Safety and Toxicity Evaluation of GonaCon™ Immunocontraceptive Vaccine in White-Tailed Deer

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ABSTRACT: GonaCon™ Immunocontraceptive Vaccine targets the reproductive hormone gonadotropin releasing hormone (GnRH) secreted by the hypothalamus of the brain. Antibodies produced in response to the vaccine inactivate endogenous GnRH, which in turn eliminates stimulation of the pituitary gland and gonads in males or females. The resulting "immunocastration" renders animals unable to produce reproductive steroids or gametes. In previous studies, we demonstrated that GonaCon™ was effective in impairing fertility for up to 3 years in female deer without apparent side effects. However, detailed post mortem evaluations were not done. To better understand the entire physiological response of deer to GonCon™ and to establish if contraindications were associated with its use, we undertook the present study. To evaluate toxicity and safety, 7 does were given the standard single injection GnRH-KLH vaccine dose (1000 µg) delivered IM in 1 ml using AdjuVac™ adjuvant and compared to 6 does given a single control saline IM injection and 6 does given 3 injections of GonaCon™ at 2-week intervals per dose. The study was conducted for 20 weeks. Does were blocked by weight and randomly assigned within blocks to treatment groups. Blood samples were drawn immediately prior to vaccination and at 5, 10, 15, and 20 weeks post immunization. Blood was assayed for LH, testosterone and progesterone, and anti-GnRH titers, as well as hematology and blood chemistry. At each sampling period, the general health of the doe was observed and the injection site was inspected for the formation of abscesses or other tissue reactions. At Week 20, all deer were euthanized and evaluated at necropsy by veterinary pathologists and samples of lymph nodes, reproductive organs, lung, liver, heart, kidney, spleen, and brain were taken for histology. Aside from granulomata formation at the injection site, there were no significant contraindications or toxic effects associated with GonaCon™.

KEY WORDS: blood chemistry, GnRH vaccine, gross pathology, histopathology, immunocontraception, *Odocoileus hemionus*, safety, toxicity, white-tailed deer

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INTRODUCTION

GonaCon™ is a single-shot GnRH vaccine that is used in combination with AdjuVac™ adjuvant to immunologically sterilize the target species. The vaccine works by stimulating antibodies against gonadotropin releasing hormone (GnRH) produced by the hypothalamus of the brain. Antibodies produced in response to the vaccine inactivate endogenous GnRH, which in turn eliminates production of the pituitary gland hormones, follicle stimulating hormone (FSH), and luteinizing hormone (LH). In the absence of FSH and LH, the gonads atrophy in both males and females. Secondary sex characteristics and reproductive behaviors dependent on gonadal steroids are also absent or greatly diminished in treated animals.

Both GonaCon™ and AdjuVac™ were developed by the USDA National Wildlife Research Center and have been tested in combination as a single-shot vaccine to effectively block reproduction in white-tailed deer, *Odocoileus hemionus* (Miller *et al*. 2000); feral and domestic swine, *Sus scrofa* (Miller *et al*. 2003, Killian *et al*. 2006); Nevada mustangs, *Equus caballus* (Killian *et al*. 2004, 2006); domestic cats, *Felis catus* (Levy *et al*. 2004); American bison, *Bison bison* (Miller *et al*. 2004*a*); as well as other species (Miller *et al*. 2004*b*). These studies clearly demonstrated that GonaCon™ is highly effective as an immunocontraceptive, with infertility lasting up to 2 or more years. Although no apparent side effects were observed with GonaCon™ use in any of the species tested, detailed evaluations were not performed to assess effects of the vaccine on target species health and safety. We undertook the present study to better understand the overall physiological response of deer to GonCon™ and to establish if any contraindications were associated with its use. The objectives of the current study were to evaluate the safety of GonaCon™ in white-tailed deer under conditions of normal recommended use and to document any effects of toxicity of GonaCon™ in white-tailed deer.

METHODS AND MATERIALS

Animals

A total of 19 female deer, ranging in age from 2-5 years and weighing 35-80 kg, were used in the study. The study deer were born, raised, and maintained at the Penn State University Deer Research Center. None of the deer were pregnant, lactating, or in estrus as the study began in late July. Does were assigned to treatments by age classes using a computerized randomization program prior to study. To evaluate the safety of GonaCon™ under conditions of normal use, 6 does were randomly assigned to a control group and received a 1-ml injection of 0.9% saline and 7 does received a single 1000-µg dose of GonaCon™. The 1× dose, delivered in a 1-ml volume, consisted of the GnRH peptide-keyhole limpet hemocyanin (KLH) conjugate combined with AdjuVac™ adjuvant. To evaluate any toxic effects of GonaCon™, 6 does received the initial injection of GonaCon™, followed by 2 additional injections of GonaCon™ at 2-week intervals. Hand injections were made intramuscularly in the semitendinosus muscle of the hind quarter, which was clipped to facilitate subsequent examinations. For the group receiving 3 injections, the injections were given on alternating hind quarters. Blood samples were taken from each doe just prior to vaccination and at 5, 10, 15, and 20 weeks post vaccination.

During the course of the study, does were chemically restrained for immunizations, taking blood samples, and veterinary examinations. For sedation, does received 2.2 - 4.4 mg/kg xylazine (100mg/ml) IM, which was reversed with 4.0 mg/kg Tolazine, either IV or IM. At the last sampling 20 weeks post vaccination, sedated does were euthanized with Euthasol IV as needed.

Animal Evaluations and Sampling

During the study, each animal was observed daily for general health, well-being, and normal behavior. When does were sedated at 0, 5, 10, 15, and 20 weeks, they were weighed and subjected to close veterinary examination of the injection site and to observe any ocular, temperature, integument, cardiovascular, or respiratory abnormalities. At the end of the study, euthanized does were transported approximately 1 mile to the Pennsylvania State University Animal Diagnostic Laboratory, where necropsies were performed by a team of veterinary pathologists within 4 h of euthanasia. Tissue samples were taken of the injection sites, draining lymph nodes, reproductive organs (mammary gland, uterus, ovaries, fallopian tubes), lung, liver, kidney, spleen, pancreas, bladder, ileum, duodenum, bladder, bone marrow, joint, heart, brain (cerebral cortex, cerebellum, brain stem, hypothalamus), pituitary, thyroid and adrenal glands, and any other organs that indicated gross pathology or abnormality. Tissue samples were fixed in buffered formalin and sent to Colorado Histo-Prep, Ft. Collins, CO for further processing and reading by a certified veterinary pathologist. The treatment identity was unknown to the pathologists collecting and evaluating the tissues. Samples collected of serum and whole blood were shipped overnight on cold packs to LabCorp PreClinical, Research Triangle Park, NC, where they were analyzed for blood chemistry and hematology. A sample of serum was also sent overnight on ice to the National Wildlife Research Center, Ft. Collins, CO, for assays of progesterone, luteinizing hormone, and antibodies to GnRH (Levy *et al*. 2004).

Statistical Analysis

Planned comparison contrast using a general linear model, repeated measure two-way analysis of variance was used. Responses of measured variable were analyzed on the basis of time and dose, with deer blocked by age.

RESULTS

Observations on Live Animals

During each data collection period, detailed observations were made on the general health status of the animals. All does were in good flesh and had normal vital signs. The only notable observation made was that small, raised areas were palpable beneath the skin at the site of injection of many treated does. These areas typically ranged in size from 0.5 to 2.0 cm and generally regressed over the course of the study. Late in the study, one animal in the control group injured its leg during recovery from anesthesia. After 3 days of observation, it was concluded that her prognosis was poor and she was euthanized.

Gross Post-Mortem Observations

At necropsy, all of the does were observed to be in good flesh and to contain moderate amounts of fat subcutaneously and in the body cavity. For 3 control does and all does treated with GonaCon™, there was some tissue reaction at the injection site in the semitendinosis muscle, typically evidenced by a tan-yellow appearance and a bulge at the cut surface. For the 2 other control does, there was a brown needle track oriented perpendicular to the surface. For 2 does receiving the 3 injections of GonaCon™ and one doe receiving 1 injection of GonaCon™, the reaction at the injection site included creamy-white exudates and additional nodules nearby. Three does receiving 1 injection of GonaCon™ and one doe receiving 3 injections of GonaCon™ had fibrous adhesions between the lung and the thoracic wall. The doe receiving the 3 injections also had fibrous adhesions between lung lobes. No such adhesions were seen in control does.

Histopathology

Many does in the study were observed with sarcocysts, in either skeletal or cardiac muscle, but there was no apparent correlation with treatment. Likewise, spleenic congestion was also noted in several controls and GonaCon™-treated does. All does treated with GonaCon™ were characterized by severe granulomatas at the injection sites, in contrast to the absence of granulomatas in control animals. Mild interstitial pneumonia was detected in one doe treated with a single injection of GonaCon™ and 2 does treated with 3 injections of GonaCon™. The one control doe euthanized after a leg injury was diagnosed post mortem with pneumonia, apparently the result of aspiration of rumen fluid during anesthesia. Minimal pleuritis, characterized by fibrous tags over the pleural surface, was also

observed in 2 does receiving 1 injection and 2 does receiving 3 injections of GonaCon™. In one doe receiving a single injection of GonaCon™, granulomatous lymphadentis was observed, indicating that the reaction at the injection site had progressed to the draining lymph node. Single incidents of myocarditis and nephrosis, and 2 incidents of hepatic or spleenic fibrous tags, were also observed, without apparent relationship to treatment.

Hematology

Baseline hematology values are presented as the mean and standard deviation for all does at the initial sampling period (Table 1). There was no significant treatment effect for any hematology parameter over all sampling points. Average values obtained were comparable to values reported previously for deer treated with PZP vaccine (Miller *et al*. 2001) and from the International Species Information System for female white-tailed deer available on the Internet (www.isis.org). For several hematology parameters, there was a significant effect ($p < 0.05$) of week of sampling (Table 2). These were all related to red blood cell parameters including RBC, HGB, HCT, MCV, MCH, MCHC, and PLT. Moreover, a significant ($p > F$; 0.02) week × treatment interaction was observed for MCH.

Table 1. Mean and standard deviation (SD) of blood hematology values for all does collected at the first sampling period. Treatment P-values are from 2-factor (treatment, week) repeated measures analyses for all deer and all sampling periods. There was no treatment effect for any of the parameters.

Parameter*	Mean (SD)	Treatment P-values
WBC × 10^3/μL	5.6 (2.1)	.17
RBC × 10^6/μL	13.7 (2.1)	.63
HGB g/dL	15.3 (1.8)	.12
HCT %	42.5 (6.0)	.40
MCV fL	30.9 (2.0)	.71
MCH pg	11.2 (0.8)	.39
MCHC g/dL	36.0 (1.3)	.19
PLT × 10^3/μL	422.1 (88.2)	.08
MPV fL	7.7 (2.5)	.29
NEUT × 10^3/μL	4.3 (2.0)	.21
LYMPH × 10^3/μL	1.0 (0.5)	.34
MONO × 10^3/μL	0.05 (0.09)	.69
EOS × 10^3/μL	0.39 (0.38)	.86
Neutrophils %	74.0 (11.4)	.64
Lympnocytes %	18.2 (7.9)	.36
Monocytes %	0.9 (1.84)	.69
Eosinophils %	7.0 (5.9)	.86

*Abbreviations: WBC – white blood cells; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelets; MPV – mean platelet volume; NEUT – neutrophils; LYMPH – lymphocytes; MONO – monocytes; EOS – eosinophils

Table 2. Mean and standard deviation (SD) for hematology parameters of white-tailed deer treated with GonaCon™ sampled at 5-week intervals post vaccination. The dates of sample collection are indicated. A significant week effect ($p < .01$) associated with seasonal changes was detected for all parameters except NEUT, LYMPH, MONO and percent neutrophils, lymphocytes, monocytes, and eosinophils.

	Time Period 0 wk July 19-20 (*n*=19)	Time Period 5 wk August 23-24 (*n*=19)	Time Period 10 wk September 27-28 (*n*=19)	Time Period 15 wk November 1-2 (*n*= 18)	Time Period 20 wk December 6-7 (*n*=18)
WBC	5.55 (2.08)	5.74 (2.39)	5.74 (2.17)	5.35 (2.94)	3.93 (1.71)
RBC	13.71 (2.12)	14.43 (1.11)	13.79 (1.89)	14.41 (1.82)	14.99 (1.48)
HGB	15.25 (1.77)	16.13 (1.15)	15.56 (1.49)	16.42 (1.54)	17.36 (1.45)
HCT	42.47 (6.03)	52.68 (9.43)	42.37 (4.51)	44.72 (4.73)	52.17 (4.66)
MCV	31.10 (2.07)	36.76 (7.66)	31.24 (2.72)	31.19 (2.26)	36.54 (7.51)
MCH	11.20 (0.80)	11.21 (0.72)	11.36 (0.97)	11.46 (0.90)	11.62 (0.91)
MCHC	36.03 (1.29)	31.61 (6.12)	36.78 (1.07)	36.76 (1.17)	33.34 (1.95)
PLT	422.0 (88.2)	376.8 (116.4)	429.4 (137.4)	377.4 (120.3)	309.7 (111.2)
MPV	7.71 (2.48)	7.52 (2.54)	8.69 (5.30)	8.29 (3.57)	12.39 (6.88)
NEUT	4.31 (1.95)	4.03 (1.88)	4.28 (2.10)	4.08 (2.69)	2.63 (1.57)
LYMPH	0.96 (0.50)	1.12 (0.46)	1.15 (0.43)	0.91 (0.42)	1.03 (0.37)
MONO	0.05 (0.09)	0.08 (0.09)	0.05 (0.07)	0.04 (0.06)	0.04 (0.06)
EOS	0.39 (0.38)	0.51 (0.37)	0.25 (0.24)	0.32 (0.30)	0.23 (0.18)
% Neutrophils	74.00 (11.37)	67.89 (11.92)	71.89 (12.56)	72.44 (14.38)	63.06 (14.62)
% Lymphocytes	18.16 (7.87)	21.42 (9.50)	22.47 (10.30)	20.00 (10.25)	29.11 (12.22)
% Monocytes	0.89 (1.41)	1.21 (1.03)	1.00 (1.29)	0.78 (1.00)	0.83 (1.30)
% Eosinophils	6.95 (5.92)	9.42 (5.91)	4.58 (4.03)	6.78 (6.14)	7.00 (6.10)
% Basophils	0.00 (0.00)	0.05 (0.23)	0.05 (0.23)	0.00 (0.00)	0.00 (0.00)

*Abbreviations: WBC – white blood cells; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; PLT – platelets; MPV – mean platelet volume; NEUT – neutrophils; LYMPH – lymphocytes; MONO – monocytes; EOS – eosinophils

Blood Chemistry

Baseline chemistry values are presented as a mean for all does at the initial sampling period (Table 3). There was no significant treatment effect for any blood chemistry parameter over all sampling points. Average values obtained were comparable to values reported previously for deer treated with PZP vaccine (Miller *et al*. 2001) and from the International Species Information System for female white-tailed deer available on the Internet. Statistical analysis revealed that there was a significant effect ($p < 0.05$) of week of sampling on all blood chemistry values except CPK (Table 4). Moreover, a significant week × treatment interaction was observed for GLOB ($p > F$; 0.055) and TBIL ($p > F$; 0.0080).

Table 3. Mean and standard deviation (SD) values for blood chemistry of all does collected at the first sampling period shown with treatment P-values from 2-factor (treatment, week) repeated measures analyses for all deer and all sampling periods. There was no treatment effect for any of the parameters

Parameter*, units	Mean (SD)	Treatment P-values
Na, meQ/L	142.2 (1.8)	.59
K, meQ/L	3.98 (0.29)	.28
Ca, mg/dL	8.55 (0.45)	.48
PO_4, mg/dL	8.10 (1.90)	.76
ALK, U/L	101.3 (35.2)	.76
GLUC, mg/dL	313.8 (41.4)	.81
TP, g/dL	6.57 (0.35)	.20
ALB, g/dL	3.16 (0.18)	.20
GLOB, g/dL	3.41 (0.42)	.081
CPK, U/L	658.3 (619.1)	.66
TBIL, mg/dL	0.66 (0.35)	.066
ALT, U/L	47.4 (26.5)	.14
CREAT, mg/dL	1.38 (0.20)	.14
BUN, mg/dL	31.7 (3.7)	.49
AMYL, U/L	122.9 (21.2)	.95

*Abbreviations: Na – sodium; K – potassium; Ca – calcium; PO_4 – phosphate; ALK – alkaline phosphatase; GLUC – glucose; TP – total protein serum; ALB – albumin; GLOB – globulin; CPK – creatine kinase; TBIL – total bilirubin; ALT – alanine aminotransferase; CREAT – creatinine; BUN – urea nitrogen; AMYL – amylase

Antibody Titers and Hormone Assays

Antibody titers to GnRH were absent in the controls, but does receiving 3 injections of GonaCon™ had significantly greater titers ($p < 0.05$) than those receiving 1 injection (Figure 1). These higher titers were sustained throughout the period of study and were predicted from earlier efficacy trials. There was no treatment effect on serum concentrations of progesterone or luteinizing hormone among the groups. There was a significant week effect on antibody titer and serum progesterone concentrations.

DISCUSSION

Immunocontraceptive vaccines are generally considered a potentially useful approach for controlling overabundant populations of wildlife and feral species.

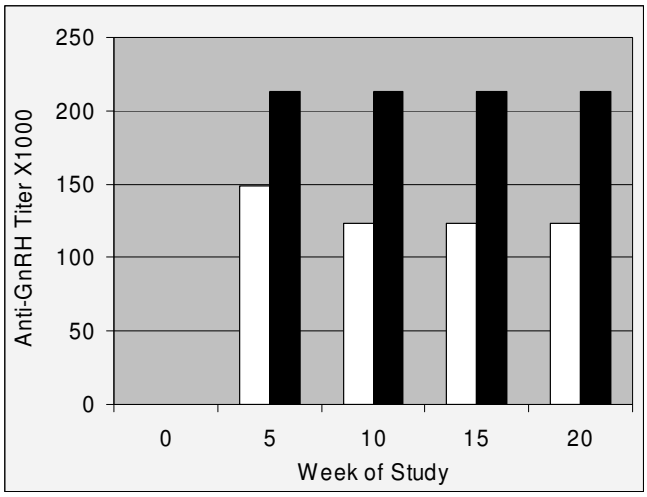

Figure 1. Anti-GnRH titers following a single vaccination of GonaCon™ (open bars) or 3 injections of GonaCon™ at 2-week intervals (solid bars). Titers were significantly greater at 10, 15, and 20 wk post vaccination in deer receiving the 3 injections.

Table 4. Mean and standard deviation (SD) for blood chemistry values of white-tailed deer treated with GonaCon™ sampled at 5-week intervals post vaccination. The dates of the sample collection are indicated. A significant week effect ($p < .01$) associated with seasonal changes was detected for all parameters except TBIL and CPK.

	Time Period 0 wk July 19-20 (*n*=19)	Time Period 5 wk August 23-24 (*n*=19)	Time Period 10 wk September 27-28 (*n*=19)	Time Period 15 wk November 1-2 (*n*=19)	Time Period 20 wk December 6-7 (*n*=18)
Na, meQ/L	142.2 (1.8)	140.3 (1.9)	139.4 (1.8)	136.5 (1.9)	144.8 (3.3)
K, meQ/L	3.98 (0.29)	4.11 (0.42)	4.15 (0.42)	4.44 (0.53)	4.83 (0.48)
Ca, mg/dL	8.55 (0.45)	9.37 (0.35)	8.32 (0.43)	8.35 (0.48)	8.93 (0.33)
PO_4, mg/dL	8.10 (1.90)	5.67 (1.43)	6.99 (1.43)	6.06 (1.27)	6.22 (1.11)
ALK, U/L	101.3 (35.2)	105.7 (32.0)	94.4 (34.8)	63.3 (19.4)	57.5 (16.0)
GLUC, mg/dL	313.8 (41.4)	292.2 (49.4)	255.3 (55.7)	284.2 (54.3)	340.8 (52.2)
TP, g/dL	6.57 (0.35)	7.09 (0.45)	6.85 (0.56)	7.16 (0.43)	6.92 (0.38)
ALB, g/dL	3.16 (0.18)	3.34 (0.18)	3.36 (0.21)	3.53 (0.23)	3.52 (0.24)
GLOB, g/dL	3.41 (0.42)	3.75 (0.50)	3.49 (0.63)	3.64 (0.54)	3.40 (0.45)
CPK, U/L	658.3 (619.1)	436.0 (229.3)	457.1 (316.1)	436.6 (297.6)	363.7 (344.7)
TBIL, mg/dL	0.66 (0.35)	0.72 (0.93)	0.61 (0.25)	0.47 (0.19)	0.41 (0.18)
ALT, U/L	47.42 (26.45)	47.42 (9.52)	50.16 (11.11)	51.74 (10.44)	45.50 (9.65)
CREAT, mg/dL	1.38 (0.20)	1.55 (0.34)	1.44 (0.22)	1.65 (0.29)	1.81 (0.29)
BUN, mg/dL	31.74 (3.72)	28.32 (4.37)	39.84 (4.39)	39.42 (5.87)	36.78 (6.04)
AMYL, U/L	122.89 (21.20)	113.26 (19.57)	91.32 (17.42)	90.05 (14.49)	97.11 (16.54)

*Abbreviations: Na – sodium; K – potassium; Ca – calcium; PO_4 – phosphate; ALK – alkaline phosphatase; GLUC – glucose; TP – total protein serum; ALB – albumin; GLOB – globulin; CPK – creatine kinase; TBIL – total bilirubin; ALT – alanine aminotransferase; CREAT – creatinine; BUN – urea nitrogen; AMYL – amylase

This notion is based on many studies demonstrating excellent contraceptive efficacy using either porcine zona pellucida or GnRH peptide as the immunogen (see review, Fagerstone *et al*. 2002). Our previous work involving the development and testing of the GnRH vaccine GonaCon™ has demonstrated that this vaccine has good efficacy in a variety of species. When research trials suggest an immunocontraceptive vaccine has potential for wide scale application, safety and toxicity data are necessary to ensure regulatory agencies that the contraceptive vaccine is safe for the target animals. While the efficacy studies we conducted gave no reason to believe that the GonaCon™ was unsafe or associated with contraindications, detailed physiological and post mortem evaluations were not performed to thoroughly evaluate the treated animals relative to untreated controls.

The current study was undertaken to evaluate GonaCon™ safety in deer that had been given a single injection as a representation of normal recommended use of the vaccine. In addition, we also conducted a parallel trial to evaluate GonaCon™ toxicity, by giving 3 injections of the vaccine spaced 2 weeks apart. The toxicity study was to simulate what may potentially occur in the field involving an unintentional revaccination of previously treated does. The design of the study was reviewed by the federal Food and Drug Administration, prior to its undertaking.

Compared to control does, the most notable parameter in treated does was the reaction which occurred in the semitendinosus muscle at the site of injection. In treated live animals, however, the reaction site was only minimally detectable by skin palpation and caused no reason for concern. The lesser tissue reaction at the injection site of control does suggests that the severity of the reaction in treated does is likely associated with the AdjuVac™ and the *Mycobacterium avium* contained within. All deer on this study had minimal Johne's antibody titers in pre-immune serum samples. However, only does treated with AdjuVac™ showed significant increases in Johne's antibody titers post vaccination. This immune response to *M. avium* in AdjuVac™ no doubt contributes to the successful contraception achieved with GonaCon™.

The results of the general veterinary exams and the extensive list of blood parameters evaluated for each time point lead us to conclude that there was no adverse affect of treatment on overall animal health. Although there were some changes in blood chemistry and hematology associated with week of the study, they were not related to treatment and not unexpected given the metabolic changes deer undergo during the breeding season and in preparation for winter in the Northern Hemisphere. Three of the 35 blood parameters sampled also had a significant week × treatment interaction. This response may have also been the result of seasonal changes occurring with rut, and the fact that compared to controls, the reproductive hormones of GonaCon™-treated females would have been diminished in the later weeks of the trial as the antibody titers reached effective levels.

In addition to the tissue reactions at the site of injection, both the gross pathology and histopathology examinations revealed a variety of unremarkable findings that one would expect to observe in captive white-tailed deer populations in Pennsylvania (Hattel *et al*. 2004). The only possible treatment effect was the mild pleuritis observed in a few treated does. However, pulmonary disease is common in deer and is the primary cause of death in captive deer herds in Pennsylvania (Hattel *et al*. 2004). While observation of fibrous lung adhesions is not uncommon and in itself not a reason for alarm, the fact that none were observed in the 6 control does is curious. Fibrous adhesions between the lung and thoracic wall are indicative of a prior infection that has been resolved. It is not possible to know whether these adhesions occurred during the study or were the result of a preexisting condition. Two years prior to the study, a large number of deer in the PSU herd, as well as domestic livestock herds in the county, were exhibiting respiratory symptoms associated with an infection. Because so many deer were showing symptoms, it was not possible to thoroughly examine each animal. Alternatively, we treated about 100 deer that were either showing symptoms or in the same paddocks with those showing symptoms. Given this history and the relatively small number of treated does exhibiting the condition, it is certainly possible that the mild pleuritis observed in the treated does was the result of a preexisting condition.

Based on the available evidence, we conclude that GonaCon™ is safe to target animals when used as a single-shot contraceptive vaccine. Moreover, based on the toxicity study, unintentional repeated vaccination over a 6-week period does not pose a serious threat to the health and well being of female deer.

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