Summary information note regarding the small scale release of genetically modified sterile male *Anopheles coluzzii* strain Ac(DSM)2 in Bana village in Burkina Faso

1 Background

This document is a summary of the information provided to the Burkina Faso authorities for the analysis of risk for the small scale controlled release of genetically modified (GM) sterile male mosquitoes at one site in Burkina Faso.

It is intended to briefly summarise the key features of the trial, the genetically modified mosquitoes and the assessment of risk undertaken both by Target Malaria, and independent risk assessment (available on the website of Target Malaria). It should not be viewed as the complete information that was submitted to the authorities on any topic. It is provided by Target Malaria in the spirit of openness.

2 General information

2.1 Title of the project

Comparative mark release recapture of *An.coluzzi* sterile male Ac(DSM)2 strain (containing homing endonuclease gene and two fluorescent marker genes) and evaluation of its persistence profile in the environment.

2.2 Proposed period of release

A single release during the rainy season (June-Oct) 2019, with field surveillance for up to one year post release.

2.3 Applicant:

Institut de Recherche en Sciences de la Santé (IRSS), Burkina Faso

GMO characterisation:

2.3.1 *Identity of the genetically modified organism (GMO)(genus and species)*

The GMO is an insect; *Anopheles coluzzii*, part of the *Anopheles gambiae* s.l . species complex comprising of eight members (see 3). Three of the members are the main vectors of the malaria parasite in Sub-Saharan Africa. These are *An.gambiae, An.coluzzii* (these two were formerly known as S and M forms of *An.gambiae* s.s) and *An.arabiensis*. The Ac(DSM)2 genetic insert has been introgressed from an *An. gambiae* laboratory reference strain (G3) in which the genetic transformation occurred (Windbichler et al, 2008), into an *An. coluzzii* background that originated from Burkina Faso.

2.3.2 *Intended outcome of the genetic modification*

Two fluorescent marker genes and a homing endonuclease gene conferring genetic male sterility. Matings of released males with wild female *Anopheles* species will result in no viable progeny. The fluorescent marker genes can be used to identify the released males both visually using specialised microscopes following recapture, and molecular techniques can also be used.

2.3.3 *Method used in the process of transformation*

Micro-injection of embryos (Windbichler et al, 2008)

2.3.4 *The vector used in the process of transformation*

piggyBac non-autonomous transposable element from the cabbage looper moth (*Trichoplusia ni).* The vector is widely used to transform a range of insect species (Handler et al, 2002). The transformation was accomplished via a conventional binary system in which the transposase necessary for the insertion event is supplied in trans and is not present in the resulting transgenic strain.

2.3.5 *Information on the genetic insert*

The composition and source of the constituent parts and their intended function is listed below. The construct was created synthetically based on sequence data, and thus the Ac(DSM)2 strain was not exposed to the living source organisms through the development process. The transgene insertion is contained within a non-autonomous *piggyBac* transposable element which has inverted repeats but not transposase-encoding regions.

Genetic element	Source	Function
pBacR & pBacL	*Trichoplusia ni piggyBac vector*	Left and right inverted repeat sequences to facilitate DNA integration
3xP3 promoter	*Drosophila melanogaster*	to direct eye tissue-specific expression of the red fluorescent reporter gene, DsRed, in order to detect genetically modified mosquitoes.
DsRed	*Discosoma sp*	Red fluorescent marker gene
SV40 3'untranslated region	Simian virus 40	Signals end of transcription of DsRed gene
Beta-2 Promoter	*Anopheles gambiae*	Directs testes-specific expression of the eGFP::I-PpoI gene fusion product
eGFP::i-PpoI gene fusion	*Aequorea victoria (eGFP)* *Physarum polycephalum (I-PpoI)*	Fluorescence from the eGFP element of the gene product enables the detection of gene expression in the mosquito. The I-Ppo-1 element of the gene fusion product recognizes and cleaves a recognition sequence in the ribosomal genes of the mosquito X chromosome and ribosomal genes in other organisms
Beta-2 3' untranslated region	*Anopheles gambiae*	3' untranslated region from beta-*tubulin* gene that signals the end of transcription of the *eGFP::I-Ppo1* gene fusion

2.3.6 *Location of the insert*

The insert has been confirmed as a single insertion on position 11872203 on chromosome 3R as described in Windbichler et al, 2008.

2.3.7 *Sequence and copy number*

The sequence of the inserted transgenes has been verified and no rearrangements identified. A single insertion was confirmed and evidence of Mendelian inheritance pattern as expected from a dominant trait (ie: approx. 50:50) observed throughout the

generations both in the parental strain Ag(DSM)2 and the introgressed strain Ac(DSM)2.

No vector backbone sequences outside of the *piggyBac* inverted repeats are present in the transformed parental strain Ag(DSM)2.

2.3.8 *Genetic stability*

The parental strain was created in 2008 and has been in continuous laboratory maintenance via multiple laboratories (UK, USA and Italy). The introduced traits have been assessed for stability through both genetic and phenotypic analysis. No signs of instability of the genetic traits have been observed in Ag(DSM)2in Burkina Faso since the parental strain was imported in 2016 and the genetic construct introgressed into the local *A. coluzzii* genetic background (currently generation 39). If the I-Ppo-I gene was lost or silenced then the strain would revert to being fully fertile and the same as an unmodified *An.coluzzii* mosquito.

The strain has behaved in a stable fashion since the transformation in 2008 and stability is not background dependent.

2.3.9 *Has the same GMO been notified for release elsewhere?*

No

2.3.10 *Summary of the potential environmental impact of the release of the GMO*

The introduced traits are either neutral (fluorescent marker genes) or confer a selective disadvantage and significant fitness cost (no progeny expected) on the mosquito. The number of mosquitoes to be released in the study area will have no significant effect short or long term on the overall size of the wild mosquito population. The released male mosquitoes are anticipated to survive for up to 2 weeks in the field based on laboratory studies. For example, the mating competitiveness for the Ag(DSM)2 strain is significantly lower than for the non-genetically modified sibling strain. Consequently, the genetic modification confers a selective and competitive disadvantage to the mosquito. Interactions between the sterile male Ac(DSM)2 strain and non-target organisms are anticipated to be transitory, due to the fitness cost and lack of progeny from mating with wild Anopheles mosquitoes. There will be no changes to existing vector control practices or malaria treatments in this trial. The proposed release is a single event and the sterile males are highly unlikely to persist in the environment longer than 2-3 generations, they are susceptible to the insecticides used in current control programmes, and therefore can be treated with insecticides to remove them from the environment as a risk management measure. Any effects would be transient. The existing parental species is already harmful to human life causing morbidity and mortality, through disease transmission.

No new species will be released and no ecological niche is being disrupted because the proposed release involves small numbers of mosquitoes (less than 10,000) in relation to the existing mosquito population, and long-term persistence in the environment is not an intended outcome. Based on modelling, elimination of the transgene is expected to occur within 2-3 generations. Background studies indicate the natural *An.gambiae* population to be around 400,000-500,000 male and female

adult mosquitoes during the wet season. *Anopheles coluzzii* is not a keystone species in the ecosystem in terms of either biomass or ecological niche, with no predator being obligate on it. The proposed field release has no intended effect on the local *Anopheles* species population size or genetic composition. The number of mosquitoes released or captured in the release area during the study will have no significant short or long-term effects on the population size of the wild mosquitoes. The transgenic males to be released have been shown to be fully sterile and thus there is no mechanism to have effects on genetic diversity on the wild population through mating. Indeed, the genetic background strain was originally sourced from VK5 in Bama.

3 Information relating to the recipient or parental organism from which the GMO is derived:

3.1 Common Name

Mosquito, Malaria Mosquito,

3.2 Scientific name

Anopheles gambiae sensu lato (s.l.) Patton (Cellia)—mosquito (Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta; Order: Diptera; Superfamily: Culicoidea; Family: Culicidae; Subfamily: Anophelinae; Genus: Anopheles).

4 Geographical distribution of the organism

4.1 Indigenous to or otherwise established in the country where the notification is made?

YES, they are widespread in the Sub-Saharan region

5 Natural habitat of the recipient or parental organism

5.1 Natural habitat of the organism

An. gambiae and *An.coluzzii* are freshwater aquatic organisms in the larval phase, laying eggs in transitory bodies of standing water such as hoof prints or tyre tracks exposed to sunlight. They can also lay eggs in damp soil at the edges of water bodies if there are no better breeding sites. Adults are terrestrial once emerged from pupae. 2-3 days after emergence, both males and females are sexually mature and begin to look for mates. Mating in *An.gambiae s.l.* mostly happens outdoor and at sunset. Males swarm at specific mating stations waiting for females. Females visit these mating stations and depart in copula with the most competitive males (Diabate and Tripet 2015). *An. gambiae* females are anthropophilic and feed predominantly on human blood. After a blood meal, the female rests indoors, most frequently in human habitations, to digest the blood meal and mature her eggs. Three days after the blood meal, she looks for a water body where she will lay eggs and the cycle will start again.

5.2 Detection techniques

Adult *An.gambiae s.l* are collected from swarms by sweep netting, or indoors by insecticide spray catches. Other adult trapping methods are available, such as traps that mimic human odors (BGS traps). Adult sampling frequently occurs at dusk when the mosquitoes are most active. Larvae are sampled from the shallow water in which they breed. Mark Release recapture methods are also used regularly, and swarm locations mapped with Geographic Information Systems (GIS).

6 Is the recipient organism pathogenic or harmful in any other way to humans, animals, plants?

Malaria is transmitted to humans principally via the bite of infected female *Anopheles* mosquitoes. In 2016, malaria affected approximately 216 million and killed 445,000 people worldwide[1], most of them African children under the age of five years. Malaria symptoms typically include fever, tiredness, vomiting, and headaches. If left untreated, the most severe form of infection can lead to permanent learning disabilities, coma, and even death. There has been a recent trend towards increasing numbers of cases per annum, for example in 2015 there were 211 million, which rose to 216 million in 2016[2], this trend is particularly visible in high burden countries such as Burkina Faso.

7 Information concerning reproduction

7.1 Generation time in natural ecosystems

Typically eggs hatch 24-36 hours after they have been laid resulting in first instar (L1) larvae. The L1 larvae undergo 3 major transformations (L2-L3-L4) before pupating. From L1 to L4 the larvae feed on microorganisms and decaying materials in their aquatic habitats. The L4 larva undergoes a last metamorphosis to reach a non-feeding but motile pupal stage before turning into adult. It takes about 6-8 days for the egg to reach the adult stage but the length of development is strongly influenced by both the availability of food and temperature in the larval habitat. Only a relatively small fraction of adult *An.gambiae* live longer than 14 days in the environment (Charlwood et al., 1997). Mating in *An.gambiae s.l.* mostly happens outdoor and at sunset. Males swarm at specific mating stations waiting for females. Females visit these mating stations and depart in copula with the most competitive males (Diabate and Tripet 2015).

7.2 Generation time in the ecosystem where the release will take place

See 7.1 – the release will be carried out in the natural ecosystem

[1] Updated in 2017 -WHO (2017), –World Malaria Report 2017. World Health Organisation to 219 million cases and 435000 deaths.

7.3 Mode of reproduction

Sexual reproduction

7.4 Factors affecting reproduction

Environmental factors such as atmospheric humidity, type of water (Yaro et al., 2006), temperature (Valencia et al., 1996, Bayoh and Lindsay 2003, Huang et al., 2006b, Impoinvil et al., 2007) and desiccation all affect the proportion of eggs that hatch. Nutrition and water temperature affect the development time of larvae. Successful completion of the egg to adult cycle occurs between ~18 and 34°C (Bayoh and Lindsay 2003). Presence of human hosts on which the female can take a blood meal are necessary for egg development in female mosquitoes. There are strong, natural reproductive barriers between members of the *An. gambiae s.l* breeding complex that limit the capacity to spread and establish in the environment by intraspecific hybridization within the species complex.

8 Survivability

8.1 Ability to form structures enhancing survival or dormancy

An. gambiae s.l are tropical species and do not enter a diapause state or survive below temperatures of 17°C. The survival structures are eggs and pupae. Eggs are not resistant to desiccation and do not survive without water or damp substrate availability.

8.2 Relevant factors affecting survivability

Temperature is a critical factor in survivability of eggs and larvae with optimum temperatures between 24 and 30°C. Rainfall and humidity are critical abiotic factors affecting survival of *An. gambiae s.l.*. Eggs hatch in response to rainfall and populations increase in the rainy season. High level of rainfall however can conversely reduce survival due to the potential flushing of breeding sites or secondary effects (Paaijmans et al., 2007).

Oviposition sites are rare in the dry-season (which can last 2-7months in Burkina Faso) and it has recently been shown that short term depravation of oviposition sites dramatically reduces reproductive output and as well as reducing longevity and bloodfeeding rates (Artis et al., 2014). Female adults require hosts on which to take bloodmeals to provide sufficient resources for egg-laying. *An. gambiae s.l* feeds almost exclusively on human hosts.

9 Dissemination

9.1 Ways of dissemination

Dissemination is by intentional adult flight to find food, hosts, mates, breeding sites or shelter and is the main form of dissemination, although passive movement in vehicles and wind assisted long distance flight is also reported.

9.2 Factors affecting dissemination

Geophysical and landscape features are known to be barriers to the movement of the mosquitoes and include vegetation height and density, surface features such as large water bodies, availability of breeding sites and large open areas act as barriers (reviewed in Verdonschot et al, 2014).

Wind assisted dispersal is mediated by the energetic reserves of the mosquito and the survival rate during wind-assisted dispersal or migration is likely to be very low due to the harsh meteorological conditions of temperature and humidity.

10 Information on the genetic modification

10.1 Is the GMO different to the recipient as far as survivability is concerned?

YES. The introduced genetic trait confers male sterility and impairs the fertility of the male mosquitoes to the extent that they do not produce viable progeny.

10.2 Is the GMO different to the recipient as far as mode or rate of reproduction is concerned ?

There is no change in the sexual nature of the reproduction but as no progeny are formed the rate of reproduction is affected. The parental transformation strain Ag(DSM)2, prior to introgression in the *An. coluzzii* background, displayed delayed pupation and reduced male adult eclosion relative to non-genetically modified siblings (Klein et al., 2012). Studies conducted within IRSS with the introgressed strain, Ac(DSM)2, confirmed there was no evidence of a higher reproductive rate than the non-genetically modified comparator, and showed that Ac(DSM)2 is less fit compared to the non-modified comparator.

10.3 Is the GMO different from the recipient as far as dissemination is concerned ?

NO, it is expected to be the same as the recipient organism. Mark-release-recapture (MRR) studies conducted with wild-type *An. coluzzii* has shown limited dispersal (on average 138 (+/- 6.9m from their release point) in the vicinity of the villages that are intended trial sites (Epopa et al, 2017). The introduced genetic trait confers male sterility and impairs the fertility of the male mosquitoes to the extent that they do not produce viable progeny therefore the trait limits establishment and spread in the environment. Additionally, there are strong, natural reproductive barriers between members of the breeding complex that limit the capacity to spread and establish in the environment.

10.4 Is the GMO different from the recipient as far as pathogenicity is concerned ?

YES, as the release will involve over 99.5% adult Ac(DSM)2 males. Male *An. coluzzii* cannot bite or transmit disease. Prior to release the mosquitoes will undergo a double sorting process in the contained laboratory to minimize the numbers of adult transgenic females. Less than 0.5% females could be co-released alongside the Ac(DSM)2 males, which will be fertile but only 50% of them will carry the transgene. The sorting procedure will aim minimise the numbers of females. Procedures are in place to ensure that mosquitoes from the containment facility are not infected with

malaria or other diseases, and prior to release they will only be fed sugar water, so there will be no potential for infection prior to release. The females will disappear from the population within 2-3 generations (2-3 months) at the height of the wet season and more rapidly in the dry season due to the unfavourable environmental conditions. This release is not expected to have any impact on malaria or the numbers of *An. gambiae* mosquitoes in the release site.

10.4.1 *Is the GMO significantly pathogenic or harmful in any way?*

NO, the Ac(DSM)2 male sterile strain has no pathogenic activity (see 10.4.). The introduced proteins (DsRed, eGFP::I-Ppo-I) has been compared for homology to known toxic and allergenic sequences and found not to have any significant homologies to known toxins or allergen sequences in accordance with international assessment criteria (Codex 2003, 2009).

11 Genetic stability of the GMO

The strain was created in 2008 (Windbichler et al ,2008) and has been in continuous laboratory maintenance via multiple laboratories (UK, USA and Italy). The inheritance of the inserted fluorescent protein traits, and I-PpoI enzyme expression and activity have been studied over at least 140 generations in the G3 background strain and the expression of the inserted genes has remained stable. The inheritance patterns of these genetic elements are as expected for a dominant male sterile trait, i.e. Mendelian inheritance. No signs of instability have been observed in Ac(DSM)2 in Burkina Faso since the parental strain was imported in 2016 and introgressed in to the *An coluzzii* genetic background (at the time of writing the colony is at generation 39). The introduced traits have been assessed for stability through both genetic and phenotypic analysis.

11.1 Describe the identification and detection methods

DsRed, one of the fluorescent marker genes in the strain, is expressed in eye tissue of both transgenic males and females and is scored visually when viewed under a fluorescence microscope. The stability, specificity and sensitivity of the DsRed marker gene was investigated and determined that no evidence of excision or failure of the DsRed marker to distinguish transgenic from non-transgenic siblings was observed. However, the sensitivity of the assay was limited by the specific primers, the potential for polymorphism in the flanking DNA, and the DNA preparation. Despite these factors the detection of the transgene was highly specific.

Released mosquitoes will be dusted with fluorescent powder prior to release. Identification of released dead male mosquitoes bearing the fluorescent powder, will be conducted with a hand-held UV illuminator.

To determine whether the marked and recaptured mosquitoes carry the transgene, Ac(DSM)2 males or their wild-type sibling males, individual molecular identification using a Polymerase Chain Reaction (PCR) technique will be performed on all marked and recaptured mosquitoes. Unmarked wild type mosquitoes captured during the primary study will be analyzed by a technique of pooled-PCR to determine whether any loss-of fluorescent marking took place in the field.

A further monitoring survey will be made using these and other methods within one month after the release. Subsequent entomological surveys will be at approximately monthly intervals for a maximum of twelve (12) months. From these surveys, and if sufficient mosquitoes are caught (numbers fall substantially in the dry season) a subsample of individual *An. gambiae* s.l. mosquitoes collected within the recapture area will be examined by PCR and other molecular techniques for evidence of the transgene in the local mosquito population.

12 Information regarding the release

12.1 Geographical location of the release and brief description of the site.

The sites were selected based on the basis of ongoing monitoring of *An. gambiae* s.l populations (as they are dynamic) and societal considerations. Two villages had originally been shortlisted as potential sites for this study: Bana and Souroukoudingan in the Kou valley of western Burkina Faso. They are approximately 5 km apart and considered medium sized for the region. The villages are located within the department of Bobo Dioulasso in the Houet province. Bana has finally been selected as the site for the limited and controlled environmental release of the Ac(DSM)2 strain. IRSS and Target Malaria have been working with the villages of Bana and Souroukoudingan for over 7 years, conducting regular entomological surveys to understand the mosquito population and engaging with the community about the project.

The site is an urban/peri urban site typical of a *An. gambiae* habitat in the region. There is a protected forest between the village sites and Bobo-Dioulasso, known as Forêt classée de Dinderesso. This is a forest reserve located in Houet Province approximately 17km west of Bobo-Dioulasso. It is used in the summer for recreational activities and swimming by the townspeople of Bobo-Dioulasso.

13 Flora and fauna which may interact with the GMO

13.1 Fauna

Anopheles gambiae s.l. adult females are highly anthropophilic (preferring human hosts to other animals), though host selection is influenced by location, host availability and the genetic make-up of the mosquito population. Female *An. gambiae* typically feed late at night and frequently feed on humans indoors during the hours of darkness, although they will bite outdoors as well. Therefore humans are the main hosts although they will feed from animals if no human hosts are available.

The human population from a survey in 2014 conducted by IRSS, recorded 130 compounds (concessions) and within these 398 houses with 580 rooms in Bana. In Bana Village itself there were 65 concessions lived-in by 443 people. Bana Marché is the commercial hub whereas Bana Village houses the administrative portion.

Pastoral activities with moderate numbers of cattle, sheep, goats, pigs and poultry can also be found. The animals are in the village and in the area around such as in peripherals farms which are inhabited seasonally during the planting and harvesting season.

Predators and competitors of *Anopheles gambiae* s.l. include those in both the larval and terrestrial habitats. The main natural enemies, or entomophagous organisms, of mosquito larvae are diverse and many, including insects, spiders, hydras, planaria, copepods, bats, bird and fish. Munga (2007) identified seven families of mosquito predators in the larval habitats, including *Hydrophilidae* (Coleoptera, water scavenger beetles), *Dytiscidae* (Coleoptera, diving beetles), *Corixidae* (Hemiptera, water boatmen), *Nepidae* (Hemiptera, water scorpions), *Notonectidae* (Hemiptera, backswimmers), *Belostomatidae* (Hemiptera, giant water bugs), and *Cordulidae* (Odonata, dragonflies). *Anopheles gambiae s.l.* larvae occur in a great variety of habitats, but the most important are small, shallow, sunlit and usually temporary pools. Because of the small size and transient nature of many of these water bodies few predator species successfully colonize them. Predators of adults include birds, bats, dragonflies and spiders.

The role of the predators in the Anopheles ecosystem was reviewed by Collins et al, (2018) who reviewed the scientific literature and assessed the strength of the ecological interactions identified. Most predators identified consume many other insect species and there is no evidence that any species preys exclusively on any anopheline mosquito. There is one predatory species with a preference for blood-fed mosquitoes including *An. gambiae s.l.*. *Evarcha culicivora* is a jumping spider, known as the vampire spider, found around Lake Victoria, Uganda. There is no evidence that these spiders require *Anopheles* mosquitoes and will readily consume blood-fed *Culex*.

13.2 Threatened and endangered and charismatic/valued species

A wide scale search was conducted using different strategies:

- IUCN Red list systematic search, including a specific search on Dragonflies
- Burkina Faso's 5th report to the Convention on Biological Diversity (CBD) (2014),
- database search of peer reviewed literature using search terms (charismatic OR threatened OR keystone OR indicator AND species AND (Mali OR Burkina Faso)
- and a general web-based search

has been used to identify threatened, endemic or otherwise culturally important species in the West African Region covered by Burkina Faso and Mali.

Whilst a number of endangered or vulnerable species were identified, including a fish, bivalves, reptiles, birds and mammals none had habitat overlap with the *Anopheles* mosquitoes. The specific IUCN query addressing dragonflies, which are known to consume mosquitoes, indicates that there are no species of conservation concern.

There are a few sacred sites ('sacred bush') and valued animals (the boa snake, the sacred caiman and long-horned beef) in the village of Bana. Fruit trees are also valued to the extent they are protected from cutting.

13.3 Flora at the trial site

In the Houet province the vegetation is composed of woody savannah, parkland savannah and shrub savannah. These various types of vegetation have undergone

significant anthropogenic change and have been replaced by agro-pastroal farmlands. The remaining native flora is mainly composed of *Vitellaria paradoxa*, *Burkea africana*, *Detarium microcarpum*, *Isoberlinia doka*, *Anogeissus leiocarpus*, *Daniella oliveri* and *Borassus aethiopium (Some 2006).*

In Bana the main crops grown are corn, millet, rice and vegetables. Harvest is conducted at the household level and there is no collective or common efforts to organise agricultural activities or access markets.

Adult male mosquitoes imbibe nectar from plants and although they visit flowers to seek nectar and may move pollen between plants there are not known in the scientific literature to be pollinators.

13.4 Method and amount of GMO to be released

Up to 10,000 genetically modified Ac(DSM)2 mosquitoes and an equivalent number of non-modified sibling species will be released. The mosquitoes will be marked with fluorescent powder prior to release to aid identification on recapture.

13.5 Objectives of the release

The objectives of the controlled mark release recapture experiment are to:

- Estimate the daily survival rate of adult male *An. coluzzii* Ac(DSM)2 sterile male strain in comparison to the non-genetically modified sibling species co-released at the same time
- Assess movement of the adult male *An. coluzzii* Ac(DSM)2 sterile male mosquitoes within the area of release.

13.6 Duration of the release

The study will be performed in the rainy season (June-Oct 2019) and there will be a single release within that period. Post release monitoring will be continued for up to 12 months post release.

13.7 Short description of average environmental conditions (weather, temperature)

Bana falls predominantly in the Sudan bio-climatic zone, with some overlap of the Sudano-Guinean Zone. The Sudan zone receives 600-1,000 mm of rain annually. The Sudano-Guinean Zone has higher rainfall of 1,000-2,000 mm annually, is humid and features heavily wooded savanna. Köppen climate classification[3] for the area of Burkina Faso that encompasses Bana is Tropical Savanna (Aw classification), typified as tropical moist climates with average temperatures greater than 18°C. The Aw classification has an extended dry season during the winter with rainfall below 60mm.

[3] http://koeppen-geiger.vu-wien.ac.at/pdf/Paper_2006.pdf

13.8 Relevant information on previous releases of the same GMO, if any

The GMO has not been released previously.

14 Interaction of the GMO with environment and potential impacts on the environment if different from the recipient organisms.

14.1 Name of target organism if applicable

The target organism is *Anopheles gambiae* s.l, species complex of which the predominant species at the site is *An.coluzzii.*

15 Anticipated mechanism and result of interaction between the released GMO and target organism if applicable

Sexual mating by the release male mosquitoes entering the swarm of wild female *Anopheles coluzzii* mosquitoes. If mating is successful, no viable progeny would be produced due to the introduced trait conferring sterility. There are strong natural reproductive barriers between members of the *An. gambiae* complex which limits capacity to spread and establish in the environment.

15.1 Other potentially significant interactions with other organisms in the environment

No other significant interactions with other organisms in the environment are anticipated as the sterile male release proposed is limited in duration, the number of individuals to be released (<10,000) is low relative to the standing population and the geographic area has features of ecological isolation. Survival from laboratory-based studies indicate an average survival time of approximately 15 days, which is expected to be less in the natural environment due to the harsher conditions. Additionally:

- There is a high fitness cost in the Ac(DSM)2 sterile males and male mosquitoes do not bite humans or transmit disease. A few females could be co-released however multiple levels of procedure are in place to minimise the potential for genetically modified females released to below 0.5% of the total male release number.
- No new species will be released, and no ecological niche is being disrupted because the proposed release involves very small numbers of mosquitoes (less than 10,000) in relation to the existing population, and long-term persistence, in the environment, or a reduction in the existing wild population are not intended outcomes. Based on modelling (Valerio et al, 2016), elimination of the transgene is expected to occur within 2-3 generations. Any interactions which have not been forseen are thus expected to be highly transient as this is a single release with an intense period of recapture.
- No predators are obligate on *An. coluzzii* as discussed in 13.1 . Predators that may consume a few of the released mosquitoes will degrade the insects in the gastric juices in the same manner as a non-modified mosquito.

- Trial sites have been chosen for their geophysical features that are known to be barriers to mosquito movements. These include vegetation height, surface features and open areas.
- Based on modelling, elimination of the transgene is expected to occur within 2-3 generations. Background studies indicate the natural *An. gambiae* population to be around 400,000-500,000 male and female adult mosquitoes during the wet season. *Anopheles coluzzii* is not a keystone species in the ecosystem in terms of either biomass or ecological niche, with no predator being obligate on it.
- The insecticide resistance profile of the Ac(DSM)2 strain has been evaluated, which confirmed that they were fully susceptible to two insecticide classes (carbamates and organophosphates) and more sensitive than the Ac(WT) mosquitoes to the other insecticides used in the study, which included the commonly used pyrethroids. The release of the Ac(DSM)2 strain would not change chemical, pesticide or herbicide use.
- Vertical gene transfer to sexually compatible organisms; while mating is expected to occur the sterile male has shown complete male sterility with no viable progeny produced in different genetic backgrounds of sexually compatible species.

16 Information on monitoring

16.1 Methods for monitoring the GMO

Monitoring methods include those described for the recipient organisms in 5.2 which are insecticide spray collections and swarm netting. Additionally molecular analysis using PCR and other molecular techniques will be used to analyse the transgene persistence. Entomological surveys will continue alongside and use standard entomological methods as described in 5.2.

16.2 Methods for monitoring ecosystem effects

None have been considered necessary following the assessment of risk of the release.

16.3 Methods for detection of the GMO to other organisms

Adult males and non-modified siblings will be dusted with fluorescent powder prior to release. Gene transfer within the species complex will be examined by screening for the fluorescence and with molecular verification.

16.4 Duration of the monitoring

Up to one year post release

16.5 Frequency of the monitoring

There will be an intensive phase of monitoring for the longer of 10 days or three consecutive days without recapture, immediately post release. This will be followed by entomological survey within one month of the release and then at approximately monthly intervals for up to a period of one year.

17 Post release information

17.1 Post release treatment of release area

There will be approximately monthly entomological surveys for up to one year post release

17.2 Post release treatment of GMO

On each recapture day swarms will be sampled at dusk using sweep nets. Captured mosquitoes will be killed by freezing the contents of the net. Insecticide spray catches will be conducted in family compounds which will be selected randomly with the owners' consent. These collections will take place each morning of the intensive recapture period.

17.3 Type of wastes generated

Cages, dead mosquitoes both modified and non-modified, ethanol for mosquito storage, insecticide cans.

17.4 Treatment of wastes

Cages and cage covers will initially be sprayed with ethanol (70%) to ensure any remaining mosquitoes post release, are knocked-down. Dead mosquitoes will be returned in cooler chests to the IRSS insectary for further analysis. On return to the insectary cages, cage covers and frames will be dipped in dilute bleach and then washed with soap and hot water. Any surplus to requirements will be destroyed in accordance with the containment insectary Standard Operating Procedures (SOPs), which includes autoclaving and incineration. Any other wastes will be returned to the containment insectary in IRSS and treated as described in the containment insectary SOP's. Once treated using these measures wastes are contracted to a biomedical waste facility for further treatments and disposal. All transport will use double containment for the genetically modified mosquitoes.

18 Information on response plans

18.1 Methods and procedures for controlling the dissemination of GMO in case of unanticipated events.

Challenges that can be anticipated include serious weather events, transport related events (accident or mechanical failures), unrelated security events that may lead to potential containment breach during transport. If containment is breached during the transport to and from the insectary, insecticide is carried in the vehicles which may be used. Events must be reported to the national competent authority, Agence Nationale du Biosécurité, within the specified timeframe.

A chain of custody is in place to sign out at the insectary and sign-in at the field site. if unanticipated disruptions occur in the field that prevent release then the mosquitoes can be retained in their locked containers.

The Ac(DSM)2 mosquitoes have an intended fitness penalty associated with the genetic transformation and are not expected to survive or have progeny due to the male sterility trait.

Use of all existing mosquito control practices and malaria prevention practices are recommended throughout the trial duration. This was regularly communicated to the communities during the pre-release preparations and will be repeated during the study period.

The insecticide resistance profile of the mosquitoes has been examined and they are fully susceptible to two insecticide classes (carbamates and organophosphates) and more susceptible than the non-modified mosquitoes to other insecticides used in mosquito control programs.

19 Socio-economic impact and public consultation

The project works in an interactive dialog with stakeholders to ensure a good understanding of the activities of the project and its approach; to ensure that the project can respond to the expectations and concerns of stakeholders in a constructive manner and in regular dialog. It is a process of co-development with all the stakeholders of the project (local communities at the study sites of the project, civil society organisations, political and administrative authorities, municipal authorities and parliamentarians, traditional and religious leaders etc. The dialog is ongoing, and the engagement is inclusive to all parts of the community. The project only operates with the acceptance and the cooperation of the impacted communities.

19.1 Social acceptance

The social acceptability on the part of local communities of the controlled environmental release of genetically modified sterile male mosquitoes in the village of Bana or Souroukoudingan was assessed. The community acceptance has been expressed by the customary leaders, officials and representatives of social groups of those villages.

19.1.1 *Impact on prosperity, jobs, incomes in the release sites?*

Target Malaria and IRSS has been generating some income for the communities through small purchases on site and short-term casual worker contracts.

19.1.2 *Impact on Cultural and spiritual values*

The field entomology and engagement activities take into account local cultural and traditional considerations in the area of work and ensure that the teams respect these. For instance, the project complies with local traditions, in terms of traditional authorities and their role. The proposed controlled release should have a limited impact on cultural, traditional, religious or ethical dimensions of the community, but these aspects will be monitored during the study period.

19.2 Ethical and scientific research approvals

All protocols and project studies were evaluated by the appropriate ethics and scientific committees of the IRSS institution of the Centre National de Recherche Scientifique et Technologique (CNRST) in order to ensure that the research activities of the Target Malaria project are carried out in a manner to protect the rights and physical and psychological integrity of individuals who participate or who are directly exposed to the project studies.

19.3 Committee for the management of complaints

At the individual study sites, committees for the management of complaints have been put in place to serve as a management framework for all complaints made against the activities of the project. The committees are composed of members of the Community and of the members of the research team.

19.4 Information exchange with civil society and public sector

Groups have been put in place at the level of civil society and public sector stakeholders to provide a framework for information exchanges regarding the project (Groupes Relais).

19.5 Stakeholder engagement

The project has been presented to stakeholders at different governmental levels and to civil society organisations composed of different non-governmental organisations and religious groups, as well as to the communities in the study sites. These included governmental level, traditional and religious leaders, scientific community, administrative leaders and municipal governments, as well as communities at the study sites. Their comments have been fed into risk assessment by the project and its independent risk assessment.

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