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EFSA guidelines on environmental risk assessment of GM animals, including insects

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Abstract: Future applications for the marketing of genetically modified organisms (GMOs) in the EU may include food/feed products derived from genetically modified (GM) animals, and the release of GM animals, including insects, into the environment. Efforts towards the development of GM insects to control insect vectors of human diseases and manage agricultural pests have progressed substantially with various GM insect × trait combinations in the development pipeline. As a proactive measure, the scientific GMO Panel of the European Food Safety Authority (EFSA) has developed guidelines on: (1) the risk assessment of food/feed derived from GM animals including animal health and welfare aspects; and (2) the environmental risk assessment (ERA) of living GM animals, including insects, released into the environment for commercial purposes. The latter assists applicants in the preparation and presentation of their applications by describing the elements and data requirements for a structured ERA of GM insects consistent with the current Directive 2001/18/EC. A dedicated Working Group (WG) was involved in the elaboration of the ERA guidelines on GM insects, which underwent a public consultation before their finalisation. Relevant comments received were considered by the WG. The WG also took into account the external scientific report on GM insects commissioned by EFSA (Benedict *et al.*, 2010). This report provided background information by mapping relevant fields of expertise and identified essential elements to be considered when performing an ERA of GM insects. Content and stakeholder involvement for the EFSA guidelines are presented.

Key words: EFSA, environmental risk assessment, GM animals, GM insects, public consultation

EFSA remit on genetically modified organisms

Genetically modified organisms (GMOs) and derived food/feed products are subject to a risk analysis and regulatory approval before entering the market in the European Union (EU). In this process, the role of the European Food Safety Authority (EFSA) is to independently assess and provide scientific advice to risk managers on risks that the consumption or the deliberate release into the environment of a GMO may pose to human and animal health and the environment. EFSA was created in 2002 in response to multiple food crises that caused considerable public concern in Europe about food safety and the ability of regulatory authorities to fully protect consumers. EFSA's remit for the risk assessment of GMOs encompasses GM plants, GM microorganisms and GM animals, and involves the assessment of their safety for humans, animals and the environment (Waigmann *et al.*, 2012). Besides ensuring a high level of protection of human and animal health and the environment, EFSA responsibilities also include communicating independent scientific advice to its principal partners, stakeholders and the public in a timely, clear, accurate and meaningful way. By communicating risks in an open and transparent manner, EFSA aims to continue to bridge

gaps between science and the consumer, and to build consumer and public confidence in risk assessment and the safety of the EU food chain (Deluyker & Silano, 2012).

The decision on whether a certain risk is acceptable and whether a GMO or a derived product can be placed on the EU market is not part of the risk assessment itself, but part of the wider risk analysis. Such decisions are taken by risk managers, such as the European Commission and EU Member States, as they involve political, socio-ethical and economic considerations on the acceptability of risks and benefits.

The EFSA scientific advice on the risk assessment of GMOs is given through its scientific Panel on GMOs (referred to hereafter as EFSA GMO Panel), which adopts scientific outputs by qualified majority. Currently (April 2016), the EFSA GMO Panel consists of 18 scientific experts who come from EU research institutes, universities or risk assessment bodies, and is supported scientifically by several Working Groups (WGs) and the EFSA GMO Unit. Besides members of the EFSA GMO Panel, each WG is composed by additional independent experts, who are invited on an *ad hoc* basis. With this pool of experts EFSA can apply a broad range of expertise for the risk assessment of GMOs.

The main focus of EFSA in the field of GMOs lies in the evaluation of GMO market registration applications (referred to hereafter as GMO applications) and in the development of risk assessment and monitoring guidelines (reviewed by Waigmann *et al.*, 2012; Devos *et al.*, 2014). These guidelines help applicants in the preparation and presentation of their applications by describing elements and data requirements for the risk assessment and monitoring of GMOs. EFSA uses these guidelines in the evaluation of risk assessments and post-market environmental monitoring plans submitted by applicants as part of their GMO applications. EFSA also provides scientific advice in response to requests from the European Commission on specific issues.

GM animals

Ongoing scientific developments suggest that future GM animal applications may be prepared across a range of species to improve traits related to disease resistance, growth enhancement, sterility, population suppression, cold tolerance, dietary performance and ornamental uses (reviewed by Benedict *et al.*, 2010; Cowx *et al.*, 2010; Henry *et al.*, 2010). Broadly, GM traits would be used to improve the efficiency of beneficial animal production, and help control harmful animals.

GM insects

From the early 2000s, GM insects have been developed to control insect vectors of human diseases such as malaria and dengue by means of vector population suppression, prevention or replacement. GM *Aedes aegypti* mosquitoes with an inherited lethality trait, which prevents offspring maturing, are presently being used in the field for dengue control in Brazil (Carvalho *et al.*, 2015), and US regulators have found no significant environmental impact, including animal and human health, for an investigational field trial linked to the development of preventative release programs in the USA, where *A. aegypti* is a potential threat (FDA, 2016). Vector control currently relies heavily on the use of insecticides around human habitations or on environmental management such as drainage, which can have significant impacts on health or environmental quality, respectively. It is also important to improve the effectiveness of vector control, as it is often required in conditions where the economic and social situation makes it difficult to apply conventional control methods consistently and well. As an alternative to vector suppression or prevention, replacement techniques could involve

introducing GM insects with traits that limit the ability of the vector population to transmit disease (Alphey, 2014). Replacement can also lead to suppression, for example with gene-drive approaches that produce male-biased progeny.

GM insects are also being developed to suppress populations of agricultural pest species (such as Mediterranean fruit fly or Medfly, *Ceratitis capitata*; olive fruit fly, *Bactrocera oleae*; and Diamondback moth, *Plutella xylostella*) in several crops (fruits, olives, cabbage) through reduced fertility, inherited pre-reproductive lethality in offspring (Benedict *et al.*, 2010). Outbreaks of Medfly have been successfully eradicated in many areas using radiation-induced sterility in the sterile insect technique for many decades (Dyck *et al.*, 2005). In areas where incursions of Medfly have regularly occurred, preventative releases of sterile male flies have greatly reduced the frequency of outbreaks. GM traits can offer an alternative to radiation or chemically induced sterility where these reduce mating competitiveness of released insects, and open new options such as strong male-bias in offspring as novel forms of sterile insect technique.

Other modifications involve GM insects with enhanced stress tolerance, performance or fitness characteristics which would contribute to the enhancement of agricultural production systems (Benedict *et al.*, 2010). Examples include cold-tolerant bees to enhance pollination at lower temperatures and disease-resistant silkworms for more efficient production of silk.

The range of potential uses of GM insects creates some challenges in giving guidance across applications. For example, some traits, such as those in replacement techniques to reduce pathogen transmission, depend on persistence. The choice of appropriate comparators needs to take into account comparisons at an individual organism level and also at a population level. Unlike in animal production systems, where the conventional cow or fish is an acceptable animal, in the case of vector or agricultural pest species, the conventional animal is itself directly harmful to humans, livestock and plants. Furthermore, GM insects used for pest or vector control will inevitably be released extensively into the wild rather than being contained and managed on farms or aquaculture enclosures in production systems. Risk assessments need to take account of these particular objectives and circumstances while adhering to the overall principles and intentions of the Directive 2001/18/EC.

EFSA guidelines on environmental risk assessment of GM animals

No GM animals or derived products from GM animals are presently approved for the EU market, nor have any GM animal applications been submitted to EFSA. However, future GMO applications may include the marketing of food and feed products derived from GM animals, and the release of GM animals, including companion animals, into the environment. Therefore, as a proactive measure, EFSA was requested by the European Commission to develop guidelines for applicants on: (1) the risk assessment of food/feed from GM animals and animal health and welfare aspects (EFSA, 2012); and (2) the environmental risk assessment (ERA) of GM animals deliberately released into the environment for commercial purposes (EFSA, 2013).

The objective of the EFSA (2013) guidelines is to provide recommendations to applicants and risk assessors on how to consider and address potential adverse effects of GM animals on the environment, human and animal health, and to outline the necessary data requirements to perform a comprehensive ERA.

General ERA principles

The EFSA (2013) guidelines are structured around two main chapters: (1) one main chapter addressing the generic issues to consider throughout the whole ERA of GM animals, and (2) one detailing the specific areas of risk to consider during the ERA of GM fish, GM insects and GM mammals and birds. Whereas the generic considerations apply to GM animals regardless of the group they belong to, the chapters concerning the areas of risk are specific to each group of GM animals in order to account for the specificities of the animal and types of applications related to each group. Applicants should take into account generic and specific considerations simultaneously throughout their ERA of GM fish, GM insects and GM mammals and birds.

The EFSA (2013) guidelines are specifically related to the objectives and general principles of the ERA of GMOs as referred to in Annex II of Directive 2001/18/EC. The guidelines advocate that ERAs are conducted in a scientifically sound and transparent manner based on the identification and analysis of the differences between the GM animal and the appropriately selected non-GM comparator. In addition, each GM animal must be assessed independently on a case-by-case basis, meaning that the supporting dataset may vary depending on the type of animal and the GM trait(s), the potential receiving environment(s) and the intended use(s).

The structure of the ERA was developed around the concept of a comparative safety assessment, based on the principles outlined in Directive 2001/18/EC. This central part of the ERA process starts with the crucial first step of problem formulation which facilitates a structured approach to identifying potential risks and scientific uncertainties. Problem formulation is then followed by five further steps to characterise and subsequently manage risks. It is also stressed that ERAs are iterative and should examine previous conclusions in light of new information in a transparent manner.

Applicants must address seven areas of potential risk: (1) persistence and invasiveness of the GM animal, including vertical gene transfer; (2) horizontal gene transfer; (3) interactions of the GM animal with target organisms; (4) interactions of the GM animal with non-target organisms; (5) environmental impacts of the specific techniques used for the management of the GM animal; (6) impacts of the GM animal on biogeochemical processes; and (7) impacts of the GM animal on human and animal health

The guidelines also highlight a number of cross-cutting considerations that should be factored into the full ERA process. These include which non-GM animals to use as comparators, the use of appropriate surrogates if necessary, and recommendations on identifying environments into which GM animals are likely to be released.

Generic ERA considerations

The EFSA (2013) guidelines describe several generic considerations throughout the whole ERA, such as:

- The identification and characterisation of the receiving environments representative of areas which are likely to be exposed to the GM animal;
- The selection of non-GM comparators that needs to be justified to support and validate the outcomes of the comparative approach;
- The possibility to use non-GM surrogates, with phenotypic characteristics similar to the GM animal, as valuable tools to gather data on possible biotic and abiotic interactions with the animal;
- An effective experimental design, modelling and general statistical principles outlined in the guidance, such as the specification of the effect size and power analysis;

- The assessment of long-term effects pertaining to the placing on the market of the GM animal, mainly through desk studies taking into consideration available information (e.g. literature, monitoring data, meta-analysis, data with non-GM surrogates, modelling);
- The qualitative and, where appropriate, quantitative assessment of uncertainties inherent to the risk assessment owing to data gaps, variability of data across environments and over seasons, and data extrapolations.

Specific ERA considerations

Potential adverse effects that a GM animal might have on its receiving environment include (EFSA, 2013):

- Changes in fitness of the GM animal that could result in changes in persistence, competitiveness and invasiveness of the GM animal, and might lead to environmental harm. Consequences of gene transfer between the GM animal and recipient (non-GM) animals in the receiving environments also require consideration;
- Effects pertaining to the transfer of recombinant DNA from the GM animal to other organisms (e.g. soil bacteria), without being the offspring of that organism;
- Effects of the GM animal on target organisms (e.g. pathogens (e.g. bacteria, virus, fungi) or pests);
- Effects of the GM animal on non-target organisms, including the effects on populations of e.g. competitors, prey, hosts, symbionts, predators;
- Impacts of the specific techniques used for the management of the GM animal (i.e. changes in the breeding, rearing and production systems such as changes of dietary regimes in aquaculture);
- Impacts of the GM animal on biogeochemical processes (e.g. incorporation of dead GM animal into soil and water systems influencing organic matter decomposition, food web structure, biological diversity in soil or water ecosystems, etc);
- Impacts of the GM animal on human and animal health through other routes of exposure than ingestion or intake (e.g. ocular, nasal, dermal contact and inhalation).

The successive steps to characterise the risk should be applied to each of the above mentioned areas of risk. For each area of risk, applicable protection goals and assessment and measurement endpoints should be specified for use in ERA.

External scientific reports

In order to gather the necessary background information for an ERA of GM animals, EFSA commissioned separate external scientific reports covering three different animal groups, with the aim of: (1) identifying GM animals or derived products that may be the subject of an EU market approval application within the next decade, and relevant scientific disciplines and fields of expertise that might support an environmental risk assessment of GM animals; and (2) defining risk assessment criteria for GM fish, insects, and mammals and birds.

The considerations provided in the external scientific reports by Cowx *et al.* (2010) for GM fish, Benedict *et al.* (2010) for GM insects, and Henry *et al.* (2010) for GM mammals and GM birds served as a basis for the identification of scientists with relevant expertise, and the development of the guidelines.

EFSA GMO Panel Working Groups

To address the complex mandate of the European Commission and elaborate the guidelines on the ERA of GM animals, EFSA established three WGs of the EFSA GMO Panel, focusing on fish, insects, and mammals and birds, respectively.

Public consultation

In line with its policy on openness and transparency, EFSA consulted EU Member States, the scientific community, stakeholders and the public during the development of the guidelines via online public consultations. Interested persons were invited to submit electronically their comments on the draft guidelines.

Given the relative novelty and complex nature of the topic, the engagement with EU Member States, the scientific community, stakeholders and the public was considerable. EFSA received 720 comments from 25 interested parties (including national risk assessment bodies, research institutes, non-governmental organisations, universities, industry, associations and individuals). The insect portion of the guidelines received the greatest number of comments in the consultation. Some contributors considered the proposed ERA guidelines useful, but in some cases insufficient. Further examples of issues and evidence were requested by some respondents. Others found the guidelines excessive or too precautionary. A recurrent comment was that the guidelines are not sufficiently prescriptive in some cases, leaving room for interpretation. There has been some criticism that consultations on highly complex topics like this lead to responses that are likely to be limited to stakeholders with considerable technical knowledge and interest, possibly failing to pick up value judgements that may be held by stakeholders without such high levels of technical knowledge.

All the scientifically relevant comments received during the consultations were considered by the three WGs when finalising the guidelines. Many of the contributions added to and improved the scientific quality and clarity of the guidelines. Along with the EFSA (2013) guidelines, EFSA published a technical report listing all the comments received from the public consultation and outlining how these were taken into account in the final guidelines (http://www.efsa.europa.eu/en/supporting/pub/428e?wtrl=01). Appendix B of the consultation report describes the comments received and the responses of the WGs.

Conclusion

The EFSA (2013) guidelines establish a harmonised framework for the ERA of GM animals, including insects. The guidelines should assist applicants in the preparation and presentation of their applications by describing elements and data requirements for the ERA and monitoring of GM animals. Moreover, EFSA will use these guidelines in the evaluation of ERA and post-market environmental monitoring plans submitted by applicants as part of their potential future GM animal applications.

Three dedicated Working Groups of the EFSA GMO Panel were involved in the elaboration of the ERA guidelines, which underwent a public consultation before finalisation. Relevant comments received from EU Member States, the scientific community, stakeholders and the public were considered by the WGs. The WGs also took into account the external scientific reports on GM fish, insects, mammals and birds commissioned by EFSA. These reports provided the necessary background information in the area of the ERA of GM animals by mapping relevant fields of expertise and identifying essential elements to consider when performing an ERA.

EFSA will continue to closely monitor the technical progress and scientific developments in the field of GM animals, and may take initiatives to further update the contents of its guidelines accordingly in the future.

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Disclaimer

The views expressed in this publication are those from the authors and do not necessarily represent the official position of EFSA. EFSA assumes no responsibility or liability for any errors or inaccuracies that may appear.

References

Alphey, L. 2014: Genetic control of mosquitoes. Annual Rev. Entomol. 59: 205-224.

Benedict, M., Eckerstorfer, M., Franz, G., Gaugitsch, H., Greiter, A., Heissenberger, A., Knols, B., Kumschick, S., Nentwig, W. & Rabitsch, W. 2010: Defining environmental risk assessment criteria for genetically modified insects to be placed on the EU market. Supporting Publications 2010, EN-71: 1-200.

Carvalho, D. O., McKemey, A. R., Garziera, L., Lacroix, R., Donnelly, C. A., Alphey, L., Malavasi, A. & Capurro, M. L. 2015: Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLoS Negl. Trop. Dis. 9(7): e0003864.

Cowx, I. G., Bolland, J. D., Nunn, A. D., Kerins, G., Stein, J., Blackburn, J., Hart, A., Henry, C., Britton, J. R., Copp, G. & Peeler, E. 2010: Defining environmental risk assessment criteria for genetically modified fishes to be placed on the EU market. Supporting Publications 2010, EN-69: 1-187.

Deluyker, H. & Silano, V. 2012: The first ten years of activity of EFSA: A success story. EFSA J. 10: 1-6.

Devos, Y., Aguilera, J., Diveki, Z., Gomes, A., Liu, Y., Paoletti, C., du Jardin, P., Herman, L., Perry, J. N. & Waigmann, E. 2014: EFSA's scientific activities and achievements on the risk assessment of genetically modified organisms (GMOs) during its first decade of existence: looking back and ahead. Transgenic Res. 23: 1-25.

Dyck, V. A., Hendrichs, J. & Robinson, A. S. 2005: Sterile insect technique. Principles and practice in area-wide integrated pest management. Springer, Heidelberg, Germany.

EFSA 2012: Guidance on the risk assessment of food and feed from genetically modified animals and animal health and welfare aspects. EFSA J. 10: 1-43.

EFSA 2013: Guidance on the environmental risk assessment of genetically modified animals. EFSA J. 11: 1-190.

FDA 2016. Oxitec mosquito – Preliminary finding of no significant impact. URL: http://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/UCM487379.pdf. Cited 7 Apr. 2016.

Henry, C., Kerins, G., Blackburn, J., Stein, J., Smith, G. C., Eyre, D., Roy, S., Parrott, D. & Hart, A. 2010: Defining environmental risk assessment criteria for genetically modified (GM) mammals and birds to be placed on the EU market. Supporting Publications 2012, EN-107: 1-187.

Waigmann, E., Paoletti, C., Davies, H., Perry, J., Kärenlampi, S. & Kuiper, H. 2012: Risk assessment of Genetically Modified Organisms (GMOs). EFSA J. 10: 1-7.