GeneWatch UK comments to USDA APHIS on the proposal to permit the field release of genetically engineered diamondback moth in New York: Environmental Assessment (December 2016): Docket No. APHIS-2014-0056

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GeneWatch UK is a not-for-profit organisation based in the United Kingdom. Our aim is to ensure that genetic science and technologies are used in the public interest.

GeneWatch UK has previously published a 2015 briefing[1] and two submissions to the 2014 consultation[2,3] regarding the proposed releases of GE diamondback moths in New York State.

In this submission, we reiterate the concerns raised in our previous briefing and consultation responses. These have not been adequately addressed by the new Environmental Assessment[4] (EA) of the proposal to release genetically engineered (GE) diamondback moths (DBM) in New York State. More detail is provided below, including updated information.

If approved, the proposed experiments would likely be the first to utilise GE insects with a female-killing trait anywhere in the world. It is therefore of particular importance to expose the environmental assessment to detailed independent scrutiny.

We therefore welcome the opportunity to respond to this consultation.

We conclude that the EA should be withdrawn and not reissued for further consultation until a number of important issues have been addressed. This will require a more thorough Environmental Impact Statement (EIS).

1. **Overview of the EA**

The EA proposes (pages 1 and 4) that USDA APHIS will approve a two year permit for open release of genetically engineered (GE) diamondback moths (DBM) produced by the UK company Oxitec (now owned by the US company Intrexon). Each release would include up to 10,000 GE males (up to 30,000 a week, i.e. three releases per week) on a release site within the grounds of the Cornell University New York State Agricultural Experiment Station (NYSAES), planted with brassica crops (e.g. broccoli or cabbages). The release site consists of an experimental field, up to 10 acres in size, within which there will be a single point at which the open air release will occur. The applicant would additionally be conducting caged field studies in the area defined as the release site, but outside of the brassica plot containing the single release point. In the second year, the specific location of the release site within the NYAES may change due to crop rotation practices. The permit is subject to conditions (EA pages 24 to 32), including Standard Permit Conditions and the following Supplemental Permit Conditions (more detail is provided in the EA):

1. Anyone working with the GE insects must sign/ initial a document containing the conditions before beginning work. All personnel must visually inspect themselves and their clothing for potential hitchhiking moths before leaving the release area and field cages.
2. A 10-meter buffer of bare ground is required, surrounded by an additional 50 meters that, excepting cages, must not be planted with crops that can act as a host for diamondback moth and any substantial clusters of plants that could serve as hosts must be eliminated.
3. Further confinement/monitoring measures: (i) Dispersal of regulated diamondback moths within and outside of the perimeter of the open release site must be monitored and if the numbers trapped are greater than anticipated this must be reported immediately to APHIS, i.e. if the numbers are greater than 1% of the released number of regulated GE moths

(calculated on a weekly basis), or any regulated GE moths are captured outside of the NYSAES. (ii) If a hurricane is expected, no regulated moths may be released within one week prior to the event OR the release site must be treated with an insecticide (per EPA regulations) to kill any existing regulated moths no less than two days prior to the event. Additionally, this must be reported immediately to APHIS. Other unusual events must also be reported immediately to APHIS and APHIS may impose additional conditions as a result.

4. Field Test Termination: (i) This is a crop-destruct trial. Plants/plant materials that can function as hosts for diamondback moth must be in double contained bags transported to the secure laboratory for examination and eventual destruction via freezing and/or autoclaving to render any insects non-viable. No plant/plant materials that can function as hosts for diamondback moth can be used for food or feed. (ii) On or before the expiration of the permit, the field test must be terminated by treating the release site out to the 10m buffer and the caged areas with an insecticide to kill any existing diamondback moths. All plants within the release site and in the cages must be devitalized by disking into the ground. Cages must not be removed until after insecticide treatment and devitalization of host plants within the cages are completed.
5. Post-termination Monitoring: Following the trial, a specified number of traps must be placed within the open release site and up to 60 meters beyond the perimeter of the release site. If the detection of GE diamondback moth occurs, APHIS must be informed immediately. The post-termination monitoring period will not be considered complete until two consecutive months conducive to diamondback moth development have passed without the detection of any GE diamondback moth.

These permit conditions all focus on measures to limit or monitor dispersal of the GE moths. However, they anticipate an expected dispersal of up to 1% of the total GE moths released per week outside the release site (i.e. up to 300 per week), and the possibility of the "unexpected" dispersal of greater numbers, including the possibility GE moths spreading beyond the NYSAES. The implications of dispersal and the (in)adequacy of these conditions are considered in more detail below. However, we note that, following the earlier (withdrawn) application, USDA APHIS added a condition to the permit for the proposed trial which states [5]: "*THIS IS A CROP DESTRUCT TRIAL. Brassicas will be destroyed at the end of the research field trial. No plants or produce shall be used for food or feed. Upon completion of the experiment, the insecticide shall be sprayed on the plants and the surrounding area within 100m radius of treated fields to kill remaining diamondback moth larvae*". It is unclear why the new permit conditions no longer include this 100m spray zone but instead state: "*On or before the expiration of the permit, the field test must be terminated by treating the release site out to the 10 m buffer and the caged areas with an insecticide to kill any existing diamondback moths*". This would appear to be a weakening of the requirements to prevent spread of the GE moths, which is not justified by any evidence provided in the EA.

Other conditions note that: fines, penalties and remedial action may be required if the permit is breached; APHIS and State regulatory officials can make inspections without prior notice; unauthorized or accidental releases and unintended effects must be reported to APHIS; Planting and Environmental Release Reports, a Field Test Report and a Post-Termination Report are required.

In addition, the EA states:

- This Permit does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including for the use of: (1) any non-genetically engineered plant pests or pathogens as challenge inoculum; (2) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; and (3) experimental use of unregistered chemical or other approval as permitted under FIFRA (EA, page 28).

- Interstate movement, release/movement, and release permits may also be subject to PPQ domestic permit and/or quarantine requirements (EA page 32).

2. Legal requirements: potential release of non-GE diamondback moths

The EA has been issued in response to an environmental release permit application (APHIS Number 16-076-101r) received on March 16th, 2016 from Dr. Anthony Shelton of Cornell University. However, since the application is not public, it is unclear how it differs from Application 13-297-102r, which was the subject of the previous EA (issued for consultation in 2014). Application 13-297-102r was released when, following the previous 2014 consultation, the Center for Food Safety (CFS) obtained copies of the permit, application and supporting documents following a Freedom of Information Act (FOIA) request. [6] Application 13-297-102r stated (on page 5) that non-GE moths may also be released as part of the experiments: "*If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental field will be artificially infested with male and female moths from a USA-derived wild-type diamondback moth strain currently maintained in the laboratory; dye-marked wild-type moths may also be used in mark-release-recapture experiments to provide a direct comparison with the GE moths*". [7]

Although the current EA indicates (page 28) that a further approval would be needed to release any non-genetically engineered plant pests, it is silent on the question of whether such a release is still proposed. In the interests of transparency, APHIS should notify the public of whether or not such a release of non-GE moths is still proposed, and, if so, provide details of the permitting and consultation process for this proposed release.

3. Legal requirements: omission of economic and social effects

In its public comment notice, USDA APHIS states:
"*The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969 (NEPA), as amended (42 U.S.C. 4321 et seq.), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372)*".

The EA (page 11) cites the Council on Environmental Quality regulation CEQ (40 CFR 1508.14)[8], but does not quote it in its entirety:

"*§ 1508.14 Human environment.*
Human environment shall be interpreted comprehensively to include the natural and physical environment and the relationship of people with that environment. (See the definition of ''effects'' (§1508.8).) This means that economic or social effects are not intended by themselves to require preparation of an environmental impact statement. When an environmental impact statement is prepared and economic or social and natural or physical environmental effects are interrelated, then the environmental impact statement will discuss all of these effects on the human environment". [Emphasis added]

According to the CEQ (40 CFR 1508.8): "*Effects includes ecological (such as the effects on natural resources and on the components, structures, and functioning of affected ecosystems), aesthetic, historic, cultural, economic, social, or health, whether direct, indirect, or cumulative*".[9]

Economic and social effects have been omitted from the EA and this is incorrect. To be compliant with legal requirements, the EA must be revised to include economic and social effects and then reissued for further public consultation. As discussed further below, there is potential for major adverse economic effects on brassica farmers, due to contamination of crops with GE larvae. This is a major omission from the EA. A full environmental impact statement (EIS) is necessary to address these impacts thoroughly.

4. Legal requirements: omission of cumulative effects

The EA should have included the issue of cumulative impact. CEQ (40 CFR 1508.7) states:
"*§ 1508.7 Cumulative impact.*
Cumulative impact is the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal or non-Federal) or person undertakes such other actions. Cumulative impacts can result from individually minor but collectively significant actions taking place over a period of time".

Since the purpose of the trial is to lead to commercial use of the GE DBM, on an area-wide scale, for pest control, the impacts of future trials or commercialisation (without the "crop destruct" requirement, and on a larger scale) should have been considered as "reasonably foreseeable future actions" to follow from the proposed open release trial. These cumulative impacts should have been considered in the risk assessment and in the comparison of the "action" and "no-action" alternative. This includes impacts on crop damage and contamination of the food chain (discussed in more detail below). Cumulative impacts should be considered in a new EIS for further consultation.

5. Implications of the GE moths not being sterile: failure to focus on a key characteristic of the GE DBM

Agencies are required to focus on the characteristics and risks of a biotechnology product (EA, pages 2 and 6): however, one important characteristic has been omitted entirely from consideration. This is the fact that the GE DBM are not sterile. In fact, after mating, they reproduce and the female GE offspring then mostly die at the late larval or early pupal stage. Compared to the sterile insect technique (SIT) this has some major downsides:

- The GE male moths will mate with wild moths which will lay GE eggs on the crop (and wild relatives) and these will produce GE larvae;
- The GE moth larvae will contaminate the crop before some of them (most of the females) die at the late larval or pupal stage;
- The GE larvae will consume the crop (and wild relatives), causing damage to crops (and related wild plants);
- A high proportion (up to 50%, i.e. all females) of larvae and pupae will contaminate the crop (and wild relatives) after most non-GE larvae would have left it as adults i.e. large numbers of dead GE (late stage) larvae will remain on the crop (and on related wild plants).

The company reports that 9% of GE females from the OX4319L-Pxy strain survived the caterpillar stage to become pupae, in laboratory experiments, with about 1% surviving until adulthood.[10] This means that about 41% of the offspring of the GE moths that are released (most of the female offspring) are expected to die as caterpillars when they are feeding on the crop, and another 8% are expected to die as pupae. Significant risks of crop damage and crop contamination (leading to economic loss) therefore arise, which are discussed further below. These risks are much greater than if the insects were sterile, because sterile insects would not produce viable larvae which damage and contaminate the crop. Further, even wild-type moths have a much lower risk of crop contamination, because most of the larvae are expected to survive to adulthood, rather than a large percentage dying in the crop. Contamination with GE larvae may also prove unacceptable to consumers and markets, and could pose a food safety risk.

6. Crop damage

Diamondback Moth (DBM) causes damage to crops in two ways: larvae chew holes in the foliage, and pupae contaminate heads. The proposed releases therefore clearly pose a significant plant pest risk. As experts advising the European Food Safety Authority have noted: "[Late lethality*] implies that the offspring of the mating between the released arthropods and the wild population carry the transgene and survive beyond the embryo stage…For fruit flies such an approach would be detrimental as it would result in significant damage of larvae to the agricultural produce*." [11] This same problem applies to Oxitec's diamondback moths.

Further, Oxitec reports that it took six weeks to achieve a considerable reduction in reproductive output of diamondback moths in a caged trial in the UK, and ten weeks to reduce the reproductive output to zero.[12] Open air use will generally be much less effective, meaning that crop damage caused by both wild and GE caterpillars is likely to continue for several months during the releases. In the open air, the wild population is unlikely to become extinct because wild moths will fly in from surrounding crops or be blown in on the wind from further afield. Further releases will need to be made each season to maintain the suppression effect (if achieved), since DBM (if present) are reintroduced each season via migration or contaminated seedlings. Therefore, any contamination and crop damage caused by the GE larvae and pupae will continue over many weeks (perhaps even the whole 3 to 4 month DBM season).

The EA admits that crop damage will occur, however it concludes that "*Damage from GE diamondback moth larvae on planted cruciferous plants is not anticipated to be substantial*…" (Table 1. Comparison of Alternatives, Section 4.3, page 34). This conclusion has not been substantiated. In particular, the applicant does not appear to have been required to submit evidence from previous caged trials regarding the extent of damage to the crop. Some relevant evidence is likely to be available but unreported from one published trial[13], and unreported and unpublished from the subsequent caged trials[14]. This is a very serious omission, since crop damage is one of the main negative impacts expected from the release. This further information should be published in a peer reviewed journal, and incorporated into a new Environmental Impact Statement (EIS) for further public consultation.

In addition, the EA admits that some crop damage is likely to also occur outside the trial site, but makes no attempt to quantify this damage. Regarding impact outside the trial location, the EA states (page 57): "*Assuming stability of the autocidal trait in the released GE diamondback moths, cruciferous crops planted on adjacent fields may experience some herbivory damage from the larval offspring of a GE diamondback moth male and a non-GE diamondback moth female. This potential impact on planted cruciferous crops in adjacent fields is not likely to be significant due to the anticipated reduction of the local diamondback moth population through a reduction in reproductive capacity (Jin et al., 2013), the ubiquity of diamondback moth within the action area (i.e., those cruciferous plants are likely already incurring diamondback moth herbivory damage) (Andaloro and Baker, 1983; Shelton, 2001a; 2001b), nor is there likely to be a future impact because of the inability of that local diamondback moth to overwinter (Talekar and Shelton, 1993; Shelton, 2001b; Nguyen et al., 2014) within the action area*". However, as noted above, crop damage is likely to be serious and ongoing, due to: (i) the fact the released GE insects are not sterile but female-killing only, and even the female offspring survive until the late larval stage; (ii) any suppression effect is expected to take many weeks, perhaps months, to achieve[15]; (iii) since a new DBM infestation arrives each year (when present), releases will be required each season. This reinforces the need to publish the existing information regarding crop damage from the caged trials (as noted above), but also to provide detailed modelling of the likely spread of crop damage further afield. Such modelling is likely to require more detail on dispersal (discussed further below). This information should be included in a new EIS for further public consultation.

Further, the statement in the EA that DBM is ubiquitous within the action area is incorrect, as DBM infestations are not present every year. Indeed, the original (withdrawn) application 13-297-103r recognises this where it states (page 5): "*If the wild diamondback moth population is not present in sufficient numbers at the trial sites, the experimental fields will be artificially infested with male and female moths from a USA-derived wild type diamondback moth strain currently maintained in the laboratory*…". [16] Since the introduction of a GE (or wild-type) pest in a year in which the wild type pest is not present clearly introduces additional (unnecessary) pest pressure, further conditions should be added that prevent such releases.

In addition, the EA should have included the issue of cumulative impact (as noted above). Since the purpose of the trial is to lead to commercial use of the GE DBM, on an area-wide scale, the impacts of future trials or commercialisation (without the "crop destruct" requirement, and on a larger scale) on the issue of crop damage should have been considered in the EA, including in the comparison of the "action" and "no-action" alternative. Beyond the risks of the current trial, future open releases of Oxitec's GE diamondback moths, perhaps on a commercial scale, are not a credible approach to tackling these pests because the GE DBM are not sterile and their dead and surviving larvae will damage and contaminate the crop, making it unlikely to be fit for human consumption. Cumulative impacts, including crop damage and crop contamination (see below), should therefore be included in a new EIS for further public consultation.

7. Implications for famers: omitted social and economic risks

In addition to failing to quantify the expected crop damage, the EA has entirely omitted any consideration of the likely socio-economic impacts of the contamination of brassica crops in the neighbouring area or region with GE larvae. As well as direct harm through contamination, this risk includes likely reputational damage to conventional and organic brassica crops grown in the area. This failure is compounded by the failure to conduct a proper food safety assessment (discussed further below): however economic damage, due to reduced consumer demand and loss of markets, will likely occur even if there is no food safety concern. As discussed above, crop contamination with large numbers of GE larvae is likely to occur, since most of the female offspring of the GE moths are expected to die at the late larval stage, whilst they are feeding on the crop.

Some relevant evidence on crop contamination is likely to be available but unreported from one published trial[17], and unreported and unpublished from the subsequent caged trials[18], but no information on crop contamination is included in the EA. This is a very serious omission, since crop contamination is one of the main negative impacts expected from the release. This further information should be published in a peer reviewed journal, and incorporated into a new Environmental Impact Statement (EIS) for further public consultation. Detailed modelling of the likely spread of crop contamination further afield is also essential for meaningful consultation. Such modelling is likely to require more detail on dispersal (discussed further below). This information should be included in a new EIS for further public consultation.

According to Cornell University, with more than 12,000 acres grown annually, New York ranks in the top three states nationally for both fresh market and kraut cabbage.[19] Fresh cabbage is sold in retail and wholesale markets and is used for coleslaw, egg rolls and other products. Broccoli is grown on an estimated 800 acres throughout New York State.[20] Multiple plantings are typically grown along with other Cole crops such as cauliflower, cabbage and kale on small-scale diversified vegetable farms. Broccoli is predominantly grown from transplants set in April and May for a spring crop and in late June through August for a fall crop.

If crop contamination with GE larvae occurs outside the test site (where the permit requires the crop to be destroyed), there would be implications for international as well as domestic markets (including organic markets), since most overseas markets (including the EU) have a regulatory approvals process without which products containing GE insects will not be accepted on the market. In the EU, foods containing genetically modified organisms (GMOs) must also be labelled.[21] This is also the case in many other countries, such as Mexico, Peru, Brazil, Russia, China, Australia, New Zealand and Japan.[22] Further, there may be cross-border issues with Canada if GE diamondback moths spread across the border (discussed further below), with implications for the canola industry as well.

This issue appears to be being taken more seriously elsewhere. In 2014, the Brazilian regulator CTNBio approved experimental releases of Oxitec's GE Mediterranean Fruit Fly (Medfly). However, the company has yet to make the transboundary notification for export of GE Medfly required by European Union law, which requires a risk assessment which meets EU standards to be reviewed and accepted by the importer.[23] The European Commission has notified Brazil that export of fruit contaminated with GE Medfly to the EU would be illegal under EU law and sought further information about the steps that will be taken to ensure such exports do not happen.[24]

Although contamination could affect all farmers, including conventional farmers, there are particular concerns about organic crops because the use of genetic engineering, or genetically modified organisms (GMOs), is prohibited in organic products.[25]

For comparison, in some cases GE crops have caused major (multi-million dollar) damage to markets for conventional or organic crops and foods.[26,27,28,29] Before any open releases of GE pests take place, it is therefore important to have clarity about who will be liable if they contaminate other crops outside the experimental area.

In addition to providing more information about the likely extent of future contamination, more information should be supplied regarding the steps that will be taken to test and monitor produce in the area and prevent contamination of the food chain (including exports). Likely socio-economic damage should be quantified and liability for contamination incidents should be clarified. This information should be provided in a new EIS for further consultation.

In addition, the EA should have included the issue of cumulative impact (CEQ (40 CFR 1508.7), as noted above). Since the purpose of the trial is to lead to commercial use of the GE DBM, on an area-wide scale, the impacts of future trials or commercialisation (without the "crop destruct" requirement, and on a larger scale) on the issue of contamination should have been considered in the EA, particularly in the comparison of the "action" and "no-action" alternative. Beyond the risks of the current trial, future open releases of Oxitec's GE diamondback moths, perhaps on a commercial scale, are not a credible approach to tackling these pests because these GE insects are not sterile and their dead and surviving larvae will damage and contaminate the crop, making it unlikely to be fit for human consumption. Thus, although the current proposed permit is for a "crop destruct" trial, commercial application in the future would require the contaminated crops to enter the food chain, including local, national and international markets. This must be considered when comparing the merits of the action and no-action alternatives. Cumulative impacts, including crop damage (see above) and crop contamination through the anticipated further steps of future trials and commercialisation should be included in a new EIS for further public consultation. This must include socio-economic impacts.

8. Dispersal concerns and lack of an adequate buffer zone

A major difference between Oxitec's GE mosquitoes and its GE agricultural pests, such as the GE diamondback moths, is that the GE trait in the agricultural pests is "female killing" only, whereas the both the male and female offspring of the GE mosquitoes die. This means that the male GE moths are not genetically programmed to die and are likely to survive for many generations, increasing the risk that they will spread widely in the environment.

In addition to most GE males surviving, some female GE moths will survive to adulthood. Cornell researchers plan to release Oxitec's GE diamondback moth OX4319L-Pxy in the proposed experiments in New York State. For this strain, female survival rates to adulthood in the absence of chlortetracycline (CTC, one of the tetracycline family of antibiotics used to breed the GE DBM in the lab) is reportedly 1%, relative to the wild moths.[30] Because very large numbers of GE moths are expected to reproduce in the environment, the numbers of female survivors could be very high even if survival rates are only 1%. The proposed permit allows the release of up to 30,000 GE males a week over a two year period. Assuming releases only take place during the four month DBM season, this is up to 1.04 million GE moths (but it could be more – up to 3.12 million - if the GE males are released all year). As a very rough estimate, if a million released GE males mated successfully once, this would lead to about 150 million eggs laid in the crop containing the GE trait (on average a female diamondback moth lays 150 eggs[31]), leading to up to 750,000 surviving female offspring, as well as up to 75 million surviving male offspring. Female survival rates may be much higher in the presence of tetracycline contamination, or if the released GE insects evolve resistance to the killing mechanism (discussed further below).

The proposed permit conditions include that dispersal of regulated diamondback moths within and outside of the perimeter of the open release site must be monitored and if the numbers trapped are greater than anticipated this must be reported immediately to APHIS, i.e. if the numbers are greater than 1% of the released number of regulated GE moths (calculated on a weekly basis), or if any regulated GE moths are captured outside of the NYSAES (EA, page 26). This means that 1% of the released number of GE moths are anticipated to escape elsewhere on the NYAES site i.e. 10,000 GE moths are expected to escape if one million are released. Further, there is no public reporting (only reporting to APHIS) if numbers are greater than this, or if they spread outside the site. The EA should be amended to include more extensive monitoring and public reporting of any dispersal of GE moths outside the buffer zone, as this is critical to monitoring the risks to farmers of crop contamination, as discussed above.

Further, one potential response to the releases is that wild-type males will disperse further from the release site, to avoid competition from the released GE males for mates. Therefore, monitoring and reporting requirements should also be sufficient to identify the increased dispersal of wild-type males.

The proposed permit requires that a 10-meter buffer of bare ground, maintained by weekly disking, must be maintained around the perimeter of the open release site. The buffer must be surrounded by an additional 50 meters that, excepting cages, must not be planted with crops that can act as a host for diamondback moth and "*any substantial clusters of plants that could serve as hosts"* must be eliminated (EA, page 25). At the conclusion of each proposed open release experiment, the release site will be treated with the EPA-registered insecticide, Coragen (chlorantraniliprole) to kill any remaining moths (EA, page 4). The proposed permit requires that the field test must be terminated by treating the release site out to the 10 m buffer and the caged areas with an insecticide to kill any existing diamondback moths (EA, page 27), but this proposed requirement does not extend to the additional 50 meters. All plants within the release site and in the cages must be devitalized by disking into the ground and cages must not be removed until after insecticide treatment and devitalization of host plants within the cages are completed. However, spraying for 100m around the site, which was a condition of the previous USDA APHIS permit (now withdrawn) is no longer a requirement in the new proposed permit.

The EA states (page 11) that the proposed release sites are generally surrounded by other agricultural fields, planted to row/vegetables crops, orchards, and vineyards. According to the EA (page 48), within-field and adjacent-field plant communities are anticipated to be similar within the action area, in that there will be a mixture of cultivated crops and weeds of those cultivated crops. Domesticated crops that may be found within the action area include fruits and field crops, including a variety of domesticated cruciferous crops, such as cabbage or broccoli (EA, Table 3, Page 50). According to the EA, weeds in the area will include some 50 species of non-domesticated cruciferous plants which may act as hosts for diamondback moth larvae (EA, Table 4, page 50).

Transport and sale of brassica produce is one mechanism through which this pest has been transported worldwide and from the south to north of the United States.[32] Other mechanisms for spread include: independent flight of adult moths to and from wild relatives; dispersal through the movement of humans, animals and birds; and dispersal assisted by the wind, including mass migration. These mechanisms are considered further below.

Diamondback moth movement within crops involves a series of short flights within the crop canopy: in experiments in Australia, 95% of released diamondback moths remained within 40 to 106m of the release point, 99% remained within 63 to 117m, and 99.9% remained within 113 to 300m of the release point.[33] If these estimates are applicable to the release site, this means that about 1% of the released moths may move outside a 100m area around the release point: however, the EA has inexplicably proposed only a 10m buffer zone. Further, although there is a proposed permit requirement for a further 50m around this not to be planted with host plants, removal of wild relatives is only required for (undefined) “substantial clusters” of host plants. It is therefore certain that some host plants will remain, increasing the likelihood of dispersal.

There can be significant movement between the crop and neighbouring flowering vegetation, and a small proportion of individuals (less than 1% based on the Australian study) may move greater distances from healthy crops to colonize neighbouring crop patches. The authors of the Australian study also cite unpublished data that small percentages of diamondback moths can move between host patches of up to 150m separation. In addition, they state they cannot rule out some moths engaging in long-distance dispersal or migration. When applying population control strategies, such as mating disruption, the authors recommend a minimal separation distance of 3km between target and non-target populations of moths, based on a safety factor of 10 applied to the 300m distance from their release point that 99.9% of moths stayed within in their study. This is far larger than the 10m proposed buffer zone.

An earlier study of diamondback moth flight paths in Japan found a mean flight distance of 615m in summer and 286m in autumn for moths captured outside the release field.[34] Although the majority of released moths, which were caught in the release field, were not included in these calculations, this research also demonstrates that released diamondback moths can fly much further than the 10m buffer zone required for the proposed trial in New York State. This study was not included in the EA, despite it being included in our submission in response to the previous application.

Longer-range movement of DBM is achieved through active migration, which explains its global distribution. Migration enables diamondback moths to move from areas that allow year-round persistence into areas that are only seasonally suitable for growth and development so that regions where diamondback moths cannot survive low winter temperatures can be invaded annually from regions where they can overwinter. [35] Ecological studies suggest that the diamondback moth is an actively dispersing species and when environmental conditions dictate, such as when host plants degrade, and the species moves large distances to colonise particular regions when climatic conditions are favourable for migration.[36] The authors of the Australian study cited above note that the dispersal pattern from harvested crops is likely to be quite different from that in healthy host crops and that, depending on the location of the closest host patches, moths from harvested crops may have to travel hundreds of meters or even kilometres. In China, there is evidence that diamondback moths have made regular long-distance migrations across the Bohai sea (approx. 100km)[37] and studies show long-distance migration routes from the lower reaches of the Yangtze River to northern China and then to northeastern China and potentially also from southwestern China to both northwestern and southern China.[38] However, there is also rare effective migration in the reverse direction.[39]

Rather than requiring evidence of migration routes in New York State (including both prevailing routes, and rarer occurrences of migration in alternative directions), the EA relies solely on statements regarding the prevailing wind direction in the area (pages 12 and 13). Further, the emphasis on risk of overwintering, whilst important, ignores the more immediate risks of crop damage and crop contamination (discussed above), which can occur during a single season, and potentially lead to major socio-economic losses (lost markets and consumer trust etc.). A new EIS should be prepared for further consultation, including extensive data on EBM migration routes and a more reliable assessment of the risk of migration of the GE DBM on to crops outside the release site.

There may be movement of released diamondback moths (GE or wild moths) from the crop to neighbouring crops or flowering vegetation, and movement might occur over much longer distances if the moths migrate. As well as brassica crops, such as cabbage and broccoli, there are numerous weedy brassicas which may provide viable habitat for diamondback moths, including *Sinapis arvensis* (wild mustard or charlock), *Raphanus raphanistrum* (wild radish), *Brassica rapus* (wild or bird rape), and *Hirschfeldia incana* (hoary mustard).[40]

In summary, the buffer zone proposed for the proposed trials is totally inadequate to prevent dispersal of GE DBM outside the trial site, which carries risks discussed elsewhere (including crop damage and contamination). A credible assessment of likely dispersal, including more data and modelling studies, should be made as part of a new EIS, for further consultation. A buffer zone of kilometres, rather than meters, would be likely to result from an in-depth consideration of the evidence, but even this may prove inadequate to deal with long-distance migration events. Assessment of the risks must include cumulative impacts of "reasonably foreseeable future actions", such as the removal of the crop destruct requirement, and future trials or commercialisation on a larger scale.

Dispersal through the movement of humans has been partially addressed by the inclusion of a proposed permit condition requiring that anyone working with these insects must sign/ initial a document containing the conditions before beginning work and that all personnel must visually inspect themselves and their clothing for potential hitchhiking moths before leaving the release area and field cages. These signed conditions must be readily accessible in the event of an APHIS inspection and presented upon request (EA, page 25). However, there is no evidence that self-inspection will really work. Self-inspection should be tested in a pilot study under contained conditions before it is proposed as an adequate means to prevent spread of GE DBM through 'hitchhiking' on workers. In addition, cumulative impacts of "reasonably foreseeable future actions" must be considered in a revised EIS, such as the removal of the crop destruct requirement and future trials or commercialisation on a larger scale, when an inspection requirement for individual workers is likely to become even less feasible to implement.

Further, dispersal through the movement of animals and birds has been entirely omitted from the EA. This means of dispersal should be included in a revised EIS for further consultation.

Transport and sale of brassica produce is another mechanism through which this pest has been transported worldwide and from the south to north of the United States.[41] This could occur if the buffer zone is inadequate, as described above, leading to the contamination of crops which are subsequently sold. This adds weight to the importance of revising the buffer zone (to the order of kilometres, rather than meters) in the light of a thorough review of the evidence plus additional modelling. Although the proposed permit includes a condition for a "crop destruct trial", a revised assessment of the risks must also include cumulative impacts of "reasonably foreseeable future actions", such as the removal of the crop destruct requirement and future trials or commercialisation on a larger scale.

It is not necessary for DBM to overwinter for the releases to propose a major risk of crop damage and contamination, as discussed above. However, although the main mechanism for crop damage in northern climates is re-infestation via long-distance dispersal by the wind, small numbers may overwinter in cold climates, potentially allowing survival of the GE trait. In Canada, Alberta's Department of Agriculture and Rural Development reports that overwintering diamondback moths were found in central Alberta in the early 1990s i.e. considerably further north than the proposed trial site.[42,43] Adults have also recently been found in spring emergence traps in Saskatchewan and have been collected (in small numbers) very early in spring in Manitoba. The average temperature in January (the coldest month) in Geneva, New York, where the proposed experiments are sited, is -8.9°C[44], compared to a lower lethal temperature where 25% survived of -15.2°C in laboratory tests.[45] This does not provide confidence in claims made in the Environmental Assessment that GE diamondback moths cannot overwinter at the release site, particularly if there is unintentional survival of females due to failure of the killing mechanism (discussed further below). The potential for overwintering should therefore be included in a revised EIS, especially in relation to cumulative impacts.

9. Food safety implications

The EA states (page 7):

"*Under this policy* [57 FR 22984-23005]*, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. The permit applicant did not undergo this voluntary consultation because GE diamondback moth is not anticipated to yield food or feed*". [Emphasis added]

However, this decision is misguided because:

(i) The GE moth (particularly its larvae) may accidentally contaminate food crops (as highlighted above);

(ii) If contamination does occur (as anticipated via the reporting requirements for unexpected and anticipated events, included in the conditions), regulators will be unable to reassure consumers or markets (including overseas markets) that consumption of contaminated brassicas is safe;

(iii) The EA should have considered cumulative impacts, including the need for the crop to enter the market should this technology ever be commercialised (as outlined above).

As noted above, death of most female GE moths at the larval stage will significantly increase the number of larvae dying in the brassica crop (and in wild relative brassica weeds), compared to current conventional or organic farming, since about 41% of the offspring (i.e. most of the females) are expected to die at this stage if the technology works correctly, rather than pupating or reaching adulthood. The dead larvae will contain the DsRed2 (fluorescent) and tTA (late lethality) genetically engineered traits. They will be consumed by all species which normally consume diamondback moth larvae or brassica crops, or wild relatives of these crops which diamondback moths may also feed on. This will include humans if any contaminated crops accidentally enter the food chain (despite the permit condition for a "crop destruct trial"). Some living GE larvae may also contaminate food crops.

Another potential exposure route for humans is through swallowing the adult moths during the releases. Journalists have reported that in Brazil "…*it's impossible to talk during the liberation sessions without accidentally swallowing a few*…" of Oxitec's adult GE mosquitoes due to the very large numbers released to try to swamp the wild population.[46] This is because use of Oxitec's technology requires wild moths to be vastly outnumbered by the GE male moths, which are repeatedly released in order to seek to suppress the population of wild moths. In caged experiments conducted at Cornell and published in 2015, initial release ratios of 20 GE male moths to one wild male moth, increased to rates of 40 to 1 in the next generation, were predicted to be sufficient for population suppression, although even higher ratios might be needed in the wild.[47]

In its application to release GE moths in New York State, Oxitec provides a commercial reference for toxicity testing of the red fluorescent marker, DsRed2, by Pioneer DuPont.[48] Oxitec also cites a 26-day feeding study in rats, using GE oil seed rape (canola) genetically modified to express green (not red) fluorescent protein (GFP), which concludes: "*These data indicate that GFP is a low allergenicity risk and provide preliminary indications that GFP is not likely to represent a health risk*".[49] Other than a bioinformatics report (discussed further below), Oxitec provides no evidence regarding the safety of the RIDL genetic mechanism and the high level expression of tTA that kills the insects at the larval stage. The mechanism of action of this killing mechanism is not fully understood and very limited safety data is available. The tetracycline transactivator (tTA) protein is created by fusing one protein, TetR (tetracycline repressor), found in *Escherichia coli* bacteria, with the activation domain of another protein, VP16, found in the Herpes Simplex Virus. This mechanism is commonly used by researchers to switch on and off different genetic traits, for example in transgenic (GE) mice used in medical research, but it is not normally present in the human food chain. Oxitec has published only one feeding study (cited in the EA), in which its GE *Ae. aegypti* mosquito larvae were fed to two different species of a type of mosquito that eats other mosquitoes (known as *Toxorhynchites*).[50] No feeding studies have been published for Oxitec's GE diamondback moths or any of its other GE insect pest species, and no feeding trials have been published which study potential impacts on birds, mammals, reptiles or amphibians, such as lizards or frogs.

Oxitec has provided a bioinformatics report by a consultant, Dr Richard E Goodman of the University of Nebraska, which compares the chemical sequences of the tTA (also known as tTAV) and DsRed2 (fluorescent) proteins with known toxins and allergens in databases. The bioinformatics report was made publicly available for the first time in 2015 in response to FOIA requests by the Center for Food Safety[51] and was not published during the 2014 consultation, nor is it provided in the current consultation. The released version is poorly copied so that it is difficult to read. The bioinformatics report considers exposure only through mosquito bites and saliva, not through the dietary route, which is more relevant to the GE diamondback moths.

In the scientific literature, there is some evidence that enhanced tTA expression can have adverse effects (loss of neurons affecting cognitive behaviour) in transgenic (GE) mice.[52] Other mice studies have detected adverse effects on the lung.[53,54] The bioinformatics report fails to identify or cite this evidence, instead claiming that these constructs have been inserted in mice and other animals without any adverse impacts. The reported adverse effects are caused by production of tTA in the cells of these mice through genetic engineering, rather than through eating tTA. However, they suggest that more evidence is needed before concerns about safety are dismissed. In the case of the rat feeding trails reported for Green Fluorescent protein (cited above), the authors state: "*Moreover, transgenic animals that constitutively express GFP have been reported as being healthy… This may differ from dietary exposure because the GFP in transgenic animals is located intracellularly and toxicity or allergenicity may function differently than when exposed extracellularly. Nevertheless, these data do suggest that GFP is minimally toxic, a conclusion that is supported by this feeding study*". However, for tTA, the opposite is true, as adverse effects have been reported in transgenic mice. These adverse effects are not cited in the EA and should be taken as a warning sign that further testing – including feeding studies - is required.

Considerably more data, based on specific feeding trials in relevant species, is therefore needed to establish that consumption of GE moth adults or larvae is not harmful to humans, farm animals, pets or wildlife.

European Union (EU) standards are relevant here because: (i) Oxitec is required by EU law to provide a risk assessment which meets EU standards before exporting its GE diamondback moth eggs to the USA or other countries[55]; (ii) future exports of crops produced using GE moths to the EU, and perhaps to other countries, will be required to meet these standards. EU Guidance on risk assessment of GE insects (known as genetically modified, GM, insects in the EU) published by the European Food Safety Authority (EFSA) requires applicants to assess the effects of toxins or allergens associated with the GE insect animals such as birds, mammals, reptiles and amphibians.[56] It also states (page 8): "…*applicants should also assess the likelihood of oral exposure of humans to GM animals or their products which are not intended for food or feed uses. If such exposure is likely and ingestion or intake will occur at levels which could potentially place humans at risk, then applicants should apply the assessment procedures described in the EFSA Guidance Document on the risk assessment of food and feed from GM animals and on animal health and welfare aspects*". To meet the requirements of the cited Guidance on risk assessment of food and feed, it is likely that repeated dose toxicity studies using laboratory animals would be required.[57]

An application by Oxitec to release GE olive flies in Spain, genetically engineered with the same female-killing trait, was withdrawn in 2013, following a request for further information from the regulator, including toxicity testing using feeding trials in relevant species.[58,59] Oxitec re-applied to release GE olive flies in Spain in 2015, without providing any further published safety information.[60] This application was also rejected.[61]

The evidence provided for the proposed releases of GE diamondback moths falls far short of the data or precautions needed to assess safety of the GE moths, for example to birds and mammals, including humans, which may eat them, especially at the larval stage when they will contaminate food crops. Farm workers and animals might also swallow adult flies due to the very large numbers involved in the releases. Further safety testing must be conducted and included in a new EIS for further public consultation. This must include cumulative impacts of "reasonably foreseeable future actions", such as the removal of the crop destruct requirement and future trials or commercialisation on a larger scale.

10. Lack of safety testing for wildlife

As noted above, Oxitec has published only one feeding study (cited in the EA), in which its GE *Ae. aegypti* mosquito larvae were fed to two different species of a type of mosquito that eats other mosquitoes (known as *Toxorhynchites*).[62] No feeding studies have been published for Oxitec's GE diamondback moths or any of its other GE insect pest species, and no feeding trials have been published which study potential impacts on birds, mammals, reptiles or amphibians, such as lizards or frogs. Therefore, there is no data to establish whether or not consumption of the GE adult moths or their larvae is harmful to wildlife.

Species which could be affected if consuming the GE moths is harmful include species which are endangered, threatened or of special concern and which may feed on diamondback moths or larvae or on brassicas in New York State: such as the Northern Long-Eared Bat (*Myotis septentrionalis*)(which is proposed as endangered without critical habitat, as noted on page 64 of the EA) and the Grasshopper Sparrow, Golden-Winger Warbler and New Cottontail Rabbit, which are identified as relevant species in the information provided by Oxitec appended to the 2014 APHIS EA (Appendix VII). Migratory birds also forage for insects in or adjacent to fields containing brassica crops (EA page 70) and require protection under EO 13186 (US-NARA, 2010), "Responsibilities of Federal Agencies to Protect Migratory Birds". The EA's conclusion (page 70) that there is no reason to believe that the release of GE DBM would have any effect on migratory birds is based on no evidence whatsoever.

The EA must be withdrawn due to lack of evidence regarding potential harm to wildlife and only reissued for further consultation after appropriate feeding trials have been conducted on relevant species and included in a new EIS for further consultation.

11. Implications of tetracycline use for the development of antibiotic resistance

Even if the genetic changes to the moths, including expression of tTA, were demonstrated to be safe for humans, animals and wildlife to consume, concerns would exist about the dead GE larvae in the crop spreading antibiotic resistant bacteria into the environment, as discussed below.

Oxitec uses tetracycline (an antibiotic which is used commonly in agriculture and medicine) as a kind of antidote to the genetic killing mechanism, allowing it to breed its insects in the laboratory or insect factory, prior to making a release of GE males. Tetracycline binds to tTA and prevents it leading to the expression of more tTA so that the genetic killing mechanism does not work. Including tetracycline in their feed allows the female insects to live to adulthood rather than dying at the larval stage.

The use of tetracycline to breed the GE diamondback moths in the lab carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health.[63] This is because insect guts are reservoirs for antibiotic resistance genes which can be spread into the environment.[64,65,66] GE insect production in factories exposed to antibiotics could lead to drug resistance in bacteria in their guts so that the insects disseminate antibiotic resistance when released into the environment.[67,68] Oxitec's GE diamondback moths are reared on an artificial diet containing 100μg/mL of tetracycline[69] or chlortetracycline (CTC, another antibiotic in the tetracycline family)[70]. These are both common antibiotics which are being phased out for uses other than treating animal diseases, according to FDA Guidance.[71,72] The Guidance is intended to ensure that antibiotics including tetracycline and chlortetracycline are only available for use in veterinary or human medicine. This is because antibiotic resistance can make antibiotics useless to treat diseases, with serious implications for human and animal health.

Cornell's earlier (13-297-103r) application to USDA APHIS states that all GE diamondback moths used in the trials will be reared as larvae on a non-tetracycline diet (its 2016 application is not publicly available). This is consistent with the report of the contained trials undertaken at Cornell to date. [73] It is not necessary to release GE males fed on tetracycline, because the next generation, from eggs produced by females which were bred on tetracycline, can be used instead. Oxitec can do this with its GE agricultural pests (but not its GE mosquitoes) because the males survive and are not killed by the genetic killing mechanism (i.e. the technology is "female killing" only). However, the parents of the released males must be fed on tetracycline for the females to survive to adulthood and be able to lay eggs. This could still allow the spread of antibiotic resistant bacteria because many bacteria in insects pass from the eggs to the next generation.[74,75] There are very limited studies of these effects in moths but there is some evidence that *Lepidoptera* (the group of insects which includes diamondback moths) can transmit their immune status, influenced by their midgut microbiota, to future generations.[76] If antibiotic resistant bacteria can spread from one generation of moths to the next, they will end up in the environment and subsequently in the food chain when the GE moths are released and reproduce.

No figures are available on the quantity of tetracycline that might be discharged from a laboratory or factory producing GE diamondback moths. However, some information is available about tetracycline use when breeding Oxitec's GE mosquitoes. Oxitec produces about 250 GE mosquito larvae in one breeding tray, creating 220 adults or 110 adult males at an 88% survival rate. Each tray contains one litre of tetracycline water (at 30 µg/ml concentration) and this water may require replacement once a week during the 7 to 10 day development of the eggs to pupae.[77] This means that to produce 2 million male GE mosquitoes a week (the current target for experiments in Brazil) Oxitec requires about 18 to 36 thousand litres of tetracycline water, which then requires disposal. Any tetracycline contaminated water released from the laboratory or GE insect-production factory could lead bacteria in the receiving environment to develop antibiotic resistance, which might spread into bacteria which cause diseases.

No public information is available about the proposed use of tetracyclines to feed GE diamondback moths at Cornell, or the proposed method of disposal. It is unclear how the proposed use of tetracycline for a non-veterinary purpose can be regarded as consistent with FDA Guidance on this issue. The EA does not consider this issue at all, nor is it mentioned in the 2014 EA or FONSI.

The EA should be withdrawn and not reissued until the issue of the use of tetracycline in breeding the GE moths has been addressed. This must include assessment of the cumulative impacts of "reasonably foreseeable future actions", such as the removal of the crop destruct requirement and future trials or commercialisation on a larger scale.

12. Impacts on other pests

If Oxitec's releases of GE moths successfully reduce the wild population, even temporarily, other pests may move in and start eating the crop. This is because Oxitec uses a "single species approach" which aims to remove only one species from the ecosystem. However, improving management of one pest can increase outbreaks of another. Other pests compete with diamondback moths for resources, so if the moths are reduced or removed, numbers of other pests are likely to increase. These risks have not been considered in the Environmental Assessment for the proposed experiments at Cornell. In contrast, they would be required to be considered in a risk assessment that met EU standards.

There are numerous other brassica pest species which compete with diamondback moths to eat such crops. They include: cabbage root maggot (*Delia radicum*); flea beetle (*Phyllotreta striolata* and

P. cruciferae); imported cabbage worm (*Pieris rapae*), also known as the small cabbage white; cabbage looper (*Trichoplusia ni*); cabbage and green peach aphids (*Brevicoryne brassicae* and *Myzus persicae*); onion thrip (*Thips tabaci*); and Swede midge (*Contarinia nasturii*). Successful Integrated Pest Management (IPM) programmes provide growers with the means to control all pests in a compatible manner, so that the control measures for one pest do not disrupt control of other pests.[78]

Imported cabbageworm, cabbage looper and thrips, as well as diamondback moths, all cause perennial pest problems in New York State and the Swede midge is also recognised as a potentially serious pest.[79] Thus a strategy which only tackles one of these pests, potentially making the crop more available to other pests by reducing competition, may not be effective in limiting damage to the crop. In New York State, the imported cabbageworm is the most common of the three *Lepidoptera* (moths and butterflies) which eat the leaves of plants and which may contaminate the marketable portion of brassica crops by either their presence or their faecal matter.[80] It overwinters locally throughout the Northeast, so it is generally a pest every year. In contrast, the diamondback moth and cabbage looper are commonly carried north on weather fronts from southern overwintering sites. Because this migration does not occur every year, populations are highly variable.

The vast majority of (non-organic) Brassica crops are treated with insecticides prior to any diamondback moth infestation.[81] However, these insecticide applications have usually been dictated by the presence of early season pests such as root maggots (*Delia* species) and flea beetles (*Phyllotreta* species), rather than by diamondback moths.[82] The imported cabbageworm is often controlled using the bacterial insecticide Bt (*Bacillus thuringiensis*) which may also be used to control diamondback moths.[83,84]

Thus, Oxitec's approach is likely to conflict with conventional methods of control for other major pests. Unless alternative management practices are used for all pests, insecticide applications are likely to continue in order to deal with these other types of pests. In such a situation, Oxitec's approach will cost extra money for no benefit, because continued insecticide spraying is likely to also kill the GE diamondback moths, so they cannot contribute to suppression of wild relatives. Alternatively, if insecticide applications cease, and no alternatives are implemented, other pests are likely to become a significant problem in the crop, and these problems may spread to neighbouring crops, potentially increasing pest damage.

The risk of increases in other pests is analogous to problems with GE insect-resistant crops (Bt crops) which have developed in China, Brazil and India. In China, secondary pests which are not affected by the Bt toxins in its GE cotton crop have become a major problem.[85,86,87] In Brazil, the Agricultural Ministry has issued warnings about massive explosions in corn ear worm (*Helicoverpa armigera*) in areas growing Bt maize.[88,89] More recently, a major outbreak of whitefly has been reported in GE cotton in India.[90] These examples show how reductions in competition can lead to an explosion in another type of pest.

<u>The EA should be withdrawn and not reissued for further consultation until impacts on other pest species have been properly considered in a full EIS</u>. <u>This must include assessment of (i) the impacts on DBM and other pests at and around the site of reducing or removing other pest control approaches in order to allow the trial to proceed; (ii) the potential for other pests to increase in response to DBM population suppression (if successful); (iii) the cumulative impacts on control of other brassica pests of "reasonably foreseeable future actions", such as future trials or commercialisation on a larger scale</u>.

13. Additional impacts on ecosystems and other species

The proposed releases will significantly increase the number of adult diamondback moths in the short-term (by an order of magnitude or more), due to the need to swamp each wild male with many GE ones. If the experiment is successful, the number of dead caterpillars in the crop will also increase, as the wild population is replaced by the GE one. In the longer term, if population suppression is successful, the number of moths should fall due to the lower survival rate of the GE moths compared to the wild ones. In practice, wild male moths might move away, in response to the releases, and cause an increase in pests in neighbouring fields. Wild moths could also move back into the site as the local population falls and competition is reduced. Other competitor pest species might also increase if the number of diamondback moths does fall. In the following season, re-infestation is likely and repeated releases of GE male moths may take place again. The changing cycle of releases implies significant changes to the ecosystem, including short-term increases in adult moths and larvae for predator species, followed by temporary falls in numbers if population suppression is successful, and movement of GE and wild-type DBM in and out of the release site. As well as possible direct adverse effects of consuming GE adult moths or larvae (considered above), significant fluctuations in the availability of the moths as food might cause some problems for some species. These risks have not been considered at all in the EA. More information regarding the anticipated fluctuations in DBM populations, including modelling studies, should be included in a new EIS, for further public consultation. This should include assessments of the ecosystem impacts.

Horizontal gene transfer (HGT) is defined as any process in which an organism incorporates genetic material from another organism into its genome without being the offspring of that organism. HGT is common in viruses and bacteria but is much rarer in plants and other animals.[91] Oxitec has introduced genetic modifications to its GE insects using something called the *piggyBac* transposon: this is a small piece of DNA that inserts itself into another place in the genome. Some scientists have expressed concerns that the *piggyBac* transposon could move from a GE insect to a virus and then from the virus to another insect. [92,93,94] Although such changes are likely to be rare, mass rearing of GE insects prior to release could provide an opportunity for this to happen.[95] Oxitec's GE insects contain a complex system of genetic elements from other species and it is unclear what would happen if these were transferred to other organisms. The EA should be withdrawn and not republished for further consultation until more information is included regarding the risks of horizontal gene transfer.

14. Release of non-native strains

In the UK, Oxitec was prevented from releasing its GE diamondback moth partly because of concerns about the use of a North American background strain, which is subject to controls under plant pest control regulations.[96] Using a non-native strain can introduce undesirable traits which might not be present in a local strain of pest e.g. the introduced strain of pest could do more crop damage, be more invasive, or be resistant to treatment with some insecticides.

The strain of diamondback moth used by Oxitec is not indigenous to New York State but originates in Vero Beach, Florida, USA. [97] According to the Oxitec document appended to the USDA-APHIS 2014 Environmental Assessment, this strain has been tested for susceptibility to the insecticide *Bt* and is unlikely to have developed resistance to other insecticides as it is a laboratory strain (page 16). However, no tests of resistance to other insecticides have been reported, nor has data been provided on other properties, such as invasiveness.

Caged experiments at Cornell have shown how releasing GE diamondback moths to mate with wild ones may slow the development of resistance in wild Bt-resistant strains of moth.[98] Whilst this could be a beneficial impact, it highlights how other traits in a non-native strain of moth could also spread into the wild moth population through mating.

The EA should be withdrawn and not republished for further consultation until tests of resistance to other insecticides have been reported, and data been provided on other properties of the strain, such as invasiveness.

15. Development of resistance and potential for increased survival when encountering tetracycline in the environment

Oxitec's technology includes the use of the common antibiotic tetracycline as a chemical switch to turn off the killing mechanism and allow breeding of its GE insects in the lab. This means that there is a risk that contamination with tetracycline and related antibiotics in the environment and could lead to significantly increased survival rates. Oxitec has reported female survival rates at different chlortetracycline (CTC) concentrations for the OX4319L-Pxy strain of GE diamondback moth (the numbers tested are not reported).[99] In these tests, no GE females survived to adulthood at CTC concentrations up to 0.01 µg/mL, while at or above 10 µg/mL CTC the female survival rate was similar to that of males. At concentrations of 0.1 µg/mL and 1 µg/mL female survival to adulthood was around 15% and 55% respectively, relative to wild moths.

Contamination with tetracycline and related antibiotics is widespread in the environment. The tetracyclines are a family of antibiotics any one of which can increase the GE female moth's survival rates. Because of their use in treating animal diseases, tetracyclines commonly contaminate animal manure. Oxytetracycline can be found at concentrations above 500 µg/g in animal manure and doxycycline at up to 78.5 µg/g dry weight in broiler manure.[100,101] A global review reports lower but still relevant concentrations of tetracyclines of up to 0.88 µg/g in pig manure, 11.9 µg/g in poultry manure and 0.208 µg/g in cattle manure.[102] These concentrations are likely to be more than enough to inactivate the killing mechanism in the female GE moths if the larvae come into direct contact with contaminated manure. Although diamondback moths do not normally lay their eggs in direct contact with manure, they might change their behaviour if this benefits their survival. Behavioural adaption beneficial for survival could be selected for in the field, leading to females seeking contaminated areas in which to lay their eggs. There is evidence of behavioural resistance developing in a SIT programme using irradiated flies, when females became unreceptive to mating with irradiated males.[103]

Tetracycline levels in industrially farmed animals may also be sufficient to increase GE female moth survival. When Oxitec's GE mosquitoes were fed cat food containing industrially farmed chicken, which probably contained the antibiotic tetracycline, their survival rate increased to 15-18%. Oxitec originally hid this information[104] but later admitted to an 18% survival rate of larvae fed on cat food in a published paper.[105] In one study, levels of tetracycline from beef carcasses at a slaughterhouse in Iran were 131.0 µg/kg in meat, 254.9 µg/kg in liver and 409.1 µg/kg in kidney.[106] In some circumstances fruit trees could be another source of exposure because oxytetracycline is sometimes used in fruit production to treat bacterial diseases of plants, especially fire blight in pear and apple and bacterial spot in peach and nectarine.[107]

In addition, resistance to the genetic killing mechanism can also develop through evolution in the wild or during mass production, when any mutations which arrive by chance to allow the insects to survive and breed will be selected for so they become common in the population .[108,109,110] Such resistance is another mechanism which could allow more GE female moths to survive and breed. This type of resistance is not a concern with traditional sterile insect technique (SIT), because irradiated sterile insects contain multiple chromosome breaks, not a single genetically engineered construct. However, there is evidence of behavioural resistance developing in a SIT programme using irradiated flies, when females became unreceptive to mating with irradiated males. [111] This type of behavioural resistance could also develop in the GE moths, and may also reduce efficacy and increase risks.

The EA should be revised to include the risk of increased survival of the offspring of the GE moths, due to encountering tetracycline in the environment, or the development of resistance. Increased survival reduces effectiveness but also increases a number of other risks e.g. risks of dispersal, contamination and crop damage, and ecosystem risks. A full EIS including these effects should be issued for further consultation. The assessment must include assessment of the cumulative impacts of "reasonably foreseeable future actions", such as the removal of the crop destruct requirement and future trials or commercialisation on a larger scale.

16. International implications

If migration occurs, there may even be potential for diamondback moths released in New York State to contaminate the Canadian canola crop[112] as well as brassica production. In Ontario, diamondback moths generally arrive from the South although they can sometimes also overwinter.[113]

This risk should be assessed as part of a thorough EIS. This must include assessment of the cumulative impacts of "reasonably foreseeable future actions", such as the removal of the crop destruct requirement and future trials or commercialisation on a larger scale.

17. Lack of information and inadequate transparency

Meaningful consultation requires transparency.

Following the previous 2014 consultation, the Center for Food Safety (CFS) obtained copies of the permit, application and supporting documents following a Freedom of Information Act (FOIA) request, although a number of these documents are heavily redacted to protect commercial confidentiality. [114]

Many concerns remain about missing and redacted information, for example:

- The current permit application is not publically available, so it is not possible to see if there are any changes;
- The information published by Oxitec in the previous (withdrawn) EA and in the FOIA documents has not been included in the current consultation, so it is unclear whether it remains valid and/or available for comment;
- Many of the documents released in response to the FOIA request remain heavily redacted.

As noted above, some relevant evidence is likely to be available but unreported from one published trial[115], and unreported and unpublished from the subsequent caged trials[116]. In particular, this includes data on the crop damage and contamination which occurred when the GE diamondback moths were released in the caged trials.

All this missing information should be provided in a new EIS for consultation to be meaningful. In particular, the results of the caged trials should also be published in a peer reviewed journal before further experiments can be properly considered.

18. Regulatory approach

Since 1986, the US government has regulated genetically engineered (GE) organisms under a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302[117], 57 FR 22984[118]). APHIS regulations at 7 Code of Federal Regulations (CFR) part 340 rely on authority granted by the Plant Protection Act (PPA), as amended (7 United States Code (U.S.C.) 7701–7772), to regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. The USDA APHIS permit for the proposed GE diamondback moth experiments was issued under 7 CFR Part 340, which covers the introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests.[119]

However, the Coordinated Framework is widely considered to be inadequate for the regulation of open releases of GE insects.

In 2002, the US National Academy of Sciences published a report on GE animals which stated that aquatic organisms and insects present the greatest environmental concerns, because their mobility poses serious containment problems, and because they easily can become feral and compete with indigenous populations.[120] The report expressed concerns about gaps in regulation. The Pew Initiative on Food and Biotechnology published a further report in 2004 on gaps in the regulatory system for GE insects in the USA, and a report of a workshop on the issues.[121,122] A central finding of the Pew report was that there are gaps in the regulatory framework in place to review the many issues raised by the potential introduction of GE insects into wild populations. There is no specific regulation on the release of GE insects, no law that clearly covers all the risks and all of the types of GE insects and no single regulatory body: the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) could all play a role.

Nevertheless, USDA APHIS initiated oversight of GE insects by issuing permits first for contained experiments and then open releases of GE bollworms containing a fluorescent marker trait.[123] There were 14 US government-funded field trials over a nine year period, beginning in 2002. The only open release experiments were conducted in Arizona in 2007 and 2008, using Oxitec's GE pink bollworms (a cotton pest), with only the fluorescent trait (not the 'late lethality' trait), and made sterile using radiation.[124]

Although they used irradiated sterile insects, with only a GE fluorescent trait, the GE bollworm experiments were halted, partly because of concerns raised by US organic farmers about contamination of their crops with genetically modified organisms (GMOs).[125,126] They also led to a highly critical report by the US Department of Agriculture (USDA) Office of Inspector General. This report argues that USDA APHIS' controls over GE insect research are inadequate and that regulations need to be strengthened.[127] The report also criticises APHIS' Center for Plant Health Science Technology (CPHST) for spending about $550,000 on developing GE plant pests such as the pink bollworm, the Mediterranean fruit fly, and the Mexican fruit fly (in collaborations with Oxitec) without any formal process for selecting which projects would receive funding. The report's recommendations were accepted by APHIS, requiring it to clarify its role, draft specific GE insect regulations, and make more transparent research funding decisions. However, no attempt to draft specific regulations appears to have been made to date.

The Environmental Impact Statement (EIS) published by APHIS in 2008 which recommended the use of GE insects was also found to be "scientifically deficient" when reviewed by scientists at the Max Planck Institute.[128] They report that the document reverses an earlier more cautious view published by APHIS in 2001, without providing the substantial body of evidence required to back up its assertions. However, this "scientifically deficient" 2008 APHIS report and later reports made under the framework criticised by the USDA Office of Inspector General continue to be cited in the current Environmental Assessment for the proposed release of GE diamondback moths.

The White House announced plans to revise the Coordinated Framework for the Regulation of Biotechnology in July 2015.[129] Rather than proceeding with open releases under the current inadequate regulatory regime, this revision could provide an opportunity to ensure that the concerns which have been raised about the regulation of GE insects are properly addressed.

Public confidence in the risk assessment will not exist unless concerns about the adequacy of the regulatory regime have been addressed. A new regulatory regime should be in place before a more comprehensive EIS is issued for further consultation.

19. Conclusions

Compared with the no action alternative, the proposed experiments pose unnecessary socio-economic, environmental and health risks. Therefore the application should be refused.

Numerous important gaps have been identified in the environmental assessment for open release of GE DBM into the environment. The proposed experiments therefore carry unnecessary risks and are premature.

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