Le directeur général

Maisons-Alfort, le 6 juillet 2020

NOTE
d'appui scientifique et technique de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

relatif à « la consultation publique de l'EFSA sur le forçage génétique »

L'Anses met en œuvre une expertise scientifique indépendante et pluraliste.
L'Anses contribue principalement à assurer la sécurité sanitaire dans les domaines de l'environnement, du travail et de l'alimentation et à évaluer les risques sanitaires qu'ils peuvent comporter.
Elle contribue également à assurer d'une part la protection de la santé et du bien-être des animaux et de la santé des végétaux et d'autre part l'évaluation des propriétés nutritionnelles des aliments.
Elle fournit aux autorités compétentes toutes les informations sur ces risques ainsi que l'expertise et l'appui scientifique et technique nécessaires à l'élaboration des dispositions législatives et réglementaires et à la mise en oeuvre des mesures de gestion du risque (article L.1313-1 du code de la santé publique).
Ses avis sont rendus publics.

L'Anses s'est auto-saisie le 10 avril 2020 pour la réalisation de l'appui scientifique et technique suivant : « Analyse de l'avis scientifique du groupe OGM sur l'évaluation des lignes directrices existantes de l'EFSA, quant à leur adéquation pour la caractérisation moléculaire et l'évaluation des risques environnementaux des insectes génétiquement modifiés par forçage génétique ».

« Public consultation on the GMO Panel scientific opinion on the evaluation of existing EFSA guidelines for their adequacy for the molecular characterisation and environmental risk assessment of GM insects with synthetically engineered gene drives".

1. CONTEXTE ET OBJET DE LA DEMANDE

Le 6 juin 2018, l'Autorité européenne de sécurité des aliments (*European Food Safety Authority*, EFSA) a reçu une demande d'avis de la part de la Commission européenne concernant les organismes génétiquement modifiés par la technique du forçage génétique[1] (FG) et les méthodes d'évaluation des risques associés. Après discussion avec la Commission européenne (DG SANTE), il a été décidé que le champ du mandat confié à l'EFSA couvrirait les aspects suivants :

- la libération dans l'environnement (en espace non-confiné) d'insectes modifiés par la technique du forçage génétique, pour d'autres usages que l'alimentation humaine et animale ;
- la caractérisation moléculaire et l'évaluation des risques pour l'environnement des insectes modifiés par la technique du forçage génétique destinés à être disséminés volontairement dans l'environnement ;

[1] Le **forçage génétique** (FG) consiste à augmenter l'hérédité d'un élément génétique par rapport à l'hérédité naturelle décrite par les lois de Mendel, conduisant à l'accroissement de la fréquence de cet élément génétique dans une population.

- l'utilisation du forçage génétique de synthèse pour contrôler les populations d'insectes tels que les moustiques vecteurs de maladies et les ravageurs des cultures.

Dans le cadre de ce mandat, il n'était pas demandé à l'EFSA de développer des documents guides pour l'évaluation des risques liés aux organismes modifiés par la technique du forçage génétique. En revanche, il lui était demandé de fournir une expertise technique et scientifique sur l'évaluation des risques liés à ces organismes, en appui à l'Union européenne dans les travaux qu'elle mène dans le cadre de la convention sur la diversité biologique et du protocole de Cartagena[2] sur la prévention des risques biotechnologiques. L'EFSA n'a pas non plus été mandatée pour fournir des recommandations sur les aspects éthiques et socio-économiques, ni sur les bénéfices potentiels associés à la technique du forçage génétique. Une partie de ces aspects doit être traitée par le groupe européen d'éthique, à qui la Commission européenne a demandé un avis concernant les organismes modifiés par la technique du forçage génétique.

Compte tenu du débat de société sur les applications potentielles du forçage génétique, de la nécessité d'un meilleur dialogue et conformément à sa politique d'ouverture et de transparence, l'EFSA a organisé deux consultations, à différents stades de développement de l'avis, afin de recueillir les contributions de ses parties prenantes (y compris les États membres de l'UE) et d'autres parties intéressées. L'une a eu lieu au début du processus, sous la forme d'un atelier pour les parties prenantes. La seconde est la consultation publique sur le projet d'avis (EFSA, 2020).

En accord avec ses missions et compétences, l'Anses a décidé de participer à cette consultation. L'objet de la présente note d'appui scientifique et technique est de présenter et d'expliciter les réponses apportées *pro forma* à cette consultation.

À l'issue de cette consultation, le projet d'avis sera révisé par le groupe scientifique sur les organismes génétiquement modifiés (OGM) (*Panel Genetically Modified Organisms* (*GMO*)) de l'EFSA. Un rapport relatif aux résultats de la consultation sera publié en même temps que l'avis validé.

2. ORGANISATION DES TRAVAUX

Afin d'instruire la présente expertise, l'Anses a fait appel à six experts (voir liste en Annexe 1). Ces rapporteurs ont été nommés pour leurs compétences scientifiques et connaissances dans les domaines de la biologie de l'évolution, des OGM et des techniques innovantes de lutte contre les vecteurs[3] et les ravageurs[4] (faisant appel au forçage génétique notamment). Ils se sont réunis une fois le 15 avril 2020, puis l'ensemble de la grille de commentaires leur a été soumis pour validation par voie électronique.

L'Anses a transmis ces commentaires à l'EFSA le 23 avril 2020, dans un format conforme aux attentes de la consultation publique (voir tableau en

Annexe 2). La présente note d'AST a ensuite été soumise à la relecture des rapporteurs et des experts du groupe de travail « vecteurs » de l'Anses, puis discutée lors de la réunion de ce dernier, le 9 juin 2020.

[2] Adopté le 29 janvier 2000 dans le cadre de l'Organisation des Nations unies (ONU), le **protocole de Cartagena** constitue le premier accord instituant un cadre réglementaire à l'échelle internationale pour concilier les impératifs commerciaux et la protection de l'environnement au regard de l'utilisation croissante des biotechnologies. https://www.cbd.int/doc/legal/cartagena-protocol-fr.pdf.

[3] Un **vecteur** est un organisme (le plus souvent un arthropode) susceptible de transmettre un agent infectieux d'un sujet à un autre (ex : les moustiques peuvent transmettre des arbovirus tels que la dengue, le Chikungunya et le Zika).

[4] Les **ravageurs** des cultures sont des prédateurs ou parasites des plantes, nuisibles pour les plantes et susceptibles de causer des pertes économiques.

L'expertise a été réalisée dans le respect de la norme NF X 50-110 « Qualité en expertise – prescriptions générales de compétence pour une expertise (mai 2003) », avec pour objectif le respect des points suivants : compétence, indépendance, transparence, traçabilité.

L'Anses analyse les liens d'intérêts déclarés par les experts avant leur nomination et tout au long des travaux, afin d'éviter les risques de conflits d'intérêts au regard des points traités dans le cadre de l'expertise. Les déclarations d'intérêts des experts sont publiées sur le site internet de l'Agence (www.anses.fr).

3. ANALYSE ET CONCLUSIONS

Les techniques de forçage génétique (FG) visent à propager un caractère génétique d'intérêt dans une population naturelle d'insectes. Elles sont le plus souvent développées sur des arthropodes vecteurs en vue d'une dissémination volontaire dans l'environnement, pour lutter contre des vecteurs de maladies telles que la dengue ou le paludisme (anophèles, *Aedes aegypti*, *Aedes albopictus*…) ou, dans le domaine agricole, pour lutter contre les ravageurs de cultures (*Drosophila suzukii…*). Elles peuvent viser deux types d'objectifs :

- FG à des fins de **modification** de populations, par exemple pour les rendre incapables de transmettre des agents pathogènes (p.ex. pour inhiber la transmission à l'être humain du virus responsable de la dengue ou réduire la dissémination du paludisme en rendant résistantes les femelles ou en inhibant la reproduction du parasite dans les moustiques : *Anopheles gambiae*, *Culex quinquefasciatus…*) ;
- FG à des fins d'**élimination** de populations, par exemple par propagation d'un allèle provoquant la stérilité à l'état homozygote ou modifiant le sex-ratio et résultant en une population de mâles uniquement.

Le projet d'avis de l'EFSA aborde les différentes techniques de forçage génétique et évalue l'adéquation des lignes directrices actuelles pour la caractérisation moléculaire et l'évaluation des risques environnementaux liés aux insectes génétiquement modifiés par la technique du forçage génétique. Il est composé des huit chapitres suivants :

1. Introduction
2. Matériel et méthodes
3. Le forçage génétique : explications
4. Écologie et dynamique des populations
5. Connaissances / expériences issues des stratégies existantes de lutte contre les insectes vecteurs & ravageurs
6. Nouveaux dangers / risques potentiels associés aux moustiques vecteurs et aux ravageurs agricoles modifiés par forçage génétique
7. Évaluation des lignes directrices de l'EFSA (2012 et 2013), quant à leur adéquation pour la caractérisation moléculaire et l'évaluation des risques environnementaux des insectes génétiquement modifiés par forçage génétique
8. Conclusions

L'analyse de l'Anses a porté sur l'ensemble de ces chapitres.

Présentation de la méthode du groupe de travail de l'EFSA

Pour produire l'avis demandé par la Commission européenne, le groupe scientifique sur les OGM de l'EFSA et son groupe de travail « forçage génétique »[5] ont pris en compte les réflexions et exigences qui figurent dans leurs avis relatifs à l'évaluation des risques liés aux animaux (y compris les insectes) génétiquement modifiés (EFSA, 2012 et 2013), la directive 2001/18/CE relative à la dissémination volontaire d'organismes génétiquement modifiés dans l'environnement et la directive (UE) 2018/350 de la Commission modifiant la directive 2001/18/CE, lorsque cela était approprié, ainsi que les informations pertinentes de la littérature scientifique.

Une approche section par section a été utilisée pour déterminer si les réflexions et exigences qui figurent dans les avis du groupe scientifique sur les OGM (EFSA 2012 et 2013) sont appropriées pour la caractérisation moléculaire et l'évaluation des risques pour l'environnement des insectes modifiés par la technique du forçage génétique, respectivement. Cette évaluation a également été réalisée sur la base des informations pertinentes de la littérature scientifique et des résultats des développements expérimentaux en matière d'insectes modifiés par la technique du forçage génétique.

Analyse du projet d'avis de l'EFSA

À la lecture du projet d'avis de l'EFSA, les experts de l'Anses ont formulé près de 150 commentaires (voir liste des commentaires dans le tableau en

Annexe 2), et soulignent les points suivants :

- Le projet d'avis de l'EFSA aborde la question de la réglementation des produits issus du forçage génétique en espace non-confiné (plein champ, écosystèmes ouverts), mais n'aborde pas celle des espaces confinés (en laboratoire). Or, il existe un vide juridique à cet égard. Par ailleurs, la possibilité de dissémination transfrontalière de moustiques génétiquement modifiés pose un problème en matière de gestion. Par exemple, il a été envisagé d'envoyer en Afrique des moustiques vivants qui seraient modifiés par forçage génétique en Italie. Les mouvements transfrontaliers intentionnels et non-intentionnels d'organismes vivants modifiés doivent actuellement être notifiés sur le fondement du Protocole de Cartagena, chaque Partie au protocole étant tenue de prendre des mesures appropriées pour empêcher les mouvements transfrontaliers non-intentionnels. Ceci étant difficilement réalisable pour des moustiques génétiquement modifiés capables de propager une modification, cette question devrait être traitée au niveau international et susciter une harmonisation réglementaire supranationale.

  Il est donc indispensable de proposer dès à présent un cadre réglementaire pour traiter les questions qui se posent lors des expérimentations de laboratoire ou des transports d'organismes issus de l'utilisation des techniques de forçage génétique.

- Le projet d'avis de l'EFSA mentionne l'importance d'évaluer les risques environnementaux associés à chaque application du forçage génétique. Il faut cependant noter qu'il est extrêmement difficile de lister de manière exhaustive tous les effets non intentionnels potentiels et d'estimer chacun des risques associés. Par exemple, la probabilité que le transgène envahisse une population non cible (ou une espèce proche) n'est pas nulle, surtout pour les transgènes dont la dissémination n'est pas limitée dans le temps ou dans l'espace. De plus, le projet d'avis mentionne que la technique utilisée peut induire des mutations hors-cibles (cela peut être le cas avec la technique CRISPR/Cas9[6] par exemple), qui sont généralement

[5] À noter que certains des membres constituant le groupe de travail ont des liens notoires avec des développeurs de techniques utilisant le forçage génétique (*Gene Drive ERA Working Group experts*).

[6] La protéine Cas9 (*CRISPR-associated protein 9*) est une endonucléase impliquée dans la réponse immunitaire bactérienne CRISPR (*Clustered Regularly Interspaced Short Palindromic Repeats).* Le système CRISPR-Cas9 permet de générer des mutations ponctuelles, dirigées ou aléatoires, ou d'insérer des fragments d'ADN, dont des transgènes. Lors de la lutte contre les

désavantageuses pour les individus qui les portent et qui seraient naturellement contre-sélectionnées. Or, certaines mutations pourraient introduire un caractère avantageux et se propager de manière non contrôlée.

Aussi, il est essentiel i) de pouvoir caractériser la persistance de l'insert dans le temps et ii) de contrôler sa propagation dans l'espace :

- Comme pour les OGM (par règlement d'exécution (UE) n° 503/2013 de la Commission du 3 avril 2013), il pourrait être demandé au pétitionnaire qui sollicite une autorisation pour utiliser une espèce modifiée par la technique du forçage génétique, de montrer la stabilité de l'insert sur plusieurs générations. Il semble également nécessaire qu'il fournisse le séquençage complet du génome de l'organisme génétiquement modifié par forçage génétique et pas seulement la séquence de la construction insérée (Anses, 2019).
- Afin de maîtriser la propagation de l'insert, il est nécessaire de prévoir dès sa conception des contre-mesures pour empêcher sa propagation. Des techniques capables de contrer ou limiter le forçage génétique, également en cours de développement, pourraient permettre d'éviter une dissémination éventuellement non souhaitable d'un tel système de modification ou de suppression de populations.

- Concernant l'évaluation des risques pour l'environnement associés aux techniques de forçage génétique, les critères listés dans la directive 2001/18/CE semblent pertinents. Toutefois, une déclinaison particulière, comme prévue par l'approche au cas par cas de la directive, doit être effectuée. À noter que le projet d'avis de l'EFSA mentionne uniquement la prise en compte des services écosystémiques pour évaluer les risques environnementaux des insectes génétiquement modifiés par forçage génétique.

  Il est essentiel d'adopter une approche globale pour évaluer les risques environnementaux et de prendre en compte différents critères tels que la conservation de la fonction des écosystèmes ou la biodiversité en sus des seuls services écosystémiques.

- Compte tenu de la controverse scientifique et sociétale sur les applications potentielles du forçage génétique et de l'importance qu'un dialogue ait lieu entre les parties prenantes (décideurs, scientifiques et société), les experts de l'Anses soulignent la nécessité de rester vigilants concernant les conflits d'intérêts et de mettre en place des lieux de débat autour de ces questions et de garantir la transparence des décisions publiques prises sur le sujet.

Conclusion

Dans le cadre de la consultation organisée par l'Autorité européenne de sécurité des aliments (EFSA) concernant le projet d'avis relatif aux lignes directrices existantes de l'EFSA, quant à leur adéquation pour la caractérisation moléculaire et l'évaluation des risques environnementaux des insectes génétiquement modifiés par forçage génétique, l'Anses a répondu en date du 23 avril par la grille de commentaire *pro forma* en annexe 2. La présente note d'appui scientifique et technique vise à formuler et expliciter l'articulation des éléments de fond de cette réponse.

Les experts considèrent que les conclusions du Haut Conseil des Biotechnologies (HCB, 2017) concernant l'utilisation de moustiques génétiquement modifiés dans le cadre de la lutte antivectorielle sont toujours d'actualité : « *Aucune technique de forçage génétique n'a encore atteint le stade*

insectes vecteurs ou ravageurs de cultures, le forçage génétique utilise un transgène comportant le gène codant Cas9 et qui cible une région d'intérêt du chromosome sauvage (p.ex. gène de stérilité). La transmission du transgène étant non Mendélienne, celui-ci peut augmenter en fréquence dans les populations même lorsqu'il diminue la survive ou la reproduction des individus qui le portent.

d'aboutissement technique permettant d'envisager une dissémination dans l'environnement – les discussions en sont plutôt au stade des bonnes pratiques de recherche en milieu confiné ».

De plus, ils soulignent le caractère intentionnellement invasif de la technique du forçage génétique, qui a le potentiel théorique d'atteindre tous les individus d'une espèce dans l'environnement, que ce soit pour l'éradiquer ou la modifier, et estiment qu'un cadre juridique est nécessaire pour encadrer les expérimentations sur le sujet. De plus, l'évaluation et la gestion des risques associés au forçage génétique doivent nécessairement être adaptées aux spécificités du forçage génétique, technique qui n'est limitée ni dans le temps ni dans l'espace.

Enfin, en termes d'évaluation de la faisabilité de la technique, tant globalement que localement (*in situ*), lorsque des expérimentations en milieu ouvert pourront être envisagées, il sera essentiel de prendre en compte la structuration génétique des populations présentes dans le milieu naturel, car c'est bel et bien la structure génétique des populations (et son évolution dans le temps) qui renseigne sur la dynamique des flux de gènes dans les populations naturelles cibles de ces actions de lutte. Ces indications permettront d'évaluer clairement tant les possibilités de succès que d'échec de la technique sur le terrain.

Dr Roger Genet

MOTS-CLES

Autorité européenne de sécurité des aliments (EFSA), consultation publique, forçage génétique, organisme génétiquement modifié (OGM), protocole de Cartagena, risques pour l'environnement.

European Food Safety Authority (EFSA), public consultation, gene drive, genetically modified organism (GMO), Cartagena protocol, environmental risk.

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Directive (UE) 2018/350 de la Commission du 8 mars 2018 modifiant la directive 2001/18/CE du Parlement européen et du Conseil en ce qui concerne l'évaluation des risques pour l'environnement des organismes génétiquement modifiés. JO L 67 du 9.3.2018, pp. 30-45.

Directive 2001/18/CE du Parlement européen et du Conseil du 12 mars 2001 relative à la dissémination volontaire d'organismes génétiquement modifiés dans l'environnement et abrogeant la directive 90/220/CEE du Conseil. JO L 106 du 17.4.2001, pp. 1-38.

EFSA GMO and AHAW Panels. 2012. "Guidance on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects." *EFSA Journal* 10(1): 2501, 43 pp. doi: 10.2903/j.efsa.2012.2501.

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Règlement d'exécution (UE) n° 503/2013 de la Commission du 3 avril 2013 relatif aux demandes d'autorisation de denrées alimentaires et d'aliments pour animaux génétiquement modifiés introduites en application du règlement (CE) n° 1829/2003 du Parlement européen et du Conseil et modifiant les règlements de la Commission (CE) n° 641/2004 et (CE) n° 1981/2006. JO L 157 du 8.6.2013, pp. 1-48.

ANNEXE 1

Présentation des intervenants

PRÉAMBULE : Les experts sont tous nommés à titre personnel, *intuitu personae,* et ne représentent pas leur organisme d'appartenance.

EXPERTS RAPPORTEURS

Christophe BOETE – Chercheur à l'Institut des Sciences de l'Evolution (ISEM) – Domaines de compétence : écologie des vecteurs, biologie évolutive, aspects sociologiques et réglementaires des technologies de lutte anti-vectorielle innovantes.

Fabrice CHANDRE – Responsable du Vectopole Sud à l'Institut de recherche pour le développement (IRD) – Domaines de compétence : entomologie, lutte anti-vectorielle, génétique de la résistance des vecteurs aux insecticides.

Virginie COURTIER – Directrice de recherche au Centre national de la recherche scientifique (CNRS), Institut Jacques Monod – Domaines de compétence : évolution génétique et génomique, biologie du développement, étude des risques associés à l'utilisation du forçage génétique.

Florence DEBARRE – Chercheuse au Centre national de la recherche scientifique (CNRS), Institut d'Écologie et des Sciences de l'Environnement (IEES-Paris) – Domaines de compétence : biologie et écologie évolutives, modélisation mathématique, notamment du forçage génétique.

Nicolas RODE – Chercheur à l'Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) – Domaines de compétence : biologie et écologie évolutive, étude des risques associés à l'utilisation du forçage génétique

Pierre ROUGE - Professeur émérite à l'Université Paul Sabatier, Faculté de pharmacie de Toulouse - Domaines de compétence : biologie végétale, biochimie, allergies alimentaires, biotechnologie.

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Secrétariat administratif

Régis MOLINET – Anses

ANNEXE 2

Comment number	Draft opinion page	Draft opinion line	Line-by-line text comments	Paragraph comments
1	n/a	n/a		The document "Evaluation of existing EFSA guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified insects with synthetically engineered gene drives" is of high quality: the scientific data is well presented, multiple relevant references are provided and the recommandations are overall sensible and well formulated.
2	n/a	n/a		Compared to previous strategies, gene drives have, by definition, non-linear effects on targeted populations: gene drive modified insects (GDMI) are expected to produce multiple GDMI progeny individuals, which will each produce GDMI progeny, and so on. Except (and it is one of the target of GDMI, if the modification is aimed at avoiding progeny). As a result, it is extremely difficult to predict all possible outcomes of a given gene drive. Because impacts are more difficult to predict for gene drives than for other measures, the current environmental risk assessment (ERA) paradigm is not fully appropriate for testing GDMIs. An additional check should be done: to make sure that effective countermeasures are available to stop the spread of a gene drive, in case it is noticed that their effect is unexpected and detrimental. Proposals for the use of GDMIs should also describe possible countermeasures that are expected to be efficient to stop the spread of the proposed gene drive. Countermeasures can be insecticides, a second gene drive which inactivates the initial gene drive (for low-threshold-dependent drives), or simply to stop releasing GDMI (for high-threshold-dependent drives). In "Spatio-temporal controllability and environmental risk assessment of genetically engineered gene drive organisms from the perspective of EU GMO Regulation. Integrated Environmental Assessment and Management." (https://setac.onlinelibrary.wiley.com/doi/pdf/10.1002/ieam.4278?casa_token=omlFzSl0LE0AAAAA:Zk1zMhW7K1DAB9_6m9EMuxjVZXRd7znbRA2j1E75YxJLnczKpY3m2yhNpOrrqyLrwd0u2ymPc9QTkQ) C., Kawall, K., & Valenzuela, N. (2020) wrote that "If there is a plausible risk that GE gene drive organisms can escape spatio-temporal controllability without effective means to control dispersal or persistence, then the authorization process could not proceed and the environmental release of the GE gene drive organisms would not be allowed." Adding a section on countermeasures may help to better assess the problem of gene drive organisms escaping spatio-temporal controllability.
3	n/a	n/a		The categories "self-limiting" and "self-sustaining", as indicated in Table 1, are indeed very relevant for assessing the environmental risks of gene drives. It should however be noted that with changes in external conditions and in genome backgrounds, a high-threshold or self-limiting gene drive may sometimes become a low-threshold, self-sustaining drive.
4	n/a	n/a		For information regarding the recipient, given that sequencing is now relatively cheap, it would be worth asking also for the genome sequence (illumina reads) of the gene drive strain that is to be released, as well as the genome sequence of a sample of the target population. Such genome data can be very useful to monitor the target population. Especially if a new, unexpected phenotype arises.
5	n/a	n/a		For gene drive projects where the final insects are planned to be released outside of Europe, but are being made in Europe, what is the regulation regarding the transportation of such GDMIs ? For example, for the Target Malaria project, GDMI are currently being produced in Italy, and the plan is then to send them via airplane to Africa. Section 4.2.6 of EFSA 2003 mentions the accidental escape of GM insects from enclosed rearing facilities or greenhouses, and the transport between rearing facilties. However, the transportation from rearing facilities to the site of release, if outside of EU, is not explicitly mentioned.
6	1	10-36	Abstract	The abstract mentions gene drive for insect/pest control especially for population replacement. This seems somehow surprising given the fact that the most advanced work done on Anopheles by a lab based in the UK is mostly dealing with population suppression. Even if the tests have not been conducted outside of the lab, recent ones have been performed in large cages in Italy (see: https://www.npr.org/sections/goatsandsoda/2019/02/20/693735499/scientists-release-controversial-genetically-modified-mosquitoes-in-high-securit) with the aim of mimicking an environment closer to the natural one than the usual small lab cages. It would have been interesting to have this point mentioned in the abstract.

Comment number	Draft opinion page	Draft opinion line	Line-by-line text comments	Paragraph comments
7	2	38-42	Keywords	There is a lack of clear information about containment in time and in space, and about the genetic pollution (non-target species). Some important keywords are missing, i.e. "Insects", "vector control", "pest management", "genetically modified organism". Some keywords seem not really relevant like "treshold dependent drive" , "treshold independent drive".
8	7-9	120-202	Introduction	EFSA has been requested by the EC but there is no information about the "why" using gene drive approach. There is mention of the Convention on Biological Diversity and the Cartagena Protocol on Liability and Redress but no mention of their possible limitation nor any mention of other documents such as the 1972 Biological Weapons Convention and the 1976 ENMOD convention that prohibits the hostile use of environmental modification techniques (see papers by Felix Beck). It is regretable that the request did not include other types of research on gene drives (e.g. laboratory experiments), for which regulation is also lacking/inadequate.
9	7	140	Biological Diversity[2] and the Cartagena Protocol on Biosafety.[3] The Cartagena Protocol and its	Line 140: for footnote #3, please define LMO ("living modified organisms").
10	8	157	endonuclease genes (HEGs) and the clustered regularly interspaced short palindromic repeats	Line 157: the discovery of HEGs (e.g. I-Sce I) is not particularly recent (B. Dujon's work in the late 80's/early 90's), so this sentence might be misleading.
11	8	158	(CRISPR) and CRISPR-associated protein 9 (Cas9) system[6], have delivered molecular and	Line 158: footnote #6, line 8, "During early development, the Cas9..." appears inaccurate, as all gene drives developped so far are mostly expressed in the germline (vasa or nanos promoters) and gene conversion can take place at other moments of the life-cycle (which gets clearer later in the note).
12	8	164	gene drives could push genes of interest through nearly 100% of a given population of yeast,	Line 164: estimates vary across species and are sometimes not that high (initial values included resistance).
13	8	165-166	fruit flies and mosquitoes (NASEM, 2016). These developments suggest that a practical application of gene drive systems could be more readily achievable than previously believed in	Lines 165-166 present a very positive (pro-GD) view of the eventual applications of GD. A more neutral redaction or balanced opinion should be adopted.
14	8	172-176	The nature of potential GDMI applications may be demonstrably different from other GMO applications, which are generally intended to be limited to specific uses in controlled environments (as is the case with genetically modified (GM) crops for agriculture or farm-raised GM fish), or limited in exposure over space and time (as is the case with the release of sterile GM insects [GMIs]).	Lines 172-176: "controlled environements" is not really applicable to "agriculture", there are some concerns about transgene dissemination with GM crops for agriculture. Proposal to replace the sentence by "The nature of potential GDMI applications may be demonstrably different from other GMO applications, which are generally intended to be limited to specific uses in controlled environments (as is the case with farm-raised GM fish), or limited in exposure over space and time (as is the case with genetically modified (GM) crops for agriculture or with the release of sterile GM insects [GMIs] (i.e Sterile Insect Technique)."
15	8	176-177	GM insects [GMIs]). Gene drive applications require the spread of genes of interest for achieving intended outcomes (e.g. fixation or high frequency in the target population). Some	Lines 176-177 are related to population replacement only and not population suppression.
16	8	179	interbreeding populations from low initial introductions, even if they incur a fitness cost on their	Line 179: replace "low initial introductions" by "low initial introduction frequency".
17	9	181	populations are locally eliminated; (3) change the genetic makeup of wild type populations	Line 181: since the sentence begins with "may enable", it should be followed by a noun, "a change in the genetic makeup...".
18	9	184	of synthetically engineered gene drive drives (Pugh, 2016; Thompson, 2018; Jones et al., 2019;	Line 184: remove "drive" before "drives".

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19	9	186	moratorium on gene drive field tests, as they argue that the deployment of synthetically	Line 186: the moratorium was also on the development of gene drives in the laboratory (http://www.synbiowatch.org/gene-drives/gene-drives-moratorium/).
20	9-10	203-222	1.1 Background and Terms of Reference as provided by the requestor	Noteworthy point: EFSA is not requested "to develop guidelines for the risk assessment of gene drive modified organisms".
21	10	218-219	Under this mandate, EFSA is not requested "to develop guidelines for the risk assessment of gene drive modified organisms".	Lines 218-219: Anses considers nevertheless that developing guidelines for the risk assessment on such topic is of high importance, and that due attention shall be paid to.
22	10-11	223-279	1.2 Interpretation of the Terms of Reference	Limitation of the scope to insects despite the fact that other groups are considered as potential targets such as mammals or plants, in the field of conservation and agriculture too. This may lead to an obsolete document once technical challenges are overcome... Why not take them as early as possible? The reflexion could be done even in the current absence of the technology... Regarding the use of GD in agriculture, there is no consideration of the coexistence with organic agriculture. The presentation of the limitations of the current tools used against vectors remains focused on the technology and doesn't take into account its deployment.
23	10	226	likely cases of GDMOs moving to practical application/for deliberate release into the	Line 226: replace the "/" by a space ("likely cases of GDMOs moving to practical application for deliberate release into the").
24	10	233	application are expected to be those that are directed at human, livestock and wildlife disease	Line 233: remove "application" at the beginning of this line ("In insects, the most likely gene drive cases for deliberate release into the environment are expected to be....").
25	10	238-239	used in areas with much lower pest concentrations and that are not easily managed, given their lower ongoing costs of implementation. Since disease vectors and agricultural pests can affect	Lines 238-239: is there a reference for these statements ("lower pest concentration" and "lower ongoing cost of implementation")?
26	10	247-250	(Feachem et al., 2019; Masterson, 2019). However, current methods of vector control, including removal of standing water, use of insecticides delivered via bed nets and indoor residual spraying, and the mass release of sterile males, have not been entirely effective in combatting the spread of mosquito-vectored diseases worldwide (Ritchie and Staunton, 2019).	Lines 247-250: this is a strong and general statement that does not consider sucessfull programs (e.g. 96% reduction in Dengue transmission; Ryan, P. A., Turley, A. P., Wilson, G., Hurst, T. P., Retzki, K., Brown-Kenyon, J., ... & Paton, C. J. (2019). Establishment of wMel Wolbachia in Aedes aegypti mosquitoes and reduction of local dengue transmission in Cairns and surrounding locations in northern Queensland, Australia. Gates open research, 3.; or population suppression and reduction in biting rate: Zheng, X., Zhang, D., Li, Y., Yang, C., Wu, Y., Liang, X., ... & Wang, X. (2019). Incompatible and sterile insect techniques combined eliminate mosquitoes. Nature, 572(7767), 56-61 or Crawford, J.E., Clarke, D.W., Criswell, V. et al. Efficient production of male Wolbachia-infected Aedes aegypti mosquitoes enables large-scale suppression of wild populations. Nat Biotechnol (2020). https://doi.org/10.1038/s41587-020-0471-x). Therefore, it is recommended to reconsider the statement, taking into account these results.
27	10	249	spraying, and the mass release of sterile males, have not been entirely effective in combatting	Line 249: yet in some parts of the world methods have been effective and have led to the eradication of mosquito populations.
28	11	260	conservation purposes or the enhancement of agricultural production systems, as no concrete	Line 260: add "direct" to "enhancement of agricultural production systems" as the use of gene drive for pest management is a way to increase, indirectly, agricultural production.
29	11	264	environment; such releases are non-confined[8] and not intended for food/feed uses. Since	Line 264: ... "though they are indirectly linked to food/feed uses, when agricultural pests are targeted".
30	11	266	interbreeding wild type/target populations occurring in the environment, the deliberate release	Line 266: add "with" after "interbreeding".

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31	11	267-269	of GDMIs will be non-confined, and not covering GMIs for food/feed uses. Consequently, the mandate excludes confined and semi-confined GDMI releases and the deliberate release of GDMIs for food/feed uses (if any).	Lines 267-269: the interpretation that the scope of the mandate is restricted to the intentional release of GDMIs in non-confined environments is surprising. Research in confined and semi-confined conditions represents a pre-requisite for the release of GDMIs in non-confined conditions. Research projects on GDMIs currently conducted in the EU should carefully address the risk of unintentional release of GDMIs in non-confined environments (e.g. following an escape from confined and semi-confined environments). It seems that the GMO Panel implicetly assumes that previous guidelines are well suited for confined and semi-confined GDMIs releases. There is a pressing need to clarify whether previous guidelines (EFSA 2012, 2013) are adequate for the MC and ERA of GDMIs in confined and semi-confined environments.
32	11	271	The non-confined release of GDMIs into the environment for non-food/feed uses;	Line 271: again, regrets are expressed that confined use is not covered.
33	12	300	biologically relevant change(s) in the GMA and/or derived food/feed, the allergenicity	Line 300: replace the coma before "the allergenicity" by a semi-colon.
34	13	350-357	2.2.3 Consultations	Some of the presenters and the persons leading the group discussions are involved in the development of the gene drive technology (FNIH, Target Malaria...). There is a need to be vigilant with the way of proceeding without preconceptions.
35	13-14	358-403	2.2.3.1 Stakeholder workshop "Problem formulation for the environmental risk	Regarding mosquitoes, work is conducted on Aedes albopictus. While this is of interest for the EU, the work in GD is mostly conducted on malaria vectors. This makes one wonder why the "exercice" has not been conducted with mammals or weeds.
36	14	373-376	1. Self-sustaining low threshold gene drives to control disease-spreading mosquitoes (Aedes albopictus, the Asian tiger mosquito); 2. Self-sustaining low threshold gene drives to control agricultural pests (Drosophila suzukii, the spotted-wing Drosophila).	Lines 373-376: "Self-sustaining low threshold gene drives" has not yet been defined. For better clarity, a link to the subsections of section 3 where these concepts are explained would be appreciated.
37	14	377	The two case studies were selected representing species relevant for the EU.	Line 377: proposal to replace "The" at the beginning of the sentence by "These", to replace "representing" by "because they represent" and to replace the full stop at the end of the sentence by a colon ("These two case studies were selected because they represent species relevant for the EU:").
38	14-15	404-411	2.2.3.2 Online public consultation	Ongoing consultation. No information about the way the document is spread and the consultation organized and the expected feedback from the public.
39	15-26	412-801	3 Explaining gene drives	Description of gene drive with some emphasis on the fact that natural ones already exist and that they can even be used for the risk assessment of GDMIs (p. 16).
40	15	417	Society, 2018; Ethics Council of the Max-Max-Planck-Gesellschaft, 2019; Hurst, 2019; North et	Line 417: remove one "Max" in "Max-Max-Planck-Gesellschaft".
41	15	419	organisms, each of the two alleles of a gene present in each parent has a 50% chance of being	Line 419: each of the two *copies.
42	15	424	and that of any genetically linked cargo/payload genes[13], even if they incur a fitness cost on	Line 424: this is incorrect. If the fitness cost is too high, the gene drive won't spread. There is a maximum fitness cost that allows the spread (Deredec et al. 2003).
43	15	425-426	their host. This is because individuals with a gene drive element will produce more offspring carrying the gene drive allele than without it (Champer et al., 2016).	Lines 425-426: this sentence is unclear. Whether or not the spread will be sucessful depends on the level of dominance of a gene drive, its conversion efficiency and its fitness cost (Deredec et al 2008 Genetics). Drives that are recessive, have high conversion efficiency and low fitness costs are more likely to spread. Proposal to change the end of the sentence, because one given individual cannot be with and without a gene drive. Proposed new sentence: "This is because... than individuals that don't carry any gene drive (Champer et al., 2016)".
44	15	435	terminology – a "standard lexicon" – if generally accepted, would help to frame gene drive	Line 435: where is this lexicon proposed?
45	15-17	437-481	3.1 Mechanisms	Some iconography would make this section easier to understand.

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46	16	456	(1) over-replication; (2) interference; and (3) gonotaxis (Burt and Trivers, 2006).	Line 456: Wolbachia are not included in this classification because they are not genetic elements (but organelles). However, they should be classified as using gonotaxis. A few gene drive strategies are currently being developped using Wolbachia, thus the risks associated with the gonotaxis-type are still worth investigating.
47	17	500-502	lifespan or bias(es) sex ratios (Buchman et al., 2018b; James et al., 2018). Modified target insects are expected to decrease to low numbers over the period of a few generations as the overall target population is reduced. This may result in population decline or even collapse.	Lines 500-502: proposal to suppress "target" after "Modified" and to add "strategy" after "This" in the second sentence, because the population decline or collapse is not the result of the decrease in the number of modified insects (modified sentences: "Modified insects are expected to decrease to low numbers over the period of a few generations as the overall target population is reduced. This strategy may result in population decline or even collapse."). Alternative proposal for this paragraph: "[...] (Buchman et al., 2018b; James et al., 2018). This strategy may result in population decline or even collapse over the period of a few generations. Suppression drives are being developed for suppressing populations of human/animal disease vectors and agricultural pests. Strategies aiming for population suppression from a single release would require the modification to persist, despite the fact that modified insects are expected to decrease to low numbers as the overall target population is reduced. [...]".
48	17	504-505	vectors and agricultural pests. Strategies aiming for population suppression from a single release would require the modification to persist. Alternatively, strategies could use self-limiting	Lines 504-505: unclear because in a population suppression approach, the modification is not expected to persist but to vanish once the population has been suppressed.
49	17	510-511	transmit disease (disease refractory/impaired vector competence), or that is more resistant to pathogen infection (Franz et al., 2006; Mathur et al., 2010; Hedge and Hughes, 2017;	Lines 510-511: "disease refractory" looks like a synonym of "resistant to pathogen infection". Therefore, the sentence could be modified as follows: "transmit disease (impaired vector competence), or that is more resistant to pathogen infection (disease refractory)[...]".
50	17	513	Pham et al., 2019). These strategies are based on the inactivation of a gene or genes involved	Line 513: proposal to add "in some cases" after "These strategies are", because in line 516, it is mentionned that "They can also involve the introduction of a new gene or genes".
51	18	518	(Lejarazú and James, 2017; James et al., 2018; Buchman et al., 2019, 2020). To perform	Line 518: other example = make mosquitoes sensitive again to insecticides.
52	19	555-557	This type of gene drive is referred to as a temporally restricted drive. Examples (see Section 3.3 for more details) are daisy-chain drives (Noble et al., 2019) and split killer-rescue drives (Gould et al., 2008).	Lines 555-557: Gould 2008 present a "killer-rescue"model. Please explain whether there is an implicit distinction between "killer-rescue" and "split killer-rescue"? (same comment line 572)
53	19	556	for more details) are daisy-chain drives (Noble et al., 2019) and split killer-rescue drives (Gould	Line 556: the report needs to be clearer about what has already been implemented in labs and what is a theoretical suggestion (daisy-drive).
54	19-20	561-584	3.2.2.2 Low vs. high threshold gene drives	Issue of reversibility (possible with high threshold via dilution) is presented as the major aspect. No info about the production of high numbers of insects and the needed production facilities. Adding a figure with the main types of drives presented would be helpful. It seems that the GMO Panel uses terms that are different from those in the original publications. Gould 2008 present a "killer-rescue" model. Please explain whether there is an implicit distinction between "killer-rescue" and "split killer-rescue" (line 572 and Table 1)? Is "interference by killer-rescue" (line 559) a different type of drive? Similarly, please explain the difference between "cleave and rescue" and "double cleave and rescue" (Table 1)?
55	19	573-574	(Webster et al., 2019). These types of drives enable local confinement and may be eliminated from a population through being diluted below the threshold frequency. Such threshold-	Lines 573-574: other types of drives have high thresholds such as CRISPR-Cas9 homing drives (especially when they are dominant and when conversion occurs in the zygote; Deredec et al 2008 Genetics, Unckless et al 2015 Genetics) or toxin-antidote recessive embryo drives (Champer, J., Lee, E., Yang, E. et al. A toxin-antidote CRISPR gene drive system for regional population modification. Nat Commun 11, 1082 (2020)). https://doi.org/10.1038/s41467-020-14960-3).

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56	19	578-580	spread, independent of whether the drive is based on over-replication by synthetic homing elements (Hammond et al., 2016; Kyrou et al. 2018), or by interference by killer-rescue elements (Oberhofer et al., 2019). These types of drives have a higher potential to spread into	Line 579: replace "by interference" by "by meiotic interference drives (Bernardini et al 2018),"?
57	20	583-584	Table 1. Overview of gene drive strategies	Table 1: split homing endonuclease drives and split killer-rescue drives can also be found in population suppression. They are not necessarily threshold independent. Depending on the fitness cost, they can also be thereshold dependent (including with high threshold), not spread at all, or even lead to coexistence between drive and wild-type (see Deredec et al. 2003, and Unckless et al as well).
58	20-21	600-603	GDMI approaches and applications will likely continue to expand as gene editing tools become more refined (NASEM, 2016; Holman, 2019). Consequently, the previously reported "prototype" gene drives may not necessarily be representative of the gene drive systems that are currently under development and expected to be more specific, stable and controllable systems.	Lines 600-603: no real justification about more specific, more stable and controllable systems that could be created in the future. The only one is the access to gene editing tools. No mention of a better understanding of the ecological and environmental determinants of a "safe", controlled deployment.
59	21	608-636	3.3.1 Homing endonuclease gene-based gene drives	Potential time for release is given: 2030 for HEG-drive for suppression and/or replacement.
60	23	682-705	3.3.4 Underdominance gene drives	There are older references about underdominance than Champer et al. 2016.
61	23	704	fitness costs and resistance due to naturally occurring genetic variation and associated	Line 704: remove "and associated" at the end of the sentence.
62	25-26	753-801	3.4 State of the art	Regarding point 4, the mitigation of the spreading is mostly considering the spatial and temporal components but little has been done concerning the off-target effects (as discussed in Courtier-Orgogozo et al. 2020, Evolutionary Applications : https://doi.org/10.1111/eva.12939).
63	25	754	To summarise, gene drive research is currently focused on the following main areas:	Line 754: add a part on assessing the efficiency of countermeasures to stop a proposed gene drive, if available.
64	26-29	802-933	4 Ecology and population dynamics	Indeed, it is important to mention the possible alteration of the environment once a GMDI is released.
65	26	804	detailed understanding of the ecology impact of species carrying these modified traits (e.g.	Line 804: replace "the ecology impact" by "the ecological impact".

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66	27	851-857	et al., 2008a,b; Alphey and Bonsall, 2014). The timing of the genetic control with respect to the intraspecific competition can influence the outcome. For example, SIT is a control intervention that acts early in the life cycle of an insect by disrupting egg production. Rogers and Randolph (1984) showed that this sort of control can even lead to enhanced vector/pest population sizes as the imposed (control-based) mortality alleviates the strength of intraspecific competition, allowing surviving individuals unrestricted access to resources and mating opportunities, which can lead to unwanted population level increases rather than decreases in abundance. Mitigation	Lines 851-857: indirect criticism of SIT by using the example of a reduced intraspecific competition is based on a model built for tse tse population and this might be different for mosquito populations. Interestingly in the conclusion of the cited paper by Rogers and Randolph, the authors declare "We believe that many past control schemes have emphasized the technological at the expense of the biological aspects, and led to a solution that neither maximised control nor minimised costs." It might be worth taking this into account too with GD.
67	27	853	that acts early in the life cycle of an insect by disrupting egg production. Rogers and Randolph	Line 853: replace "disrupting egg development" by "disrupting embryo development" (might be less misleading).
68	28	892	and can dependent on the optimal timing of the genetic-based control (Yakob and Bonsall,	Line 892: replace "can dependent on" by "can depend on".
69	28	914	Invasiveness is the ecological concept that allows a species to spread from rare as the species	Line 914: a word seems to be missing in "allows a species to spread from rare as the species has positive population growth".
70	29	926-933	4.2 Heterogeneity of receiving environments	Add also the possibility of external conditions influencing the rate at which a drive will spread in a target population: the fitness value associated with the gene drive alleles might change in different environmental conditions (temperature, humidity, etc.), so that a gene drive can be expected to spread with various dynamics in different geographical regions. This point might be considered an advantage regarding the limitation of the spread of a gene drive but the question of "isolated populations" remains quite vague. Considering the way insecticide resistance has spread easily in mosquito populations globally, this point is questionable.
71	30-31	956-1003	5.1.2 Release of artificially reared males with dominant/female specific lethality	No mention of the strong limitation of the GM mosquitoes that have been released (RIDL by Oxitec). The recent release of GM mosquitoes in Burkina Faso could also be mentionned: https://targetmalaria.org/target-malaria-proceeded-with-a-small-scale-release-of-genetically-modified-sterile-male-mosquitoes-in-bana-a-village-in-burkina-faso/
72	30	993	radiation sterilisation, they typically require inundative releases of large numbers of sterile	Line 993: the statement is inaccurate. Releases are not done with "sterile individuals", but with fertile individuals whose progeny is not viable. However for OX513A strain (Aedes aregypti), it is known that a small proportion (2-3%) of hybrid progeny is viable and can reproduce under field conditions (Evans et al. Scientific Report, 2019, 9:13047). Then genome parts of the released RIDL strain may be incorporated into the target populations. This is a strong difference in terms of risk assessment and may be a limitation compared to classic SIT.
73	32	1048	and/or in open release trials (e.g. De Barro et al., 2011; Hoffmann et al., 2011; Walker et al.,	Line 1048: so far, has there been open release trials in Europe? It would be good to mention it.
74	33	1073-1074	insect population (Champer et al., 2016). Wolbachia should be seen as a natural gene drive that is cytoplasmically inherited, and thus would not fall within the GMI category.	Lines 1073-1074: Wolbachia may be cytoplasmically inherited, but if Wolbachia themselves are genetically modified, at least one of the two actors (Wolbachia, host insect) is genetically modified and has to be regulated as such. Please note that in some scientific publications, Wolbachia is considered as a natural GD.
75	33	1085	EU, Wolbachia could be regulated as a microbial agent under the appropriate biocide legislation.	Line 1085: mosquitoes transfected with Wolbachia from Drosophila spp. could be considered as GMI by the public. It might be worth explaining why they would not be considred GMI under the EU legislation?

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76	33	1089	control insect pests is an important pest management tool. There are two principle applications	Line 1089: the conservation of natural enemies is usually considered as a third method for biological control.
77	33	1092	In augmentative biological control, native or exotic species are mass-reared and repeatedly	Line 1092: using the term "augmentative" when introducing an exotic species that is not present in the environment does not seem appropriate. Replace "native or exotic species" by "large numbers of a natural enemy of the pest".
78	33	1098-1101	potential environmental effects caused by such releases are likely to be irreversible. However, since classical biocontrol is generally used against exotic pests, this irreversible effect of reducing the target organism is to revert to the ecosystem back to a state without the insect pest species. A major consideration in risk assessment and regulatory approval for classical	Lines 1098-1101: research on trophic webs has shown that reverting the ecosystem back to its original state is not always possible, especially when the exotic species now plays an important role in the food web (e.g. Zavaleta, E. S., Hobbs, R. J., & Mooney, H. A. (2001). Viewing invasive species removal in a whole-ecosystem context. Trends in Ecology & Evolution, 16(8), 454-459; Ehrenfeld, J.G., 2010. Ecosystem consequences of biological invasions. Annu. Rev. Ecol. Evol. Syst. 41, 59–80).
79	33	1102	biocontrol is the host specificity of any biocontrol agent to ensure that the CBC agent will not	Line 1102: impact on the ecosystem through the modification of trophic webs is also an important factor to consider (cf comment on lines 1098-1101).
80	33	1103-1104	adversely affect any native host (Shaw et al., 2011; Marchante et al. 2017). Therefore, the application of CBC could thus serve as a model for ERA of GDMIs. These experiences provide a	Lines 1103-1104: the fact that GDMIs are very specific to the target species do not justify that their ERA could be based on application for Classic Biological Control. 1) if some tools for biological control are very specific, like parasitoids or some insect viruses, others are much less specific like predators or fungus. 2) biological methods aim only to population reduction/suppression but not to population replacement. 3) biological control do not modify insect genome for which these modifications should be a major side of an ERA for GDMIs. Therefore this sentence has to be rephrased to avoid any confusion between ERA for CBC and ERA for GDMI.
81	34-35	1116-1161	6 Potential new hazards/risks associated with gene drive modified disease-spreading mosquitoes and agricultural pests	The list of existing and potential new hazard associated with GDMIs should be more explicit.
82	34-35	1116-1161	6 Potential new hazards/risks associated with gene drive modified disease-spreading mosquitoes and agricultural pests	No information about the risks for non-target species. No mention of the potential inefficacy. The potential epidemiological impact in the case of GD used against insects transmitting vector-borne diseases should be considered.
83	34	1128-1130	However, similar forms of environmental harm are anticipated from the deliberate release into the environment of GDMIs that have been encountered before, whether from the use of non-GDMIs or other existing insect vector/pest control strategies. These include among others: the	Lines 1128-1130: a difference with previous strategies is the non-linear, exponential expected effect of gene drives. Impacts are thus more difficult to predict. It is recommended that proposals should also include a description of countermeasures, to stop the gene drive from spreading.
84	34	1131	potential negative consequences of removing the target organism from the environment (e.g.	Line 1131: explicetly listing theses negative consequences would be helpful.
85	34	1136	No additional unintended effects due to the genetic transformation process are expected for	Line 1136: the argument that unintended effects regarding the use of either GDMIs or non-GDMIs are the same is not supported. Some hazards could be considered similar (effect of the food chain, vertical or horizontal gene transfer to non-target species, etc.), but there are also new unintended hazards. For example, the complete eradication of Drosophila suzukii in both its native and invasive range represents a new hazard that does not exist when using non-GDMIs or biological control.

Comment number	Draft opinion page	Draft opinion line	Line-by-line text comments	Paragraph comments
86	34	1136-1151	No additional unintended effects due to the genetic transformation process are expected for GDMIs than for non-GMIs, as similar approaches (e.g. based on transposable elements or CRISPR-Cas9) are typically used for genetic transformation in insects (e.g. Alphey and Alphey, 2014; Macias et al., 2017; Anderson et al., 2019; Paulo et al., 2019; Sim et al., 2019; Zhao et al., 2019; Li et al., 2020b). Unintended effects could also occur through mutations on the gene drive sequence, the cargo/payload sequence, or some related or unrelated off-target sequences. Random off-target mutations are likely to disappear naturally in a gene drive if they do not confer any fitness advantage. Mutations biased to occur with greater frequency when the drive mechanism occurs, however, could be maintained in a population. For replacement drives, there may be such off-target effects, but the likelihood, viability and impact of any such mutations is not known. The rate of phenotype and genotype changes in GDMIs could be checked by whole genomic sequencing if reference genome data are available. NASEM (2016) indicated that the optimisation of gRNA design, endonuclease cutting efficiency, and homology directed repair (HDR) vs. non-homologous end joining (NHEJ) activity may enable to achieve high specificity and thus reduce the potential for off-target effects (see also Thomas et al., 2019).	Lines 1136-1151: it is not clear if the authors recommend the evaluation of unintended effects due to the transformation process, like mutations on the gene drive sequence or of target mutation. Even if they are "likely to disappear" or their "likelihood, viability, and impact are not known", the risks have to be evaluated (at least partially) before releasing.
87	34	1137	GDMIs than for non-GMIs, as similar approaches (e.g. based on transposable elements or	Line 1137: replace "non-GMIs" by "non-GDMIs".
88	34	1140	al., 2019; Li et al., 2020b). Unintended effects could also occur through mutations on the gene	Line 1140: this sentence suggests there actually are additional unintended effects, please clarify. Cassette translocation through ectopic recombination is a special form of mutation that should also be mentioned.
89	34	1143	do not confer any fitness advantage. Mutations biased to occur with greater frequency when	Line 1143: this sentence is unclear, do the author refer to new mutations in wild-type individuals confering resistance to gene drive? Maybe replace "any fitness advantage" by "any fitness or transmission advantage".
90	34	1146	mutations is not known. The rate of phenotype and genotype changes in GDMIs could be	Line 1146: genome sequencing cannot always inform on phenotypic changes (e.g. due to epigenetic changes).
91	34	1150	high specificity and thus reduce the potential for off-target effects (see also Thomas et al.,	Line 1150: need to distinguish between different kinds of off-target effects (another population of the same species, other species).

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92	34-35	1152-1156	While the molecular complexity of some GDMIs may be higher than that of non-GDMIs, especially for multi-locus gene drive approaches, tools and approaches from computing and engineering such as mathematical modelling and computer-aided design are typically employed to inform and predict the outcomes of different engineering strategies. These tools and approaches might similarly aid and improve the MC and ERA of GDMIs.	Lines 1152-1156: this section does not explicitely refer to the description of potential new hazards.
93	35	1157-1160	Table 2. Overview of existing genetic and biological vector/pest control strategies	Table 2: suggestion to replace "Ability of the gene drive to establish..." by "Ability of the modification to establish", because some of the strategies indicated in this table are not gene drives.
94	36-42	1176-1399	7.1.2 Strategies for the environmental risk assessment of genetically modified animals	Idea of a case-secific approach for the information about EFSA for the ERA of the GDMIs. Absence of an eventual larger framework. How can the ERA be sustainable if this is done for each technique? How could developers work under some particular guidelines by the EU? Could a mixed approach be considered?
95	36-42	1185-1384	7.1.2.1 Different steps of the environmental risk assessment [Section 2.1]	A more balanced presentation of all the potential environmental risks would be appreciated.
96	37	1212	Consequently, in the problem formulation process, more weight needs to be given to ecological	Line 1212: this point is considered important.
97	37-38	1237-1238	under the relevant regulations. This requires the delineation of the environmental components that are valued and must be protected (e.g. species, ecosystem services, habitats), where and	Lines 1237-1238: it would be good to examine not only the environmental components but also the relationships of the various environmental components.
98	38	1240	the context for ERA by describing the components of ecosystems and the environment that	Line 1240: replace "components" by "components and relationships".
99	38	1248	Brink et al., 2018). EFSA has recommended the use of an ecosystem services (ES) approach for	Line 1248: the use of an ecosystem services approach could appear as reponding only to anthropocentric values. Complementary approaches such as the preservation of ecosystem functions or the conservation of biodiversity could be useful for the ERA of GDMIs.
100	39	1299	target release area and the potential for transboundary movements.	Line 1299: it clearly has to include transboundary movements.
101	42	1385	7.1.2.2 Information to identify potential unintended effects [Section 2.2]	Line 1385: unintended effects are not always on the GDMI itself, they might be found on other species which interact, directly or indirectly, with the GDMI. So section 2.2 of EFSA 2013 does not cover fully all the issues raised by GDMIs.
102	42	1418	on the context of the deliberate release. Yet, for GDMIs, this may be unfeasible. It will depend	Line 1418: this may be feasible at least for self limiting GDMI theshold dependent and probably also treshold independent.
103	43-44	1446-1485	7.1.3.3 Choice of comparators [Section 3.3, including subheading 3.3.2]	There is here the evocation of the difficulties to select "comparators". The section on malaria vectors (lines 1471-1475) mentionng the difficulties regarding the species complex is not clear at all. While the document focused initially on Aedes albopictus the discussion on malaria vectors is quite odd. One major point here is also the lack on any epidemiological endpoints (which is the expected measurement for VBD).
104	44	1468	As GDMI systems will operate at an ecosystem level the definition of comparator may need to	Line 1468: another comparator could be naturally occuring gene drives.

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105	44	1479-1485	Consideration should be given to the selection pressure on a colony based on the nature of the gene drive, so for example a male bias colony will have very high selection pressure from low proportion of females each generation. There may need to be a comparison between an early generation colony and later generations (to test potential effects early in release vs. later after release when many generations have passed). Any changes in trait expression over generations is likely to mainly affect interactions with target organisms that relate to efficacy, but indirectly may affect (non-)target organism interactions.	Lines 1479-1485: the meaning of this paragraph could be clarified.
106	44-45	1486-1504	7.1.3.4 The use of non-genetically modified surrogates [Section 3.4]	Bednets cannot be considered as species-specific. Alternatively, another surrogate to a GDMI might be a GMA, i.e. a classical GMO which does not carry a gene drive construct.
107	45	1522	population suppression versus population replacement. Further, given the expected increase of	Line 1522: risk to have an approach that favours monitoring post-release instead of an evaluation in advance.
108	45	1523	spatial and temporal extent of these organisms, the use of small-scale physically and/or	Line 1523: this should not mean that "small-scale physically and/or ecologically confined field trials" should not be used. They are complementary, important as well.
109	46	1558-1579	7.1.3.7 Further guidance on modelling [Section 3.7]	The importance of mathematical modelling remains unclear. Examples would be appreciated.
110	46	1566	complexity of empirical studies. As there may be difficulties in validating model predictions,	Line 1566: after "due to the complexity of empirical studies", add "and the non-linear effects of gene drives on the target population parameters".
111	47	1593-1596	The scope of the adequacy assessment of EFSA (2013) is limited to the use of synthetically engineered gene drives to control harmful insect species such as disease-transmitting mosquitoes and agricultural pests, and excludes the use of such gene drives for biodiversity conservation purposes or the enhancement of production systems.	Lines 1593-1596: GD to control "harmful insect species such as disease-transmitting mosquitoes and agricultural pests". Does that imply that rodents are excluded despite being potential pests for agriculture? Idem for plants - exclusion for biodiversity conservation or enhancement of production systems.
112	47-49	1597-1691	7.1.4.1 Persistence and invasiveness of genetically modified insects, including vertical	Vertical transfer is not considered.
113	47	1600	flow, given in Section 4.2.1 of EFSA (2013) are not adequate for the GDMIs considered in this	Line 1600: "are not adequate...", no justification.
114	49	1657	spread the intended traits. In most scenarios, GDMIs are likely to be less persistent and invasive	Line 1657: there is a confusion about levels of selection. A given individual with a gene drive construct has a reduced fitness, but the fitness of the gene drive itself is higher because of biased transmission. So the construct itself may actually be more persistent and invasive.
115	49	1660	Regarding the potential of GMIs to hybridise with compatible relatives to produce viable and	Line 1660: the cross-fertilization is considered rare but no information is given about the genetic exchange between insect species belonging to species complex (very often in Anopheles). That point does not seem to be taken with much consideration.

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116	49	1661	fertile offspring, it should be noted that cross-species fertilisation is rare in insects and hybrids	Line 1661: this is a very general statement, that does not seem to apply to Anopheles mosquitoes (Fontaine, M. C., Pease, J. B., Steele, A., Waterhouse, R. M., Neafsey, D. E., Sharakhov, I. V., ... & Mitchell, S. N. (2015). Extensive introgression in a malaria vector species complex revealed by phylogenomics. Science, 347(6217), 1258524.). Transfer to related species might also be made easier by the separation of the gene drive cassette from its genetic background (i.e. compared to non GDMIs, hybrid incompatibilities won't be as efficient as preventing introgression of the transgene to related species).
117	49	1666	increase its spread (Courtier-Orgogozo et al., 2019). Laboratory experiments could be	Line 1666: yes, this is necessary, but it is an herculean task. This requires an exhaustive list of all the species the GDMIs will encounter in the release environment (and beyond). We rarely have such an exhaustive naturalis knowledge of an environment.
118	49	1675	thus form part of the rationale of release in self-sustaining drives. The risk to biodiversity may	Line 1675: no justification of the difference in risk for biodiversity.
119	49	1682	drive will remain limited with self-limiting high threshold drives despite the release of a high	Line 1682: note that the threshold value might change temporally or spatially depeding on ecological conditions (Backus G. A., and J. A. Delborne, 2019 Threshold-dependent gene drives in the wild: spread, controllability, and ecological uncertainty. BioScience 69: 900–907.), so that self-limiting drives might become more invasive than previously anticipated.
120	50	1715-1716	target population may facilitate the establishment and persistence of the gene drive in the new host population.	Lines 1715-1716: add the following "If it is possible that a gene drive cassette will insert into the genome of an unrelated species, the probability that it will exhibit gene drive characteristics (i.e. spread within the new species) should be assessed."
121	50	1733-1734	It is unlikely, following gene drive modification that species would become susceptible to new pathogens or symbionts as host-pathogen interactions are so complex. The close	Lines 1733-1734: this statement is speculative and de facto not supported by any reference. This is also the case of the following sentence, lines 1734-1738. Some GDMI technologies for vector control are based on genes involved in insect immunity and then may have an impact (positive or negative) on the vectorial competence for different species of pathogens. Both sentences should be removed and replaced by a statement emphasizing the need for an evaluation of the vectorial competence of the GDMI against a significant range of pathogens.
122	51	1739-1742	Since GDMIs may operate at large scale and over a long term, the problem formulation should consider whether all diseases that can be transmitted by a vector should be taken into account or only the ones circulating in the particular receiving environment and when species relationship justify this possibility.	Lines 1739-1742: it seems not appropriate to envisage only the diseases circulating in a particular receiving environment to evaluate the risk, since recent outbreaks demonstrated the capacity of some arboviruses for circulating at a global level like Zika virus or the different serotypes of dengue virus, etc.
123	51	1749-1750	For disease vectors a comparator system for a replacement strategy could be a widespread vaccine campaign that reduces disease transmission.	Lines 1749-1750: the use of vaccine campaign for comparator is usually not possible in public health since no vaccine are available for most diseases.
124	51	1757	gene drives, as stated in EFSA (2013). Target organisms may include a species complex or a set	Line 1757: this requires knowledge that may be lacking.
125	52	1804	target population, but also the penetrance of the gene drive construct, in addition to the	Line 1804: a definition of the term "penetrance of the gene drive construct" would be appreciated.
126	53	1821	(c) An extreme result of narrow diversity in the deliberate release step for GDMIs is that	Line 1821: assortative mating is mentioned but only as a case of "narrow diversity" but no mention of the results of the genetic modification. This point is not discussed nor its epidemiological impact.
127	53	1842	For the assessment of effects on the target organism population the comparator should be	Line 1842: for the assessment on the target organism population the idea is to use a "classical method" as comparator such as bednet. Is that realistic and ethically acceptable, because this would mean removing the bednets to test the GD approach?
128	54	1857-1869	7.1.4.5 Interactions of genetically modified insects with non-target organisms	The use of a comparator can be considered here as a "non very ambitious" manner because its aim is to consider environmental risks in a manner relative to other methods. How could this take into account risks usually not encountered with "classical' methods"?

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129	54	1862-1863	EFSA (2013) lists potential impacts, rather than focussing on potential quantifiable harm to protection goals. The challenge is to distinguish between ecological change and harm to	Lines 1862-1863: it is unclear what is meant by "harm to protection goals".
130	54-55	1884-1902	7.1.4.7 Impacts of GM animals on human and animal health [Section 4.2.7, including	Some unintended effects to humans could result from the biting capacity of the Gene-Drive Modified Insects (GDMIs). The allergenic risk falls under this category: depending on the insect species used as GDMIs, either mosquitoes, bees, wasps or ticks, the allergenic risk relies on the occurrence of various allergens in the insect salivary glands, which could be introduced in humans upon biting. These allergens could subsequently trigger some anaphylactic response in previously sensitized people. The list of potentially harmfull allergens occurring in biting insects has been well documented, essentially for hymenoptera (bees and wasps) and, to a lesser extent, for mosquitoes and ticks. Considering the possible use of widely consumed insects (entomophagy), such as locusts or caterpillars, as GDMIs, the potential allergenic risk associated to the deliberate release of GDMIs would turn out to be an important issue in the evaluation process of the potential harmfull effects of GDMIs for humans. As a mandatory task, the potential allergenicity of GDMIs should be evaluated using the same guidelines as for genetically modified plants GMPs (EFSA, 2017), namely 1) the allergenicity of the newly expressed protein(s) if appropriate, including the 5 subheadings of the weight of evidence approach: the absence of allergenicity for the gene source organism(s), the absence of global and local identities with known allergens, the resistance to in vitro simulated stomacal and intestinal digestions, the resistance to thermal denaturation, and the minute amount(s) of the newly expressed protein(s) measured in the GDMIs, 2) the allergenicity of the insect(s) used as GDMI(s) and, 3) the possible adjuvancity and immunotoxicity (non IgE-mediated anaphylaxis towards celiac diseased people) of the newly expressed protein(s). The allergenic risk resulting from the unintended (swallowing) ingestion of GDMIs seems more anecdotal and might be evaluated using the same guidelines (EFSA, 2017) on a case-by-case basis.
131	55-57	1903-1984	7.1.5 Post-market environmental monitoring [Section 5]	The monitoring after an eventual release is called "post market" and not post-release. The scientifically significant event is not the post-market phase, but the post-release phase, all the more as the respective responsabilities are not yet legally defined. Recommandation : replace "post-market" by "post-release".
132	56	1949-1950	The transboundary issues of monitoring and response need to be addressed, planned and resourced (Rabitz, 2019).	Lines 1949-1950: the transboundary issue is mentioned but in a very vague manner. No clear and precise operating procedure is given. Only on lines 1963-1966 is mentioned the need for an organization to be liable. No information about the budget, etc...
133	57-58	1991-2023	7.2.1 Information relating to the recipient or (where appropriate) parental animals	Given the fact that sequencing is now relatively cheap, I think that it would be worth asking also for the genome sequence (Illumina reads) of the gene drive strain that is to be released and of a sample of the target population. Such genome data could be useful to monitor what happens to the target population. Especially if the phenotype is not as expected.
134	60	2096	off-target activity of the gene drive (e.g. Sander and Joung, 2014; Taning et al., 2017). Any	Line 2096: minimization of the mutation occuring by Site-specific nuclease (SDN) because of off-target (again with a comparision with SIT with irradiation) but CRISPR-Cas9 has been shown to induce unexpected mutations in vivo (Schaefer et al; 2017 Nature methods).
135	60	2097	sequence changes in the genome of the target population induced by off-target activity of the	Line 2097: it is true that it is "less" at the level of the DNA sequence, but this does not mean that it will also be "less" at the level of the phenotype. For example, a replacement drive (e.g. malaria-resistant mosquitoes) might lead, due to off-target mutations, to individuals which are more active and attracted to humans.
136	60	2100	occur, they would be of the same nature as spontaneous mutations. Taking these	Line 2100: off-target mutations are more likely to be insertions or deletions than nucleotide substitutions.

Comment number	Draft opinion page	Draft opinion line	Line-by-line text comments	Paragraph comments
137	60-61	2126-2142	Inheritance and genetic stability of the inserted/modified sequence and phenotypic stability of the genetically modified insect [Section 2.1.2.2.4] Several considerations/requirements given in Section 2.1.2.2.4 of EFSA (2012) and Section II C.2 of Annex III A of Directive 2001/18/EC are not adequate for the GDMIs considered in this GMO Panel Scientific Opinion. In particular, due to the super-Mendelian inheritance of gene drives and linked cargo/payload gene(s), the concepts of inheritance and genetic and phenotypic stability as outlined in Section 2.1.2.2.4 of EFSA (2012) need further consideration to address the broad array of possible GDMI applications and their intended outcomes. For example, phenotypic stability of a suppression gene drive will be linked to reduced fitness (leading to mortality) of the individuals bearing the gene drive module, whereas for replacement drives the phenotypic stability will be linked to the trait(s) conferred by the cargo/payload gene(s). In addition, some gene drive systems can be designed to target multiple genes and the products of those genes themselves may interact to produce the desired trait. In some cases, genetic elements can be segregated out intentionally as part of the gene drive strategy (e.g. daisy-chain strategy). These features will complexify the definition of genetic and phenotypic stability as stated in EFSA (2012) and can also challenge the concept of "transformation event" as currently	Lines 2126-2142: when feasible, information should be provided to demonstrate the inheritance and genetic stability of the locus/loci altered by the genetic modification and the phenotypic stability and inheritance pattern(s) of the introduced/modified trait(s), as mentioned in EFSA (2012). If the genetic stability can be demonstrated while the phenotypic stability cannot, then the information concerning the genetic stability should be provided, and vice versa. The sentence « In addition, some gene drive systems can be designed to target multiple genes and the products of those genes themselves may interact to produce the desired trait. » is misleading, because it suggests that the genetic stability cannot be demonstrated in that case, while the genetic stability of each targeted gene can be studied. And in the case described in the following sentence « In some cases, genetic elements can be segregated out intentionally as part of the gene drive strategy (e.g. daisy-chain strategy). », it is also feasible to check if the desired segregation is indeed observed.

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138	61	2134-2137	outcomes. For example, phenotypic stability of a suppression gene drive will be linked to reduced fitness (leading to mortality) of the individuals bearing the gene drive module, whereas for replacement drives the phenotypic stability will be linked to the trait(s) conferred by the cargo/payload gene(s). In addition, some gene drive systems can be designed to target multiple genes and the products of those genes themselves may interact to produce the desired trait. In some cases, genetic elements can be segregated out intentionally as part of the gene drive strategy (e.g. daisy-chain strategy). These features will complexify the definition of genetic and phenotypic stability as stated in EFSA (2012) and can also challenge the concept of "transformation event" as currently implemented for GMOs.	Lines 2134-2137: phenotypic stability could also apply to the rate of conversion that can vary depending on the genetic background (Champer, J., Wen, Z., Luthra, A., Reeves, R., Chung, J., Liu, C., ... & Clark, A. G. (2019). CRISPR gene drive efficiency and resistance rate is highly heritable with no common genetic loci of large effect. Genetics, 212(1), 333-341.), on the age of the individual (Preston, C. R., Flores, C., & Engels, W. R. (2006). Age-dependent usage of double-strand-break repair pathways. Current Biology, 16(20), 2009-2015.) or on the environment (Chan, Y. S., Huen, D. S., Glauert, R., Whiteway, E., & Russell, S. (2013). Optimising homing endonuclease gene drive performance in a semi-refractory species: the Drosophila melanogaster experience. PloS one, 8(1)).
139	62	2181-2185	Although the scope of this GMO Panel Scientific Opinion focuses on the use of synthetically engineered gene drives to control harmful insects such as disease-transmitting mosquitoes and agricultural pests, some of its principles would be applicable to the potential use of synthetically engineered gene drives for biodiversity conservation or the enhancement of agricultural production systems.	There is some discrepancy between the idea of a case-by-case approach and the fact of obtaining principles produced here for disease-transmitting mosquitoes and agricultural pests that could be valid for biodiversity conservation or the enhancement of agricultural product in general. This seems contradictory. Some cautions should be taken before generalizing.
140	62-63	2186-2224	8.1 Role of problem formulation for the environmental risk assessment of gene drive modified insects for deliberate release into the environment	Mention of the role of societal engagement but no societal (NGO....) involved in the preparation of this document by mostly scientists, including several involved in the development of gene drive systems. It is important to remind how potential conflicts of interest are taken into account.
141	63	2227	Similar forms of environmental harm are anticipated from the deliberate release into the	Line 2227: the text only mentions "similar forms of environmental harm" than "from the use of non-GDMIs or other existing insect vector/pest control strategies". A difference with previous strategies is the non-linear, exponential expected effect of gene drives. Impacts are thus more difficult to predict. Description of countermeasures to stop a gene drive from spreading is recommended.
142	63	2234	GDMIs aimed at population replacement are not intended to have a direct impact	Line 2234: this is inexact. It is likely there will be a fitness effect (e.g. if a payload gene carries a fitness cost), which may be small indeed, but will have an impact on population density (which may be small).
143	64-65	2285-2299	8.5 Specific areas where updated guidance is needed	An additional section of the ERA could describe potential mitigation strategies to recall a gene drive, including the release of wild-type individuals for high-threshold gene drives or gene drive based countermeasures for CRISPR gene drives (Vella, M. R., Gunning, C. E., Lloyd, A. L., & Gould, F. (2017). Evaluating strategies for reversing CRISPR-Cas9 gene drives. Scientific reports, 7(1), 1-8.; Can a population targeted by a CRISPR-based homing gene drive be rescued? Nicolas O. Rode, Virginie Courtier-Orgogozo, Florence Débarre bioRxiv 2020.03.17.995829; doi: https://doi.org/10.1101/2020.03.17.995829).
144	81	2904	López Del Amo V, Bishop AL, Sánchez HM, Bennett JB, Feng X, Marshall JM, Bier E and Gantz	Line 2904: this is the preprint of the Nat. Comm. paper listed below line 2907, please cite only one of the two studies.