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Applicant: Fort Dodge Saúde Animal.
BQC: 244/08
Corporate Taxpayer Identifying Number: 43,588,045/0001-31
Address: Rua Luis Fernando Rodriguez, 1701 Vila Boa Vista – Campinas, São Paulo, CEP 13064-798; Phones: (19) 37456240 Fax: (19) 37456189.
Subject: Request for opinion on the biosafety of genetically modified organism for activities involving import, transport, storage, and marketing.
Previous extract: 2368/10 published on 17 May 2010
Meeting: 138th Regular meeting of CTNBio held on 17 November 2010
Decision: GRANTED

SYLLABUS: The person legally responsible for the institution requested to CTNBio technical report on the biosafety of genetically modified organism called Poulvac ST – a live vaccine against Salmonella typhimurium to import, store, transport, and market. This product will be imported ready and finished, and the stages of production, purification and bottling performed in the Iowa (USA) by the company Fort Dodge Animal Health. This product is registered in the country of origin (USA) since 16 May 2001, and since then had a history of safe use. CIBio from the institution states that the operative unit has adequate infrastructure and competent technical staff to safely develop the proposed activities. The institution and owner of the Biosafety Quality Certificate No. 244/08 issued by CTNBio in 2008 for the purposes of import, trade, and stockpiling of biological veterinary products containing genetically modified organisms of biological risk class I, as CTNBio Opinion No 1270/2008. The product in question is a vaccine, from lineage of S. typhimurium LT2 strain 1545, in which aroA gene and SerC gene are deleted. Demand aromatic conferred by a deletion of aroA results in an inability to grow in defined media without aromatic supplements, such as tyrosine, phenylalanine, tryptophan and p-aminobenzoate (PABA). The need for PABA, a metabolite not found in the tissues of vertebrates results in an attenuation of growth in vivo.

1. General information
Food contamination with Salmonella is a serious public health problem worldwide. The bacterium belongs to the family Enterobacteriaceae, and is widely distributed in nature, having as natural reservoirs intestinal tract of humans and other animals. Among these, there are the birds; they are asymptomatic and constantly eliminate Salmonella in faeces. Transmission to humans occurs by eating food contaminated with faeces of carrier animals (cattle and poultry) and through eggs and milk. Typically, cases of salmonellosis are associated with the consumption of undercooked products of avian origin such as eggs and meat. In humans, the appearance of symptoms caused by infection with Salmonella starts 6~48h after ingestion and include severe diarrhoea, vomiting, nausea, abdominal pain, and severe headaches, which persisted 2~7 days. Currently there are traditional markets vaccines composed by formalin-inactivated whole bacteria, which are used for vaccination of birds and who have limited protection for not confer significant immunity in the intestinal tract. Live attenuated vaccines temporarily colonize the intestine and stimulate local and systemic immunity of the vaccinated animal, preventing infection.
The vaccine proposed for commercial release, the subject of this opinion, contains the bacterium Salmonella typhimurium, genetically modified to be used as live vaccine for

poultry. The vaccine is administered with coarse spray, with 1.1 x 10 7 CFU per dose to birds with only one day of life. The aim is to protect the birds against infection with S. enteridídis, S. typhimurium, and S. Heidelberg, only occasionally pathogenic for young birds, but can cause serious health problems.

2. Describing the GMO:
The parent organism is an isolated poultry farm of Salmonella typhimurium, strain 82/6915, isolated from a chicken in Victoria, Australia in 1982. The genetic modification was performed by using bacteriophage transduction of p22 AroA-554:Tn 10 S. typhimurium LT2 strain 1545 to the wild type strain. Initially, allele aroA-554:Tnl0 strain S. typhimirium 1545 was transferred to the strain S. typhimurium 82/6915, and transducers resistant to tetracycline were selected. The transducers aroA-554:Tnl0 grown in selective medium, containing tetracycline. Colonies were isolated and screened for loss of tetracycline resistance. A tetracycline-sensitive mutant that remained auxotrophic for aromatic metabolites were identified. This mutant was shown to have also undergone SerC gene deletion adjacent to the aroA. Therefore, the deletion mutant organism has two genes, aroA- and serC-, being denominated strain STM-1. The aroA gene encodes the protein 3-phosphoenolpyruvylkimate-5-phosphate synthase, an enzyme in the biosynthesis of aromatic amino acids. The serC gene encodes phosphoserine aminotransferase, an enzyme in the biosynthesis of serine. The loss of aroA gene function prevents the biosynthesis of aromatic metabolites, such as tyrosine, phenylalanine, tryptophan, p-aminobenzoate (PABA), and 2,3-dihydroxybenzoate. The culture medium must be supplemented with these metabolites to be bacterial growth. The requirement for PABA, a metabolite not found in vertebrate tissues, results in retardation of growth in vivo, since it is required for bacterial growth. The mutation at serC results in a requirement of serine for growth. Outside the host, the mutant organism is not able to replicate due to the need for the presence of serine, aromatic amino acid, and PABA. Deficient lineages in genes of this metabolic pathway remain in host tissue for several days, but without causing symptoms and eventually be eliminated by immunological defence mechanisms.

3. Product biosafety
Assessment of GM micro-organism as normative resolution No 5, 12 March 2008, Annex III
Virulence of master seed
The master seed was produced from an original fiasco of culture STM-1 stored at -70°C. The culture was confirmed and tested for purity by Gram stain, PCR, profile plasmid, antigen tests, growth on blood Agar, XLD Agar, Agar of MacConckey and IST, and minimal broth. Studies of reversion to virulence have shown that the organism S. typhimurium STM-1 is not virulent after a series of five recta in chickens. Genotypic and phenotypic stability is high. The rate of reversion to the mutant aroA is estimated to be <10 -11 and the rate of reversion to the mutant aroA is serC <10 - 18.
Risk evaluation to human and animal health
Study was conducted to assess the possible reversion to virulence, demonstrating that the strain S. typhimurium STM-1 is not after a series of virulent recta in chickens. At five days after inoculation, the contents of the caeca of birds were collected and cultured, showing a recovery of up to 60% for each rectum. There was no recovery of the micro-organism of any of the uninoculated birds kept in touch with any of the inoculated recta. In comparison study of 21 days, the birds inoculated with the caecal contents collected from 5" rectum or with the working seed, did not show any clinical

signs of disease typical of S. typhimurium. It was concluded that the vaccine strain did not revert to virulence through 5 recta in live birds and poultry can be given safely. The virulence of the strain was tested in BALB/c mice by intraperitoneal injection at 10 8 CFU. There were no adverse effects to the mice for up to 10 days after inoculation. However, mice that received an oral dose of 42 CFU of the parental strain died within eight days. The strain was used to vaccinate cattle and pigs with a dose of 10 10 CFU, subcutaneously proved to be safe on its use. There were no deaths or clinical signs of disease attributable to administration of the vaccine during the study. Assessing the information that comprise the process, studies of attenuation of virulence of the mutant strain, the finding of no reversal of virulence in the target animal (chickens) and non-target animals (mice, pigs, cattle), we can conclude that the risk of this vaccine is low, both for animals and for the environment. Although no human studies done with this strain, work in the literature with strains of Salmonella typhimurium to the aroA gene showed no adverse reactions in human volunteers. It leads us to conclude that the vaccine strain for poultry does not present significant risks to human health.
- Animal safety
The isolation of the micro-organism by environmental scanning of a test aviary and commercial poultry farms showed that the vaccine strain does not persist in the environment or in the birds for more than 21 days after vaccination. The organism vaccination was not spread among birds when kept in isolation. There was no recovery of the micro-organism of any of the uninoculated birds kept in touch with any of the inoculated recta. This indicates that the micro-organism vaccine does not spread to nearby birds, and cannot be isolated after 21 days of inoculation in birds held on the first day of life. There were no deaths or clinical signs of disease attributable to administration of the vaccine during the study. Thus, it is believed that the live vaccine with a deletion aroA and serC of S. typhimurium (1.1 x 10 9 CFU per dose) is safe. Tests to prove the safety of the product revealed that it is not detrimental to public health, environment or infected animals. The product is already approved and used in Australia and the USA as being registered for sale included in the copy process. So far there were no reported harmful effects for the use of the vaccine.
- Safety monitoring plans for post-trade liberalisation
In compliance with the additional information requested to the company, they explain in this document sent on 10 Nov 2010 that the marketing of the vaccine POUVALC ST - Salmonella typhimurium live vaccine will be made through the company's distributor partners, and Fort Dodge Saúde Animal Ltd. has the ability to track vaccine use in the field. Fort Dodge has a program that accounts for overall monitoring of their use of poultry products in the area called MISA (Integrated Monitoring for Avian Health), which consists of visitation with the creations poultry veterinary consultant, clinical, and necropsy examinations and laboratory diagnosis when needed. This system MISA results in reports and analysis that are filed in a database that could be used to identify challenges and need for control.
The company points out that, despite having concluded that vaccination with POULVAC – ST against Salmonella thyphimurium no take risk to animals, environment, and humans, has overall monitoring capacity through the system of pharmacovigilance, monitoring the field performance of the product . The company also has toll-free number, available on the product package, through which can be received complaints and inquiries. If there are reports received by this number, investigative processes will be initiated.
Regarding the procedure for monitoring case – specifically, the company states that assesses and filters the possible complaints and conducts investigations for future cases,

which includes a visit to affected farm and diagnostic tests. Severe cases may be reported to the Ministry of Agriculture, Livestock and Supply, as well as procedures for collecting the product and stop distribution.

4. Final opinion

The technical advice on the biosafety of genetically modified organism called Poulvac ST – Live Vaccine against Salmonella typhimurium and based on the following points: (1) the vaccine is produced at the site of Fort Dodge Health in the USA and provides safe history of use in this country and in Australia; (2) during the construction of the vaccine organism genetically modified were deleted the genes aroA and serC, which makes the micro-organism dependent of nutrient supplements such as PABA and aromatic amino acids for its multiplication, preventing it settles in the environment; (3) the parental strain does not display attenuated virulence in humans and other mammals tested (such as pigs, cattle, mice); (4) the data submitted by the company on construction and safety demonstrate a low risk to public health, animal health, and the environment; (5) the company sent a monitoring program through general pharmacovigilance, monitoring the product in the field and throughout its commercial life.

Thus, the cultivation and consumption of MON 89034 x NK 603 maize are not potentially causer of significant environmental degradation or risk to human and animal health.

Edilson Paiva,PhD

President of CTNBio