Technical Opinion no. 1300/2008

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Applicant: Fort Dodge Saúde Animal Ltda.
CNPJ: 43.5880.045/0001-31
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Matter: Requests technical opinion on biosafety of a product derived from genetically modified organisms – inactivated vaccine against Porcine Circovirosis – Suvaxyn PCV2 one Dose – for commercial use.
Previous extract: 993/2007. Published in the Federal Official Gazette no. 86, of May 07, 2007.
Meeting: 111th Regular Meeting held on March 13, 2008.
Decision: GRANTED.

SYNOPSIS: CTNBio, following examination of the request for import and marketing of the inactivated vaccine against Porcine Circovirosis – Suvaxyn PCV2 One Dose, GRANTED the request on the terms of the within Technical Opinion. Mr. Cristopher Roger White, manager of regulatory issues, Fort Dodge Saúde Animal Ltda., holder of CQB 224/08, requests from CTNBio a technical opinion regarding the biosafety of the Porcine Circovirosis inactivated vaccine genetically modified organism – Suvaxyn PCV2 One Dose – for the activities of import, storage, transportation and marketing. The product shall be imported in a finished form, being the phases of production, purification and packaging conducted in the United States (Iowa, USA) by the company Fort Dodge Animal Health, and shall be used to vaccinate four week aged pigs. The result of the voting was: One (1) abstention and twenty-two votes for approval of the request to release the inactivated vaccine against Porcine Circovirosis – Suvaxyn PCV2 One Dose, on the terms of the within Technical Opinion. In the context of the competences granted by Law no. 11,105/05, regulated by Decree no. 5,591/2005, CTNBio held that the product follows the applicable rules and legislation aimed at securing biosafety in what relates to the environment, agriculture and human and animal health.

1. General Information

Porcine Circovirosis is an infectious disease of viral etiology caused by porcine circovirus type 2 (PCV 2), family Circoviridae, genus Circovirus. The infection of piglets and young pigs (five to twelve week old) may determine the behavior of a syndrome. Clinical signs include loss of weight, emaciation, tachypnea, dyspnea, jaundice or mucosa paleness, lymphadenopathy (inguinal lymphonodes) and diarrhea. PCV 2 was also isolated (1994) in newly-born pigs with congenital tremors and, in 1996, in Canada, the infection was related to the Swine Post-weaning Multisystemic Wasting Syndrome. PCV 2 antigens were already identified in association with proliferative and necrotic pneumonia, in reproductive failures and abortions in swine females and in the Porcine Dermatitis and Nephropathy Syndrome, this virus has also been isolated in sub-clinical cases and asymptomatic animals.
Economic losses caused by circovirosis may be significant and are mainly due to progressive thinning of infected animals, reduction in weight gain and increased food

conversion. Mixed infections (co-infection) of PCV 2 jointly with other microorganisms causing respiratory, enteric and reproductive infections are common. The weakened immunologic system of affected pigs infected by PCV 2, which may cause reduced immunity, is another area currently under studies. Compared with other viral infections affecting hog farming, both the PCV 2 identification and the probable clinical signs of porcine circovirosis syndrome have been described in the recent past. However, serologic and mainly etiologic studies show that PCV 2 infection is largely disseminated in world swine herds, especially in countries where production features high technical level.

PCV was first identified in 1974, as a contaminant of PK-15 swine kidney cell cultures. This virus, currently known as PCV 1, is held as non-pathogenic. The first isolation of PCV 2, which is antigenic and genetically distinct from PCV 1, occurred in 1996, in animals displaying Swine Post-weaning Multisystemic Wasting Syndrome.

In Brazil, the first PCV 2 identification report occurred in 2000. Later, new diagnostic descriptions were made. Currently there are at least five research teams dedicated to the study of PCV 2 in this country. Special emphasis shall be given to the pioneering team, still active in the field, of researchers linked to the Swine and Bird National Research Center (EMBRAPA, Concórdia, SC). Besides, research teams in the states of Rio Grande do Sul, São Paulo, Rio de Janeiro and Minas Gerais have spent time studying porcine circovirosis. Preliminary results from these studies have shown that porcine circovirosis is highly spread in Brazilian herds. Undoubtedly, the disease is currently a major concern in animal health, afflicting hog farmers and professionals in the area. The reduction caused by the disease in productivity entails significant economic losses in the important sector of Brazilian hog farming and reduces the competitiveness of Brazilian pork in the international market due to increased production costs.

GMO description:

Porcine Circovirus Type 2 (PCV 2) is the primary, though not exclusive, cause of PMWS (Postweaning Multisystemic Wasting Syndrome). It has a genome of 1.768 pb (GenBank AF264042) that contains the gene codifying the replicase (945 pb, between positions 822 and 1766) and the gene of the capsid protein (702 pb, between positions 37-738 of the complementary genome). Porcine Circovirus Type 1 (PCV 1) is a mammal virus, unenveloped, icosaedric, the genome of which is formed by a single stranded DNA molecule (ssDNA). Two important genes have been identifies in PCV 1 and PCV 2 genome: the one encoding the replicase (ORF 1) and the gene of the capsid protein (ORF 2). The vaccinal organism is a chemical virus, with part of the genome coming from a non-pathogenic PCV 1 (using a non-virulent strain) isolated from PK-15 (ATCC CCL-33) swine renal calculi, which had no evidence of causing cytopathological lesions. Both PCV 1 and PCV 2 are small viruses, with a diameter of about 17 nm, icosaedric morphology and are devoid of envelope. Nucleic acid is made of single stranded DNA, with about 1759 nucleotides, negative polarity and circular structure covalently closed.

In order to construct the vaccine, a chimera was produced using PCV 1 as a receptor virus. PCV 1 genome is made of two regions of open reading frames (ORF). OFR 1 is a replicase encoder while OFR 2 encodes the viral capsid protein. This sample is not pathogenic. The OFR 2 of this virus was replaced by ORF 2 of PCV 2.

The Porcine Circovirus Type1 – Type 2 Chimera (cPCV1-2) is made of a capsid gene (702 pb, encoding the immunogenic protein of 30-32 kDa) of PCV 2 (originated from the viral PCV 2 genome no. 40895, isolated in swine spleen tissues displaying Swine Post-weaning Multisystemic Wasting Syndrome) that was cloned in the PCV 1 genomic structure, replacing the capsid gene of this genoma, generating the

vaccine organism denominated cPCV1-2. Regulating and selecting genes were not introduced in this construction, and the chimera has just viral genes (Fenaux, et al. J. Virol. 78:6297-6303, 2004; Fenaux et al. J. Virol. 77:11232-43,2003).
Following, a vector was generated containing two copies of the chimera virus (cloned in the pBS vector) that was used for transforming PK-15 cells to generate viruses (cPCV-1 MSV 1227-63-052004) used as vaccine. The chimera denominated cPCV1-2 was inactivated by BEI (binary ethylenimine) (J. Clin. Microbio. 3:209-10, 1976) and confirmation of the inactivation was conducted in PK-15 cells by immunofluorescence after the third multiplication cycle using PCV 2 anticapsid monoclonal antibody. Methods are shown to determine cPCV1-2 identity. The test to determine purity of the master seed virus (MSV) demonstrates non-contamination by bacteria, fungi and mycoplasma. Tests evidenced the innocuousness of MSV for pigs and mice. Contaminating viruses in tests using MA104, MDBI, ST, SPK cell lines and Vero cells. The virus kept stable in X, X+5 and X+7 passes (tests conducted with sequencing of the seed virus and by indirect immunofluorescence).
Product Biosafety:
Being an inactivated (dead) GMO and the phases of production, inactivation and packaging conducted in facilities abroad, there is no risk of introducing microorganisms in Brazil. There is no risk in using this recombinant virus as an immunogen to animal health – both pigs and any other animal species, public health – through infection of humans, and also to the environment.
High dose vaccine security tests were conducted in newly-born susceptible piglets. Application of the vaccine failed to bring any toxic reactions and, according to the studies produced, the vaccine was held safe for pigs over two weeks old.
Environmental Safety:
As the vaccine is an inactivated (dead) organism, the risk of leakage to the environment is very low, and may be held acceptable, requiring little care regarding this aspect. The likelihood of an inactivated vaccine to establish itself in the environment is even remoter, requiring the concurrent materialization of rare events, thus lowering the probability of occurrence.
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2. Final Opinion by CTNBio:
CTNBio is favorable to the granting of commercial release of the product styled Suvaxyn PCV 2 One Dose – inactivated vaccine against porcine circovirosis, considering that:
(1) There are not reports on problems associated to the protein of the PCV 2 capsid (insulated from insect and recombinant Escherichia coli cells);
(2) PCV 1 is not pathogenic and the chimera PCV 2 gene is avirulent and noninfectious, making little likely that cPCV-2 is pathogenic, as shown in controlled essays (innocuous for pigs, the main PCV 2 hosts);
(3) The vaccine with PCV1-2 is manufactured in the Fort Dodge Animal Health facilities in the USA;
(4) The cPCV1-2 used to produce the vaccine is inactivated (in the form proposed to be marketed), and has been innocuous to pigs, mice and guinea pigs in both laboratory and field conditions.;
(5) There are no expected conditions for the inactivated virus to establish in the environment;

(6) There are no reports of human diseases associated to this porcine circovirus, though antibodies against such virus have been found in serum; and
(7) The risks to public health, animal health and environment are low.
Twenty-two (22) CTNBio members voted for the release of the product, and one (1) member abstained from voting.

Walter Colli
President of CTNBio