OXITEC LTD

SUMMARY NOTIFICATION INFORMATION FORMAT (SNIF)

PREPARED FOR THE RELEASE OF OX3097D-BOL OLIVE FLY IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC.

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Contents

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS

in accordance with Article 11 of Directive 2001/18/EC

A. General Information

1. Details of notification

a) Member State of notification: Spain
b) Notification number: **B/ES/13/07**
c) Date of acknowledgement of notification 3rd Jan 2013
d) Title of the project: Evaluation of the mating competitiveness, longevity and persistence of the OX3097D-Bol Olive fly in a field setting.
e) Proposed period of release: April 2013 – April 2014

2. Notifier

Name of institution or company: Oxitec Ltd. 71 Milton Park, Abingdon, Oxfordshire OX14 4RX UK tel. +44 (0) 1235 832 393

3. GMO characterization

a) Indicate whether the GMO is a:	Viroid	☐
	RNA virus	☐
	DNA virus	☐
	bacterium	☐
	fungus	☐
	animal	☐
	- mammals	☐
	- insect	☒
	- fish	☐
	- other animal	☐
please specify phylum, class other, please specify (kingdom, phylum and class)		
b) Identity of the GMO (genus and species):		

Bactrocera (Dacus) oleae
c) Genetic stability – according to Annex IIIa, II, A(10) The Olive fly strain OX3097D-Bol was developed over 3 years ago (approximately 45 generations). Since the insertion event the strain has been continuously maintained in the laboratory at Oxitec Ltd with no signs of instability in the genetic trait (stability has been assessed through morphological evaluation, PCR analysis with the known flanking sequences and through assessment of the female lethality trait, when reared off the dietary antidote).

4. <u>Is the same GMO release planned elsewhere in the Community [in conformity with Article 6 (1)], by the same notifier</u>?

Yes ☒	No ☐
If yes, insert the country code(s): IT	

5. <u>Has the same GMO been notified for release elsewhere in the Community by the same notifier?</u>

Yes ☐	No ☒
If yes: – Member State of notification - Notification number	

6. <u>Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier</u>?

Yes ☐	No ☒
If yes: – Member State of notification - Notification number	

7. Summary of the potential environmental impact of the release of the GMOs

The introduced traits are either neutral (fluorescent marker) or confer a selective disadvantage (female-conditional lethality) to the Olive fly. The Olive fly does not exchange gametes with other species and exhibits mating behaviours that prevent outcrossing with other species. Interactions between the OX3097D-Bol strain and non-target organisms are expected to be equivalent to those of the unmodified Olive fly. The species-specific nature of the inserted lethality trait (due to the requirement for mating) means there are potentially beneficial effects on non-target organisms compared to the use of chemical insecticides for olive fly control. Olive fly is monophagous and only a pest of olive. The experimental design uses netted plots, is time-limited and post-trial monitoring will be conducted. There are no short term changes to land or management use, with the exception of the use of nets. Potential environmental impact is considered negligible given the conditions of use in the trial.

B. Information relating to the recipient or parental organisms from which the GMO is derived

1. Recipient or parental organism characterization:

a) Indicate whether the recipient or parental organism is a:

Viroid	☐
RNA virus	☐
DNA virus	☐
bacterium	☐
fungus	☐
animal	☐
- mammals	☐
- insect	☒
- fish	☐
-other animal	☐ (please specify phylum, class)

other, please specify

2. Name

(i) Order and/or higher taxon (for animals) Diptera
(ii) Genus Bactrocera
(iii) Species *Bactrocera (Dacus) oleae*
(iv) Strain OX3097D- Bol (Oxitec name)
(v) pathovar (biotype, ecotype, race, etc.) The parent lines were originated from Greece and have been outcrossed into additional strains from the Mediterranean basin.
(vi) common name Olive fly

3. Geographical distribution of the organism

a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes ☒ No ☐ Not known ☐

b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes ☒

If *yes*, indicate the type of ecosystem in which it is found:

Atlantic ☐
Mediterranean ☒
Arctic ☐
Alpine ☐
Continental ☐

(ii) No ☐

(iii) Not known ☐

c) Is it frequently used in the country where the notification is made?

Yes ☐ No ☒

d) Is it frequently kept in the country where the notification is made?

Yes ☒ No ☐

4. Natural habitat of the organism

(a) If the organism is a microorganism

Water ☐
soil, free-living ☐
soil in association with plant-root systems ☐
in association with plant leaf/stem systems ☐
in association with animals ☐

other (specify)

(b) If the organism is an animal: natural habitat or usual agroecosystem:

Olive plantations, wild Olive plants

5. a) Detection techniques

The modified Olive fly can be detected by the fluorescent marker DsRed2 under the appropriate wavelengths of excitation and emission using a stereomicroscope (e.g. Leica MZFLIII or Olympus SZX12). Molecular biology techniques can also be used to identify the insect.

5. b) Identification techniques

See 5a)

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes ☐	No ☒
If yes, specify	

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes ☐	No ☒	Not known ☐

If yes:

a) to which of the following organisms:

- humans ☐
- animals ☐
- plants ☐
- other ☐

b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

The Oxitec Olive fly has no pathogenic activity.

The tTAV protein which confers the female conditional lethality trait has been reviewed in a study commissioned by Oxitec Ltd. where it was compared to known toxic and allergenic sequences and was found not to encode any sequence homologous to a toxin or allergen. (Goodman 2011).

The fluorescent marker DsRed2 has no known toxicity or allergenicity.

The inserted genetic traits are not expected to confer the Olive fly with any ability to colonise other host plants. No abnormal behaviour has been observed in the Olive fly during its 3 years of development in the laboratory, through evaluation of its behaviour in cage trials in a semi-natural environment and during mass rearing.

8. Information concerning reproduction

a) Generation time in natural ecosystems:

Generation time is temperature dependent and at 25°C is approximately 30 days.

b) Generation time in the ecosystem where the release will take place:

See above. The releases will be carried out in the natural ecosystem of the Olive fly.

c) Way of reproduction: Sexual ☒ Asexual ☐

d) Factors affecting reproduction:

Temperature, photoperiod, humidity and presence of host plants (olives) and presence of Olive fly.

9. Survivability

a) Ability to form structures enhancing survival or dormancy:

(i) endospores	☐
(ii) cysts	☐
(iii) sclerotia	☐
(iv) asexual spores (fungi)	☐
(v) sexual spores (fungi)	☐
(vi) eggs	☐
(vii) pupae	☒
(viii) larvae	☐

(ix) other, please specify

Adult Olive flies are also capable of sexual diapause where the adult Olive flies can survive without reproduction for up to 5 months. This has not been observed in the developed strain OX3097D-Bol to date.

b) Relevant factors affecting survivability:

The olive fly is completely reliant on the host plant, as olives are required for egg laying sites and larval development. The climate at the release site can affect the survival of the Olive fly with temperatures between 20-30°C being optimum. High humidity or irrigation of the release site can also lead to an increase in adult survival as the adults require a source of water.

10. a) Ways of dissemination

Dissemination is by adult flight or movement of infested fruit.

10. b) Factors affecting dissemination

Factors affecting dissemination of the adult olive fly include the presence of the host plant and the presence of other Olive flies. Factors affecting other life stages include fruit movement and climatic conditions.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

None

C. Information relating to the genetic modification

1. Type of the genetic modification

(i) Insertion of genetic material ☒
(ii) Deletion of genetic material ☐
(iii) Base substitution ☐
(iv) Cell fusion ☐

(v) Other, please specify

2. Intended outcome of the genetic modification

Two traits have been introduced on a single inserted DNA segment: female-specific conditional lethality and a fluorescent marker.

Matings of released males with wild females will result in the survival of male OX3097D-Bol olive flies however all the females will fail to develop further than the late larval stages; evaluation of the fluorescent trait in the male larvae will indicate mating ratios.

3. a) Has a vector been used in the process of modification?

Yes ☒	No ☐
If no, go straight to question 5.	

3. b) If yes, is the vector wholly or partially present in the modified organism?

Yes ☒	No ☐
If no, go straight to question 5.	

4. If the answer to 3 b) is yes, supply the following information

a) Type of vector

plasmid ☐
bacteriophage ☐
virus ☐
cosmid ☐
transposable element ☒

other, please specify

b) Identity of the vector

piggyBac transposable element from the Cabbage looper moth *(Trichoplusia ni)*

c) Host range of the vector

PiggyBac can be used to transform a range of species, particularly insects. Functional piggyBac elements have only been isolated in *Trichoplusia ni* (Handler 2002).

d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes ☒ No ☐

Antibiotic resistance ☐

Other, specify: conditional lethality trait (tTAV), DsRed2

Indication of which antibiotic resistance gene is inserted

No antibiotic resistance gene is used in the modification

e) Constituent fragments of the vector piggyBac, conditional lethality trait (tTAV) and the fluorescent marker DsRed2.
f) Method for introducing the vector into the recipient organism (i) transformation ☐ (ii) electroporation ☐ (iii) macroinjection ☐ (iv) microinjection ☒ (v) infection ☐ (vi) other, please specify

5. <u>If the answer to question B.3 (a) and (b) is no, what was the method used in the process of modification?</u>

(i) transformation ☐ (ii) microinjection ☐ (iii) microencapsulation ☐ (iv) macroinjection ☐ (v) other, please specify

6. <u>Information on the insert</u>

a) Composition of the insert: b) Source of each constituent part of the insert: c) Intended function of each constituent part of the insert in the GMO
Nls: synthetic DNA: nuclear localization of DsRed2 protein into the nuclei of cells
DsRed2: Discosoma Sp (Coral): Expression of a fluorescent protein which acts as a marker
Intron: Drosophila melanogaster: Stabilizes mRNA and required for translation of mRNA
TetOx14/TetOx7: Synthetic: Enhancer region to control gene expression
Tra intron: Ceratitis capitata: Gives female specificity
tetR/tTAV: synthetic sequence based on domains from E. coli/Herpes simplex virus: conditional lethality trait
VP16: Herpes simplex virus: Component of synthetic transcription factor tTAV.
d) Location of the insert in the host organism - on a free plasmid ☐ - integrated in the chromosome ☒

- other, please specify
e) Does the insert contain parts whose product or function are not known? Yes ☐ No ☒ If yes, please specify

D. Information on the organism(s) from which the insert is derived (Donor)

Taxonomy of the Donor Organisms	**Pathogenicity of the donor organisms**	**Are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?**	**Is The Donor Organism Classified Under Existing Community Rules Relating To The Protection Of Human Health And The Environment, Such As Directive 90/679/Eec On The Protection Of Workers From Risks Related To Exposure To Biological Agents At Work?**	**Do the donor and recipient organism exchange genetic material naturally?**
Discosoma spp. Anthozoa, Hexacorallia, Corallimorpharia	Discosoma spp. are not known to be pathogenic	No	No	No
Drosophila Melanogaster Insect, Diptera,	*Drosophila melanogaster* is not known to be pathogenic	No	No	No
Escherichia Coli Bacterium, Proteobacteria, Enterobacteriales,	The donor organism was taken from a non-pathogenic laboratory strain	No	Non-pathogenic strains of *E. coli* are not classified in the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC).	No
Herpes Simplex Virus Type 1 DNA Virus, Group 1 (dsDNA) Virus, Simplexvirus,	Herpes is a known pathogen	No	Herpes simplex virus type 1 is classified as group 2 biological agents in the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC).	No
Trichoplusia ni Insect, Lepidoptera, Noctuidae Cabbage looper moth	The cabbage looper moth is not a known pathogen	No	No	No

Ceratitis capitata Insect, Diptera, Tephritidae Medfly	The Medfly is an invasive plant pest but is not pathogenic	No	No	No

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

a) Is the GMO different from the recipient as far as *survivability* is concerned?

Yes ☒ No ☐ Not known ☐

Please specify:

The modification encodes a female-specific conditional lethality trait.

b) Is the GMO in any way different from the recipient as far as mode and/or rate of *reproduction* is concerned?

Yes ☐ No ☒ Not known ☐

Please specify:

The mode and rate of reproduction has not been altered, however the intended effect of the modification is to confer a selective disadvantage on the females (i.e. death) in subsequent generations.

c) Is the GMO in any way different from the recipient as far as *dissemination* is concerned?

Yes ☐ No ☒ Not known ☐

Please specify

d) Is the GMO in any way different from the recipient as far as *pathogenicity* is concerned?

Yes ☐ No ☒ Not known ☐

Please specify

2. Genetic stability of the genetically modified organism

The Oxitec Olive fly was developed over 3 years ago (approximately 45 generations). Since the transformation the strain has been continuously maintained in the laboratory at Oxitec Ltd with no signs of instability in the genetic traits (stability has been assessed through phenotypic assessment, analysis using molecular techniques and through assessment of the female-specific lethality trait when reared in the absence of the dietary antidote).

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes ☐ No ☒ Not known ☐

If *yes*,

a) to which of the following organisms?:

- humans ☐
- animals ☐
- plants ☐
- other ☐

b) give the relevant information specified under Annex III A, point II (A) (11) (d) and II (C) (2) (i)

The Oxitec Olive fly has no pathogenic activity.

The tTAV lethality trait has been reviewed in a study commissioned by Oxitec Ltd. where it was compared to known toxic and allergenic sequences and was found not to encode any sequence homologous to a toxin or allergen, (Goodman 2011).

The fluorescent marker DsRed2 has no known toxicity or allergenicity.

The inserted genetic traits are not expected to confer the Olive fly with the ability to colonise other host plants.

No abnormal behaviour has been observed in the Olive fly during its 3 years of development in the laboratory or during cage trials in a semi-natural environment.

4. Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

Standard fly trapping techniques such as McPhail traps or Yellow sticky traps will be used to detect the released olive flies as well as collecting infested fruit to assess emerging offspring for the presence of the genetic traits.

b) Techniques used to identify the GMO

The modified olive fly can be detected by the fluorescent marker DsRed2 under the appropriate wavelengths of excitation and emission. PCR techniques can also be used to identify the insect.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The primary objectives of the study are to:

- Establish the performance of the OX3097D-Bol olive flies when competing with wild males for wild females
- Gather information on the longevity of the OX3097D-Bol olive fly in a field environment
- Evaluate different release methods.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes ☐	No ☒
If yes, please specify	

3. Information concerning the release and the surrounding area

a) Geographical location (administrative region and where appropriate grid reference):

Tarragona

b) Size of the site (m^2): The total release area will be less than 1000m^2

(i) actual release site (m^2): The total release site will be split into 6 small treatment sites all totalling an area less than 1000m^2.

(ii) wider release area (m^2): The releases will only be within the netted areas.

c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:

The release site is approximately 8 km from the port at Tarragona. In Catalonia there is one National Park, Aigüestortes i Estany de Sant Maurici and this is 157km from the release site.

d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

The releases will be in managed agricultural land in which there will be no grazing livestock. The majority of agricultural crops in the area are olive and hazel trees however there is also almonds, walnuts, carobs, pistachios, figs, apples, pears, peaches and vineyards. There will be other insects within the netted release site which could be predators or parasitoids of the olive fly present at the release site. Although understory herbage is controlled at the release site with herbicide there is the potential for unknown plant species to grow throughout the release period. The presence of a number of insectivorous species is likely at the release site and whilst erection of the netting is likely to repel many larger animals there could still be reptiles, small mammals and birds present at the release site.

4. Method and amount of release

a) Quantities of GMOs to be released:

Release numbers will be proportional to the field population in order to achieve a target inheritance ratio[1] of (e.g.) 50-90% initially. The numbers needed for this will be estimated and may then be adjusted during the release period as the actual mating frequency is determined. Therefore absolute numbers of OX3097D-Bol males to be released cannot be determined in advance.

b) Duration of the operation:

The releases will last for a period of 8 weeks.

c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release:

The release site will be netted. Trapping is an essential component of insect releases and traps will be deployed before, during and post release both inside and outside the netting.

[1] The inheritance ratio is (progeny inheriting at least one copy of the inserted construct)/(total progeny). It can readily be estimated during a release program by monitoring fluorescence in immature stages. The mating ratio, which is the proportion of females mating a released OX3097D-Bol male, is also useful and is obviously related to the inheritance ratio, though with a time delay. Note also that this is the initial ratio – given constant release numbers and where releases are sustained for more than one insect generation, as the local population declines over time (e.g. due to the impact of the sterile male releases), this ratio will increase.

5. Short description of average environmental conditions (weather, temperature etc.)

The average temperature in Catalonia varies from lows of (4^{o}C in January, 16^{o}C August) to highs of (13^{o}C January, 28^{o}C August). The average precipitation (rainfall) is at its lowest in July with 20mm and at its highest in October with 91mm. Hours of sunlight per week range from 138 throughout December to 310 in July.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release

Caged trials and life history analyses' have been carried out to establish the mating competitiveness of the Ox3097D-Bol olive fly (Ant, Koukidou et al. 2012). Further cage trials have established proof of principle that the OX3097D-Bol olive fly is capable of eliminating a caged population of wild type olive flies, under semi-natural conditions.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organisms (if applicable)

order and/or higher taxon (for animals) Diptera
genus *Bactrocera*
species *Bactrocera (Dacus) oleae*
common name Olive fly

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The released male OX3097D-Bol olive flies are expected to mate with wild female olive flies at the release site. All female offspring resulting from such a cross will not survive to adulthood as there is a lack of the dietary supplement required to suppress the lethality trait in the release environment. Males from such crosses will not live beyond their own short lifespan.

3. Any other potentially significant interactions with other organisms in the environment

No significant interactions are anticipated. The modification is limited to the olive fly by reproductive barriers. In the event that the OX3097D-Bol olive fly is eaten by predators present at the release site the inserted genetic traits are not anticipated to have any toxic effect (Richards, Han et al. 2003; Pavely and Fedorova 2006).

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes ☐	No ☒	Not known ☐
Please give details: the introduced trait confers a selective disadvantage.		

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

The Olive fly is a monophagus species which lives only on olive trees. Any dissemination from the release site is likely to result in olive flies moving into other managed agricultural environments containing olive trees. The presence of netting is expected to prevent the movement of the olive fly however in the event that the OX3097D-Bol olive fly does move from the release site the presence of a lethality trait prevents the fly from establishing in the environment. Ecosystem services are unlikely to be affected by the presence of the OX3097D-Bol olive fly. Olive flies are not known to be pollinator species.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

Non-target organisms are not expected to be harmed by the releases of the genetically modified organism. The risk assessment has determined that there will be a negligible risk to animal health associated with the release of the OX3097D-Bol olive fly.

7. Likelihood of genetic exchange in vivo

a) from the GMO to other organisms in the release ecosystem:

The only anticipated genetic exchange is from the OX3097D-Bol male olive flies to the wild female olive flies at the release site "vertical transmission".

b) from other organisms to the GMO:

None expected

c) likely consequences of gene transfer:

The gene transfer from the OX3097D-Bol males to the wild females is likely to result in the death of the female progeny in the release site as the conditional lethality trait female-specific. Males from such crosses are not anticipated to have an altered lifespan in comparison to the unmodified olive fly.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc.):

Detailed life history analyses of the behaviour of the olive fly have been carried out in the laboratories at Oxitec Ltd, (Ant, Koukidou et al. 2012). The thorough evaluation of the Olive fly also included studies in contained semi-natural conditions in collaboration with University of Crete. Studies evaluating the mating competitiveness of the OX3097D-Bol olive fly in comparison to the wild olive fly collected from infested olives found in Crete indicated that there is a high mating competitiveness between the OX3097D-Bol olive fly and the wild olive fly. Further studies indicated that the OX3097D-Bol olive fly was capable of a population suppression of a stable wild-type population in a caged environment. No adverse effects were noted during any of these studies.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

The majority of decomposer species are considered to be generalists that will feed opportunistically on organic matter found in their habitats, an influx of dead adult males and any change in the overall olive fly population is not considered to have a lasting detrimental impact on the decomposer population.

The OX3097D-Bol olive flies have been altered to produce two novel proteins relative to unmodified olive flies which are likely to breakdown into constituent amino acids at the release site; these are not anticipated to have any different effect on the biogeochemical processes in comparison to the non-modified organism.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Standard olive fly traps will be set up within the release sites and will be checked periodically to monitor the population of the olive flies. Immature stages may be monitored by sampling infested olive fruit. Fluorescent scoring of samples of olive flies from the traps will detect the persistence of the OX3097D-B olive fly in the environment.

Standard olive fly traps will be set up at intervals outside of the netted release sites to monitor for the presence of any escaped OX3097D-Bol olive flies in vicinity of the release sites.

2. Methods for monitoring ecosystem effects

None have been considered necessary following the risk assessment

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

The vertical transfer of genetic material can be detected in the olive fly by screening for fluorescence; the exchange of gametes is limited to Olive fly by mating behaviours there have been no reports of mating between Olive flies and any other flies.

Predators which consume the OX3097D-Bol Olive flies cannot transfer DNA by ingestion; presence of the genetic material may be detected by molecular methods.

4. Size of the monitoring area (m^2)

As the release site is contained within a netted enclosure there will be no monitoring beyond the release sites and control sites: a total area of no greater than 1000m^2.

5. Duration of the monitoring

Pre-release monitoring will commence after approval is given and in advance of the trial. Monitoring will continue throughout the trial and post-release monitoring will continue until no fluorescent adults have been trapped for a period of 4 successive weeks.

6. Frequency of the monitoring

Traps will be monitored weekly

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Monitoring of the release site will continue until the OX3097D-Bol Olive flies are not detected for a period of 4 successive weeks. If the Oxitec Olive flies remain at the release site at the end of the monitoring period then an approved insecticide will be used. The OX3097D-Bol males are not expected to persist in the environment and there is not anticipated to be any treatment to eliminate the olive fly from the release site.

2. Post-release treatment of the GMOs

Any GMOs not used in the trials will be deep frozen at -15^{o}C or below for 12 hours and disposed of according to the usual procedures at the research facility where the olive fly will be eclosed.

3. a) Type and amount of waste generated

Adult diet, fly remains and eclosion waste – up to 5kg

3. b) Treatment of waste

Any eclosion and release materials used at the eclosion facility will be frozen at -15^{o}C or below for 12 hours prior to disposal in the usual waste channels as specified at the eclosion facility.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

The proposed releases are within a netted area and will be double contained when transported to the release site. In the event that any OX3097D-Bol olive flies escape during transport they are not capable of establishment in the environment due to the presence of the conditional lethality trait. Any large scale inadvertent release can be treated with an approved insecticide.

In the event that OX3097D-Bol olive fly is detected in the traps which are placed outside of the release site the olive fly contains a conditional lethality trait which will prevent establishment of the flies in the environment. Any unexpected persistence of the OX3097D-Bol olive fly will be controlled with approved insecticides; the released olive flies are expected to be susceptible to insecticides as they do not carry any genetic mutations associated with insecticide resistance.

2. Methods for removal of the GMO(s) of the areas potentially affected

The OX3097D-Bol olive fly contains a female specific conditional lethality trait which will limit its persistence in the release environment.

The release site will be monitored for up to 4 weeks post-release in which time-frame it is anticipated that the olive fly populations will have been depleted. Any unexpected persistence of the olive fly will be detected by the release site monitoring and if necessary approved insecticides may be applied in order to remove any remaining olive flies.

3. Methods for disposal or sanitation of plants, animals, soils etc. that could be exposed during or after the spread

No particular measures are envisaged.

Any of the released olive flies which will remain in the environment are anticipated to break down in the environment into non-toxic components as per other insects at the release site.

Larvae emerging from infested olives collected during the trial will be scored for fluorescence in order to assess the mating competitiveness of the OX3097D-Bol male olive flies. These olives will then be frozen at -15^{o}C and disposed of in the usual waste channels

4. Plans for protecting human health and the environment in the event of an undesirable effect

The olive fly has been reared and used in the laboratories at Oxitec Ltd for over 3 years (approximately 45 generations) with no perceived undesirable effect on the laboratory workers. There is not anticipated to be any necessary actions to protect human health or the environment during the release however should unforeseen problems arise during the trial the releases will be stopped and if necessary the residual population of olive fly will be eliminated using an approved insecticide.

References:

Ant, T., M. Koukidou, et al. (2012). "Control of the Olive fruit fly using genetics-enhanced Sterile Insect Technique." BMC Biology **10**: 51.

Goodman, R. (2011). Bioinformatics Evaluation of transgenic protein tTAV from mosquito. Unpublished report.

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