse reactions or to obtain a copy of the Material Safety Data Sheet (MSDS) call 1-800-336-5288.

PRECAUTIONS: Subcutaneous injection in intact male pigs can cause a transient local injection site reaction that may result in trim loss at slaughter.

ADVERSE REACTIONS: The field study observations from field effectiveness studies were consistent with the observations made during the target animal safety studies of transient inflammation at the injection sites. IMPROVEST did not cause unusual clinical signs or an unexpected frequency or severity of injection site reactions. Adverse events, as reported, were not uniquely attributable to IMPROVEST.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that IMPROVEST, when used according to the label, is safe and effective for the temporary immunological castration (suppression of testicular function) and reduction of boar taint in intact male pigs intended for slaughter. Additionally, data demonstrate that residues in food products derived from intact male swine treated with IMPROVEST will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian. Adequate directions for lay use cannot be written because profession expertise is required to properly administer the injection and due to the significant impact on human reproductive function if self-injected.

B. Exclusivity:

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the animal drugs has previously been approved.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.

VII. ATTACHMENTS:

None