Bioveiligheidsraad
Conseil de Biosécurité

16-04-2012

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0382

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/11/V3 of the company HIPRA Laboratorios for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/11/V3 has been submitted by HIPRA Laboratorios to the Belgian Competent Authority (CA) in November 2011 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development, according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a veterinary clinical trial and the title of the notification is: "Evaluation of the safety and efficacy of the vaccine PB-116 in the control of porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae.*" The purpose of the study is to assess in the field the safety and the efficacy of the vaccine for pigs. This last step in the development of a veterinary medicinal product follows trials under contained laboratory conditions and is required by the EU legislation. The trial will be undertaken in different countries in Europe. In Belgium 3 farms for fattening of pigs located in Flanders and in which acute *Actinobacillus pleuropneumoniae (App)* outbreaks took place in the previous fattenings will be selected.

The vaccine PB-116 – *Actinobacillus pleuropneumoniae* HP-3276 strain - or matched placebo is administered by intramuscular injection to fattening pigs from minimum 8 weeks of age and a second dose will be administered 3 weeks after. A total of maximum 600 pigs will receive the GM vaccine. The dissemination of the *App* HP-3276 strain after its administration to pigs by intramuscular route is considered negligible.

The notification has been officially acknowledged by the Competent Authority on 12 January 2012 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Two experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier while the Platform for Molecular Biology and Biotechnology of the Scientific Institute of Public Health evaluated the analytical procedure for the detection of *App* HP-3276 strain submitted by the notifier.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism for its intended use, would not raise any problems for the environment, animal health or human health (people coming in contact with the treated animals and/or with the GMO).

On 19 January, on 21 February and on 27 March 2012, based on questions prepared by the Biosafety Advisory Council, the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority respectively on 26 January 2012, on 9 March and on 4 April 2012 and transmitted to the secretariat of the Biosafety Council. This complementary information was reviewed by the coordinator and the experts.

For the purpose of this evaluation, the following legal basis has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.
The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated animals, as well as aspects related to social, economical or ethical considerations, are outside the scope of this evaluation.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. The comments, questions and reaction of the public relevant for the environmental and/or public health safety of the GMO have been taken into account in the elaboration of the advice of the Biosafety Advisory Council.

Summary of the Scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

The parental organism from which the GMO is derived corresponds to a strain of *App* isolated from an outbreak in Spain, and identified as strain HP-3179.
The Biosafety Council asked to the notifier to give more information about the origin and the reason of choice of this strain.
A characterization of antibiotic susceptibility of strain HP3179 and of the vaccine strain HP3276 has also been requested.

Toxins produced by *App* (RTX-toxins) are responsible of the pathogenicity of this bacteria. More information was required about the molecular structure, size and stability of these toxins. Also details were requested on the composition and structure of the capsule, on the mechanisms of survival of *App* and the possibility that *App* may be a carrier of other pathogens.

The requested information was received and judged satisfactory. However regarding the antibiotic susceptibility there is no study on the Belgian situation and the experts suggest that during the planned trial strains isolated from the treated pigs should be fully characterized.

2. Information related to the vector

No risks were identified regarding the plasmids vectors used to produce the genetically modified *App*.

3. Information related to the characteristics of the GMO

HP-3276 is a live attenuated *App* strain characterized by two deletions in one segment of a specific gene . Due to this deletion the exotoxins of *App* have lost their haemolytic and

cytolytic activity. The deleted strain is apathogenic for the target species but it keeps all the antigens that are required to induce a protective immune response.
To the question why only one gene is modified in the GMO, although also another gene is involved in active production of the toxins, the notifier explains that the deletion is the minimum modification required to disable the toxic effects of the toxins without suppressing the immunogenic properties of the vaccine. This answer was judged satisfactory.

The experts requested more information regarding the biological controls performed by the notifier to check the identity and purity of the vaccine product. The control protocols provided by the notifier are judged satisfactory for a correct quality control of the product. But in addition the experts advice that in case this control shows aspecific results adequate analysis should be performed to discard impurities or unknown genetic modification.

The notifier was also requested to submit a clearer and more detailed analytical procedure regarding the method allowing the detection of the vaccine strain in control samples.
The information and needed document were provided by the notifier and deemed acceptable.

4. The condition of release

The notifier was requested to precise where the euthanization and dissection of some of the pigs included in the trial will occur. This info was received and judged satisfactory.

A clear information for persons working with the GMO was requested. This info was received and judged adequate to protect and inform the personnel that will handle and administer the vaccine.

5. The risks for the environment and human health

With the exception of the risks for personnel handling and administering the product to the pigs (see above), no major risks were identified for the general population or people coming into contact with the treated animals. Pathogenicity of *App* has never been demonstrated in humans. Moreover horizontal transfer of genetic material between *App* genetically modified and other bacteria species is highly improbable.. Although transmission of the vaccine strain to non-vaccinated pigs cannot fully be excluded, from the data available and in the conditions of the release it should be rare and without adverse consequences for the environment.

Concerning other animal species, only wild boars are known to be sensible to infection by *App*. The notifier was requested to document the possible presence of wild boars in the proximity of the selected farms. The notifier satisfactorily stressed that in Flanders wild boar occurs at very low densities and that, in the event of the presence of wild boar in the region, only farms sufficiently isolated as to prevent the entrance of wild animals will be chosen for the field trial.

6. The monitoring, control, waste treatment and emergency plans proposed by the applicant

The monitoring and control of the trial as well as the emergency plans and the treatment of the waste have been judged adequate. In addition, access to the farms where the trial will be performed will not be permitted unless authorisation given by the main investigator.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that the genetically modified *Actinobacillus pleuropneumoniae* HP-3276 strain, a life attenuated vaccine for the control of porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae*, will have any adverse effects on human health or on the environment in the context of the intended clinical trial.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the trial protocol, as described in the dossier.
- Before the start of the trial the notifier provides the precise identification and location of the farms selected in Belgium (Flanders Region) and precisions should be given regarding their isolation from wild boar populations.
- Access to the farms will be restricted to authorized persons.
- Any protocol amendment has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that personnel handling and administering the product to the pigs is qualified and experienced in handling infectious material.
- Strains isolated during the trial from the treated pigs should be fully characterized.
- The competent authority and the Biosafety Advisory Council should be informed within 2 weeks when the first and the last pigs are vaccinated.
- At the latest six months after the end of the trial, the notifier must send to the competent authority at the attention of the Biosafety Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - the total number of farms and pigs included in the trial and the number of farms and pigs included in Belgium;
    - a report on the accidental releases, if any, of the recombinant *Actinobacillus pleuropneumoniae;*
    - the characterisation of the strains isolated from the treated pigs.

p.o. Dr. Philippe Herman

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex 1: Compilation of comments of experts in charge of assessing the dossier B/BE/11/V3 (ref: BAC_2012_0241)

Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

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Compilation of Comments of Experts in charge of assessing the dossier B/BE/11/V3

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 16 december 2011
Coordinator: Dr. Bruno Urbain
Experts: David Fretin (CODA-CERVA), Erick Vandamme (UGent), Nicolas Willemarck (WIV-ISP)
Domains of expertise of experts involved: Microbial genetics, molecular genetics, molecular microbiology, molecular characterisation, microbial ecology, infectiology, bacterial vaccines, bacterial toxins, biosafety, containment measures, risk management, workers protection
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/11/V3** concerns a notification of the company Hipra Laboratorios for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 12 January 2012. It concerns a veterinary clinical trial and the organism that is the object of this application corresponds to a strain of *Actinobacillus pleuropneumoniae* (referenced as HP-3276) with modification of specific genomic sequences corresponding to the pathogenic activity of this gram-negative bacterium. This attenuated strain has been developed as a live attenuated vaccine against swine pleuropneumonia.

♦ INSTRUCTIONS FOR EVALUATION

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments received from the experts

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 21-02-2012 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1

The information provided is sufficient and adequate . Some comments /questions are :
1).In "Summary notification information ..":
-p.3/23 :The HP-3276 strain belongs to serotype 2 and it produces, next to the common toxin ApxIV and II and III, also 2 "additional" toxins: no information is given about these toxins or their possible action? Apart from the LPS as endotoxins, all these RTX-toxins are all exotoxins: little information is given about their molecular structure and size (105 and 120 kD resp., less 2kD), long time stability.
...
Only gene A is deleted in the GMO, although also gene C is involved in active production of the toxin : is deletion of gene C not needed ?

-p.7/23 :As to the pathogenicity of *Actinobacillus pleuropneumoniae* (App), it is mentioned that disponibility of essential nutrients in the respiratory tract, especially iron, is important : does App produce "siderophores", or other molecules to capture/store iron from the environment ?

2). In "Technical dossier ":
-p.3/167 : 3th alinea ,line 6 : ... *Pasteurellaceae* ..
-p.11 : item 8 : does it occur in Africa ???
-p. 12: item 9 : the example of genomic recombination given here relate to viral strains and diseases, rather than bacterial ???
-p14: ...enormous genetic variability ...(see first comments above in 1) .
-p.15. 4th alinea : ...which mechanisms are used to obtain iron ????(see also above in 1).
-p.16. 2nd alinea : more details on "...persistent infections ,...dormant " mechanisms must be given !
-p.18. bottom alinea : nature of capsule : only polysaccharide or also proteins ...??
-p. 19. top line : App is a carrier of other pathogens ?? : explain !
-p. 20 . literature referencing to antibiotic resistence plasmids is quite old : (Hirsch et al. , 1982) ???
-p.20. bottom alinea : how to speed up development of "third generation vaccines" ??
-p.63/167 : 3th alinea : ...virus ..?? ; correct into :....bacteria !!

3).In " Environmental risk assessment...":
-p./3/12 : ...endotoxins ApxII and ApxIII ... : is there some confusion/error here ?? since these RTX-toxins are all exotoxins !?

Comment 2

The different strains of *Actinobacillus pleuropneumioniae* (HP3179 & HP816) used in the development of the vaccine strain (GMO) are not well documented in the dossier. The dossier should at least introduce those different strains used to make the IMP (origin?; reason of choice?,….) // HP3179 short introduction p3 AnnexIIIa

Comment 3

Cfr point 3.1

2. INFORMATION RELATED TO THE VECTOR

(e.g. description, sequence, mobilisation ...)

Comment 1

The information provided is quite detailed, accurate and sufficient . The protocol for the construction of the 2 vectors is clearly and logically described and illustrated , and is discussed against pertinent literature data. Potential use of other microorganisms (*E.coli, Saccharomyces cerevisiae*) in which the genomic sequence encoding one or more immunogenic Apx toxins has been inserted, is also discussed .
Following the homologous recombination event in vivo, no foreign genetic matter is incorporated into the recombinant strains obtained .

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment 1

The information provided is accurate and very detailed in experimental procedures used. The transformation /mating protocol, results of Southern blots, haemolytic tests are described in detail and illustrated as well.
The genetic modification procedures used to obtain the attenuated vaccine strain allowed to avoid the need to incorporate any antibiotic resistance marker in the final strain, which is a crucial positive element, while eliminating haemolytic and cytolytic activity and pathogenic activity.

Comment 2

Idem 1.; origin and choice of parental strains HP3179 & HP816.

Comment 3

The GMO is derived from a Spanish strain of Actinobacillus pleuropneumoniae; this strain is isolated in an outbreak. No data about antibiotic resistance of this strain are presented. In case of vaccination of a previously infected animal a transfer of genetic material (Antibiotics resistances) is possible. Transfers of genetic traits present in Spanish strain (for example Antibiotic resistance) to a Belgian strain of Actinobacillus must be avoided. A characterization of antibiotic susceptibility of the GMO is needed.

Additional comment SBB:
The parental App strain HP-3179 belongs to serotype 2 – In the technical dossier (Annex IIIA, p.19) it is said that antibiotic resistance is very rare in serotype2. Can it be completely excluded ?

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

Comment 1

The molecular characteristics of the final GMO are accurate and well described in detail. The deleted strain maintains its range of toxins without haemolytic and cytolytic activity and keeps all the antigens, that are required to induce a protective immune response.
The genetic stability has been proven by 5 serial subcultures and genomic extraction after each step, showing no reversion to the original phenotype. The rate and expression level of the new genetic material is experimentally detailed as is the activity of the expressed and exported proteins, lacking haemolytic and cytolytic activity.
Strain HP-3276 displays a characteristic restriction pattern, due to the 2 deletions in its genome, allowing a rapid and easy fingerprint detection and differentiation between the vaccine strain and the wild type.

Comment 2

The notifier described different assays in the dossier, such as Southern blot, PCR and subcultures on platelets of TSYN medium to control genetic stability.

1. Genetic stability assay // Although Southern blot analyses are not sensitive to detect point mutations or mutation outside the targeted genes, the notifier suggests with this assay that no additional modifications are introduced into the targeted sequence.

2. Genetic reversion towards the original // The dossier describes an assay of serial subcultures on platelets of SYN to test haemolytic activity and a PCR-assay targeting the deletion on genomic DNA to proof genetic stability of the strain. Given the lack of control on mutations (cfr. wrong choice of control assay_Southern Blotting) in the genome (with possible epidemiologic enhancing effects) and given that the deletion is a main factor in pathogenicity (transmembrane domain), those described stability

tests are not the right assays to test genetic stability but are rather proofs of loss of pathogenicity (of the vaccine strain).
In that way a genetic reversion between virulent field strains and a vaccine strain with additional mutations towards more pathogenic strains can not be excluded (cfr/ study of Henderson, 1991).
(PCR analyses described on p40 IIIa to check the deletions have no positive control)

3.The described study _ PE-2008-CB-07 _ to measure the capacity to genetic reversion is the study was only based on *Actinobacillus pleuropneumoniae* sero-negative pigs and not in combination with already infected pigs (with virulent *Actinobacillus pleuropneumoniae.* → Such a test is not mimicking the natural circumstances of vaccination in the future (when it should be marketing authorized vaccine)

The notifier should comment this…

Additional comment from the SBB:
In the Method IT-00062-BM (supplementary information provided on 25 January 2012) on page 22 it is said that "When samples containing the App strain with Apx III modified gene are analysed, it is possible, in some cases, that in round 2 A an unspecific band of 500 bp appears, which does not interfere with the 678 bp band considered as positive." . Has the applicant an explanation for this ? Have they sequenced this unspecific band ? Can it be excluded that this reveals that the expected deletion did not occur?

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

Comment 1
Based on the solid experimental data provided, the vaccine strain is not transmitted to unvaccinated animals, housed in close contact with vaccinated animals, despite the fact that it is detected about 4 weeks post-vaccination on the tonsils and in the inoculation point of vaccinated animals. The vaccine strain does not show reversion to virulence after serial passages in the target animal.
In the pathogenicity comparative tests, (Technical dossier :Annex III p.45/167 ,table), the parental strain HP-3179 was applied in a lower dose (10x7 cfu) than the vaccine strain (10x9 cfu): this is a factor 100 ?? Why is the same dose not used in these animal trials ?

Additional comment coordinator:
The lower dose used for the infectious parental strain already killed 100% of the animals injected.

Comment 2

Transmission from vaccinated to unvaccinated animal was evaluated in a previously experiment (PE-2008-CB-03), in L3 facility. This experiment is not presented in dossier. It is important to test this aspect to the new trial in field's conditions with more animals.

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

Comment 1

This information about GMO release conditions is accurate and sufficiently detailed. The purpose of the deliberate release is to confirm the lack of adverse effects (safety) and to test the protection of vaccinated animals (efficacy of the vaccine). The site of release will be three farms in Flanders, Belgium. The quantities of GMO released will be about 2x10 exp8 cfu up to > 1.2x10 exp11 cfu, administered via intramuscular injections to pigs, housed in isolated farms . The GMO will be released during 2 days: on vaccination day and a few weeks(3) later on revaccination day. No spread or release of the GMO is expected to occur due to the inoculation via intramuscular injection and since the GMO did not spread from inoculated animals in previous tests. Lab and field waste, in contact with the GMO, will be sterilized and incinerated on the farm. Emergency response plans are considered in case of an unexpected spread and consist of sacrifice and incineration of all the animals and disinfection/fumigation of the facilities.

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment 1

This information is given in a sufficient and accurate way (see a.o. Technical Dossier Annex III : p. 56/167).
App infection occurs via airborne spread or direct animal contact only between pigs . Infection of pigs between farms occurs via introduction of carrier animals. No reports are available on the fact that App can affect humans. Still, attention will be paid to avoid accidental ingestion and/or inoculation of people. Upon accidental release, the vaccine strain is not able to survive in the environment for over more than 8 to 10 days.

Comment 2

The (horizontal) transmission tests_PE-2008-CB-04&PE-2008-CB-06_ with pigs vaccinated and non-vaccinated (sentinels) (p55_IIIa) // The results of these assays are disputable because the positive control is missing → there were no animals infected with virulent wild type *Actinobacillus pleuropneumoniae* in the herd during the simulation experiment.

How sure are you that the setup of your transmission model/assay is fulfilled?

Precise non-inoculated animals housed “in direct contact”.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment 1

See above : sufficient attention is given to possible health effects on people in close contact with the GMO.
It is understood that immunocompromised people should not be involved in the overall process of developing, producing and applying the GMO.

Comment 2

Actinobacillus pleuropneumoniae is not a zoonotic agent but risk of human contamination is possible (Rycroft et al 2011). The Inhalation and the ingestion are not the only risks ; injury with material containing the GMO, is also a risk .A clear information and formation for persons working with the GMO is needed.

5.3. Information on possible effects on animal health or on the environment.

Comment 1

These aspects are addressed in sufficient detail. The beneficial effects of the vaccine on animal health are described in sufficient detail. The possible negative effects (due to wind-and contact spread , GMO-survival , ..) on the environment are discussed as well.

Comment 2

Wild boars are also sensible to infection by Actinobacillus pleuropneumoniae. For the choice of farm, the evaluation of presence of wild boars in the proximity is needed.

Additional comment from the SBB:
In Annex II the applicant says that App does not affect animal species other than pigs.
In the Human Health and Environmental risk assessment the applicant says that no seroconversion has been demonstrated in any wild species.
According to Vengust et al, 2006, in Slovenia App seroprevalence in wild boar is as high as 52%. It seems that wild boar are subclinical carriers of App (Reiner, 2010)

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1

The GMO has the desirable vaccine properties, is genetically stable, and develops its life cycle in the inoculated animals. No reversion to the wild type has been observed in vitro, under lab as well as field conditions. This is clearly outlined throughout the proposal.

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

Comment 1

See above : The GMO is genetically very stable and is unlikely to reconvert to the wild type. Methods to verify and ensure are indicated (Annex III p. 57/167).

Comment 2

See 3.2

Comment 3

I agree with the authors of the dossier, the risk of the reversion towards original genotype is very low.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1

See above.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1

See above.

Comment 2

Transfer of genetic material between Actinobacillus pleuropneumoniae genetically modified and other bacteria is highly improbable.

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment 1

An adequate tracing and monitoring plan is presented and risk assessment is included. Differential PCR is the method of choice to identify the vaccine strain under different circumstances.

6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

Comment 1

The farms where the vaccine is used and tested have adequate isolation and containment facilities.

Comment 2

- There is no disinfection of the stalls at the end of the trial described, neither a 'wait' period following the trial before reusing the pig stall is demanded, although shedding of GMO cannot be completely excluded. Please comment this and justify the decision to not disinfect the stall or to not include a certain 'wait' period?

- In section III (Information concerning the condition of release and the receiving environment) annex IIIa, it is written "To date, there have been no reports of any signs that *Actinobacillus pleuropneumoniae* can affect humans. Despite that, special attention will be paid to avoid accidental ingestion and/or inoculation"; Is it possible to precise those precautionary measures taken to prevent accidental ingestion and/or inoculation?

- There are no procedures described to protect the site from intrusion by unauthorized persons.

Comment 3

The procedures of disinfection (type of disinfection and the condition of use) are not described. A clear document is needed.

6.3. Information on the waste generated by the activity and its treatment.
(e.g. type of waste, amount ...)

Comment 1

The only type of waste to be generated is the inoculation material. If needed, incineration and disinfection techniques will be applied.

Comment 2

Contradiction in material waste management: autoclave (technical dossier IIIa p60) >< incineration (Public dossier Dutch/French/English p14)

Precise the end-use of the test animals (p26_EC-2008-CB-003) // Body waste management
- What will happen with the test animals at the end of the trial?

Where will be done the euthanization and dissection?

Additional comment coordinator :
This is the competence of the AFMPS-FAGG to propose an adequate withdrawal period so that animals enter the food chain. There are also discussions ongoing with the Belgian Food Agency (FAVV – AFSCA) about the end use of the animals.

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment 1

Emergency plans are given in case of uncontrolled release, and consist in slaughtering of all animals involved, fumigation with formaldehyde of the premises and UV-sunlight exposure.

6.5 Information related to the identification of the GMO and the detection techniques
(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1

See above.

Comment 2

The PCR method for the detection proposed by notifier is a good method.

7. OTHER INFORMATION

7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1

This is a solid proposal, well written in sufficient detail and scientifically sound. The molecular characteristics of the GMO and the potential impact of the GMO on human health and on the environment is adequately evaluated. The information provided is sufficient, accurate and adequate to state that the deliberate release of the GMO for its intended use will not raise any problem for the environment, animal and human health.
Some comments, questions to be addressed and corrections needed are indicated above .

References Not in Application

Reiner G. et al (2010) Prevalence of Actinobacillus pleuropneumoniae Infection in Hunted Wild Boars (Sus scrofa) in Germany. Journal of Wildlife Diseases, 46(2), pp. 551–555

Rycroft AN, Assavacheep P, Jacobs M, Langford PR. (2011) Necrosis from needlestick injury with live Actinobacillus pleuropneumoniae porcine vaccine. BMJ. 2011 Oct 4;343.

Vengust G. et al (2006) A Serological Survey of Selected Pathogens in Wild Boar in Slovenia. J. Vet. Med. B 53, 24–27