PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Belgium
(b)	Notification number	B/BE/07/BVW1
(c)	Date of acknowledgement of notification	../../….
(d)	Title of the project	Phase 1b and Phase 2a clinical trials with an hIL-10-expressing *Lactococcus lactis* (*L. lactis*).
(e)	Proposed period of release	From 01/07/2008 until 01/07/2011

2. Notifier

Name of institution or company: ActoGeniX N.V.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(.)
bacterium	(X)
fungus	(.)
animal:	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class: …

(b) Identity of the GMO (genus and species)

Lactococcus lactis

(c) Genetic stability – according to Annex IIIa, II, A(10)

L. lactis strains have been used in food production. No particular factors have been identified. The growth of *L. lactis*, in particular of MG1363, is largely determined by the specific ecological niche.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (X) No ( )

If yes, insert the country code(s):

Depending on availability of subject following countries are explored: FR, GB & NL.

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

The GMO is a biologically contained strain of *Lactococcus lactis*. It is only able to grow in artificial laboratory cultures and is totally dependent on supplementation of thymine/ thymidine to the medium.
The GMO will be administered orally or via enema to patients with moderately active ulcerative colitis. The organism does not colonize the gastrointestinal tract. Live organisms are likely shed in stools at low levels for about 3 days. Shedding will constitute the release of the organism and potentially, it could be released into the sewage system. Normal hygiene (hand washing) is considered sufficient to prevent transmission from person to person.

The GMO has no selective advantage in the environment. It is not invasive and does not persist in the environment. The potential for exchange of genetic material is extremely low,

as the organism does not harbor plasmids or conjugative transposons and phage replication is severely hindered as it is not able to produce thymidine.

A previous clinical study in Crohn´s disease patients with a highly similar strain showed good tolerability and safety, and demonstrated not only that the biological containment strategy was safe but also provided indications for clinical efficacy.

In summary, the risk assessment for this study shows a very low risk associated with administering the GMO to patients. The risk to other humans is negligible and the risk to the environment is estimated to be practically zero.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:
(select one only)

viroid	(.)	
RNA virus	(.)	
DNA virus	(.)	
bacterium	(X)	
fungus	(.)	
animal:		
- mammals	(.)	
- insect	(.)	
- fish	(.)	
- other animal	(.)	
specify phylum, class: …		
other, specify	…	

2. Name

(i)	order and/or higher taxon (for animals)	Lactobacillales
(ii)	genus	*Lactococcus*
(iii)	species	*L. lactis*
(iv)	subspecies	subsp. *lactis*
(v)	strain	MG1363
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	common name	

3. Geographical distribution of the organism:

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (X) No (X) Not known (.)

While the wild type *L. lactis* is indigenous and globally present, MG1363 is a strain incapable of survival outside of artificially supplemented laboratory conditions.

(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes (X) *idem as 3.A*

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (X) *idem as 3.A*

(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?

Yes (X.) No (.)

(d) Is it frequently kept in the country where the notification is made?

Yes (X) No (.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	

L. lactis strain MG1363 can only grow in artificially supplemented media and is restricted to laboratory cultures.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques

Standard microbial techniques.
Molecular techniques based on 16sRNA PCR and sequencing.
Specific culture media requirements.

(b) Identification techniques

PCR analyses and sequencing to identify sAGX0037 (16sRNA genetic material for *L. lactis* and
hIL-10 gene)
hIL10-production is measured by a specific quantitative ELISA.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes ( ) No (X)

If yes, specify: /

EFSA introduced the concept of "Qualified Presumption of Safety" (QPS) in relation to a generic approach for safety assessment of micro-organisms used in food/feed and the production of food/feed additives. As the first evaluations of candidate microorganisms are ongoing, *L. lactis* has been included in the list of gram positive non-sporulating bacteria.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (X) Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

L. lactis are non-pathogenic bacteria, critical in manufacturing dairy products such as buttermilk, yogurt and cheese. In spite of the widespread use and massive discharge in the environment, *Lactococci* have not been identified as invasive or disruptive. Although they can be found in very diverse sources (soil, manure, waste water), the bacteria depend on particular nutritional components for growth. MG1363 is restricted even more and as such confined to artificially supplemented culture conditions.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

MG1363 is restricted to artificial laboratory growing conditions. In optimal culture circumstances, the generation time is 30 minutes.

(b) Generation time in the ecosystem where the release will take place:

The GMO will be released in the sewage system after administration to patients. MG1363 is not able to grow outside the laboratory.

(c) Way of reproduction: Sexual: Asexual: X

(d) Factors affecting reproduction:

MG1363 can only grow in artificially supplemented culture conditions.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (fungi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	none

(b) relevant factors affecting survivability:

MG1363 can only grow in artificial laboratory conditions.

10. (a) Ways of dissemination

Dispersal of the bacteria is essentially passive.

(b) Factors affecting dissemination

No specific factors.
Passive dissemination with medium. The survival time outside of the laboratory is very short.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

No previous notifications.

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify	…

2. Intended outcome of the genetic modification

The gene for human interleukin-10 (*hIL-10*) has been inserted in the bacterial chromosome, replacing the *thyA* gene and promoter encoding thymidylate synthase. The accompanying regulatory sequences are aimed at secreting human Interleukin-10 (hIL-10).
Upon administration to patients, the protein is targeted to reduce inflammatory responses in ulcerative colitis.
Deleting the *thyA* gene resulted in strict thymine/thymidine dependency, not only for growth but also for survival of the GMO (*thymine-less death*).

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (.) No (X)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector:

plasmid	(.)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector.
…

(c) Host range of the vector
…

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (.) No (.)

antibiotic resistance (.)
other, specify: …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	…

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(X)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify	…

6. Composition of the insert

(a) Composition of the insert

The insert consisted of a bacterial promoter, a secretion sequence leader, the *hIL-10* gene and a non-coding sequence downstream of the *thyA* coding sequence.

(b) Source of each constituent part of the insert

The promoter is of bacterial origin.
hIL-10 is a synthetic gene derived from the human gene with codon optimization for expression in *L. lactis*.
The secretion sequence leader and the non-coding sequence downstream of *thyA* are from *L. lactis* MG1363.

(c) Intended function of each constituent part of the insert in the GMO

The promoter is used to drive expression of the *hIL-10* gene. The secretion leader sequence encodes an extracellular secretory protein that enables the GMO to secrete hIL-10 in the gastrointestinal tract after administration to the patient.

The hIL-10 protein is aimed at reducing inflammatory responses in ulcerative colitis.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify …

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify: …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal:
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  specify phylum, class: …

other, specify: man

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	…
(iii)	genus	*Homo*
(iv)	species	*Homo sapiens*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	man

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (X) Not known (.)

If yes, specify the following:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (X)

If yes, specify: …

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (X) No (.) Not known (.)

If yes, specify:

The GMO is dependent on addition of thymine/thymidine to the growth medium.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (X) Not known (.)

If yes, specify: …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (X) Not known (.)

If yes, specify: …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (X) Not known (.)

If yes, specify: …

2. Genetic stability of the genetically modified organism

The insertion has occurred on the bacterial chromosome, which was confirmed by PCR amplification. The modified strain was entered in the ActoGeniX collection in January 2007 and has been maintained in culture since.

Analysis of the genetic stability of *L. lactis* strain sAGX0037, obtained by repeated sequential dilution and growth to saturation, was performed after a minimum of 55 and again after a minimum of 75 generations of growth. The genetic stability was analyzed by three parameters:

- Inability of sAGX0037 to grow in thymidine-deficient medium (showing the efficiency of the biological containment system).
- Unchanged hIL-10 secretion by sAGX0037.
- Stability of the modified locus using PCR analysis with specific oligonucleotides.

The experiment concluded that genetic stability was absolute for all of these parameters.
Genetic stability during manufacture of the GMO is demonstrated for each batch

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (X) Not known (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

L. lactis are non-pathogenic bacteria, critical in manufacturing dairy products such as buttermilk, yogurt and cheese. In spite of the widespread use and massive discharge in the environment, *Lactococci* have not been identified as invasive or disruptive. Although they can be found in very diverse sources (soil, manure, waste water), the bacteria depend on particular nutritional components for growth. MG1363 is restricted even more and as such confined to artificially supplemented culture conditions. On top of that, the GMO is dependent on thymine/thymidine/ supplementation.
There is no indication that the GMO itself is toxic, allergenic or pathogenic. The changes that were induced in the recipient strain MG1363 as well as in the GMO, do not affect the basic toxic or allergenic features.
Systemic injection of high doses of hIL-10 has been documented to cause important side effects. The delivery system in this study avoids the exposure to high systemic doses by localized expression in the gastrointestinal tract. The doses are much lower and act only locally. The exposure is also limited in time as the bacteria are evacuated from the gastrointestinal tract in a period of a few days following administration.
L. lactis does not colonize the gastrointestinal tract.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

- Through PCR amplification of 16sRNA and subsequent sequencing of the PCR fragment, the species identity of sAGX0037 was established as *Lactococcus lactis* subspecies *cremoris* MG1363 during the manufacturing of the master cell bank. In addition, the presence of the *hIL-10* gene and the absence of the *thyA* gene was also demonstrated by sequencing.
- Enzyme-Linked ImmunoSorbent Assay (ELISA) was used to quantify the levels of hIL-10 secreted by the sAGX0037.

(b) Techniques used to identify the GMO

- PCR and sequencing methods result in clear-cut identifications.
- Detection limit of the hIL-10 ELISA is 10 pg/ml; the assay is specific with respect to various other cytokines, growth factors, etc. For a detailed list of factors tested for cross-reactivity, please refer to the BD OptEIA™ Human IL-10 ELISA Kit II Instruction Manual (Cat. No. 550613).

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The proposed clinical development program comprises two early-Phase trials establishing the framework for investigating ulcerative colitis:

- A Phase 1b Randomized Placebo-Controlled Double-Blind Multi-Centre Study to Evaluate the Safety, Tolerability and Efficacy of AG011 in Subjects with Moderately Active Ulcerative Colitis.
- A Phase 2a Randomized Placebo-Controlled Double-Blind Multi-Centre Study to Evaluate the Safety, Tolerability, Pharmacodynamics and Efficacy of Multiple Dose Levels of AG011 in Subjects with Moderately Active Ulcerative Colitis.

AG011 is the lyophilized powder of the GMO, formulated for oral and rectal administration.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (X) No (.)

If yes, specify:

The parental strain MG1363 can only grow in laboratory conditions. The GMO will be administered to patients orally or rectally and will follow the normal flow of faeces. As these are outpatient studies, the shedding will occur at the patient's home or elsewhere.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):

While the location of the clinical trial centers is known, the identity and coordinates of the patients will not be known to the notifier. In addition, shedding will occur mainly during the evacuation of stool. This is not necessarily limited to the home of the patient. In consequence, the national territory is considered as the wider potential release area. Patients will be recommended not to leave the country during the treatment due to the constraints imposed by the design of the clinical trials.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Not applicable

(c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:

The proximity of significant biotopes, protected areas or drinking water supplies can not be excluded as possible sites of release. However, the only route for exposure would be via the disposal of stool, which would in any event not be expected to reach such areas. In addition, if this would be the case, one can expect that already today exposure to *L. lactis* is occurring as it is a natural component of dairy products. The GMO has no additional features that make exposure more likely, on the contrary, the strict dependence on specific components and the self-eliminating thymine/thymidine dependency makes any exposure even more limited in time.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

L. lactis does not interact with fauna and flora. No involvement in particular environmental processes is known.

4. Method and amount of release

(a) Quantities of GMOs to be released:

A total of 120 patients are involved in the studies. Two ways of administration are foreseen; capsules for oral intake and enema for rectal application. In the Phase 1b study, a single dose or a placebo will be administered rectally. In the second trial, capsules at 3 different concentrations or placebo will be formulated, in combination with enema (single dose).

(b) Duration of the operation:

Recruitment of the first patients is expected to start in July 2008. Completion will depend on availability of patients fulfilling the selection criteria and could take unitl July 2011. In the Phase 1b study, each patient will be treated during 4 weeks; in the Phase 2a study, the treatment will last 8 weeks.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

Each patient will receive a treatment package containing ready-to-use doses for one week. At the time of intake, there is no contact with the bacterial powder in the case of capsules. In the case of the enema formulation, brief contact with the solution is

possible when the applicator is opened for immediate application. Standard hand washing hygiene is sufficient to avoid transmission.
In the event that the packaging would be disrupted, the powder quickly degrades when in contact with moist or warmth. The organism is sensitive to temperatures above 40°C, low pH, air drying, direct sunlight, UV, soap, bleaching agents, antibiotics and high salt. The quantity of a spillage will be limited (one dose) and the affected area can be decontaminated with a standard detergent (soap) or bleach.

Patients are examined regularly. Normal hygiene conditions for clinical staff handling patient's body fluids (in particular stool) should be sufficient. Disposable gloves and disposable wipes should be used when handling devices for analysis and biopsies. All waste material should be handled as hazardous medical waste.
While shedding of live bacteria during the treatment period and up to a few days after the last application is realistic, the biological containment and the absence of relevant impact is deemed sufficient not to warrant any specific treatment of the shedding environment.

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

5. Short description of average environmental conditions (weather, temperature, etc.)

Environmental conditions will be those of the sewage system.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

A similar hIL-10 producing strain was studied in a Phase 1 clinical trial in Crohn's disease patients.
Presence and kinetics of the strain release in the stool of patients were assessed by conventional culturing and quantitative PCR. Compared to the amount of intake, a significant decrease in amount of culture forming units (CFU) was detected in faeces.
The results obtained demonstrated the efficacy of the chosen biologic containment strategy.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable):

The target organisms are a specific group of patients (with Crohn's disease and ulcerative colitis).

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	…
(iii)	genus	*Homo*
(iv)	species	*Homo sapiens*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	man

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Human patients will ingest the GMO orally or administer the bacteria rectally. The organism will reach the gastrointestinal tract and will produce hIL-10. The protein is expected to alleviate disease.

3. Any other potentially significant interactions with other organisms in the environment

hIL-10 expression only triggers an effect on human cells that have the appropriate receptors. These receptors are highly specific and other organisms that might react to IL-10 have specific receptors with little or no cross-reactivity towards hIL-10.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (X) Not known (.)

Give details:

Compared to the wild type *L. lactis* and the parental strain MG1363, the GMO is reduced in its capacities.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

Once administered, the GMO passes the intestines and will be evacuated via stool and eventually via the sewage system. The GMO is not able to survive, let alone establish, in this environment.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i)	order and/or higher taxon (for animals)	No specific interactions with non-target organisms have been identified.
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

MG1363 does not contain plasmids or conjugative transposons. The GMO is thymine/thymidine dependent, severely hindering phage replication. Therefore, transduction of modified genetic material via phages is very 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.

(b) from other organisms to the GMO:

MG1363 can only act as a recipient of conjugative transposition.
The only relevant risk is transfer of an intact *thyA* inwards. In the *Bacteriae* and *Archaeae*, *thyA* genes do not reside on plasmids, so plasmid borne mobility of *thyA* inwards is impossible. 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.
Importantly, in the unlikely event of acquisition of an intact gene for thymidine production, the transgene would be lost (double homologous recombination).

(c) likely consequences of gene transfer:

In the highly unlikely event that *hIL-10* is transferred to other organisms, it would give no selective advantage to those organisms. Acquisition of an intact *thyA* gene via homologous recombination would return the bacterium to the non-modified state.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

Information not available.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

No potential interactions with biogeochemical processes have been identified.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The *hIL-10* gene in the GMO is a unique, synthetic gene that can be distinguished from the native *hIL-10* gene and detected via PCR. A method has also been developed to distinguish between live and dead bacteria.

2. Methods for monitoring ecosystem effects

Not planned.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

To detect the hypothetical transfer of donated genetic material to other organisms, PCR aimed at the *hIL-10* gene can be used.

4. Size of the monitoring area (m^2)
. m^2

Not relevant.

5. Duration of the monitoring

The last time point for, monitoring of the GMO in stool samples is 7 days after the end of the treatment.

6. Frequency of the monitoring

Monitoring is planned at baseline (before the start of the treatment), at 7 days of treatment and at 7 days after treatment cessation.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The clinical trial centers will disinfect equipment and surfaces according to standard medical procedures.
Patients will leave the clinical setting during treatment. Although shedding of live bacteria will occur, the biological containment and the absence of relevant impact are deemed sufficient not to warrant any specific treatment of the shedding environment.

2. Post-release treatment of the GMOs

Given the biological containment which combines several inherent inactivation factors, no additional inactivation is foreseen. If required, a standard antibiotic treatment would suffice to inactivate the bacteria.

3. (a) Type and amount of waste generated

Two types of waste possibly carrying living GMOs are identified:

- Disposable materials that have been exposed to bacterial material (e.g. empty containers, patients' disposable gloves, wipes, etc.).
- Faeces and faecal traces, hygienic wipes, disposed of in sewage system.

(b) Treatment of waste

Disposable items will be inactivated as hazardous medical waste by incineration or other validated method.
Faeces end up in the sewage system. The biological containment does not require additional treatment. Moreover, the sewage treatment system is designed to eliminate bacteria.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

Unexpected spread would mainly be limited to accidental opening of the packaged materials, releasing the lyophilized powder or the suspended enema liquid. Application of standard detergent (soap) or bleach would be sufficient to eradicate the GMOs and decontaminate the affected area. Special instructions will be provided to the patient.

2. Methods for removal of the GMO(s) of the areas potentially affected

Idem.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Idem. No specific sanitation measures are foreseen.

4. Plans for protecting human health and the environment in the event of an undesirable effect

The bacteria can be inactivated with several treatments. Furthermore, the biological containment system is expected to eliminate the bacteria in a short period after the release. In addition, there are no indications of possible undesirable effects on the environment.
L. lactis bacteria, and thus the GMO, are sensitive to all groups of commonly used antibiotics.