

FORMAT FOR THE PRESENTATION OF THE RESULT OF DELIBERATE  
RELEASE INTO THE ENVIRONMENT OF GENETICALLY MODIFIED  
HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 10  
OF DIRECTIVE 2001/18/EC

## 1 General information

**1.1 European notification number: B/ES/12/16**

**1.2 Member State of notification: SPAIN**

**1.3 Date of consent and consent number: 20/04/12. Consent by the “Presidente del Consejo Interministerial de Organismos Modificados Genéticamente”.**

## 2 Report status

**2.1 Please indicate whether, according to Article 3 of the present Decision, the current report is:**

- **the final report**
- a post-release monitoring report
  - final
  - intermediary

## 3 Characteristics of the release

**3.1 Scientific name of the recipient organism:**

*Nicotiana tabacum*

**3.2 Transformation event(s) (acronym(s) or vectors<sup>1</sup> used (if transformation event identity not available):**

pL3-thioredoxin f

**3.3 Unique identifier, if available:**

There is not

**3.4 Please provide the following information as well as the field(s) layout:**

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<sup>1</sup> In the case of small-scale field trials where several lines may be tested, the vectors used should be mentioned, which gives insight into the introduced traits and/or genetic elements. In the case of large-scale trials, the number of events notified is limited to only one or a few events.

edge (WT)					
edge	VIR	TRX-503	503	TRX-VIR	edge
edge	503	TRX-VIR	VIR	TRX-503	edge
edge	TRX-VIR	503	TRX-503	VIR	edge
edge (WT)					

Geographical location(s) (administrative region and, where appropriate, grid reference)	Size of the release site(s) ( <sup>2</sup> ) (m <sup>2</sup> )	Identity ( <sup>3</sup> ) and approximate number of GM higher plants per event actually released (number of seeds/plants per m <sup>2</sup> )	Duration of the release(s) (from ... (day/month/year... until... (d/m/y)
Sartaguda (Navarra)	120 m <sup>2</sup> + 40 m <sup>2</sup> non- GM border	300 plants: 80 plants wt and 220 transgenic plants (18500 plants /ha)	From 23/05/2012 to 01/09/2012

(<sup>2</sup>) Specify the size of the GM area and, where appropriate, the size of the non-GM area (e.g. non-GM border)

(<sup>3</sup>) Vectors used

#### 4 Any kind of product that the notifier intends to notify at later stage

4.1 Does the notifier intend to notify the released transformation event(s) as product(s) for placing on the market under Community legislation(s) at a later stage?

Yes (by another juridical entity of the group)  No  Unknown to date

#### 5 Type(s) of deliberate release(s)

Please select the main type(s) (in boxes) as well as subtype(s) of the release(s). In the case of multi-sites, multi-events and/or multi-annual release(s), please provide a general overview of the type(s) of deliberate release(s) which has/have been carried out for the full duration of the consent. Please tick the appropriate type(s):

5.1 Deliberate release(s) for research purposes

5.2 Deliberate release(s) for development purposes

Not applicable

5.3 Official testing

Not applicable

- Variety registration on a national variety catalogue

- DUS (=Distinctness, Uniformity and Stability)
- VCU (=Value of Cultivation and Use)

- Others: (specify): .....

**5.4 Herbicide authorization**

Not applicable

**5.5 Deliberate release(s) for demonstration purposes**

Not applicable

**5.6 Seeds multiplication**

Not applicable

**5.7 Deliberate release(s) for biosafety/risk assessment research**

Not applicable

**5.8 Other(s) type(s) of deliberate release(s):**

(describe) : .....

Not applicable

**6 Method(s), result(s) of the release, management and monitoring Measure(s) in respect of any risk to human health or the environment.**

**6.1 Risk management measure(s)**

**It has not been necessary to apply any risk-management measures. Everything went on as expected.**

*6.1.1 Before the sowing/planting:*

- Clear labelling of the GM seeds (distinct from other seeds/tubers/etc.) (describe).
- **Seeds were germinated in trays at the Institute of Agrobiotechnology. Control plantlets (VIR y 503) and transgenic plantlets (TRX-VIR y TRX-503) of the two varieties were transplanted to the experimental field as previously notified.**
- Segregation during the processing and transport of the seed/planting material (describe the method involved; provide example(s) of containment to prevent spillage during the processing and transport).

All the material was transported to the field in the private car of the principal investigator. No other material was transported at the same time.

- Destruction of superfluous seeds/planting material (describe the method involved).

**Seed formation in transgenic plants was avoided by manual techniques and chemical treatments. Therefore, there was no need to destroy superfluous seed material of transgenic plants.**

**Plants remaining in the plot at the end of assay were manually pulled up. The field was ploughed up for the destruction of plant material and to bury them. Later, field irrigation was performed to promote germination of control seed plants. Several weeks later these plants were destroyed by herbicide treatment.**

- Temporal isolation (specify).

**Not applicable**

- Rotation (specify the previous crop).

**In the previous year (2011) corn plants were growing there**

- Other(s): (specify) .....

**Not applicable**

#### *6.1.2 During the sowing/planting activities:*

- Method of sowing/planting.

**Planting was manually**

- Emptying and cleaning of the sowing machinery on the field of release.

**Not applicable**

- Segregation during the sowing (provide example of containment to prevent spillage during the sowing/planting).

**Not applicable**

- Other(s): (specify)

**Not applicable**

#### *6.1.3 During the period of release:*

- Isolation distance (x meters)

- From sexually compatible commercial plant species.

**There were no other tobacco plants in the surrounding plots.**

- From sexually compatible wild relatives.

**Not applicable. There are not compatible wild type plants.**

- Border rows (with the same crop or a different one, with a non-transgenic crop, x meters, etc).  
**The rows between lines was 0.9 m**  
**The rows between repetitions was 0.6 m**  
**The distance from the closet cultivated plants (corn and fruit trees) was 5 m**
- Cage/net/fence/signpost (specify).  
**Each repetitons was marked by using wooden sticks.**  
**Each experimental unit was indicated by singpost in which the name of the lines was written.**
- Pollen trap (specify).  
**Not applicable**
- Removal of GM inflorescences before flowering (indicate the frequency of removal).  
**Inflorescences from transgenic plants were removed on 07-07-12 and plants were treated with Stomp to avoid lateral sprouting. New developed inflorescences were removed every two weeks until the end of the experiment (01-09-12).**
- Removal of bolters/relatives/hybrid partners (indicate the frecueny of the removal, x metres around the GM field, etc).  
**Not applicable**
- Other(s): (specify).....  
**Not applicable**

*6.1.4 At the end of the release:*

- Harvest/destruction methods (of crop or part of it) / other means (e.g.: sampling)  
**Harvest was performed manually.**  
**Leaves and stalks were harvested, stored in sealed and labeled paper bags. This material was dried in ovens at 45°C in the laboratory and stored for further assays.**
- Harvest / destruction before the ripeness of the seeds.  
**Not applicable**
- Effective removal of plant parts.  
**Not applicable**
- Segregated storage and transport of crop/waste (provide examples of containment to prevent spillage of collected seeds/crops/wastes).  
**Leaves and stalks were harvested, stored in sealed and labeled paper bags. The harvested plant material was transported to the Institute of Agrobiotechnology in the private car of the principal investigator. Only this plant material, properly labeled, was transported in the car.**
- **This material was dried in ovens at 45°C in the laboratory and stored for further assays.**

- Clean up of machinery on the release site.  
**Not applicable**
- Destination of the waste, treatment of waste/ surplus yield/plant residues (describe).
- **Seed formation in transgenic plants was avoided by manual techniques and chemical treatments. Therefore, there was no need to destroy superfluous seed material of transgenic plants.**
- **Plants remaining in the plot at the end of assay were manually pulled up. The field was ploughed up for the destruction of plant material and to bury them. Later, field irrigation was performed to promote germination of control seed plants. Several weeks later these plants were destroyed by herbicide treatment.**
- Post-harvest treatment and cultivation measures on the release site (describe the method for preparing and managing the release site at the end of the release, including cultivation practices).  
**See above.**
- Other(s): (describe):  
**Not applicable**

#### *6.1.5 Post-harvest measures:*

Please indicate which measures were taken on the release site after harvest:

Frequency of visits (average) **Weekly by the Experimental Field personal**

- Subsequent crop (specify).  
**Fallow land**
- Crop rotation (specify).  
**Corn- potato-fallow land**
- Fallow/no crop (specify).  
**Fallow land**
- Superficial soil work / no deep ploughing.  
**Pass of Rotavator**
- False-sowing beds.  
**Not applicable**
- Control of volunteers (specify intervals and duration).  
**Every week the personal working in the experimental field will control the development of tobacco plantlets. If they appear, these plantlets will be destroyed manually. If it is so, they will inform us.**
- Appropriate chemical treatment(s) (specify).  
**Not applicable**
- Appropriate soil treatment(s) (specify).

**Not applicable**

- Other(s) (specify)

**Not applicable**

6.1.6 *Other(s) measure(s): (describe)*

6.1.7 *Emergency plan(s).*

Indicate: **Not applicable**

a) If the release applicable as planned:

- **Yes**
- No (describe for which reason, e.g. vandalism, climatic conditions, etc.)

b) if measures according to the emergency plan(s) (Article 6(2)(a)(vi) and Annex III.B of Directive 2001/18/EC) had to be taken:

- **No**
- Yes (describe)

## **6.2 Post-release monitoring measures**

Due to the fact that the current report format can be used for the final and post-release monitoring report(s), the notifier is asked to clearly make the difference between both types of report through this section 2 of Chapter 6. Please indicate whether

- **The post-release monitoring plan will start** (in the case of a final report, after the last harvest of the GM higher plants) **the week after harvest**

The results of this monitoring are meant to confirm or invalidate earlier assumptions in the risk assessment.

According to the aforementioned cases, please indicate which monitoring measure(s) will be/are/were taken and where (on the release site/near the site (e.g. on fields edges)). Please be aware that all post-release monitoring measures taken during the whole post-release period shall be indicated here.

**Mechanical and chemical treatments have been undertaken to avoid the formation of seeds in transgenic plants. To prevent the improbable situation of permanence of transgenic seeds in the field, measures for seed germination and further plantlet destruction have been undertaken. Additionally, every week the personal working in the experimental field will control the development of tobacco plantlets. If they appear, these plantlets will be destroyed manually. If it is so, they will inform us.**

Specify:

- Monitoring measures within site

Duration: **from September 2012 to May 2013**

Frequency of visits (average): **weekly**

- Observation of resistant relatives. **Not applicable**
- Observation of resistant insects. **Not applicable**
- Control of volunteers (specify intervals and duration). **Every week the personal working in the experimental field will control the development of tobacco plantlets. If they appear, these plantlets will be destroyed manually.**
- Monitoring of gene flow (specify). **Not applicable.**
- Appropriate chemical treatment(s) and/or soil treatment(s). **Not applicable**
- Others (specify). **Not applicable**

- Monitoring measures of adjacent areas:

**Not applicable. In the surrounding, there were not sexually compatible plants.**

Duration:

Frequency of visits (average):

Area monitored:

- Observation of resistant relatives.
- Observation of resistant insects.
- Control of volunteers and/or monitoring of feral populations (specify intervals and duration).
- Monitoring of gene flow (specify).
- Appropriate chemical treatment(s) and/or soil treatment(s).
- Others (specify).

### **6.3 Plan for observation(s)/methods(s) involved**

**We have not observed any adverse effect of the GM on weeds seeds either pests.**

**All the weeds and pests detected were the typical ones for tobacco and affected in the same way to the control and the GM plants.**

**We have not observed any effect of the GM on human health. No one persons handling with those plants (stems and leaves) had any symptom (no rash, no allergy).**

**We have not observed any effect of the GM on animal health.**

### **6.4 Observed effect(s)**

#### *6.4.1 Explanatory note.*

All results of the deliberate release(s) in respect of any risk for human health or the environment shall be stated, without prejudice to whether the results indicate that any risk is increased, reduced or remains unchanged.

The main objectives of the information given in this section are:

- to confirm or invalidate any assumption regarding the occurrence and impact of potential effect(s) of the GMO(s) which was/were identified in the environmental risk assessment,
- to identify effect(s) of the GMO(s) which was/were not anticipated in the environmental risk assessment.

The observed **effect(s)/interaction(s)** of the GMO(s)

- with respect to any risk to human health,
- with respect to any risk to the environment

shall be reported under this section.

Particular attention shall be drawn to unexpected and unintended effect(s).

Indications as regards the effects, that the notifier may have to report, are provided hereunder. The effects have obviously to be considered in the light of the crop, the new trait, the receiving environment as well as the conclusions of the environmental risk assessment, which is carried out on a case-by-case basis.

In order to structure the information and to facilitate an efficient search within the given information, the notifier shall use, as far as possible, specific keywords to fill in the text fields under Chapter 6, especially sections 6.4.2, 6.4.3 and 6.4.4. A most updated list of those specific keywords is available on the Internet at: <http://gmoinfo.jrc.it>.

**As already mentioned, no effect of the GM plants on environment or on human health were detected during the growing period.**

#### 6.4.2 *Expected effect(s)*

**No effects were observed, as expected.**

#### 6.4.3 *Unexpected effect(s)*<sup>2</sup>

**We have not observed any unexpected effect.**

#### 6.4.4 *Other information*

## 7 Conclusion

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<sup>2</sup> Without prejudice to Article 8 OF Directive 2001/18/EC as regards handling of modifications or new information.

**Taking in account the results obtained in 2012 we can conclude that:**

- 1. We have not observed differences between control plants (growing both in the border and in the experimental field) and GM ones.**
- 2. We have observed no effects of the GM plants on environment, human health and animal health. The effect of weeds and pests was the same in both control (WT) and transgenic lines (GM). The handle of the GM did not affected human health.**

**DATE:**

**Pamplona, 8<sup>th</sup> of October 2012**