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	The Netherlands
(b)	Notification number	B/../../….
(c)	Date of acknowledgement of notification	../../….
(d)	Title of the project	Production of organic acids using cyanobacteria
(e)	Proposed period of release	From 2020 until 2025.

2. Notifier

Name of institution or company: Photanol BV

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 *Cyanobacteria, cyanophyceae*

(b) Identity of the GMO (genus and species)
Synechocystis, sp. PCC6803

(c) Genetic stability – according to Annex IIIa, II, A(10)
GMO remains stable over >100 generations and is expected to remain stable.

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

Yes (.) No (X)

If yes, insert the country code(s) …

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 risk for possible environmental effects of the GMO is considered very low, based on the following points. The GMO is not actually introduced into the environment but is in a bioreactor system. The bioreactor system is designed and used as a contained system and the chance of GMO being released into the natural ecosystem is very small. Leakage can only occur in extreme cases. However, should leakage occur, the organism is unable to grow, because the GMO requires higher CO2 concentrations than standard atmospheric values. The GMO cannot grow under CO2 concentrations as they are found in nature. Since no outgrowth is possible, the chance that the GMO becomes persistent and invasive is negligible. With survival tests in water and soil samples from the environment of the facility, we have also confirmed that outgrowth is not possible, in contrast to the parent organism. These results also showed that in soil samples it may take a while before the GMO is no longer viably detected, but it will never grow out, except when it is put back into the reactor. Therefore, there can never be persistent and invasive growth in the natural ecosystem around the facility. Therefore, no effects are expected on biogeochemical processes.

Since the GMO is placed inside a bioreactor, there is little contact with other types of (micro) organisms. Reactor sterility is ensured through chemical sterilization and application of stringent inoculation procedures and specific growth conditions. Because of this restriction, the chance is very small that selective advantages or disadvantages are transferred to the GMO from other (micro) organisms. The same applies to gene transfer to other species, because the GMO does not encounter other species. If this contact does occur in the event of a leak, the chance that the GMO will transfer the inserted genes is very small, since the inserted genes give the GMO a selective disadvantage. Inserted genes are also harmless, are comparable to genes that also occur in the parent organism and occur in

nature as well. The GMO also contains no potentially harmful genes (genes involved in antibiotics, virulence or sporulation). No effects on human health of animals from interaction with the GMO are to be expected. The GMO has no toxins or pathogenic properties, like the parental organism. Therefore, there is no expectation that the GMO will influence people and the environment.

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)	*Cyanobacteria, cyanophyceae*	

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	*Synechococcales*
(ii)	genus	*Synechocystis*
(iii)	species	sp. PCC6803
(iv)	subspecies	GT
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	*Synechocystis* sp. PCC6803

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)
The species *Synechocystis* is found virtually anywhere.

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

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

	Atlantic	(X)	..
	Mediteranean	(X)	..
	Boreal	(X)	..
	Alpine	(X)	..
	Continental	(X)	..
	Macaronesian	(X)	..

(ii) No (.)
(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	(X)
soil, free-living	(X)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	

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

5. (a) Detection techniques
Culture in specific medium

(b) Identification techniques
PCR, Genome sequencing, 16S rRNA

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
…

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
…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Dependent on temperature, light, CO2 and presence of nutrients. Although growth rate could be 0.09 h^{-1} in laboratory conditions, it is not expected to exceed 0.01 h^{-1} in natural ecosystems.

(b) Generation time in the ecosystem where the release will take place:
Dependent on temperature, light and CO2, but otherwise comparable to above.

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

(c) Factors affecting reproduction:
Growth of cyanobacteria is affected by the temperature of the water, the quantity of light and CO2 present, as well as macronutrients such as iron, phosphate and nitrate.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:
not applicable

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:
Growth of cyanobacteria is affected by the temperature of the water, the quantity of light and CO2 present, as well as macronutrients such as iron, phosphate and nitrate. Survival might on the presence of cyanophages

10. (a) Ways of dissemination
Binary fission (cell division)

(b) Factors affecting dissemination
Growth of cyanobacteria is affected by the temperature of the water, the quantity of light and CO2 present, as well as macronutrients such as iron, phosphate and nitrate. Dissemination might also be affected by the current in water.

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)
…, B/../../…

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
Increased production of organic acid glycolic acid.

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
Promoter: Ptrc or Pcpc
The inserted gene in vector # 4 is a phosphatase, the inserted gene in vector # 5 is a carboxylase.

(b) Source of each constituent part of the insert
Promoter: derived from parental organism or synthetic (Ptrc).
Gene inserts is derived from gene synthesis or from amplification of the gene of interest from the donor organism.

(c) Intended function of each constituent part of the insert in the GMO
Promoters are intended to drive gene expression. The phosphatase and carboxylase function in the metabolism of the parental organism and increase production of the organic acid glycolate.

(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	(X)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…
other, specify	algae

2. Complete name

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

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:

(b) 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 (.) Not known (X)

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 (.)
Specify The GMO cannot grow in atmospheric CO2 levels, but needs increased CO2 concentration to grow. This is a well-mentioned risk mitigation strategy for large scale algae growth, as no growth is possible outside the bioreactor. Moreover, it exhibits higher sensibility to high light

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify The GMO cannot grow in atmospheric CO2 levels, but needs increased CO2 concentration to grow. This is a well-mentioned risk mitigation strategy for large scale algae growth, as no growth is possible outside the bioreactor.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (x) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism
GMO remains stable over >100 generations and is expected to remain stable.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(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)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Culture on specific medium.

(b) Techniques used to identify the GMO
PCR with specific primers for the inserted genes

F. Information relating to the release

In fact, there is no release into the environment. The GMO is placed inside a closed bioreactor (contained system), and this system also remains closed during sampling and GMO centrifugation. On this scale, however, containment can no longer be 100% guaranteed, and that is why we opt for the "release to the environment" permit process. However, it remains the case that the chance of actual introduction into the environment is very small. Additionally, the reactor is placed on top of a liquid-tight container on an asphalt site surrounded by a fence, and a dike around it. This means that the level of containment is quite high.

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The large-scale growth and testing of a process to produce organic acid using specific genetically modified (GM) cyanobacteria. The GM cyanobacteria are grown in a contained system, the photobioreactor, and there will therefore be no release into the environment. Only if an incident occurs, namely leakage of the photobioreactor, can the GM cyanobacterium end up in the environment. However, the process is designed so that leakage is avoided.

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 (.) No (X)
If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Oosterhorn 4, 9936 HD Farmsum, The Netherlands

(b) Size of the site (m^2): 5000 m^2
(i) actual release site (m^2): 2500 m^2
(ii) wider release site (m^2): … m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
The site is on an industrial area that is close (~200m) to the Zeehavenkanaal, which is connected to the Eems water. However, we have shown that our GMO cannot

grow in water (see point 8) and number of viable bacteria rapidly declines. Therefore, we do not think any of these waterbodies will be affected.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Unknown, but most likely (aquatic) micro-organisms, but only if the GMO is released into the environment

4. Method and amount of release

(a) Quantities of GMOs to be released:
Unknown. The aimed quantity is zero.

(b) Duration of the operation:
Maximum of 5 years

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

The GMO is placed inside a closed bioreactor (contained system), and this system also remains closed during sampling and GMO centrifugation. On this scale, however, containment can no longer be 100% guaranteed, and that is why we opt for the "release to the environment" permit process. However, it remains the case that the chance of actual introduction into the environment is very small. Additionally, the reactor is placed on top of a liquid-tight container on an asphalt site surrounded by a fence, and a dike around it. This means that the level of containment is quite high.

5. Short description of average environmental conditions (weather, temperature, etc.)
Climate is moderate. Temperature ranges between 0 and 20°C average, depending on the season. There is quite a lot of rainfall; mean monthly precipitation ranges between 40 and 80mm.

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.
Not available

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism
Not applicable

1. Name of target organism (if applicable)

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

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Not applicable

3. Any other potentially significant interactions with other organisms in the environment
Not applicable

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
The GMO cannot grow in atmospheric CO2 levels and needs increased CO2 concentration to grow. Therefore, it cannot grow in the environment. It is unlikely that the organism will revert to the genome of the parental organism as 4 gene deletions have been applied.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
The GMO cannot grow in atmospheric CO2 levels and can therefore only become established inside a photobioreactor or any aquatic environment with light as well as increased CO2 levels. To our knowledge there are no such environments around the site of release.

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
Not applicable

(i)	order and/or higher taxon (for animals)	…
(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

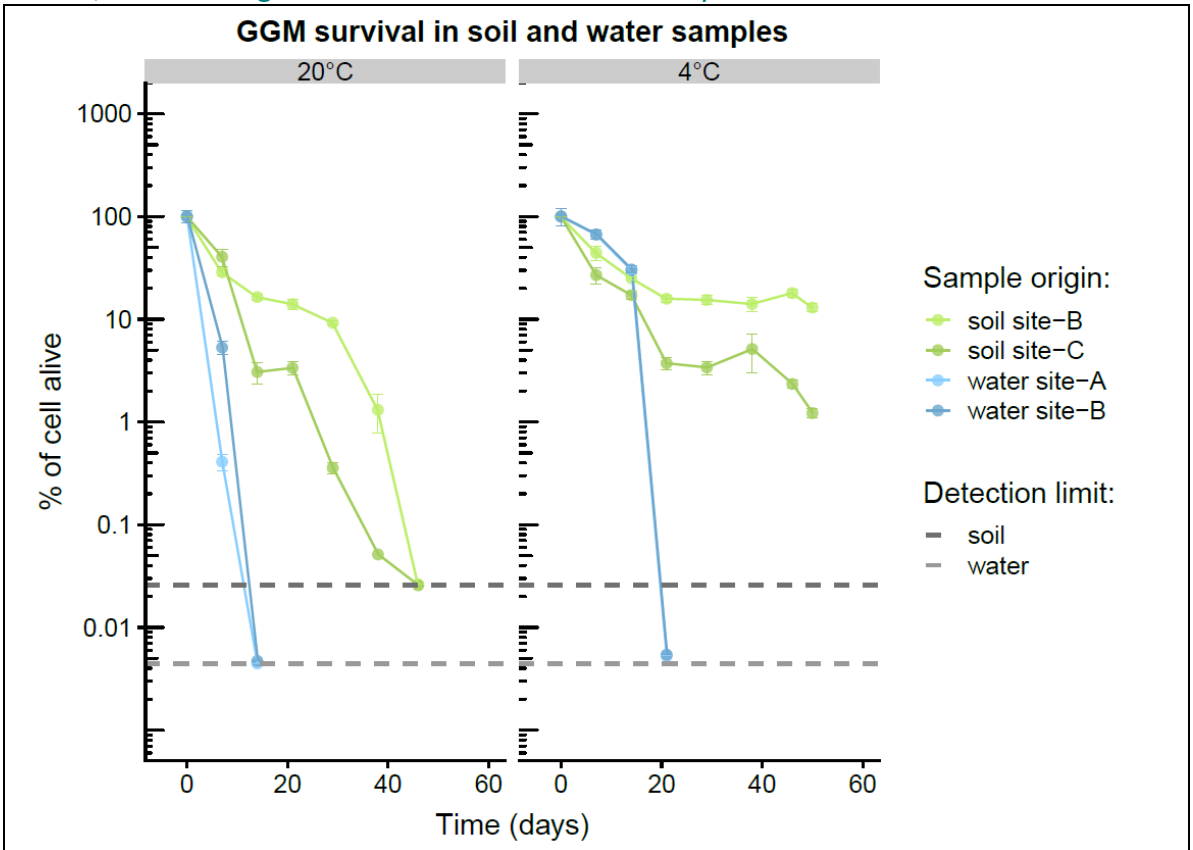
(a) from the GMO to other organisms in the release ecosystem:
Possible for micro-organisms, but unlikely to happen as the GMO is in a closed reactor system which is kept axenic. Any biomass derived from the reactor will be sterilized.

(b) from other organisms to the GMO:
Possible for micro-organisms, but unlikely to happen as the GMO is in a closed reactor system which is kept axenic. Any biomass derived from the reactor will be sterilized.

(c) likely consequences of gene transfer:
likely none.

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.):

We tested the survival of the GMO in different samples from the site of release. A summary of this is shown in figure 1. Inoculation has taken place with a very large number of bacteria, assuming leakage of the total system in a 200m radius (50x), not considering dilution in the water body. The number of bacteria leaking into the environment will likely be many times smaller, if this leakage occurs at all. That chance is very small.



Figuur 1. Survival experiments with the GMO. This figure shows the survival of the GMO as a percentage of the inoculum in various water and soil (soil) samples around the site of release. These samples are placed at room temperature (20 °C) or at 4 °C, with a light supply to enable growth. A GMO with expression of a kanamycin resistance cassette was used for better detection during the test. This cassette has no effect on the growth of the organism and has been removed in the final GMO.

It must be noted that viable cells could only be counted on agar plates grown in an environment of 130x atmospheric CO_2 levels as no colonies could be detected if the plates were placed in a reactor with atmospheric CO_2. From the data in figure 1 it becomes clear that the GMO in the water samples disappears below detection level in a short time (15-20 days), slightly faster at 20 °C than at 4 °C. This is in sharp contrast to the parental organism that shows significant outgrowth in water, especially at 20°C. In the soil samples, the GMO survives around 40-50 days at 20 °C. At 4 °C the GMO persists a little longer, but since no outgrowth is possible, the number of viable bacteria is slowly decreasing. Starting from this inoculum, it will take approximately 290 days to stop detecting a viable cell.

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

Likely none.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
The GMOs will be monitored for growth inside the reactor. Monitoring outside of the reactor is not considered necessary, unless some unexpected event occurs, such as significant leakage of the reactor.

2. Methods for monitoring ecosystem effects
N/A

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

4. Size of the monitoring area (m^2)
2500 m^2

5. Duration of the monitoring
After significant leakage of the reactor, monitoring is performed weekly until no GMO is detected twice in a row in all samples.

6. Frequency of the monitoring
After significant leakage of the reactor, monitoring is performed weekly until no GMO is detected twice in a row in all samples.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
In principle, the site is free from GMOs. The GMO in the bioreactor is killed by a validated method. Each batch is tested for effective inactivation before being discharged into the environment. This data is collected in a logbook

2. Post-release treatment of the GMOs
the GMOs are killed during and after the experiment with a chlorine-containing, an oxidative or a strong alkaline chemical for inactivation. This is a validated method to inactivate cyanobacteria.

3. (a) Type and amount of waste generated
waste will mainly consist of killed GMOs, but since these are concentrated (via centrifugation) it will be a limited volume. We expect to produce approximately 10 m3 of concentrated GMO waste per year and <500 m3 of water without GMO

3. (b) Treatment of waste
The GMOs are killed during and after the experiment with a chlorine-containing, an oxidative or a strong alkaline chemical for inactivation. This is a validated method for killing our GMO, which is also used in our contained use facility. Each batch is tested for killing before being discharged into the environment. This data is collected in a logbook. After this, it is discharged according to guidelines to the rainwater drainage

if permitted. If after sampling it appears that this is not possible then the waste will be processed in the local treatment facility or by an external party.

J. Information on emergency response plans

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

The GMO cannot grow in ambient conditions (20.95% O2 and 0.04% CO2). Nevertheless, during an unintended event, namely leakage of the bioreactor, an attempt will be made as far as possible to limit the release of the GMO into the environment through the following actions:

1. Stopping the flow of liquid in the leaking tube, which limits further release
2. Collect the leaked liquid as well as possible using a vacuum cleaner
3. Inactivation of the GMO in the collected liquid by validated method, addition of 0.06% hypochlorite
4. Monitoring of the possible site of introduction within 24 hours using PCR analysis. This analysis is performed weekly until the GMO is not detected (anymore) twice in succession

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

Collect spilled liquid and inactivate GMO with validated method.

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

It is very unlikely that any plants, animals or soils will become exposed. However, should such events occur, sterilization by autoclave could be performed.

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

The GMO cannot grow in ambient conditions (20.95% O2 and 0.04% CO2). Nevertheless, during an unintended event, an attempt will be made as far as possible to limit the release of the GMO into the environment through the following actions:

1. Stopping the flow of liquid in the leaking tube, which limits further release
2. Collect the leaked liquid as well as possible using a vacuum cleaner
3. Killing of the GMO in the collected liquid by validated method, addition of 0.06% hypochlorite
4. Monitoring of the possible site of introduction within 24 hours using PCR analysis. This analysis is performed weekly until the GMO is not detected (anymore) twice in succession