GeneWatch UK response to the EFSA consultation on Guidance on the environmental risk assessment of genetically modified animals

August 2012

TITLE
Line 2, footnote 3.
The Working Group on Insects is heavily influenced by the company Oxitec, making EFSA open to allegations of conflicts-of-interest. This does not inspire public confidence. Panel member Michael Bonsall includes his collaboration with Oxitec in his declaration of interests but states incorrectly that Oxford University receives no financial benefit from its relationship with the company: the University is in fact an investor in Oxitec (GeneWatch UK, 2010). Mike Bonsall and Jeff Bale are both members of the UK Advisory Committee on Releases to the Environment (ACRE), where they will presumably both comment on the Guidance they have drafted. It is unclear why Dr Bonsall was required to leave the room when Oxitec's genetically modified diamond back moths were discussed by ACRE (ACRE, 2011) whilst he is allowed to play a central role in drafting EFSA's guidance for the same GM insects. Panel member John Mumford declares his role in the risk assessment project Mosqguide for GM mosquitoes, but does not mention that Oxitec is a partner in this project. Panel member George Christophides declares his role in the FP7 INFRAVEC project, but does not mention that Oxitec is a partner in this project; Romeo Bellini is also a partner in the INFRAVEC project (undeclared). Luke Alphey (an advisor to the panel) declares his role as Chief Scientific Officer at Oxitec and that he has investments in the company and patents on its technology. His declaration notes that Syngenta is funding Oxitec to develop GM Lepidoptera (a large order of insects that includes moths and butterflies). Ex-Syngenta staff who are now working for Oxitec include Oxitec's CEO, Regulatory Affairs Manager and Head of Business Development (http://www.oxitec.com/who-we-are/our-team/). Oxitec's Chair and one of its other Board members are also ex-Syngenta. Oxitec and Syngenta appear to have unduly influenced the draft Guidance, see comments on line 176.

ABSTRACT
Line 17: It is unclear why other animals e.g. amphibians, molluscs, crustacea are omitted: this means the Guidance is far from comprehensive, even for GM animals envisaged in current patent applications (e.g. AquaBounty, 2011). Due to the extensive errors, omissions and inconsistencies noted in this response (including a need to identify mechanisms through which the many issues which fall outside EFSA's remit can be addressed), there will be a need for re-consultation once revisions have been made. The vast extent of the animal kingdom means that revised guidance should not attempt to encapsulate more than one genus at a time. The scale of the task required to provide meaningful guidance on even a small proportion of possible applications is enormous. For example, there is a current project to sequence the genomes of 5,000 insect and related arthropod species over the next 5 years (i5k: http://arthropodgenomes.org/wiki/i5K). This will create the potential for all these species to be genetically modified in a wide variety of ways.

SUMMARY
Line 48: It is unclear to the reader why other animals, e.g. amphibians, molluscs, crustacea, are omitted, despite their inclusion in patent applications (AquaBounty , 2011). The draft Guidance should be clear about whether it is attempting to cover all GM animals or not.

Line 57: The summary refers to selection of receiving environments but there is virtually no content in the consultation relating to this or any description of how this might be controlled. For example, the UK company Oxitec is working on genetically modified (GM) *Aedes albopictus* mosquitoes (Labbé et al. 2012) which are an invasive species currently being monitored due to concerns they will spread tropical diseases in the EU (ECDC, 2009). There is no discussion of whether releases of GM *Aedes albopictus* would be allowed in parts of the EU but not others and if so, whether they could possibly

be restricted to particular receiving environments. There are concerns about how in practice this could be achieved (Angulo & Gilna, 2008a &b).

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Lines 109-111: A section on Choice of comparators for GM mammals and birds is missing: this should be included.

Lines 138 to 144. Specific sections on Pathogens, infections and diseases; Abiotic interactions; and Impact on non-GM animal health and welfare have been omitted from the insects section, despite being included in other sections (fish, mammals and birds). There is no scientific justification for omitting these sections since insects are vectors for many human and animal diseases. In addition, applications such as Oxitec's RIDL (Release of Insects carrying a Dominant Lethal genetic system) insects will result in large numbers of dead GM larvae in the environment, since this is a late-acting lethality system which works mainly at the larval stage (Phuc et al., 2007). There is clearly potential to impact on abiotic processes, as well as on both human and animal health: these sections should therefore be added to the contents. Further, these issues were identified as important in the Expert report to EFSA (Umweltbundesamt, 2010) and their assessment is required by Annex II of Directive 2001/18/EC (EC, 2001).

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

Line 165; It is unclear to the reader why EFSA has produced draft guidance relating to issues so far outside its mandate and expertise, which is to assess and communicate on all risks associated with the food chain. The release of GM insects, fish or mammals (e.g. rabbits) to alter ecosystems through population suppression or altered disease transmission or pollination (bees) may have some impacts on the food chain, but many impacts (such as on disease incidence in humans, or on endangered species or environmentally protected areas) go way beyond this. The Guidance is so long and poorly written, with many inconsistencies between sections, that it is difficult to comment fully on it: the number gaps in content (see above comments on Table of Contents), missing references, and framing of the content (particularly problems with applying the concept of toxicological exposure assessment, rather than an ecosystem approach) need to be addressed and a new consultation exercise then needs to be conducted. Although there is useful detail provided on some aspects in some sections, this does not feed through to the document as a whole, and the quality of the GM insects section is particularly poor.

Line 176: The establishment of the working groups did not follow an open and transparent process and the Insects Working Group is unduly influenced by Oxitec (see comments on Line 2). Mike Bonsall, a member of the working group who is one of Oxitec's collaborators "*admitted he was an author of the GM animal draft safety guidelines. He confirmed there had been pressure from the biotech industry to get the rules written so that work on the safety case could begin*" (Clover, 2012). There are issues regarding public trust in EFSA being so closely associated with a company that has been widely criticised for not following existing regulations and ethical requirements. Oxitec has failed to correctly follow the process required by Regulation (EC) 1946/2003 on transboundary movement of genetically modified organisms for its exports of GM mosquito eggs for open release to date, or to obtain informed consent for its experiments (GeneWatch UK, 2012). Oxitec did not send the transboundary notification documents to either the UK or EC authorities in a timely way, with the result that the risk assessments were not publically accessible before the experiments took place: the dates of receipt by the UK authorities are documented in GeneWatch UK (2012); GeneWatch UK received an email from DG SANCO on 28th November 2011 which states: "*It seems that at the beginning Oxitec was not well aware of the obligation to copy this information not only to*

the UK authorities but to the Commission as well (it should not be the UK authorities forwarding this information to the Commission but rather for the exporter to transmit it directly not only to the competent authorities of the country from which the GMO is exported but also to the Commission)". This oversight seems rather surprising given that the company's (ex-Syngenta) head of regulatory affairs has been actively involved in the Cartegena Biosafety Protocol discussions. The risk assessments are of a poor standard and provide inadequate information (GeneWatch UK, 2012; Reeves et al., 2012). The company has not succeeded in publishing its results from its population suppression experiments in the Cayman Islands, despite submitting them to Science in January 2011 (Enserink, 2011): only the results of its small preliminary trial have been published. Oxitec has been strongly criticised for failing to seek informed consent for its releases of GM mosquitoes overseas (Enserink, 2010): it is widely recognised that informed consent is needed for releases of genetically modified disease vectors (Macer, 2003; Macer, 2005). Oxitec's influence on the draft Guidance has clearly been substantive. "Sterile" GM insects (a term favoured by Oxitec in its PR materials) have been referred to more than 20 times, despite the fact that this term is misleading (Reeves et al., 2012): Oxitec's GM insects contain a lethality trait that is partial (i.e. not fully penetrant), conditional (dependent on the absence of tetracycline which is used as a chemical switch to allow breeding in the lab) and late-acting (normally at the larval stage) and many applications are female-killing only (there is also a flightless-female mosquito): further, resistance to the trait may develop over time. Transgenic "sterile" insects are referred to in Guidance on "Confined Field Release" of transgenic arthropods issued by the North American Plant Protection Organisation (NAPPO, 2007a), written with assistance from Oxitec (NAPPO, 2007b). The NAPPO guidance and the use of the term "sterile" appears to be part of an attempt by Oxitec to claim that its insects have "biological containment" and therefore that open releases of the insects should not count as open releases of GMOs for the purpose of regulation (despite the facts that they mate with wild females; and that some of the transgenic insects will survive). Oxitec has also made a failed attempt to release a US strain of GM diamond-back moth in the UK under contained use regulations on the spurious claimed grounds that the genetic trait amounted to biological containment (Oxitec 2011b; ACRE, 2011; HSE, 2011a&b; DEFRA, 2012; FERA, 2012). The concept that many risks are only or mainly relevant to replacement strategies and not to Oxitec's population suppression approach is also reiterated in this draft Guidance more than 20 times, rarely with any scientific justification. Only 13 scientific references are mentioned in the GM insects section (and six of these relate only to horizontal gene transfer). In lines 4092-4094 a sentence has been inserted which completely changes EFSA's remit and the purpose of Environmental Risk Assessment (presumably this sentence was added at Oxitec/Syngenta's request). It is surprising that other members of EFSA's GM insects working group (such as the Vice Chair of the EFSA GMO panel, Patrick Du Jardin) did not seek to prevent this attempt to change EFSA's remit through the back door.

Lines 185-186: Issues relating to traceability, labelling and co-existence are a key element of risk management. For example, in the US, the fact that large-scale releases of Oxitec's GM fluorescent bollworms are incompatible with organic standards appears to have led to this programme being halted: this would suggest that open releases which are compatible with co-existence rules are unlikely to be achievable in Europe (Reeves et al., 2012). Traceability of food crops containing GM insect eggs and GM larvae is also critical to monitoring human health effects and to preventing dispersal into receiving environments where releases have not been authorised.

Line 187: Whilst it is correct to state that ethical and socio-economic issues are outside EFSA's remit, the issuing of draft Guidance before such issues are addressed is premature. The production of GM mammals, including pets and farm animals, raises many important ethical issues (GeneWatch UK, 2002) and much of the harm to animal welfare (e.g. aborted foetuses) is caused at the production stage of GM mammals. For example, in the case of production of transgenic pigs with increased levels of omega-3 fats in their meat, a total of 1,633 reconstructed embryos were transferred into 14

pigs; 12 early pregnancies were established, and five of them went to term leading to 12 (ten alive and two dead) male piglets being born by either caesarean section or natural delivery (Lai et al., 2006). Ethical concerns about this process have been completely ignored. In the case of GM fish, the North Atlantic Salmon Conservation Organisation (NASCO) states in the Williamsburg Declaration: "*In view of the current lack of scientific knowledge on the impact of transgenic salmonids on wild salmon stocks, the use of transgenic salmonids should be considered a high-risk activity. There should be a strong presumption against any such use*" (NASCO, 2006). There is strong opposition to the introduction of GM fish from fishing organisations and producers in the EU. Yet EFSA's starting point seems to be that the production and deliberate release of GM animals is ethical and acceptable. Oxitec (which is acting as an advisor to the Working Group on Insects) has already been strongly criticised for failing to seek informed consent for its releases of GM mosquitoes overseas (Enserink, 2010) and it is widely recognised that informed consent is needed for releases of genetically modified disease vector species (Macer, 2003; Macer, 2005). Yet the Guidance does not even mention informed consent as an issue that must be addressed. Food safety, consumer acceptability and trade issues associated with the use of GM agricultural pests have also been ignored (see comments on lines 267-272) as have the implications for plant pest control regulations.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

Lines 195-211: It is hard to understand why the EC has requested much of this report which falls so far outside EFSA's food safety role. It is clear that the biotech industry has exerted pressure to adopt guidelines which would allow the introduction of GM fish and insects to the EU market (see comment on Line 176) but EFSA's focus is on risks to the food chain (EC, 2002). Whilst the original remit was to build on work done in the context of Codex Alimentarius (i.e. covering food safety standards) the later revisions to the mandate for the report extend way beyond this e.g. to population suppression techniques intended to engineer whole ecosystems, or alter disease transmission or pollination. The release of GM mosquitoes in an attempt to alter disease transmission, for example, has nothing to do with the free movement of food and feed within the EU. Although some aspects (e.g. potential contamination of the food chain with GM eggs, larvae or adult insects) do fall within EFSA remit, these aspects have been deliberately ignored and explicitly excluded from any form of consultation: see comments on lines 185-186 (traceability and labelling) and 267-272 (risks of ingestion of GM insects).

Lines 228-230: Whilst non-food/feed uses may clearly impact on the food chain in various ways, many impacts may not be on the food chain but on ecosystems, disease transmission, pollination etc. EFSA's mandate (EC, 2002) states that in order to avoid duplicated scientific assessments and related scientific opinions on genetically modified organisms (GMOs), the Authority should also provide scientific opinions on products other than food and feed relating to GMOs as defined by Directive 2001/18/EC (1) and without prejudice to the procedures established therein. However, the entire mandate is predicated on the basis that EFSA exists in order to ensure the effective operation of the internal market for food and feed. The link between some proposed applications (especially the release of GM disease vectors and pets) and EFSA's remit is tenuous at best. Further, EFSA has not established expertise in many of the relevant areas.

ASSESSMENT

Lines 231-236: Numerous important requirements such as plant pest regulations are omitted here (see also comments on Section 2.2.1).

1. **Scope of this document**

Lines 239-241: The mind-set of the entire document appears to be based on extending an approach applied to GM plants to GM animals. The underlying concept appears to be that GM animals may replace non-GM animals in specific production systems by individual commercial producers; and that the aim of the risk assessment is to ensure that these do not introduce harms above certain levels (to be determined by the applicant). However, the broadening of the remit of the guidance (see comments on Terms of Reference) means this may not be appropriate to many of the potential applications. For example, large scale experimental releases of Oxitec's GM fluorescent bollworms have already taken place in the USA: these are not constrained to a single farm or production system. Releases of Oxitec's GM mosquitoes (*Aedes aegypti*) are also now taking place on a large scale in Brazil. At what point do these types of applications count as "placing on the market" (the releases are being conducted via third parties in Brazil)? How will transboundary impacts be dealt with? How will potentially very different impacts in different receiving environments be dealt with (for example, releasing a species of GM insect pest in an area where it is not established may risk it becoming established there)? Similar population suppression approaches may be applied to fish, molluscs, amphibians and other animals in future (Aquabounty, 2011) yet the Guidance is largely silent on the issues raised.

Lines 248-250: The guidance is full of contradictory information regarding the distinction between commercial and experimental uses: this needs to be clarified with reference to the requirements of Directive 2001/18/EC (EC, 2001). For example, lines 1226-1229 imply that open release experiments will not be allowed prior to commercial approval; whereas line 5859 implies that open field studies should be undertaken for mammals and birds, provided the potential environmental risks of such studies are considered. A whole Section (3.5) discusses experimental studies and Section 3.4 emphasises the use of GM surrogates in order to avoid open experiments with the GM animal itself. Yet, since no guidance is envisaged for experimental purposes it is unclear how these environmental risks will be considered and taken into account when applications for open releases for experimental purposes are made. Reference should be made to the 'step by step' principle in paragraph (24) of EC (2001) which requires containment to be reduced only gradually step-by-step "*only if the evaluation of earlier steps in terms of the protection of human health and the environment allows the next step to be taken*". Member states should be aware that large numbers of animals could potentially be released as part of experiments: open release experiments using Oxitec's GM mosquitoes in Brazil have to date used 10 million GM mosquitoes, and larger numbers are planned. It would be helpful to have clarified here whether such releases would count as placing on the market in the EU: Directive 2001/18/C seems to suggest that they would since "*placing on the market means making available to third parties, whether in return for payment or free of charge*" (Article 2, paragraph (4), EC, 2001). If so, the claim that "*release for experimental purposes*" is not covered by this Guidance may need to be revised, since some experiments may count as placing on the market. It is worth noting that Part B notifications (deliberate release for any other purpose than for placing on the market) under Directive 2001/18/EC are decided by Member States but insects, fish and many mammal or bird species may become widely dispersed and potentially move into the territory of another Member State as the result of an experimental release. In view of Oxitec's repeated attempts to claim that its RIDL technology is equivalent to "biological containment" (see comments on line 176) it is perhaps worth reiterating here that the requirement for regulation as a contained use application i.e. that stringent containment limits contact with the population and the environment (Paragraph (4), Article 2, EC, 2001) is not met by Oxitec's RIDL insects, nor conceivably by any other population suppression approach that might be attempted using GMOs (for insects, fish, birds or mammals). Mating with a wild species cannot be regarded as limited contact.

Line 250: Transboundary notification requirements (EC, 2003) require the exporter to provide "*A previous and existing risk assessment report consistent with Annex II of Directive 2001/18/EC*". This Guidance should state explicitly whether or not it is intended to apply to these requirements.

Oxitec's compliance with the transboundary notification requirements to date has been extremely poor (GeneWatch UK, 2012).

Lines 250-252: Some aspects of traceability, labelling and co-existence are an essential part of risk management, see comments on lines 185-186. Risk management is repeatedly discussed in this document, as is post-market monitoring (which also requires traceability).

Lines 259-263: Why are crustacea, molluscs, amphibians not included? (see comment on line 48). Why are issues included which fall outside EFSA's remit? (see comments on lines 195-211).

Line 266: Why is the use of GM animals for production of pharmaceuticals excluded? Whilst EMEA may approve pharmaceutical products from GM animals it does not consider environmental impacts (which still require an Environmental Risk Assessment) or accidental impacts on the food chain. The guidance should clarify whether all GM animal products e.g. low-lactose or high omega-3 or human proteins in cows' milk will require approval by EMEA, and clarify more specifically which traits could as "pharmaceutical production" for the purposes of this guidance (e.g. where is the line drawn between nutraceuticals and pharmaceuticals?). If some or all of these applications are to be included, re-consultation is necessary so that consultees know what they are being consulted about.

Lines 267-272: In its previous consultation on risk assessment of food and feed from genetically modified animals EFSA stated explicitly that "*Insects and other invertebrates <u>were not taken into account</u>, with the exception of honey bees that are used in agricultural practice*" (EFSA, 2011). This statement was repeated in the final Guidance (EFSA, 2012a). In its response to the consultation, GeneWatch highlighted that GM insects and invertebrates (including GM bees) raised a whole range of additional issues which could not be properly considered in this document and required separate in-depth consideration (GeneWatch UK, 2011). Yet now, EFSA appears to be implying that accidental intake (ingestion) of GM insects not intended for food and feed <u>is</u> included under this guidance. This is an important issue because the ingestion route may be significant in many GM insect applications. For example, Oxitec's RIDL insects will give rise to very large numbers of GM insect eggs and larvae potentially entering the food chain, since the late-acting lethality system causes most of the offspring to die at the late larval stage (Phuc et al., 2007): this has already been a concern with Oxitec's proposal to release GM diamond back moths in the UK, because of concerns that GM eggs and larvae will contaminate food crops such as cabbages and broccoli (GeneWatch UK and GM Freeze, 2012; Spelman, 2012). Oxitec's GM olive flies contain a late-acting lethality trait which means that they are expected to die mostly at the pupal stage (Ant et al., 2012), when olive flies remain within the olive. Oxitec expects these dead pupae to be treated as an 'adventitious presence' under EU law (Ant et al., 2012) but it is hard to see this being either publicly acceptable or compatible with food safety legislation. Details are not yet published for Oxitec's GM tomato leaf borers (Morrison et al., 2011) but it is likely that dead GM larvae will also remain within the tomato fruit. Failure to consider food safety and trade issues for GM insects is a particularly important omission because international guidelines do not cover this either (Codex Alimentarius, 2008): this means there has been no discussion of the implications for consumers and international trade. These issues were highlighted in the expert report to EFSA (page 98) which expressly mentioned the prospect of food such as fruits being contaminated with GM eggs and larvae (Umweltbundesamt, 2012). Further, the population suppression approach involves the release of very large numbers of mostly male GM insects to mate with wild females: release ratios to date have been up to 54 GM mosquitoes to wild mosquitoes and production in Brazil is being scaled up to 2.5 million mosquitoes a week (PAT, 2012). If the population suppression strategy is applied to e.g. Mediterranean fruit flies (*Ceratitis capitata*) – one of the species on which Oxitec is working, and which it might wish to release in the EU – releases of millions of flies could contribute to the transfer of human pathogens from faeces to fruit (Sela et al., 2005). This aspect (increased ingestion of transferred pathogens), as

well as ingestion of GM insects directly, is completely ignored in the EFSA Guidance (EFSA, 2012a) despite the claim here that ingestion risks have been dealt with there. Other proposed uses of population suppression (for fish, insects, crustacea, molluscs, amphibians or mammals) could also pose risks to the food chain; as could insects released for other purposes (e.g. pesticide-resistant or pest-resistant GM bees; disease-resistant mosquitoes). EFSA guidance on risk assessment of food and feed from genetically modified animals considers whether the GM animal may be more susceptible to pathogens, but ignores the potential for the releases of large numbers of GM pests (whether GM insects, fish or mammals e.g. carp, rats or the theoretical 'sterile rabbit' included in the consultation) to spread pathogens: this is a result of the failure to adopt an ecosystem approach. It is difficult to understand why EFSA has focused much of this guidance on issues outside its remit and/or expertise whilst failing to provide any guidance on issues that <u>are</u> within its remit.

2. Strategies for the ERA of GM animals

Line 304: Should also refer to the requirement for an ecosystem approach (CBD, undated). This is essential to consider the risks of deliberate or accidental releases. A comparative approach plays a role but is more important in the comparison of a single GM animal with a single non-GM animal and may not help to ascertain the risks of e.g. a large release of GM fish or insects.

Line 307: Intended effects may include significant effects on ecosystems, such as a significant reduction in the population in the population of a particular pest species or disease vector (in population suppression approaches), or the replacement of a population (e.g. replacing a mosquito population with one which is a less effective vector of disease).

Line 312-314: In the case of large-scale deliberate (or accidental) releases (e.g. of GM insects, fish), secondary effects may include altered populations of competitors, predators or prey and effects on human or animal immunity due to altered disease transmission. Longer-term effects can include development of resistance to the GM trait or evolution of viruses in response to changes in the disease vector.

2.1 Different steps in the Environmental Risk Assessment

Line 329: Should say: on the receiving environments and human health.

Line 338: Should cite the conclusions required in the case of GMOs higher than plants (D.1) from Annex II of Directive 2001/18/EC (EC, 2001): 1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s). 2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s). 3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species. 4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable). 5. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens. 6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s). 7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed. 8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in

the vicinity of the GMO release(s). 9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs.

Lines 338: The proposed approach pays insufficient attention to points 4, 5 and 6 in part D.1 Annex II of Directive 2001/19/EC, cited above, which refer to direct and indirect interactions between the GMO and target and non-target organisms and humans, and effects which may be immediate or delayed. Addressing these issues requires an ecosystem-based approach (CBD, undated).

2.1.1 Step 1: Problem formulation (including identification of hazard and exposure pathways)

Lines 357-357: This section should refer at the outset to the need for an ecosystem-based approach (CBD, undated).

Lines 360-361: Should also refer to health-related legislation since many relevant organisms are vectors of human and animal diseases.

Line 362 and 370-372: Table 1 is deficient in many respects. Examples of major omissions include: (1) the omission of the International Plant Protection Convention (IPCC) and related EU legislation (EC, 2000); (2) the Helsinki Declaration, which requires informed consent to medical experiments, and the Oviedo Convention: both relevant to releases of GM disease vectors (Macer, 2003; Macer, 2005). Oxitec has also run into difficulties with its plans for open releases of GM diamond-back moths in the UK, due to its failure to consider plant pest regulations (HSE, 2011a&b; DEFRA, 2012; FERA, 2012). Many relevant conventions e.g. covering marine protection, are also omitted from Table 1, as are animal health requirements (e.g. EC, 2006). In the UK (as an example) the Health Protection Agency has as one of its functions to prevent the spread of infectious disease, and any programmes to control vectors of disease using GM approaches are likely to require scrutiny by it. Plans and programmes by public authorities to release large numbers of GM animals e.g. insects e.g. for population suppression across multiple farms or fields may require a Strategic Environmental Assessment (EC, 2001b)

Lines 363 to 369: It is not only the characteristics of the GM animal but the characteristics of the programme for its deliberate release (e.g. numbers, location) that can cause harm or adverse effects on human health or the environment. A GM animal may be less harmful than its wild counterpart (e.g. it may be partially sterile and less fit, and therefore less invasive) but still cause significant harm because (1) if it is a harmful organism (e.g. disease vector, invasive species) releasing a less-fit version may still cause harm; (2) the impacts of releases on ecosystems are intended to be significant (a large change to the population) and can have harmful knock-on effects (including increases in harmful competitors, or rebounds in numbers due to complex interactions). These concerns are in addition to any differences between the GM and non-GM organism that may in themselves be harmful. These issues are recognised in Annex II, part D.1 of Directive 2001/18/EC (EC, 2001) but not adequately covered here.

Lines 386-391: The phrase “exposure pathways” makes sense in toxicology but becomes meaningless in complex ecosystems. Directive 2001/18/EC (EC, 2001) requires consideration of complex interactions between the GMO, predators, prey, competitors, pathogens, humans etc. (see comment on line 338). This requires an ecosystem approach (CBD, undated) which cannot be reduced to a simple question of exposures. For example, release of GM *Aedes aegypti* mosquitoes, a vector for dengue and other viruses, can have complex effects on populations of *Aedes albopictus* mosquitoes (also a vector of dengue and other viruses), including possible increases in the population of the latter, due to reduced competition between larvae (Bonsall et al., 2010). This

poses a potential risk, since *Aedes albopictus* is in invasive species which can cause dengue epidemics (Beech et al., 2009; GeneWatch UK, 2012). However, the term "exposure pathway" is not really meaningful to encapsulate this risk: what does the term exposure mean in this scenario? Possible pests and pathogens associated with the ecosystem as a whole (not just the GM animal) need to be considered, as for example in the scenario described a disease carried by a competitor species (rather than the GMO) might increase as a result of the releases and thus cause adverse impacts on human health.

Lines 412-417: This paragraph underpins a fundamental misconception that permeates this entire document i.e. that it is the difference between the GM animal and its wild counterpart that matters ("a minimum level of difference between the GM animal and its conventional counterpart that may lead to harm"). This may be relevant for some applications (e.g. replacing a GM cow with a non-GM cow), but many applications for open release are likely to involve GM animals which would not normally be allowed to be released into the wild and which are intended to alter entire ecosystems (not just to replace non-GM animals in a particular production system with GM animals). Examples include a wide variety of species intended to be used in the population suppression approach to reduce numbers of disease vectors, plant pests and invasive species: e.g. GM mosquitoes (Phuc et al., 2007); GM agricultural pests (Morrison & Alphey, 2012); GM fish, crustacea, molluscs and amphibians (Aquabounty, 2011; Nowak, 2002; Thresher, 2008) and perhaps mammals (the theoretical example of 'sterile' GM rabbits given in this document, but perhaps also other pest species such as rats). These animals will not in general be sterile but have a genetically engineered form of conditional lethality: i.e. sterility may be partial, late-acting and conditional (because a system which over-rides lethality is needed to breed the animals in the lab). Many insect applications are also female-killing only (i.e. partial, late-acting, conditional and sex-specific). Other potential applications include insects or other animals with altered disease transmission properties; non-native species of bees engineered to be pest- or pesticide-resistant or to have other supposedly useful characteristics (e.g. pollination). With many such applications it is not the difference between the GM and non-GM animal that causes the potential harm but the complex response of the entire ecosystem (including both unintended survival and spread of the GM organism and knock-on effects such fluctuations in species numbers or increased transmission of viruses). For example, extinction of one species can have knock on effects on other species (Sanders & van Veen, 2012). It is vital that consideration of any such releases takes account of (1) existing legislation on the release of invasive species (e.g. plant pest regulations) and disease vectors; (2) the need for an ecosystem-based approach to any assessment, which recognises that a dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit (CBD, undated).

Lines 418 to 440: This section seems to be cut-and-pasted from a similar approach used for plants where, for example, exposure of non-target organisms to toxins from Bt plants is one of the main considerations. These steps are a poor fit to deliberate or accidental releases of large numbers of GM fish, insects, birds or mammals, which may disperse and mate with wild species and potentially cause adverse effects via interactions with ecosystems. The ultimate objective of this list should be to enable conclusions to be reached regarding all the potential impacts from the release of GMOs other than higher plants identified in part D.1 of Annex II of Directive 2001/18/EC (EC, 2001).

Line 423: Annex II of Directive 2001/18/EC (EC, 2001) requires identification of the characteristics of GMO and releases (C.1), including the intended release or use including its scale; the potential receiving environment; and the interaction between these. By omitting the characteristics of the receiving environment and release programme from consideration here, the Guidance risks missing important aspects of the analysis. For example, a population suppression approach for a particular pest, using releases of partially/conditionally sterile GM fish or insects would almost certainly not be

considered in receiving environments were the pest was not already established. Further, many of the risks would depend on interactions between multiple species, rather than on some direct characteristic (such as toxicity) of the GMO itself.

Line 427: The use of the term “exposure pathways” is too restrictive as it does not identify the potential harms due to ecosystem interactions identified in part D.1 of Annex II of Directive 2001/18/EC (EC, 2001). See comment on lines 386-391.

Lines 429-440: These steps are over-simplistic for many of the relevant applications and take no account of the potential hazards of conducting the experiments to measure the envisaged endpoints. See comments on lines 248-250. Annex II of Directive 2001/18/EC (EC, 2001) requires the ERA to assist in drawing conclusions on, *inter alia*, potential immediate and/or delayed environmental impact on interactions affecting levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens. GM fish and insects will not sit still in a field but swim or fly and mate. Predicting the consequences of releases will require a thorough understanding of natural ecosystems (including humans and viruses) to develop and validate computer models of such systems. Taking a precautionary approach (as required by the Directive) means that it will not be good enough simply to set endpoints, conduct releases, and wait see if the predictions are correct. A pre-requisite to conducting any releases must be a good understanding of the natural system and how it might be disrupted by the introduction of the GMO.

2.1.2 Step 2: Hazard characterisation

Line 457: Should also refer to harm to human and animal health.

Line 459: Should refer to potential adverse impacts on human and animal health, not just on the environment.

2.1.3 Step 3: Exposure characterisation

Line 468: Change “Exposure characterisation” to “Characterisation of potential impacts”.

Lines 468-486: As noted in comments on lines 386-396 the concept of exposure characterisation is too narrow to capture the wide range of potential adverse effects on the environment and human and animal health associated with significant changes to ecosystems: a term such as “Characterisation of potential impacts” would be better. For example, releases of GM mosquitoes in a population suppression approach may be only partially or temporarily effective at suppressing populations. In the case of dengue vectors, a partial reduction in mosquito numbers in dengue-endemic areas can lead to an increase in cases of dengue hemorrhagic fever (the more severe and often fatal form of the disease) (Thammapalo et al., 2008; Nagao & Koelle, 2008). Such potential negative impacts on human health are not captured in a methodology which focuses on exposure to the GM organism: they are not caused by exposure to the GM organism but by the complex interactions between the release programme for the GMO, ecosystems, humans and disease. Annex II of Directive 2001/18/EC (EC, 2001) is very clear that such interactions must be considered (see list in D.1, especially points 4, 5 and 6), but the process adopted here is too narrow to do this. Exposure characterisation is only a part of the characterisation of potential impacts. This issue is recognised to some extent in Section 3.2 but this recognition of complexity is not reflected here.

2.1.4 Risk characterisation

Lines 496-500 provide an inadequate characterisation of uncertainties. Characterising the potential direct and indirect interactions between the GMO and target and non-target organisms (including competitors, prey, hosts, symbiots, predators, parasites and pathogens) and human health (as required by Directive 2001/18/EC) is a potentially mammoth task for mobile organisms such as insects and fish, and mobile mammals (such as rats or rabbits) and birds. It is inconceivable that such a task can be based merely on "extrapolations": it is likely that complex environmental models will be needed, with multiple alternative assumptions explored in alternative scenarios and a need for a thorough understanding of natural ecosystems to define concepts (i.e. write the model equations) and determine input parameters (see comments on Section 3). Further research will then be needed to validate the models i.e. to establish that they have sufficient predictive value to be fit for purpose. The use of unvalidated models that are not fit for purpose would likely give rise to wrong predictions with potential adverse consequences.

Lines 501-504: Multiple scenarios will need to be explored, including more than one worst-case scenario. This is because different model assumptions will give very different answers. For example, dengue virulence in mosquitoes can be selected for by release mosquitoes genetically-modified to block transmission, reduce biting, or increase mortality, but the evolutionary trade-offs that lead to the virus become more virulent as a result of the GM mosquito releases depend on the assumptions in the model (Medlock et al., 2009). The potential for such adverse impacts on human health would need to be ruled out before any releases were allowed (on the ethical basis of "do no harm"), but doing so would be a major task due to the extent of the uncertainty. This paragraph again focuses misleadingly on "level of exposure" which is only one part of problem formulation if major risks are indirect or result from interactions rather than direct exposure to the GMO.

Line 506: "Exposure characterisation" is too limited. Add: characterisation of interactions between the GMO and target and non-target organisms (including competitors, prey, hosts, symbiots, predators, parasites and pathogens) and human health (as required by Directive 2001/18/EC).

2.1.5 Risk management strategies

Line 518: Infertility can reduce direct risks (e.g. exposure to any toxin in the GM animal) but methods of limiting reproduction are also envisaged as a means to make a major change to ecosystems e.g. by releasing large numbers of GM insects with a conditional lethality trait (Oxitec's RIDL mosquitoes and agricultural pests) or by releasing GM invasive species e.g. carp (Thresher, 2008) to breed with and reduce wild populations. This method could potentially be applied large numbers of other species (AquaBounty, 2011). This paragraph incorrectly implies that infertility is only a method of reducing risks: in reality it may reduce some direct risks related to survival of the GM organism, but introduce or increase other indirect risks such as the potential to crash populations of some species, with knock-on effects on the rest of the ecosystem. The Guidance is generally poor on recognising the risks associated with such population suppression approaches, which might be applied in future to insects, fish, mammals and birds. Directive 2001/18/EC requires that these risks due to ecosystem interactions are assessed.

Line 527: There is no such thing as GM sterile mosquitoes: this term should be avoided. Oxitec's GM mosquitoes have a conditional lethality trait: this is conditional because it relies on tetracycline as a chemical switch to allow breeding in the lab; partial because it does not have full penetrance; and late-acting i.e. the insects are not sterile but mate and reproduce with most dying at the late larval stage (in the absence of tetracycline) (Phuc et al., 2007). Many applications are also female-killing only (i.e. sex-specific). The use of the term sterile is misleading because it implies there is no exposure to female biting GM mosquitoes or prospect of survival and breeding of the GM mosquitoes in the environment, which is incorrect.

Line 545: It is questionable whether the applicant is best placed to devise its own worst-case scenarios when its aim is to get its product on the market. Alternative conceptual models, which might identify unexpected risks (e.g. Medlock et al., 2009) require time and resources to develop, which requires independent funding. It is not clear where such capacity and expertise currently resides: clearly not with EFSA.

2.2 Information to identify potential unintended effects

Line 2.2 Add: In addition, Directive 2001/18/EC requires effects due to direct and indirect interactions between the GMO and target and non-target organisms (including competitors, prey, hosts, symbiots, predators, parasites and pathogens) and human health to be characterised.

Line 590: Add: biotic interactions include those between the GMO and target and non-target organisms (including competitors, prey, hosts, symbiots, predators, parasites and pathogens) and human health.

Line 615: Information on the GMO itself and its comparator is only a small part of the information required to meet the regulatory requirements: the information listed here is insufficient to fulfil the claimed purpose of the heading i.e. to identify potential unintended effects. Add: for both types of applications information on the intended release or use including its scale; the potential receiving environment; and the interaction between these is also required (Annex II, EC, 2001). The Directive's requirements in Annex IIIA should also be cited here. For example, information required on the receiving environment includes that listed in Annex IIIA of Directive 2001/18/EC: 1. geographical location and grid reference of the site(s); 2. physical or biological proximity to humans and other significant biota, 3. proximity to significant biotopes, protected areas, or drinking water supplies, 4. climatic characteristics of the region(s) likely to be affected, 5. geographical, geological and pedological characteristics, 6. flora and fauna, including crops, livestock and migratory species, 7. description of target and non-target ecosystems likely to be affected, 8. a comparison of the natural habitat of the recipient organism with the proposed site(s) of release, 9. any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

2.3 Structural overview of this Guidance Document

Lines 619 to 623: As noted in earlier comments, these specific areas of risk should have been listed much earlier in the document and a process should have been adopted which encompassed all of them from the outset (rather than being restricted by the concept of "exposure assessment"). As noted in comments on the contents, specific sections on Pathogens, infections and diseases; Abiotic interactions; and Impact on non-GM animal health and welfare have been omitted from the insects section. There is no justification for issuing a consultation with these sections missing: further consultation is likely to be needed once they have been included.

3. Cross-cutting considerations

3.1 Receiving environments

Lines 640-646: The specific information required by Annex III A of Directive 2001/18/EC (EC, 2001) should be listed here.

3.1.1. Definition of receiving environments

Lines 648-657: It would be helpful to consider both intended and unintended environments. Some GMOs, especially fish, insects, some birds and mammals (e.g. rats) and eggs or sperm of any species, may spread easily outside the intended receiving environment, either inadvertently through transport on clothing or in ships or tyres; or through poorly regulated marketing (e.g. sale of GM bull sperm). Impacts of accidental releases will have to consider potential impacts outside the intended receiving environment. For example, GM salmon produced by the company Aquabounty are intended for production in on-land facilities, but might escape via water outflows and/or appear in EU waters as a result of poorly controlled marketing or shipment of eggs. The potential impact on wild salmon populations will therefore need to be assessed.

3.1.2 Identification and characterisation of the receiving environments

Lines 700-701: Interactions with humans should be added.

Lines 713-714: Pests and pathogens associated with the GM animal and its non-GM comparators and its competitors need to be considered, for the reasons outlined in comments on lines 386-391 above.

Lines 722: Add: Including interactions with humans.

Lines 726-727: Table 2; Pests (e.g. pathogens and parasites) and diseases should be added to the 2nd column (as well as the first), under "Biotic and abiotic ecosystem sub-factors interacting with the GM animal".

3.1.3 Selection of the relevant receiving environment

Lines 733-734. This paragraph should be clear that it is not advocating open release experiments in the highest risk areas: or is it? See comments on lines 248-250. In general, the Guidance fails to recognise that many proposals for releases (at least for a population suppression approach) will be for invasive species, on the grounds that releasing the GM organism will reduce the numbers of an undesired non-GM organism (a pest, disease vector or invasive species). In such cases there may be no receiving environments where the risks are considered acceptable or there may be some restricted environments, with characteristics such as: a severe problem due to the pest, lack of alternatives to tackle it, and expected low adverse impacts on non-target organisms and human health. The Guidance seems to imply that authorisations will be granted for placing on the market across the whole of the EU, which is not remotely realistic given the potential risks of releasing GM insects (mosquitoes or plant pests) or fish in the wrong areas. Insufficient attention has also been paid to how spread into non-authorised environments will be avoided. For example, Oxitec lists more than 50 species of insects it wants to genetically modify in its patent (Oxitec, 2011a): how will these be restricted to areas where the target pest is actually a problem and not allowed to spread to other receiving environments? The same problems apply to other highly mobile species, such as GM salmon or GM bees.

Lines 737-739: Again, this appears to advocate causing potentially irreversible harm to non-target organisms in order to conduct experiments on safety: the need for a step-by-step approach, focusing on contained experiments in the first instance, should be emphasised. This is particularly important where there are potential adverse impacts on human health: for example, contained trials and trials in non-inhabited areas should be prioritised over trials in inhabited areas. For example the risk identified in comments on lines 468-486 is associated with open releases of GM mosquitoes (vectors for dengue) in inhabited areas where dengue is endemic: these should be the last places where tests

are conducted if the efficacy of the technology is uncertain. For mosquitoes, to answer questions about impact in nature requires field experiments to manipulate species densities under realistic conditions; to answer questions about biological details requires more-complex experiments to manipulate other factors in addition to population density; whilst some questions about biological details can be answered using experiments under less realistic, but more precisely controlled, laboratory conditions (Juliano, 2009). However, important questions about e.g. competition between species and the effects ecological interactions can be assessed in the first instance without releasing GM insects: this helps to establish a baseline level of understanding for the step-by-step approach as required by paragraph (24) of Directive 2009/18/EC (EC, 2001).

Lines 762-767: A major proposed application is to combine GM agricultural pests (Oxitec's RIDL technology) with GM crops (pest-resistant Bt crops) in an attempt to tackle the growing problem of the emergence and spread of resistant pests (Alphey et al., 2007; Alphey et al. 2009; Oxitec, 2011b). The Guidance should therefore refer not only to combinations of GM animals, but also combinations of GM animals with GM plants. Taking into account all GMOs already in the environment (not just other GM animals) is a requirement of Directive 2001/18/EC Annex II (Part B, General Principles).

Line 781: Add: Human populations, including relevant characteristics e.g. age, disease status.

Lines 782-788: Modelling will be required not only for persistence and invasiveness, but also to predict impacts on other species and diseases.

3.2 Experimental environment

Line 803: Add: Many animals are also vectors for existing pathogens and potential future reservoirs for new viruses to develop that may be transferred from animals to humans. This introduces a new level of complexity because transport and evolution of viruses, including their interaction with human and animal hosts becomes an important part of the risk assessment process.

Lines 808-810: Add humans.

Lines 825-828: The potential for irreversible effects (due to hysteresis) to occur even if the GMO is removed from the environment should also be considered: for example, ecological replacement by a more invasive competitor during the use of a population suppression approach; the evolution of a more virulent virus due to the release of GM virus-resistant mosquitoes or birds. Again, the Guidance is too focused on the idea of "exposure" (as if a toxicological assessment were being conducted and removing the exposure would remove the problem), rather than an ecosystem approach.

Line 834: The presence of humans and pathogens must also be considered.

Line 849: Add: Interactions with humans should be limited until potential adverse effects of the GMO and its behaviour and interactions in the environment are fully understood. Releases may be premature if the baseline receiving environment has not been sufficiently characterised or understood.

3.3 Choice of comparators

Line 854: The comparative approach is only one aspect of the assessment, as outlined in Directive 2001/18/EC and section 2.2 (see also the comments on that section). There will be no comparable use, for example, of non-GM mosquitoes or agricultural pests which would not be released in their

millions into the environment because they are harmful organisms. There may of course be some potential to make a comparison with the Sterile Insect Technique (SIT) but there are important differences which will need to be considered as part of the assessment (see comments on insects section, below). The impacts of large-scale releases of GM insects for example are not predictable from a straightforward comparison between the GM and non-GM insect (see comments above on e.g. potential for increase in invasive competitor species, interactions with human immunity leading to more serious cases of disease, evolution of viruses).

Lines 867-877: An important issue missing here is that different strains of the same species (e.g. a mosquito or agricultural pest) can vary significantly in their ability to transmit diseases (Aitken et al., 1997; Bonizzini et al., 2012; De Oliveira et al., 2003; Lima & Scarpassa, 2009; Scarpassa et al. 2008; Tabachnick et al., 1985; Van Den Hurk et al., 2011) and their resistance to insecticides (Martins et al., 2009, Ocampo & Wesson, 2004). This raises concerns about how introgression may effect both persistence and disease transmission (GeneWatch UK, 2012). Release of non-native strains will not be compatible with plant pest regulations, as highlighted by Oxitec's failed attempt to release a North American strain of GM diamond-back moth in the UK under contained use regulations (on the spurious claimed grounds that the genetic trait amounted to biological containment) (Oxitec 2011b; ACRE, 2011; HSE, 2011a&b; DEFRA, 2012; FERA, 2012). This issue has not been fully resolved since backcrossing the North American GM strain with a native strain will still not make the strain identical to a native one. In the case of disease vectors, such problems are exacerbated by the fact that disease transmission characteristics may vary significantly between strains and more than one strain may exist in the EU. Using strains that are different from the background strain (in a particular area) risks introducing new diseases to that area.

Lines 888-903: It is unclear why EFSA is considering recommending the release of GM fish or insects into areas where no conventional counterpart exists. Re-interpreting Directive 2001/18/EC to allow such releases would appear to be stepping even further outside its remit than it already has. Further, such releases are unlikely to be compatible with other legislation, such as plant pest regulations.

Lines 904-908 recognise that releasing a GM species into a receiving environment where it does not currently exist would amount to introducing an alien species but fails to mention any of the legislation or conventions which would prevent such introductions.

Lines 908 to 914 amount to a misreading of Directive 2001/18/EC: the Directive is clear that effects such as interactions with other species must be considered: the difference between the GM and non-GM animal are only a part of this assessment as discussed extensively above.

Lines 915-924 appear to be a correct interpretation.

Lines 945-949: This paragraph again makes no sense for population suppression approaches (as explained in comments above). Even where there is a non-GM version of a pest in the environment, assessing the environmental impacts of large scale releases of the GM version do not depend on a comparison between the GM and non-GM animal, because large scale releases of the non-GM pest would certainly not be allowed. The overall environmental consequences of the release must be assessed. The comparison between the non-GM and GM animal is only one aspect of this. The aspects which must be assessed are listed in part D.1 of Annex 2 of Directive 2001/18/EC. This is the starting point, not the comparative approach, which is merely one aspect of the assessment.

3.3.1 Choice of comparators for ERA of GM fish

Lines 984-985: This section should recognise that some proposed applications involve population suppression of invasive fish species through the release of GM fish that mate with the wild ones and limit future reproduction. A specific proposed application is to produce GM carp that only produce male offspring and hence crash the population (Nowak, 2002; Thresher, 2008). Although this Australian project has recently lost funding (ABC, 2012); a different transgenic approach to producing all male fish and other animals has been patented recently (Aquabounty, 2011). Population suppression approaches need to be treated consistently in this Guidance, including in the insects and mammals and birds sections (the theoretical example of the "sterile" rabbit). These proposed applications could have very significant impacts on ecosystems since population suppression may be partial or temporary and give rise to complex interactions with competitors, predators, prey, symbiots, pathogens and humans. It is unclear to the reader whether the release of GM invasive species as part of a population suppression approach is consistent with existing legislation (since the release of the non-GM animals in such cases would normally be inconceivable) and, if so, how such applications would be assessed.

3.3.2 Choice of comparators for ERA of GM insects

Lines 1039-1048: Annex II of Directive 2001/18/EC, part B states that "*identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations*" (EC, 2001). For applications involving pests this poses a problem with a lack of "corresponding situations" since mass releases of non-GM mosquitoes or agricultural pests would not be contemplated. As suggested elsewhere in this Guidance, for population suppression one approach might involve a comparison with the Sterile Insect Technique (SIT). Comparisons with wild populations should also certainly be made. There is no requirement in Directive 2001/18/EC for a comparison with other management techniques (e.g. insecticides) although the impact of altered management techniques must be considered. GM insects are likely to be released as part of an Integrated Pest Management (IPM) programme which will include continued use of insecticides and other control methods: assessment of any changes to this management regime falls under step 8 of the nine step process, not under the selection of comparators. Release programmes run by authorities may require a Strategic Environmental Assessment, which involves considering alternatives (EC, 2001b), but this does not alter the need to produce an ERA to meet the requirements of 2001/18/EC. Multiple alternative management approaches are likely to be available and used in complex combinations in different locations and will not be limited to the use of insecticides alone (e.g. agro-ecological approaches to controlling pests; mosquito control programmes including public health approaches to reducing breeding sites and early surveillance for disease). Any comparison of GMO releases with alternatives would need to consider efficacy of the releases as well as risks and how the system might change with time e.g. as resistance develops.

Lines 1049-1050: The implication that a GM pollinator would replace a non-GM pollinator of a different species is extremely worrying. The Guidance needs to be clear under what circumstances release of a different species of pollinator might be allowed (taking account of plant pest regulations and other relevant legislation). The strain must also be considered (see comments on lines 867-877). Annex II of Directive 2001/18/EC, part B states clearly that "*identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations*" (EC, 2001). There is no suggestion that a GM species can be used to replace a non-GM organism of a different species.

Line 1052: A section on choice of comparators for ERA of GM mammals and birds is missing. This should also include guidance on comparators for the population suppression approach (the theoretical example of the “sterile” rabbit).

3.4 The use of non-GM surrogates

Lines 1053-1107: This section should also emphasise the need to fully understand the baseline characteristics and behaviour of the target wild organism and non-target organisms in the receiving environment. For example, a full understanding of density-dependent effects on mosquito populations is critical to understanding responses to the release of GM insects in a population suppression approach (Juliano, 2007; Gould & Schliekelman, 2004; Walsh et al., 2011; Walsh et al., 2012; Barclay, 2001).

3.5 Experimental design and statistics

Line 1133: Reference to EFSA (2011) should state whether this is 2011a or 2011b.

3.5.1 General principles

Lines 1110-1118: This section should refer to the principle in paragraph (24) of EC (2001) i.e. that the introduction of GMOs should be carried out according to the ‘step by step’ principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken.

Lines 1139-1140: Reference to EFSA (2010) should state whether this is 2010a,b,c or d. Presuming it is EFSA (2010c) it should be noted that this report was developed for application to GM plants. As noted in Section 3.2 (see also comments on this) the ERA of a GM animal would be more varied and complex, and encompasses a wider range of issues than the ERA of a GM plant or substance. Some of the issues have been highlighted in comments on Lines 386-391 (potential increase in a competitor species that is harmful), Lines 468 to 486 (potential harm to human health due to interaction with human immunity), and Lines 501-504 (potential for evolution of viruses in response to releases of GMOs), above. It is completely inadequate to base the assessment on the idea of ‘limits of concern’ as applied to plants and a basically toxicological approach: an ecosystem based approach is needed, otherwise steps 4,5 and 6 in Part D.1 of Annex II of Directive 2001/18/EC will not be adequately addressed. In the population suppression approach, for example, the primary impact on other species will not be through toxicity but through the fall in population of the target species (or potential fluctuations or even increases due to density-dependent effects, should the approach prove ineffective) combined with the response of the system of the whole (e.g. influx of the target species from surrounding areas, possible increases in competitors and reduction in predators, changes in the age or size structure of the population etc.). Addressing these issues would benefit from a new methodological report, since they are more complex than have been considered to date for plants. Multiple scenarios will need to be considered (see comments on Lines 501-504) and these may vary considerably in different receiving environments (for example, depending on the presence or absence of a particular agricultural pest). For disease vectors, the equivalent of clinical trials may be needed to assess impacts on human health (James et al., 2011): this lies way outside EFSA’s expertise and certainly cannot be addressed merely by extrapolating from risk assessments for GM plants. There are also important ethical issues associated with conducting trials of releases of disease vectors, which are widely recognised to require informed consent (Macer, 2005).

Lines 1151 and 1157: Heard et al., 2003 is missing from the reference list.

Lines 1167-1173: Complex modelling will be needed to predict the interactions required to be assessed in part D.1 of Annex 2 of Directive 2001/18/EC (EC, 2001), steps 3, 4 and 5. Demonstrating the validity of these models must be part of the ERA.

3.5.2 Principles of experimental design

Line 1219-1220: Mead (1990) is missing from the reference list.

Line 1271: There are two Sundström et al. (2007) references in the reference list, these should be individually identifiable.

Line 1353: The assumption that there are no interactions will not be valid in all cases. Ecosystem responses to large-scale open releases of GMOs may be non-linear.

3.5.3 Statistical analysis

Line 1388: A new section is needed after this one: a section on Computer Modelling. Modelling is mentioned in the Guidance on lines 77, 81, 782, 845 (which states that applicants deploying mathematical or other modelling techniques should seek to verify those models and justify explicitly their validation), 1105, 1106, 1146, 1225, 1522-1526, 1542, 1589-1592 (regarding uncertainty), 1603, 1625, 1629-1632 (assumptions), 1654-1656 (validity/uncertainty), 1689-1707 (choice of models, model structure effects, uncertainty and variability), lines 2183-2186 (fish), 2294-2296 (fish), 2516-2520 (pathogens in fish), 2745-2747 (modelling fish production systems), 3268-3271 (horizontal gene transfer in insects), 3891 and 3392-3394 and 4004 (insects and non-target organisms), 4106 (insect release management), 4123 (mosquito vector control dynamics: incorrectly described as validated models when they are not), 4294 (human immunity), 4522 (persistence of mammals and birds), 4529 (bioclimatic and species distribution models for mammals and birds), 4543 (inclusion of biotic and abiotic factors in models of mammals and birds), 4552, 4639-4671 (further requirements for modelling mammals and birds), 4677, 4837, 4998-5001, 5246-5250 and 5301 and 5307 and 5313-5317 and 5444-5446 (modelling of pathogens in mammals and birds), 5895 and 5946 (effects of GM mammals and birds on non-target organisms), 6134 and 6178 (management systems for mammals and birds). It would be helpful to readers if all the concepts referred to were outlined in one section. This would also help to ensure that principles outlined in one section (e.g. Lines 4639-4671) are applied to other sections (i.e. to insects and fish).

3.5.4 Information required

3.6 Long-term effects

Lines 1436-1440: This is a misunderstanding of Directive 2001/18/EC, as explained above. The comparison required in the Directive is with the “non-modified organism from which it is derived and its use under corresponding situations”. There is no justification for releasing GM animals which are not present as non-GM animals in the same environment: in most cases these will be regarded as alien species and releases would not be allowed under other legislation/conventions. The release of GM pests, disease vectors and alien species has been proposed as part of a population suppression or population replacement approach (e.g. to reduce disease transmission): in this case the problem is not the absence of a non-GM comparator (as in its absence, the GMO would almost certainly not be authorised for release) but the absence of a “corresponding situation” in which the non-GM organism (which is harmful) would be released in similarly large numbers. In all these scenarios the GM organism is expected to be (or at least intended to be) less harmful than its non-

GM comparator, but this does not mean that its release (which, for many applications, will greatly outnumber the wild population by e.g. a factor of ten or more for GM mosquitoes) will not be harmful. Assessment is therefore not of whether the GMO is more or less harmful than the non-GM organism (this is merely a step in the process): it must include an ecosystem assessment designed to fulfil the requirements of the Directive. There must also be a recognition that the ecosystem as a whole may change in ways that are not reversible (see comments on Lines 825-828).

3.6.1 Categories of long-term effects

Line 1464: There is an implication here that long-term effects do not have to be assessed before placing on the market: they do. Short-term effects may also differ from predicted or measured effects before placing on the market. The assessment is supposed to include both long-term and short-term effects, although uncertainties may be greater for long-term effects.

Line 1474: Interactions with pathogens (including possible evolution of viruses) and human or animal immunity can also result in long-term effects on human or animal health.

Line 1482: Williams & Jackson, 2007 is missing from the reference list.

3.6.2 Guidance to applicants

Line 1505: Add: Modelling of alternative scenarios under different assumptions and with a variety of conceptual models will also play a role in identifying potential long-term effects (e.g. Alphey et al., 2011, Medlock et al., 2009).

Line 1526: The "verification" of a model is not the same as its "validation": verification consists of verifying that equations are solved correctly while validation consists of verifying that the equations implemented provide an acceptable representation of reality (e.g. Hemez & Doebling, 2001). Validation of computer models is essential, as highlighted elsewhere in the Guidance e.g. line 8444-847.

Line 1546: The defining characteristics of the receiving environment and any conditions on these should also be outlined: for example GM daughterless carp would be expected to be released (if at all) only in areas where invasive carp were a problem.

Line 1548: Add: Potential long-term adverse effects on health (e.g. due to altered transmission of pathogens, effects on human immunity etc.) and any resulting ethical requirements e.g. informed consent from persons who might be affected.

3.7 Uncertainty analysis

3.7.1 Introduction

Line 1579: Applicants and regulators should recognise that environmental models are generally mathematically ill-posed or ill-conditioned, meaning that the information content available to define a modelling problem does not allow a single mathematical solution (Baveye, 2003, Beven, 2002, 2003 and 2006). Even well-calibrated models (i.e. models fitted to the data at a particular site) can have no predictive value if the equations and structure of the model do not adequately represent processes that occur in the real world: this is true even for physical systems (e.g. Carter et al., 2006) but uncertainties and unknowns will be greater for biological systems. It is therefore critical to explore alternative conceptual models and assumptions which may lead to very different

conclusions about risk (e.g. Medlock et al., 2009). Scientific bias can be classified into five types: confirmation bias, rescue bias, mechanism bias, "time will tell" bias and orientation bias (Kaptchuk, 2003) and the existence of bias in technology assessment has been well-documented, especially in the medical literature (e.g. Bhandari et al., 2004). It is therefore important to be transparent about subjective judgments contained in model assumptions or data analysis methodology and to explore a variety of alternative conceptual models and scenarios. For example, outputs of population models of a wide variety of species change significantly if the effects of environmental fluctuations are included. Failure to anticipate unexpected events can be exacerbated by the use of complex models which are only comprehended within a small expert group, because they are then less likely to be open to scrutiny or challenge by outsiders (Beken et al., 2010). Failure to act on early warnings and anticipate unexpected events (resulting in e.g. collapses in fish stocks, the effects of CFCs on the ozone later, and the harm to health caused by X-rays and asbestos) underpins the adoption of the precautionary principle in Directive 2001/18/EC and elsewhere (European Environment Agency, 2001).

Line 1621: There is extensive evidence that quantitative as well as semi-quantitative assessments are vulnerable to subjective bias (see comments and refs above).

Lines 1698-1704: A variety of conceptual models should be presented, exploring multiple scenarios including worst-case scenarios, since alternative concepts can give very different answers whilst all being consistent with the available data. The analysis should not be limited to sensitivity analysis (i.e. testing the effects of altering parameters within a single model) because conceptual model uncertainty is often greater.

3.7.3 Interplay between ERA conclusions and PMEM

Line 1727: Reversibility of effects and their potential seriousness should be considered (e.g. possible establishment of a more invasive disease vector, evolution of a virus, or adverse human health impacts due to effects on immunity).

Line 1737: Monitoring methods should be tested for robustness e.g. Oxitec's transgenic fluorescent marker in bollworms begins to fail in ovitraps after as little as four days in hot weather (Walters et al., 2012).

3.8 Aspects of GM animal health and welfare

Line 1752: The first production stage for GM animals involves establishing the transgenic trait. The process of obtaining eggs is invasive if taken from live mammals, and implanted genetically modified eggs lead to many stillbirths, miscarriages or invasive surgery on the mother (GeneWatch UK, 2002). Ethical issues are similar to those associated with cloning mammals (EGE, 2008) but have been entirely neglected here. Loss of genetic diversity (due to the production of genetically identical herds of cows or farmed chickens or fish) also needs to be considered as it may increase vulnerability of the animals to infection.

3.8.1 Health and welfare aspects for GM mammals and birds

Line 1797: Loss of genetic diversity needs to be considered as mass production of identical GM mammals or birds may increase vulnerability to infection.

3.8.2 Health and welfare aspects for GM fish

Line 1817: Loss of genetic diversity needs to be considered as mass production of identical GM fish may increase vulnerability to infection.

3.8.3 Health and welfare aspects for GM insects

Line 1821: There are a wide variety of other beneficial insects e.g. butterflies, ladybirds, not just bees.

4. Specific areas of risk to be addressed in the ERA

Lines 1825-1826: The inconsistencies between the three different sections are deeply problematic, particularly in the insects section where many important issues required in the ERA by Directive 2001/18/EC are not properly addressed. This section should firstly lay out in full the information on which conclusions need to be drawn as listed in Annex II D.1 of the Directive (EC, 2001). This would make clear, for example, that "Interactions with non-target organisms" means "Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens". All sections (Fish, insects, mammals and birds) should have subsections which match the requirements in D.1. It makes sense, however, to subdivide some of these, for example to include a subsection on "Pathogens, infections and diseases": the omission of this from the insects section is inexcusable since many insects are disease vectors. Subsections on impact on biogeochemical processes and impact on animal health also need to be included in the insects section: these are major areas of potential impact and there is no excuse for omitting them. A subsection on animal health is also needed in the fish section. These are significant gaps and it is likely that further consultation will be needed once they have been filled.

Lines 1832-1843: The definition of target organism in the glossary creates considerable confusion between the sections: is it the organism that is genetically modified (as used in the insects section) or the parasites, pathogens or other species which are displaced or consumed by the animal (mammals and birds section)? In the latter case there may be multiple "target organisms" leading to further confusion: further, a population suppression approach might be used for mammals (the theoretical "sterile" rabbit example) in which case the target is presumably the animal itself. Again, the concept seems to have been borrowed from the risk assessment process for plants without proper thought. It would greatly help the clarity of the document if target organisms were defined as animals of the same species that is genetically modified, throughout. Alternatively, at least a consistent definition must be used between all the sections.

4.1 Specific areas of risk for the ERA of GM fish

4.1.1 Gene transfer and consequences

Step 1: Problem formulation (including identification of hazard and exposure pathways)

Lines 1899-1900: Information on the parent fish strain must be provided.

Lines 1864-1936: This entire section neglects consideration of potential applications of deliberate releases of GM fish, engineered to be partially sterile or create only male offspring which are intended to mate with and crash an invasive (unwanted) population of fish e.g. carp. This analogous to the population suppression approach discussed for GM insects and the theoretical example of the GM "sterile" rabbit, yet no equivalent example has been included for fish (except as an accidental outcome of escapes, i.e. the Trojan gene effect, lines 1947-1959). There are proposals for this type

of application (i.e. the use if this and similar effects in a deliberate programme to remove unwanted species) and relevant technology has been patented (e.g. Nowak, 2002; Thresher, 2008; Aquabounty, 2011). The Guidance should be clear about whether this type of application is prohibited by existing legislation and conventions or whether it might be subject to an environmental risk assessment. If the latter, the flow chart needs to be much more sophisticated: the question "Will GM fish reproduce?" might lead to an answer: yes, but produce only (or, mostly) male offspring; or no, most or all offspring will not survive. In the latter case this means there is a reduced chance of the GM fish itself becoming invasive but this does not mean the ERA should be limited by the survival period of the GMO in receiving environments, as indicated in the flow chart (this is an error due to the mistaken focus on exposures, see comments on lines 386-391). In other words, the flowchart must recognise that production of infertile offspring (or reduced fitness, or single-sex offspring) can also lead to adverse effects. The main environmental impact of a population suppression programme using GM fish will be a significant reduction in the target species (or fluctuations in numbers, influx from surrounding areas, or other potential adverse effects if unsuccessful) and effects on non-target species could be significant (e.g. increase in competitors, reductions in prey, complex interactions etc.): these issues will need to be assessed, as this is a requirement of the Directive (EC, 2001). It is confusing for the reader to understand which subsection these impacts should be included in, due to the inconsistencies of the different sections on fish, insects and mammals and birds (see comments on lines 1825-1826). In the insects section, vertical gene transfer has been considered only in the context of persistence and invasiveness, and effects on population suppression have been considered (albeit poorly) in the section of effects on the target organism. This way of organising the information works but only if it is consistent between the different sections (fish, insects and mammals and birds) and the population suppression approach is considered in all sections. Further, it requires a consistent definition of "target organism" so that the reader is not confused (see comments on lines 1832-1843).

Step 2: Hazard characterisation

Step 3: Exposure characterisation

Line 1998; Mitigation measures to reduce gene transfer such as reduced fertility can exacerbate other effects e.g. a population suppression effect on wild species (see comments on lines 1864-1936). There is a problem with the whole concept of "exposure characterisation" as it deals only with direct effects (analogous to toxicological effects) not with complex ecosystem interactions (see comments on lines 386-391).

Step 4: Risk characterisation

Lines 2025-2026: Reduced reproductive fitness may mean negative impacts on wild population through mating and the production of less fit wild populations.

Lines 2034-2043: If population changes are to be included here, the above comments on population suppression approaches must be taken into account. It would also be helpful, as noted above, if the sections on insects and mammals and birds were consistent so the reader knew which subsection these effects would appear in.

Step 5: Risk management strategies

Line 2059: Lowered sexual fertility of the GM fish may have negative impacts on wild populations if mating success is high but reproductive fitness low.

Step 6: Overall risk evaluation and conclusions

Line 2067: Should include the extent to which the GM fish and offspring of matings are more or less successful.

4.1.2 Horizontal gene transfer

Step 1: Problem formulation (including identification of hazard and exposure pathways)

Line 2128: Bensasson et al., 2004 is missing from the reference list.

4.1.3 Impacts on biotic components and processes

Lines 2220-2222: Including all biotic effects (target and non-target) in one section, with a separate section for pathogens and diseases, makes it easier to follow an ecosystem approach (CBD, undated), which acknowledges complex interactions (for example, a reduction in the numbers of the target organism could reduce non-target predators and then increase the numbers of the target organism again). However, this approach differs from that used in the insects and mammals and birds sections: a consistent approach should be used throughout the guidance. The definition of target organism should also be consistent (see comments on lines 1832-1843): in the insects section it is taken to mean the organism that is genetically modified: it would be clearer if this definition is used throughout. For example, in the case of AquaBounty's GM salmon, what is the target organism (presumably it is Atlantic Salmon, *Salmo salar*?). This should be clear to the reader throughout.

Step 1: Problem formulation (including identification of hazard and exposure pathways)

Lines 2229-2256: It is unclear to the reader why the detail provided in the mammals and birds section (e.g. Figures 7, 8 and 9 and accompanying text) is not provided here.

Step 2: Hazard characterisation

Line 2263: Decreased competition (for example, if releases of the GMO reduce the numbers of the target species) can also be a problem if it allows numbers of an invasive or harmful species (e.g. a disease vector) to increase due to reduced competition for food, breeding sites etc.

Lines 2275-227: Mating of the GM fish with wild fish can also reduce numbers if the offspring have reduced fitness or reproductive capacity (e.g. are all male): indeed this is one intended application for GM fish (a population suppression approach), as described above.

Line 229: Should say changes in fish characteristics or fish populations and associated ecosystems. For example , if GM daughterless fish were released in order to try to remove an invasive species through mating and producing an all-male population which cannot breed, each individual fish may have the same relationship with symbiots as its wild counterpart, but the effects of the release programme as a whole could still have a significant effect on symbiots. The same problem might occur if an accidental release occurred of a GM fish which gave rise to the Trojan effect, or similar adverse effects, on wild populations. As is clear in Directive 2001/19/EC it is the characteristics of the GMO and the conditions of its release and its interactions with the receiving environment that are important.

Lines 2294-2296: Models must be validated and the effects of alternative conceptual models must be explored. See comments on modelling above.

Lines 2297-2303: Impacts on animal health merit an entire subsection, as has been included in the mammals and birds section. A single paragraph, whilst important to draw attention to the issue, is insufficient to address the requirements of the Directive, which includes this issue in step 7 of part D.1, Annex II (EC, 2001). Further, it is incorrect to imply that such assessments are less important for fish that are destined for human or animal consumption. This new subsection must include the impacts of the pathogens and diseases identified in Section 4.1.4 on other animals: with a focus on their possible transfer to other fish and/or transfer to or consumption by other animals. It should be noted that an assessment of food/feed safety of the GM fish under EFSA's other guidance does necessarily not apply to (1) other species eating the GM fish (i.e. not the species intended to be fed with it); or (2) any species eating other fish that have been infected with pathogens from GM fish.

Step 3: Exposure characterisation

Lines 2315-2316: correctly identify that it is the fish and its influences that must be considered. However, there is still a tendency to characterise exposure in a narrow sense which may exclude some of the hazards. For example, a GM fish that escapes and decimates the wild population due to a Trojan gene effect may have limited survival in the environment, but so will the wild species! In this scenario, even when all the GM fish are dead there may still be irreversible adverse effects on the ecosystem (including e.g. the loss of an endangered species). The same is true of population suppression approaches: whilst intended to remove an unwanted species (such as invasive carp) by mating with them, there could be unintended consequences on other species which might be irreversible (e.g. a loss of a predator species, or establishment of a competitor invasive species). These problems stem from the concept of "exposure characterisation" which has been borrowed from toxicology and is too narrow to encompass all the effects required to be considered in the ERA by Directive 2001/09/EC (EC, 2001). See comments on lines 386-391.

Step 4: Risk characterisation

Step 5: Risk management strategies

Line 2344: Should say biota and ecosystems and key ecological functions. For example an increase in an invasive competitor species is not an adverse impact on that species or on an ecological function but may nevertheless be regarded as an adverse impact on the ecosystem.

Step 6: Overall risk evaluation and conclusions

Line 2353-2354: It is not just long-term exposure but long-term impacts (e.g. potentially irreversible impacts as discussed in lines 2339-2342) that need to be considered.

4.1.4 Pathogens, infections and diseases

Lines 2380-2383; A new section on animal health is also needed, which should include impacts of pathogens on animal health (i.e. not just humans). This should also consider contact with and consumption of other fish or aquatic organisms that may carry the pathogen as a result of the release or escape of the GM fish (see lines 2428-2431).

Step 1: Problem formulation

Line: 2481: Add: Can the GM alter the spread of pathogens via its interactions with other components of the ecosystem e.g. an increase in competitor species that carry pathogens as the result of a population suppression effect?

Step 2: Hazard characterisation

Lines 2491-2496; Add: d) any altered ecosystem effects expected as a result of the GM fish release or escape (see Section 4.1.3) that might change the spread of pathogens, even if they are not spread by the GM fish itself (e.g. this could be by a competitor species whose population has increased due to the impact of the GM fish releases on the target species).

Lines 2497-2500: Information on infectivity of pathogens to competitor species is also required because harm could be caused by an increase in a competitor species.

Step 3: Exposure characterisation

Lines 2525: Potential increased exposure to pathogens due to indirect effects, such as increase of an infected competitor species due to the effects of the GM fish on wild wish, should be included.

Step 4: Risk characterisation

Step 5: Risk management strategies

Step 6: Overall risk evaluation and conclusions

4.1.5 Abiotic interactions

4.1.6 Environmental impacts of the specific techniques used for the management of GM fish

Line 2711: Data should be provided on changes in diet or feed consumption. For example, Aquabounty has conducted experiments during which transgenic salmon had rates of consumption that were approximately five times that of the control fish (Abrahams & Sutterlin, 1999).

4.1.7 Impact on human health

Lines 2776-2778: It should be clarified whether GM fish not intended to be marketed as food or feed but which might nevertheless be inadvertently eaten will require a food safety evaluation e.g. GM daughterless carp released with the aim of reducing the wild carp population might be eaten even though the application is not for food/feed. A section on impacts on animal health is also needed: this should include impacts e.g. on pets or other wild species that might eat the fish (which are not necessarily included in the food safety assessment) or which might be exposed to it or incur risks via the environment (for example, pathogens transferred from the GM fish to other fish and then to another animal).

Line 2780: Pathogens and parasites might also enter the food chain in increased levels in other species due to complex ecosystem interactions caused by the release of the GM fish.

Step 1: Problem formulation (including identification of hazard and exposure pathways)

Line 2800: Reference Veenstra et al., 1992 is missing from the reference list.

Step 2: Hazard characterisation

Step 3: Exposure characterisation

Lines 2897-2901: It is not only exposure to the GM fish itself that must be considered. Escapes or releases of the GM fish could alter ecosystems in a way that is harmful to human health through indirect pathways e.g. an increase in a harmful competitor species.

Step 4: Risk characterisation

Step 5: Risk management strategies

Line 2928: Reducing exposure to the GM fish itself will not help in situations where the pathogen is transmitted by another organism (e.g. non-GM fish which have been infected by the GM fish).

Step 6: Overall risk evaluation and conclusions

4.2 Specific areas of risk for the ERA of GM insects

2937: As noted above whole subsections are missing from this section, including: Pathogens, infections and diseases; Abiotic interactions; Impacts on animal health. In general, it is very unclear which issues should be addressed in which subsections, due to poor correspondence with the requirements of the Directive and inconsistencies throughout the Guidance (see comments on Lines 1825-1826). There is frequent mention of tropical diseases: it should be clear at the start whether the Guidance is intended to apply to EU applications only or also to the risk assessments required for transboundary notifications for exports of GMOs from the EU to overseas (see comments on Line 250). Oxitec has already made three transboundary notifications, for experimental releases of GM *Aedes aegypti* mosquitoes in Cayman, Malaysia and Brazil. The notifications and associated risk assessments were not made publicly available in advance of the trials (except in Malaysia where a summary risk assessment was published) and the risk assessments provide inadequate information (GeneWatch UK, 2012; Reeves et al., 2012). This issue of whether the Guidance covers transboundary movements or not should have been clarified prior to consultation, otherwise it is hard to give meaningful responses, since different insect species and applications are likely overseas from those expected in the EU.

Lines 2941-2944: The word 'several' is misleading: Oxitec's patent lists more than 50 species of insect including many agricultural pests that it wishes to genetically modify (Oxitec, 2011a). More than 500 species of insects and related arthropods are now being sequenced as part of the i5K project: these will all have very different life cycles and roles in ecosystems and in transmission of human and animal diseases. The expert report commissioned by EFSA lists six species of agricultural pest of possible interest in the EU over the next ten years (olive fruit fly, Mediterranean fruit fly, green bottle fly, stable fly, codling moth and cotton pink bollworm) (Umweltbundesamt, 2010), however this may be an underestimate. Oxitec has published research papers about GM Mediterranean fruit fly (Morisson et al., 2009), Mexican fruit fly (Leftwich et al., 2012), diamond back moth (Martins et al., 2012; Morrison et al., 2011b), and olive fly (Ant et al. 2012) and has other GM pest species under development: such as the tomato leaf miner/borer (*Tuta absoluta*) being developed jointly with Certis Europe, funded by the UK Technology Strategy Board (Morrison et al., 2011b; Oxitec, 2012). The company has received research and development funding from Syngenta for genetic transformation of *Lepidoptera* (Alphey, 2011). *Lepidoptera* include 174,250 known species of butterflies, skippers and moths (*Lepidoptera* Taxome Project, 1999). Oxitec has already sought to make open releases of GM diamond back moths (*Plutella xylostella*) under contained use

regulations in the UK on the spurious grounds that its RIDL (Release of Insects carrying a Dominant Lethal genetic system) technology (based on a conditional lethality trait) is equivalent to "biological containment" (Oxitec 2011b; ACRE, 2011; HSE, 2011a&b; DEFRA, 2012; FERA, 2012). Diamondback moths are not even mentioned in the draft Guidance. Pink bollworms genetically modified to contain a fluorescent marker have been released in open trials in the USA (Simmons et al., 2011), yet this application (for bollworms or other species) is not mentioned either. A major proposed application is to combine GM agricultural pests (Oxitec's RIDL technology) with GM crops (pest-resistant Bt crops) in an attempt to tackle the growing problem of the emergence and spread of resistant pests (Alphey et al., 2007; Alphey et al. 2009; Oxitec, 2011b). The issues associated with this proposed application (GM insects combined with GM crops) have not been discussed at all in the draft Guidance.

Lines 2949-2950: It is misleading to state that chemical insecticides are the current primary means of controlling insects causing public health concerns, although they can certainly play an important role. For example, in the case of *Aedes aegypti* (a vector for dengue), destruction of breeding sites by government programmes and/or community programmes is one of the main interventions, although this is often accompanied by the routine use of larvicides and by the use of adulticides during epidemics, as well as mosquito traps (Florida Mosquito Control, 2009; Baly et al., 2009; Egger et al., 2008). Provision of piped water, because water storage containers used by households without tap water supply provide mosquito breeding sites, is also an important intervention (Schmidt et al., 2001). Some biological control programmes have been successful in Vietnam (Nam et al., 2005; Kay & Nam, 2005). It is also unclear the extent to which species-specific population suppression approaches could replace insecticide use (even if population suppression approaches using GM insects are effective, which is questionable) because there are often multiple species involved in disease transmission or simply present as a nuisance species which need to be controlled. For example, in Cayman, where Oxitec has conducted open release experiments using GM *Aedes aegypti* mosquitoes, aerial spraying is mainly not related to this dengue-transmitting species but to the swamp species *Aedes taeniorhynchus* (Anon, 2012a). The possibility of competitive displacement of one disease transmitting mosquito for another has been highlighted in risk assessment workshops for GM mosquitoes (Beech et al., 2009), although largely ignored in practice (GeneWatch UK, 2012): the issue of multiple species will also be important for malaria in many regions (Kiszewski et al., 2004) and will limit the role of species-specific GM approaches in reducing insecticide use even if the GM approach is effective at reducing disease transmission by the target species (which currently remains highly questionable).

Lines 2954-2955: This description is inadequate to give the reader any insight into proposed applications. The concept of GM bees engineered to be more efficient pollinators is mentioned several times throughout the text but no references are given. Other potential applications, such as pest-resistant or pesticide-resistant bees (or other beneficial insects, such as butterflies: Marcus et al., 2004) are not mentioned at all. Research on the honey bee (*Apis mellifera*) has included some laboratory research on insecticide-resistance (cited in Beech et al., 2012) and genetic modification may one tool considered by researchers who wish to create bees that are more resistant to pests or diseases (Zakaib, 2011). Insecticide-resistant beneficial insects, including bees, might lead to increased use of insecticides. Transgenic silkworms with a high antiviral capacity have recently been created (Jiang et al., 2012): this raises a whole set of new questions about interactions between viruses and silkworms: for example, would viruses evolve to become more virulent? Could similar traits be applied to other insects (e.g. bees)?

4.2.1 Persistence and invasiveness, including vertical gene transfer

Line 2957: The title and content of this section could be taken to imply that only GM insects that are more invasive or persistent that their wild counterparts can have adverse effects on the

environment or human or animal health. This is because the specific issue in D.1 of Annex II of the Directive (Likelihood of the GMO to become persistent and invasive) is being conflated with the whole issue of vertical gene transfer. The first three issues listed in Annex II D.1 of Directive 2001/18/EC are: 1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s); 2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release(s); 3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species [emphasis added]. The population suppression approach relies on releasing insects that are less able to reproduce than their wild counterparts (via a conditional lethality or female-killing approach), which are intended to mate with the wild insects and significantly reduce the wild population: many of the environmental and human health issue with this approach associated with vertical gene transfer are not associated with persistence and invasiveness, but relate to the impacts of the releases on the target species (covered in Section 4.2.3) and non-target species. However, the disadvantage conferred to the GMO (or other species if cross-mating occurs) need to be considered here, including whether it will be realised under the release conditions. Issues that need to be discussed in this subsection include: (1) circumstances under which the conditional lethality approach may fail, leading to breeding and survival of future generations of GM insects; (2) adverse effects of expected male insect survivors under a female-killing approach: (3) adverse effects of unintended female GM insect survivors. However, it should also be noted that releases of GM insects in a population suppression approach are intended to disrupt the population dynamics of the target species (discussed further in Section 4.2.3) and that this will have further knock-on effects on non-target species (discussed further in Section 4.2.4) and pathogens (missing section). It is important that the definition of "target organism" is clear throughout the guidance so that readers are aware that impacts of the GMO on the wild-type organism are considered in Section 4.3.2.

Line 2983-2984: GM insects currently being considered for release in population suppression approaches are not sterile: the term sterile should be avoided. Oxitec's GM mosquitoes have a conditional lethality trait: this is conditional because it relies on tetracycline as a chemical switch to allow breeding in the lab; partial because it does not have full penetrance; and late-acting i.e. the insects are not sterile but mate and reproduce with most dying at the late larval stage (in the absence of tetracycline) (Phuc et al., 2007). The use of the term sterile is misleading because it implies there is no exposure to female biting GM mosquitoes or prospect of survival and breeding of the GM mosquitoes in the environment, which is incorrect. This section also requires a description of female-killing approaches, such as female-specific flightless *Aedes albopictus* (Labbé et al., 2012) and female-specific lethality in Diamond back moth (*Plutella xylostella*) (Annex 1 to HSE, 2011a; Oxitec, 2011b; Martins et al., 2012) and tomato borer (*Tuta absoluta*) (Morrison et al., 2011) as these approaches to population suppression are also significantly different from "sterility". Note that the flightless-female mosquito application does not directly kill the insects, but the females die due to inability to seek out blood to feed. Oxitec's GM *Aedes albopictus* could in theory be used in the EU; Oxitec has already attempted to obtain permission release GM diamond back moths in the UK (arguing that release in open field or polytunnels could be treated as a "contained use" application due to claimed "biological containment") and has cited 2013 as its target date for trials of GM tomato borers: such trials could take place in the EU.

Line 2994: This section should also consider the risks associated with the introduction of non-native parent species and strains, which may have altered capacity to transmit diseases or insecticide-resistance, see comments on lines 867-877. Applicants should be required to specify the parent strain and test its properties: any proposed releases will also need to be compatible with plant pest regulations.

Lines 2995-2998: This section should mention disease-resistance and pesticide-resistance as traits that could enhance fitness (see comments on Lines 2954-2955). Traits that could reduce fitness (e.g. a conditional lethality trait) will also be passed to the same species through mating (vertical gene transfer): applicants should provide information on: (1) penetrance of the loss-of-fitness trait in a variety of experimental conditions; (2) tests on conditionality i.e. on whether insects bred in the lab could also breed in the wild in the presence of contaminants e.g. Oxitec's RIDL technology relies on the common antibiotic tetracycline as a chemical switch to allow breeding in the lab, but feeding the mosquitoes on cat food presumed to be contaminated with tetracycline allowed a 15% survival rate, compared to 3-4% survival rate on a normal diet (Nimmo et al., undated). It should be clear to applicants that this kind of information should not be withheld as commercially confidential (House of Lords Hansard, 2011). Experimental data should be provided regarding the penetrance of the trait in the presence of different levels of tetracycline and its analogues.

Lines 2999-3003: Other traits could also presumably cause negative impacts as a result of cross-mating.

Line 3004: the use of the word sterile should be avoided, see comments on Lines 2983-2984. Other issues on which data needs to be provided are: (1) stage of the life-cycle at which any conditional lethality trait acts, because e.g. late acting lethality in pests may lead to substantial damage to crops at the caterpillar stage (see e.g. page 26 of Umweltbundesamt, 2010); (2) for female-killing approaches (including flightless females), possible harms may be caused by surviving males e.g. whilst male mosquitoes do not bite, male *Aedes Aegypti* infected with the chikungunya virus can infect female *Aedes Aegypti* during mating, and may mate with multiple females (Mavale et al., 2010; Bargielowski et al., 2011); male flies may transfer pathogens from faeces to food.

Lines 3011-3016: Insects with limited reproductive capacity, developed for use in population suppression approaches, can also have significant effects on ecosystems (these issues should be discussed further in Sections 4.2.3 and 4.2.4.). Mating fitness is also an important parameter in such programmes (to be discussed further in Section 4.2.3).

Line 3018: Should mention pest-resistance and pesticide-resistance as well as drought tolerance.

Line 3027-3030; It is unclear why intentional releases into environments other than those already inhabited by the species of interest are being contemplated: even non-native strains (let alone non-native species) need strict control. Attempts by Oxitec to release a genetically-engineered North American strain of diamond back moth in the UK have already caused problems due to plant pest regulations (HSE, 2011a&b; DEFRA, 2012; FERA, 2012).

Step 2: Hazard characterisation

Lines 3037-3039: Restriction of this section to considering only "enhanced fitness" is wrong. Penetrance and survival of GM insects with reduced fitness traits such as conditional lethality or female-killing traits must also be considered in a range of conditions (including the conditions used to breed the insects in the lab e.g. presence of tetracycline). See comments on line 2957 above: the Directive (EC, 2001) refers explicitly to advantage or disadvantage. This section should also consider the risks associated with the introduction of non-native parent species and strains, which may have altered capacity to transmit diseases or insecticide-resistance, see comments on lines 867-877. Applicants should be required to specify the parent strain and test its properties: any proposed releases will also need to be compatible with plant pest regulations. Data must also be supplied on the stability and persistence of the trait and the development of resistance should to be considered. For example, radiation-induced sterility (which involves multiple chromosome breaks) has built-in

redundancy that is not provided by molecular genetic approaches: this raises the possibility that any genetic or molecular event that allows the GM mosquitoes to survive and breed successfully could therefore be rapidly selected for during mass production (Benedict & Robinson, 2003; Robinson et al., 2004; Alphey et al., 2011a). If this happens, a conditional lethality effect could rapidly disappear as resistance develops in production facilities or in the field and vertical gene transmission in the field would increase significantly. Other mechanisms of resistance include wild females appearing that are unreceptive to mating with the transgenic males, as occurred in one study with SIT (Hibino & Iwahashi, 1991). Loss of gene expression in a virus resistant GM mosquito has also been reported (Franz et al., 1991). Stability of the trait must also be demonstrated (Adelman et al., 2004).

Lines 3047-3051: The use of the word sterility is misleading. Oxitec's GM insects have a conditional lethality trait (Phuc et al., 2007) and/or a conditional female-killing or female-sorting (Morrison et al., 2010) or female-flightless trait (Labbé et al., 2012), usually combined with a heritable fluorescent marker. Vertical gene transfer therefore occurs to the next generation, via mating in the wild. This requires data to be provided on (1) conditionality (i.e. the extent to which the conditions used for breeding in the lab may occur in the wild); (2) life stage of late-lethality or other non-sterile traits (for example, percentage that die as larvae or pupae etc.); (2) penetrance under different conditions (i.e. the numbers that express the trait and die prematurely or are flightless etc.). If genetic markers are used to establish frequency of survival, the reliability of the market itself must be established (Walters et al., 2012). In the case of female-only traits (flightless females or female-killing) the dispersal of males must be considered.

Lines 3052-3054: Vertical gene transfer within the greenhouse to the target species (with which the GM insect will mate) also needs to be described. Escape may occur during production, transport and use and may occur through a variety of mechanisms at all life stages (e.g. flying, transport of eggs or larvae on workers or materials carried in or out, including the crop).

Line 3066-3069: Escape is also relevant at the production and transport stages for GM insects which are intended to be released into the wild, as problems may occur in particular receiving environments e.g. if GM *Aedes albopictus* intended for release in a population suppression programme in Italy escape in France this could lead to the establishment of this invasive species. Information on conditionality, penetrance, stability, potential to develop resistance etc. is required as described above (see comments on Lines 3037-3039).

Step 3: Exposure characterisation

Line 3061: The use of the term sterile should be avoided as it implies the insects do not reproduce: a conditional-lethality trait allows the insects to reproduce in the lab and also in the wild, although the intention is that the majority of the progeny die at the larval stage; female-killing or flightless-female approaches are also intended to have population suppression effects but are not sterile. "Sterility" implies no vertical gene transfer, which is not the case. See comments on lines 3047-3051.

Step 4: Risk Characterisation

Lines 3073-3076: Where uncertainties exist multiple conceptual models must be considered (e.g. Alphey et al., 2011a) and requirements for model validation followed.

Step 5: Risk management strategies

Lines: 3090-3092: Specific conditions may be required in terms of receiving environments or geographical areas, as noted in paragraph 1, Article 19, Directive 2001/19/EC. In particular, possible

establishment of a species or strain of a pest or disease vector in an area where that species or strain of pest or disease vector is not present must be avoided.

Step 6: Overall risk evaluation and conclusions

4.2.2 Horizontal gene transfer

[No comments]

4.2.3 Interactions of the GM insects with target organisms

Line 3318: The use of the phrase "commonly applied" in relation to SIT programmes is misleading. SIT has been used successfully with some agricultural pest species, but has been less successful with others because different insect species have very life histories and behaviours. In general SIT is not effective at reducing large populations of insects without other interventions, but may be effective at reducing or eradicating smaller, isolated populations (Klassen, 2005). SIT has not generally been successful for mosquitoes, where population suppression has been achieved only in a few experiments with very large "release ratios" of sterile to wild mosquitoes (Spielman, 2003; Asman et al., 1981; McDonald et al., 1977).

Lines 3324-3325: The use of the term sterile should be avoided as it implies the insects do not reproduce: a conditional-lethality trait allows the insects to reproduce in the lab and also in the wild, although the intention is that the majority of the progeny die at the larval stage. See comments on lines 3047-3051. Large-scale releases of GM insects with conditional lethality or female-specific traits (e.g. female killing, flightless-female) are intended to have significant impacts on the target organism by suppressing or eliminating the population. Changes in the size, distribution and age structure of the target population will then have knock on effects on other organisms via interactions with predators, prey, competitors etc. (see Section 4.2.4) and potentially on pathogens, infections and diseases (missing section) and human and animal health (Section 4.2.6). Effects on non-target organisms may in turn alter the population dynamics of the target organism. For population suppression approaches the efficacy of the approach will need to be considered (i.e. whether the target population is suppressed and whether this is sustained), including the release ratios to achieve a given effect on population and any unintended or unwanted effects on the target population e.g. fluctuations in target population, increases in the target population outside the release area, influx of wild-type insects from surrounding areas. Indirect effects will include interactions with non-target species e.g. if a predator population falls as a result of an initial population suppression effect, the target population might rebound.

Line 3326: Add: pest-resistance and pesticide-resistance.

Lines 3333-3334: Adverse effects may not be reversible even if the GM insect population dies out, due to changes in population dynamics (including the elimination of a species, or alterations in numbers of predators, competitors or prey).

Step 1: Problem formulation (including identification of hazard and exposure pathways)

Line 3342: The term "sterility" should be avoided, see comments on line 3061. Lethality may be female specific in some applications.

Lines 3352-3356: are based on claims of efficacy, rather than demonstration of efficacy. For example, Oxitec has claimed it achieved an 80% reduction in the wild-type mosquito population

during experiments in the Cayman Islands in 2010, but the company has not published these results so the release ratio and details of the experiment are not open to independent scientific scrutiny (GeneWatch UK, 2012). Results of Oxitec's experiments in Brazil are poor, with a release ratio of fifty-four to one being required to achieve any noticeable effect in a small suburban area (PAT, 2012). Information on both short-term and long-term efficacy is critically important for population suppression programmes, especially for disease vectors (see comments on Section 4.2.6). Failure to publish results is not consistent with the step-by-step approach required by paragraph (24) of Directive 2001/19/EC (EC, 2001). The applicant should be required to: (1) Provide evidence on mating fitness and on the expected release ratio of GM to wild-type insects required for a given population suppression effect in a given receiving environment; (2) Consider unintended effects on population dynamics such as fluctuations in target insect populations; influx of insects from surrounding areas; potential increases in target insects in surrounding or target areas: some models exist (e.g. Atkinson et al., 2007; Yakob et al., 2011; White et al., 2010) but have not been validated; (3) Consider any changes to population structure, especially where these may have impacts considered in other subsections e.g. transmission of some diseases may be related to mosquito size (Alto et al., 2008); (4) Consider the mechanism and impacts of developing resistance (see comments on lines 3037-3039) and other mechanisms that may limit efficacy in either the short or long term, such as multiple mating (Helinski et al. 2012; Patil et al., 2012) and loss of mating fitness through the colony effect (IAEA, undated); (5) Quantify numbers of dead and surviving GM insects in a variety of scenarios, including numbers of dead larvae or pupae using a late-acting lethality trait, numbers of surviving males and dead adult females in each generation using female-specific approaches; numbers of surviving progeny, including females, taking account of limitations in penetrance conditionality etc. (e.g. exposure to tetracycline where this is used as a chemical switch for conditional lethality).

Lines 3356-3357: Should not refer to "sterile" insects: there are no sterile GM insects currently being considered for release, see comments on Lines 2983-2984, 3061 etc. etc.

Lines 3363-3365: The claim that population suppression of a non-native pest "should help to restore the environment to the state prior to the establishment of the non-native pest" is speculative: firstly, this depends on efficacy and whether the pest is really eradicated or e.g. only temporarily suppressed; secondly, there is hysteresis in environmental systems and non-linear effects meaning a return to some kind of former state is an over-simplification; thirdly, removing or reducing a pest (even if non-native) can cause increases in competitor pest species or reductions in beneficial species, or complex effects on human immunity i.e. adverse effects may also occur (see later comments). For native and non-native species the implications of reducing one component of a complex ecological effect may be difficult to predict. In the case of preventative release, there is a lack of current harm from the target pest and plans to introduce releases of a GM pest must consider the potential for survival and introduction of the pest (Section 4.2.1) due to incomplete penetrance or other mechanisms through which it might survive and breed. The issue of introduction of non-native strains as parent strains of the GM insect, as well as non-native species, should be fully considered in this section, along with the compatibility of such proposals with plant pest regulations, see comments on Lines 867-877.

Lines 3369-3373: This section should be clear that a step-by-step approach to releases must be followed, so open release experiments are not conducted prematurely, see paragraph (24) of Directive 2001/19/EC (EC, 2001). Predictions should be made using computer models, calibrated with data from the lab and caged trials and validated at each stage before moving to open releases: multiple conceptual models must be considered to develop worst-case scenarios. Care must be taken to establish baselines of wild populations (which will fluctuate in different conditions, seasons etc.): since one of the predicted potential harms of SIT is a possible increase in target populations in

surrounding areas (Atkinson et al., 2007; Yakob et al., 2011; White et al., 2010): a simple comparison of population levels in the target area with a neighbouring area is insufficient to establish a beneficial effect. Adverse effects which occur outside the release area also need to be identifiable and distinguishable from natural fluctuations. Population density as a result of GM insect releases may fluctuate with time, suffer an increase due to reduced effectiveness of the releases (e.g. due to developing resistance) and vary in and around the release site. Conditional lethality will result in large numbers of dead larvae, the numbers and distribution of these should be established in order to assess their potential impacts on biotic and abiotic processes. Female-killing or female-flightless approaches may also result in the survival of multiple generations of GM males and dead females: the numbers and distribution of these should be established. For disease prevention, the ultimate endpoint is disease incidence and severity (James et al., 2011): it is important that this is assessed because successful population suppression does not necessarily mean less, or less severe, disease , due to issues such as disease transmission thresholds and human immunity and cross-immunity (see comments on Section 4.2.6). Again, multiple conceptual models need to be considered to identify worst-case scenarios: for example, Oxitec's models of dengue transmission (Yakob et al., 2008; Alphey et al., 2011b) omit the important effects of cross-immunity on the incidence of dengue haemorrhagic fever and thus assume that only beneficial impacts on disease impacts can occur, when in practice there could be significant harm if population suppression in high-transmission areas is only partially effective (Thammapalo et al., 2008; Nagao & Koelle, 2008; GeneWatch UK, 2012). If the right concepts and mechanisms are not in the model in the first place, potentially serious risks may not be identified. For agricultural pests, crop damage will need to be assessed. Endpoints will also be needed for other potential applications such as enhanced pollinators.

Line 3375: Markers must be tested for reliability: for example Oxitec's fluorescent marker began to disappear after 4 days in ovitraps at high temperatures (Walters et al., 2012).

Lines 3379-3382: Stability should be assessed (not merely considered).

Lines 3382-338: It is unclear how easily colonies can be renewed with local wild-types in the case of GM insects because the wild-type cannot be simply irradiated (as is the case with SIT) but a new transgenic line will have to be developed: any such lines must be fully tested to ensure they are consistent with the Environmental Risk Assessment (ERA) and account must be taken of pest control regulations and the potential differences in disease transmission and insecticide resistance between strains (see comments on lines 867-877). All potential means of developing resistance should be assessed (see comments on lines 3037-3039) and other mechanisms that may limit efficacy in either the short or long term, such as multiple mating (Helinski et al. 2012; Patil et al., 2012) and loss of mating fitness through the colony effect (IAEA, undated). Failure of conditional lethality or female lethality mechanisms can also occur in the presence of e.g. tetracycline contamination, in the case of Oxitec's technology, because tetracycline is used as a chemical switch which allows the breeding of insects in the lab. Data must be presented show penetrance of the conditional lethality or female killing trait in the presence of tetracycline; and levels in the environment (e.g. in sewage, industrially farmed meat etc.) must be established.

Lines 3389-3396: Initial success of suppression followed by subsequent failure can lead to irreversible effects (such as establishment of an alternative invasive pests, see comments on Section 4.2.4; or reduction in human immunity, leading to a rebound in cases of disease, see comments on Section 4.2.6). There may be implications of introducing population suppression approaches for insect population management techniques more broadly, such as the need to prevent the use of some techniques which could interfere with the release programme, see comments on Section 4.2.5. These changes in management could affect the control of disease vectors and pests.

Lines 3397-3399: A step-by-step approach must be taken to any experiments, so that open release experiments are not conducted prematurely, see paragraph (24) of Directive 2001/18/EC (EC, 2001).

Lines 3402-3413: The introduction of new parent strains can be problematic, due to altered disease transmission or insecticide resistance, see comments on lines 867-877. The 'colony effect' can have severe impacts on male mating fitness, considerably reducing the efficacy of population suppression programmes (IAEA, undated).

Line 3425: If mass releases of infected male mosquitoes occur this can be problematic even though male mosquitoes do not bite, because e.g. male *Aedes Aegypti* infected with the chikungunya virus can infect female *Aedes Aegypti* during mating, and may mate with multiple females (Mavale et al., 2010; Bargielowski et al., 2011). Releases are also likely to include some females due to imperfect sorting (Reeves et al., 2012).

Lines 3429-3430: Strain and size may also be important for disease transmission and other properties.

Lines 3448-3451: Properties such as pest-resistance and pesticide-resistance should also be mentioned.

Line 3455: Markers should be tested for reliability in a range of conditions.

Lines 3487-3492: The introduction of new parent strains can be problematic, due to altered disease transmission or insecticide resistance, see comments on lines 867-877.

Lines 3495-3499: Strain and size may also be important for disease transmission and other properties.

Lines 3502-3508: The introduction of new parent strains can also be problematic, due to altered disease transmission or insecticide resistance, see comments on lines 867-877. The 'colony effect' can have severe impacts on male mating fitness (IAEA, undated): this may affect gene drive mechanisms and the ability to replace wild-type populations with the GM trait.

Step 2: Hazard characterisation

Lines 3522-3525: Expected outcomes in release sites and neighbouring areas are both needed (because there is a possibility that populations increase in areas surrounding the release site): expected release ratios must be reported. Markers must be tested.

Step 3: Exposure characterisation

Lines 3527-3529: This section should also recognise the importance of interactions between effects on target and non-target organisms i.e. the importance of an ecosystem approach. For example, an initial reduction in the target organism using a population suppression approach could reduce the abundance of predators and increase the availability of food supplies, breeding sites or prey, but these initial effects could create further feedbacks on the target population, e.g. reduced predators and reduced competition for resources could lead to a rebound in the target population.

Lines 3530-3532: Expected and actual release ratios and mating competitiveness should be reported: for example, the release ratio for Oxitec's experiments in the Cayman Islands has not been published and release ratios in experiments in Brazil have reached up to fifty-four GM mosquitoes to one wild

mosquito (GeneWatch UK, 2012; PAT, 2012). The mating competitiveness was only 0.03 (3 in 100) on average and dropped to 0.012 (1.2 in 100) in the final phase in the Brazil experiments. This is an indication of poor efficacy in suppressing the wild mosquito population and could be used to make a comparison with similar parameters expected for the Sterile Insect Technique (SIT) as suggested in Lines 447-450. Tests should be conducted on conditional lethality and other traits to assess the penetrance of the trait under varying laboratory and environmental conditions (e.g. the dose-response curve to tetracycline is an important parameter which varies for different lines of Oxitec's GM insects, see e.g. Ant et al., 2012). Mechanisms through which laboratory conditions which allow breeding and survival in the lab may be encountered in the wild must be reported, e.g. tetracycline (which acts as a chemical switch for Oxitec's conditional lethality trait) is widely used in medicine and agriculture and can be found in sewage, slurry and food products. An Oxitec laboratory protocol reports a 15% survival rate of its GM mosquitoes when fed cat food containing industrially farmed chicken, which contained sufficient levels of tetracycline (or an analogue of tetracycline) to overcome the lethality trait despite heat treatment (Nimmo et al., undated). It is also important to report the strain released (which may influence important properties such as disease transmission and insecticide resistance).

Line 3535: Stability is not only important for replacement strategies but also for population suppression strategies, as loss of efficacy can result in a rebound in the numbers of pests etc. In the case of disease vectors this can cause a rebound in cases of disease (Curtis et al., 2003; Scott & Morrison, 2003; Egger et al., 2008). A key difference between the Sterile Insect Technique (SIT) using irradiated insects and the release of genetically modified (GM) insects is that radiation-induced sterility involves multiple chromosome breaks, whereas the RIDL system relies on a specific genetic modification. Radiation-induced sterility therefore has built-in redundancy that is not provided by molecular genetic approaches. A number of authors have therefore speculated that any genetic or molecular event that allows the GM mosquitoes to survive and breed successfully could therefore be rapidly selected for during mass production (Benedict & Robinson, 2003; Robinson et al., 2004). If this happens, the conditional lethality effect could rapidly disappear as resistance develops in production facilities or in the field. Experimental data are therefore needed on resistance. Mechanisms other than selection for mutations during mass production may also be important such as female insects developing strategies to avoid mating with GM males or increased multiple mating (Hibino & Iwahashi, 1991; Helinski et al., 2012). Strains must be reported and re-tested if new GM strains are introduced periodically to counter the "colony effect" as new strains may have different properties (e.g. disease transmission or insecticide resistance).

Lines 3550-3553: Markers should be tested.

Lines 3556-3558: The type and extent of density-dependence in populations plays an important role in determining whether a population suppression approach will have a positive, neutral or negative effect (Juliano, 2007; Gould & Schliekelman, 2004; Walsh et al., 2011; Walsh et al., 2012; Barclay, 2001). Density-dependent effects at all life stages (e.g. larvae, pupae, adult) must therefore be reported for the receiving environment. Density dependence e.g. the effects of larval interactions on mosquito populations are different in different contexts, because they may be altered by ecological conditions (Juliano, 2009).

Lines 3565-3568: It is questionable whether preventative releases would be compatible with plant pest control regulations since the concept of a "preventative" release implies that a GM pest would be released where the wild pest does not currently exist. This risks establishment of the pest in the release area: e.g. because conditional lethality is not fully penetrant, resistance develops, or the necessary conditions to ensure lethality are not met (e.g. in Oxitec's case, through exposure to tetracycline in the environment, which acts as a chemical switch for the lethality trait). The concept

of preventative releases is even more questionable in the case of disease vectors such as *Aedes albopictus*, currently present in Italy (by far the most heavily infested country in Europe) and posing a potential health hazard to the rest of the EU (Hansford et al., 2010; ECDC, 2009). Is EFSA really suggesting that preventative releases of GM *Aedes albopictus* would be allowed in areas where this mosquito is not yet established? Why is there no discussion of the receiving environment in this section? Establishment of the baseline of the target species and of non-target species, as well as the presence of humans who may be bitten is critical. It is not clear how an EU-wide market approval can be applied to mass releases of GM insects given the major problem that target species will be established in some environments and not others (and at varying densities) and that the response to population suppression will depend on the ecosystem (e.g. density dependent effects). Thus, even if it were possible to establish that GM insect releases might have a beneficial effect in one area (e.g. reduction of a pest species or disease vector with a genuine sustained reduction in crop damage or disease incidence) the same GM insect releases might have a harmful effect elsewhere (e.g. establishment of a new pest species or disease vector in an area whether this species or strain had not been a problem). More complex effects might also occur in some areas but not others (e.g. competitive displacement by a more invasive pest species, effects on human immunity etc.).

Step 4: Risk characterisation

Lines 3586-3590: Lines 3591-3591: The use of the word “sterile” should be avoided: GM insects to date are not sterile but have a late-acting lethality trait that is partial and conditional. The possibility that GM insects with such traits become self-sustaining is only one aspect of impacts on target populations (and hence on ecosystems and endpoints such as human disease and crop damage). Even if the GM insects to do not become self-sustaining they are intended to have a significant effect on the target population (a population suppression effect) which can pose risks through a variety of mechanisms. There does not seem to be any consideration here of the dispersal of insect eggs and the timeframe for releases: it is unclear to the reader why these issues do not crop up until the section on non-target organisms, see comments on lines 3724-3725.

Lines 3596-3597: It is not in principle correct to assume that ecosystems will revert to the original status after releases are stopped since they may exhibit hysteresis e.g. target populations could rebound after an initial suppression effect; other species may move into the ecological niche and become established; viruses could evolve, extinctions could occur etc. Some effects may not be reversible.

Line 3598: Inherited lethality is partial and conditional in the case of Oxitec’s RIDL insects.

Lines 3601-3602: In some circumstances, loss of efficacy can increase adverse impacts beyond original levels e.g. via a rebound in populations or disease impacts.

Lines 3603-3605: Oxitec’s fluorescent marker fails in ovitraps in hot weather (Walters et al., 2012). Markers should be tested.

Lines 3607-3610: Expected loss of fitness (e.g. through the “colony effect”) and all mechanisms for development of resistance should also be considered.

Lines 3614-3615: Resistance is expected to develop and loss of fitness will occur through the “colony effect”: these are not unexpected effects.

Lines 3616-3617: Preventative releases are problematic: see comments on lines 3565-3568.

Lines 3620-3621: An ecosystem approach is needed: see comments on lines 3527-3529. Population suppression approaches can also lead to fluctuations in target populations and/or increases in populations in neighbouring areas (Yakob et al. 2008).

Lines 3624-3625: These issues also apply to population suppression strategies.

Step 5: Risk management strategies

Lines 3627-3629: There is a complete absence here of any discussion of the importance of restricting receiving environments (see for example comments on Lines 3565-3568). It is virtually inconceivable that a GM insect will be authorised for release across the whole of the EU because of the risk of establishing agricultural pest or disease vectors species where they do not currently exist. See also the discussion of strains above e.g. lines 867-877 i.e. introduction of non-native strains is also problematic and not compatible with plant pest regulations. The idea of introducing non-native beneficial insects such as bees is also deeply problematic. This means that risk management strategies MUST include measures to restrict transport and dispersal of eggs (deliberate or accidental), larvae and adults, and to limit the spread of the releases to the authorised receiving environment only. Whether this is any way practical or achievable is of course questionable, but this issue cannot be simply ignored. For example, controls are likely to be needed on fruit and vegetables containing GM eggs or larvae as 100% penetrance of lethality traits cannot be guaranteed. If an eradication approach were really achievable this might be less problematic as the marketing of fruit and vegetables could be suspended during the release programme, the crops could be destroyed, and it might be possible to allow resumption of marketing once sufficient monitoring had established the absence of the pest. But Oxitec's concept of ongoing releases to achieve population suppression implies that fruit and vegetables containing GM eggs and larvae would continue to be marketed throughout perhaps decades of releases (see also comments on Lines 3724-3725). See also comments on lines 185-186, regarding traceability and labelling.

Lines 3636-3638: Not only the numbers but also population structure (e.g. age, size) can affect disease transmission so these need monitoring too.

Line 3648: Applicants should also indicate how loss of efficacy would be detected and managed.

Step 6: Overall risk management and conclusions

4.2.4 Interactions of the GM insect with non-target organisms

Line 3677: There is nothing about pathogens at all in this section, despite the citation given from the Directive: this is an extraordinary omission given the many roles that insects play as disease vectors and transmitters of pathogens e.g. from faeces to fruit, and that some of the proposed applications specifically involve disease vectors with a potentially major impact on public health (e.g. mosquitoes that are vectors for dengue and chikungunya) and on animal health. Insects may also transfer pathogens to animals and plants. For comparison the GM fish subsection 4.1.4 includes five pages on pathogens, infections and diseases. The GM mammals and birds section includes eight pages on pathogens, infections and diseases in subsection 4.3.3 and then subsection 4.3.4 also (confusingly) treats the pathogens carried by birds and mammals as target organisms (this problem with inconsistent definitions needs to be addressed, see comments on lines 1832-1843). An entire new section on pathogens, infections and diseases needs to be included in the GM insects section, similar to the ones for fish and mammals/birds. This should address the multiple roles that insects play in spreading viruses and bacteria to humans, animals and plants and how these processes might be affected by deliberate or accidental releases of GM insects. As noted in comments on lines 267-272

it is not correct to claim that ingestion risks have already been considered in existing EFSA Guidance (EFSA, 2012a) as risks of ingestion of GM insects have been specifically excluded from that Guidance document: an entirely new Guidance document therefore needs to be developed to address this issue, taking account of the fact that many of Oxitec's proposed applications will result in large numbers (many millions) of dead or dying GM larvae or pupae in or on fruit or vegetables for human consumption. However, the non-ingestion route is also important: for example transmission of viruses or parasites via mosquito bites or ticks; feeding on wounds by blowflies. Important issues include: potential for target and non-target insects to evolve in response the GM releases to become more efficient vectors of disease; potential for viruses to evolve in response to genetically engineered changes to the disease vector (Medlock et al., 2009); potential for non-target pests or disease vectors to increase as a result of competition effects (discussed further below) and to continue or enhance disease transmission and/or be more invasive; the possibility that large-scale releases of GM organisms themselves transmit diseases or pathogens (e.g. fruit flies released to reduce pest damage might nevertheless spread pathogens from faeces to fruit); potential for introgression from GM strains into wild strains altering disease transmission properties (e.g. introducing a more effective vector for the target disease or a non-target disease, see GeneWatch UK, 2012). Although many insects transmit disease mainly at the adult stage, some parasitic larvae can also feed on human or animal tissue. Since many aspects of these issues do not relate to food safety it is highly questionable whether EFSA has the remit or expertise to cover them: nevertheless this gap must clearly be addressed before any releases actually take place. All relevant issues e.g. measurement endpoints, risk management need to be included in this new section on pathogens, infections and diseases and it must be re-issued for consultation. Impacts on plant pathogens must also be assessed as many insects can also transmit pathogens to plants or cause damage which makes plants vulnerable to attack. For example tomato leaf borer (*tuta absoluta*) causes both direct and indirect damage to tomatoes, with the latter being caused by secondary infections, with pathogens developing on the infested plant and fruit tissues. Releases of large numbers of GM *tuta absoluta* (as proposed by Oxitec as part of a population suppression approach) could therefore play a role in plant infections. Finally, the creation of virus-resistant silkworms (Jiang et al., 2012) (followed, perhaps, in the future by other species such as bees) raises a further set of questions e.g. whether viruses might evolve to overcome resistance and pose a greater problem to these species than before. The development of disease-resistant traits has been discussed in some detail for mammals and birds (Subsections 4.3.3 and 4.3.4) but ignored for insects.

Lines 3699-3703: Impacts on animal health must also be considered, see proposal for a new section on this (comment on line 4154). Why are impacts on crops also completely omitted? For releases of GM agricultural pests a key endpoint will be crop damage: this must be considered both in terms of efficacy (i.e. whether the releases are achieving their intended purpose of reducing crop damage) and in terms of unintended consequences. For example, the GM releases might allow plant pests to become established in new areas and increase crop damage: for example, this might be either be through the GM insects or hybrids becoming established and directly causing damage, or through competition effects allowing new plant pests to be established. See also comments on plant pathogens (comments on line 3677).Compliance with plant pest legislation will be essential.

Line 3719: Oxitec envisages continued releases of its GM mosquitoes for more than 50 years (Alphey et al., 2011b) and its business plan depends on repeated payments for ongoing releases (GeneWatch UK, 2010). This 50-year timescale now seems rather over-optimistic given the poor performance in the field (PAT, 2012; GeneWatch UK, 2012). It is therefore difficult to understand why the draft Guidance refers to "eradication" (although in theory eradication might be feasible with a different technology, such applications do not appear to be close to market).

Lines 3724-3725: Fifty years is not really a "limited time" (see comments on line 3719 above) and, in any case, eradication is not envisaged over this time frame, merely continued population suppression (this in itself is questionable, given the many mechanisms for loss of fitness or development of resistance). There are also many mechanisms through which adult (flying) insects and particularly eggs might be transported to areas other than the release site. For example, the invasive species *Aedes albopictus* is thought to have spread worldwide via ships and tyres and agricultural pests spread via shipments of fruit, vegetables and other plant material etc. Because conditional lethality is partial and conditional, it is therefore deeply questionable whether GM insects will remain restricted limited area. For example, many mosquito species breed in septic tanks (Barrera et al., 2008) where sewage may be contaminated by tetracycline, allowing Oxitec's GM mosquitoes to survive and breed, perhaps for multiple generations (GeneWatch UK, 2012). Depending on the species, some insects eggs can remain dormant and survive dessication with larvae re-emerging at a later date (see e.g. Reiter et al., 1995: CDC, undated).

Line 3725: It is difficult to understand why this sentence is restricted to "natural enemies" when the Directive (as cited in line 3678) is clear that many other species and interactions must be considered e.g. competitors. The argument that short-term presence of the GM insects cannot lead to long-term effects is incorrect: for example, if an invasive competitor where to become established due to competitive replacement whilst the population of the target organism is suppressed this effect might not be reversible. Similarly, if it were true that the target pest is likely to be eradicated, this could have irreversible effects (including other extinctions). Effects on competitors are very important because many diseases are spread by more than one vector and many crops have more than one pest. If the ecological niches of these species overlap, i.e. if they are competitors, the use of the population suppression approach (which is species-specific) could lead to increases in competitors with potentially harmful (and possibly irreversible effects). Although this is discussed in lines 3739-3756, this is not reflected in this paragraph, which downplays the risks and implies all such effects would be reversible.

Line 3769: However, <u>indirect</u> effects of the population suppression approach on pollinators should be considered.

Line 3775: Should refer to potential increases in competitors.

Line 3783: See comments on "limited in space and time", lines 3724-3725.

Lines 3786-3787: See comments on "preventative releases", lines 3565-3568.

Lines 3788-3790: Suppression of a non-native species can still have significant effects on biodiversity, e.g. if numbers of another non-native species increase.

Lines 3791-3804: It is unclear why a separate section on biogeochemical processes and abiotic interactions has not been included, to parallel the requirements of the Directive in Section D.1 of Annex 2 (point 8) (EC, 2001). This would aid consistency within the Guidance i.e. with GM fish and GM mammals and birds (Subsections 4.1.5 and 4.3.6), both of which extend to several pages. The numbers of dead larvae and pupae introduced into the environment if Oxitec's RIDL technique is used commercially will number many millions per week (e.g. PAT, 2012 reports scaling up production to 2.5 million GM male mosquitoes a week: although perhaps only 10% of these will mate successfully each female lays multiple eggs which are expected to hatch and die at the late-larval stage). Large numbers of dead female adults may also arise from some female-specific approaches such as flightless female *Aedes albopictus* mosquitoes (Labbé et al., 2012). Dead GM insect larvae, pupae or adults (whether disease vectors or agricultural pests) might have effects on e.g. water

quality or soils. Some insects can lay very large numbers of eggs. Applicants should estimate the number of dead larvae etc. likely to enter the environment as a result of the proposed release programme and quantify their expected fate (e.g. percentage eaten by predators, rotting on the ground, in fruit or vegetables, or in water supplies etc.).

Lines 3819-3820: The ERA is required to take into account GMOs already in the environment (EC, 2001, Annex II). This might include other GM insects, GM crops or GM fish, mammals or birds. Multiple GM insect species may be released where there is more than one disease vector or agricultural pest. For example, Oxitec has responded to concerns that the dengue-transmitting species of mosquito *Aedes albopictus* might increase in response to its releases of GM *Aedes aegypti* by saying that it could introduce a GM *Aedes albopictus* population suppression programme (presumably based on its prototype flightless-female technology) in combination with a GM *Aedes aegypti* release programme (Alphey et al., 2010). A major proposed application is to combine GM agricultural pests with GM crops (pest-resistant Bt crops) in an attempt to tackle the growing problem of the emergence and spread of resistant pests (Alphey et al., 2007; Alphey et al. 2009; Oxitec, 2011b). If a non-target pest increased (as has been observed e.g. in association with Bt cotton in China : Zhao et al., 2011), a different species of GM insect might presumably then be introduced to tackle that.

Line 3826: See comments on "temporary", lines 3724-3725.

Lines 3831-3835: This discussion of receiving environments is extremely poor, see e.g. comments on lines 3565-3568. Issues to be considered include: (1) density of target species (presence or absence, likely efficacy of suppression etc.); (2) other species e.g. presence or risk of introduction of competitor pest or disease vector species; (3) human habitation. Implying that human-made habitats are lower risk is completely wrong: for example human immunity effects can create increased risks to human health if population suppression approaches to disease vectors are used in inhabited areas where disease transmission is high (see e.g. GeneWatch UK, 2012 and comments on Section 4.2.6).

Lines 3836-3842: This paragraph ignores the risks of introducing non-native GM species and strains to areas where they are not currently established.

Lines 3843-3845: It is not correct to state that some GM insects will only be present at specific life stages: for example Oxitec's GM insects mostly die at the larval stage but some survive to adulthood and large numbers of adults will be continually released (including a small percentage but potentially large number of females due to imperfect sorting). Female-killing approaches will also obviously allow multiple generations of males to survive in the environment.

Step 2: Hazard characterisation

Lines 3854-3855: The concept of environmental endpoints that "need to be protected from harm" is too restrictive as it ignores the possibility of increases in harmful competitor species.

Lines 3867-3870: Pest regulation is critical and needs to be properly quantified, not ignored as "too difficult". If it is too difficult a precautionary approach means that releases should not be allowed.

Lines 3871: Restriction to "potential hazard to NTOs" is too restrictive a definition: it does not encompass potential increases in harmful non-target species (e.g. pests or disease vectors, which might then cause damage to crops, endangered species or human health).

Line 3873: The strain as well as the species is important.

Lines 3892-3896: Population dynamics of the target and non-target species and their interactions (e.g. larval competition) must be understood, otherwise risks such as increase of non-target pests due to competitive displacement cannot be assessed. Information on ability of species to recover is important, but so is information on the ability of competitor disease or pest species to persist.

Line 3889: Not just "hazards for non-target species", also potential increases in non-target disease vectors or pests.

Line 3900: Properties of the strain e.g. disease transmission must also be measured.

Line 3909: It is not correct to say that susceptibility to pesticides (and disease transmission properties) are mainly relevant for replacement strategies as there may be introgression into wild relatives in population replacement strategies and the GM insects may also survive as a result of incomplete penetrance, failed conditionality, resistance etc.

Line 3913: Distribution of competitors is also important (as this relates to potential increase in non-target pests or disease vectors).

Line 3923: Delete the word "especially": some issues, e.g. increases in competitors, are more likely to be a problem with population suppression approaches.

Line 3926: The word "sterile" is misleading: this should refer to partial, conditional, late-acting lethality. The reference to adult-only life stages is misleading: see comments on lines 3843-3845. (This is an especially problematic claim for female-killing approaches).

Line 3929-3933: "Sterile" is misleading. This whole section is again confused about whether open experimental releases can be made or not. Reference should be made to the step-by-step approach required by the Directive (EC, 2001), see comments on lines 248-250. For example, surveys of competitor species and studies of inter-species competition can be made in the undisturbed proposed release environment (wild target and wild non-target species) and in the lab and caged trials (GM target, wild target and wild non-target) and can be combined with modelling approaches to seek to predict likely effects of competitive displacement. Applications for open release experiments should only follow if these earlier studies suggest that such releases will not to lead to an increase in harmful competitors and if it has been established that releases will not spread to other environments where such effects might pose a problem.

Line 3934: Replace "can" with "should".

Lines 3937-3941: This paragraph must include competitor species and methods to establish that harmful competitor species will not increase or become established in new areas.

Step 3: Exposure characterisation

Line 3944: The term "exposure pathways which may harm the environment" is too narrow. "Exposure pathways" implies direct toxicological effects (excluding, for example, increases in competitor species) and human health should also be included here (for example, an increase in a competitor disease vector may harm human health).

Line 3945: Not only adverse effects on NTOs, also increases in harmful NTOs.

Lines 3948-3953: Release ratios are also needed. Note: the structure of the Guidance risks being repetitive here: all these parameters are also needed to assess the effects on target organisms too. There should be a better way to organise this information.

Line 3968: The RIDL population replacement strategy involves repeated large scale releases for decades. Hence the sentence claiming that climatic changes are of particular importance for replacement strategies should be deleted.

Line 3970: Should refer not just to the GM insect but to competitors, prey, hosts, symbionts, predators etc. as this is what this section is supposed to be about and their ranges can also change with climate. The same is true of pathogens but a whole new section needs to be added to deal with these, as noted above.

Step 4: Risk characterisation

Lines 3977-3978: Not just risks caused to NTOs, risks caused by interactions with NTOs, including e.g. increases in non-target pests or disease vectors. Implications for crop damage need to be considered here and implications for human and animal health in future sections (a subsection on animal health is missing and needs to be included).

Lines: 4002-4003: It may be impossible to reverse adverse effects even if the released GM insect population can be eradicated (e.g. if an invasive competitor has become established).

Step 5: Risk management strategies

Lines 4020-4021: The comment regarding receiving environments is important: for example, a GM species-specific approach might be approved for an area where the crop or disease is eaten or spread by only one pest or vector, but not approved where there is more than one pest or vector, due to concerns about potential increases in these species. However, the issue of how spread of GM eggs, larvae and adults into unapproved receiving environments requires a lot more thought than is given here: see comments on lines: 3627-3629.

Lines 4024-4025: The sentence “This is of particular importance when applying replacement strategies” should be deleted.

Line 4028: Mitigation might also be needed for non-target species not just the GM insect, e.g. to tackle an increase in a non-target pest.

Lines: 4032-4033; A step-by-step approach to is also important in minimising risks. See comments on lines 248-250.

Step 6: Overall risk evaluation and conclusions

Lines 4035-4044: The concept of assessing feedback between effects on non-target organisms and effects on target organisms is missing here e.g. a reduction in target organism might lead to a reduction in predators which might lead to a rebound in numbers of the target organism. For example, for a population suppression approach, this could have adverse effects on the intended endpoints e.g. crop damage or human health. NTOs can have direct, indirect and multitrophic effects with GM animals, see Section 4.3.5 (in GM mammals and birds) and e.g. Figure 9. It is difficult for the reader to understand why such effects are discussed in detail for mammals and birds (where current

proposed applications are mainly semi-contained) but ignored for insects (where proposed applications will involve large-scale open releases).

Line 4044: An entirely new section must be added here on pathogens, infections and diseases, to parallel subsection 4.1.4 for GM fish. See comments on line 3677.

4.2.5 Environmental impact of the specific techniques used for the management of GM insects

Line 4054: There will not be one "comparable non-GM insect system" but many, because there are a wide variety of ecosystems in the EU and also multiple approaches to tackling pests, e.g. for agricultural pests agro-ecological systems versus more intensive systems, large-scale and small-scale farming systems, open fields, polytunnels and greenhouses, monocultures etc. etc. The same is true for disease vectors, see comments on lines 2949-2950. Management practices and any changes to them will be context-specific (i.e. depend on the ecosystem at the target site and a wide range of alternative management practices) and vary with time (e.g. as resistance develops, or as new tools become available e.g. vaccines or better monitoring for diseases). This likely diversity of management systems is recognised in EFSA's Guidance on the environmental risk assessment of genetically modified plants (EFSA, 2010a) which states that the ERA shall: describe the potential range of GM-based management and production systems likely to occur across receiving environments and how they differ from current management systems; identify the potential adverse environmental impacts associated with these systems; assess to what extent the environmental impacts overlap those of the range of non-GM systems; determine which conditions (receiving environments, management and production systems) are related to potential higher adverse effects than current systems; assess to what extent the range of GM management and production systems would meet the assessment endpoints identified in the other chapters. Similar wording should be used here. Management regimes are likely to be complex: for example, Oxitec and co-authors state: "*our analysis leads us to conclude that in many instances the optimal strategy is likely to be an IVM* [Integrated Vector Management] *program with a significant SIT* [Sterile Insect Technique] *component but also using other methods, especially insecticides*" (Alphey et al., 2010).

Line 4062: It should be recognised here that management systems are likely to change with time e.g. as resistance develops to the GM trait, ecosystems change (e.g. in response to the GM releases or to climate change) and new technologies are developed (e.g. new larvicides or mosquito traps, better disease interventions such as vaccines) or farming practices change. Multiple GM insects might be introduced into the same management system in future. For example, Oxitec has responded to concerns that the dengue-transmitting species of mosquito *Aedes albopictus* might increase in response to its releases of GM *Aedes aegypti* by saying that it could introduce a GM *Aedes albopictus* population suppression programme (presumably based on its prototype flightless-female technology) in combination with a GM *Aedes aegypti* release programme (Alphey et al., 2010). A major proposed application is to combine GM agricultural pests with GM crops (pest-resistant Bt crops) in an attempt to tackle the growing problem of the emergence and spread of resistant pests (Alphey et al., 2007; Alphey et al. 2009; Oxitec, 2011b): discussion of the implications of this strategy should have been included in this draft Guidance document. One proposal, for example is to reduce the size of non-Bt-crop refuges and use GM insect releases to slow resistance instead: this clearly has implications that should have been discussed. Again, long-term risks must be considered (e.g. potential increased use of more hazardous pesticides when neither the Bt plant nor the GM crop is any longer effective, due to development of resistance in both systems). If a non-target pest increased (as has been observed e.g. in association with Bt cotton in China : Zhao et al., 2011), would a different species of GM insect then be introduced to tackle that? How would the complexity of this system be addressed? Presumably more than one company could become active in this area in future: therefore the possibility of multiple applications being released or escaping into the same

receiving environment also needs to be considered. Other potential applications that have not been discussed include the possible development of pesticide-resistant bees or other beneficial or useful insects (e.g. silkworms): such applications might result in increased use of pesticides.

Lines 4063-4080: Need to add here: changes in management system (e.g. suspension in use of larvicides, adulticides or public health approaches to removing breeding sites) may be needed during a GM insect release programme but changes to these measures could reduce controls on other GM disease vectors or GM pests. Continued use of control measures such as insecticides during release programmes could affect population dynamics in complex ways: impacts on efficacy and safety of the programme therefore need to be considered (Thomé et al., 2010).

Lines 4081: Should include protection of human health.

Lines 4092-4094: The sentence "*Alteration to management practices might provide both environmental benefits as well as harm so that the net environmental impact of the overall production system needs to be considered*" must be deleted. EFSA's Guidance on the environmental risk assessment of genetically modified plants (EFSA, 2010a) states clearly: "*The overall risk/benefit is out of the remit of the EFSA mandate. The ERA should primarily focus on potential environmental risks arising from the GM plants*". The sentence added here (presumably inserted at the request of Oxitec/Syngenta) is not consistent with EFSA's mandate and is a blatant attempt to change EFSA's mandate and the entire purpose of the ERA process through the back door. The addition of this sentence raises a number of serious concerns: (1) The Guidance is intended to assist applicants to produce an environmental risk assessment as defined in Article 2, paragraph 8 of Directive 2001/18/EC (EC, 2001), this does not include an assessment of potential benefits; (2) this proposal amounts to a significant proposed change in the purpose and role of environmental risk assessment, which should not be buried on page 97 of a draft Guidance document; (3) EFSA has no competence to assess claimed environmental benefit: its remit is safety of the food chain (EC, 2002); (4) net environmental impact will be context-specific (i.e. depend on the ecosystem at the target site and a wide range of alternative management practices) and vary with time (e.g. as resistance develops): it is therefore unlikely that claimed benefit can be quantified in a manner that is meaningful in the context of the single market; (5) claimed benefits are likely to be contentious and disputed: if environmental net benefit were to be assessed, relevant guidance and jurisdiction over such assessments would need to be developed and an appropriate body would need to be allocated this task; (6) programmes for large-scale releases of GM insects for pest control or public health purposes may well be subject to a Strategic Environmental Assessment (SEA) under EC Directive 2001/42/EC, however the role of the EC in evaluating net environmental impact at an individual farm level is less clear: even where an SEA is required, this does not replace the legal obligation to conduct an ERA consistent with the requirements of 2001/18/EC.

Step 2: Hazard characterisation

Lines 4099-4101: Multiple management systems need be considered, including changes over the short- and long-term.

Line 4106: Alternative conceptual models must be explored in order to identify worst-case scenarios and models must be validated.

Step 3: Exposure characterisation

Lines 4114-4115: Short-term and long-term changes must be considered e.g in response to the development of resistance.

Lines 4121-4125: It is not correct to state that these models have been validated.

Step 4: Risk characterisation

Lines: 4131-4133: Multiple receiving environments and management practices must be considered. Changes over time must be considered e.g. taking into account loss of efficacy might (e.g. due to development or resistance, loss of fitness). The focus should be on risks, this Guidance is for risk assessment (see comments on lines 4092-4094), this section is about risk characterisation.

Step 5: Risk management strategies

Line 4137: It is not clear what is meant by "compared to non-GM related outcomes" (especially when there may be other GMOs in the environment e.g. GM insects combined with GM crops): this phrase should be deleted.

Line 4154: An entire section i.e. Impact on animal health has been omitted here. Insects transmit many pathogens to animals, via many routes e.g. ingestion, biting, transfer of pathogens from faeces to food. Proposed applications might in future include GM ticks or midges, with a view to reducing the impacts of animal diseases. The relevant issues need to be considered in detail. A new section therefore needs to be added here and then consulted on. This should build on the proposed new pathogens section (see comments on line 3677). Issues discussed below that are relevant to human health (e.g. impacts on immunity, increase in alternative vectors or disease transmission routes, evolution of viruses) may also be relevant to animals.

Section 4.2.6 Impact on human health

Line 4165: The wording in the Directive (EC, 2001) is coming into contact with *or in the vicinity of* the GM release(s). The rewording given here implies that it is only direct health impacts (e.g. from being bitten by the GM insect) that are important. In the case of population suppression approaches adverse health impacts can also occur indirectly, especially via: (1) the impacts of the GM releases on the non-GM target species (e.g. due to poor or temporary efficacy, rebounds in numbers, increases in the area surrounding the release site); (2) the impacts of population suppression on other disease vector species, especially increases in competitor disease vectors (see comments above and GeneWatch UK, 2012). These issues are largely ignored here in favour of issues relating to direct contact only. For disease prevention applications, the ultimate endpoint is disease incidence and severity (James et al., 2011): it is important that this is assessed because (i) population suppression may not be effective (or may be only temporary); and (ii) successful population suppression does not necessarily mean less, or less severe, disease, due to issues such as disease transmission thresholds and human immunity and cross-immunity (see comments on lines 4220-4227). It is important that informed consent is obtained for studies involving disease vectors.

Lines 4166-4168: The ingestion route will be important, especially for GM agricultural pests, and has been completely ignored and excluded from any consultation process (see comments on lines 267-272).

Line 4182: Toxicity testing should be required for all exposure routes (e.g. ingestion, biting): the introduction of toxic proteins could clearly have adverse impacts on human health and could be introduced via bites (e.g. mosquitoes or ticks) as well as ingestion or inhalation.

Line 4192: Allergenicity must be assessed for all exposure routes.

Lines 4203-4204: Changes to population structure e.g. age, size can also affect disease transmission. Strains must be tested and non-native strains must be avoided: otherwise there is potential to introduce enhanced transmission via new strains of disease vectors e.g. for Yellow Fever when trying to tackle dengue (GeneWatch UK, 2012).

Lines 4218-4219: Development of resistance, loss of fitness etc. need to be considered here. Contamination of production facilities with pathogens must also be considered. The potential for a different species of disease vector to increase or become established as a result of population suppression is of recognised importance and must be included in this section (Beech et al., 2009; GeneWatch UK, 2012). This is because GM approaches are species-specific, unlike many other approaches (e.g. removing breeding sites, or using traps or larvicides).

Line 4205: Possible evolution of pathogens in response to GM insect releases needs to be considered (e.g. Medlock et al.2009).

Lines 4420-4227: This section needs to recognise that temporary or partial efficacy in terms of population suppression can harm: it is not simply a question of changing human behaviour. For example, a rebound in cases of disease can occur (Curtis et al., 2003; Scott & Morrison, 2003; Egger et al., 2008). If population suppression is ineffective it may have no impact on disease transmission (if transmission thresholds are low) and it is also possible that mosquito populations increase in areas neighbouring the release site. Further, long-term suppression may fail due to effects discussed above such as loss of fitness or development of resistance. Cross-immunity as well as immunity may be important: for example, Oxitec's models of dengue transmission (Yakob et al., 2008; Alphey et al., 2011b) omit the important effects of cross-immunity between multiple serotypes of dengue fever on the incidence of dengue haemorrhagic fever and thus assume that only beneficial impacts on disease impacts can occur, when in practice there could be significant harm if population suppression in high-transmission areas is only partially effective (Thammapalo et al., 2008; Nagao & Koelle, 2008; GeneWatch UK, 2012). The most serious and often fatal form of dengue, dengue hemorrhagic fever (DHF), appears to be more likely when a person is infected by a second serotype of dengue fever, having already been infected by one of the other serotypes. This is thought to be due to immunological mechanisms including antibody dependent enhancement (ADE), in which the antibodies developed against the first infection make the second infection more severe. However, if the two infections with different serotypes occur in quick succession (within weeks) cross-immunity can develop which has the opposite effect, reducing the risk of DHF. Many of the individuals in areas of high vector mosquito abundance would be infected by, and acquire immunity against, multiple serotypes while they are protected by this cross-immunity and develop resistance to DHF unknowingly. One concern about partially effective interventions to reduce mosquito numbers is that as the mosquito abundance decreases, an increasing number of individuals would experience secondary infections after the protective cross-immunity has waned, and the incidence of DHF would then increase. One study in Thailand has suggested that in regions of intense transmission, insufficient reduction of mosquito populations may increase long-term incidence of DHF, because of the existence of this complex cross-immunity effect. This analysis suggested that reducing Aedes aegypti abundance from the highest level in Thailand to a moderate level would increase the incidence of DHF by more than 40%. Further computer modelling of this data has confirmed this finding. If correct, this has major implications for dengue control programmes, including the use of Oxitec's GM mosquitoes. It suggests that ineffective programmes may be worse than useless because they can actually increase the harm due to the disease, at least in high risk areas.

Step 2: Hazard characterisation

Line 4232: This section needs to be amended to take account of the comments above. The possible increase of other (non-target) disease vectors is a particularly important omission.

Line 4259: Strains are important, not just species. Viruses may evolve in response to altered properties of GM insects (Medlock et al. 2009): it is not clear how it is proposed this issue should be dealt with in the ERA.

Line 4265: Delete "might", replace with "should".

Line 4267: Delete "In case a replacement strategy is proposed": it is important to test vector competence for population suppression approaches also, as lethality is partial, conditional etc. and there will be some introgression of traits into the wild population.

Line 4286: This sentence is not about "SIT" it is about populations suppression using GM insects. Development of resistance, loss of fitness etc. must be considered.

Line 4290: A new section on health hazards due to increases in non-target disease vectors must be added.

Lines 4291-4295: Modelling alone is insufficient and models must include all relevant effects or they are useless (see comments on lines 4420-4227). Models should be developed and validated so that they represent real-world effects in the absence of releases first (a step-by-step approach). Releases in endemic areas where human immunity plays an important role should only be considered as the final step in a step-by-step approach as required by the Directive (EC, 2001). Cross-immunity as well as loss of immunity must be considered. Other problems with suppression must also be considered e.g. potential to increase disease vectors in surrounding areas, fluctuations in populations as a result of interactive effects (see comments on impacts on target and non-target organisms above). Loss of efficacy of populations suppression must also be considered as well as loss of efficacy of other traits in the population replacement approach (e.g. if disease transmission properties are reduced, will these be maintained?). There must be baseline monitoring of health and antibodies etc. before any open releases. Impacts of releases on disease must be evaluated following appropriate protocols (James et al., 2011).

Step 3: Exposure characterisation

Lines 4301-4346: This section does not consider any of the indirect hazards described above (i.e. hazards which come not from contact with the GMO but from the effect of releases on target and non-target species). The reader is inclined to feel that all input to the above sections on impacts on target and non-target species have been a waste of time as these effects are then totally ignored when it comes to the important endpoint of impacts on human health.

Line 4326: Risk of escape is also important.

Step 4: Risk characterisation

Lines 4333-4346: see comments on lines 4301-4346. Also: where is the discussion of receiving environment (including for example, which vectors are present there)? This is of critical importance because of the possibility of establishing new vectors in areas where they don't currently exist (this might be the target vector or a non-target vector).

Step 5: Risk management strategies

Lines 4348-4362: Perhaps the principle "first, do no harm" should be recalled at this point. Again, everything is focused on direct exposure, although indirect effects could cause significant harm by e.g. increasing transmission of diseases. Virus evolution is also missing. This section should be rewritten and re-consulted on.

Step 6: Overall risk evaluation and conclusions

Lines 4364-4366: Particular attention should be paid to risks to the health of individuals living in disease-endemic areas, due to adverse impacts on disease transmission (by target or non-target species), evolution of viruses, or impacts on immunity or cross-immunity. Experiments with disease vectors require informed consent and should not be conducted until these risks have been assessed.

4.3 Specific areas of ERA of GM mammals and birds

Description of the case studies

Lines 4370-4409: The scope of the Guidance should be clarified: see comments on Line 266: why is the use of GM animals for production of pharmaceuticals excluded from ERA? Transgenic goats that produce ATryn (an antithrombin drug for human therapeutic use) in their milk already exist on a farm in Massachusetts: ATryn was authorised for use in the EU in 2006 and in the US in 2009; applications involving the production of other pharmaceuticals in the milk of cattle, sheep and goats and the production of recombinant protein in birds eggs are being developed (FERA, 2010). The deliberate release of any of these GM animals in the EU should require an ERA. Further, the guidance should state more specifically which traits count as "production of pharmaceuticals" (lines 30, 226 and 596) for the purposes of this guidance. For example, are cows (or other animals e.g. sheep, goats, pigs, rabbits) genetically engineered to produce low-lactose or high-omega-3 milk or human proteins such as lysozyme or lactoferrin in milk included or not (Yang et al., 2011; Gray, 2012; FERA, 2010; Anon, 2012b)? Where is the line drawn between nutraceuticals and pharmaceuticals? If these applications are to be included, re-consultation is necessary so that consultees know what they are being consulted about. Presumably production of high omega-3 meat in transgenic pigs (Lai et al., 2006) is included in the remit of the Guidance, but it is odd that this application is not discussed. In general, the rationale for the choice of GM animal and bird examples is very unclear. The so-called Enviropigs at the University of Guelph were ordered to be destroyed in April 2012 (Nickel, 2012): it seems unnecessary to waste time on them. US company Exemplar Genetics aims to sell GM pig models for use in academic and pharmaceutical laboratories and there are concerns these might enter the food chain accidentally (Maxmen, 2012), but it is not clear whether these animals fall within the scope of this draft guidance. Would there really be a market in the EU for an enhanced growth cat (given both the environmental concerns and ethical and animal welfare issues especially surrounding the production process) and is any such product really being developed? Is it really the case that the release of GM 'sterile' rabbits "*can be forseen in the near future*"? None of the references in FERA (2010) suggest that such an application is close to being developed and it is unclear how sufficient numbers could be released for a population suppression application unless the sterility trait is somehow conditional (allowing breeding in the lab): more information is needed to allow informed comment. And if rabbits, why not other species, e.g. rats, grey squirrels? Rabbits as bioreactors producing bioactive chemicals in their milk are highlighted in the FERA report (FERA, 2010) and the Dutch company Pharming is now focusing on protein production in transgenic rabbits (Anon, 2012b), but these applications are not discussed, and nor is the escape of transgenic lab rats, although this issue was highlighted in page 32 of the expert report (FERA, 2010). The "avian flu resistant chicken" is at the proof-of-concept stage and impacts and disease transmission are as yet unknown (see discussion in Section 4.3.3).

General differences among captive, semi-captive, and non-captive GMOs relevant for ERA

Line 4439: Escape is important but so is human error or ignorance of regulations or failure to follow them. For example, GM chickens authorised for contained use in intensive production might still be sold to free-range chicken farmers, or smuggled out of factories etc. Traceability will be important for all species as e.g. eggs, sperm, embryos and adults (e.g. a male sire). For example, cloned cattle were exported to Scotland as embryos, and ended up in the food chain (Poulter & Bruce, 2012).

4.3.1. Persistence and invasiveness of GM mammals and birds and vertical gene transfer to wild and feral relatives

Line 4446: The title of this section is confusing: it implies vertical gene transfer to wild relatives is included, but only covers issues of persistence and invasiveness. It is then very unclear to the reader what aspects of vertical gene transfer are included in Section 4.3.2. However, the theoretical 'sterile' rabbit application is intended to crash the wild population of this species and could also have unintended consequences for whole ecosystems. It achieves this through the opposite of being persistent: it is engineered to mate with the wild population and cause that population to die out (through vertical gene transfer of the sterility trait). Issues associated with population suppression approaches such as this have in general been treated poorly throughout the Guidance (see comments on fish and insects) and a consistent approach is needed to capture these risks.

Line 4451: Remedial action may not reverse damage.

Line 4468: Keller et al. (2011) is not in the reference list.

Line 4621: Wheeler et al. (2001) is not in the reference list.

Line 4634: Shears et al. (1991) is not in the reference list. Is this also relevant to the fish section?

Line 4753-4755: The recommendation that sterile releases should always be considered has not been properly thought through: the theoretical 'sterile' rabbit application is intended to crash the wild population of this species and could also have unintended consequences for whole ecosystems. Whilst sterility may minimise the issues of concern considered in this section (i.e. invasiveness and persistence) it can exacerbate other concerns through its potential impact on wild populations and ecosystems. In particular, any GM animal that mates with wild animals can be regarded as having close contact with the wild species and a potentially large impact on it, whether the offspring survive or not: greater fitness in the offspring means more potential for persistence and invasiveness, but reduced fitness (or sterility) means more potential to suppress or even wipe-out the wild population, with potentially significant effects on other species due to interactions.

4.3.2 Vertical and horizontal gene transfer

Lines 4787-4884: As noted in comments on line 4446, the hazards associated with vertical gene transfer of 'sterility' traits or similar (i.e. population suppression approaches) have been completely omitted here. This problem is compounded by the different definition on "target organism" being used for mammals and birds compared to insects (see comments on lines 1832-1843). If the same definition is used in this section as for insects then impacts on population dynamics of the wild species could be included in the "target species" section (see Section 4.2.3 and comments on this above).

Line 4806: Reference EFSA (2011e) is missing from the reference list.

Line 4811: What about genes that are unadvantageous e.g. sterility but also any loss of fitness? Vertical gene transfer from the GMO to the wild species could then harm that species. See comments on population suppression approaches throughout this document.

Line 5031: EFSA (2009g) is missing from the reference list.

4.3.3 Pathogens, infections and diseases

Line 5033: It is confusing for the reader to see some of the information on pathogens deferred to Section 4.3.4, due to the conflicting definitions of "target organism" used in the document (see comments on lines 1832-1843). In addition, there is insufficient attention paid to the possible hazards of the population suppression approach (as represented by the 'sterile' rabbit). This type of approach might conceivably be applied with a view to reducing transmission of diseases (including those that might be transferred from animals to humans). However, this requires a full understanding of how this form of culling (reduction of the population through inherited forms of sterility or loss of fitness) might affect disease transmission. Such effects can be counter-intuitive leading to increased disease where a reduction was expected. For example, a recent study of the effect of culling bats on rabies transmission in Peru found that the prevalence of the virus was not reduced by culling and that the programme may even have been counter-productive (Streicher et al., 2012). This study confirms other findings in badgers and bats that suggest culling in wildlife disease systems can sometimes increase disease prevalence when it stimulates the recruitment of susceptible individuals or increases host dispersal. Theoretical modelling of a population of game has shown that culling can increase disease prevalence in animals and mortality (Choisy & Rohani, 2006).

Lines 5134-5140: This paragraph does not seem to adequately reflect the variety of hazards identified below e.g. evolution of viruses, compromised immunity etc.

Lines 5201-5212: Suggested additional reference: Greger (2011).

Line 5231: reference Velthuis et al. (2007) is missing from the reference list.

Lines 5294-5334: Reference should be made throughout this section to the need to validate computer models and the need for a variety of alternative conceptual models to be developed to ensure that worst-case scenarios are captured.

Line 5344: If a particular farm must produce only GM animals in order to mitigate risks this reinforces the need for traceability (see line 4439) and mechanisms for the enforcement of any authorisation conditions.

4.3.4 Interactions of the GM mammals and birds with target organisms

Lines 5365-5370: This definition of target organism is extremely confusing, see comments on lines 1832-1843. A better approach would be to define the target organism as the species that is being genetically modified (as is done in the insects section). Whilst the issues included here are important they could be included in Section 4.3.3. An entirely new section is needed to address the impact of releases of the GM on the population dynamics of the wild species (compare Section 4.2.3 for insects and comments above on this section). This is particularly important for the 'sterile' rabbit or other population suppression approaches (e.g. 'sterile' rats) but also for any application with altered

fitness which may change the population dynamics of the wild species (and hence of other species as discussed in Section 4.3.5).

4.3.5 Interactions of the GM mammals and birds with non-target organisms

Lines 5559-5563: As noted above, due to definition of target organism used in this section, there is no section of the guidance dealing with the impacts of population suppression approaches for pests on the pest population. This needs to be considered first, then extended to the interactions with NTOs. For example, the release of 'sterile' GM rabbits may or may not succeed in reducing the target population of rabbits and there could be fluctuations in time or increases in rabbits in the surrounding area, and changes in the rabbit population structure (more males, less young etc.). Once the population dynamics of these aspects (i.e. interaction between the GM pest and the non-GM pest) are understood, it is the possible to look at interactions with NTOs i.e. predators, competitors and prey. It is not only loss of an endangered prey species that needs to be considered: for example, there could be an increase in a competitor, which might have adverse consequences if it is a pest. Further, there will be feedbacks between the population dynamics of the different species: for example, population suppression of the target pest might be successful initially but reduce a food source for predators, resulting in a loss of predators followed by a rebound in pests. See the extensive comments on the population suppression approach in the GM insects section. Whilst this omission is most obvious for population suppression approaches (which are intended to reduce or eliminate the wild population) it should also be considered here whether any loss of fitness in any GM mammal or bird could impact on wild populations, following mating and poor survival of the offspring. For example, if a sterility (or partial-sterility) trait were introduced into a GM chicken with the aim of reducing the risks of persistence or invasiveness should it escape an intensive production system, what would happen if such chickens were inadvertently introduced into free-range chicken farms?

Lines 5599-5609: Competitor species should not be forgotten.

Line 5616: It is confusing to have non-GM individuals of the same species described as non-target organisms here, when they are regarded as target organisms in the insects section. Also, population dynamics of non-GM individuals of the same species needs to be considered more thoroughly than it is here, see comments on lines 5559-5563.

Lines 5656-5671: The population suppression approach needs to be considered more carefully here (i.e. the 'sterile' rabbit example, but bearing other invasive species in mind e.g. rats).

Lines 5698-5699: Increases in harmful competitor pest species, disease vectors or predators as a result of the introduction of the GM animal also need to be considered: these may then have a secondary effect on a vulnerable species or on pathogens or humans.

Lines 5775-5776: Different effects may occur at different life stages.

Lines 5841-5841: It is not just question of data but also adequate understanding of a complex, dynamic system: this includes the need for theoretical concepts that adequately describe the necessary ecosystem processes.

Line 5965: GM animals that mate with wild animals and fail to reproduce, or produce offspring with reduced fitness, can also have devastating effects on the wild population and non-target organisms c.f. the 'sterile' rabbit example or similar population suppression approaches i.e. limiting reproduction is not always a measure to reduce harm. GM animals may also exhibit "conditional

lethality" (see GM insects section): otherwise how will they be reproduced in the laboratory? Can the mechanism by with they are produced in the lab also occur in the wild? If so, under what circumstances? What is the penetrance of the 'sterility' trait i.e. no offspring or just a reduced number? Are there also aborted foetuses, stillbirths, deformed offspring? If so, what are the impacts of this (including on animal welfare and the environment)?

Lines 5993-5994: Reducing persistence and invasiveness does NOT necessarily reduce indirect risks: see comments on line 5965: population suppression approaches can have major impacts on ecosystems. If approved, how will releases be restricted to specific receiving environments? For example, the GM 'sterile' rabbit would presumably not be released in Spain, where the European rabbit is endangered and rabbits are the main diet of the endangered Lynx. Were GM 'sterile' rabbits considered suitable for authorisation for release elsewhere in the EU, how would their receiving environments be restricted? Would there be penalties for individuals taking rabbits from one part of the EU to another unless they could certify that they were not GM?

4.3.6 Abiotic interactions

4.3.7 Environmental impacts of the specific techniques used for the management of GM mammals and birds production systems

4.3.8 Impact on non-GM animal health and welfare

Line 6215: "If considered necessary" by whom?

4.3.9 Impact on human health

Lines 6381-6383: Not only direct exposure to the GM animal but also indirect risks must be included in the assessment and risk management must address these too. For example, if GM chickens act as a reservoir for infection as discussed in Section 4.3.3, there may be increased disease transmission in non-GM chickens and the human health risk may come from contact with the non-GM chickens. Similarly, if rabies from a GM animal were transferred to pets as described in Section 4.3.8, the risk to humans would arise indirectly via the pet not via contact with the GM animal.

5. Post-Market Environmental Monitoring plan

Line 6447: Environment and health risks must both be considered, not only of the GM animal but of its uses. Health risks must be monitored, especially for interventions involving disease vectors (James et al., 2011).

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