

## SCIENTIFIC DISCUSSION

### a) PRODUCT INFORMATION

<b>Product name:</b>	RABIGEN SAG2 oral suspension
<b>Procedure No.:</b>	EMEA/V/C/043/01/0/0
<b>Applicant company :</b>	Virbac
<b>Active substances:</b>	Suspension of SAG2 modified rabies virus strain
<b>Proposed International Non-proprietary Name:</b>	Not applicable
	QJ57H A (vaccine for fox)
(1)	ATCv et code
<b>Pharmaceutical form:</b>	Liquid virus suspension of live-attenuated rabies virus
<b>Strength:</b>	Minimum of 8 log <sub>10</sub> CCID <sub>50</sub> <sup>1</sup>
<b>Presentation/s:</b>	PVC/aluminium sachets embedded in a bait matrix
<b>Package size:</b>	200 Units (4x50) and 400 Units (2x200)
<b>Target species:</b>	Red foxes ( <i>Vulpes vulpes</i> )
<b>Withdrawal period:</b>	Not applicable
<b>Route of administration:</b>	Oral use
<b>Product type:</b>	Immunological
<b>Therapeutic indication:</b>	For the active immunisation of red foxes to prevent infection by rabies virus.

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<sup>1</sup> CCID<sub>50</sub>: Cell Culture Infective Dose 50 %  
CVMP/256/00

## II SCIENTIFIC DISCUSSION

### III 1. Introduction

Rabigen SAG2 is a single-strain, live modified rabies virus vaccine indicated for the oral vaccination of free-ranging red foxes (*Vulpes vulpes*) against rabies. The liquid virus suspension is presented within a PVC/aluminium sachet coated with a bait matrix containing biological and artificial attractants, biological and chemical binding material and tetracycline as a biomarker. Since tetracycline is deposited in the teeth, bait uptake can be evaluated. The product is intended for use by authorised personnel only, within the framework of national rabies eradication programs.

Rabigen SAG2 vaccine has been used so far on an experimental basis in the field, in France since 1993 and in Switzerland since 1994.

The live modified rabies virus strain SAG2 is a natural double mutant of the attenuated first generation oral rabies vaccine strain SAD-Bern (originally isolated from a naturally infected rabid dog in the United States), from which it has been selected and cloned using monoclonal antibodies. The original strain belonged to serotype I of the genus Rhabdovirus. Serotype I rabies viruses are characterised by an antigen homology of over 98 % and, therefore, serotype I derived vaccines confer excellent cross protection against all existing terrestrial street rabies virus strains.

A similar technique has been used to select an earlier single mutation rabies vaccine strain, SAG1 that has been registered under the name of "SAG1 Oral Fox Vaccine" outside the European Community. The two-step selection process by monoclonal antibodies, followed by cloning, make SAG2 a new active ingredient which qualifies for registration under the centralised EEC procedure. Rabigen SAG2 has so far not been licensed elsewhere.

The CVMP provided scientific advice with respect to quality, safety and efficacy testing of the SAG-2 rabies vaccine.

## 1. Part II A

### QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

The quality part of the dossier complies with EU directives and guidelines, with Ph. Eur. monographs and with the scientific advice provided by the CVMP. The quality part is comprehensive and no major deficiencies were observed.

The main quality issues specific for this vaccine are as follows:

- the possibility of reversion of the vaccine strain to wild-type virus;
- the possibility of contamination by extraneous agents (from animal cell substrates, virus seed; excipients and media components of animal origin, contamination during production);
- the stability during production (especially during embedding into hot bait paste at 65°C);
- the stability during storage of intermediates and final vaccine and during use.

#### 3.1 1. Composition of the veterinary medicinal product

The liquid preparation consists of live attenuated rabies virus, strain SAG2 (titer at least of  $10^8$  CCID<sub>50</sub>/ per dose (CCID<sub>50</sub>: Cell Culture Infective Dose 50 %), suspended in excipient and contained in a PVC/aluminium sachet which is embedded in a bait. The SAG2 vaccine virus strain was further attenuated from its direct parental strain SAD-Bern by selection of point mutations under immunological pressure from monoclonal antibodies directed against an epitope of the rabies virus glycoprotein.

#### 2. Container

The vaccine virus is contained in a PVC/aluminium sachet, which is coated with appetent matrix (bait). The weight of the sachet is about  $0.420 \pm 0.020$  g (empty) and  $2.25 \pm 0.15$  g (full).

#### 3. Baits

- 3.2 An appetent matrix made partly from substances of animal origin is used to incorporate the PVC/aluminium sachet.

#### 3.3 4. Product Development Studies

##### 4.1 Vaccine baits:

###### *Mechanical Resistance*

The mechanical resistance was investigated in two trials. In one the distribution of the baits was made by helicopter at a speed of 180 km/hour and at altitude of 60 to 100 m. In a second trial the baits were dropped by helicopter at a speed of 160 km/hour and an altitude of 130 m, onto a solid ground (paved airfield) with baits already weakened by a partial thawing.

By calculation of the fall speed with respect to the height and the speed limit of the fall it was demonstrated that for a bait measuring 4.9 x 4.4 x 1.5cm and weighing 28.1 g (maximal weight), and considering that the surface of rubbing is minimal (worst case), the maximum speed of fall of 58 km/h is reached from a height of 13 m thus much inferior to that of the helicopter. It was concluded that the mechanical resistance of the bait has been established.

### ***Water Resistance***

In one study the baits were plunged into water during 4 days (twice the duration of the activity claim) at a temperature of 25°C. A mean increase of 2.7 % of the dimensions of the bait, a weight gain of 3.7 % and partial bleaching were observed.

### ***Heat Resistance***

The temperature of pouring of the paste is of 65°C±5°C. The melting temperature is slightly lower, at 56°C; its exact determination being difficult since the bait softens progressively before turning into the pasty state. The temperature of 56°C is considerable higher than the temperatures found in the European climate during the vaccination campaigns of spring and autumn and moreover above the temperatures tested concerning the stability of the vaccine after distribution.

#### **4.2 Choice of the packaging material**

The vaccine is packaged in carton boxes which are stored on pallets. Each carton box contains 2 bags in polyethylene comprising 200 baits each (i.e., 400 baits per carton box) and a package insert.

#### **4.3 Choice of the vaccine virus strain**

The vaccine virus strain was initially selected on the basis of its avirulence and protective capacity in mice and its growth potential in cell culture. The SAD-strain was originally isolated from a street virus (1935), adapted to hamster kidney cells and passaged in chicken embryo and porcine kidney cells (ERA-strain, 1966), passaged in canine and bovine kidney cells (SAD-Bern-strain, 1966) and selected by CNRS under Mab pressure to the SAG2 strain (1993).

The SAG2 vaccine strain of Rabigen was generated from the SAD-Bern strain by inducing two point mutations under immunological pressure: a first nucleotide change of the codon for arginine (AGA) in position 333 of the glycoprotein gene changed the codon into one for lysine (AAA) and reduced virulence in mice; from this mutant, a further attenuated mutant with two changes in codon 333 was generated with the sequence GAA coding for glutamate.

The genetic stability of SAG2 was demonstrated in mice (2 intracerebral passages in suckling mice and intracerebral testing of pathogenicity in adult mice) and during multiplication in cell cultures (10 consecutive multiplications in BSR cells and testing of pathogenicity in adult mice and nucleotide sequencing).

Studies were performed on the highest production passage + 5 series of 5 back passages.

- The mouse model is documented to have a sensitivity of 1-3 pfu/inoculum causing 50% lethality. This allows the conclusion that the master seed contains less than  $1.7 \times 10^{-4}$  revertants.
- Immunofluorescence with a monoclonal antibody specific to virulent arginine/lysine 333 revertants allows the conclusion that the probability of double reversion is around  $10^{-12}$ .
- Sequencing studies can detect 20% revertants, which is deemed sufficient to detect faster growing mutants after cell culture passages.

#### **4.4 Choice of the cell line for virus propagation**

The rodent BSR cell line, a BHK 21 clone, was chosen for its virus growth supporting characteristics and because it is a well known cell line used for the production of other veterinary vaccines.

#### **4.5 Choice of the excipient**

The excipient was chosen to ensure stability at freezing and storage at low temperature.

## **2. II B DESCRIPTION OF METHOD OF PREPARATION of the finished product**

### **3.1 1. Manufacturing process**

#### **(a) 1.1 Preparation of production cells**

Thawing and inoculation of working cells, change of medium, cell passage, are done according to specified conventional techniques under GMP conditions.

#### **(b) 1.2 Preparation of virus inoculum**

Working viral seed is thawed and expanded on cell culture. Viral harvests are immediately pooled, clarified and concentrated. Specifications for the concentrate are provided.

Starting materials are tightly controlled. Proof was provided that all materials of bovine origin are in compliance with the CVMP “Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal product” (EMEA/CVMP/145/97 – Revision). The master cell seed and the working cell seeds have been tested in accordance with Eur. Ph. and CVMP requirements. Selection of specific virus testing is based on cell species, culture media and target species. The master virus seedlot and the working virus seedlot have been tested in accordance with the guidelines. A full description of the selection of the strain master seed lot under monoclonal antibody pressure and genetic stability data have been provided.

#### **(c) 1.3 Vaccine production**

Cells are inoculated with inoculum of vaccine virus. Some roller bottles may be set aside for further cell multiplication using the procedures used for the original passages, up to the maximum allowed total passage number of 20. Viral harvests are immediately pooled, clarified and concentrated. After addition of freezing excipient, the harvest is aliquoted and frozen for further storage.

### **1.4 Manufacturing of final product**

The manufacturing of the final product concerns formulation, filling and freezing of sachets (formulation is based on targeted virus titre).

Data are presented demonstrating sufficient stability of inocula, concentrated suspension and sachets at relevant defined storage temperature.

The preparation of the bait is given, where it appears that the blending of all the raw materials is done at high temperature. A report of a stability study on tetracycline-chlorhydrate is presented. After 2 hours at +60°C and after 2 hours at +80°C, activity losses are 10% and 80% respectively. The manufacturing process causes a significant activity loss. The remaining activity expressed in live weight of fox is still significantly larger than the minimum dose detectable by fluorescence, and marker capacity is therefore maintained.

Later on, the frozen vaccine sachets are inserted in the bait. Data showed no significant titre loss before and after coating.

### 3.2 2. Validation studies

As validation data, more than 10 batches produced between December 1993 and November 1996 were investigated for cell seeding, rate of viral inoculation, date of harvest, and other parameters for downstream process. Concentrates were diluted to attain target titres of formulation. Dilution factors used for formulation are based on observed titres in the concentrates.

Furthermore, results on three final lots, embedded in bait, are presented. Titres observed in bait embedded product are equal or higher than titres in sachets and no titre loss was observed.

As inocula, concentrates and sachets can be stored, the stability of these intermediates has been evaluated.

The stability of the storage is checked. Stability data showing very slight decrease. Variations are attributed to variability of titration method.

### 3. IIC CONTROL OF STARTING MATERIALS

#### 1. Starting materials listed in pharmacopoeia

For all the starting material listed in a pharmacopoeia, a certificate of analysis was provided with a statement of conformance.

#### 2. Starting materials not listed in pharmacopoeia (European)

##### 2.1 Starting materials of biological origin

##### 2.1.1 Seed Material

##### 2.1.1.1 BSR (Kidney Syrian Hamster) cells

The master cell seed (MCS) (= “master strain”) and the working cell seed (WCS) (=“working strain”) have been submitted to the following tests.

Test	Ph. Eur. & CVMP requirement	MCS	WCS	Max. Passage
General microscopy (characteristics of culture)	MCS, WCS and Max. Passage	+	+	-
Species identification (DNA fingerprinting, isoenzymes)	MCS and Max. passage	+	n.r.	+
Karyotype	MCS and Max. passage	+	n.r.	+
Detection of mycoplasma	MCS and WCS	+	+	n.r.
Detection of bacteria and fungi	MCS and WCS	+	+	n.r.
Leptospira spp. (CVMP, canine list)	MCS	-	+	n.r.
General detection of extraneous viruses (direct microscopy, hemadsorption, staining, tests on cell cultures: monkey, pig, bovine, canine & hamster cells)	MCS and WCS	+	+	n.r.
Specific detection of extraneous viruses* (by general detection tests, specific IF or staining):	MCS and WCS +	+/(1)	+	n.r.
Retroviruses (reverse transcriptase)	MCS and max. passage	+	n.r.	-
Tumorigenicity**	MCS	+	+	-

Oncogenicity	MCS	+	-	-
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(1) depending of the agent see hereafter

- n.r. not required
- + is required or has been tested at this stage
- not performed or not clear if performed or not
- \* the CVMP scientific advice agreed that compliance with the canine guidelines should be sought for foxes as target species
- \*\* tumorigenicity of BHK21 cells for hamsters has been reported and was detected for intact BSR cells. A study was performed on 3 stages of the vaccine preparation and showed no tumorigenicity.

Testing for virus contamination, although deviating in some aspects from EU requirements, can generally be regarded as satisfactory. In particular the characterisation of the MCS for specific virus contamination through testing of the WCS is acceptable.

Three different techniques are provided for the detection of mycoplasmic contamination of the MCB and 2 different ones for the WCB. Two techniques are used simultaneously for seeding strains. The culture technique is documented to be in conformity with the 1999 Ph. Eur. Monograph and is used for working cell seeding strains, intermediate products and final product.

Three different techniques are provided for the detection of bacterial and fungal contamination of the MCB and the WCB.

Furthermore, two different techniques for the detection of any viral contamination are applied for viral seeding strains and for cell seeding strains.

Since intact BSR cells were found tumorigenic in hamsters, proof was provided that, with a high degree of confidence, neither intact BSR cells nor fragments of these cells will be left in the viral concentrated suspension. A validation of a sequence of filtration steps in the manufacturing process was provided.

### 2.1.1.2 SAG2 rabies virus

The master virus seedlot (MSL) (= “master strain”) and the working virus seedlot (WSL) (=“working strain”) have been subjected satisfactorily to the following tests:

Test	MSL	WSL
Identification	+	+
Virus titre	+	+
Detection of mycoplasma	+	+
Detection of bacteria and fungi	+	+
Detection of extraneous viruses on cell cultures (monkey, pig, bovine, canine, feline and hamster cells) using positive controls	+/(1)	+/-

(1) depending of the agent see hereafter

In general, testing for virus contamination, although deviating in some aspects from EU requirements, can be regarded as satisfactory. In particular the characterisation of the MSL for some of the specific virus contamination through testing of the WSL is acceptable. The passage history of the virus seed at and before its laboratory of origin include passages in mice, hamster kidney tissue, chicken embryo’s, porcine kidney cells, canine and bovine kidney cells and hamster kidney cells. There was exposure to mouse ascites monoclonal antibodies and to foetal calf serum from New Zealand.

Details have been provided on the antiserum used to neutralise the vaccine virus strain.

A full description of the selection of the strain Master Seed Lot under monoclonal antibody pressure; genetic stability has been provided.

Master and Working Seed Lot: data for identity, bacterial and fungal sterility, mycoplasmic sterility, extraneous agents (with the list of the specific viruses, which are searched, and a complete description of the technique used) and titration have been provided.

### **2.1.2 Other products**

For all the starting material of biological origin, a certificate of analysis was provided with a statement of conformance with the internal specification of the applicant.

Proof is provided that all materials of bovine origin are in compliance with the CVMP 'Note for Guidance for minimising the risk of transmitting animal spongiform encephalopathy agents via veterinary medicinal products' (EMEA/CVMP/145/97-REVISION).

## **IIC 2.2 Starting materials of non biological origin**

Information is provided concerning function, identification, storage and testing. A certificate of analysis was provided with a statement of conformance with the internal specification of the applicant.

### **(a) IIC 2.3 In House Media preparation**

Water for Injection is used for the preparation of all solutions.

Growth medium 1 is used for the expansion of the BSR cell cultures, growth medium 2 is used for the final passage of BSR cell cultures prior to inoculation, maintenance medium is used throughout the virus propagation phases. The preparation and formulations of the 3 media are described. All are prepared in a similar manner and all are sterilised by filtration through a 0.1- $\mu$ m membrane. The composition of the different media, expressed as concentration per litre is given.

The following preparations were also discussed:

- Solutions for pH adjustment
- Buffer, used to rinse the systems of filtration and concentration.
- Saline solution, used for dilution of the concentrate.
- Proteolytic enzyme for the removal of cells from their support and their dissociation.
- Freezing Excipient and Sachet Excipient, which are used as cryoprotector.

## **4. IIC 2.4 CONTROL TESTS During production**

Control tests during production are carried out on:

- Cell passages used for production on extraneous viruses (haemadsorbant and haemagglutinating agents, parvoviruses, rabies virus, mycoplasma and intracellular anomalies).
- Inoculum 2 for sterility, titre, safety in mice, PH, identity (immunofluorescence and mice I.C. innocuity) and mycoplasma.
- Bulk vaccine for PH, sterility, mycoplasma, extraneous agents in BSR cells, titre and safety in mice.
- Sachets on volume, watertightness, titre, extraneous agents in BSR cells, safety in mice, pH, sterility, mycoplasma and identity (immunofluorescence and mice I.C. innocuity).



Batch analysis data demonstrate consistency.

In compliance with the Ph. Eur. Monograph, an unequivocal identity test using neutralizing monospecific monoclonal antibodies has been performed.

## 5. II E CONTROL TESTS OF THE FINISHED PRODUCT

### (a) II E 1 General characteristics

The bait is tested for weight, physical integrity and biomarker. Extraneous agents of the finished product are tested. Batch analysis data demonstrate consistency. Furthermore, a satisfactory model production and control protocol is presented.

### (b) II E 2 Identification of active ingredient

The embedded vaccine is tested for its titre. The activity test was performed with baits with a titre close to the minimum of 7.8 log, manufactured from a suspension of 9.5.

### (c) II E 3 Identification of excipient constituents

Tetracycline is used as a biomarker.

A low but detectable level suitable to allow a correct reading of the dental sections, was established. This level is 10 times inferior to the residual rate found at the end of the manufacturing process.

### (d) II E 9 Batch to batch consistency

Data confirming batch to batch consistency have been provided. The melting point of several subsequently produced batches of baits is  $> 50^{\circ}\text{C}$ .

## 6. II F STABILITY

Results of 3 finished product batches kept at  $-20^{\circ}\text{C}$  and  $-40^{\circ}\text{C}$  for up to 27 months are presented. Parameters studied were volume, pH, sterility and virus titre. No significant changes were observed for any parameter at both temperatures. The proposed shelf life of 2 years at either  $-20^{\circ}\text{C}$  or  $-40^{\circ}\text{C}$  is acceptable.

Results of 3 finished product batches kept at  $+4^{\circ}\text{C}$  and  $+25^{\circ}\text{C}$  for up to 7 days have been provided. Parameters studied were volume, pH, and virus titre. No significant changes were observed for any parameter at both temperatures. Sterility has also been studied after exposure to conditions for use.

The stability data of the bait indicate maximum losses of  $0.16 \log_{10} \text{CCID}_{50}$  after 2 years at  $-20^{\circ}\text{C}$  and  $0.16 \log_{10} \text{CCID}_{50}$  after 2 days at  $+25^{\circ}\text{C}$ .

A validation study of the titration method and an appropriate statistical analysis has been provided to determine the release titre as sum of the minimum active titre, the max. loss at  $-20^{\circ}\text{C}$  2 years and the max. loss at  $+25^{\circ}\text{C}$  2 days.

### 3.1 Conclusions on quality

The quality part is comprehensive and no major deficiencies were observed.

## IV III Overview of part III of the dossier: SAFETY and Residues

### 1. Part III A Safety

#### 2. III A Introduction

The performed studies with regard to administration of a single, an overdose and a repeated administration of one dose comply with the specific requests from the CVMP. In these studies, all foxes remained healthy over the period of observation and furthermore sero-conversion was observed from 30 days after vaccination.

None of the single dog studies provided complies exactly with the requirements of overdose testing in dogs by the European monograph. However, it is acceptable that the deviations in these studies were considered of minor importance, because of the different dog studies, which as a whole prove the safety of an overdose administration to dogs.

The safety of the vaccine after administration of an overdose to cats is considered as being proven. SAG2 has been demonstrated to be safe for most common European non-target species.

Sufficient evidence is provided of a very small risk of dissemination of the vaccine virus to the brain or excretory organs and that reversion to virulence of the attenuated vaccine is very unlikely in field conditions.

### 3. III C Laboratory Tests

#### III C 1. Safety of the administration of a single dose

In a first trial 10 foxes, at age  $\pm$  12 months, were orally vaccinated with the SAG2 strain, 5 received a dose of  $10^7$  PFU and the other 5 a dose of  $10^8$ . The study included also 5 control animals. No abnormal behaviour or signs of illness were observed during 29 days after vaccination. Seroconversion occurred by day 29. All vaccinated animals were protected against an intramuscular challenge (day 29) with a vulpine street rabies virus (GS7), to which all five unvaccinated control foxes succumbed within 25 days. The vaccinated individuals remained healthy until the day of euthanasia, 84 days after vaccination, and no viral antigen was detected in brain samples collected post mortem.

In a second trial 17 adult seronegative foxes ( $\pm$  9 months age) were inoculated with  $10^{7.5}$  PFU of SAG2 virus. No vaccine was isolated from saliva swabs collected 1 and 3 days after vaccination. All foxes were observed daily until the day of challenge. A lethal dose of vulpine street virus was administered 85, 220 or 303 days after oral vaccination to the foxes grouped in 6, 5 and 6 individuals. Twenty-four days after vaccination, all vaccinates had seroconverted. One vaccinated fox died on day 111, tests applied on its brain tissues produced negative results, and meanwhile *Streptococcus faecium* 2 was isolated in a fibrous muscle near kidneys. All other vaccinated individuals developed rabies virus neutralising antibody titres and survived the challenge. All the unvaccinated controls succumbed to rabies (days 85 - 202).

The vaccine dose used to inoculate the 38 foxes was in all but five animals, inferior to the minimal recommended vaccine concentration. The Ph. Eur. requires 40 foxes be given a single dose and 10 foxes be given a 10 times overdose to be studied for 180 days following vaccination. On the requirements for safety testing, the CVMP agreed that a 10-time overdose study, using vaccine suspension at maximum titre on 10 foxes, kept under observation for 180 days, would be sufficient to demonstrate vaccine safety in the target species.

## C III 2 Safety of an administration of a single overdose

### (a) 2.1 Safety of an administration of a single overdose in foxes

The vaccine has not been administered in its final presentation (vaccine filled bait) but as a highly concentrated vaccine suspension in the overdose study.

A study was performed on request of the CVMP and in accordance with the Ph. Eur. between October 1996 and May 1997. Ten foxes were inoculated by instillation of 10 times the recommended dose. After vaccination with 2 ml 109,8 CCID<sub>50</sub> SAG2 vaccine, the foxes were clinically observed for a period of 183 days. They were all found seronegative with respect to the rabies antigen at day 0 before vaccination. Seroconversion was detected in all foxes at the first blood sample. An increase of the antibody titres was shown until day 91. All foxes remained in good health during the entire observation period. For the detection of virus in saliva, all samples collected at day 0, 1, 3 and 7 were negative. All tests for the detection of the antigen (direct immuno-fluorescence (IF)) or the rabies virus (inoculation in a neuroblastoma cell line (N2a)) were carried out (positive control included in series) on the encephalon (hippocampus, medulla oblongata, cortex) and the salivary glands of the 10 foxes: all these examinations were negative.

A similar study was performed earlier in 1994. Ten foxes ( $\pm$  9 months old) were treated orally (by drip instillation of 1 ml) with exactly ten times ( $10^9$  PFU) the recommended dose and observed for 175 days. A SAG2 suspension at a passage level prior to the industrial MSV (origin CNRS) was used. Blood sample and dosage of the rabies antibodies were performed on day 0, 31, 111 and 175. Saliva was sampled at day 1, 3 and 6 for the detection of rabies virus (immuno-fluorescence (I.F.) after culturing). After euthanasia, it was looked for rabies antigen in the hippocampus, the submaxillary salivary glands and the submaxillary and parotid lymph nodes were examined for rabies antigen (I.F.). All post-euthanasia I.F. tests were negative and no life virus indicative of excretion was isolated from saliva. The mean neutralising antibody titres before and after vaccination were on day 0 negative, on day 31 5.46 IU/ml, day 111 7.81 IU/ml and day 175 8.13 IU/ml.

The studies performed on a total of 20 foxes monitored up to 175 - 183 days indicate the safety of the SAG2 suspension given orally as a ten fold overdose. With regard to administration of a single and an overdose the studies comply with the specific requests from the CVMP.

### 2.2 Safety of an administration of a single overdose in dogs and cats

The European monograph for live oral rabies vaccine for foxes requires testing of a ten times recommended dose given to 10 dogs and 10 cats with no sign of rabies within 180 days of administration. Several studies in dogs and cats therefore, have been provided.

In one study, three groups of 5 beagle dogs, randomly allocated ( $\pm$  age 3 months) were given orally  $10^7$ ,  $10^8$  or  $10^9$  PFU/ml, 5 animals remained untreated controls. All vaccinated dogs did seroconvert at day 39. All vaccinated dogs survived the challenge (at day 39), while 4 of the five control animals succumbed.

In a similar study in cats, 5 cats received orally  $10^9$  PFU/ml of SAG2 vaccine. No pathological signs were reported during the 33 days of follow-up but also antibody titres at day 33 were below the threshold level considered as positive. The challenge infection was not relevant since no signs of rabies lesion were observed in the control cats (n=3).

Both studies deviate in animal numbers and duration of the observation period from the relevant guidelines. Furthermore, the vaccine suspension used came from a passage level prior to the MSV and was not produced by the manufacturer according to the industrial procedure.

In another study, ten European short hair cats, free of rabies antibodies prior to oral inoculation with  $10^9$  CCID<sub>50</sub> of SAG2 suspension did not exhibit any clinical signs during the 189 days following vaccination.

A bibliographic study concerning the administration of the vaccine to dogs was provided. Ten beagles remained free of clinical signs during an observation period of 180 following oral inoculation with SAG2 vaccine. The vaccine suspension used was the first industrial batch produced by the manufacturer (S2/1) and titrated in the hands of the principle investigator at  $10^{8.5}$  MICLD<sub>50</sub> (Mouse Intracerebral Lethal Dose). This titre lies 0.5 log below the 10 times recommended dose required by the legislation.

Another safety study over 90 days in dogs aged 3 months and younger documents the safety of SAG2 (1 ml,  $10^{7.2}$  MICLD<sub>50</sub>/ml  $\approx 10^{9.0}$  CCID<sub>50</sub>) when inoculated by oral (n=11) and intramuscular route (n=10) to dogs of various origins. While none of the puppies developed clinical signs, virus was detected in the saliva in dogs inoculated by intramuscular route (7 out of 10). All animals seroconverted by day 7 with a peak on day 14. It is stated by the Applicant that antibody titres of the orally vaccinated dogs show a much lower protection level than intramuscular vaccinated puppies and that “the sudden drop” in antibody titre suggests that there may be a need to boost the vaccination if the puppies are given vaccine at such a young age. However, supporting data were not provided. The analysis of blood and organ samples (brain, salivary glands and tonsils) did not allow detecting live virus in any of the tissues harvested at the end of the study.

### 2.3 Safety of an administration of a single overdose in non-target species

Directive 92/18/EEC requires that the potential risk to unvaccinated animals of the target or any other potentially exposed species shall be evaluated. The release of vaccine baits into the environment inevitably leads to contamination of non-target species. By definition, any omnivorous or carnivorous animal sharing the habitat of foxes and occurring in sufficient numbers in the vaccination area has to be considered a significant non-target species. The most important West-European target species have been identified by photographic capture. In a trapping experiment 21 % of baits were consumed by a carnivore (fox, beech marten, wild cat, dog), 43 % by hedgehogs and 36 % by cows. Only 3 % of all photographs showed foxes. However, the total number of photographs (62) was rather low. In absence of any specific directives, the manufacturer carried out non-target species safety testing according to WHO recommendations (WHO/Rab.Res./89.32). These recommendations request that the most common wild rodent species be inoculated by the oral and intramuscular route and that 5 of the most relevant non-target species be studied. The vaccine is considered safe if no more than 10% of the rodents and none of the orally vaccinated non-target species develop rabies.

The company has tested the SAG2 vaccine strain in approximately 30 species, at least 14 of which are widely distributed in the Western-European ecosystem, by the oral and in certain cases, by the intramuscular and intracerebral route. The following table provides a partial overview.

Species	Duration of trial	Vaccine dosage	Route of administration and sample size
Wild rodents	42 – 78 days	$10^{7.44}$ CCID <sub>50</sub> – $10^{7.7}$ CCID <sub>50</sub>	Oral: 52, IM: 50, Intracerebral: 10, controls: 14
Wild boar	93 days	$10^{8.8}$ UFPF	Oral: 5, controls : 1
Badger	37 days	$10^{8.17}$ UFPF	Oral: 5, controls : 1
Goat	35 days	$10^{8.8}$ UFPF	Oral: 6, controls : 1
Ferret	37 days	$10^{8.17}$ UFPF	Oral: 4, IM: 4, controls: 2
Hedgehog	57 days	$10^{7.87}$ UFPF	Oral: 6, controls: 3
Rook	33 days	$10^{8.66}$ UFPF	Oral: 8, controls: 8
Crow	33 days	$10^{8.66}$ UFPF	Oral: 7, controls: 3
Buzzard	41 days	$10^{8.18}$ UFPF	Oral: 8, controls: 2
Owl sp.	41 days	$10^{8.18}$ UFPF	Oral: 4, controls: 2

No adverse effects, signs of pathology, excretion or dissemination were detected during the observation periods. Instead, SAG2 induced a specific humoral response in most species which, if occurring under natural conditions, could contribute favourably to vaccination success. However, in goats no specific neutralising antibodies were detected. No live virus or viral antigen was found in the tissues primarily affected by virus multiplication and dissemination (brain and salivary glands).

### **2.3.1 Safety in racoon dogs.**

It is considered that the manufacturer has included all relevant species with the exception of racoon dogs (*Nyctereutes procyonoides*). This species has gradually extended its home range from Eastern Europe to Western Europe, similarly to another species, the raccoon (*Procyon lotor*), which originates from Eastern European fur farms and has consistently migrated westward. Both species have reached the North-East of France, are potential rabies vectors and bait consumers and should be tested, if the population densities reach critical levels. The Applicant was asked to provide additional data on safety testing in racoon dogs due to the fact that this species may be rabies vector in Northern Europe.

A complete study was provided including ten racoon dogs. Five received the suspension by direct oral instillation and 5 received each one bait to eat. Quantity of virus was  $10^{9.8}$  or  $10^{9.9}$  CCID<sub>50</sub>/racoon dog. None of the racoon showed any clinical signs that could suggest rabies or any change in behaviour. After 2 months observation, the vaccine virus could not be re-isolated neither from the brain nor from the sub-maxillary salivary glands. Seroneutralising antibodies were detected at day 60. In conclusion, the SAG2 vaccine was considered safe for racoon dogs.

### **2.3.2 Safety of oral administration of the bait to cows.**

Based on the results of the photographic trapping experiment showing that 36 % of the consumers of the bait were cows, the Applicant was asked to provide further safety data of oral administration of the bait to cows. Following aerial bait distribution in a rural environment, the likelihood for cows, which are known to exhibit interest in any foreign object, to encounter baits is high. Due to their role as food producing animals, they represent some risk of contamination for human beings and safety studies were considered. Although not easily transferable between species, the trial results obtained on goats however, provide already some evidence for the safety in ruminants.

The test vaccine suspension ( $10^{9.8}$  CCID<sub>50</sub>) was directly administered by oral route to 5 calves. The animals were observed daily for 60 days. No rabies clinical signs were observed. Virological isolation (immunofluorescence and cell cultures) from cerebral tissues were negative. Serological determination revealed low titres on day 60.

#### **(b) III C 3. Safety of a repeated administration of a single dose**

Following the suggestion by the CVMP, the Applicant has carried out a study, using a study protocol from published literature. Fifteen foxes (10 adults and 5 juvenile), seronegative for rabies virus prior to the study, consumed 1 (group 1), 2 (group 2) or 3 baits (group 3) containing the recommended dose of SAG2 vaccine on three consecutive days. During the 32 days following the last vaccination, all animals developed anti-rabies antibody titres and none developed any clinical signs. Mean titres of anti-rabies antibody at day 32 were 3.6 IU/ml (group 1), 4.2 IU/ml (group 2) and 7 IU/ml (group 3). Encephalon, salivary glands and tonsils, analysed post-mortem (immunofluorescence and inoculation on N<sub>2</sub>a cells) were free of live rabies virus and rabies antigen.

This study shows that the repeated administration of the product is not harmful to foxes.

(c) III C 4 Examination of reproductive performance

Rabies virus and attenuated rabies vaccine viruses do not usually accumulate in reproductive organs and are not known to directly affect reproductive functions. During 4 years of large scale use of SAG2 in the field in France and Switzerland, no difference in fox population structure other than an increase was detected in areas lying within or without the vaccination zones. Therefore, it is justified that no specific studies have been conducted on reproductive performance despite the fact that consumption of baits by pregnant vixen must be expected.

**III C 5 Examination of immunological functions**

One of the parameters consistently studied in the experiments presented in the safety and efficacy dossier of this application is the development of a humoral immune response following vaccine inoculation. The kinetics of the neutralising antibody response to the SAG2 vaccine strain has, with few exceptions, followed the expected pattern for a first contact with the antigen: Seronegative at day 0, appearance of detectable levels (0.32 - 5.46 IU) 14 to 30 days after inoculation, persistence of titres until the end of the experiment (day 30, 175 and 183 resp.).

(d) III C 6 Special requirements for live vaccines

**6.1. Spread of the vaccine strain**

Although rabies has been transmitted to experimental animals by aerosol and blood, these and all other non-bite exposures are extremely unlikely to occur in nature and for attenuated rabies vaccine viruses, much less likely to result in clinical rabies than with fully virulent street or laboratory strains. The morphology and particularly the chemical composition render the rabies virus, as most enveloped viruses, sensitive to inactivation by acids and bases, ultraviolet light, heat, repeated freeze-thaw-cycles and disinfectants as mild as soap solution. Release into the environment is therefore inevitably accompanied by viral inactivation. Spreading from animal to animal is most likely to occur through infective saliva.

While persistence of SAG2 virus in the oral cavity for few hours after oral inoculation has been documented in genets (*Genetta tigrina*), entry into the acid environment of the stomach will quickly inactivate the virus. Consequently, no live virus can be expected in the faeces and no testing is required.

The formation of virions is most common within neurons and spreading occurs essentially in relation to the central nervous system (CNS) and the cerebrospinal fluid (CSF). The only other tissue regularly affected, even before clinical signs of rabies develop, are the salivary glands.

For the purpose of studying the risk of spread of the vaccine virus among animals, the Applicant deviates from the suggested protocol of housing animals together and instead addresses this issue by collecting saliva or salivary gland samples at one to three occasions during any experiment presented in the safety or efficacy documentation. Firstly, within hours after inoculation when input virus might be transmitted to an unvaccinated animal via the saliva; secondly, within days of inoculation, when the vaccine virus can theoretically be shed from mucosal cells into the saliva after having locally completed one or more cycles of replication and thirdly, later in the course of a successful infection of the CNS when virus is spread back to the periphery and is actively excreted by the salivary glands, in the context of clinical or latent rabies.

In conclusion, no isolation of SAG2 or any variant of SAG2 from saliva or salivary glands or the detection of viral antigen in the brain or salivary glands was made, with the exception of the published information cited above and the results of the safety trial in young dogs (the positive detection of virus in saliva of puppies after intramuscular and oral vaccination).

Convincing evidence has been accumulated, suggesting an infinitely small risk of spreading of the vaccine virus from animal to animal.

#### (b) 6.2 Dissemination in the vaccinated animal

The exchange of aminoacid arginine by glutamic acid in position 333 of the rabies virus glycoprotein is thought to have significantly modified the tropism of the SAG2 virus and inactivated the most important virulence factor of rabies virus. As suggested by the reduced growth rate and growth modalities in tissue culture, SAG2 varies from pathogenic rabies virus strains at least in its capacity to enter cells and by the rate at which it completes a cycle of multiplication. As a consequence, the entry into neurons and the rabies virus specific retrograde migration towards the central nervous system inside the cytoplasm of nerve cells should also be inhibited. If this were the case, one would not expect to observe centrifugal contamination of the submaxillary salivary glands either.

To prove these assumptions, the Applicant has systematically subjected foxes and non-target species to post mortem analysis of brain and salivary glands. No live rabies virus (SAG2 or a pathogenic revertant strain) or rabies virus antigen was isolated from brain samples, salivary glands or local lymph nodes of foxes or non-target species in any of the experiments assembled in the safety or efficacy part of this application. Furthermore, not even when injected intracerebrally to adult mice did SAG2 cause rabies and the virus and viral antigen was rapidly cleared from the brain.

However, in suckling mice SAG2, inoculated by the intracerebral route, not only multiplies to high titres but also causes rabies, although after a slightly prolonged incubation period and with milder clinical manifestation than less attenuated rabies virus strains. The reason for these results can most likely be found in the immature immune system of these young animals and an anti-rabies immune response, which develops too slowly or at insufficient levels to contain viral development and disease. A situation resembling this highly experimental model is unlikely to be of relevance in a natural context.

Sufficient evidence for the absence of viral dissemination has been provided of a very small risk of dissemination of the vaccine virus to the brain or excretory organs.

#### 3.2 6.3 Reversion to virulence of attenuated vaccines

Amongst all the studies performed with SAG2, virus was recovered three times from saliva swabs of vaccinated animals, once from a genet, once from a black-backed jackal at day 1 and 3 post vaccination and from puppy dogs that were vaccinated orally or intramuscularly. SAG2 virus was never recovered from foxes vaccinated by the oral route and the virus also fails to replicate by any other than the recommended route. The Applicant did not perform reversion to virulence studies in foxes and proposed a mouse model using suckling mice to carry out five consecutive passages and adult mice for the assessment of the pathogenicity of the strain. The CVMP agreed to this proposal.

Mice are the species most susceptible to rabies virus in general and even the parent strain of SAG2, SAD-Bern, is highly pathogenic for adult mice by the intracerebral route. The vaccine strain SAG2, at its lowest and any previous passage levels does not, in contrast, produce clinical or latent rabies in adult mice, even when injected by the intracerebral route.

Rabies viruses have a spontaneous mutation frequency of approximately  $10^{-4}$ . The proposed model is based on the assumption that the phenotype of the vaccine strain should eventually switch from apathogenic to pathogenic, should sufficiently high concentrations of virulent revertant viruses

accumulate in the SAG2 suspension during viral replication. This change would be detectable in intracerebrally inoculated adult mice.

Since passaging of SAG2 in cell culture would more likely lead to further attenuation of the strain, the model of choice for the passages was suckling mice. Suckling mice either do not yet possess a sufficiently mature immune system to control rabies virus infection in their brains or they are, for other reasons, particularly receptive to rabies virus multiplication. To increase the chances of detecting pathogenic revertants, five groups of suckling mice (total n=143, 4 days old) were inoculated in parallel for each of the five consecutive passages. Brains were harvested and pooled. The virus titres of the pools ranged consistently around  $10^8$  PFU per ml, indicating successful replication of the SAG2 strain in the suckling mice. The pathogenicity of each pool was then assessed by intra-cerebral inoculation of adult mice (n=125, 6 weeks old). None of the adult mice developed rabies nor could antigen be found in their brains (double fluorescence testing).

It was noticed, however, that if apathogenic SAG2 particles exceed a certain percentage in a contaminated suspension, they confer protection to adult mice and clinical rabies will not develop, unless the proportion is clearly in favour of the virulent particles. Additional *in vitro* testing, however, helped to rule out any detectable reversion to the pathogenic genotype.

Since five consecutive passages of SAG2 under most favourable conditions did not allow for the generation of any pathogenic revertant particles, sufficient proof has been provided that reversion to virulence of SAG2 is extremely unlikely to occur. Given that revertants could not be detected under highly sophisticated experimental conditions, chances for their appearance under field conditions must be considered extremely limited. Moreover the vaccine complies with the Eur. Ph. Monograph for the safety studies in immunocompetent mice.

In conclusion, it was agreed that reversion to virulence of the attenuated vaccine is unlikely in field conditions.

### **3.3 6.4. Biological properties of the vaccine strain**

The single, most characteristic biological property of SAG2, the apathogenicity for adult mice after intracerebral inoculation, has already been explained. This property serves as a biological marker and is repeatedly controlled throughout the manufacturing process as described in detail in the chemical, pharmaceutical and biological documentation.

### **3.4 6.5. Recombination or genomic reassortment of strains**

Rabies virus does not possess a segmented genome and rhabdoviridae are not known to undergo genomic reassortment. As no natural recombinational event has been described with rabies virus and SAG2 is not a product of recombination, but has been selected from a pool of natural mutants, this point should not be addressed.

### **3.5 7 Residues**

Rabigen SAG2 cannot be traced in the body of vaccinated animals, does not contain any adjuvants or preservatives and is not intended for use in pets or food-producing animals. However, the bait contains small amounts of tetracycline HCl (not more than 146.88 mg/ bait), the use of which is contraindicated for ruminants and the photographic trapping experiment showed cows approaching the bait in 36 % of the pictures.

In view of the low concentration of tetracycline, there is no safety concern. Also a residue problem is not expected for tetracycline when a cow accidentally consumes a bait. The company's decision not to undertake studies of residues is therefore justified.



### 3.6 8 Interactions

There are no known interactions between the active ingredient SAG2 and other medicinal products and chances are small that interaction would be of relevance when the product is used as indicated.

#### (a) III D Field studies

No specific field trial results on safety are provided in this report. However, the distribution of almost 4 million baits of the product during the field trials of the last 4 years have been followed by an intensive surveillance of the vaccination areas. Each year, between 3 to 8 foxes are collected per 100 square kilometre and submitted to rabies diagnosis. As mentioned in the part on ecotoxicity, no case of vaccine induced rabies has been reported, confirming the safety of the vaccine in the field.

#### III E Ecotoxicity

Inside the undisturbed bait and even less, when exposed to environmental conditions, the vaccine does not persist for extended periods of time in nature. The titre of the vaccine suspension inside the sealed sachet was shown to decrease by approximately  $10^{0.4}$  CCID<sub>50</sub> per week when stored at 25°C. When administered by the recommended and other routes, it does not disseminate in the vaccinated animal and can generally not be recovered from the oral cavity within hours of vaccination, thus limiting the chances for shedding and spreading.

Although exposure of non-target species is likely to occur, the vaccine is not expected to be a safety hazard. The degree of attenuation of the SAG2 vaccine strain is high and it is unlikely to cause rabies and establish itself in nature. The safety of SAG2 has so far been demonstrated in almost 30 species belonging to the most relevant potential bait consumers. Contact with the vaccine has never led to clinical signs and was shown to induce immunity, which in the case of dogs was sufficient to confer protection against a virulent rabies virus challenge.

Human contact with baits and vaccine cannot totally be avoided, but it remained without clinical consequences during the 4 years of approved field trials. In contrary to the rabies vaccine (VVTGgRAB, based on a vaccinia recombinant), in case of an accidental contamination of a person with the bait carrying the SAG2 strain, a classical anti-rabies treatment remains necessary since the risk of rabies cannot be completely eliminated although it is deemed low for humans.

The frequency of contact with the vaccine can be significantly reduced through the placement of warnings on every vaccine capsule and through public information. In view of safety to the public it was therefore requested to put the following warning on the capsule and the bait: "RABIES VACCINE DO NOT TOUCH". Since this vaccine will predominantly be used in vaccine campaigns, organised with local governmental parties, it was also asked to a phone number on the capsule and the bait to allow direct information to persons who by accident encounter a bait.

The vaccine is presented in an aluminium/PVC-sachet coated with an appetent matrix of animal origin containing a biomarker. The components of the bait and the liquid vaccine are biodegradable and non-toxic.

### 3.7 Conclusions on safety

The performed studies with regard to administration of a single, an overdose and a repeated administration of one dose comply with the specific requests from the CVMP. In these studies, all foxes remained healthy over the period of observation and furthermore seroconversion was observed from 30 days after vaccination.

Although none of the single dog studies provided complies exactly with the requirements of overdose testing in dogs by the European monograph, it was accepted that these deviations were only of minor importance and that the different dog studies as a whole prove safety of an overdose administration to dogs. The Applicant was, however, requested to provide further information on the safety study in dogs and cats. This information was provided and presented at the oral explanation. Vaccine was administered orally at 10 times the recommended dosage to 10 dogs and 10 cats. No mortality and no morbidity was found that can be related to rabies within 180 days of vaccination. There was no effect on the growth of the animals. It was concluded that a 10 times maximum dosage is well tolerated by dogs and cats.

SAG2 has been demonstrated to be safe for most common European non-target species.

Sufficient proof is provided of a very small risk of dissemination of the vaccine virus to the brain or excretory organs and that reversion to virulence of the attenuated vaccine is very unlikely in field conditions.

The main criticism of the data is the use of pre-industrial vaccine virus for roughly half the safety studies and the failure to properly identify batches provided. However, lack of identification of certain batches is of little practical importance, because the Master Seed Virus and all resulting vaccine batches since the arrival of the strain in 1992 have been produced by the production department according to the industrial procedure described in the analytical part.

#### **4. IV OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY**

##### **3.1 A INTRODUCTION**

It was considered more important to see the effect of the herd immunity on a rabies epidemic than measure the immunological effect on an individual level; however, as indicated by the trials hereafter, this accompanies an increasing amount of uncontrollable variables. As no guidelines are available, the efficacy trials focussed on the three following topics:

- Immunogenicity of SAG2 suspension in the fox
- Efficacy of SAG2 in the pharmaceutical form adapted for oral vaccination
- Efficacy of SAG2 in controlling a rabies epidemic

##### **3.2 IV C Laboratory trials**

##### **3.3 1 Immunogenicity of SAG2 suspension in the fox**

In a first trial 10 foxes, at age  $\pm$  12 months, were orally vaccinated with the SAG2 strain, 5 received a dose of  $10^7$  PFU and the other 5 a dose of  $10^8$ . The study included also 5 control animals. All foxes were daily observed. No abnormal behaviour or signs of illness were observed during 29 days after vaccination. Seroconversion occurred by day 29. Mean titres were 0.48 IU/ml and 0.32 IU/ml for foxes given  $10^7$  and  $10^8$ , respectively. All vaccinated animals were protected against an intramuscular challenge (day 29) with a vulpine street rabies virus (GS7), to which all five unvaccinated control foxes succumbed within 25 days. The vaccinated individuals remained healthy until the day of euthanasia, 84 days after vaccination (59 days after the death of the last control fox), and no viral antigen was detected in brain samples collected post mortem.

In another trial 17 adult seronegative foxes ( $\pm$  9 months age) were inoculated with  $10^{7.5}$  PFU of SAG2 virus. No vaccine was isolated from saliva swabs collected 1 and 3 days after vaccination. All foxes were daily observed until the day of challenge, with a lethal dose of vulpine street virus, which was administered 85, 220 or 303 days after oral vaccination to the foxes grouped in 6, 5 and 6 individuals. Twenty-four days after vaccination, all vaccinates had seroconverted. The mean titre at day 24 was 1.5 IU/ml. One vaccinated fox died on day 111, FA tests applied on its brain tissues produced negative results, and meanwhile *Streptococcus faecium 2* was isolated in a fibrous muscle near kidneys. All

other vaccinated individuals developed rabies virus neutralising antibody titres and survived the challenge. They were euthanased 40 to 60 days after challenge and FA tests applied on the brain tissues were negative. All unvaccinated controls succumbed with rabies clinical signs 16 to 22 days after challenge. FA tests applied on their brain tissues were positive.

All foxes vaccinated with a  $10^8$ ,  $10^{7.5}$  or a  $10^7$  PFU SAG2 solution survived a challenge, even up to 303 days after vaccination (for the  $10^{7.5}$  PFU group). The inclusion of the control group showed that the challenge model was effective to induce rabies in non-vaccinated animals. The overall immune response at day 29 in both animal groups of experiment 1 is considerably lower than the antibody titre of the foxes in the second trial at day 24 post vaccination.

### **3.4 2. Efficacy of the pharmaceutical form adapted for oral vaccination**

#### **3.5 2.1 Determination of the minimum effective titre of a single bait in foxes**

Three groups of 5 adults (13 to 14 months old), seronegative (for rabies antigen) red foxes, were offered in the evening a single fox bait containing approximately  $10^9$ ,  $10^8$  or a  $10^7$  CCID<sub>50</sub> of SAG2 respectively, while being deprived of food for one day. The purpose of the study was to determine the minimum effective titre of a single bait in foxes. This form of vaccine presentation and the route of administration generally require between 0.5 to 1.5 log more vaccine virus to be as efficacious than suspension. Thirteen foxes out of 15 consumed the bait within the hour following the presentation. Twelve out of 15 chewed directly into the sachet. Three foxes were given a new bait after which also these foxes perforated the sachets. However, only six sachets were perforated properly out of all punctured sachets (14). All animals seroconverted until day 30 in a dose correlating fashion (mean 5.1, 2.5 and 1.7 IU level in the  $10^9$ ,  $10^8$  and  $10^7$  CCID<sub>50</sub> dosage groups). The foxes of the group receiving the highest vaccine concentration and 4 of 5 individuals vaccinated with the two doses survived a lethal challenge with a vulpine street rabies virus isolated, carried out 30 days after vaccination. Two unvaccinated controls died 17 and 24 days, respectively after inoculation. Forty-eight days after the death of the last control, the survivors were sacrificed and their brains were harvested. The brain samples were free of rabies virus antigen. The expert concluded that the minimal effective titre was situated below any of the vaccine concentrations used and that it was not possible to identify the ED<sub>50</sub>.

The experiment indicates that even at the lowest concentration of  $10^7$  CCID<sub>50</sub>, a protection against vulpine rabies virus can be observed, however strongly depending on whether the fox punctured the sachet or not. It is clear that the uptake of the bait by the fox is a difficult to control variable that could interfere with efficacy, independently of the vaccine concentration contained in the bait. It is also difficult to generalise the results of the bait acceptance from the laboratory to field conditions since the animals were deprived of food for one day before having offered the bait.

#### **3.6 2.2 Efficacy of different pharmaceutical forms of the bait**

A study was provided in which two bait prototypes, characterised by a higher fusion point of their coating, were tested at the laboratory and in a small field trial and compared to a previously used bait. The tests were conducted within the framework of the development of the definitive formula of the bait. The 2 prototypes tested, differ from the previous bait produced by a higher melting point. Both contain EVA (Ethyl Vinyl Acetate) but one contains fish liquid and the other fish aroma. Since this trial was not performed with the definitive bait formulation, this study supports the efficacy of the product only partially.

In the laboratory trial, 18 foxes that had already been used for appetite tests with other vaccine baits were used. The foxes received alternatively a bait of prototype 1 or 2 until all baits were used (54 baits were distributed in total over three days). The baits were given together with the food and their food ration was not modified during the test. All baits were accepted by the foxes, but 6 vaccine sachets remained intact (= 11%). The foxes continued receiving their normal diet while offered the baits, however they were experienced in appetite tests with baits, so the results from this trial are difficult to translate to field conditions. The trial proves, however, that in general the bait is well accepted by the foxes.

In a small field study, from 238 baits distributed on marked places (flags) few baits, nor the capsule could be found in or near the bait site. For 33 sites the flags were removed and it was also considered that 10 to 15 % of the baits hidden under dry leaves or grass escaped the attention of the controlling person during the control. Most of the capsules found were punctured or empty (40/44) whatever the type of bait. Approximately 50 of each of the both prototypes were thrown onto a paved surface (airfield) from a helicopter in stationary flight at 130 meters altitude. The baits were completely thawed. Only 2 baits were split in half during the experiment, the others were slightly out of shape but without consequences for the bait and capsule. However, it is difficult to interpret the data from the small field study since it is not known how many baits were consumed by non-target species.

### **3.7 2.3 Acceptance of the baits**

Seven foxes, which had never received a bait before, age 1 to 2 years, were given baits in the afternoon with the food (3 baits/day). Their behaviour was recorded on video until the consumption of the bait (36 hours maximum). The cages were lightened with two 500 W projectors during the night. Four foxes out of seven consumed the baits during the first night. The mode of bait consumption is complicated. Behaviours were seen, suggesting hiding and storage behaviour, in nature still to be quantified, which could be of importance considering the conservation limit of the vaccine. The animals consumed the baits at different moments and often dissociating the different constituents. Under these conditions, the possibility of rejecting the capsules is higher. At the end of the experiment, 4 capsules out of 21 were found unperforated (3 baits distributed to one fox were left intact as one fox was excluded). The small number of animals and the great behaviour variations observed make any generalisation difficult. Since all results were obtained from captive animals, which are well fed and not used to food variations (chicken heads and apples), the interest for the baits has to be considered carefully.

### **3.8 2.4 Efficacy study**

A study, complying with the requirements of the European monograph (0746), was performed. Thirty-five foxes (15 foxes of age 6.4 to 7.5 months and 20 foxes between 1 and 4.5 years) were at random allocated to two groups. In the first group, 25 animals received previously thawed bait at the recommended titre of vaccine suspension included. The foxes did not receive food since the day before and were observed to keep track of bait consumption and capsule perforation. The 10 foxes in group II did not receive any treatment. At day 180 post vaccination, each animal received an intramuscular injection with challenge rabies strain. All animals were observed for 90 days following the challenge. Blood samples were taken for the detection of neutralising antibodies at regular interval from the day of vaccination until the end of the trial. The foxes having survived the challenge infection were euthanized. After death, the encephalon and submaxillary salivary glands were sampled and tested by direct immunofluorescence on smears and upon inoculation to N<sub>2a</sub> cells (neuroblastoma cell line). In group I, 3 foxes bit the capsule without chewing it, much of the vaccine therefore remained within the capsule, or was lost. Three other foxes left the capsule apparently intact. All the control foxes died of rabies during a period of 18 to 26 days following challenge. In group I, three foxes out of 25 died of rabies. Among the 19 foxes, which ate the bait and completely crushed the capsule, only one died of rabies after a period of 23 days. All 3 foxes that damaged the capsule of vaccine without completely chewing it up, survived the challenge infection. Among the three foxes, which left a capsule apparently intact, 2 died of rabies 18 and 19 days later. The foxes less than 1 year of age from group I did not appear more sensitive to the challenge than the older ones: only one of the 3 foxes having died of rabies was less than 1 year old. All control foxes remained free of anti-rabies antibodies until the day of the challenge. Ten days after the challenge, they displayed very low titres. In group I, all foxes that survived except one displayed seroconversion as of day 28 after having been presented a bait. The three foxes from group I that died due to rabies never displayed any seroconversion. There was no significant difference in antibody titres between the foxes, younger or older than one year old.

Three of 25 vaccinated foxes, instead of the only 2 foxes allowed by the European Monograph, developed signs of rabies and succumbed to the disease. The challenge virus dose was sufficient to kill all unvaccinated control animals, one more than requested by the European Monograph. Although this study did not comply to all Ph. Eur. requirements (severity of the challenge), it can be accepted, as the requirements were hardly conceivable due to the difficulties of provisioning and duration of acclimatisation period.

In conclusion, consumption of the bait induces sufficient high antibodies to protect foxes against a rabies challenge up to at least 180 days. A determining factor is whether the fox did or did not puncture the vaccine capsule.

### **3.9 Conclusions to Laboratory tests**

Overall, the protective effect of the SAG2 bait can be considered proven in laboratory conditions. However, several parameters, such as the bait acceptance, sachet perforation, bait competition with non-target animals are known to interfere with the efficacy of the vaccination procedure and were shown to be difficult to assess in laboratory conditions. It is therefore clear that the efficacy of this particular kind of vaccine can be best assessed with the use of epidemiological data from a field trial.

## **IV D Field trials**

### **3.10 1. Identification of species consuming the bait (photographic capture system) in France**

A photographic capture system was developed in order to identify the species consuming baits in the fields in France. The traps were combined in series, each series containing 2 baits, one from the Applicant and one control bait (chicken head). They were set up in the same habitat 10 to 20 meters apart and hidden from one another. The baits are put out on the first morning of day 1, picked up and exchanged on the morning of day 3 and 5 and collected on day 7. Thirty photographs were taken from 234 baits (15 containing the product and 15 control baits) during a vaccination campaign in autumn 1991. Thirty-two photographs were taken from 288 baits during another vaccination campaign in spring 1992. Most of the photographs were obtained with chicken heads (48 out of 62) and showed carnivores (wild cats, weasel), insectivores (hedgehog), domestic animals (cows, dog) and birds. Only 2 photographs (one vaccine bait, one control bait) showed a fox. So only in 1.6% of all photographs a fox figured on a photograph with a vaccine bait. Of all vaccine baits put out during the spring campaign, 13% were found melted after two days.

### **3.11 2. Comparison of 3 different oral rabies vaccines in France**

The efficacy of three different rabies vaccine baits was compared. The incidence of rabies was compared between areas in which exclusively a SAD-B 19 strain, a vaccinia recombinant vaccine (VRG) or the SAG1 baits were used. The cases of rabies were counted between 1989 and 1991, regardless of the species, for a period of 6 months (Spring or Autumn campaign). Rabies cases were also counted 12 months preceding the first campaign as indicated below:

Bait type	Surface (km <sup>2</sup> )	12-6 months before	During 6 months preceding vaccination	6 months after 1 <sup>st</sup> campaign	6 months after 2 <sup>nd</sup> campaign	6 months after 3 <sup>rd</sup> campaign	6 months after 4 <sup>th</sup> campaign
SAD-B 19 strain	8096	38	120	63	37	8	
	9140	113	117	56	26		
VRG	15090	295	384	26	2	0	0
SAG1	2039	32	5	18	26	13	
	4051	92	125	76	37	31	
	3802	64	126	72	15		

For all baits a reduction in the number of rabies cases was seen after the different treatment campaigns. It was shown that the treatment with Virbac baits was more efficient in the areas treated in the order Spring-Autumn-Spring than the Autumn-Spring-Autumn campaigns.

A difficulty in the evaluation of these figures is the difference between the departments with respect to the collection of rabid animals, which depends on the motivation of the public, veterinarians, as well as the workload and human resources of the departmental directions of veterinary services. Too many variables specific to each area are not controllable as the level of fox population, density, partitioning of these populations, stage and specific dynamics of the epidemic at the beginning of the vaccination. Considering that a rabies epidemic being the results of stochastic phenomena, even in the assumption of the absolute similarity of two close areas, the epidemic is likely to yield different results in the two areas.

However, it was concluded that the SAD-B 19 bait gave the least convincing results. The SAG1 bait was given the second place in efficacy, acknowledging the possible improvement to examine for a more protective excipient and a more heat-resistant coating. The VRG bait was considered the most efficient since it induced the reduction of the occurrence of rabies to approximately 99.5 % after the two campaigns and led the apparent extinction of rabies after three campaigns.

The tested bait in this report was the single mutation rabies vaccine strain SAG1. Therefore, this report provides no direct information of the efficacy of the SAG2 strain, subject of this application. However, it indicates that a vaccination with the single mutation rabies vaccine included in a bait, with a lower thermal and mechanical stability than the present application, decreased the incidence of rabies cases. The efficacy was lower than the reduction of rabies seen in the areas treated with the bait including the recombinant vaccinia vaccine (VRG).

### 3.12 3. Comparison efficacy of a recombinant rabies vaccinia (VRG) and SAG2

A report was presented comparing the efficacy of baits with the recombinant vaccinia anti-rabies vaccine (VRG) with baits containing SAG2, in vaccination campaigns from 1993 to 1995 in France and Switzerland. The following was investigated:

- The rate of bait consumption indicated by tetracycline marking (e.g. tetracycline deposit in the teeth of foxes shot in the vaccinated area);
- The efficacy of intake (antibody detection);
- The elimination of rabies in the area considered.

#### The rate of bait consumption

Foxes were examined after 4 vaccination campaigns conducted from Autumn 1993 to Spring 1995 in France. The number of tetracycline-marked and -unmarked foxes in areas vaccinated with VRG or SAG2 baits was determined and the serum investigated for anti-rabies antibodies (ELISA).

The total number of adult and young (less than 1 year) foxes marked with tetracycline in areas vaccinated with SAG2 was 124 and 16 (anti-rabies antibodies positive) and 46 and 52 (anti-rabies antibodies negative), respectively. This number in areas vaccinated with VRG was 162 and 50 (anti-rabies antibodies positive) and 228 and 117 (anti-rabies antibodies negative). In adult foxes with anti-rabies antibodies 25 unmarked-foxes were found in areas vaccinated with SAG2 versus 42 foxes in the area vaccinated with VRG.

#### Field efficacy study (France )

From spring 1994 until May 1999, the SAG rabies vaccine bait has been used in France for vaccinating foxes by oral route. The vaccinated areas ranged from 15642 to 32709 km<sup>2</sup>. The areas vaccinated have been changed for adapting the strategy as more areas appears freed from the disease, while other area remained under the threat of remnant rabies foci. Baits have been distributed by helicopter with a density ranging from 13 to 25 baits/km<sup>2</sup>. For taking into account the vaccinated areas changes, the rabies incidence was re-evaluated in each case during the 6-month period before the vaccination campaign and during the 6 month period that begun one month after the end of the same campaign.

Out of a total of 8 campaigns, no increase in rabies incidence when comparing results before and after vaccination. In 6 situations a decrease in rabies incidence was observed. In autumn 1996, one rabies case was observed before and after the campaign. No rabies case was observed before and after the campaign of autumn 1997.

Vaccination campaigns were performed using concomitantly 2 vaccines (VRG and SAG2), one in each subsections. Subsections of treated territory varied from year to year for each vaccine. However, vaccine efficacy was evaluated without consideration of environmental factors, geomorphology or history of pre-treatment with other vaccines. The lowest rabies incidences (0.0) after a vaccination campaign were recorded in spring 1997 and autumn 1997. The number of baits used were 24.8 and 18.8 baits/km<sup>2</sup> respectively. This observation could suggest the use of a higher number of baits (25/km<sup>2</sup>) than recommended (13 to 20 baits/km<sup>2</sup>). This opinion on the density of baits to be used is reinforced by the fact that tetracycline results in foxes younger or aged one year were the highest for the 2 last vaccination campaigns. This is in accordance with the observation, that the increase of the overall bait density and consequently abundance in the field increases the chance of young and/or previously disadvantaged foxes to consume at least one bait. The recommended number of baits to be distributed depends on the foxes density.

In conclusion, it is obvious that rabies incidence clearly decreased in France from spring 1994 until autumn 1997 under vaccination campaigns performed with SAG2 Rabigen vaccine.

#### **(a) Field efficacy study in Switzerland (1995 and 1996)**

In autumn 1994, the vaccine strain SAG1 was replaced by SAG2. Use of SAG2 coincided with the implication of the new rabies control program in which immunisation programs were carried out in

critical areas in early summer to immunise a higher proportion of young foxes. In 1995 and 1996, this method covered an area of 2000 km<sup>2</sup> in the Western Jura. In the regular vaccination zones, the bait density was increased from 15 to 20-25 baits/km<sup>2</sup>. In this report, only data from 1995 and 1996 are discussed because in 1994 results of SAG1 and SAG2 cannot be distinguished.

The adaptation of the new vaccination strategy to the changed fox population parameters had an immediate impact on the rabies incidence. The number of total rabies cases dropped from 225 in 1994 to 23 in 1995 and 6 in 1996 corresponding to 167, 11 and 1 cases in foxes respectively. In 1997 only one case was diagnosed in a dog imported from Morocco. Therefore, no case related to the vulpine epizootic has been diagnosed in 1997, 1998 and 1999 so far, although the number of baits used and the vaccination area covered were reduced significantly. In 1999, Switzerland was considered free of terrestrial vulpine rabies. Overall about 70% of foxes were marked with tetracyclines and approximately 50% exhibited a detectable antibody response.

In combination with a vaccination strategy well adapted to the prevailing population densities and vector biology in Switzerland, Rabigen SAG2 has therefore demonstrated its efficacy in the field by achieving rabies elimination within 3 years of its application.

Summaries of parameters such as rabies incidence, surface treated, baits density, tetracycline detection, rabies antibodies detection are provided. The decrease in rabies is evident and can mainly be ascribed to the vaccination strategy.

### **3.13 Conclusions on efficacy**

The immunogenicity of SAG2 suspension is proven in the fox up to the lowest dosage used of 10<sup>7</sup> PFU. When incorporated into the bait, even at the lowest concentration of 10<sup>7</sup> CCID<sub>50</sub>, a protection against vulpine virus strain can be observed depending on whether the foxes break and open the sachet. In one laboratory study 11 % of the sachets remained intact. The protective effect of the SAG2 bait is proven under laboratory conditions. However, several parameters such as bait acceptance, sachet perforation, bait competition with non-target animals interfere with the efficacy of the vaccination procedure and were difficult to assess under laboratory conditions.

The Committee considered that the efficacy of Rabigen SAG2 can be best assessed with epidemiological data from field trials. Only one report was provided including efficacy data regarding the bait containing the actual SAG2 strain. A great number of foxes carrying anti-rabies neutralising antibodies were observed in the areas in which foxes were treated with the Rabigen SAG2 bait. The incidence of rabies in areas, where foxes were treated, gave no conclusive answer on the efficacy of Rabigen SAG2 under field conditions. The number of rabies cases were low in those areas, however, it can be concluded that the use of Rabigen SAG2 baits under field conditions enables maintenance of the rabies incidence in foxes at low levels.

Rabigen SAG2 induces adequate anti-rabies antibodies and protection against rabies virus in challenge studies, but further information to prove the ability of Rabigen SAG2 to reduce the incidence of rabies in field conditions was considered necessary. Consequently, more results from field trials were presented at the oral explanation. It was shown that when distributed in the wild, the SAG2 rabies vaccine bait was readily taken by free ranging foxes: according to campaigns (Spring or Autumn) and years, 69 to 92 % of adult foxes and 37 to 58 % of fox cubs had consumed baits after campaigns. The mean number of bait meals was 2.6 to 2.1 in adult and fox cubs. Following bait uptake, adult and fox cubs showed significant antibody response. As a result, rabies decreased significantly in the vaccinated areas until its complete elimination. Moreover for several years until now, the use of this vaccine has been efficiently preventing the vaccinated area from re-infection from still contaminated neighbouring areas.



## 5. Risk-Benefit Assessment and conclusion

Rabigen SAG2 is a single-strain, live modified rabies virus vaccine indicated for the oral vaccination of free-ranging red foxes (*Vulpes vulpes*) against rabies. The liquid virus suspension is presented within a PVC/aluminium sachet coated with a bait matrix containing biological and artificial attractants, biological and chemical binding material and tetracycline as biomarker.

The product is intended for use by authorised personnel only within the framework of national rabies eradication programs. Rabigen SAG2 vaccine has been used so far on an experimental basis in the field, in France since 1993 and in Switzerland since 1994.

The quality part of the dossier complies with EU directives and guidelines, with Ph. Eur. monographs and with the scientific advice provided by the CVMP. The genetic stability of SAG2 was sufficiently demonstrated. Data showed no significant titre loss before and after coating the vaccine sachet into the bait.

The performed safety studies with regard to administration of a single, an overdose and a repeated administration of one dose comply with the requests given in scientific advice offered by the CVMP. All foxes remained healthy over the period of observation and furthermore sero-conversion was observed from 30 days after vaccination. Sufficient proof was provided of a very small risk of dissemination of the vaccine virus to the brain or excretory organs and that reversion to virulence of the attenuated vaccine is very unlikely in field conditions so that the chances of the virus establishing itself in nature is very unlikely. When administered orally, it does not disseminate in the vaccinated animal and can generally not be recovered from the oral cavity within hours of vaccination, thus limiting the chances for shedding and spreading. Convincing evidence has been accumulated, suggesting an infinitely-small risk of spreading of the vaccine virus from animal to animal. The safety of SAG2 was demonstrated in almost 30 non-target species belonging to the most relevant potential bait consumers, including racoon dogs and cattle. Contact with the vaccine has never led to clinical signs and was shown to induce immunity, which in the case of dogs was sufficient to confer protection against a virulent rabies virus challenge. Human contact with baits and vaccine cannot totally be avoided, but it remained without clinical consequences during the 4 years of approved field trials. In case of an accidental contamination of a person with the bait carrying the SAG2 strain, a classical anti-rabies treatment remains necessary since the risk of rabies cannot be completely eliminated although it is deemed low for humans. With regard to safety to the public, the Committee considered it necessary to put a warning (“RABIES VACCINE DO NOT TOUCH”) and an informative telephone number on both, the vaccine capsule and the bait.

Rabigen SAG2 is shown to induce an adequate level of anti-rabies antibodies and protection against rabies virus in challenge studies in foxes. Consumption of the bait protects foxes against a rabies challenge up to at least 180 days. A determining factor is whether the fox having eaten the bait did or did not puncture the capsule that contains the vaccine. Different epidemiological studies show that following vaccination campaigns from 1993 to 1995 in France and Switzerland the use of the SAG2 vaccine efficiently has prevented re-infection of foxes in vaccinated areas from neighbouring still contaminated areas.

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC and supported the claim ‘For the active immunisation of red foxes to prevent infection by rabies virus.’

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- 8. Part III: Toxicopharmacological aspects**
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- 10. Overall conclusions and benefit/risk assessment**
  - Benefit/risk assessment**

Based on the CVMP review of data on quality, safety and efficacy, the CVMP considered <by consensus/by majority decision> that the benefit/risk profile of <insert product name> was favourable in the treatment of <insert approved indication> in <insert target species>.