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CVMP assessment report for Rabitec (EMEA/V/C/004387/0000)

Common name: rabies vaccine (live, oral) for foxes and raccoon dogs

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

On 20 October 2016 the applicant IDT Biologika GmbH submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Rabitec, through the centralised procedure falling within Article 3(1) and point 1 of the Annex of Regulation (EC) No 726/2004 (product developed by means of a biotechnological process).

The eligibility to the centralised procedure was agreed upon by the CVMP on 21 January 2016 as Rabitec is developed by recombinant DNA technology.

The rapporteur appointed is Esther Werner and the co-rapporteur is Kristina Lehmann.

Rabitec is a live, genetically modified organism (GMO), attenuated rabies vaccine. The vaccine strain SPBN GASGAS is a recombinant rabies virus which has been derived by site directed mutagenesis from the most widely used oral rabies vaccine for wildlife, the passage attenuated vaccine strain SAD B19. The recombinant virus has been further attenuated compared to the SAD B19 strain by three targeted genetic modifications.

The applicant applied for the following indication: For the active immunisation of foxes and raccoon dogs against rabies to prevent infection and mortality.

The vaccine is presented as a bait of fishmeal containing a PVC-aluminium blister filled with 1.7 ml of the vaccine suspension in packs of altogether 800 baits (1x800, 2x400, or 20x40).

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC.

On 5 October 2017, the CVMP adopted an opinion and CVMP assessment report.

On 1 December 2017, the European Commission adopted a Commission Decision granting the marketing authorisation for Rabitec.

Scientific advice

The applicant received scientific advice from the CVMP on 13 August 2015 (EMA/CVMP/SAWP/553740/2015). The scientific advice pertained to non-clinical and clinical aspects of the dossier. The applicant followed the scientific advice given by CVMP.

MUMS Status

The applicant requested MUMS classification for this product by the CVMP, and the Committee confirmed at their 8-10 April 2014 meeting that, where appropriate, the data requirements in the appropriate CVMP guidelines on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS status was granted for the following reasons:

As the target species foxes and raccoon dogs are minor species, the product is indicated for MUMS/limited market.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (version 8, 23 July 2014) which fulfils the requirements of Directive 2001/82/EC, as amended. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the

necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Rabitec is manufactured in the EU by IDT Biologika GmbH, Germany.

The active substance is manufactured at IDT Biologika GmbH, Am Pharmapark, Dessau-Rosslau, Germany or by IDT Biologika (Riems) GmbH & Co. KG, Greifswald-Insel Riems, Germany.

Batch release will be carried out by IDT Biologika GmbH, Am Pharmapark, Dessau-Rosslau, Germany.

For all the sites listed above, appropriate and valid manufacturing authorisation and GMP certificates were presented. Specific inspections are currently not required.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites were considered in line with legal requirements.

Part 2 - Quality

Composition

The finished product is presented as bait for oral suspension containing a live, attenuated rabies virus strain SPBN GASGAS as active substance.

The titre per vaccine dose (1.7 ml) is $10^{6.8}$ – $10^{8.1}$ FFU (Focus Forming Units).

The excipients used for the vaccine are: water for injection as diluent for the stabiliser, sucrose and gelatine as stabiliser, disodium phosphate di-hydrate and potassium dihydrogen phosphate as stabiliser and buffer and further the antibiotic neomycin. The bait of fishmeal is containing a PVC-aluminium blister filled with the vaccine suspension.

A stabiliser to protect the vaccine during freezing and storage is included. No adjuvant is included. The composition of the vaccine is considered acceptable. The presence of the antibiotic neomycin in the vaccine was initially considered as a major objection; however its function in the vaccine suspension was described in detail and the reason for its use was justified. A detailed description on the measures applied to avoid any possible contamination during production of the blister and filling is provided, as well as a risk assessment taking into account the risks for humans, target animals, non-target animals, the environment and antibacterial resistance. It is not possible to employ commonly used preservatives as the product is a live viral vaccine. In fact the use of antibiotics as preservative in vaccines should be avoided in general as indicated in Ph. Eur. monograph 0062 “Vaccines for veterinary use”; however in this case where the vaccine is not administered by a veterinarian under controlled conditions, but is deployed in the environment and possibly taken up by an animal not until several days later, an exception is justified.

The bait comprises of fish meal as attractant, palm fat, coconut fat and paraffin as matrix material and oxytetracyclin (OTC) as biomarker. Ingredients are listed as excipients in section 6.1 of the SPC. The excipients are compliant with European Pharmacopoeia (Ph. Eur.) standard, where applicable.

Container

The vaccine is filled into a PVC-aluminium blister and is then embedded into a bait matrix which contains

fishmeal as attractant for the wild animals for which the vaccine is intended. 1.7 ml of the vaccine suspension is filled into one blister. This blister consists of a PVC thermo-forming foil and an aluminium cover foil.

For preparation of the blister the PVC foil is heated and formed into a mould. For manufacture of the blister the aluminium foil is pressed on the thermo-form filled with vaccine suspension using a hot press. Heat-seal-lacquer connects both foils by welding.

Sterilisation of the foils by the usual methods is not possible due to the properties of the materials. However, the applicant describes the measures taken to prevent contamination in detail.

Furthermore every batch of the final product is tested for sterility in the routine batch release testing and the batches included in the stability studies remained sterile so far, as long as they were stored frozen.

In summary, the measures taken for prevention of contamination are considered appropriate.

The primary packaging materials used for the manufacture of the blister comply with the respective requirements in the Ph. Eur.

Development pharmaceutics

An explanation and justification for the composition and presentation of the vaccine was provided.

The presented vaccine is an advancement of the oral vaccine Fuchsoral registered in several EU countries. The aim was to further enhance the safety profile of a known virus strain in combination with a well-established vaccine-bait system. The applicant gives a short summary on the development of measures on rabies control over the last 60 years and concludes that the increasing number of raccoons, which are highly susceptible to the virus and live in high densities especially in urban areas, enhances the risk of rabies outbreaks, due to the possibility of spread from foxes.

The final formulation was chosen as the vaccine is intended to be taken up by foxes or raccoon dogs. During chewing, the virus is released into the oral cavity, thus leading to the active immunisation to prevent infection by rabies virus. The intake of one single bait is sufficient to ensure active immunisation to prevent infection by rabies virus. The baits are distributed by land or by air within the framework of vaccination campaigns against rabies.

The description of the development of the vaccine is satisfactory. A detailed report on the pharmaceutical development of the vaccine was provided.

Data on the mechanical stability of the bait under laboratory and environmental conditions were provided. Furthermore the applicant complemented the dossier with data on the acceptance of the bait by the target species, providing literature data and an additional study performed in 2016. The results were satisfactory.

Method of manufacture

The maximum passage level in the finished product is MSV+5.

The manufacturing process of the antigen is based on the seed lot system.

For the production of the vaccine, the vaccine virus is cultivated in a continuous BHK21 CL13 cell line in cell culture flasks and/or roller bottles. Cells are infected with the vaccine virus and harvests are performed after infection. Harvests are pooled, clarified by filtration to remove cell debris and filled into sterile bags. A concentration step by ultrafiltration may be applied to the harvests. After blending the vaccine is stirred for homogenization. 1.7 ml vaccine solution per dose is filled into blisters moulded from PVC foil and sealed with aluminium foil. Filled blisters are then printed.

The components of the bait are added to a heated mixing vessel under constant stirring until a homogenous mixture is obtained. This mixture is then transferred to a semi-automated machine with two dosing units, a conveyor with casting moulds which are partly filled with the bait mass and the frozen blister containing the vaccine is put onto it and is covered by further bait mass. Then the baits are immediately cooled in the water bath. Afterwards the vaccine baits are dried and packed.

In-process control tests are considered in line with the requirement of current guidelines.

The method of manufacture is described satisfactorily; some details concerning different steps of manufacture were added for clarification.

Control of starting materials

Active substance

The active substance is the genetically modified live rabies virus strain SPBN GASGAS. The modification comprises of two amino acid substitutions in the G-protein, a second integration of the modified gene for the G-protein and the deletion of the pseudogene. No foreign genetic material was introduced into the genome.

The virus strain has three stable genetic markers that discriminate the vaccine strain from other rabies virus strains.

Specifications of the active ingredient (SPBN GASGAS) are defined. Analytical methods and validation data are provided and satisfactory.

The stability of vaccine strain SPBN GASGAS and the absence of any increase of virulence potential was investigated by genotypic analysis of the passaged vaccine strain via PCR (confirmation of nucleotide exchanges and double G-protein integration) and comparison of the genome of the 5th passage with the genome of the master seed virus (MSV) by next generation sequencing (NGS).

The MSV was produced from a cell passage in cell factories of the research virus seed obtained from Bernhardt Dietzschold from Thomas Jefferson University in Philadelphia. The working seed virus (WSV) is produced accordingly in cell factories using the MSV as seed material.

MSV and WSV are stored frozen in 5 ml vials. Identity, purity (by gene sequencing) and extraneous agents testing are controlled in line with requirements of Ph. Eur. monograph 0062 on vaccines for veterinary use.

Production and control of MSV and WSV are described in sufficient detail.

Excipients

Specifications of excipients and other starting materials (e.g. materials of biological and non-biological origin, media) are defined and analytical methods are provided. Where applicable, the starting materials are in compliance with Ph. Eur. or other respective regulations.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

An assessment was conducted in order to demonstrate that the risk of TSE transmission and propagation is minimised by the documented and recorded sourcing of animals (animal-derived material of known and controlled origin) and by the nature of the animal tissues used in manufacturing (low or no detectable infectivity).

The starting materials including the seed materials for virus and cells comply with the "Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMEA/4010/01- Rev.3).

It is concluded that the risk of transmitting transmissible spongiform encephalopathy (TSE) infectivity through the use of this vaccine is negligible. EDQM certificates of suitability are provided and valid.

Control tests during production

During manufacture the following in-process control tests are carried out to ensure the quality parameters:

Vaccine suspension:

During cell culture before infection and later during virus propagation: morphology of cells, sterility, absence of mycoplasma.

Blister:

Filling volume (by weighing).

Bait:

Weight of raw materials, temperature control mixing vessel and dosing unit, bait mass, visual blister control, temperature control of the water bath, appearance of the bait.

Test descriptions and limits of acceptance are presented. The in-process controls are appropriate, adequately performed and described. Results of the testing of four consecutive vaccine bait batches are provided and well within the specifications.

Control tests on the finished product

The control on the finished product is performed on the filled product and is carried out to ensure the quality parameters. The following tests are performed:

Blister:

Sterility, absence of mycoplasma, identity, purity, extraneous viruses, virus titre, pH, appearance

Bait:

Organoleptic test (texture, colour, odour, bait stability and form), melting point, biomarker stability.

The description of the methods used for the control of the finished product and the specifications were provided.

The specifications proposed at release and at the end of shelf-life are appropriate to control the quality of the finished product. The results of the analysis of four consecutive batches of vaccine baits were presented and comply with the required specification.

Validation data for all tests were provided and considered satisfactory.

Stability

Stability studies are provided for the following intermediates of production with satisfactory results:

Live virus harvests may be stored for up to 7 days at 2 – 8 °C.

Clarified filtrates of the virus harvests may be stored for up to 24 months at -15 to -45 °C.

Blended vaccine bulks may be stored for up to 4 days (96 hours) at 10 – 20 °C.

The applicant has provided results of long term stability studies on the blister alone and on the blister bait combination. At present, data are available up to 27 months for four batches, thus currently a shelf life of 24 months is justified when stored at -15 °C to – 25 °C. The justification provided for the increase in pH value over storage time is satisfactory. The increased pH value is not considered to have an impact on quality, safety or efficacy.

Vaccine batches were also subjected to a stress test for 7 days at 25 °C or 40 °C, respectively. Within 5 days storage at 25 °C the titre of all analysed batches does not decrease below the minimum titre of $10^{6.6}$ FFU/ml. Only one of four investigated batches showed a result slightly under the minimum titre after 7 days ($10^{6.4}$ FFU/ml). However stability in the environment for up to 7 days at temperatures below 25 °C can be assumed. The vaccine fulfils the requirement of Ph. Eur. monograph 0746 on bait stability. At 40 °C the titre is still kept for several hours.

A cold chain transport is required for the vaccine bait. Baits should be distributed immediately after thawing. The thawed vaccine bait may be stored for 7 days between 2 – 8 °C before use. The minimum titre of $10^{6.6}$ FFU/ml is necessary to ensure full protection over the shelf life. The release titre of $10^{7.0}$ FFU/ml ensures the efficacy of the product over the proposed storage period. Further data was provided employing aged batches. The batches used in the in-use stability study (2 - 8 °C for 5 days after thawing) were 30 months old at time of testing and all results were satisfactory.

The SPC and package leaflet reflect the necessary storage and transport conditions adequately.

Data on bait stability are also provided. Testing of the bait stability is described in detail. The same bait blister system is already used for over 20 years in the distribution of the vaccine Fuchsoral which is authorized in several EU countries. All results during the several tests to mimic environmental conditions (including thermo-stability, randomized temperature changes every 8 hours over 5 days; simulation of rainfall and mechanical stability after distribution by plane) were satisfactory. The suitability and integrity of the blister bait system was sufficiently evaluated and the results are satisfactory.

Environmental risk assessment for products containing or consisting of genetically modified organisms

The application is compliant to Directive 2001/18/EC. For details please see Part 3.

Overall conclusions on quality

The qualitative and quantitative particulars of the vaccine suspension, the bait matrix and the containers are described adequately. The necessary certificates are provided. The addition of the antibiotic neomycin to the vaccine suspension at blending of the bulk was justified satisfactorily by the applicant. The justification also includes a detailed risk assessment. The use of antibiotics as preservative in vaccines should generally be avoided; however in this case where the vaccine is not administered directly by a veterinarian under controlled conditions, but is deployed in the environment an exception is justified. The applicant gives a detailed description of the development of the vaccine strain and its manufacturing process. Summaries of studies which were performed in the course of the development of the vaccine, and studies on genetic stability are provided.

The production process of vaccine blister and bait is described in detail and a validation report on the manufacturing process is provided. The production system is a BHK21 cell culture.

All starting materials comply with the provisions of Ph. Eur. and the TSE risk assessment is adequate. The respective certificates of suitability are provided.

The in-process and finished product controls performed should ensure a consistent production of Rabitec and are considered valid. A visual inspection (appearance test) of the vaccine blister was added as finished product control. Furthermore a specification for the pH value was added.

Data on the mechanical stability of the bait under laboratory and environmental conditions were provided; data on the acceptance of the bait by the target species were complemented and are satisfactory.

The production process results in a reproducible composition of the vaccine. Four batches were analysed and batch to batch consistency was demonstrated.

Stability data were provided for the finished product, intermediates of vaccine production and the bait material. The provided stability data are satisfactory.

The proposed shelf life of 24 months is considered demonstrated. Baits should be distributed immediately after thawing. The thawed vaccine bait may be stored for 7 days between 2 – 8 °C before use.

In conclusion, the production and quality of Rabitec are adequately described and controlled and comply with the respective legal requirements including the TSE risk assessment.

Recommendation for further quality development

Not applicable.

Part 3 – Safety

Introduction and general requirements

The active substance SPBN GASGAS of Rabitec is a live attenuated rabies virus strain which has been derived by site directed mutagenesis from the attenuated vaccine strain SAD B19. SPBN GASGAS has been further attenuated by three targeted genetic modifications.

As strain SAD B19, which belongs to the first generation of live modified oral rabies vaccines together with all other SAD Bern derivates, are known to have a certain level of residual pathogenicity in rodents under experimental conditions, and as also few cases of vaccine associated rabies cases in not yet immune competent or immune compromised animals were reported, the new vaccine strain SPBN GASGAS was developed to improve the safety profile.

Safety was demonstrated in compliance with Directive 2001/82/EC, Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010) and the relevant monographs of the Ph. Eur. chapter 5.2.6., and the specific Ph. Eur. monograph (0746: Rabies vaccine (live, oral) for foxes and raccoon dogs) and VICH GL41 Target Animal Safety - Examination of Live Veterinary Vaccines in Target Animals for Absence of Reversion to Virulence.

Safety documentation

A total of 14 safety laboratory studies were conducted to investigate the safety of the product. Two laboratory studies investigating the safety of the administration of one and repeated dose, two overdoses, one reproductive performance studies, eight spread of the vaccine strain and one reversion to virulence of attenuated vaccine studies were included. Seven studies were carried out in foxes and raccoon dogs, seven studies in non-target species such as dogs, cats, several mouse species, pigs and guinea pigs. The vaccine was administered via oral route, as recommended. Laboratory studies reported to be Good Laboratory Practise (GLP) compliant were carried out in foxes and raccoon dogs.

No field trials were performed. The MUMS guideline allows the omission of safety field trials when

sufficiently justified. Provided that the laboratory data are fully supportive of vaccine's safety, the collection of data from the field could be handled as a follow-up measure. The following agreements were achieved in the Scientific Advice:

- Due to the further attenuation of SPBN GASGAS non-target animal safety data which were generated with the parent virus strain SAD B19 will be accepted, provided that the homology between SAD B19 and SPBN GASAS (except the nucleotide sequence change in G-protein sequence to further reducing the residual pathogenicity) can be demonstrated.
- Fulfilment of requirements of Directive 2001/18/EC, in particular of Annex III A (part 3.E of the dossier).
- Collection of field safety and field efficacy data after granting the marketing authorization; thus dossier submission was made without safety and efficacy data from the field.

In addition, for vaccine candidate selection or confirmation that the selected seed material retained its properties several (pre-) development studies have been performed in different categories of mice and in other species. These studies are not described in detail but an overview is presented. The results do not deviate from the results of the pivotal safety studies included in the present application dossier.

Data from an extensive range of non-target species collected with the predecessor vaccine strain SAD B19 (Fuchsoral) are included in the dossier to further support the safety of vaccine strain SPBN GASGAS.

Pivotal safety studies were performed with SPBN GASGAS using MSV, MSV+1 or MSV+2 administered via oral route except for reversion to virulence (intracerebral application).

Study Type	Target Species		Non-Target Species						
	Fox	Raccoon Dog	Pig	Dog	Cat	Field Mice	House Mice	Guinea Pig	NMRI Mice
One Dose/ Overdose									
Repeated One Dose									
Reproductive Performance									
Spread									
Dissemination									
Reversion									

Grey shadowed: species and type of study that were conducted.

The three batches used in the safety studies were produced according to the outline of production. Information is presented on the diagnostic methods used in the safety studies. The following test methods were used:

- Polymerase chain reaction (PCR) was used for screening issues for the detection of viral RNA fragments in various samples (saliva, faeces and other tissues) to be performed prior to the RTCIT (see below). It should be noted that the majority of PCR screening testing was done at two different laboratories using one method, which targets two different locations of the N genome ("double check") and therefore limits the chance of false-negative results. A third laboratory used two RT-PCR methods, one detecting a short fragment of the N-gene (77bp), the other detecting a longer fragment (659 bp).
- Rabies tissue culture infection test (RTCIT) was used for the detection of replication competent

vaccine virus to be utilized following PCR-positive screening results.

- Fluorescent antibody test (FAT) was used for the detection of rabies virus antigen (SPBN GASGAS vaccine virus, rabies challenge virus) in the brain.
- Rapid fluorescence focus Inhibition test (RFFIT) and fluorescence antibody virus neutralization (FAVN) were used for the detection of neutralizing antibodies against rabies virus.
- Enzyme-linked-immuno-sorbent-assay (ELISA) was conducted to detect rabies virus antibodies in some sera using a commercial test kit (BioPro) for information only purposes.

The laboratory methods decisive for a valid conclusion in view of dissemination in the body and spreading are WHO/OIE prescribed/recommended methods: PCR, RTCIT, FAT, and RFFIT. Test descriptions are submitted. Validity of the test methods is claimed by referencing the proficiency tests which are organized by the European Union reference laboratory (EURL) for rabies and regularly performed at all national rabies reference laboratories (NRL). The test methods have been proven suitable to generate reliable results. The key validity criteria such as specificity and sensitivity as well as limit of detection were sufficiently addressed.

Laboratory tests

Safety of the administration of one dose

The safety of the "administration of one dose" was part of the study to investigate the safety of the "repeated administration of one dose". The "safety of the administration of an overdose" (section B.2) is also covered in this section.

Safety of one administration of an overdose

Two overdose laboratory studies were provided. In these studies a high titre vaccine of $10^{9.1}$ FFU per dose was used. Therefore, the maximum dose is set at $10^{8.1}$ FFU/dose. At least 1 ml was used in foxes and raccoon dogs allowing setting this maximum titre per dose.

The animals were observed daily for at least 180 days (as required by Ph. Eur. monograph 0746) using a scoring system for clinical symptoms, measurement of body temperature (except for raccoon dogs), body weight, determination of feed intake, *post mortem* histology of the brain (raccoon dogs: in addition p.m. histology of the gonads) including FAT analysis to confirm absence of rabies virus (RABV) in the brain and serology.

<u>Foxes</u>

Twenty-six (26) seronegative foxes (122 to 138 days old) were orally vaccinated with 1 ml SPBN GASGAS MSV+1; 10 control foxes received 0.9% NaCl solution.

No clinical symptoms related to typical signs of rabies occurred until day 183, the study end. There was no impact on body weight in any of the groups after the start of the study. No notable increase in body temperature occurred in the vaccinated animals. The control animals developed slightly higher values; this is explained by their handling at the study end that caused additional stress for the animals. *Post mortem* histology and FAT were negative. All 26 vaccinated animals seroconverted (RFFIT ≥ 0.5 IU/ml) by day 42. No seroconversion occurred in the 10 control animals.

The study complies with the requirements of Ph. Eur. monograph 0746; the results clearly indicate the safety of the 10-fold overdose in the target species fox. A maximum titre of $10^{8.1}$ FFU per dose is justified.

<u>Raccoon dogs</u>

Twenty-six (26) seronegative raccoon dogs (225 to 265 days old) were orally vaccinated with 2 ml SPBN

GASGAS MSV+1; 10 control animals received 0.9% NaCl solution.

No clinical symptoms related to typical signs of rabies occurred until day 182, the study end. There was no impact on body weight in any of the groups. Although *post mortem* histology revealed mild micro-gliosis in 7 animals of the treatment group (29.1%) and in 2 of the control group (20.0%), this was not associated with changes typical for a rabies infection. There was no significant difference in the frequency of these findings between the treatment groups ($p = 0.6921$). Overall, no rabies related histopathological finding occurred. No RABV antigen was detectable by FAT in the brain. All gonad samples of the treated animals were RABV RNA PCR negative. Serum samples were only examined at the study start to confirm the seronegative status of the animals.

The study complies with the requirements of Ph. Eur. monograph 0746; the results clearly indicate the safety of the 10-fold overdose in the target species raccoon dog. On the basis of the results no safety concerns arose following the administration of an overdose ten times higher than the recommended dose to the foxes and raccoon dogs.

Safety of the repeated administration of one dose

Two repeated dose laboratory studies were provided. The animals were observed daily for general health for at least 14 days (as required by Ph. Eur. monograph 5.2.6) using a scoring system also taking into account feed intake and defecation. Body temperature and body weight were measured. Saliva and faecal samples were examined by RT-PCR for the presence of RABV RNA and, if applicable by RTCIT for the presence of replication competent RABV as the aspect of potential vaccine strain spread was also covered by the study design. *Post mortem*, the brains were tested by FAT to confirm absence of RABV. Serology was performed too.

Foxes

Eight (8) seronegative European foxes, younger than 1 year, received 1 ml of the vaccine (MSV+2, $10^{7.5}$ FFU/ml) orally distributed on the mucosa of both cheeks under the tongue and on the palate at day 0, 3 and 7.

The 3-day-interval simulated the field conditions and is therefore acceptable even though it deviates from the Ph. Eur. monograph. As it is unlikely that the total volume of 1.7 ml is consumed, the dose volume was decreased to 1 ml by direct oral administration. This is also considered acceptable.

The 4 direct in-contact control foxes remained untreated; the animals were subdivided into 4 sub-groups of 3 animals each (2 vaccinates and one contact control per cage). One of them was accidentally vaccinated at 3rd application.

All animals remained in good general health except for one treated animal that died at day 23 due to an injured front leg. Reduced feed intake could be observed for one day at the 2nd administration and for few days after the 3rd administration (presumably due to the sedation needed for the vaccine administration). There was no effect on defecation. Neither body weight loss nor increase in body temperature occurred.

Overall, no replication competent virus could be determined in any of the saliva samples though RABV RNA was detectable at least once (day 0+2 hours and/or day 0+4 hours post administration) from treated as well as from contact animals. All faecal samples were already tested negative by PCR. No RABV antigen was detectable in the brain of any of the animals.

All vaccinated animals seroconverted by day 28. The one in-contact control animal accidentally vaccinated at the 3rd application also seroconverted. 2 out of 3 contact controls developed an antibody titre ≥ 0.5 IU/ml. As regards this result, the applicant assumes that the animals got into contact with the vaccine virus shortly after 1 or more of the 3 administrations. This argumentation is deemed reasonable

as sedation and administration of the vaccine was performed inside the cages in the presence of the also sedated in-contact foxes. Due to the sedation, the swallowing reflex is reduced and any vaccine virus will be present in the oral cavity for a longer period of time than when not sedated. Thus, it seems possible that contact animals may have taken up vaccine remnants either from the floor or the still sedated vaccinated foxes directly after one or more of the repeated vaccinations.

Raccoon dogs

Eight (8) seronegative raccoon dogs, younger than 1 year, received 1 ml of the vaccine (MSV+2, $10^{7.5}$ FFU/ml) orally distributed on the mucosa of both cheeks under the tongue and on the palate at day 0, 3 and 7.

The 4 direct in-contact control raccoon dogs remained untreated; the animals were subdivided into 4 sub-groups of 3 animals each (2 vaccinates and one contact control per cage).

All animals remained in good general health. There was a reduced feed intake over the whole observation period; it is assumed that this was due to relocation and handling. A loss of weight of > 15% was observed in two animals (1 in-contact control and 1 treated animal) by day 31; it was assumed that the animals lost weight for seasonal reasons. No effect on defecation. No increase in body temperature occurred throughout the entire trial.

Overall, no replication competent virus could be determined in any of the saliva samples though RABV RNA questionable results were found in 7 treated animals and 1 control (day 0+2 hours and/or day 0+4 hours post administration). All faecal samples were already tested negative by PCR. No RABV antigen was detectable in the brain of any of the animals.

All vaccinated animals seroconverted by day 31 (RFFIT/FAVN ≥ 0.5 IU/ml). Two in-contact controls also seroconverted but this observation was not consistent in both assays tested (RFFIT/FAVN). According to the applicant, this is most probably due to contact with the vaccine virus shortly after one or more of the three administrations. Two contact animals remained seronegative in both serological assays.

In conclusion, the safety of the repeated administration of 1 dose was satisfactorily evaluated in 2 studies in the target species fox and raccoon dog in compliance with Ph. Eur. 5.2.6 (Evaluation of safety of veterinary vaccines and immunosera). Furthermore, active shedding via the saliva as a result of local replication did not take place as none of the saliva swabs taken more than 24 hours after the first vaccine administration tested positive for the presence of the vaccine virus (PCR). No replication competent virus was found. No RABV RNA was detected in the faeces of the animals. Brain samples of all individuals showed no presence of the rabies vaccine strain, thus indicating that there is also no vaccine virus dissemination to the brain.

On the basis of the results no safety concerns arose following the administration of the recommended dose one additional time after the primary vaccination schedule to the foxes and raccoon dogs.

Examination of reproductive performance

One study on examination of reproductive performance was provided in foxes.

Three pregnant vixens (European fox) in the first trimester of gestation received the vaccine ($10^{7.2}$ FFU/ml; MSV+2) via bait or drinking bowl.

General health, feed intake and defecation were assessed daily for 126 days using a scoring system including clinical symptoms of the 14 cubs born around day 39 to day 43. Body temperature and body weight post parturition were recorded. Saliva samples from vixens and cubs were examined by RT-PCR for the presence of RABV RNA and, if applicable, by RTCIT for the presence of replication competent RABV.

Post mortem the *glandula (G.) submandibularis* of each cub was tested by RT-PCR; the brains of the vixens and cubs were tested by FAT. Serology was performed to confirm the vaccine intake by the vixens and exclude an immune response in the cubs.

No clinical symptoms occurred in the 3 vixens and the 14 cubs. The duration of the vixens' gestation was in the normal range. There was no impact on body temperature, feed intake and defecation; no effect on body weight could be observed either in the vixens (apart from an interim decrease after parturition) or the cubs. No viral RNA was detectable in any of the animals at any occasion.

Post mortem, no viral RNA was detectable in the *G. submandibularis* as well as no RABV antigen was detectable by FAT in the brain of any of the 3 foxes and 14 cubs. Whereas the vixens showed seroconversion (≥ 0.5 IU/ml), all 14 cubs remained at < 0.5 IU/ml but with a clear evidence for a warning level of maternally derived antibodies.

According to Ph. Eur. monograph 5.2.6., reproductive performance should be examined in not fewer than 8 animals in each stage of pregnancy unless otherwise justified. Here, 3 vixens were used in the first trimester of their pregnancy. The reduced number of animals is justified by the difficulties in holding pregnant vixens.

As none of the treated vixens or untreated cubs showed any clinical signs of rabies or died after vaccination from causes attributable to the vaccine and no adverse effects on the pregnancy or the offspring were noted, the vaccine can be regarded as safe after consumption by pregnant vixens.

According to the applicant, for raccoon dogs 2 vertical transmission studies were performed as well but had to be invalidated due to lack of pregnancy and stress conditions. Ph. Eur. monograph 5.2.6. requires to perform the examination of reproductive performance in the most sensitive category. Fox and raccoon dog belong to the family *Canidae* and, thus, both are susceptible to rabies. However, the raccoon dog required an approximately 100-fold higher challenge dose of the rabies field virus strain FLI ID 148 (Fox Krefeld) than the fox. This indicates that the fox seems to be a more sensitive species regarding rabies susceptibility. As it is extremely difficult to perform such studies in wildlife, this justification is acceptable all the more as in the overdose safety study in raccoon dogs it is shown that the vaccine virus does not disseminate to the gonads. This is of further supportive evidence that the vaccine virus does not affect the reproductive organs of raccoon dogs.

Based on the results in foxes and the findings in raccoon dogs, no negative impact on pregnancy (and lactation) is to be expected. However, the uptake of vaccine blisters by pregnant or lactating animals is not controllable in wildlife.

Examination of immunological functions

No specific tests on immunological functions were carried out since there are no specific data and no reasons to suspect that the vaccine (virus) might adversely affect the immune response of vaccinated target or non-target species or its progeny. In contrast, the vaccine has shown to elicit a fast antibody response and rapid onset of immunity, showing that the immunological functions are not impaired.

Special requirements for live vaccines

The safety parameters chosen to evaluate the spread and dissemination potential of the vaccine were general health condition including body temperature, feed intake and defecation as well as body weight. The detection of RABV RNA by RT-PCR in saliva and faecal samples, in lymphatic tissues and inner organs, if applicable, was studied. RTCIT was used to investigate the presence of replication competent RABV. *Post mortem*, the brains were tested by FAT to confirm absence of RABV. Serology was also performed.

Spread of the vaccine strain

The spread of the vaccine strain for target and non-target species was investigated in 8 studies. In addition, supportive safety data in target species with parent strain SAD B19 was submitted.

As the vaccine is distributed in form of baits, it cannot be excluded that the vaccine strain is consumed by non-target animal species. Therefore, the spread and dissemination potential of vaccine strain SPBN GASGAS was investigated in several non-target species (dogs, cats, swine, field mouse, house mouse and wild guinea pigs).

SPREAD IN TARGET SPECIES

Foxes

One of the 2 studies conducted in foxes belongs to the already discussed repeated dose study, the other was designed to investigate dissemination and spread of the vaccine after oral administration of a single dose ($10^{7.5}$ FFU/ml, MSV+2) to adult foxes.

Fourteen (14) seronegative adult European foxes were included; 12 were vaccinated, 2 served as in-contact controls.

All animals stayed in good health throughout the trial and showed no clinical signs, supported by normal body temperatures, feed intake and defecation.

Viral RNA was detectable in 3 saliva samples from 2 treated animals (2/12) at 2 and/or 4 hours post administration. No vaccine virus was found in saliva of the in-contact animals. No viral RNA was detected in any of the faecal samples. Out of 17 tissues and organs, replication competent virus was detectable in the *tonsilla palatina* up to 3 days post vaccination in 5/11 animals. No viral RNA was detectable in the in-contact foxes.

Post mortem, no RABV antigen was detectable in the brain tissue of any of the animals. Seroconversion ($\geq$ 0.5 IU/ml) was observed 7 days post vaccination. The two direct in-contact animals remained seronegative throughout the trial (until day 84) and thus confirmed absence of spread.

As indicated above, dissemination of the vaccine virus was restricted to the site of vaccine uptake, the *tonsilla palatina* where replication competent virus could be detected for a maximum of up to 3 days in 5 out of 11 foxes. As the *tonsilla palatina* has been identified as the predominant site of vaccine uptake, the detection of vaccine virus in this tissue is not an unexpected finding. In saliva samples replication competent virus was found in only 2 out of 12 animals during the day of oral administration. So, in the rare case a naive animal is in direct contact with an animal that consumes a vaccine bait, this contact animal may also vaccinate itself. Nevertheless, 1 in-contact animal was housed in the same cage as 1 of the positive animals but did not become (sero)positive.

Thus, it is considered very unlikely that an in-contact animal infects itself under normal field circumstances. SPBN GASGAS is not expected to spread to direct in-contact animals.

Raccoon dogs

One of the 2 studies conducted in raccoon dogs belongs to the already discussed repeated dose study, the other was designed to investigate dissemination and spread of the vaccine after oral administration of a single dose ($10^{7.5}$ FFU/ml, MSV+2) to adult raccoon dogs.

Fourteen (14) seronegative adult raccoon dogs were included; 12 were vaccinated, 2 served as in-contact controls.

All animals stayed in good health throughout the trial and showed no clinical signs, supported by normal body temperatures. Feed intake varied but had no effect on defecation. The body weight of the 2 controls decreased initially but increased until study end.

None of the saliva samples that were detected RABV RNA positive by PCR at D0+2hrs and/or D0+4 hrs in 9 (3 questionable) out of 12 vaccinated animals contained replication competent virus. No viral RNA was detectable in the direct in-contact controls. No viral RNA was detectable in any of the faecal samples. Out of the 16 tissues and organs, replication competent virus was detectable up to 3 days post vaccination in 5 raccoon dogs in the *tonsilla palatina* and for 1 day in 1 of these 5 animals in the *velum palatinum*.

Post mortem, no RABV antigen was detectable in the brain of any of the animals. Seroconversion (≥ 0.5 IU/ml) was first observed 10 days (RFFIT) and 7 days (ELISA) post vaccination. The two direct in-contact animals remained seronegative throughout the trial (until day 85) and thus confirmed absence of spread.

As regards the vaccine's potential to spread from one raccoon dog to another, no SPBN GASGAS vaccine virus antigen was found in the brain using FAT and other tissues by RT-PCR; saliva and faeces of the contact animals were also free from vaccine virus. Furthermore, the contact animals remained seronegative for the entire duration of the study. It can therefore be concluded that the vaccine virus was not spread horizontally.

SPREAD IN NON-TARGET SPECIES

Swine

Ten (10) seronegative pigs, at an age of 6 weeks, were orally inoculated with $10^{7.5}$ FFU/ml SPBN GASGAS (MSV+2) and 2 in-contact controls remained untreated.

At study days 1, 2, 4 and 8, each time body weight from 2 treated animals was recorded; subsequently the animals were euthanized to investigate dissemination of the virus and virus spread. The 2 remaining vaccinated animals and the 2 direct in-contact controls were monitored for 8 weeks (day 56), then euthanized and also tested for dissemination and spread.

All animals remained in good general health throughout the trial and showed no clinical signs supported by normal feed intake, normal defecation and good bodyweight gain of the 4 animals that stayed in the trial for the total of 8 weeks.

RABV RNA was detectable in saliva samples for 4 hours in 4 out of 10 vaccinated animals but none of the samples contained replication competent virus. No viral RNA was detectable in the direct in-contact controls. No viral RNA was detectable in any of the faecal samples. In none of 20 tissues and organs tested RABV RNA was detectable.

Post mortem, no RABV antigen was detectable in the brain of any of the animals. Seroconversion (RFFIT ≥ 0.5 IU/ml) occurred in both remaining vaccinated animals by day 28 as confirmed by ELISA. Both contact animals had an antibody titre above 0.5 IU/ml (RFFIT) at either of the two sampling time points post vaccination. These results could not be confirmed by ELISA, where both animals were found to be antibody negative. According to the applicant, it is therefore questionable whether these 2 contact animals were truly seropositive. It is known that the quality of some sera may have a deceptive effect on biological test systems causing false positive results in rare cases. In such cases the ELISA method can contribute to clarify questionable results as it is known to be less susceptible to interfering substances (such as bacterial contamination or autolysis) because the serum dilution used in the assay is generally higher (Moore et al, 2010). This explanation seems reasonable all the more as no replication competent virus was detectable in any of the samples of the treated pigs. If finally a possible vaccine uptake by the contact animals had occurred, this would have been restricted to the time immediately after vaccine application as the contact controls were housed in the same cage as 4 treated pigs for 2 days.

Unfortunately, the *tonsilla pharyngea* of 4 treated pigs euthanized on days 1 and 2 after vaccine application were not sampled and examined.

Altogether, the study results indicate that the vaccine strain did not disseminate in the body or spread to in-contact animals, thus fulfilling the requirements of the corresponding monograph.

Dogs

Twelve (12) seronegative beagle dogs, at an age of 12 to 14 weeks, were orally vaccinated with $10^{9.1}$ FFU/ml (MSV+1); the 4 direct in-contact controls remained untreated.

In this study not only the spreading capability of the vaccine strain via saliva and faeces was studied by placing naïve animals in direct contact to the vaccinated puppies but also the safety of the administration of a 10-fold overdose.

All animals stayed in good general health throughout the trial and showed no clinical signs. Comparable and consistent body weight gain was observed in the groups, feed intake and defecation were normal.

Depending on the fragment detected by the PCR method, viral RNA was detectable in saliva samples for 6 hours up to 1 day in 10 - 12 out of 12 vaccinated animals. Replication competent virus was detectable in 3 out of 12 vaccinated dogs (25%) at 6 hours post vaccination. It can be assumed that this result most probably represents residual vaccine inoculum. Also depending on the fragment detected by the PCR method, RABV RNA could be detected in faecal samples for up to 3 days but in no case replication competent virus was detectable.

Post mortem, no RABV antigen was detectable by FAT in the brain of any of the animals. Seroconversion (≥ 0.5 IU/ml) could be observed in 11 out of 12 vaccinated animals at 16 days post vaccination. Some control animals showed a positive reaction in the RFFIT but not in the FAVN assay. These positive results of the contact controls were considered unspecific neutralization.

In conclusion, the vaccine is safe for young dogs free from maternally derived antibodies against rabies. Shortly after vaccination, residual vaccine virus might be detected in saliva but this vaccine virus level deteriorates rapidly and does not infect dogs that were put in direct contact (ratio 1 to 3). This was shown by the absence of seroconversion in all 4 in-contact control dogs, whereas 11 out of 12 treated dogs seroconverted.

Cats

Twelve (12) seronegative European SPF kittens, at an age of 12 to 16 weeks, were orally vaccinated with $10^{9.1}$ FFU/ml (MSV+1); the 4 direct in-contact controls remained untreated.

In this study not only the spreading capability of the vaccine strain via saliva and faeces was studied by placing naïve animals in direct contact to the vaccinated animals but also the safety of the administration of a 10-fold overdose.

All animals stayed in good general health throughout the trial and showed no clinical signs. Comparable and consistent body weight gain was observed in the groups, feed intake and defecation were normal.

Viral RNA was detectable in saliva samples for up to 9 days in all (12 out of 12) vaccinated animals. Replication competent virus was detectable in 5 out of 12 vaccinated cats (42%) at 2-4 hours post vaccination. All saliva samples taken at 6 hours and later were tested negative for replication competent virus by RTCIT. Viral RNA could be detected by the small fragment PCR (IDT) in faecal samples for up to 2 days but in no case was the virus replication competent.

Post mortem, no RABV antigen was detectable in the brain of any of the animals. Seroconversion (≥ 0.5 IU/ml) occurred in 2/12 vaccinated cats by RFFIT and in 9/12 on D14 by FAVN. It increased by FAVN to 11/12 animals by D56. By RFFIT more animals remained seronegative for a longer period but by

day 91 10 animals had seroconverted as well. All four in-contact control animals remained seronegative by RFFIT (three positive values observed by FAVN on day 126 were considered false positive as they were negative again at the next testing point on day 182; RFFIT remained completely negative at this time point (and all others like FAVN). Thus, the in-contact animals were concluded to be seronegative at study end.

The vaccine is concluded to be safe for kittens free from maternally derived antibodies against rabies. Shortly after vaccination, residual vaccine virus might be detected in saliva but does not spread to other not treated in-contact kittens as it deteriorates rapidly. This was confirmed by the absence of seroconversion in all 4 in-contact control cats whereas 11 out of 12 cats that received the vaccine by oral application seroconverted.

Field mice

Seventy-five (75) adult field mice (between 163 and 957 days old) were split into three groups: 30 vaccinated mice (orally 0.03 ml SPBN GASGAS/MSV+1, $10^{9.1}$ FFU/ml), 24 direct untreated in-contact controls and 21 negative controls.

The in-contact mice served to determine the effect of potential vaccine spread. As it was expected that some mice would die during the course of the experiment (88 days), the negative control group served to establish the normal back-ground mortality in the mice.

Here, general health was assessed using a scoring system specific for rabies development in mice. On all observation days the general health condition was scored 0 (no clinical signs of rabies). However, in total 12 mice were found dead during the observation period with the following group distribution: 3/30 treated mice, 4/24 in-contact mice and 5/21 negative control mice. The mortality rate in both the treated group and in-contact group was not higher than compared to the negative control group.

No viral antigen was detectable in the brain of any of the 12 mice that were euthanized or died, nor in any of the remaining 20 direct in-contact control mice (according to study design).

This study, too, was conducted to fulfil the requirements of the relevant Ph. Eur. monographs (0746 and 5.2.6.). In natural and experimental conditions, the virus strain does not spread from one animal to another in wild rodents. The study design also reflects WHO requirements. According to the requirements, the study complies if none of the orally treated mice dies due to a rabies infection and the mice remain healthy; no virus should be detectable in the brain and no spread to other mice should occur.

The daily observation of the animals indicated that the mice were generally in good health independent of groups. None of the surviving contact control mice, vaccinated mice or negative control mice showed any abnormal behaviour or signs of a rabies infection within the 87 day observation period. The death of 12 mice out of the 75 mice is interpreted as probably age related as the normal life expectation of *Microtus arvalis* in the field is 10-12 months and only in exceptional cases such as laboratory conditions *Microtus arvalis* may reach an age of 2-3 years. In this study the oldest mouse was 957 days old (=2.6 years). This explanation is considered acceptable all the more as it is in line with the FAT negative results of the 3 vaccinated dead/euthanized mice. No RABV was detectable in any brain of the other 9 mice that died or were euthanized during the observation period.

It is also acceptable that none of the orally vaccinated mice which reached the study end and did not show any abnormality were examined by FAT.

Furthermore, no RABV was found in the brains of the contact animals or in any brain of the remaining 20 contact mice. It can therefore be concluded that the vaccine virus is safe and does not spread to contact animals following oral administration of a tenfold overdose in rodents (*Microtus arvalis*).

House mice

Sixty-eight (68) house mice (285 – 339 days old) were split into three groups: 20 vaccinated mice (orally 0.03 ml SPBN GASGAS/MSV+1, $10^{9.1}$ FFU/ml), 32 direct untreated in-contact controls and 16 negative controls.

The in-contact mice served to determine the effect of potential vaccine spread. As it was expected that some mice would die during the course of the experiment (90 days), the negative control group served to establish the normal back-ground mortality in the mice.

General health was assessed using a scoring system specific for rabies development in mice. On all observation days the general health condition was scored 0 (no clinical signs of rabies related disease). One mouse in the negative control group was found dead on day 71. No other mortality was observed.

No viral antigen was detectable in the brain of any of the 32 direct in-contact control mice (according to study design). Thus, the vaccine is also safe for house mice via oral route; there are no indications for spread to direct in-contact control mice.

Wild guinea pigs

Nine (9) adult (ca. 250 days old) male guinea pigs were split into two groups: 5 vaccinates (orally 0.5 ml SPBN GASGAS/MSV+1, $10^{9.1}$ FFU/ml) and 4 direct in-contact controls. Due to the aggressive character of wild male guinea pigs, the animals were housed in individual cages in the same room; the nest material was mixed daily to simulate contact.

On all observation days (90 days) the general health condition was scored 0 (no clinical signs of disease). No mortality occurred. No viral antigen was detectable in the brain of any of the 9 guinea pigs.

Ph. Eur. monograph 0746 requires testing in wild rodents. Two mice species and wild guinea pigs were tested for the absence of pathogenicity and spread. No pathogenicity was observed after direct oral administration, neither in the two mice species nor in the wild guinea pigs, during an observation period of 88-90 days. These data support the initially performed study in NMRI mice performed to select the candidate vaccine strain for this product.

Supportive safety data in target and non-target species with parent strain SAD B19 (vaccine name: Fuchsoral) is presented below.

	Species	**N = (total)**	**Conclusion**
Supportive safety data in non-target animal species obtained with the SAD B19 strain.	Fox	68	Safe
	Dog	39	Safe
	Cat	34	Safe
	Jackal	13	Safe
	Raccoon	32	Safe
	Raccoon dog	18	Safe
	Domestic pig	10	Safe
	Wistar rat	34	5.8 % developed rabies
	Mouse (NMRI)	150	2-4.5 % developed rabies
	Brown rats	9	Safe
	Mus musculus	17	18 % developed rabies
	Apodemus sylvaticus	29	3.5 % developed rabies
	Apodemus agrarius	13	Safe
	Microtus epiroticus	13	7.7 % developed rabies
	muskrats	11	Safe
	mink	5	Safe
	ferrets	5	Safe
	marten	4	Safe
	pigeons	10	Safe
	young chickens	10	Safe
	magpies	10	Safe
	chimpazees	10	Safe
	baboons	12	Safe

Except for the directed modifications, SAD B19 and SPBN GASGAS are identical. Therefore, the safety data in non-target animal species obtained with the SAD B19 strain are suitable to support the safety of Rabitec in non-target species. The SAD B19 data presented above reflect the safety profile of this rabies virus vaccine strain. The low level of residual pathogenicity in rodents is documented but also the safety for other non-target species including non-human primates.

The critical studies in wild rodent species as required by Ph. Eur. monograph 0746 were successfully repeated with vaccine strain SPBN GASGAS. The studies in field mice, house mice and wild guinea pigs show that the vaccine is not pathogenic for these species and does not spread within these species. Therefore, it is justified that the applicant omitted these studies from the SAD B19 dataset as they are overruled by the new data obtained with Rabitec itself.

The latter data clearly indicate that the SPBN GASGAS vaccine strain is indeed further attenuated in comparison to SAD B19. The applicant has not repeated the study in Wistar rats as these laboratory animals do not represent a wild rodent as required by Ph. Eur. monograph 0746 all the more as the studies in mice provided sufficient evidence for the further attenuated property of SPBN GASGAS, thus fulfilling the requirements of Ph. Eur. monograph 0746.

It is concluded that the vaccine virus does not spread to in-contact unvaccinated animals. Studies were carried out to investigate spreading to other non-target species.

Dissemination in the vaccinated animal

Dissemination in the vaccinated animal (fox and raccoon dog) was investigated in combination with spread (section B.6.1) and/or with the administration of an overdose (section B.3) and the repeated administration of one dose (section B.2). As swine may also consume baits, may be hunted and the meat may enter the human food chain, this non-target species was also covered by the dissemination issue (section B.6.1). Study details and results are not repeated here as they are provided in detail above.

Below the summarizing conclusions are presented with regard to the dissemination results in the target species fox and raccoon dog and the non-target species swine:

Fox

- Dissemination of the vaccine virus is predominantly restricted to the site of vaccine uptake i.e. the *tonsilla palatina*. At this site, replication competent virus could be detected for a maximum of up to 4 days.
- No RABV antigen was detectable in the brain by FAT.
- No RABV RNA was detectable in faecal samples by RT-PCR.
- In saliva samples replication competent virus was found in 2 out of 12 animals during the day of oral administration.

Raccoon dog

- Dissemination of the vaccine virus is predominantly restricted to the site of vaccine uptake i.e. the *tonsilla palatina*. At this site, and on one occasion in the *velum palatinum*, replication competent virus could be detected for a maximum of up to 3 days.
- No RABV antigen was detectable in the brain by FAT.
- No RABV RNA was detectable in faecal samples by RT-PCR.
- RABV RNA but no replication competent RABV was detectable in saliva samples.

Swine

- Vaccine virus could not be detected in any of the organs or tissues tested. Only in some saliva samples some viral RNA could be detected by RT-PCR but this did not represent replication

competent virus.
- It is concluded that the vaccine virus did not disseminate in the body of swine.

In conclusion, dissemination of the vaccine strain is predominantly restricted to the site of vaccine uptake, the *tonsilla palatina* where viable virus could be detected for a few days in the target species only. RABV was never detectable by FAT in the brain of any animal.

Reversion to virulence of attenuated vaccines

The reversion to virulence of the vaccine strain was investigated in mice.

Five (5) groups of NMRI mice were used, the last group consisting of 30 mice to collect sufficient material for:

- Genotypic analysis of the passaged vaccine strain by PCR (groups A-E).
- Phenotypical testing by intracerebral inoculation of 16 adult mice (+ 4 direct in-contact mice + 20 negative control mice).

For passaging, one-day-old NMRI mice (≥6 and ≤24 hours) were used each receiving 0.01 ml intracerebrally, for phenotypical analysis 6-8 weeks old NMRI mice (24-33 gram) each received 0.03 ml intracerebrally.

As first inoculum MSV SPBN GASGAS/ID03:011012 (100% virus harvest filtrate) at a titre of $10^{7.6}$ FFU/ml (= $10^{8.5}$ TCID50/ml) was used, the passage material consisted of virus extracted from pooled brain (at least 9 mice) collected 5 days after inoculation.

A group of at least 15, one-day-old suckling mice were intracerebrally inoculated. After 5 days, the mice were humanely killed, the brains collected from at least 9 mice. The brain material was pooled and the virus extracted by homogenization of the brain material, diluting it with 4 parts tissue culture medium, centrifugation and clarification. The virus containing material was aliquoted and stored frozen until virus titration and sterility testing was performed. When satisfactory, the process was repeated with Px (x = passage 1 to 5) material used as intracerebral inoculum until a total of 5 sequential passages in one-day old mice were made. The 5th passage was performed in 30, one-day-old mice to collect sufficient material for the subsequent genotypic and phenotypical analysis.

Group & passage step		No of animals	Test material	Administered quantity	Titre after re-isolation
A	1st	15	MSV	0.01 ml	$10^{7.6}$
B	2nd	15	brain suspension 15 mice pooled 1st passage		$10^{7.1}$
C	3rd	15	brain suspension 11 mice pooled 2nd passage		$10^{8.0}$
D	4th	15	brain suspension 10 mice pooled 3rd passage		$10^{8.3}$
E	5th experimental group	30	brain suspension 9 mice pooled 4th passage		$10^{7.4}$
F-1	Phenotypical characterisation experimental group	16	brain suspension 29 mice pooled 5th passage	0.03 ml	$10^{7.3}$
F-2	Phenotypical characterisation contact controls	4 mice (together kept with the experimental group F-1 (4:1 split)	Not treated		-

G	Negative controls to F	20	Not treated	-

The following parameters were assessed:

- Confirmation of successful passage.
- Observation period of 5 days for passaging regime and genotypic characterization; 28 days for the phenotypical characterization. The general health status of all mice was observed and documented daily.
- For genotypic analysis (primary study aspect), confirmation of nucleotide exchanges at amino acid position 194 (AAT-> TCC) and 333 (AGA -> GAG) and full length integration of the GASGAS construct of the 5th passage material.
- For phenotypical testing (secondary study aspect), inoculation of adult mice with the 5th passage material; mice that were euthanized or found dead would be submitted for FAT testing of brain material.

At all 5 passages live virus was present in the brain extract (titre ranged between $10^{7.1}$ and $10^{8.3}$ FFU/ml, see table above). The phenotypic analysis showed no clinical symptoms or mortality during the 28 days lasting observation period in adult mice and thus no evidence for spread to direct in-contact mice. Therefore, no mice brain tissue was examined by FAT. The genotypic analysis revealed the presence of nucleotide exchanges at G-protein amino acid position 194 (AAT-> TCC) and 333 (AGA -> GAG); confirmation of full length integration of the GASGAS construct in the 5th passage material was given.

The absence of reversion to or increase in virulence is requested by Ph. Eur. monograph 0746 in section 2-3-2 as proof of stability of the genetic marker. The test must be carried out in suckling mice not vaccinated against rabies. The vaccine virus is sequentially passaged through 5 groups via intracerebral route. Five mice of the 1st group receive a quantity (not more than 0.02 ml) of the vaccine virus that will allow virus recovery. When the mice show signs of rabies, but not later than 14 days after inoculation, the mice are euthanized. A suspension is prepared from the brain of each mouse; the samples are pooled. Not more than 0.02 ml of the pooled samples are administered to each mouse of the next group. This passaging should be done not fewer than 4 times. The presence of the virus is verified at each passage. The genetic marker in the vaccine virus recovered from the last passage should be demonstrable. The vaccine virus complies, if the genetic marker remains stable.

All these conditions are clearly fulfilled by the applicant. The stability of vaccine strain SPBN GASGAS and the absence of any increase in virulence potential was investigated by genotypic analysis of the passaged vaccine strain via PCR (confirmation of nucleotide exchanges) and comparison of the genome of the 5th passage with the genome of the MSV by next generation sequencing (NGS). Furthermore, phenotypic testing was done by intracerebral application of brain suspension (5th passage material) to adult mice.

As Ph. Eur. monograph 0746 specifically deals with the stability of the genetic marker by using suckling mice and the applicant gave unambiguous proof of the vaccine's stability, there is no need to conduct a reversion to virulence study in the target species as required by Ph. Eur. monograph 5.6.2.

It is concluded that no reversion to virulence was observed following five passages *in vivo*.

Biological properties of the vaccine strain

Comparative results were observed in dissemination studies with the GMO and its parental vaccine strain SAD B19 (without the change at amino acid position 333 and 194 in G-protein) after administration by the recommended oral route. In the SPBN GASGAS dissemination studies conducted in 12 foxes and 12 raccoon dogs (on day 1, 2, 3, 7 and 10 post administration 2 animals each were sacrificed) selected tissues were collected and investigated for the presence of the vaccine strain.

Only 7 samples taken from the raccoon dogs were tested positive for RABV RNA by PCR; 1 velum (day 3),

1 lung (day 7) and 5 palatine tonsil (day 1-3). However, infectious virus was only isolated from the 5 palatine tonsil samples.

In foxes, RABV RNA was detected in 11 of the 12 palatine tonsils, all other tissue samples tested negative. Replication competent virus was only detected in these tonsils during the first 3 days, just like in the raccoon dogs.

Similar results were obtained previously with the parental strain, SAD B19; here too, virus could predominantly be isolated from the tonsils and only a few samples taken from the oral upper mucosa tested positive (Vos et al., 1999). The vaccine strain and other conventionally attenuated rabies vaccines appear predominantly to be taken up by the palatine tonsils, where local replication takes place after which the virus is rapidly cleared by the immune response. Hence, changes in tropism compared to the parental strain have not been observed during the dissemination studies performed with SPBN GASGAS in the target species. The risk of any alteration in the expected route of transmission of the vaccine strain after oral administration is negligible.

This is further confirmed by the studies in the non-target species (cats, dogs, swine, mice and guinea pigs). In none of these species the vaccine virus was disseminated in the body. Only in rare cases vaccine virus could be recovered from saliva collected on the day of vaccine administration; it most probably represented the vaccine inoculum.

On the basis of the data presented the safety profile of the vaccine strain can be considered acceptable.

Recombination or genomic reassortment of the strains

Homologous recombination and horizontal gene transfer is considered rare in negative-stranded, non-segmented RNA-viruses (NNSV) to which the rabies virus belongs. A literature search was performed to find published evidence of horizontal gene transfer between rabies virus and other viruses or recombination between rabies viruses. No publications were found with the exception of some publications suggesting evidence for recombination in rabies virus based on phylogenetic sequence analysis (Geue et al., 2008, Liu et al., 2011). However, for recombination to happen a single cell needs to be infected with two different rabies viruses and this has never been shown *in vitro*. Several factors limit virus co-infection in case of a fatal disease like rabies. Wild-type rabies virus is predominantly transmitted through biting with limited replication at the site of injection. Only in the CNS, extensive replication and subsequent dissemination occurs. The GMO is offered via oral route and taken up predominantly by the palatine tonsils, where after an initial round of limited replication the virus is rapidly cleared. Therefore, an extreme low risk of co-infection is present.

It can be concluded that adverse environmental effects from spontaneous recombination of the GMO with wild-type RABV or any other circulating lyssavirus are exceedingly rare.

As rabies virus belongs to the order *Mononegavirales* (see above: viruses with a non-segmented, negative-stranded RNA genome) genomic re-assortment does not occur.

Study of residues

This section would be in principle not applicable as the vaccine is not intended for a food producing animal. Nevertheless, the bait matrix may contain OTC as marker substance added to control the success of rabies elimination programs. Via bait competitors such as wild swine OTC may enter the food chain and potentially harbour a risk for the consumer. However, the risk for human consumers caused by OTC residues is considered negligible as the amount of OTC in a bait matrix is far below the dose which is used for the tetracycline (TC) treatment of pigs in veterinary medicine. Furthermore, after bait distribution, no hunting is allowed for a period of 14 days which would cover the 12 day withdrawal period established for

a product containing OTC and authorised for pigs. TC is quickly eliminated and would be degraded by cooking. Thus, this aspect of potential OTC hazard for humans by consumption of game is satisfactorily addressed.

Interactions

Not applicable, wildlife is not treated with any other veterinary medicinal product.

Field studies

No field studies have been provided. Following the outcome of the scientific advice it was agreed to submit the licensing documentation without safety data obtained from the field and collect the corresponding data after granting the marketing authorisation.

Guidelines to design an EU co-financed programme on eradication and control of Rabies in wildlife (Sante/10201/2015/rev1) emphasize the need for licensed oral rabies vaccines. Neither OIE nor WHO require any efficacy proof for a rabies wildlife product in the field, prior to granting the marketing authorisation. Furthermore, the MUMS guideline allows the omission of safety and efficacy field trials when sufficiently justified.

As the laboratory data are fully supportive of the vaccine's safety, the submission of data from the field after granting the marketing authorization as initially proposed in the scientific advice appears not necessary and is therefore not requested.

User safety

The User Safety Risk Assessment has been conducted in accordance with EMEA/CVMP/IWP/54533/2006 – Guideline on User Safety for Immunological Veterinary Medicinal Products.

The active ingredient is an attenuated RABV strain which has lost its ability for immune evasion. The vaccine strain is safe for a range of animal species and does not disseminate in the body of any of the species tested. Its level of attenuation is stable as assessed by genotypic and phenotypical characteristics. Therefore, the active ingredient does not constitute a hazard. The excipients (sucrose, porcine gelatine, disodium phosphate dehydrate, potassium dihydrogen phosphate, and neomycin sulphate) are generally considered as safe since there are no known hazards relating to these starting materials contained in the finished product.

Any exposure to the professional user to any of the vaccine components is negligible as the active ingredient and the excipients are contained frozen in a blister, the blister is subsequently contained in a bait. Personnel are trained in the distribution of the baits and must wear protective gloves. The risk that a person involved in a vaccination campaign comes into contact with the vaccine virus during administration is absent.

In case a person involved in the distribution of the baits still comes into contact with the vaccine (Assessment of the consequence of a hazard occurring), it is most probably by contact to intact skin which is of no consequence. If the contact is via mucosal route or damaged skin, the recommendations of the WHO must be followed as outlined in the Post Exposure Prophylaxis (PEP). Based on the above, in principle no risk management and/or communication are deemed necessary. Nevertheless, as a precautionary measures the following advice is included in SPC section 4.5.:

Special precautions to be taken by the person administering the veterinary medicinal product to animals:

Handle the baits with care. It is recommended to wear disposable rubber gloves when handling and distributing baits. In the event of direct human exposure to the active ingredient, seek medical advice

immediately and show the package leaflet or the label to the physician.

Proposed first aid measures immediately after direct human exposure to the vaccine fluid should follow the recommendations of the WHO as outlined in Post Exposure Prophylaxis (PEP).

To ensure the public safety a warning as a pictogram with the text “Rabies vaccine” is included on the blister. On the bait a warning pictogram with the text “Rabies vaccine” and “Do not touch!” is included.

Environmental risk assessment

Ecotoxicity is evaluated according to the CVMP guidance “Environmental risk assessment for immunological veterinary medicinal products” (EMEA-CVMP/074/95).

The risk of ecotoxicity has been evaluated following the use of the vaccine as negligible for the following reasons:

- The immunological active component is the attenuated live Rabies vaccine virus strain SPBN GASGAS.
- Further components incorporated into the vaccine are commonly used in daily life, and they are safe taking into consideration the proportions they are present in the vaccine.
- Control of the active ingredient and of the finished product (safety, efficacy) complies with the requirements of the European guidelines and Ph. Eur.
- The excretion from vaccinated animals of metabolites from the vaccine components that would contaminate the environment is negligible.

For all these reasons only phase I of the risk assessment was carried out.

With regard to the hazard identification, the vaccine strain has been shown to be safe for a range of target and non-target species including oral administration to adult mice. In younger mice (and thus not fully immunocompetent) some pathogenicity could be observed after oral or intracerebral administration; these experimental conditions do not occur in normal ecological situations.

The potential of transmission to non-target species was examined in dogs, cats, swine, mice and wild guinea pigs as well; naïve animals were placed in direct contact (except for guinea pigs) to assess spread. In no case evidence was found that the vaccine virus was transmitted to the animals that were in direct contact. As regards the shedding potential, the vaccine virus was tested in foxes, raccoon dogs (target species) and, as mentioned before, in several non-target species. Only in rare cases vaccine virus could be recovered from saliva collected on the day of vaccine administration. Most probably this represented the vaccine inoculum itself.

The capacity of rabies virus to survive, establish and disseminate in the environment is negligible as it is very unstable in the open environment and stays infectious outside the host for a restricted period of time only; rapid antigen degradation occurs. Drying and sunlight rapidly deactivate rabies virus. It has been suggested that RABV can survive for prolonged times in frozen conditions. However, as baits are distributed in spring and autumn, the risk that the vaccine virus remains frozen and thus remains infectious in the environment is negligible. If such a bait were to be consumed by a non-target species, this would remain without any consequences except for an immune response.

The vaccine’s excipients are generally considered as safe and non-toxic. Thus, these components have no toxic effect on the environment. The product contains a very small amount of neomycin. After oral consumption of the vaccine, the antibiotic concentration is rapidly diluted and inactivated in the gastro-intestinal tract of the animal. This will end up a concentration that is far below the therapeutic effect of such antibiotics. It has been clarified to what extent OTC contained in the bait matrix may reach the food chain and that there is no risk for the human consumer (see also *Study of residues*).

The likelihood of hazards is discussed focussing on the risk of pollution of the environment in case not all baits are consumed by an animal and thus remain in the environment. Also, after bait consumption the empty, non-biodegradable blister may remain in the environment. As unveiled by a literature research the major cause for OTC contamination of the environment (water and soil) is the use of livestock excretion and sewage sludge as fertilizer. The amount of OTC as biomarker of a rabies vaccine which had been introduced in the environment over the last decades is negligible compared to the OTC-introduction which is caused after antibiotic treatment of humans and animals (ingested antibiotics - i.e. OTC - are excreted partially non-metabolised via urine and faeces).

Even though the likelihood of the identified hazard to occur is high (the blister will remain in the environment) the consequence of the hazard is considered low, the overall level of risk is "Medium/Low". No phase II assessment is considered to be necessary as the applicant.

The applicant would prefer an optional incorporation of OTC into the bait matrix as it is known that bait up-take rates, assessed by proof of the bait marker, are consistent over the years and any added value over the seroconversion rate is very rare. Thus, the question was raised if there is an actual need to incorporate OTC into bait matrices. As some European countries require the use of a biomarker, while other European countries would prefer baits without any antibiotic marker, the applicant proposes this optional incorporation of OTC into the bait matrix to be able to react flexibly depending on the respective national competent authority.

Based on the data provided the ERA can stop at phase I. Rabitec is not expected to pose a risk for the environment when used according to the SPC.

Environmental risk assessment for products containing or consisting of genetically modified organisms

The technical dossier supplying information as requested by Annexes IIIA and IV of Directive 2001/18/EC has been provided. The presentation of the data has been done in accordance with the Note for 'Guidance on environmental risk assessment for veterinary medicinal products consisting of or containing GMO as or in products (ENTR/F2/KK D(2006)'. The environmental risk assessment has been provided in detail and the overall risk to humans and to the environment was considered negligible.

Overall conclusions on the safety documentation

As Rabitec is a GMO product, but obtained a MUMS/Limited market status, it was decided to accept a reduction of data (Scientific Advice /EMA/CVMP/SAWP/553740/2015).

A total of 14 pivotal laboratory safety studies with SPBN GASGAS using MSV, MSV+1 or MSV+2 administered via oral route except for reversion to virulence (intracerebral application) are available.

The test methods have been proven suitable to generate reliable results. The key validity criteria such as specificity and sensitivity as well as limit of detection were sufficiently addressed, the validity of the test results is considered unquestionable.

The safety of the administration of one dose was covered by the tenfold overdose studies in foxes and raccoon dogs. The tenfold overdose is safe for both target species; furthermore, the vaccine virus does not disseminate to the brain. As a batch was used containing $10^{9.1}$ FFU/ml, the maximum titre of $10^{8.1}$ FFU/dose is justified.

The safety of the repeated administration of one dose was combined with the evaluation of potential horizontal transmission in foxes and raccoon dogs. The vaccine is safe for both target species when repeatedly administered at a short interval of time. RABV antigen was not detected in brain samples.

The vaccine was found to be safe when used in pregnant vixens and their cubs. Raccoon dogs were also considered as not affected in their reproductive performance as no RABV RNA was detectable in the gonads of animals originating from the overdose study.

As regards special requirements for live vaccines, potential spreading of the vaccine strain was investigated in both target species and several non-target species (dogs, cats, swine, field mice, house mice, wild guinea pigs). The risk from the vaccine virus replicating or spreading in target and non –target animals was found to be minimal and of acceptable level.

The dissemination potential of the vaccine strain is well reflected in the studies conducted in combination with repeated dose administration and spread in the fox and raccoon dog. As swine may also consume baits this non-target species was also covered in a dissemination study. Dissemination of the vaccine strain is predominantly restricted to the site of vaccine uptake, the *tonsilla palatina,* for a few days in the target species only. In none of the investigated species RABV antigen was detected in brain samples.

Supportive safety data generated with the parent strain SAD B19 were submitted reflecting the safety profile of this rabies virus vaccine strain with its low level of residual pathogenicity in rodents and other non-target species including non-human primates.

The stability of vaccine strain SPBN GASGAS and the absence of any increase of virulence potential was investigated by genotypic analysis of the passaged vaccine strain via PCR and comparison of the genome of the 5th passage with the MSV by next generation sequencing (NGS). Furthermore, phenotypic testing was done by intracerebral application of brain suspension (5th passage material) to adult mice with a 100% identity compared to the MSV, in compliance with the Ph. Eur. monograph 0746. In addition, the 5th passage material was safe upon administration in adult mice and there was no evidence for spread to direct in-contact mice.

As regards the potential for recombination and re-assortment, it can be concluded that spontaneous recombination of the GMO with wild-type RABV or any other circulating lyssavirus is exceedingly rare; genomic re-assortment does not occur as rabies virus has a non-segmented, negative-stranded RNA genome.

The user safety risk assessment has been performed in accordance with the Guideline on User Safety for Immunological Veterinary Medicinal Products (EMEA/CVMP/IWP/54533/2006). All sections are satisfactorily discussed; the information needed for the safe use of the product is provided.

The risk for human consumers caused by TC residues is considered negligible. Furthermore, after bait distribution, no hunting is allowed for a period of 14 days which would cover the 12 day withdrawal period established for a product containing OTC and authorised for pigs. Moreover, TC is quickly eliminated and would be degraded by cooking.

Rabitec is not expected to pose a risk for the environment when used according to the SPC. The amount of OTC as biomarker of a rabies vaccine introduced in the environment is negligible compared to the OTC-introduction after antibiotic treatment of humans and animals.

No field studies were conducted. This was in accordance with the scientific advice and was considered acceptable as the laboratory data are fully supportive of the vaccine's safety.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended for active immunisation of foxes and raccoon dogs against rabies to prevent infection and mortality.

Duration of immunity: The duration of protection is at least 6 months.

Detailed information and guidance on bait vaccination (vaccination area, distribution density, aerial and manual baiting, emergency vaccinations etc.) are given in section 4.9 of the SPC.

The testing procedures were performed at appropriate laboratories.

An overview of the methods used in the efficacy trials is presented and the methods are shortly described, their suitability and validation outlined.

The diagnostic methods used in the efficacy trials are considered acceptable. SOPs and validation reports of FAVN and RFFIT are provided.

Efficacy was demonstrated in compliance with European Directive 2001/82/EC, Annex I, Title II (as amended 2004/28/EC and 2009/9/EC), the CVMP guidelines and the Ph. Eur. monographs:

- Ph. Eur. monograph 0062: Vaccines for veterinary use, specific chapter 5.2.7 "Evaluation of Efficacy of Veterinary Vaccines and Immunosera".
- Ph. Eur. monograph 0746: Rabies vaccine (live, oral) for foxes and raccoon dogs.

Scientific advice was given concerning field studies and it was considered acceptable that no field trials were performed. The MUMS guideline allows the omission of efficacy field trials when sufficiently justified. Provided that the laboratory data are fully supportive of vaccine's efficacy, the collection of data from the field could be handled as a follow-up measure.

Laboratory trials

In total, 6 laboratory studies were conducted to evaluate the efficacy of Rabitec:

- One study for the determination of the lethal dose of the rabies challenge strain FLI ID 148 in foxes (Good Clinical Practice - GCP study).
- One study for the determination of the lethal dose of the rabies challenge strain FLI ID 148 in raccoon dogs (GCP study).
- One study for the evaluation of the minimum effective dose of SPBN GASGAS for the administration of baits to foxes (GCP study).
- One study for the evaluation of the immunogenicity of the rabies vaccine strain SPBN GASGAS in foxes (Good Scientific practice – GSP - study).
- One study for the evaluation of the immunogenicity of the rabies vaccine strain SPBN GASGAS in raccoon dogs (GSP study).
- One study for the evaluation of the immunogenicity of the rabies vaccine strain SPBN GASGAS in raccoon dogs (GSP study).

The batch protocols of the batches used in the efficacy studies are presented.

The vaccine virus used was at passage level WSV + 1 (corresponding to MSV + 2). This passage level does not represent the highest passage level intended for the commercial vaccine (MSV + 5). However, sequence data show that there is no difference between the MSV and MSV + 5.

The efficacy parameters (signs of rabies according to a scoring system, i.e. apathy, reduced reactions on manipulations, neuropathological symptoms, paralysis, coma, stupor, death) in the efficacy studies are in the line with Ph. Eur. The parameters chosen are considered appropriate for evaluating the efficacy of the product. The tests performed to detect rabies challenge virus in the brain was FAT and to evaluate neutralising antibodies were RFFIT, FAVN and BioPro Blocking ELISA.

Establishment of a challenge model

The challenge virus strain (FLI ID 148) used in the efficacy studies was isolated in 1998 from the brain of a naturally infected rabid fox in Krefeld, Germany and was obtained from the FLI. It was passaged on MNA-cells (mouse neuroblastoma cells) in 2012. This challenge virus strain was chosen because it was isolated from a European target species individual, which means that it is suitable to infect other European foxes and raccoon dogs. A molecular characterization of the challenge virus was performed by RT-PCR. It is part of the Western European lineage of fox mediated RABV. Therefore, it is a relevant challenge strain for the European situation. It should be noted that the challenge strain does not have any relation to the vaccine strain and sequencing data show that the vaccine and challenge strain are not closely related (91% homology on a nucleotide level, compared to a 95% homology between the most representative European rabies strains of the last 30 years).

In order to develop a challenge model for the target species foxes and raccoon dogs two studies were performed:

Foxes

In this study the lethal dose of a rabies challenge strain FLI ID 148 (Fox Krefeld) in the fox was determined.

Eighteen (18) eight-month-old foxes (*Vulpes vulpes*, breed: silver fox), which were naïve for RABV, were challenged intramuscularly with strain FLI ID 148 in different dilutions (group 1 to 5 with each $10^{4.7}$, $10^{3.7}$, $10^{2.7}$, $10^{2.3}$ and $10^{2.0}$ $MICLD_{50}$/ml [mouse intracerebral lethal dose 50%]. Animals were observed for clinical signs, food intake, defecation and serum was analysed for antibodies. All of the 18 animals (100%) showed clinical signs of rabies and were consequently euthanized within 10 to 16 days after challenge. All animals were seronegative (0.07 - 0.22 IU/ml).

The challenge dose of $10^{2.0}$ $MICLD_{50}$/ml for foxes is considered acceptable.

Raccoon dogs

In this study the lethal dose of a rabies challenge strain FLI ID 148 (Fox Krefeld) in the raccoon dog was determined.

Twenty-two (22) nine- to ten-month-old raccoon dogs (*Nyctereuts procyonoides*), which were naïve for RABV, were challenged intramuscularly with strain FLI ID 148 in different dilutions (group 1 to 3 with each $10^{3.0}$, $10^{2.7}$ and $10^{2.3}$ $MICLD_{50}$/ml). The animals were observed for clinical signs, food intake, defecation and serum antibodies for 47 days. All animals which had received the highest dose (group 1) died within 12 to 29 days after challenge infection. 88.9% of the animals of the other both groups (group 2 and 3) died within 10 up to 26 days after challenge. All animals stayed seronegative during the experiment except one animal from group 3 which had survived.

The challenge dose of $10^{3.0}$ $MICLD_{50}$/ml for raccoon dogs is considered acceptable. It is noted that there is a remarkable difference between the sensitivity of foxes and raccoon dogs for this challenge strain. Therefore it is justified to use a higher challenge dose in raccoon dogs than in foxes.

Determination of the vaccine dose

One study was performed to determine the minimum immunization dose for the target species foxes:

In this study the minimum effective dose of SPBN GASGAS for the administration of baits to foxes was established.

Twelve (12) adult foxes (*Vulpes vulpes*), which were naïve for RABV were vaccinated orally with Rabitec. Group A and B were vaccinated using two different presentations of 1 vaccine bait, group D was

immunized directly via oral route and group C was left untreated. Approximately 55 days after vaccination animals were challenged with strain FLI ID 148 intramuscularly (challenge dose $10^{4.7}$ $MICLD_{50}$/ml). Animals were observed daily for clinical signs, food intake and defecation for 41 days. Body weight and serum antibodies were measured one day before vaccination, on day 28 and day 56. After vaccination no clinical signs or body weight change were observed. Rabies specific antibodies were measured in vaccinated groups from day 28 onwards.

After challenge infection animals in group A and B did not show any clinical signs of rabies and all animals survived. Two out of three animals of study group D survived and one animal had to be euthanized. All of the control animals in study group C showed clinical signs of rabies and died.

In conclusion, from the results the minimum immunization dose of $10^{6.8}$ FFU/dose (i.e. $10^{6.6}$ FFU/ml in 1.7 ml) for foxes is considered acceptable.

Onset of immunity

Three studies were carried out in foxes and raccoon dogs in compliance with Ph. Eur. requirements to investigate the seroconversion post vaccination. The administration route was orally.

Foxes

This study was performed to investigate the immunogenicity of the rabies vaccine (SPBN GASGAS) in foxes.

Forty-two (42) six to seven months old foxes (*Vulpes vulpes*), which were naïve for RABV, were either vaccinated with a vaccine bait containing SPBN GASGAS or stayed untreated as controls. One hundred ninety (190) days after vaccination all animals were challenged with rabies virus (strain FLI ID 148, challenge dose $10^{0.7}$ $MICLD_{50}$/ml) via intramuscular route. Animals were observed for clinical signs, food intake, defecation and serum antibodies for 91 days. Additionally brain tissue was examined at the end of the experiment for the presence of rabies virus.

No clinical signs were observed after vaccination. Twenty-seven (27) of 30 vaccinated animals seroconverted 15 days post vaccination. Only one of the vaccinated animals did show clinical signs of rabies and was euthanized 34 days post challenge. All other vaccinated animals stayed healthy and survived. In contrast all control animals showed clinical signs of rabies and died. Examination of brain tissues revealed rabies virus in all animals which died.

The study fulfils the requirements of Ph. Eur. monograph 0746. It was shown that 90% of the vaccinated animals seroconverted 15 days post vaccination.

Raccoon dogs

This study was performed to investigate the immunogenicity of a rabies vaccine (SPBN GASGAS) in raccoon dogs.

Forty-two (42) six to seven months old raccoon dogs (*Nyctereus procyonoides*), which were naïve for RABV, were either vaccinated with a vaccine bait containing SPBN GASGAS or stayed untreated as controls. One hundred ninety (190) days after vaccination all animals were challenged with rabies virus (strain FLI ID 148, challenge dose $10^{0.7}$ $MICLD_{50}$/ml) via intramuscular route. Animals were observed for clinical signs, food intake, defecation and serum antibodies for 91 days. Additionally brain tissue was examined at the end of the experiment for the presence of rabies virus. After vaccination no clinical signs were observed in any animal. Twenty-three (23) of 30 of the vaccinated animals (~ 80%) seroconverted.

After challenge infection 28 out of 30 vaccinated animals did not show any clinical sign of rabies. Two vaccinated animals did show clinical signs of rabies and had to be euthanized approximately three weeks

after challenge infection. Only 50% of the control animals did develop rabies-specific clinical signs and died. Examination of brain tissues of the succumbed animals revealed rabies virus. As the study does not fulfil the requirements of Ph. Eur. monograph 0746 it is considered invalid.

A further study evaluated the immunogenicity of a rabies vaccine (strain SPBN GASGAS) in the target species- raccoon dog (*Nyctereutes procyonoides*).

Forty-eight (48) raccoon dogs, 7 to 8 months old, RABV and rabies antibody free, were included in the trial. 36 animals received the vaccine containing $10^{6.8}$ FFU/dose via bait. As 8/36 animals did not consume the bait, they received 1.7 ml of the vaccine strain by direct oral administration (results of these animals are presented for information only). Twelve (12) raccoon dogs remained as untreated controls. The animals were daily observed for clinical signs using a scoring system for 183/184 days. Food intake and defecation were also assessed. Serology was performed at defined intervals. One hundred eighty-three (183)/184 days after the vaccination the raccoon dogs were challenged by injection into the *musculus masseter* using $10^{3.0}$ $MICLD_{50}$/ml FLI ID 148 followed by a 90 days lasting observation period. At the end of study all brains were examined by FAT.

The raccoon dogs remained in good health after vaccination and did not develop any sign attributable to rabies. Seroconversion was confirmed (≥ 0.5 IU/ml) in 25/28 vaccinated animals 15 days post vaccination. After the challenge, none of the vaccinated raccoon dogs developed any clinical sign of rabies; all of them survived. No RABV could be detected in the brain of any animal. In contrast, all control animals developed clinical signs attributable to rabies and died. RABV was detectable in the brain of each control animal.

This study fulfils the requirements of Ph. Eur. monograph 0746 and is considered acceptable. About 90% of the vaccinated animals (by bait) seroconverted 15 days post vaccination.

Duration of immunity

Two studies were carried out. The pivotal efficacy trials in foxes and raccoon dogs were used to determine both onset and duration of immunity. The interval between vaccination and challenge in these studies was at least 180 days as required by Ph. Eur. monograph 0746. Therefore, duration of immunity of 6 months is justified. To combat rabies in wildlife, vaccine baits should be distributed preferably twice yearly, in spring and autumn as stated in the SPC. Hence, this vaccination protocol is fully compatible with the documented duration of immunity of at least 6 months in both target species. Duration of immunity was investigated in rabies antibody free animals.

The influence of maternal antibodies on the efficacy of the vaccine

No studies were performed to examine the possible influence of maternally derived antibodies (MDA) on the efficacy of the vaccine.

The applicant points out that in experimental studies with the parent vaccine strain SAD B19 it was shown that MDA were present in cubs born from previously orally vaccinated vixens. However, the antibody titres were generally low (<0.5 IU/ml). It was determined that the half-life of these MDAs was 9.3 days; and on average 23 days post parturition no MDA were detectable anymore. Nevertheless, a positive correlation between the level of MDA in cubs and previously vaccinated vixens was found (Müller et al 2002). However, under field conditions, the effect of MDA will be limited due to the fact that cubs do not start to eat solid food till they are 3 to 4 weeks old. Hence, by the time young foxes have access to the vaccine by consuming baits, the MDA have already disappeared in most situations. Furthermore, cubs less than 8 weeks old will not wander far away from the den site and therefore have only limited access to vaccine baits distributed.

Finally, vaccination campaigns are most of the time carried out during early spring and in autumn while cubs are usually born in March and April. Thus, the earliest time that cubs may have access to the baits is in autumn, which means 5-8 months after their birth. It can be concluded that although maternally derived antibodies may influence the immune response of young cubs the relevance of this issue under field conditions is negligible. Therefore, it is considered acceptable that no corresponding studies were performed.

Additional pre-development studies

In the dossier the applicant refers to several pre-development studies that were performed for candidate vaccine strain selection. An overview of these studies is included. The studies were conducted in raccoons, raccoon dogs, striped skunks, foxes, domestic dogs and mongooses. In most cases a Research Virus Seed (RSV = pre-master seed) was used but sometimes also a passage derived from the MSV. These studies were not performed with Rabitec itself and additional target species (raccoons, skunks, domestic dogs and mongooses) were used. Therefore, the data are not relevant for the assessment of the efficacy of Rabitec and can only be regarded as supplementary information.

Field trials

No field studies have been provided. Following the outcome of the scientific advice it was agreed to submit the licensing documentation without efficacy data obtained from the field and collect the corresponding data after granting the marketing authorisation.

Guidelines to design an EU co-financed programme on eradication and control of Rabies in wildlife (Sante/10201/2015/rev1) emphasize the need for licensed oral rabies vaccines. Neither OIE nor WHO require any efficacy proof for a rabies wildlife product in the field, prior to granting the marketing authorisation. Furthermore, the MUMS guideline allows the omission of safety and efficacy field trials when sufficiently justified.

As the laboratory data are fully supportive of the vaccine's efficacy, the submission of data from the field after granting the marketing authorization as initially proposed in the scientific advice appears not necessary and is therefore not requested.

Overall conclusion on efficacy

Four laboratory studies were conducted to evaluate the efficacy of Rabitec.

The lethal dose of the rabies challenge strain was determined in two studies for the target species foxes and raccoon dogs. An epidemiologically relevant challenge strain for the evaluation of the benefit of the vaccine in the field has been used in laboratory studies. The challenge model was considered acceptable and therefore appropriate for using in the efficacy trials in order to mimic the natural conditions for infection.

The minimum protective dose of $10^{6.8}$ FFU could be demonstrated.

About 90% of vaccinated foxes and raccoon dogs seroconverted 15 days after vaccination. Duration of immunity of 6 months was demonstrated in foxes and raccoon dogs in laboratory studies.

No field studies were conducted which is considered acceptable.

The following indications are supported by the data provided: For the active immunisation of foxes and raccoon dogs against rabies to prevent infection and mortality.

No studies were performed to examine the possible influence of maternally derived antibodies (MDA) on

the efficacy of the vaccine. In experimental studies with the parent vaccine strain SAD B19 it was shown that MDA were present in cubs born from previously orally vaccinated vixens and that these MDA may influence the immune response of young cubs. However, the relevance of this issue under field conditions is negligible. Therefore, it is considered acceptable that no corresponding studies were conducted with the vaccine Rabitec.

Detailed information and guidance how to vaccinate animals via bait (vaccination area, distribution density, aerial and manual baiting, emergency vaccinations etc.) are given in section 4.9 of the SPC.

Part 5 – Benefit-risk assessment

Introduction

Rabitec is a live, attenuated rabies vaccine intended for active immunisation of foxes and raccoon dogs to prevent infection and mortality due to infection with the rabies virus.

It is presented as a bait of fishmeal containing a PVC-aluminium blister filled with the vaccine suspension. The active substance is the genetically modified rabies virus SPBN GASGAS. The modification comprises of two amino acid substitutions in the G-protein, a second integration of the modified gene for the G-protein and the deletion of the pseudogene. No foreign genetic material was introduced into the genome.

The proposed vaccination scheme is the uptake of one dose (one bait).

The product has been classified as MUMS and therefore reduced data requirements apply that have been considered in the assessment.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full dossier).

Benefit assessment

Direct therapeutic benefit

The benefit of Rabitec is its efficacy in the treatment of foxes and raccoon dogs against rabies to prevent infection and mortality and thus reduce the transmission of rabies virus, usually via the bite of an infected animal, to other animals and humans. Efficacy was shown in a number of laboratory studies.

Well-designed clinical trials conducted in accordance with GCP and GSP demonstrated that the product is efficacious and that the following SPC claims are supported: for the active immunisation of foxes and raccoon dogs against rabies to prevent infection and mortality. About 90% of vaccinated foxes and raccoon dogs seroconverted 15 days after vaccination. Duration of immunity is at least 6 months.

Additional benefits

The additional benefit of Rabitec in comparison to first generation oral rabies vaccines is the high attenuated phenotype of the vaccine virus with an improved safety profile and decreased risk of vaccine associated rabies in immune competent or very young animals.

Risk assessment

Main potential risks are identified as follows:

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. The addition of the antibiotic neomycin to the vaccine suspension was satisfactorily justified. A detailed risk assessment covering the risk for humans, the target animal species, non-target animal species, the environment and the risk of antibacterial resistances is provided.

Safety:

Risks for the target animals and non-target animals:

The product is generally well tolerated in the target animals. No adverse reactions were observed after a tenfold overdose of Rabitec. The vaccine strain was apathogenic in target and non-target species. The biological properties (safety, dissemination, spread and shedding) of the parental strain were even improved by the genetic modifications, reversion to virulence could not be demonstrated. The chance of recombination with other strains or other viruses occurring is considered to be negligible.

Risk for the user and consumer:

In case a person comes into contact with the vaccine it is most probably by skin contact and should be of no consequence. However, as this is a live rabies virus vaccine, exposure to the vaccine fluid should follow the recommendations of the WHO as outlined in the "WHO Guide for Rabies Pre- and Post-Exposure Prophylaxis (PEP) in humans". The user safety for this product is acceptable when used as recommended. Appropriate risk mitigation measures are described in the SPC. Appropriate warning is included on both the blister and the bait.

Risk for the environment:

The risk of oxytetracycline for the environment has been satisfactorily assessed. As unveiled by a literature research the major cause for OTC contamination of the environment (water and soil) is the use of livestock excretion and sewage sludge as fertilizer. The amount of OTC as biomarker of a rabies vaccine which had been introduced in the environment over the last decades is considered to be negligible compared to the OTC-introduction which is caused after antibiotic treatment of humans and animals (ingested antibiotics - i.e. OTC - are excreted partially non-metabolised via urine and faeces).

Even though OTC as biomarker for rabies vaccines is considered of low hazard level for the environment and non-target species including humans, the applicant's proposal to incorporate OTC only on request is appreciated.

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user and environment and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Rabitec is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.