Intrexon T1D Partners, LLC.
20374 Seneca Meadows Parkway

Germantown, MD 20876

GMO Deliberate Release Notification

ENVIRONMENTAL RISK ASSESSMENT

A PROSPECTIVE, MULTI-CENTER, PHASE 1B/2A STUDY TO ASSESS THE SAFETY AND TOLERABILITY OF DIFFERENT DOSES OF AG019 ADMINISTERED ALONE OR IN ASSOCIATION WITH TEPLIZUMAB IN SUBJECTS WITH CLINICAL RECENT-ONSET TYPE 1 DIABETES MELLITUS (T1D).

European notification number
B/BE/18/BVW5

Edition No.:	**01.1**
Issue (Release)/Report Date:	**2018-06-08**
Replaces Previous Edition No. (Date):	**NA**
Document No.:	**AG019-GMO-BE-ERA-EN-20180608**
Prepared by:	**Sven Blomme** **Clinical Project Manager** **Intrexon Actobiotics N.V., d/b/a ActoBio Therapeutics**

GMO Deliberate Release Notification Part 3
ENVIRONMENTAL RISK ASSESSMENT

ENVIRONMENTAL RISK ASSESSMENT CONCERNING THE DELIBERATE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS[1]

This environmental risk assessment is based on information provided in Part 1 A Technical Dossier and Part 1B Confidential Annexes.

TABLE OF CONTENTS

[1] according to annex II of the Royal Decree of 21 February 2005.on the deliberate release in the environment and the placing on the market of genetically modified organisms or of products containing such organisms. (MB/BS - 24.02.2005 –p. 7129)

TABLE OF CONTENTS

Page

TABLE OF CONTENTS

Page

STATEMENT OF DATA CONFIDENTIALITY CLAIM

This document is submitted by Intrexon T1D Partners, LLC. as part of a notification for a deliberate release of a GMO.

I. THE ENVIRONMENTAL RISK ASSESSMENT

A. Identification of characteristics which may cause adverse effects

1. Disease to humans including allergenic or toxic effects;

a. Evaluation of characteristics of the deliberate release leading to the potential adverse effects

Neither *Lactococcus lactis* nor *L. lactis* sAGX0407 are hazardous.

The GMO is a biologically contained strain of *Lactococcus lactis*. *L. lactis* is commonly found in and added to food products. It is not classified as a hazardous organism.
Due to the nature of the IMP (i.e.; a live bacterium), there may be a risk for clinically significant bacteremia or sepsis attributable to the sAGX0407 bacteria. However, it is important to keep in mind that:

- In the nonclinical studies, only negligible amounts of bacteria have been found in a few individual blood samples; further investigation suggested that this may have been due to the blood sampling method
- No clinical effects suggesting bacteremia or sepsis have been observed in any of the nonclinical studies
- An in vitro study performed with sAGX0407 confirmed that the bacteria are unable to survive in complement preserved human serum
- As part of the development of another ActoBiotics®, IV inoculation of the *L. lactis* bacteria in neutropenic rats revealed that the bacteria were rapidly cleared, without any signs of sepsis.
- The antibiotics resistance profile of sAGX0407 was determined; in the unlikely event of bacteremia the bacteria can easily and quickly be inactivated by a broad range of commonly used antibiotics

Therefore, the risk of clinically significant bacteremia or sepsis is considered to be highly unlikely.

The only new proteins that are produced and secreted by the genetically modified *L. lactis* sAGX0407 are hIL-10 and hPINS.

The protein sequence of the secreted hIL-10 is identical to natural hIL-10, except for one amino acid change at position 2.

The principle function of hIL-10 delivered by *L. lactis* sAGX0407 is to steer the immune system towards a more tolerant state.

Human interleukin-10 has been studied extensively in several different preclinical models as well as in humans. Systemic administration of high doses of subcutaneously and intravenously administered hIL-10 in healthy volunteers has been documented to cause side effects (Bijjiga, 2013). These effects mainly include lowering the number of red blood cells (anemia), lowering the number of blood platelets (thrombocytopenia), headache and/or fever. In addition, while hIL-10 has proven to be a potentially promising therapy in autoimmune disorders, hIL-10 therapy may also be associated with an increased risk for infections due to its immune suppressing capabilities.
As hIL-10 is delivered locally by *L. lactis*, only minuscule doses are applied to the gastrointestinal mucosa, without systemic exposure. Therefore, the occurrence of adverse events linked to hIL-10 is expected to be very unlikely. For more details please read the next sections.

Human proinsulin is the prohormone which is cleaved in to mature insulin and C-peptide. hPINS has no or little biological and pharmacological activity. The predominant function of hPINS delivered by *L. lactis* sAGX0407, is to induce an antigen specific immune tolerance.
hPINS has been studies as a potential standalone treatment in several clinical trials. hPINS was administered systemically via different routes and in different forms (intact proinsulin, proinsulin peptides, or proinsulin DNA vaccines). Despite the fact that some of these trials showed modest success, treatment effects were generally not long lasting. Nevertheless, treatment with systemic hPINS was generally safe and well tolerated.
As hPINS is delivered locally by *L. lactis,* only very low doses are applied to the gastrointestinal mucosa, without systemic exposure. Therefore, the occurrence of adverse events linked to hPINS is expected to be very unlikely.

Locally administered sAGX0407 doses are expected to be too low to trigger side effects.

A comparison between optimal secretion capacity of hIL-10 by sAGX0407, and previously published data on systemic exposure levels corresponding to the no-adverse-effect-dose-level (NOAEL) in mice treated with systemically administered recombinant hIL-10 (rhIL-10; Tenovil), allows to determine the safety margin.
A previously published report on data from preclinical toxicology studies with systemically administered recombinant hIL-10, demonstrated that the NOAEL in mice

treated subcutaneously with recombinant hIL-10 for one month, was 2 mg/kg daily (Rosenblum, 2002).

Hence, taking into account the NOAEL of 2mg/kg/day, the maximum amount of hIL-10 secreted under optimal circumstances by L. lactis will provide a more than 100-fold safety margin to any participant in this study.

For hPINS, no literature data is found on NOAEL. However, in clinical investigations involving systemically administered hPINS, no increase in the frequency of adverse events could be noted in the higher dose groups versus placebo. In one study, in which plasmid encoded proinsulin was administered at a dose of 6mg/week, no increase in the frequency of adverse events could be observed when compared to placebo.
The secretion capacity of hPINS by *L. lactis* sAGX0407 also provides an almost 100-fold safety margin compared to this high dose regimen of 6mg/week.It is expected that, at such low levels, the occurrence of adverse events linked to hPINS will be very unlikely.

Exposure remains very limited.

In the outpatient study, AG019 will be administered as capsules for oral intake. The patient only receives the necessary material for the next treatment period (up to 1 month). The pre-packaged medication will include a blister with capsules packaged in individual alveoles. The clinical trial centers store all packages for the complete treatment period.

Direct contact with the lyophilized powder is under normal procedure excluded. Single doses are very low compared to systemic administration.

Once administered, the GMO will become metabolically active and produces and secretes hIL-10 and hPINS. *L. lactis* is not able to replicate or colonize in the gastrointestinal tract. Live bacteria will be present in the stool up to a few days after administration. Faeces will eventually be released in the sewage system. The number of living bacteria will quickly decline.

b. Mechanisms through which adverse effects may occur directly or indirectly

The spread of the GMO(s) in the environment,

- The GMO is disseminated passively in the environment, essentially in the faeces and sewage system. The GMO will not be able to survive due to the nutrient requirements (auxotrophy).

- It is only able to grow in artificial growth cultures and is totally dependent on thymine/thymidine. In addition, the mechanism of thymine-less death ensures an irreversible elimination process.
- No impact on human health during the limited spread is expected due to the unlikelihood of relevant exposure, low concentration and short survival time.

The transfer of the inserted genetic material to other organisms, or the same organism whether genetically modified or not,

- The potential for exchange of genetic material with other *Lactococci* is extremely low as sAGX0407 does not harbor plasmids or conjugative transposons. Phage replication is severely hindered as sAGX0407 is not able to produce thymidine.
- The GMO is not able to transfer genetic material to other organisms or the same organism whether genetically modified or not.
- In the repeat dose toxicity study, no signs of incorporation of the expression cassettes into the host genome were observed.

Phenotypic and genetic instability

- The GMO has been shown to have the insert stably integrated for at least 62 generations.
- Genetic instability –if present– would result in the inactivation of the expression cassettes. Removal of the *il-10* and/or *pins* gene(s) by homologous recombination -a phenomenon which has never been observed so far– would result in loss of the transgene. Neither the presence of the transgenes, nor the loss thereof, would give the bacteria a competitive or selective advantage over other (micro)organisms.

Interactions with other organisms,

No interactions with other organisms are identified.

Changes in management,

- Instead of one dose, patients might accidentally take several doses at a time. The quantity of product that one person will receive at a time is limited to the next treatment period (up to 28 days treatment), as well as the number of size 1 capsules which can easily be ingested by a subject. Even if all administrations are applied at the same time (which is extremely unlikely; a 1-month supply of medication for the high dose groups would be 168 capsules), the quantities are expected not be sufficient to trigger adverse events. In addition, should such overdosing occur, the GMO can easily be killed by oral and/or intravenous antibiotics.

- Persons may get in contact with the GMO when administering the capsule (if it is damaged or opened) (patient), when examining the patient (clinical staff), through empty materials, laundry, etc; (relatives, others) and via the sewage system: The quantities are too low to induce any effect. In addition, survival time of the GMO outside the human body or the specific lab cultures is very short.

Influence on the immune system

hIL-10 is known to steer the immune system towards a more tolerant state. Especially when combined with a systemic immune modulating drug (such as teplizumab), one could expect that there is a risk of increasing susceptibility to infection. However, as stated before, the doses of hIL-10 are very low. In addition, as hIL-10 is applied locally and not systemically, an increased risk for infection is unlikely. Lastly, preclinical studies in the NOD mouse model have demonstrated that this treatment strategy induces an antigen specific immune tolerance; the immune response to antigens other than hPINS was not altered by the treatment. Nevertheless, monitoring of potential signs of viral infection (EBV, CMV, HIV, HCV) is included in the protocol. In the unlikely event of an infection, this will be picked up fast.

2. Evaluation of the potential consequences of each adverse effect, if it occurs.

L. lactis is non-pathogenic, non-toxic and does not cause allergic reactions. There is a long history of safe use of *L. lactis* in food products. The modified strain has not changed in that respect.

Repeated systemic administration of recombinant hIL-10 (Tenovil, Schering-Plough) has been extensively studied in both healthy volunteers and patients, for different indications including ulcerative colitis (UC) and Crohn's disease (CD). The main clinical adverse events were dose-related (flu-like syndrome with headache, fever, and myalgia). Hematological changes included transient, mild to moderate increase of neutrophil counts, decrease of lymphocyte counts, and a delayed decrease of platelet counts.

A Phase 1 study in CD patients which studied a genetically modified *L. lactis* strain secreting hIL-10, administered daily at a dose of 2×10^{11} CFU for 7 days, was well tolerated. The main adverse event observed was flatulence. There were no deaths or other serious adverse events. One patient was withdrawn from the study on the second day because of persistent vomiting.

Finally, should a person be accidentally exposed to sAGX0407, it is possible to treat with an antibiotic, as the GMO is sensitive to all groups of commonly used antibiotics.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

The potential adverse effects only occur following an overdose of hIL-10. The ActoBiotics® delivery system avoids the exposure to systemic high doses by localized expression in the gastrointestinal tract. The doses are very low and act only locally. The exposure is also limited in time as the bacteria are evacuated from the gastrointestinal tract within a few days following administration.

As explained in Section I.A.1.a., the high dose to be administered to subject provides a more than 100-fold safety margin compared with the NOAEL observed in preclinical studies.
No systemic exposure to hIL-10 has been measured in the animals that received the GMO.

This means that accidental intake of all supplied capsules that a patient can receive (168 capsules), will lead to an exposure level that is still far below the NOAEL for systemically administered hIL-10.
Even if all doses provided to one clinical trial centre would be taken, the risk would be very low as each centre treats and observes only a limited number of patients (multi-centre study, patients do not start the treatment all at once, on average 2 patients per site are expected).

The likelihood of exposure to high levels of hIL-10 is practically non-existing for persons that come into contact with the investigational medicinal product, with patients or with the sewage system; the bacterium is strictly biologically contained and dies quickly once released from the capsules or via faeces.

Also, indirectly the likelihood to get in contact with other organisms expressing hIL-10 is practically zero, as the GMO is not able to transfer genetic material.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

The risk of suffering from the adverse effects after being exposed to a high concentration of sAGX0407 is very low.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

Only a limited number of doses are provided to the patients. The clinical trial centers only receive the whole package on a “per patient enrolled” basis. Unauthorized individuals will not have free access to the material. Furthermore, patients will be questioned and examined at regular basis so that any adverse effect will be noticed very quickly. In addition to these regular examinations, subjects will be asked to keep an electronic diary and to record any potential adverse event. As this is a web based diary, realtime monitoring of adverse event data will ensure that any adverse effect will be noticed very quickly.

Given the biological containment which combines several inherent inactivation factors, no additional inactivation is foreseen at the clinical trial centers on top of normal practice. Any material that is collected during the trial and that has potentially been in contact with the bacteria will be disinfected or inactivated as hazardous medical waste (disposable materials, packages, etc.).

At the patient’s home, normal hygiene is sufficient.

If required, a standard antibiotic treatment would suffice to inactivate the bacteria.

6. Determination of the overall risk of the GMO(s)

The overall risk of the hIL-10 and hPINS producing *L. lactis* for human health is extremely low.

B. Disease to animals and plants including toxic, and where appropriate, allergenic effects

1. Identification of characteristics which may cause adverse effects:

a. Evaluation of characteristics of the deliberate release leading to the potential adverse effects

The basic argumentation is similar to that presented for human health. The GMO is a biologically contained strain of *Lactococcus lactis*. It is only able to grow on artificial cultures and is totally dependent on thymine/thymidine. The potential for exchange of genetic material is extremely low, as the organism does not harbor plasmids or conjugative transposons. Phage replication is severely hindered as the GMO is not able to produce thymidine.

Human IL-10 expression only triggers an effect on human cells that have the appropriate receptors. These receptors are highly specific to human species. Most other mammalian IL-10 specific receptors have little or no cross-reactivity with hIL-10 except for some simian and murine receptors. No effect at all is expected on animals or plants.
Human PINS by itself has very little biological activity. Its sole purpose is to induce and expand those Treg cells which are specific for this antigen. No effect at all is expected on animals or plants.

In the planned studies, the investigational medical product supply is stored at the patient's home where it is administered most likely. Following administration, the GMO follows the normal intestinal flow. The bacterial population is not multiplying and depending on the circumstances, will be eliminated within a few days due to combination of both the biological containment features and gastrointestinal motility. Via the sewage system, the bacteria are released into the environment.

b. Mechanisms through which adverse effects may occur directly or indirectly

At the patient's home, pets, rodents or insects might get in contact with the GMO. During storage, the exposure would be very limited, even in cases of accidental release from the package. Once administered, they may get exposed by contact with exposed materials (empty packages, laundry, etc.), or with the sewage system.
Also, animals in the wider environment might be exposed via the sewage system.

The GMO is disseminated passively in the environment, but will not be able to survive due to the nutrient requirements (auxotrophy; biologically contained).
The GMO is not able to transfer genetic material to other organisms or the same organism whether genetically modified or not.
The GMO has been shown to have the insert stably integrated for at least 62 generations.
No interactions with other organisms are identified.

2. Evaluation of the potential consequences of each adverse effect, if it occurs

L. lactis is non-pathogenic, non-toxic, and does not cause allergic reactions. There is a long history of safe use of *L. lactis* in food products. The modified strain has not changed in that respect.

The hIL-10 expression only triggers an effect on human cells that have the appropriate receptors. These receptors are highly specific to human species. Most other mammalian IL-10 specific receptors have little or no cross-reactivity with hIL-10 except for some simian and murine receptors. .
Human PINS by itself has very little biological activity. Its sole purpose is to induce and expand those Treg cells which are specific for this antigen.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

No adverse effects are identified.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

No adverse effects are identified.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

At the clinical trial centre, standard precautions are in place to avoid other organisms (pets, insects, rodents, etc.) from entering the site and the storage equipment.
At the patient home, standard hygiene is adequate.

6. Determination of the overall risk of the GMO(s)

The overall risk of the hIL-10 and hPINS-producing *L. lactis* for animal health is practically non-existing.

C. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations;

1. Identification of characteristics which may cause adverse effects:

a. Evaluation of characteristics of the deliberate release leading to the potential adverse effects

Growth of *L. lactis* can only be sustained in a selected number of nutritionally favorable areas such as milk, specifically prepared meats, vegetable fermentations and laboratory culture broths. *L. lactis* cannot successfully propagate outside these very specific ecological niches. This is underscored by the fact that despite ample opportunity –globally, living *Lactococci* are consumed and released in soil and sewage waters in tremendous amounts– no colonization of any other niche has ever been reported.

As all plasmids were removed during the isolation of recipient strain MG1363, the strain has essentially lost the capacity to access its main energy and amino acid sources. In consequence, the habitat of MG1363 is confined to artificially supplemented culture conditions.

The genetically modified sAGX0407 is highly biologically contained. It can only grow in artificial cultures. The organism is extremely sensitive to temperatures above 40°C, low pH, air drying, direct sunlight and UV. The lyophilized powder formulation is extremely sensitive to air moisture and ambient temperatures.

The patients are treated outside of the clinical trial centre. The receiving environment is therefore the sewage system, due to the evacuation of stool from treated patients, most probably at the patient's home or elsewhere. This would be comparable to the normal disposal of wild type *L. lactis.* However, given the biological containment system, a rapid elimination is expected.

b. Mechanisms through which adverse effects may occur directly or indirectly

See I.A.1.b. of this document

2. Evaluation of the potential consequences of each adverse effect, if it occurs

No adverse effects are identified.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

No adverse effects are identified.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

No adverse effects are identified.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

No specific management is foreseen. Moreover, the sewage treatment system is designed to eliminate bacteria.

6. Determination of the overall risk of the GMO(s)

The overall risk of the hIL-10 and hPINS-producing *L. lactis* for species in the receiving environment and their genetic diversity is practically non-existing.

D. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;

1. Identification of characteristics which may cause adverse effects:

a. Evaluation of characteristics of the deliberate release leading to the potential adverse effects

The GMO will produce hPINS. However as already explained, no adverse events are expected from hPINS.
The GMO will also produce hIL-10. IL-10 was initially characterized as cytokine synthesis inhibitory factor (CSIF) from concanavaline A (conA)-stimulated Th2 cells. IL-10 inhibits the production of cytokines such as interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) by Th1 cells, but not Th2 cells, in response to antigens presented by antigen-presenting cells. Therefore, it has been proposed that IL-10 plays a key role in modulating the host immune system. The principle function of IL-10 is to modulate the magnitude of a pro-inflammatory immune response.

In healthy individuals, tight regulation of the mucosal immune system prevents excessive inflammatory responses toward normal intestinal bacteria.

The ActoBiotics® delivery system avoids the exposure to systemic high doses by localized expression in the gastrointestinal tract. The doses are very low and act only locally. The exposure is also limited in time as the bacteria are evacuated from the gastrointestinal tract in a few days following administration.

A Phase 1 clinical trial with ActoBiotics®-delivered IL-10 in CD patients provided an indication for clinical efficacy, meaning that administration of hIL-10 in this way is likely suppressing the inflammatory response in the intestines.
Preclinical studies with sAGX0407 in combination with a short treatment of low-dose anti-CD3 has demonstrated that an antigen specific immune response is induced, the immune response to non-disease related antigens remains unchanged.

b. Mechanisms through which adverse effects may occur directly or indirectly

See I.A.1.b. of this document

Healthy persons may accidentally swallow entire capsules or ingest material from accidentally opened capsules.

2. Evaluation of the potential consequences of each adverse effect, if it occurs

It has been argued that, due to the immunosuppressive effect of hIL-10, a healthy person might show increased susceptibility towards pathogens active in the gastrointestinal tract. However, as hIL-10 is delivered locally by *L. lactis*, only minuscule doses are applied to the gastrointestinal mucosa, without systemic exposure. Therefore, the occurrence of adverse events linked to hIL-10 is expected to be very unlikely.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

In the clinical trial centre and at the patient's home, the lyophilized product will be stored at 4°C. At the hospital, unauthorized persons will not have access to the product and all manipulations are extremely limited: direct contact to biological material is limited to the medical observations of treated patients. During the observations, standard hygiene procedures are applicable.

At the patient's private home, the biological material should be stored in the refrigerator. This could lead to inadvertent use. During opening of blisters, additional routes for exposure are created. This could lead to exposure of the patient's relatives and visitors.

There is no indication that transmission could occur via toilets, especially when standard hygienic rules are followed.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

Only low amounts of the GMO will be ingested and these will only be temporarily active. The bacteria will leave the body within a few days, and their survival time outside the human body is very short.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

In the clinical setting, the GMO will be stored out of reach of unauthorized individuals. The patient will be advised to store the doses in a lockable container.
In case of accidental ingestion, an antibiotic treatment will inactivate the GMO.

6. Determination of the overall risk of the GMO(s)

Based on data available today on systemically administered IL-10 in patients, the risk of becoming susceptible to pathogens in the intestines is estimated to be very low.

E. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine

1. Identification of characteristics which may cause adverse effects:

a. Evaluation of characteristics of the deliberate release leading to the potential adverse effects

The transgenic *L. lactis* only contains the gene for hIL-10, replacing the *thyA* gene. In addition, the GMO contains a gene coding for hPINS, which is secreted in very low quantities and has little or no biological or pharmacological effect. No genes conferring resistance to antibiotics are present.

b. Mechanisms through which adverse effects may occur directly or indirectly

No adverse effects are identified.

2. Evaluation of the potential consequences of each adverse effect, if it occurs

No adverse effects are identified.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

No adverse effects are identified.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

No adverse effects are identified.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

No adverse effects are identified.

6. Determination of the overall risk of the GMO(s)

No adverse effects are identified.

F. Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material.

1. Identification of characteristics which may cause adverse effects:

a. Evaluation of characteristics of the deliberate release leading to the potential adverse effects

The modified *L. lactis* strain only grows in artificially supplemented cultures. The GMO has no chance of survival in the environment, due to the total dependency on thymine/thymidine (thymine-less death). Therefore, it is not expected to have any effect on biogeochemical cycles.

b. Mechanisms through which adverse effects may occur directly or indirectly

No adverse effects are identified.

2. Evaluation of the potential consequences of each adverse effect, if it occurs

No adverse effects are identified.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

No adverse effects are identified.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

No adverse effects are identified.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

No adverse effects are identified.

6. Determination of the overall risk of the GMO(s)

No adverse effects are identified.

II. CONCLUSIONS ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE DELIBERATE RELEASE OF GMOS

A. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).

L. lactis can be found in a whole range of environments, but these are not ecological niches. In spite of the widespread use and massive discharge in the environment, it has not been identified as invasive or disruptive. Growth can only be sustained in a selected number of nutritionally favorable areas such as milk. *L. lactis* does not multiply in or colonize humans or animals.

L. lactis strain MG1363 (parental strain) only grows in artificially supplemented culture conditions as the genes to metabolize milk are removed. MG1363 does not produce antibiotics, but is sensitive to a large range of antibiotics. The growth capacity of GMO sAGX0407 is even more restricted as it also lacks the ability to produce thymidine, without which it will die.

Therefore, the likelihood of the GMO to become invasive is practically zero.

B. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s).

The GMO is very much biologically contained. It can only survive in artificial laboratory cultures. Compared to the non-modified parental strain, it has an important additional selective disadvantage: it is totally dependent on the addition of thymine/thymidine to the culture medium (thymine-less death).

While there is little information available on the natural presence of free thymine/thymidine in natural environments, it is generally seen as a limiting factor resulting in irrevocable elimination of the strain.

C. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.

The potential for exchange of genetic material is extremely low as the organism does not harbor plasmids or conjugative transposons. The GMO is thymine/thymidine dependent, severely hindering phage replication. Therefore, transduction of the GMO's genetic material via phages is highly unlikely.

Genetic elements could be released in the environment upon lysis and might be taken up by other bacteria. In the case of the GMO, the likelihood of release of intact naked DNA is reduced as thymine-less death triggers the degradation of DNA before the actual cell lysis.

The genetic modification is based on targeted gene replacement by double homologous recombination: the *L. lactis* MG1363 *thyA* gene and promoter have been replaced by the hIL-10 expression cassette. Theoretically, the gene for thymidine production might be regained via homologous recombination with a natural strain. This has not been demonstrated to be possible. Also, once released in the environment, the bacteria no longer grow or replicate. Hence, no selection for *thyA* is possible.

The *hIL-10* or *hpins* genes do not confer any selective advantage.

D. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).

None. The only organisms that are targeted are a specific group of human patients.

E. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.

No interactions with non-target organisms have been identified. Bacteriophages cannot replicate in *thyA*-deficient hosts. Moreover, after leaving the patient's body, the remaining live bacteria will survive only for a very short period.

F. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).

L. lactis is non-pathogenic, non-toxic, and does not cause allergic reactions.

If people come into contact with the GMO, it will be in very little amounts. Exposure is possible at administration of the enema, when examining patients and when in contact with faeces samples, empty materials, laundry, sewage system, etc., where a reduced amount of living bacteria might be present that declines rapidly in time.

This limited exposure to the GMO will not cause any adverse immediate or delayed effect on the human health.

G. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.

The GMO is not intended to be used as animal feed. Exposure of animals will be extremely limited in quantity and time. No immediate or delayed effect is foreseen.

H. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

No interactions with any other organism are identified. The GMO is not expected to have any effect on biogeochemical processes. The GMO is not able to survive outside the laboratory.

I. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the

management of the GMO where these are different from those used for non-GMOs.

Given the biological containment that combines several inherent inactivation factors, no additional techniques are put in place for the management of the GMO. Normal hygienic practices are sufficient.

III. OVERALL CONCLUSIONS ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE DELIBERATE RELEASE OF GMOS

In summary, the environmental risk assessment confirms that the specifics of the product and the measures that are used during this clinical trial provide a high level of control on the possible impact of the GMO. Although the GMO will be released in the sewage system and is thereby disseminated beyond a well-specified release site, the biological containment features guarantee elimination of the organism and the absence of any harm during the short time that it may be present.

BIBLIOGRAPHY REFERENCES

Bijjiga E, Martino AT. Interleukin 10 (IL-10) Regulatory Cytokine and its Clinical Consequences. Journal of Clinical & Cellular Immunology 2013.

Rosenblum IY, Johnson RC, Schmahai TJ. Preclinical safety evaluation of recombinant human interleukin-10. Regul Toxicol Pharmacol 2002; 35(1): 56-71.