GeneWatch UK comment on Animal and Plant Health Inspection Service Docket No. APHIS–2014–0056: Environmental Assessment for the Field Release of Genetically Engineered Diamondback Moths

September 2014

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 undertaken extensive investigations of the UK company Oxitec and has documented a poor level of compliance with environmental regulations, particularly the transboundary notification of exports of living genetically engineered (GE) organisms from the UK to other countries. Numerous important issues have been omitted from the relevant environmental risk assessments (ERAs) for export of Oxitec's GE mosquitoes, or in some cases the ERA has not been supplied at all. [1,2] GeneWatch has previously highlighted concerns with a proposed release of Oxitec's GE Diamondback moth (DMB) in England in 2011/12. These concerns have not yet been resolved and no formal application for release has been submitted. In 2013, Oxitec applied to release GM olive flies in Spain, however this application has also been withdrawn following a request for further information from the regulators, particularly in relation to the toxicity of the tTA protein expression in Oxitec's GE insects (adults and larvae). Proposed releases of GE Medfly in Brazil have been delayed amid concerns about the implication for the market for contaminated fruit.

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.

Oxitec: background

Oxitec is a spin-out company from Oxford University[3], with close links to multinational seed and agrochemical firm Syngenta. Ex-Syngenta staff who joined Oxitec since 2006 Oxitec include Oxitec's CEO, Chief Scientific Officer, Regulatory Affairs Manager and Head of Business Development.[4] Oxitec's Chair is also a former employee of Syngenta. Oxitec consultants include Colin Ruscoe, former site manager at Syngenta Crop Protection, who is Chairman of the British Crop Production Council.[5] Oxitec's former Head of Business Development (from 2006 to 2010), Ann Kramer, is another ex-Syngenta employee who continues to act as a consultant for the company.[6] The aim of the company is to establish a new method of pest control, involving the release of GE insects (including mosquitoes and agricultural pests). From March 2009 to June 2011, Oxitec received research funding directly from Syngenta for genetic transformation of *Lepidoptera* (an order of insects which includes the diamondback moth, *Plutella xylostella*, henceforth referred to as DBM). More recent information on funding from Syngenta is unavailable.

Open release experiments using Oxitec's GE *Aedes aegypti* mosquitoes are ongoing in Brazil and Panama. The GE mosquitoes being released in these experiments differ from Oxitec's GE agricultural pests in that both sexes of the GE mosquitoes are genetically engineered to die at the late larval/pupal stage. For Oxitec's GE agricultural pests, only the female insects are genetically engineered to die at the late larval stage and males will survive to adulthood (this is known as a "female-killing" approach).[7, 8,9,10,11] Oxitec's male GE agricultural pests therefore have the potential to survive for multiple generations, even in the absence of problems with the genetic killing mechanism for the female moths. Further, dead GE larvae and a smaller number of live insects can potentially contaminate the food chain via transports of crops produced using this method of GE

pest control. To date, no GE insects utilising the female-killing approach have been released anywhere in the world.

Open release experiments using a strain of Oxitec's GE pink bollworms were previously conducted in the USA, however the strain used only the fluorescent trait (not the 'early lethality' trait), and was made sterile using radiation. These experiments were halted, partly because of concerns raised by organic farmers about contamination of their crops, and they also led to a critical report by the US Department of Agriculture (USDA) Office of Inspector General. This report argued that USDA APHIS' controls over GE insect research were inadequate and that regulations needed to be strengthened.[12] The report also criticised 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 GM insect regulations, and make more transparent research funding decisions. However, no attempt to draft specific regulations appears to have been made. The Environmental Impact Statement (EIS) published by APHIS in 2008 was also found to be "scientifically deficient" when reviewed by scientists at the Max Planck Institute.[13] 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 EA.

Oxitec has made a number of attempts to release GE agricultural pests in other countries but so far no such releases have taken place.

In 2011, Oxitec sought to make open releases of GE DBM in the UK under "contained use" regulations by claiming that its RIDL technology is equivalent to "biological containment".[14,15,16,17,18,19,20,21] These proposed releases were controversial and did not go ahead. Subsequently, no formal application has been submitted by Oxitec to make open releases of DBM into the environment in the UK or elsewhere.

In 2013, Oxitec withdrew an application to release GE olive flies in Spain, following a request for further information from the regulator.

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 mosquitoes required by European Union law, which requires a risk assessment which meets EU standards to be reviewed and accepted by the importer.[22] 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.[23]

Regulatory framework and guidance

In 2002, the US National Academy of Sciences published a report on GM 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.[24] The report expressed concerns about gaps in regulation. The Pew Initiative on Food and Biotechnology published a report in 2004 on gaps in the regulatory system for GM insects in the USA, and a report of a workshop on the issues.[25,26] A central finding of the report was that there are gaps in the regulatory framework in place to review the many issues raised by the potential introduction of GM insects into wild populations. There is no specific regulation on the

release of GM insects, no law that clearly covers all the risks and all of the types of GM 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. Despite the criticism in the Pew report and the 2011 USDA Office of Inspector General Report cited above, APHIS appears willing to proceed with consideration of an application without addressing these widely held concerns.

In the absence of a coherent regulatory framework or any published Guidance on how to assess the risks of open releases of GE insects in the USA, it is worth noting that the European Food Safety Authority (EFSA) has published guidance for environmental risk assessment under the EU's Deliberate Release Directive for genetically modified organisms (GMOs), although this does not yet cover the important area of food safety assessment. The EFSA Guidance outlines the evidence that Oxitec would need to provide for its GE insects to be placed on the EU market (placing on the market means making available to third parties, whether in return for payment or free of charge).[27] Pages 73 to 107 of the EFSA Guidance provide details on the following specific areas of risk for GE insects:

- Persistence and invasiveness of GE insects, including vertical gene transfer (VGT);
- Horizontal gene transfer;
- Pathogens, infections and diseases;
- Interactions of GE insects with target organisms;
- Interactions of GE insects with non-target organisms (NTOs);
- Environmental impacts of the specific techniques used for the management of GE insects;
- Impacts of GE insects on human and animal health.

Although the USA is not a Party to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, as a UK company, Oxitec is still obliged to make a transboundary notification compliant with the Protocol prior to exporting GE diamondback moth eggs to the USA for open release (under Regulation 1946/2003/EC). This notification must include a prior, existing environmental risk assessment which meets EU standards. Thus the EFSA Guidance is of more than academic interest in the context of the current application.

Specific issues of importance to the environmental assessment

Potential adverse effects of tTAV on non-target organisms

Release ratios of GE to wild-type DBM males are currently unknown but can expected to be of the order of ten to one or higher. The aim is to replace wild-type offspring with GE offspring which are genetically engineered so that the (majority of the) females die at the larval stage. Of the strains to be released, Jin et al. (2013) reports that OX4319A-Pxy females exhibited substantial survival to pupation (17% relative to wild-type females) with lower female survival to pupation in OX4319L-Pxy, and OX4319N-Pxy (9% and 0%, respectively). For all strains, death of most female DBM at the larval stage will significantly increase the number of larvae dying in the brassica crop (and in wild relative brassica weeds), compared to the no action alternative, since about 50% of the offspring (i.e. all the females) are expected to die at this stage, rather than reaching adulthood. The dead larvae will contain the DsRed (fluorescent) and tTAV (early lethality) genetically engineered traits. They will be consumed by all species which normally consume DBM larvae or brassica crops, including humans should the crop enter the food chain. It is therefore surprising that no safety data is provided in the EA for consumption of GE DBM larvae. Instead, a statement claiming that the DsRed and tTAV proteins expressed in Oxitec's GM mosquitoes is made (with no data provided) in the bioinformatics report by Goodman (Appendix VIII), commissioned by Oxitec, plus one published study by Oxitec is cited, in which its OX513A strain of GE *Ae. aegypti* mosquito larvae were fed to two different species of *Toxorhynchites* (*Tx. splendens and Tx. amboinensis*).[28] This falls far short of the data or precautions needed.

The presence of large numbers of dead (and some living) GE larvae in the crop is a significant difference between Oxitec's technology and the Sterile Insect Technique (SIT), which prevents the insects reproducing through the use of radiation, rather than genetically programming the offspring to die at the larval stage. Another downside of Oxitec's approach, in addition to contamination of the crop with large numbers of dead larvae, is that considerable crop damage is expected before the intended population suppression effect is observed in the wild population.[29]

Although a reference has been provided for toxicity testing of the red fluorescent marker, DsRed2, no evidence exists 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 is not fully understood and no safety data appears to be available. There is some evidence that enhanced tTA expression can have adverse effects (loss of neurons affecting cognitive behaviour) in transgenic mice.[30] Other mice studies have detected adverse effects on the lung.[31,32] Considerably more data, based on specific feeding trials in relevant species, is therefore needed to establish that consumption of GE DBM adults or larvae is not harmful to humans or wildlife.

Consistent with this need, an application by Oxitec to release GE Olive Flies is 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.[33,34]

Adverse ecosystem effects cannot be ruled out without testing the impacts of consuming GE DBM on all the main predator species for adult and larval DBM, plus species which will consume DBM larvae primarily by eating brassicas. These include species which are engendered, threatened or of special concern and which may feed on DBM moths or larvae or on brassicas: such as the Northern Long-Eared Bat (*Myotis septentrionalis*), Grasshopper Sparrow (*Ammodramus savannarum*) and the New Cottontail Rabbit (*Sylvilagus transitionalis*).

As noted in the EA, page 59, migratory birds may be found in fields containing cruciferous crops, where they may forage for insects and weed seeds found in and adjacent to the field. Assessment of larval and adult GE DBM toxicity to migratory birds must therefore also be considered, to comply with Executive Order EO 13186 ("Migratory birds").

No information is provided in the EA on whether or how brassica crops in the proposed experimental area will disposed of and prevented from entering the human food chain. This is a major omission, both in terms of potential risk to human health and the risk of dissemination of GE DBM off-site (discussed further below). Even if the (unstated) intention is to guarantee no human consumption of the crop, there remains a concern that it could enter the food chain unintentionally, as has been the case with many past field trials of GE crops.[35,36] Further, since the aim of the experiments is to assess the suitability of GE DBM releases as a pest control measure, it makes little sense to proceed unless the safety of any DBM larvae entering the human food chain has been fully tested. Under EO 13045 ("Protection of Children from Environmental Health Risks and Safety Risks", referred to on page 58), this must include testing safety for children consuming brassica crops. Further, when referencing the Goodman report, Oxitec notes (p 28-29 of the Oxitec report appended to the EA) that two matches were identified using the FASTA bioinformatics tool and the Food Allergy Research and Resource Program (FARRP) Allergenonline.org database: *Tromomyosin* from *Neptunia polycostata* (a gastropod); and a salivary protein of *Aedes albopictus*. It is unclear why these matches did not appear to merit further investigation.

The EA incorrectly states (page 7 and 8) that no FDA consultation is necessary because the GE DBMs released are not food or feed, and no EPA review is needed because they are not a pesticide. In

reality GM DBM will be present in large numbers as a contaminant in food and feed and potential future use of this approach on the commercial market would certainly lead to widespread human consumption of GE DBM. Further, a wide variety of wildlife will consume GE DBM either directly as food (adults or larvae) or as contaminants on brassicas.

Failure to conduct human safety tests prior to conducting open release experiments, and to ensure that contaminated crops do not enter the market, could damage markets far more widely than in the local area of the trial, due to frequent difficulties in tracing the source of contamination incidents. This will have 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. Further, there may be cross-border issues with Canada if GE DBM spread across the border (see discussion of off-site dissemination below), with implications for the canola industry as well.

Journalists have reported that in Brazil "…*it's impossible to talk during the liberation sessions without accidentally swallowing a few*…" due to the very large numbers of GE mosquitoes being released to try to swamp the wild population.[37] Therefore, the risk posed to workers or passers-by of swallowing adult GE DBM also needs to be assessed. It is of particular concern that, whilst staff wear masks during contained production, members of the public may be exposed to large numbers of GE DBM during open releases. For example, during Oxitec's experiments with GE mosquitoes in the Cayman Islands, local residents complained about the nuisance caused by the very large number of GE mosquitoes released, which was far higher (by an order of magnitude or more) than the normal expected population density of the wild species.[38]

Off-site dissemination of GE DBM

The EA relies heavily on claims that the GE DBM cannot be disbursed offsite and will not overwinter. These are implausible assumptions.

Firstly, consideration of dispersal via the food chain has been completely omitted, although transport and sale of brassica produce is the main mechanism through which this pest has been transported worldwide. To prevent spread of GE DBM via the deliberate or accidental marketing of crops, or transfer of seedlings, it must be made explicit that no food crops from the site will be allowed to enter the food chain and a credible process for destruction of the crop to destroy any GE DBM onsite must be provided.

The intention to clear all brassica crops and weeds for 10m around the site is stated on page 10 of Oxitec's report (appended to the EA), followed by spraying over 100m around the site. However, no mention is made in the EA itself of the need to destroy the crop and all wild relatives at the site to prevent dissemination and no specific, enforceable conditions to this effect appear to be proposed. Nor has any justification been given for the assumption that the GE DBM will not travel further from the field than 10m. Mechanisms for spread include transfer on human clothing or by wildlife moving through the crop or wild relatives, as well as independent flight of adult DBM and dispersal by the wind. As well as strict conditions for full destruction of the test crop and wild brassicas, prior study of the dispersal of existing wild type DBM at the site is essential prior to any trial. Without such a detailed study it is impossible to confirm whether the seemingly implausible assumption that DBM will not disperse further than 10m is in any way adequate.

One of the more questionable assumptions in the EA is the claim that the strong wind currents which facilitate dispersal of DBM across geographic regions do not occur at the proposed release site. In fact, migration into Canada from the proposed release site is not implausible, given that one predominant direction for wind currents in the area is south to north (as cited on page 11). There

may therefore be potential for GE DBM to contaminate the Canadian canola crop[39] as well as brassica production. In Ontario, DBM generally arrive from the South although they sometimes also overwinter.[40]

Claims regarding overwintering are also incorrect. 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.[41,42] 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. Thus, although the main mechanism for crop damage in northern climates is re-infestation via long-distance dispersal by the wind, it is clear that small numbers may overwinter in cold climates, allowing survival of the GE trait. The average temperature in January (the coldest month) in Geneva, New York, where the proposed experiments are sited, is -8.9^{o}C[43], compared to a lower lethal temperature where 25% survived (LLT_{25}) of -15.2^{o}C in laboratory tests.[44] This does not provide confidence that GE DBM cannot overwinter, particularly if there is unintentional survival of females due to failure of the killing mechanism (discussed below). The application is clearly premature in the absence of a study of DBM overwintering at the site, as the applicant relies heavily on claims that DBM do not overwinter to mitigate risks of dispersal beyond the trial area. These claims appear implausible in the light of the current literature.

A further consideration must be the potential for wild-type DBM to move to surrounding areas in response to the releases (discussed further below).

Unintentional survival of female GE DBM

Oxitec's female GE mosquitoes are genetically programmed to die at the late larval stage. However, there are several mechanisms which could allow many more of the female DBM to survive to adulthood. There is a fundamental flaw in Oxitec's approach in using tetracycline as a chemical switch to allow breeding of the GE DBM in the laboratory because tetracycline and related antibiotics are widespread in the environment.

The EFSA Guidance includes: "*Reduction in efficacy of the GM insect mediated trait that may result in adverse effects*".

Unintentional survival of female GE DBM can occur due to failure of the genetic killing mechanism. This can occur if resistance develops to the trait or if the GE DBM encounter sufficient levels of the antibiotic tetracycline, or its derivatives, to inactive the killing mechanism.

The applicant wishes to undertake open experimental releases of three of Oxitec's GE DBM strains: OX4319L-Pxy, OX4319NPxy, and OX4767A-Pxy. Jin et al. (2013) give female survival rates to adulthood in the absence of chlortetracycline (CTC, a tetracycline analogue) of 1%, 0% and 5%, relative to wildtype, for these GE strains (Figure 2c). This means, at least for two of the strains, some females are expected to survive to adulthood, even in the absence of tetracycline. However, contamination with tetracycline and related antibiotics is widespread in the environment and could lead to significantly increased survival rates.

Jin et al. (2013) investigated female-specific lethality at different chlortetracycline (CTC) concentrations for the OX4319L-Pxy strain of GE diamondback moth (although numbers tested are not reported). In these tests, no OX4319-Pxy-heterozygous females survived to adulthood at CTC concentrations up to 0.01 µg/mL, while at or above 10 µg/mL CTC OX4319L-Pxy heterozygous female survival to adulthood, relative to wild-type, 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 (Figure 6),

relative to wild-type. Oxitec claims that the level of CTC needed for survival far exceeds that which diamondback moth might be expected to encounter in the wild: this claim is incorrect.

When Oxitec's GE mosquitoes were fed cat food containing industrially farmed chicken, the survival rate increased to 15-18%. Oxitec originally hid this information[45] but later admitted to an 18% survival rate of larvae fed on cat food (assumed to contain industrially farmed chicken contaminated with tetracycline or related antibiotics) in a published paper.[46] The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow Oxitec's GE insects to breed. 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.[47,48] 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.[49] These concentrations are likely to be more than enough to inactivate the killing mechanism in the female GE DBM if the larvae come into direct contact with contaminated manure.

It would not be surprising if behavioural adaption beneficial for survival was selected for in the field, leading to females seeking contaminated areas in which to lay their eggs.

The percentage of surviving GE DBM could also increase if resistance to the genetic killing mechanism evolves over time. This concern is dismissed as unlikely in the EA (page 44) despite prior evidence of behavioural resistance developing in a SIT programme (i.e. females unreceptive to mating with irradiated males). [50] This evidence is dismissed as rare but there has been little investigation of this phenomenon, which shows the expected development of an evolutionarily advantageous behaviour in the field. Resistance can also develop through the evolution of resistance alleles.[51] This risk must be considered because radiation-induced sterility (using in the traditional Sterile Insect Technique) has built-in redundancy that is not provided by molecular genetic approaches.[52] A number of authors have therefore speculated that any genetic or molecular event that allows the GE DBM to survive and breed successfully could therefore be rapidly selected for during mass production.[53] No laboratory or caged studies have been published to investigate the potential development of resistance through either of these mechanisms. These studies should have taken place before making an application for an open release trial.

Oxitec claims that there is no adverse impact if female lethality fails (page 13 of the Oxitec report appended to the EA), but such failure could facilitate the establishment or spread of GE DBM offsite (see comments on dispersal and overwintering, above). This would exacerbate any adverse impacts such as toxicity or allergenicity to humans or wildlife (discussed above) and make it impossible to retrieve GE DBM or reverse any unintended effects.

Target organisms: response of DBM population to the proposed releases

The EFSA Guidance includes: "*Changes in TO* [target organism] *populations caused by the GM component of the releases (size, age structure, sex ratio, fertility, mortality) that may result in adverse effects leading to environmental harm*" (page 87).

Whilst the intention of the releases is to reduce crop losses by suppressing the target population of DBM, in practice the response of the target population is likely to be complex.

One issue which has not been addressed in the EA is whether or not releases of GE DBM could cause an increase in the numbers of DBM in surrounding areas. This effect is predicted by some models for the release of sterile insects.[54] For releases of GE mosquitoes, Oxitec's Cayman Islands' paper[55] and its graph from Mandacaru, Brazil (details are unpublished but the graph is in a company brochure[56]) both show increases in *Aedes aegypti* mosquitoes in the control area, as population suppression in

the target area begins to occur. In the Cayman Islands the control area was next to the target area for the releases, but for Mandacaru there is no public information about the location of the control area. The number of mosquitoes trapped in the untreated area also increased in the final phase of the experiments conducted in Iteraba, Brazil according to the PAT report, which provides the only published information on these experiments[57]. Thus, there appears to be a real possibility that wild-type males, when swamped by very high releases of GE males, simply migrate to mate in the surrounding area. More information is needed to either confirm or rule out this possibility. Since Oxitec calculates population suppression based on the difference between the target area and the control area, it is possible that claims of significant drops in population partly reflect significant increases being caused elsewhere. In the context of the EA, it is important to consider the risk that wild-type DBM will cause increased damage outside the target area. Assessment of this risk requires prior modelling of this potential effect and an altered trial protocol and monitoring to establish whether or not this adverse effect occurs. Further, long-term monitoring of DBM populations is required in advance of any trials (in the presence of brassica plots) to establish the baseline for assessment of efficacy and to avoid reliance on a neighbouring control that might itself be affected by wild-type DBM dispersal from the target site.

Risk of increase in non-target pests in response to GE DBM releases

The EA incorrectly claims that introduction of the GE DBM will only effect the target pests. It is a requirement under the Plant Protection Act to consider whether the proposed releases of GE DBM will facilitate the dissemination and establishment of other (non-target) pests. To do this correctly the EA must include not only exposure of wildlife to direct effects such as potential toxicity, but ecosystem responses to the releases (i.e. indirect effects on the population dynamics of non-target species).

The EFSA Guidance states: "*Considering the aim and type of GM insect releases, and also accounting for possible accidental releases, potential impacts on NTO [non-target organisms] that may cause adverse effects include:…(b) a change in abundance or species composition of competitors (e.g. insects exploiting the same ecological niches) of GM insects and the ecological functions they provide*" (p.94) and adds "*Other pest species (e.g. secondary pests) might exploit the available resource and build up high populations which might have an adverse effect on the environment and on human health*" (p.98).

This situation could be regarded as analogous to problems with GM insect-resistant crops (Bt crops) which have developed in China and Brazil. In China, secondary pests which are not affected by the Bt toxins in its GM cotton crop have become a major problem.[58,59,60] In Brazil, the Agricultural Ministry has issued warnings about a massive explosion in corn ear worm (*Helicoverpa armigera*) in areas growing Bt maize.[61] These examples show how reductions in competition or natural enemies can lead to an explosion in another type of pest. These concerns arise as a result of the proposed "single species" approach and do not apply to methods that are effective against multiple pest species.

Potential increases in competitor species have been a major concern in debates about the risk of releasing Oxitec's GE *Aedes aegypti* mosquitoes. [62,63,64] However, such effects have been omitted from the EA for GE DBM altogether, despite the use of a single-species approach in the likely presence of numerous other brassica pest species such as those listed on page 34: cabbage root maggot (*Delia radicum*); flea beetle (*Phyllotreta striolata* and *P. cruciferae*); imported cabbage worm (*Pieris rapae*); cabbage looper (*Trichoplusia ni*); cabbage and green peach aphids (*Brevicoryne brassicae* and *Myzus persicae*); onion thrip (*Thips tabaci*); and Swede midge (*Contarinia nasturii*). In some cases, these competitor species are invasive species and the impact of the proposed releases on their populations therefore requires consideration under EO 1311 ("Invasive species", referred to on page 58 of the EA) as well as the Plant Protection Act.

Should releases of GE DBM lead to the expansion or establishment of other pests, these adverse effects may be difficult to mitigate or reverse. Prior knowledge of the distribution and population dynamics of other pests, including any competitive effects, at the proposed field site is therefore essential before the proposed experiments are conducted. Without such data (combined with credible attempts to model likely population responses), open releases of GE DBM are premature.

Potential transfer of antibiotic resistance via DBM microbiota

The use of tetracycline to breed the GE DBM in the lab carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health.[65] Insect guts are reservoirs for antibiotic resistance genes with potential for dissemination.[66,67] Insect production in factories exposed to antibiotics could lead to drug resistance in their microbiota so that the insects disseminate antibiotic resistance when released into the environment.[68,69] This issue has been omitted entirely from the EA, despite growing recognition that antibiotic resistance poses a serious, worldwide threat to public health.[70]

Reliance on antibiotics for breeding the GE DBM in the lab is a serious downside compared to the use of the traditional Sterile Insect Technique (SIT) based on the use of radiation, or compared to the no action alternative which does not contribute to the spread of antibiotic resistance. In its Guidance for Industry #209[71], the FDA recommends that "*the administration of medically important antimicrobial drug to entire herds or flocks of food-producing animals would represent a use that poses qualitatively higher risk to public health than the administration of such drug to individual animals or targeted group of animals*". Combined with the potential for survival of female DBM in the presence of tetracycline contamination in the environment (discussed above) this suggests a fundamental flaw in Oxitec's technology.

Strain of DBM

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.[72] Using a non-native strain can introduce undesirable traits such as pesticide-resistance.

The strain described in Jin et al (2013) is not local to New York State but originates in Vero Beach, Florida, USA. According to the Oxitec document appended to the EA, this strain has been tested for susceptibility to *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. This information should have been provided and considered in the EA.

Restricted purpose, inadequate monitoring and lack of prior studies

The stated purpose of the requested field release is to assess the efficacy of GE DBM strains OX4319L-Pxy, OX4319N-Pxy, and OX4767A-Pxy in reducing pest populations of non-GE DBM. However, biosafety issues are still not yet fully understood for this new technology and must also be assessed. This requires greater prior assessment of the release environment, especially background populations and fluctuations in both target and non-target organisms, and of the GE DBM strains proposed for release, as detailed above (in particular, thorough safety testing of the impacts of ingestion on humans and animals, prior to any release). The application for open release is therefore premature. Further, were the releases to proceed following the provision of this important additional data, additional monitoring would be required to detect potential adverse effects i.e. the purpose of the experiment would need to be extended to include additional monitoring. This should include for example, monitoring to detect potential adverse effects on beneficial insects, predators and wildlife; monitoring to detect any migration of DBM to neighbouring areas and persistence or

dispersal of GE DBM; monitoring of non-target pests to detect any unintended increases in such pests due to population suppression of a competitor; and monitoring of antibiotic resistance and its spread through gut bacteria.

Lack of prior studies on all transgenic strains

Jin et al (2013) report tests of longevity on OX4319L-Pxy and OX4319N-Pxy and tests of mating competitiveness on OX4319L-Pxy. Survival to adulthood of the OX4319L-Pxy strain is reported for different concentrations of CTC in larval diet and Harvey-Samuel et al. (2014) report caged trials of fitness costs for OX4319L-Pxy. However, no such trials have been reported for OX4319N-Pxy and OX4319A-Pxy. The proposed open releases are premature in the absence of more extensive laboratory and caged testing of all strains.

Conclusions

There are a number of fundamental flaws with Oxitec's technology:

(i) The use of late-acting lethality (rather than sterility) means food supplies for humans and animals will become contaminated with large numbers of dead female GE larvae;

(ii) The large numbers of GE adult males required to swamp the wild population pose a risk of swallowing them to farm workers and passersby, as well as wildlife, and may also cause wild-type adult DBM to disperse to surrounding areas;

(iii) Impacts on non-target pests are poorly understood and may include increases in the numbers of such pests or establishment in new areas: this may include invasive pests;

(iv) The use of tetracycline as a chemical switch for the genetic killing mechanism is risky because contamination with tetracycline and related antibiotics is widespread in the environment, meaning the killing mechanism may be inactivated;

(v) In addition, the use of tetracycline to breed the GE DBM in the lab is likely to facilitate the spread of antibiotic resistance via gut bacteria, in breach of FDA Guidance;

(vi) The use of a female-killing only approach is likely to lead to the dispersal of GE males over significant distances in the longer term, especially if contaminated crops enter the food chain;

(vii) Resistance to the genetic killing mechanism is likely to evolve over time, facilitating greater off-site dispersal.

Compared with the no action alternative, the proposed experiments pose unnecessary risks to human health and the environment. Therefore the application should be refused.

Numerous important gaps have been identified in the environmental assessment for open release of GM DBM into the environment. The proposed experiments therefore carry unnecessary risks and are premature. Prior to considering any application for open release, the following additional information should have been required:

- Safety testing for consumption of GE DBM adults or larvae by humans and wildlife, including children and threatened species;
- Prior baseline assessment of DBM and non-target baseline pest populations over several years in the presence of brassica crops;
- Modelling of population responses of target and non-target species to the proposed releases;
- Studies of overwintering of DBM in the proposed test area;
- Studies of dispersal of DBM from the test site to other sites;
- Studies of dose responses of all strains proposed for release to tetracycline and its analogues;
- Studies of insecticide resistance in the parent strain;
- Caged trials of all GE DMB strains;

- Laboratory studies of resistance mechanisms;
- Laboratory studies of antibiotic resistance;
- Caged studies of competitive effects (target and non-target pests).

A strict protocol for the destruction of all contaminated or potentially contaminated crop plants and wild brassicas is also essential for any trial, to avoid contamination of the food chain.

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