PRODUCT PROFILE

Name of the veterinary medicinal product:	Nobilis OR inac
Marketing Authorisation Holder::	Intervet International B.V Wim de Körverstraat 35 5831 AN, Boxmeer The Netherlands
Active substance:	*Ornithobacterium rhinotracheale* inactivated whole cells, serotype A strain B3263/91
Pharmaco-therapeutic group (ATCvet Code):	Inactivated bacterial vaccine (QI 01AB07)
Therapeutic indication:	Passive immunisation of broilers induced by active immunisation of female broiler breeders to reduce infection with *Ornithobacterium rhinotracheale* serotype A when this agent is involved. Under field conditions passive immunity is transferred during lay for 43 weeks after the last vaccination of broiler breeders, resulting in a duration of passive immunity in broilers of at least 14 days after hatching.
Target species:	Chickens
Withdrawal period:	Zero days

INTRODUCTION

The company Intervet International BV submitted an application to the EMEA on 4 January 2000 for the granting of a Community marketing authorisation for Nobilis OR inac in accordance with Council Regulation (EEC) No 2309/93.

The product qualifies for Part B status of the Annex to Council Regulation (EEC) 2309/93 and was therefore considered eligible for the granting of a Community marketing authorisation via the centralised procedure.

Nobilis OR inac is an inactivated bacterial vaccine, containing *Ornithobacterium rhinotracheale* serotype A cells and a mineral oil adjuvant. The product is presented as a water-in-oil emulsion for subcutaneous or intramuscular injection.

Nobilis OR inac is indicated for the passive immunisation of broilers induced by active immunisation of female broiler breeders to reduce infection with *Ornithobacterium rhinotracheale* when this agent is involved. A withdrawal period of 0 days was supported.

II. QUALITATIVE AND QUANTITATIVE PARTICULARS

II.A QUALITATIVE AND QUANTITATIVE PARTICULARS

Each bottle of the product contains 250 or 500 ml of a water-in-oil emulsion, corresponding with 1000 or 2000 doses of 0.25 ml.

II.A.1 Composition per dose

Name of ingredient	Quantity	Function	Reference
Antigen concentrate (containing 1 x 10^7 cells of *Ornithobacterium rhinotracheale* strain B3263/91 serotype A)	25.00 mg	Induction of immunity	Monograph II.C.2.1.
Adjuvant: Light liquid paraffin Polysorbate 80 Sorbitan oleate	107.21 mg	 Adjuvant Emulsifier Emulsifier	 Ph.Eur. Ph.Eur. Ph.Eur.
Excipients: KH_2PO_4 Na_2HPO_4 KCl NaCl		 Buffer Buffer Isotonicity Isotonicity	 Ph.Eur. Ph.Eur. Ph.Eur. Ph.Eur.
Formaldehyde		Remnant of production	Ph.Eur.
Diluent: Water for injections		 Solvent for water soluble excipients	 Ph.Eur.

The microscopical count of the antigen concentrate was validated.

II.A.2 Containers

Details of the containers and closures have been provided for 250 ml and 500 ml glass and polyethylene terephthalate (PET) containers.

The containers are polyethylene terephthalate (PET). There is no specific monograph on PET containers in the Ph.Eur.. The PET containers are manufactured from a thermoplastic polymer granulate which is composed of the monomers, terephthalic acid, ethylene glycol and 1,4 cyclohexane dimethanol. The Applicant states that the containers meet the requirements for food contact applications and the requirements of the United States Pharmacopoeia (USP) specific monograph and that regular tests based on the requirements for plastic containers for injectables and liquid oral dosage forms (USP) are performed.

The PET containers are sterilised by ionising radiation. A dose of 25 kGy is applied to the containers. This dose is controlled by dosimeters. 25 kGy is accepted to give an SAL 10 - 6 which is accepted by the Ph.Eur. to be adequate for terminal sterilisation

Although there is no specific monograph on PET containers in the Ph.Eur., the Applicant has demonstrated that the PET bottles comply with the Ph.Eur. 3.2.2..

The vials are closed with a halogenobutyl rubber stopper and sealed with a coded aluminium cap. The stopper complies with Ph.Eur. requirements (3rd edition 3.2.9) for a type I closure. The stoppers are sterilised by autoclaving at 121ºC for at least 15 minutes. The aluminium closures are sterilised by gamma irradiation dose of at least 25 kGy, which is a generally accepted method (Ph.Eur. 5.1.1.).

II.A.3 Development Pharmaceutics

The active ingredient is inactivated cells of *Ornithobacterium rhinotracheale* strain B 3263/91, serotype A. The vaccine strain was isolated from a diseased chicken in South Africa. References are provided to support the statement that serotype A is the most prevalent serotype. After harvesting of cells, inactivation is achieved by the addition of formaldehyde. The inactivated culture is concentrated by filtration, diluted to the desired concentration and mixed with mineral oil adjuvant. The mineral oil adjuvant solution consists of light paraffin oil, polysorbate 80 and sorbitan oleate. The latter two components are included as emulsifiers. Buffering is achieved by the addition of sodium and potassium hydrogen phosphate. The vaccine is prepared as a water–in-oil emulsion. A constant amount of antigen of 10^7 cells/dose is present in each batch of vaccine.

II.B METHOD OF PREPARATION

II.B.1 Flow Charts

Flow charts of the preparation of the antigen and final product have been provided.

II.B.2 Manufacturing process and in process controls

Working seed is rehydrated in physiological salt solution, inoculated onto two blood agar base plates, incubated and several times subcultivated. Once the stationary phase of the culture has been attained, the bacterial suspension is inactivated by means of formalin. Cells are washed and concentrated and a cell count (IPC test) is performed after concentration and washing of the cells assuring that the final vaccine will always contain 1 x 10^7 cells per dose of washed cells of *Ornithobacterium rhinotracheale* strain B3263/91.

The final product is prepared by mixing the water phase (diluted antigen) to the oily part and emulsifying. Both, oil phase and the buffering solution are sterilised by filtration. The antigen is added to this solution. After emulsification of the final product, the product is stored under stirring in a bulk tank until filling and kept at 2 - 8ºC. The bottles are filled aseptically and the pumps and tubing are autoclaved before use.

II.B.3 Validation of the manufacturing process

a) Validation of the production method

The Applicant has presented acceptable results of three consecutive batches falling within the set specifications.

b) Validation of the inactivation method

Two validation studies have been presented demonstrating that the organism was inactivated within the requirements of the Directive. The bacterial suspension is inactivated by means of formalin. Validation of the inactivation method and the limit of detection of the test for residual infectivity were provided. Control tests for inactivation are performed on each batch of antigen and on each batch just before preparation of the final product.

II.C PRODUCTION AND CONTROL OF STARTING MATERIALS

II.C.1 Starting materials listed in a pharmacopoeia

A list of starting materials complying with a pharmacopoeia have been provided for:
Light liquid paraffin (Ph.Eur. 0240), potassium dihydrogen phosphate (Ph.Eur. 0920), Disodium phosphate dihydrate (Ph.Eur. 0602), Potassium chloride (Ph.Eur. 0185), Sodium chloride (Ph.Eur. 0193), Formaldehyde solution, 35% (Ph.Eur. 0826), Water for injections (Ph.Eur. 0169), Sodium hydroxide (Ph.Eur. 0677), Hydrochloric acid (Ph.Eur. 002), Polysorbate 80 (Ph.Eur. 0428) and Sorbitan oleate (Ph.Eur. 1041).

Furthermore, the rubber closures have been shown to comply with monographs of the Ph.Eur..

Compliance was demonstrated by internal specifications. Polysorbate 80 and sorbitan oleate are of vegetable origin.

II.C.2 Starting materials not listed in a pharmacopoeia

II.C.2.1 Starting materials of biological origin

Ornithobacterium rhinotracheale - production strain
Strain B-3263/91 serotype A is used. This was obtained from South Africa after isolation from a diseased hen. The strain was passaged and sent to Intervet, USA. The master seed was prepared at Intervet, Millsboro, USA. There the seed was passaged and lyophilised. The working seed was prepared from this master seed at Intervet, the Netherlands. Details of the preparation, and testing of the master seed and the working seed were provided and considered compliant. Procedures for the preparation and quality control and the batch protocols have been provided.

Todd Hewitt Broth (THB)
THB is used as a medium for culturing the production strain. It contains three components of biological origin. A certificate of suitability for Todd Hewitt Broth has been provided according to the provisions of Directive 2001/82/EC confirming compliance with the current TSE requirements.

It is stated that the manufacturer of the broth treats these materials as follows: All materials of animal origin are either heated at 100°C for at least 15 seconds, or sterilised at 134°C for at least 15 seconds. However, it should also be noted that it is stated on the certificate of animal origin that the manufacturer does not perform procedures validated to remove or inactivate infectious agents during the manufacturing process. In order to ensure inactivation of any possible agents, the Todd Hewitt broth is 25 kGy gamma irradiated. After preparation of the liquid Todd Hewitt broth, the culture medium is sterilised by filtration.

Blood Agar Base no. 2
Blood Agar Base no. 2 is used as a medium for subculturing the *Ornithobacterium rhinotracheale* production strain from the working seed. Quality Assurance certificates and a certificate of biological origin have been provided. Details about the components of biological origin have been provided confirming compliance with the current TSE requirements according to the provisions of Directive 2001/82/EC.

II.C.2.2 Starting materials of non-biological origin

Not applicable.

II.C.2.3 In-house preparation of media

Todd Hewitt Broth (THB)
THB is used for growing the strain in the pre-culture and main culture. All components are dissolved in water. The constituents of the culture media are not of Ph.Eur. quality, which is also not required.

Todd Hewitt Broth, which is used for the preparation of the inoculum and for the production culture, is sterilised and is checked for sterility and visually inspected for contamination before use.

Blood Agar Base no. 2
Blood Agar Base no. 2 is used for sub-culturing the seed material. The powder is dissolved in 1 litre of distilled water and brought to boil. The constituents of the culture media are not of Ph.Eur. quality, which is also not required. It is sterilised by autoclaving. Blood Agar Base no. 2 plates are checked for sterility immediately after production. A number of plates of each batch are incubated and thereafter checked for contamination. Before use, the medium is visually inspected for contamination.

Phosphate buffered saline (PBS)
Phosphate buffered saline is used to wash the antigen concentrate and produced by dissolving all the components in water. PBS is sterilised by filtering through a 0.22 µm filter. The constituents of the PBS (50 mM) are of Ph.Eur. quality. According to Ph.Eur. 5.1.1 steam sterilisation is the method of choice. Filter sterilisation should only be used where it is not possible to steam sterilise preparations such as solutions containing proteins. The Applicant provided a sufficient justification of the filtration method. This included a rationale that the method would provide adequate sterility.

Lyophilisation medium
Lyophilisation medium is used for freeze drying the master seed and working seed. Details of quantitative composition have been provided. All components are dissolved in water at 20 - 80ºC. The solution is sterilised by autoclaving at 121ºC for 20 minutes. The constituents of the Lyophilization Medium are of Ph.Eur. quality or of USP quality (Monosodium Glutamate). Certificates of analysis have been provided.

Conformity with Commission Directive 1999/104/EC
The Applicant has provided a risk assessment regarding the starting materials of animal origin in Nobilis OR inac. This includes a form summarising the materials and their origin. Certificates of Suitability for four suppliers of gelatin have been provided.

II.D IN-PROCESS CONTROL TESTS

D.1 Flow charts

The flow charts presented by the Applicant for Part II.B.1. of the dossier are applicable to this part too.

D.2 Tests performed

Sterility of the medium
A sterility test is conducted on each batch of medium to check for contamination. The sterility is evaluated by incubation. No growth should be observed.

Purity of the inoculum
A purity test is conducted on every inoculum to check for bacterial contamination. Two tests are conducted: gram stain and agar plate incubation. There should be no non-specific colonies on the agar plates or presenting in the gram stain.

Purity of culture
A purity test is conducted on each culture just before inactivation to check for bacteriological contamination of the culture. Two tests are conducted identical to those conducted for testing the purity of the inoculum.

Control of inactivation
A control test of inactivation is conducted on each batch immediately after finalising the inactivation process to check that all the bacteria have been killed by inactivation. Two blood agar plates are

seeded with product. Agar plates are used as a positive and negative control. All plates are incubated at 37ºC and 5 - 10% CO_2 for at least 72 hours. The plates are observed daily. No growth may occur.

Sterility of antigen concentrate
A sterility test of antigen concentrate is conducted on each batch of antigen to check the sterility.

Determination of antigen content
A test for determination of antigen content is conducted on each batch of antigen after concentration in order to bring the antigen to the desired concentration in the final product.

Second control of inactivation
A second control of inactivation is performed as soon as possible after the sample is taken on each batch, just before preparation of the final product, to check that all bacteria are killed by the inactivation method.

Test on filling volume
This is conducted at regular intervals during filling. Pre-weighed vials are introduced at random in the filling line and weighed after filling. The volume of vaccine is calculated.

Batch results
Results of in process control tests on 3 consecutive batches have been provided. The cell count per ml of all batches is above the minimum stated.

II.E Control tests on the finished product

Sterility test
Sterility is tested before the release of every product according to Ph.Eur. 2.6.1. An SOP and the validation of the test have been provided. Validation has been conducted in the presence of the product and shows that the product does not have any effect on the test.

Physico chemical tests
Physico chemical tests are conducted before the release of each batch to check the quality of the emulsion and adjuvant.

Free formaldehyde test
A test for free formaldehyde according to the requirement in Ph.Eur. monograph 0062 and Ph.Eur. 2.4.18 will be performed on every released batch.

Safety test
A detailed SOP has been provided. The test is performed before the release of each batch. Ten, 2 to 4 week old SPF birds are vaccinated subcutaneously in the neck with a double dose of the vaccine (0.5 ml). The animals are observed for abnormal reactions for a period of 21 days. Dissection is performed on dead birds.

Potency test
A potency test is performed before the release of each batch as described in detail in the SOP.

Validation of Potency test
Validation of the potency test has been performed and considered adequate.

Final Inspection
Each vial is checked before shipment by visual inspection. Only correctly coded vials are released.

Results of 3 consecutive batches
The results of control tests on the finished product for 4 batches of vaccine are provided. The batch quality protocols are presented.

II.F STABILITY

II.F.1 Stability of the Finished Product

Stability data for three batches filled in PET has been presented. The Applicant proposed a shelf life of 15 months for PET vials. The parameters studied for demonstration of stability are potency and physico-chemical tests. The results provided by the Applicant support the shelf life of 15 months for product stored in PET vials.

II.F.2 Stability of the antigen

Three final batches prepared from three 15-month-old antigen batches were tested in the potency test after production and found to meet the release requirement. To justify a 6-month shelf-life of antigen a batch has been prepared using 9 month old antigen for blending. Only the time 0 results were available. The Committee decided that until further data can be presented the antigens should not be stored prior to blending. However, the Applicant confirmed that further stability data on stored antigens would be provided allowing an antigen shelf-life of at least 6 months.

II.F.3 Stability of the Reconstituted Product

The vaccine is presented as a multi-dose product without a preservative. No data to support the stability of the product over this period of time once the seal has been broached has been provided. Therefore, it is advised to use the whole content of a vial immediately and entirely after it has been opened.

III. SAFETY

III.A INTRODUCTION

The vaccine is indicated for use in broiler breeders to provide passive immunity of progeny through transmission in the egg. The vaccination schedule stated in the SPC is for primary vaccination at 6 - 12 weeks of age followed by a second injection, after an interval of at least 6 weeks, at 14 - 18 weeks of age. The vaccine can be administered subcutaneously in the neck or intramuscularly in the breast using a volume of 0.25 ml. All safety studies were conducted in the relevant category of the target species, broiler breeders. Laboratory studies and field trials were conducted. The Applicant has clarified that the safety laboratory trials were conducted to GLP. The field trial was conducted in accordance with GCP.

Adequate laboratory safety studies of the safety of a single, double and repeat dose using a batch of standard antigen content and above minimum potency have been conducted. Amendments have been made to the SPC to describe the local reactions seen, particularly after repeat vaccination. Reactions observed after repeat dose vaccination were not followed until they had resolved, therefore, there is no information on the time period it takes these reactions to resolve. Noticeable reactions were observed during the field study conducted in South Africa after the schedule had been administered intramuscularly. No such reactions were observed in the later field trial conducted in Belgium. The SPC reflects the sporadic nature of these observations.

The effect of vaccination by the subcutaneous route on egg production, hatchability and the survivability of progeny over the first week of life has been investigated using a batch of standard antigen content but greater than minimum potency. Use of the vaccine during lay is contra-indicated.

The interaction of Nobilis OR inac with Nobilis *E. coli* vaccine was investigated. The Applicant has not provided enough information to demonstrate that both vaccines will be protective if administered

simultaneously and therefore simultaneous use has not been recommended. The standard CVMP statement regarding interactions is recommended with the appropriate time period proposed as 14 days during which vaccination with any other vaccine is contra-indicated prior to and after vaccination with Nobilis OR inac.

The overall risk to the environment is assessed as minimal.

Due to local reactions well known for mineral oil adjuvanted vaccines, the Committee agreed to modify section 5.11 of the SPC (Special precautions to be taken by the person administering the veterinary medicinal product to animals) as follows:

> To the user:
> This product contains mineral oil. Accidental injection/self injection may result in severe pain and swelling, particularly if injected into a joint or finger, and in rare cases could result in the loss of the affected finger if prompt medical attention is not given.
> If you are accidentally injected with this product, seek prompt medical advice even if only a very small amount is injected and take the package insert with you.
> If pain persists for more than 12 hours after medical examination, seek medical advice again.
> To the physician:
> This product contains mineral oil. Even if small amounts have been injected, accidental injection with this product can cause intense swelling, which may, for example, result in ischaemic necrosis and even the loss of a digit. Expert, PROMPT, surgical attention is required and may necessitate early incision and irrigation of the injected area, especially where there is involvement of finger pulp or tendon.

III.B GENERAL REQUIREMENTS

Not Applicable.

III.C LABORATORY TESTS

The Applicant has presented one overall study to address the safety of the administration of one dose, overdose and a repeated dose of *Ornithobacterium rhinotracheale* vaccine after subcutaneous or intramuscular vaccination of broiler breeders at an age of 6 and 8 weeks. The vaccine batch contained 1 x 10^7 cells and was presented in 250 ml PET bottles. Commercial broiler breeder hens, 1 or 2 day old, were obtained and placed in one unit. Before the first treatment the birds were individually marked and placed in 12 subgroups. At the day of vaccination the subgroups were assigned at random to 1 of 7 treatments: single dose (subcutaneously or intramuscularly), repeated dose (subcutaneously or intramuscularly), overdose (subcutaneously or intramuscularly) and an untreated control.

The animals were controlled for local and systemic reactions and any other abnormalities daily during the 2 weeks after each vaccination. One, 2 and 3 weeks after subcutaneous or intramuscular administration of the single dose, chickens of these groups and of the control groups were killed. The injection sites were examined and inspected for any local tissue reactions or vaccine remnants. In the case of any abnormality, samples were taken for histological examination.

III.C.1 Safety of the Administration of a single Dose (1 x 10^7 cells)

Subcutaneous use:

No clinical abnormalities were observed after vaccination. 10% of the birds showed local reactions one day after vaccination which were hard or hard/diffuse of 5 mm cross sectional area. Most reactions were still present on the third day post vaccination. All reactions had resolved by the 6th day post vaccination.

Post-mortem examination one, two and three weeks after vaccination showed that the size of reactions varied from 0.15 up to 0.75 cm^3 (one week), 1.8 cm^3 (two weeks) and 2.1 cm^3 (three weeks). The

reactions were of white colour and contained remnants of the vaccine. Hard abscesses were found around two injection sites. This may suggest poor vaccination technique resulting in the introduction of contaminants to the injection site. The "myxoid" layer noted was considered to be a typical reaction seen in chickens vaccinated with oil adjuvanted vaccines. Dispersed remnants of the vaccine were also found in the thymus of one bird. This was examined microscopically and shown to consist of multiple large granulomas, a "myxoid" layer and a fibrous capsule. The examiner's conclusion was that these reactions were low in severity and comparable with reactions seen with other oil adjuvanted bacterial vaccines.

Intramuscular use:

No clinical abnormalities or local reactions were observed.

Post mortem examination one, two and three weeks after vaccination of the injection site showed that reactions occurred in all animals, varying in size from 0.075 up to 0.2 cm^3 (one week), 2.4 cm^3 (two weeks) and 1 cm^3 (three weeks). In up to 90% of the animals, these reactions contained more than 10% of the volume of vaccine as remnants. Other reactions were greenish tissue of 1 cm^3 in size at the injection site, minor haemorrhages and a hard abscess was seen (one bird each).

It was concluded that the local reactions were of low severity and similar to those seen with other bacterial adjuvanted vaccines.

III.C.2 Safety of One Administration of an Overdose

Double dose of 0.5 ml (1 x 10^7 cells), subcutaneous use:
No clinical abnormalities were noted, but 9 days after vaccination local reactions were recorded in 20% of the birds. The reactions were 0.5 and 1 cm cross sectional size respectively and were diffuse and hard/diffuse, respectively. Both reactions had resolved by 13 days post vaccination.

Double dose of 0.5 ml (1 x 10^7 cells), intramuscular use
No clinical abnormalities or local reactions were noted.

Ten times overdose (1 x 10^8 cells), subcutaneous use:
In another study, the Applicant presented data to address the local reactions after subcutaneous vaccination and to investigate the reproduction performance (see III.C.4). Local reactions were scored by palpation. The size and the type of reaction (hard, diffuse, warm and /or painful) were estimated and recorded.

Local reactions were found in 70% of the birds. The reactions had diameters of 1 - 5 cm and were hard, sometimes painful and peaked at 2 weeks post vaccination.

Conclusion:
Since the subcutaneous administration of an overdose caused injection site reactions in the majority of birds, the following sentence was added to the SPC at section 5.9 "Overdose":
"No other undesirable effects have been observed after administration of a double dose when compared with a single dose of vaccine. Occasionally hardened minor local swellings (0.5 – 2.0 cm) were observed which disappeared within 21 days after vaccination."

III.C.3 Safety of the Repeated Administration of a single Dose (2 x 10^7 cells, at 2 weeks interval)

Subcutaneous use
No local reactions were observed after the initial vaccination, however reactions were observed in 60% of the birds after the second vaccination. The number of reactions peaked on day 7 post second vaccination with 50% of birds showing reactions. . However, some birds still had reactions on day 27, 13 days post second vaccination. The size of reactions varied from 5 to 30 mm cross sectional area with hard and diffuse types recorded. The reactions on day 13 post second vaccination were still quite

sizeable, ranging from 5 to 30 mm, with 3 being greater than 10 mm. These reactions did not appear to be resolving on day 27, however, observation was not continued beyond day 27.

Intramuscular use
No local reactions were observed after the initial vaccination, however, one local reaction (hard, 10 mm size) was seen 13 days after the second vaccination in the left breast in 1 animal.

Conclusion
After single and double dose subcutaneous vaccinations, local reactions resolved within 14 days, however, after the second vaccination in a repeat dose schedule a number of birds still had sizeable reactions 13 days post vaccination.

The Committee, therefore, considered that under "Undesirable effects" of the SPC the following wording should be added: "In laboratory studies, a local transient swelling was found at post mortem examination in up to 40% of the birds for at least 14 days after subcutaneous vaccination. Under field conditions, sporadic local and systemic clinical reactions have been reported."

III.C.4 Examination of Reproductive Performance

The Applicant has presented one overall study to address the local reactions after subcutaneous vaccination and to investigate if vaccination with Nobilis OR inac has any effect on the reproduction of broiler breeders, the hatchability of the eggs or the performance of the progeny.

Six groups of broiler breeders were vaccinated subcutaneously at 6 and 18 weeks of age with 0.25 ml vaccine containing different amounts of cells/ml of *Ornithobacterium rhinotracheale* strain B3263/91. One group of the broiler breeders was kept as unvaccinated controls. All broiler breeders received several standard vaccinations.

Blood samples and egg yolk samples were taken at 6 weeks intervals and the egg yolks were tested for antibodies against *Ornithobacterium rhinotracheale* serotype A. Eggs were collected from all the groups and the number of eggs per group were counted, weighed and 150 eggs per group were used for incubation. The number of fertile eggs was counted and the ratio of fertile/non fertile eggs was calculated. The hatched broilers were counted, observed during 7 days and weighed at 1 and 7 days of age. The reproduction was scored by calculating the fertility and hatchability rates of the eggs and by weighing the eggs, one day old broilers and 7 day old broilers.

Results:
A comparison of the total number of eggs per group was not possible because eggs were picked and eaten by birds before they could be sampled. The remaining eggs were sampled from the groups, which received a dose of 1 x 10^7 cells and the control group, at 28 - 29 and 46 - 47 weeks of age.

(a) Eggs sampled at 28 - 29 weeks of age of the breeders:

The average weight of the eggs of the vaccinated group was significantly higher than the average weight of the eggs of the control group. From both groups the 150 most freshly laid eggs were hatched. 7% of the eggs of the vaccinated group and 8% of the eggs from the control group were found to be infertile or to have died off. The hatchability of the remaining eggs was 93.5% in the vaccinated group and 90.6% in the control group.

The average weight of the 130 day old broilers in the vaccinated group was significantly higher than the average weight of the broilers in the control group. At 7 days of age there was still a difference between the average weight of the broilers in the vaccinated group and the birds in the control group, however, it was not significant.

(b) Eggs sampled at 46 - 47 weeks of age of the breeders:

The average weight of the eggs of the vaccinated group was significantly higher than the average weight of the eggs of the control group. 23% of the eggs of the vaccinated group and 63% of the eggs of the control group were found to be infertile or to have died off. The hatchability of the remaining eggs was 61.9% in the vaccinated group and 73.3% in the control group. No reason could be found for these low fertility and hatchability rates. The Applicant suggests that perhaps a field infection occurred because the antibodies increased significantly at this time point. The average weight of the one day old broilers in the vaccinated group was higher than the average weight of the broilers in the control groups.

The summary data supports the claim that the vaccine does not affect the reproductive capacity of broiler breeders vaccinated with a batch of vaccine of standard antigen input (1 x 10^7 cells) but higher potency. Production of eggs either at the beginning, or towards the end of the egg laying cycle seems to be unaffected by vaccination. The progeny of vaccinated hens seem to be unaffected during the first week of life.

The vaccine is contra-indicated for use in laying birds.

III.C.5 Examination of Immunological Functions

Due to the composition of the vaccine, no further effects on the immunological functions are expected and no further studies were performed.

III.C.6 Special Requirements for Live Vaccines

Not applicable.

III.C.7 Study of Residues

For the active principle of biological origin, Council Regulation (EEC) No 2377/90 does not apply. The adjuvants and excipients are included in Annex II of Council Regulation (EEC) No 2377/90.

III.C.8 Interactions

During safety and efficacy trials broiler breeders were vaccinated with a series of vaccines which cover the normal vaccinations received by chickens. No direct data supporting safety or efficacy have been presented.

Although a study submitted by the Applicant in the original dossier indicated that simultaneous vaccination with Nobilis *E. coli* did not show a negative effect on the safety and efficacy, no further results of the interactive effect of Nobilis OR inac with any other vaccine are available. Therefore, the following statement will be added to the SPC at section 5.7:
"No information is available on the safety and efficacy from the concurrent use of this vaccine with any other. It is therefore recommended that no other vaccines should be administered within 14 days before or after vaccination with this product."

III.D FIELD STUDIES

The Applicant provided results of 2 field trials in broiler breeders. One trial was performed in South Africa and the second one, which was submitted later, in Belgium.

III.D.1 South African Field Trial

Healthy broiler breeders were placed at monthly intervals in adjacent farms of the same design. Each breeder flock raised on a single site in climate controlled housing. At 19 weeks of age the flocks were transferred to open side facilities. The birds were vaccinated at 9 and 18 weeks of age intramuscularly with 0.25 ml vaccine containing 1 x 10^7 inactivated cells/dose. The vaccinated flocks were placed on one and unvaccinated on the other farms. A number of other standard vaccinations were given.

Birds were monitored daily after vaccination. From each vaccinated flock, at least 15 birds were palpated at 7 and 14 days post vaccination for the assessment of the reaction site of injection. Mortality was recorded during early production. Production parameters for unvaccinated and vaccinated flocks were recorded for the first part of the egg laying round.

Local reactions:
Seven days post first vaccination, 20% of the birds showed mild to moderate swellings in the breast muscle. In one bird vaccine oil could be seen subcutaneously. Fourteen days post first vaccination, 13% of the birds in one flock had moderate swellings of the breast muscles.

Seven days post secondary vaccination, 22% of the birds examined across all 3 vaccinated flocks, showed mild diffuse swelling of the breast muscle and/or a discrete abscess in one bird to circumscribed lumps in 7 birds. These were probably due to a previous vaccination. Fourteen days post secondary vaccination, 50% of the birds across 2 flocks were found to have circumscribed swellings often in the anterior of the breast and nodular lumps. Since about 80% of cockerels not

vaccinated with Nobilis OR inac had lesions similar to the vaccinated hens, it was concluded that the reactions were caused by poor vaccination technique and/or other inactivated vaccines which had been administered.

Mortality rate
The mortality rates in 3 flocks, one control and 2 vaccinated ones, were appreciably higher than in the other 2 flocks. This was due to infections with Newcastle Disease infection at 35 weeks or Infectious Coryza.

Egg production
The vaccinated flocks came into production slightly earlier than unvaccinated flocks. There was little difference in egg weights between the flocks. The vaccinated flocks had a slightly better hatchability than unvaccinated flocks; however, since hatchability rates fluctuate and this result was considered not to be due to vaccination.

III.D.2 Belgian field trial

In order to support the results of the first field trial, another field trial was performed in Belgium, using 16 healthy breeder broiler parent flocks. Eight of these flocks were vaccinated and the remaining 8 flocks served as unvaccinated controls. All groups received standard vaccination with several vaccines.

Up to 9 broiler flocks were derived from each parent flock and included in this study. The broiler flocks were slaughtered at around 6 weeks of age.

Broiler breeders were vaccinated subcutaneously at the age of 12 and 18 weeks. Seven days after the first and second vaccinations, birds were checked for systemic and local reactions. Blood samples were collected from all broiler parent flocks at different ages until 58 weeks. Performance parameters were recorded during the whole production period.

In broilers, blood samples were taken from 22 birds per flock at day one and at slaughter (6 weeks age). Antibodies against *Ornithobacterium rhinotracheale* were determined. Production parameters were collected from broilers during rear. At slaughter tracheal swabs were taken from broilers of each flock and examined for the presence of *Ornithobacterium rhinotracheale*.

Local reactions:
In none of the 8 vaccinated broiler breeder flocks local or systemic reactions were observed 7 days post first or second vaccination. No statistically significant differences in the performance parameters were found between vaccinated and unvaccinated broiler breeder flocks.

The broiler flocks derived from the vaccinated breeder flocks had a significantly lower mortality and a significantly higher production index compared to the progeny of the unvaccinated breeders. A statistically significant lower incidence of *Ornithobacterium rhinotracheale* isolations was found in broilers derived from vaccinated breeders as compared to broilers derived from unvaccinated breeders.

Serological data
At week 12 antibody titres in broiler breeders were very similar in all groups . By week 18 the titres had increased in the vaccinated groups and remained high to the end of the study.

Broiler flocks derived from vaccinated parents had higher titres at one day of age than chicks from unvaccinated parents. See also section IV.D.1.

There was no significant correlation between the serological data and the various performance parameters of the broiler flocks. No significant differences were seen between groups with respect to production, use of antibiotics or isolation of *Ornithobacterium rhinotracheale* at slaughter. No negative influence was observed on the reproductive performance and the mortality of vaccinated birds.

Conclusions:
Noticeable reactions were observed during the South African field study after the schedule had been administered intramuscularly. The Applicant attributes the degree of these reactions to the administration of other oil based vaccines. These may have contributed to the degree of reaction, however, as reactions after repeat doses by the subcutaneous route in laboratory studies were also of some duration, the Committee considered a warning in the SPC necessary. The following sentence was added to section 5.4 "Undesirable effects" of the SPC: "Under field conditions, sporadic local and systemic clinical reactions have been reported."

III.E ECOTOXICITY

The Applicant conducted a phase I assessment and concluded that no further assessment was necessary.

Possible hazards were identified as:

- Spread of incompletely inactivated *Ornithobacterium rhinotracheale* to other animals and humans.
- Contamination of the production process by organisms which may spread to the target animal, other animals and humans.
- The substance used for inactivation, formaldehyde.

The Applicant assessed the exposure to the hazard and the likelihood that the hazard may occur and concluded that:

- The procedure for the inactivation has been validated and each inactivation procedure is tested by a validated test method. Therefore any spread of a partially inactivated *Ornithobacterium rhinotracheale* is highly unlikely. *Ornithobacterium rhinotracheale* is not pathogenic to humans or mammals. The method of administration makes the likelihood of spread to the environment unlikely.
- The master seed and working seed have been demonstrated to be free of contamination. The production is performed under GMP in appropriate facilities. The sterility of the final product is tested according to the Ph.Eur. Therefore, any contamination of the final product is extremely unlikely.
- The Applicant presents the results of the final formaldehyde concentration of 3 batches. These are below the Ph.Eur. requirement of 0.05%. The method of production ensures that formaldehyde is only present at trace levels.

The Committee agreed with the assessment of the Applicant.

IV. OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY TRIALS

IV.A INTRODUCTION

Two laboratory studies were carried out. In both studies, progeny of vaccinated birds were challenged with an aerosol of *Ornithobacterium rhinotracheale*.

IV.C LABORATORY TRIALS

IV.C.1 Challenge study with Ornithobacterium rhinotracheale

The chickens used in the study were broiler breeders and broilers hatched from eggs laid by these broiler breeders.

Six groups of breeders were vaccinated subcutaneously at 6 and 18 weeks of age with 0.25 ml vaccine containing different amounts of cells/ml of *Ornithobacterium rhinotracheale* strain B3263/91. One group of broiler breeders was kept as an unvaccinated control group. All broiler breeders received several standard vaccinations. Eggs were taken at 6 weeks intervals and hatched for serology and for challenge tests in broilers. Blood samples and egg yolk samples were taken every 6 weeks to measure serotype A antibody levels. Blood was also taken at 50 weeks of age because of an increase in titres at 48 weeks of age.

Broilers were hatched out of eggs laid at a breeder age of 26 - 27 weeks, 35, 40 and 54 weeks. The birds were primed (9 or 22 days of age) by spray with Newcastle Disease Virus and then challenged by aerosol or intravenously with *Ornithobacterium rhinotracheale* at 14, 15 or 28 days of age.

Blood samples were taken from chicks at different time points. Additionally, birds were hatched to serve as Newcastle Disease controls (10 per challenge) and negative controls for challenge. All broiler breeders received vaccinations with various standard vaccines.

Systemic and local reactions as well as reproduction parameters were recorded. Post mortem investigations on challenged birds were performed 7 or 8 days post the *Ornithobacterium rhinotracheale* challenges. Birds having received the aerosol challenge were investigated for abnormalities in the thoracic airsacs, abdominal airsacs and trachea as well as whether pneumonia was present. Broilers with the intravenous challenge were investigated for the degree of abnormalities in the joints and liver.

Serology

Broiler breeders
By the time of first vaccination at 6 weeks of age, the birds were free of antibodies. Broiler breeders vaccinated at 6 and 18 weeks of age with standard antigen content (10^7 cells) had significant antibody levels up to 54 weeks of age.

Six weeks post initial vaccination, groups vaccinated with 10^7 cells had distinct mean titres. Repeat vaccination at 18 weeks caused a significant increase in titre in the groups vaccinated with 10^7 cells. This dropped off over the following 6 weeks to levels seen prior to the second vaccination and remained at about this level for the remainder of the study (54 weeks). The levels of antibodies in the control group rose slightly over the course of 24 weeks. However, the increase was more significant over weeks 42 - 48. This increase was also observed in the antibody titres of the groups vaccinated with the lowest dose. This increase was considered the result of an infection at 42 - 48 weeks of age thus invalidating the results after this time point.

Eggs
Eggs from breeders contained significant antibody levels over the period of laying to 54 weeks. There was a very slight decrease in levels of antibodies from birds as the age of the breeders increased to 42

weeks. A significant increase in the titres of antibodies found in eggs from breeders at 42 weeks of age was probably due to an infection with *Ornithobacterium rhinotracheale.*

Broilers
The levels of antibodies in birds at one day of age, which had been hatched from breeders vaccinated with 1 x 10^7 cells or more, were significantly higher than the control group. Responses were gradually declining by 2 weeks of age, but were still statistically significantly different at 5 weeks of age from the control group. The antibody levels in chicks from birds vaccinated with 10^8 cells were slightly higher than that of those vaccinated with 10^7 cells and this became more pronounced with the age of the chicks. The antibody levels in chicks from breeders vaccinated with less than 10^7 cells were only slightly above levels in control birds and were not statistically significant.

Challenge

Aerosol challenge
Reduction of both pneumonia and airsacculitis was seen in 6 of the 7 challenges in the groups hatched from breeders that were vaccinated with 10^7 cells or more. The group vaccinated with 10^8 cells showed slightly better results, while the groups vaccinated with less than 10^7 cells showed little reduction in lesions. On challenge of birds hatched from eggs of chickens vaccinated with batches of standard antigen content and above minimum potency, a reduction in pneumonia and airsacculitis was seen for at least 14 days for broilers from breeders aged 40 weeks and up to 28 days for broilers from breeders up to 35 weeks of age.

Intravenous challenge
The reduction of lesions in the joints and livers caused by intravenous challenge was not always significant and varied between batches. Some reduction of lesions in the joints and livers was seen for broilers at 21 (54 weeks of age breeders) and 28 days of age (40 and 35 weeks of age breeders) from birds vaccinated with 10^7 cells.

Conclusions:
The Applicant has shown a dose response relationship using batches of lower than standard antigen input and below minimum potency, batches of standard input and above minimum potency and a batch of higher than standard antigen input and higher than minimum release potency. An infection in the group of broiler breeders at 42 - 48 weeks of age invalidated the results after this time point.

In broilers, antibody levels were gradually declining after hatching. The period of immunity demonstrated by challenge was a minimum of 14 days; therefore, the Committee considered the maximum period of efficacy 14 days as stated in the SPC under section 5.2 “Indications”: “Under field conditions passive immunity is transferred during lay for 43 weeks after the last vaccination of broiler breeders, resulting in a duration of passive immunity in broilers of at least 14 days after hatching.”

IV.C.2 Efficacy trial after subcutaneous and intramuscular administration

Two groups of hens received 2 single dose vaccinations subcutaneously or intramuscularly, whilst a third group remained as unvaccinated controls. All animals received various vaccinations with standard vaccines. Blood samples were taken at regular intervals. Yolk samples were taken from eggs laid at 27 - 28 weeks of age.

Broilers were hatched out of eggs laid at 27 - 28 weeks of age. Broilers were challenged with an aerosol of *Ornithobacterium rhinotracheale* at 14 days of age. Another group of broilers was challenged intravenously with *Ornithobacterium rhinotracheale* at 3 weeks of age. Ten broilers per group were used for blood sampling up to 5 weeks of age.

Post mortem investigations on challenged birds were performed 7 days post *Ornithobacterium rhinotracheale* challenge. Birds of the aerosol challenge group were investigated for abnormalities of the thoracic airsacs, abdominal airsacs and trachea as well as whether pneumonia was present, whilst

birds from the intravenous challenge group were investigated for abnormalities of the joints and the liver.

Breeder broilers
Over 30 weeks the antibody levels in vaccinated birds were significantly different to those in unvaccinated birds. There was no statistically significant difference between antibody levels in birds vaccinated subcutaneously or intramuscularly.

Eggs
The levels in eggs from vaccinated birds were statistically significantly different to those in unvaccinated birds. There was no significant difference between antibody levels in eggs sampled from birds of 27 weeks of age vaccinated by either route.

Broilers
There was no significant difference in the reduction of lesions in progeny of breeders vaccinated by either route when they were challenged at 14 days (aerosol) and 21 days (intravenous) of age. Both groups showed reduction of lesions which was statistically significant when compared to unvaccinated challenged birds.

Based on these results it is concluded that there is no difference in efficacy between subcutaneous and intramuscular application of Nobilis OR inac in broiler breeders until at least 27 weeks of age.

IV.D FIELD TRIAL

IV.1 Belgian field study

Immunity in breeder parents over whole period of lay (58 weeks of age)

Data provided in the laboratory study IV.C.1 did not confirm that immunity transferred from the breeders covers the whole of the laying period because of an infection during the experimental period. Therefore, the Applicant has addressed this issue by providing data from an extensive field trial conducted in Belgium **(**see also section III.D.2).

Results from the Belgian field study confirm that antibody titres against *Ornithobacterium rhinotracheale* increased in the vaccinated groups. Four weeks after the second vaccination, at 22 weeks of age, high antibody titres were measured in the vaccinated broiler breeders, which were significantly higher than the antibody titres in the unvaccinated birds. The difference in antibody titres between the 2 groups remained significant up to the end of lay, at 58 weeks of age and remained stable during the whole production period.

An *Ornithobacterium rhinotracheale* infection occurred in one of the control flocks at week 40 resulted in seroconversion in that group. In the other unvaccinated group the levels remained stable over the study.

Furthermore a significant positive correlation was found between the mean *Ornithobacterium rhinotracheale* antibody titres in the parent flocks and the mean *Ornithobacterium rhinotracheale* antibody titres found in one-day-old broilers hatched during the whole laying period. The titres of broiler flocks at day-old were significantly higher in the progeny of vaccinated birds. This can be explained by the transfer of maternal antibodies.

The Committee therefore, agreed to the indication at section 5.2 of the SPC as follows:
"For passive immunisation of broilers induced by active immunisation of female broiler breeders to reduce infection with *Ornithobacterium rhinotracheale* serotype A when this agent is involved.
Under field conditions passive immunity is transferred during lay for 43 weeks after the last vaccination of broiler breeders, resulting in a duration of passive immunity in broilers of at least 14 days after hatching."

Immunity in broilers over lifespan (6 weeks)

In the broiler flocks derived from vaccinated breeders, significantly higher antibody titres were found at one day of age, in comparison with the antibody titres found in broilers derived from unvaccinated breeders.

Furthermore, the titres in progeny from vaccinated birds can be related to significantly lower number of *Ornithobacterium rhinotracheale* isolations at slaughter and a significantly lower mortality rate and a higher production index as compared with the progeny of unvaccinated broiler breeders.

Spearman rank correlation calculated that there was a significant correlation between the mean antibody titre of the parent flocks and the mean antibody titre of the broiler flocks at day old, however, 6 weeks later at the point of slaughter there was no difference between these 2 groups. Flocks originated from different ages of parents, showed no significant differences. A higher titre in birds from one unvaccinated flock after week 40 was the result of an infection of the parent flock.

V. RISK – BENEFIT ASSESSMENT AND CONCLUSIONS

Nobilis OR inac is an inactivated, oil-adjuvanted vaccine indicated for use in broiler breeders to provide passive immunity of progeny through transmission in the egg in order to reduce infection with *Ornithobacterium rhinotracheale* serotype A.

Originally there were a number of quality issues which were outstanding and needed to be resolved. In many cases the questions were requests for more detailed information which arose due to a lack of sufficient information in the dossier but the issues of the inactivation kinetics, the ability of the potency test to detect sub potent and over potent batches, and the absence of a final product test for formaldehyde were also required to be addressed. Most issues have been resolved, however, there remain three post-authorisation commitments to be met by the Applicant regarding quality issues.

The vaccine can be administered subcutaneously in the neck or intramuscularly in the breast using a volume of 0.25 ml. GLP laboratory studies and GCP field trials were conducted. Adequate laboratory safety studies of the safety of a single, double and repeat dose using a batch of standard antigen content and above minimum potency have been conducted in the relevant category of the target species, broiler breeders.

Noticeable reactions were observed during the field study conducted in South Africa after the schedule had been administered intramuscularly. No such reactions were observed in the later field trial conducted in Belgium. Amendments have, therefore, been made to the SPC to describe the local reactions seen, particularly after repeat vaccination.

The effect of vaccination by the subcutaneous route on egg production, hatchability and the survivability of progeny over the first week of life has been investigated using a batch of standard antigen content but greater than minimum potency. Use of the vaccine during lay is contra-indicated.

The overall risk to the environment is assessed as minimal.

The disease caused by *Ornithobacterium rhinotracheale* infection has only been identified relatively recently. The Applicant has justified the use of the strain and addressed the incidence in a number of European countries.

Laboratory efficacy studies were carried out demonstrating that vaccination with a standard antigen content vaccine batch results in production of relevant antibodies in broiler breeders up to the end of the laying period (53 weeks of age) and in progeny for up to the first 14 days of life. However, there were several remaining issues such as the duration of immunity in broiler breeders. The Applicant has addressed this issue by providing serological data from an extensive field trial conducted in Belgium. High antibody titres were measured in vaccinated broiler breeders remaining statistically significant higher than the antibody titres in unvaccinated birds over 43 weeks until the end of lay. Higher antibody titres were observed in progeny of vaccinated breeders at one day of age compared to broilers derived from unvaccinated breeders. The broiler flocks derived from vaccinated breeders had significant lower mortality and higher production index compared to the progeny of unvaccinated breeders.

The efficacy of both recommended routes of administration has been proven in broilers from breeders of 27 weeks of age by challenge of the progeny.

The vaccination schedule stated in the SPC is for primary vaccination at 6 - 12 weeks of age followed by a second injection, after an interval of at least 6 weeks, at 14 - 18 weeks of age. Data have only been provided for vaccination of broiler breeders in the age of 6 – 12 weeks (primary vaccination) and 18 weeks (secondary vaccination). However, since the immune status of chickens at 14 weeks of age is not considered different from the immune status at 18 weeks of age, the Committee agreed to accept the proposed vaccination schedule of a second vaccination at an age of 14 – 18 weeks.

It was considered that there was insufficient evidence in the efficacy studies presented in the dossier to suggest that the *Ornithobacterium rhinotracheale* acted as a primary pathogen and the claim was amended accordingly:
"For passive immunisation of broilers induced by active immunisation of female broiler breeders to reduce infection with *Ornithobacterium rhinotracheale* seroptye A, when this agent is involved.
Under field conditions passive immunity is transferred during lay for 43 weeks after the last vaccination of broiler breeders, resulting in a duration of passive immunity in broilers of at least 14 days after hatching."

Due to local reactions well known for mineral oil adjuvanted vaccines, the Committee agreed to modify section 5.11 of the SPC (Special precautions to be taken by the person administering the veterinary medicinal product to animals) in line with the standard wording for such warnings.

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 Directive 2001/82/EC of the European Parliament and of the Council. One member expressed a divergent opinion (see appendix).

APPENDIX: DIVERGENT OPINION

Demonstration of efficacy of the vaccine Nobilis OR inac can only be appreciated through two laboratory and one field trial.

In laboratory trials, positive effect of the vaccination was only demonstrated in birds receiving a double challenge: inoculation of Newcastle virus then *Ornithobacterium rhinotracheale* 5-7 days later (immunity is demonstrated in birds at 14 days of age). It seems according to the applicant, that *Ornithobacterium rhinotracheale* effectively acts as a primary pathogen as shown by success in reproducing the disease in experimental conditions after a single challenge (*Ornithobacterium rhinotracheale*, a primary pathogen in broilers by L. van Veen, P. van Empel and T. Fabri in Avian disease, 44: 896-900, 2000). So, it is justified to have a laboratory trial demonstrating efficacy of the vaccine against a simple challenge.

In the field trial, the positive effects which could be evidenced after vaccination with Nobilis OR inac are decrease of mortality, increase of production index and decrease of the number of isolations of the *Ornithobacterium rhinotracheale* bacteria in infected flocks from vaccinated flocks (passive immunity). All these positive effects are not specific and are not necessary linked to a protection against *Ornithobacterium rhinotracheale* infection. Antibodies against *Ornithobacterium rhinotracheale* are found in vaccinated flocks at levels comparable to those demonstrated as protective in laboratory trials (but, as said previously, this demonstration is very limited: double challenge and age of 14 days of the challenged birds).

The dossier as a whole may be considered in the light of the few data available (known) on the epidemiology and the pathogenicity of *Ornithobacterium rhinotracheale* in chickens.

- *Ornithobacterium rhinotracheale* infection is a recent infection whose impact in the poultry production is not still evaluated and whose role as a primary pathogen is still not very clear (first identification of the disease in 1991, identification of the causal agent in 1994 in turkeys, infection in chickens described more recently);
- The few data available mention an infection which develops principally in broiler chickens between 3 and 4 weeks of age and appear to be more common in breeders between 24 and 52 weeks especially during the egg production peak and is associated with respiratory disease, decrease of growth, mortality and eventually effects on the egg production.

(*Ornithobacterium rhinotracheale* infection by R.P. Chin and R. Droual in Disease of Poultry 10th edition // http://www.bacterio.cict.fr/bacdico/oo/ornithobacterium.html).

Considering the limitations in the data provided (limits of the demonstration of efficacy of the dossier and limits of knowledge on the disease) and the intended indication (passive immunity to protect broilers during 14 days only), the efficacy of the vaccination cannot be considered to have been satisfactory addressed.

2 October 2002
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