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This is to certify that I, Marco Antônio Rochadel, Official Public Translator, designated and installed in Office according to The Official Gazette of June 23, 1982, page 5428, have received and translated, to the best of my knowledge and belief, a document with the following contents:

Ministério da Ciência e Tecnologia

GOVERNO FEDERAL BRASIL PAÍS RICO É PAÍS SEM POBREZA

Ministry of Science and Technology – MCT

National Biosafety Technical Commission – CTNBio

comissão técnica nacional CTNBio de biossegurança

Office of CTNBio Secretary

SPO, Área 05, Quadra 03, Bloco B, Térreo, Salas 08 a 10

70610-200 Brasília, Distrito Federal, ☎ +55 61 3411 5516 • +55 61 3317 7475

Technical Opinion no. 2872/2011

Proceedings:	**01200.003421/2010-89**
Applicant:	**Intervet do Brasil Veterinária Ltda**.
CNPJ:	**07.954.091/0001-43**
Address:	**Intervet do Brasil Veterinária Ltda. Avenida Sir Henry Wellcome 225, Prédio Administrativo, 1º andar, Ala "E", Moinho Velho, 06714-050 Cotia, SP, ☎ (11) 4613-4000, 4613-4006.**
Matter:	**Application for Opinion on biosafety and activities of import, transportation, storage and marketing of INNOVAX® ILT – recombinant vaccine for poultry, a biologic product of veterinary**.
Meeting:	**141st Regular Meeting held on April 14, 2011**
Previous Extract:	**2516/2010, published in the Federal Official Gazette no. 167, of August 31, 2010**.

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Decision: **GRANTED**.

CTNBio, following examination of the proceedings related to the request for Technical Opinion on biosafety of a Class I biologic risk genetically modified organism regarding its biosafety and activities of import, transportation, storage and marketing of the veterinary use biologic product styled INNOVAX® ILT – Recombinant Vaccine for poultry, reached a conclusion favorable to granting the request on the terms of this Technical Opinion.

The President, the Internal Biosafety Commission of Intervet do Brasil Veterinária Ltda, Mr. Leonardo Bruno Costa, requests a technical opinion on activities of import and marketing of the product derived from a genetically modified organism, INNOVAX® ILT (Live recombinant vaccine against Marek's disease and infectious Laryngotracheitis on poultry). Phases of product manufacturing and bottling shall be conducted at Intervet Inc. laboratories located in 29160 Intervet Lane, P.O. Box 318, Millsboro, Delaware 19966, United States of America. The product is a live recombinant vaccine for use in poultry and intends to protect the birds against Turkey HerpesVirus (THV) and Infectious poultry Laryngotracheitis Virus. The company forwarded the documents required for the request. Regarding the competences provided by Law no. 11105/2005 as regulated by Decree no. 5591/2005, the Commission found that the information given and remaining biosafety measures proposed comply with CTNBio rules and applicable legislation aimed at securing environment, agriculture, human and animal health biosafety.

1. Technical Grounds of Report Findings

General Information

Intervet do Brasil Veterinária Ltda., holder of CQB no. 248, requests a technical opinion on

activities of import and marketing of a product derived from genetically modified organism, INNOVAX® ILT (Live recombinant vaccine against Merkel disease and poultry Infectious Laryngotracheitis). Marek's disease (EM) is a lymphoproliferative disease caused by turkey herpesvirus (THV) of the *Herpesviridae* family and characterized by infiltration in cells in one or more peripheral nerves, gonad, iris, muscles and skin. The disease morbidity ranges from 10% to 50% and its mortality is 100%. Marek's disease is caused by the Marek's disease virus belonging to the Herpesviridae virus known as Marek's disease virus (MDV), classified as a non-oncogenic, serotype 3 virus. Turkey herpesvirus (THV) is a virus that has been used commercially for over 25 years for immunization against Marek's disease since 1972. The sample used is the FC-126 of THV, obtained from blood of turkeys with no clinical symptoms. THV is a non-pathogen virus and its hosts are limited to bird species. THV does not cause any clinical diseases in chicken and its dissemination by contact is rare.

Turkey herpesvirus is a virus the genome of which is a double strain DNA with about 160 kb that has been commercially used for vaccination against Marek's disease since 1972. The strain used is the FC-126 of THV, obtained from asymptomatic turkey's blood. THV is a non-pathogenic virus and its hosts are limited to avian species. THV does not cause any clinical diseases in chicken and dissemination by contact is rare. This strain has been used as a vaccine strain for immunization against Marek's disease for over twenty-five years and its use as a vector of vaccine antigens is due to its numerous safety advantages.

Infectious laryngotracheitis (ILT) is a highly contagious chicken disease caused by ILT virus (ILTV) classified as a member of the *Herpesviridae* family subfamily *Alphaherpreviridae*. The virus has the gD and gI glycoproteins genes, which are the structural genes coding for proteins found in the virion envelope and are the ideal vaccine candidates, since they are able to induce

a strong immune response against ILTV.

Main symptoms displayed by infected animals are forcible breathing, upright head, half open beak and blood expectoration. This is a mucohemorrhagic inflammation, sometimes causing larynx and trachea hemorrhage. It causes bloody nasal discharge, repeated sneezing and difficult breathing. Propagation of the virus is by inhalation or intra-ocular. Transmission occurs by contact between contaminated and sensitive birds, through equipment and bed. Control is made through vaccinating birds with 10 weeks of life.

GMO Description

ILTV gD and gI genes, containing the regulating regions, were inserted in the THV FC-126 sample by homologue recombination through fragment superposition, resulting in the THV/ILT-138 sample. In this construct, gD and gI genes were inserted in 711-92.1A vector of *Pac*I previously inserted through cloning of a DNA synthetic sequence in site *Xho*I of the vector. This vector, with the inserted ILTV genes, was used in a co-transfection with the THV FC-126 sample to generate the THV/ILT-138: in this process, the homologue recombination between the vector and the receptor sample took place. The only difference between the recombinant THV/ILT-138 sample and the parental THV FC-126 sample is the insertion of the ILTV fragment containing the gD and gI genes. Recombinant sequences of plasmids and markers are not present in the virus.

Biosafety Aspects

Studies on the effect of the vaccine sample on non-target species, distribution of the virus on target species and dissemination and multiplication of the virus showed that the THV/ILT – 138 fails to behave differently from the THV FC-126 sample. To assess genetic stability, two studies

were conducted. In both cases, after five passages in chicken, there was no record of lesions specific for Marek's disease or ILTV. DNA and RNA were extracted from the birds' blood samples and the viral DNA was detected by Southern blot and viral RNA by RT-PCR. The techniques enable showing that gD and gI genes are stable in the THV/ILT-128 virus genome and that there was neither loss nor rearrangement of genes. Other tests were conducted *in vitro* and no evidence of genetic alteration was noticed.

The INNOVAX® ILT vaccine is indicated to be administered to 18 day embryos through *in ovo* vaccination, or to one day chicks by subcutaneous way, to protect the birds against Marek's disease and Infectious Laryngotracheitis. Several studies were performed with the purpose of observing whether the recombinant virus was different from the parental vaccine strain. All parameters assessed showed that the biologic characteristics of THV/ILT-38 do not differ from the biologic characteristics of the parental virus, in terms of *in vitro* replication and tissue tropism.

Regarding post-commercial release monitoring, after the INNOVAX® ILT vaccine is registered, all batches imported intended for marketing in Brazil shall be permanently monitored by Intervet do Brasil Veterinária Ltda. through the pharmacomonitoring system and any adverse effects reported to CTNBio.

CTNBio Final Opinion

Whereas:

1. The risk classification of the genetically modified organism that is present in the vaccine belongs to risk class 1 (low individual risk and low collective risk);
2. the data submitted show that the vaccine fails to imply additional risks to the

environment and animal production;

3. the history of safe use of a similar recombinant vaccine (Vectormune THV-IBD), using the same virus with the viral protein VP2 of the Gumboro disease virus strain, was already analyzed by CTNBio and the vaccine considered to be safe for commercial use, therefore favorable to the release of this vaccine for marketing and use in poultry;

4. there is no evidence that the use of this viral strain may offer risks to human health, vaccinated animals and the environment;

CTNBio is for the granting of the request for technical opinion on import, transportation, storage, marketing and commercial release of the product styled INNOVAX® ILT vaccine.

Twenty-one CTNBio members voted favorably to the release and two members abstained from voting.

Dr. Edilson Paiva

CTNBio President

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In Witness Whereof, I have hereunto set my hand and seal in this City of Brasília, Federal District, Brazil, this Wednesday, April 15, 2015.

Fees according to

Official Gazette of 04/15/2011 Marco Antônio Rochadel

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