

Spanish Commission on Biosafety (CNB): Strategy and Challenges in Assessing a Trial with a GM Olive Fly



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Legal Framework for GMOs in the European Union

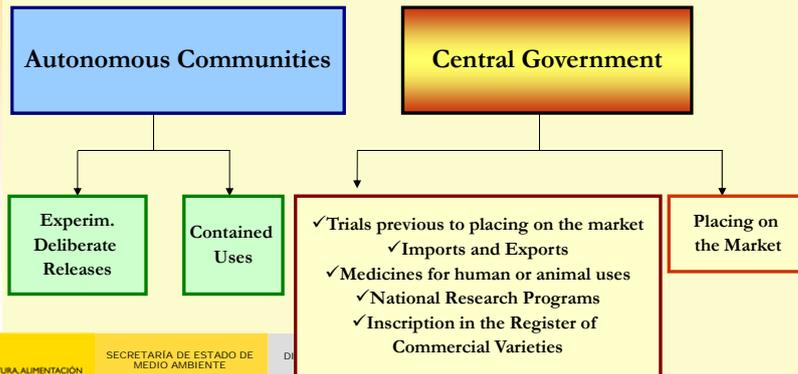
- ✓ Directive 2009/41/EC, on contained use of GMMs.
- ✓ Directive 2001/18/EC, on the deliberate release into the environment of GMOs.
- ✓ Regulation (EC) N° 2309/93: authorization and supervision of medicinal products for human and veterinary use.
- ✓ Council Directive 98/95/CE, on commercialization of seeds.
- ✓ Regulation (EC) N° 1829/2003, on GM food and feed.
- ✓ Regulation (EC) N° 1830/2003, on the labelling and traceability of GMOs.
- ✓ Regulation (EC) N° 1946/2003, on transboundary movements of genetically modified organisms.
- ✓ Regulation (EC) N° 65/2004, establishing a system for the development and assignment of unique identifiers for GMOs.

GMO: “An organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination, using those techniques which are foreseen in the regulations”

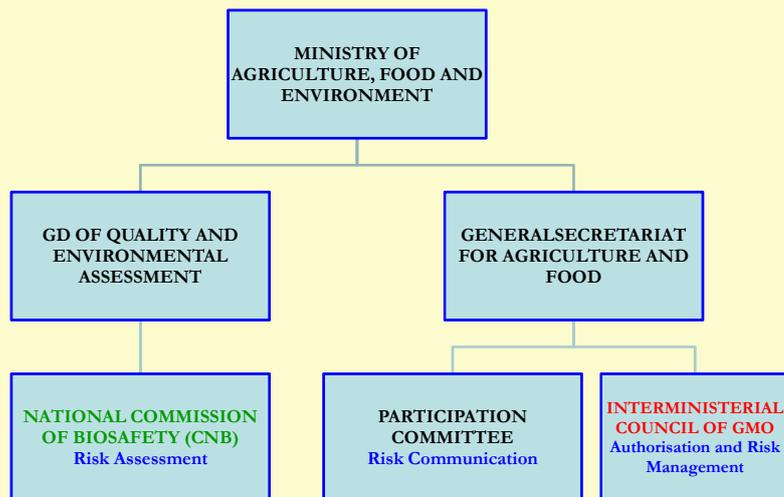


Spanish legislation and Competent Authorities

- ✓ National Act 9/2003 establishing the legal regime governing the contained use, deliberate release and marketing of genetically modified organisms (GMOs).
- ✓ Royal Decree 178/2004 approving the General Regulations governing the development and implementation of Act 9/2003.



Central Government: Competent Bodies



ERA for Field Trials with GMOs in Spain

- Requirements of Annex II of Directive 2001/18/EC and Commission Guidance notes 2002/623/EC
- *Step-by-step* and *case-by-case* approaches
- Direct, indirect, immediate and delayed effects
- EFSA Scientific Opinion on Guidance on the environmental risk assessment of genetically modified animals (Section 4.2. GM Insects):
 - Persistence and invasiveness of GM insects, including VGT
 - Horizontal gene transfer
 - Pathogens, infections and diseases
 - Interactions of GM insects with target organisms
 - Interactions of GM insects with NTOs
 - Environmental impacts of the specific techniques used for the management of GM insects
 - Impacts of GM insects on human and animal health
- Control and Monitoring
- The results of these trials have to be reported to the CNB (any effects not previously identified during the ERA?)



Trial with a GM Olive Fly (strain OX3097D-Bol)

- ❖ **Deliberate release of a GM insect:** applied twice (December 2012 and May 2015)
 - ✓ **Title of the project:** 'Evaluation of the mating competitiveness, longevity and persistence of the OX3097D-Bol Olive fly in a field setting'.
 - ✓ **Notifier:** Oxitec Ltd. Oxfordshire (UK)
 - ✓ **The primary objectives** of the study were to:
 - ✓ Establish the performance of the OX3097D-Bol olive flies when competing with wild males for wild females.
 - ✓ Gather information on the longevity of the OX3097D-Bol olive fly in a field environment.
 - ✓ Evaluate different release methods for adapting release rates to wild prevalence.
 - ✓ **Proposed period of release:** April 2013-April 2014; July 2015-July 2016
 - ✓ **Duration of the operation:** The releases would last for a period of 8 weeks (once or twice a week).
 - ✓ **Aim of the trial:** Matings of released GM males with wild females would result in the survival of male GM olive flies and the death of all females. The evaluation of the fluorescent trait in the male larvae will indicate mating ratios.

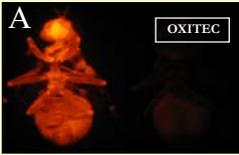
- ❖ **Why to test GM olive fly in Spain?**



Trial with a GM Olive Fly (strain OX3097D-Bol)

❖ **Characteristics of the GMO:** Two traits have been introduced on a single inserted DNA segment:

- The female-specific conditional lethality by the expression of the **tTAV protein** (*E.coli/Herpes simplex virus*).
 - The tTav protein confers the conditional genetic sexing trait because is lethal in absence of tetracycline.
 - The tTav expression repressing normal cell activity.
- The **DsRed2 protein** as a fluorescent marker (from *Discosoma Sp.*; Coral).



OX3097D WT

Red Filter



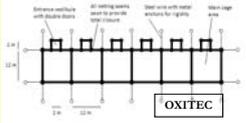
OX3097D 1 2 WT 3 4

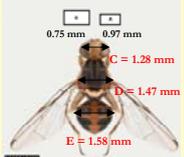
Bright Field

Trial with a GM Olive Fly (strain OX3097D-Bol)

❖ **Characteristics of the field trial:**

- Containment site in Tarragona (Catalonia Region)
- The total release site would be split into 6 small treatment sites, all totaling an area less than 1000 m².
- The release site would be in managed agricultural land in which there will be **no grazing livestock**.
- The releases of GM olive flies would only be within the netted areas (6 small cage areas, 144 m² each, with 4 olive trees inside). Initial proportion of releases 1:1 for GM olive fly and wild populations.
- The net perimeter was buried 0.5 m into the ground to avoid the entrance of insects or little mammals.
- The entrance (only authorized personnel) would be through a vestibule with double doors where personnel change its clothes.
- Traps would be deployed before, during and post release both inside and outside the netting.
- They proposed networks recommended to prevent the movement with the greater insects length of 1 mm: Openings in these networks (0.75 mm x 0.39 mm).

A = field netting
B = lab netting
C = head width
D = thorax width
E = abdomen

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Trial with a GM Olive Fly (strain OX3097D-Bol)

❖ Control and monitoring of the release:

- Pre-release monitoring would commence after approval is given and in advance of the trial.
- Monitoring during the trial
 - ✓ Standard olive fly traps would be set up at intervals outside for the presence of any escaped OX3097D-Bol olive flies in vicinity of the release sites and within of the netted release sites.
 - ✓ Fluorescent scoring of samples of olive flies from the traps would detect the persistence of the GM olive fly in the environment.
 - ✓ Immature stages may be monitored by sampling infested olive fruit.
 - ✓ The vertical transfer of genetic material can be detected in the olive fly by screening for fluorescence.
 - ✓ Presence of the genetic material in predators which consume the GM Olive flies may be detected by molecular methods.
- Post-release treatment of the site
 - ✓ The release site would be monitored for up to 4 weeks post-release.
 - ✓ If GM olive flies remain at the release site at the end of the monitoring period then an approved insecticide would be used.
 - ✓ The GM males are not expected to persist in the environment and they would not live beyond their own short lifespan.
- Waste management
- Emergency measures



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Trial with a GM Olive Fly (strain OX3097D-Bol)

❖ Environmental Risk Assessment by the notifier:

- Genetically stable over 3 years (approx. 45 generations).
- Persistence and invasiveness: the mode and rate of reproduction has not been altered. The intended effect of the modification is to confer a selective disadvantage on the females (i.e. death) in subsequent generations.
- Gene transfer from the GM males to the wild females is likely to result in the death of the female progeny in the release site.
- Pathogenicity: the tTAV protein and fluorescent marker DsRed2 have been compared to known toxic and allergenic sequences and it didn't find any sequence homologous to a toxin or allergen (bioinformatic analysis).
- Interactions with target organisms: all female offspring resulting from crosses will not survive to adulthood as there is a lack of the dietary supplement required to suppress the lethality trait in the release environment.
- Interactions with non-target organisms: no toxic effects are anticipated in the event that the GM fly is eaten by predators present at the release site.
- Impact on biogeochemical processes: the two novel expressed proteins are likely to breakdown into constituent amino acids at the release site.
- Human and animal health: there will be a negligible risk associated with this release.

Conclusion by Oxitec: 'No significant interactions are anticipated. The modification is limited to the olive fly by reproductive barriers'.

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Trial with a GM Olive Fly (strain OX3097D-Bol)

- ❖ **Risk Assessment by the National Commission on Biosafety (CNB)**

Other additional information supplied by the notifier:

 - Likelihood of horizontal transfer of genetic material due to the mobility of the transposable element (TE): **additional scientific literature supplied on the lack of mobility of *piggyBac* TE** (Silva and Kidwell, 2000; Handler, 2001; Arensburger et al., 2011; Kidwell, 1992; Thibault et al (2004), etc.): **negligible risk or very low.**
 - Additional scientific literature which support the lethality of the 100% of the females offspring: **only 1 study (Ant et al., 2012) carried out with few hundred flies. A new study with at least a thousand of insects was requested to estimate better the efficacy: 100% homozygous penetrance and 99.9% heterozygous penetrance.**
 - More information about levels of tetracycline into the environment: **difficult to assess, very low levels and very sensitive to light and temperature: negligible risk or very low.**
 - Additional data on the safety of the t-TAV protein for non-target organisms: **The initial data supplied on non toxic effects was related to experiments with other species (mosquito and guppy fish), although they expressed the same tTAV and DsRed2 proteins. Additional studies on specific olive fly terrestrial predators and parasitoids: no significant differences.**

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Trial with a GM Olive Fly (strain OX3097D-Bol)

- ❖ **Contacts with Stakeholders:**
 - **Spanish Authorities on Plant Health and Farmers Associations:** they considered these GM olive flies like a very interesting alternative strategy for a biological pest control (with the exception of organic farmers)
 - **Sector of olive oil:** they showed deep concern about the possible impact on the market by introducing this transgenic fly (remnants of GM flies inside the olives, EU labeling requirements would be needed?)
 - **NGOs:** environmental organizations were strongly opposed to the release of GM insects into the environment under research and marketing purposes



Decision awaited on genetically modified insect trial
By Victoria Gill
Science reporter, BBC News
© 20 November 2015 | Science & Environment

Oxitec Still Pursuing Trial Release of GM Olive Flies in Spain
By Julia Wiltse
Olive Oil Times

Quiéren liberar en España una mosca modificada genéticamente
La liberación de insectos modificados genéticamente en el medio es un experimento polémico que conlleva a toda Europa en un laboratorio al aire libre

Spain Turns Down Oxitec GM Flies Release Again
Published on Aug 7 2015 - 11:37pm by Sustainable Pulse

TEST BIOTECH
UK company plans to release genetically engineered flies in Spain

Reactions in the Media

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Trial with a GM Olive Fly (strain OX3097D-Bol)

❖ **CONCLUSIONS:**

- **The CNB ask for additional requirements:** double net, management in the case of incident during transport, use of a vacuum cleaner in the vestibule and heat treatment of bags, one year post-release monitoring, measures for removal and decontamination of olives, etc.
- **The CNB would request additional studies during the trial:**
 - Molecular analysis of olive flies inside the trial net to detect potential genetic trait inefficacy or resistance.
 - To repeat the study carried out with parasitoids to confirm that no significant adverse effects are expected.
 - To consider additional studies on the potential effects of this GM insect on specific olive fly predators and parasitoids.
- **The Catalonian C. A. was concerned about the reaction in the media and public opinion, the potential impact on the environment and commercial consequences.**
- **On 5 August 2015, Oxitec formally withdrew the application.**
- **In October 2015 the Catalonian C. A. sent to Oxitec a notice of application's withdrawal and asked the CNB to close the assessment procedure.**

Psytalia concolor

More debate among Authorities and transparency with Stakeholders is needed



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'Good news! GM fly will not fly in Spain'

LA VANGUARDIA

'Rejected the plan to release transgenic flies in Tarragona'

Rechazado el plan para liberar moscas transgénicas en Tarragona

El plan de la empresa británica Oxitec era usar estos insectos modificados genéticamente contra la plaga de las moscas del olivo

La empresa británica ha decidido retirar a solicitud tras las exigencias y los obstáculos puestos por las administraciones españolas.

GREENPEACE Greenpeace España

Inicio Por dentro Trabajamos en ¿Qué puedes hacer tú? Multimedia Actualidad Noticias

Inicio > Blog > ¡Buenas noticias! La mosca transgénica no volará en España

¡Buenas noticias! La mosca transgénica no volará en España

Entrada de blog por Leticia Ferrerías - agosto 7, 2015 a las 9:38 ➤ Agregar un comentario

Después de una semana en las frescas Llanas pensaba que el resto del agosto iba a ser sofocante, pero, ha llegado una enorme brisa de aire fresco. Aquí la Generalitat de Catalunya desmaga, aplicando el principio de precaución, **el plan de la empresa británica Oxitec de liberar miles de moscas del olivo transgénicas**. ¡Bueni! ¡un fuerte aplauso por esta valiente y sensata decisión!



La OC30970-BOL, la mosca transgénica, debería quedarse de una vez en el laboratorio que la concibió. No podemos seguir jugando con la naturaleza y las inversiones deben dirigirse a buscar soluciones que no pongan en peligro la biodiversidad, el equilibrio ecológico del planeta y, por ende, la salud de las personas.

La empresa quería llevar a cabo un experimento durante un año y que ocuparía un área, cubierta con redes, de un máximo de 2.000 metros cuadrados, cerca de Tarragona. **¡Según los planes se liberarían hasta 3.000 moscas transgénicas por semana!** La OC30970-BOL incorpora en su ADN genes de organismos marinos, bacterias, virus y otros insectos. **Una mosca del olivo más**



Thank you!