A Risk Assessment of the Potential Use of Gene Drives in Infectious Diseases

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Executive Summary

Gene drives are being explored for alleviating vector-borne infectious diseases however, the risks of employing them need to be understood.

This document assesses the potential use of gene drives in India by performing a stage-wise risk assessment of deploying gene drive.

Gene drive mosquitoes are an application of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology. The application is to develop mosquitoes that decrease the incidence of mosquito-borne diseases.

Current research into engineering these mosquitoes is being funded by the Bill and Melinda Gates Foundation, Defense Advanced Research Projects Agency (DARPA) and more recently, the Tata Trusts. A British company, Oxitec has also been experimenting with CRISPR in Cayman Islands, Brazil and Maharashtra.

Research into gene drives should be promoted however, there are risks associated with their potential use in India. Given the nature of the technology, it is recommended that robust monitoring mechanisms for disease incidence, mosquito burden and ecological impact be implemented before deploying these mosquitoes.

Data driven decisions on identifying the type of gene drive and deployment locations will ensure effective use of the technology.

Gene drives and infectious diseases

- In India, infectious diseases have a high economic burden.[1]
- Gene drives refer to Gene Edited Mosquitoes (GMM) which can pass on their gene modification to future generations of mosquitoes.
- Scientists are currently developing GMMs to help alleviate incidence of malaria and dengue, amongst other vector-borne diseases.
- Given the economic burden of vector-borne infectious diseases in India, it is prudent to explore gene drive technology for eradicating the diseases. However, the technology is still in its nascent stages and an appropriate risk assessment is necessary to make an informed decision and initiate public dialogue over its use.

Steps and stages involved in using a gene drive

Stage 1: CREATE GMM

1. Select the disease to target through GMM
2. Identify the pathogen strain(s) present locally causing the disease
3. Identify the local vector species
4. Choose the most effective GMM strategy to target the disease (see on Page 7)

Stage 2: DEPLOY

1. Choose target area for field trial
2. Evaluate disease burden/mosquito population prior to trial
3. Evaluate disease burden/mosquito population post trial
4. Calculate and deploy GMM based on incidence data and efficacy of GMM as evaluated through trials

Stage 3: MONITOR

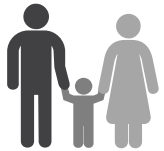
1. Continued surveillance of GMMs and ecosystem to ensure early detection of any unintended consequences

Risk determination matrix

Parameters	Low Risk	Medium Risk	High Risk
Ease of Mitigation	Best practices available	Best practices available	Best practices not yet established
Number of stakeholders involved (Ease of implementing mitigation measures)	Only one (laboratory level)	Multiple (Lab + governmental agencies)	Multiple (Lab + governmental agencies)
Probability of Occurrence	Low	Medium	High

Glossary

Ecosystem – includes humans, animals and plants.



Humans.

Laboratory/Institution: The organisation (private or government) that will create the GMMs.

Government: Governmental agencies (Local, State and Union) responsible for monitoring disease burden (National Vector Borne Disease Control Programme) and approving gene drive research and release.

Key participants and their role in gene drive development and usage

Stage 1: Create GMM

GMMs are engineered in laboratories of either private or public institutions. The designated laboratory would have to demonstrate containment facilities that meet the required safety standards for the containment of mosquitoes and the pathogen under study. Trained personnel will be required to perform experiments and laboratory trials.

There are two known strategies in designing the GMM:

Population Suppression - Decrease fertility of mosquitoes[2]

This approach designs a modified male mosquito that upon mating with a wild-type female will result in an unviable offspring. Thus, over time, the mosquito population will decrease and subsequently, the gene drive will disappear. The disadvantage of this approach is that modified male mosquitoes will need to be released into the environment every season till the entire species is wiped out.

Population Replacement - Reduce capability of GMM to carry pathogen[3]

This approach introduces an anti-pathogen peptide in the mosquito, so that it can no longer host the pathogen. So the mosquito species survives and continues to pass the modification to its offspring. The disadvantage of this approach is that the genetic modification will remain in the environment forever, increasing the likelihood of unintended consequences.

Risk 1a: GMMs have non-target mutations

LOW RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

Gene editing can lead to non-target mutations which may have physiological effects. GMMs may become resistant to insecticide, may harbor other pathogens or may not be effective at preventing the disease they were designed against. Both population suppression and replacement approaches have similar potentials of carrying non-target mutations.

Risk Evidence

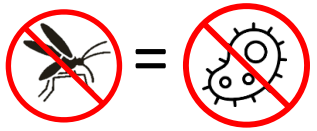
In 2017, Schaefer et al[4], reported the presence of unexpected mutations in mice edited using CRISPR. However, the paper was retracted[5] in March 2018 following criticism that all mutations may not have been caused directly by CRISPR. The presence of non-target mutations is still being debated and hence, as a precaution, should be tested in GMMs.

Potential Mitigation

Laboratory should conduct exhaustive studies to identify non-target genetic changes through gene sequencing across GMM generations. Phenotypic changes in GMM as a result of the mutations should be documented. All non-target mutation data studies should be done prior to deployment of the GMM.

Who will be affected? 

Who is responsible? 

Risk comparison 

Recommendations

- Submission of genetic sequencing data for GMM should be obligatory for deployment approval. Data should highlight any changes in gene sequences and resulting physiological changes in GMM.
- Government approval to field trials and environmental release of the GMM should be contingent on demonstration of no other functional change than that described in the proposal.

Risk 1b: GMM or pathogen becomes resistant to the genetic modification

LOW RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

GMM or pathogen may become resistant to the genetic modification. As species evolve, they may find a way to escape modifications if it does not provide a significant survival advantage. The population suppression approach may be susceptible to resistance development, since the approach is lethal to mosquitoes and evolution pressures will select for resistant mutations.

Risk Evidence

Scientific reports suggest that GMM created to lose viability may develop resistance to the modification.[6,7] A similar selection-based resistance has been observed with the boll worm which developed resistance to the toxin secreted by BT cotton plants.[8]

Potential Mitigation

Exhaustive studies must be conducted to assess when resistance develops across GMM generations. Equivalent resistance studies in pathogens would be difficult because of the requirement of human host for completion of life cycle. Monitoring mechanisms should be put in place to sample the deployed GMM for development of resistance.

Who will be affected?

Who is responsible?

Risk comparison >

Recommendations

- Include a molecular marker to identify GMM for sampling.
- Approval for deployment should be contingent on demonstration of resistance studies. A threshold for number of generations within which resistance may appear should be preset to avoid those GMMs which would be vulnerable to development of resistance quickly.
- Ensure proper monitoring mechanisms are set to capture GMMs for sampling.

Risk 1c: GMMs or pathogens escape from the Laboratory/Transport

LOW RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

If experimental mosquitoes are released, they may influence the environment by mating with other mosquitoes. However, given the low number of GMM that may escape, their impact will be minimal. If the pathogen escapes, it may infect lab personnel and residents in the leak's vicinity. However, given that the spread of the pathogen requires a vector, risk is low.

Risk Evidence

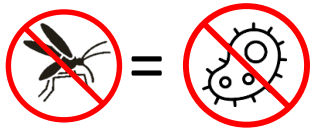
Pathogens have been known to escape from laboratories and infect individuals in the past.[9] The main cause has been poor infrastructure and containment facilities. Escape of mosquitoes is also possible and the Food and Agriculture Organization of the United Nations has issued guidelines for colony maintenance of mosquitoes.[10]

Potential Mitigation

Handling and containment of GMM and pathogens should follow best practices established worldwide. Access to GMM and pathogen should be restricted to trained personnel. Pathogen cultures should be maintained in GLP-compliant facilities under appropriate containment measures.

Who will be affected? 

Who is responsible? 

Risk comparison 

Recommendations

- Government approval for gene drive research should be contingent on demonstration of necessary infrastructure and Good Lab Practices (GLP) compliance.
- Appropriate training for personnel working in laboratory or transport facilities and periodic assessment of the training should be obligatory.
- Funding for gene drive research should include resources for developing and maintaining the infrastructure and best practices.

Create GMM - Recommendations

For Laboratories:

- Appropriate training for personnel working inside laboratory or transport facilities and periodic assessment of the training should be obligatory.
- Funding proposals should include resources for developing and maintaining the infrastructure and best practices.
- Laboratories wishing to do gene drive research should follow GLP guidelines and best practices as outlined by the government to ensure containment of mosquitoes and pathogens.

For Government:

- Government should set standards for institutions wishing to conduct gene drive research. Such standards should include the demonstration of necessary infrastructure, GLP compliance and training of personnel.
- Government should set standards for GMMs to be deployed in the environment. These should include the presence of a molecular marker for identification, absence of functional non-target mutations and minimum number of generations tested for resistance development.

Stage 2: Deploy

Once a GMM has been designed and tested under laboratory conditions, field trials would be conducted in select locations. Deployment of GMMs would be approved contingent upon satisfactory results from the field trials.

Risk 2a: GMMs get deployed at inappropriate spaces because of inaccurate burden data

HIGH RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

Infectious disease burden has been underreported in official records and current inefficient mechanisms of reporting could lead to misidentification of disease hotspots. Absence of robust real-time/predictive data could lead to deployment of GMMs in incorrect spots thereby reducing their efficiency to combat the disease.

Risk Evidence

Studies have reported that dengue incidences in India are nearly 300 times higher than official reports.[11] The incidence of malaria has been inconsistent with WHO estimating 15,000 casualties/year, while other studies suggest a plausible casualty range between 125000 – 277000 people annually in India.[12]

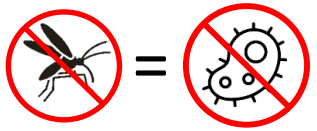
Potential Mitigation

Monitoring mechanisms should be set up to determine disease and mosquito burden before deploying GMMs. A comprehensive study report of the area to be targeted along with analysis of disease burden, mosquito burden and demographic studies should be performed before deploying GMMs.

Who will be affected?

Who is responsible?

Risk comparison



Recommendations

- The first step for GMM deployment would be to capture and validate accurate data on disease burden through improved reporting systems.
- Data on hotpots for mosquito breeding sites would be necessary to ensure appropriate deployment of mosquitoes.
- Analysis of these data to generate predictive models for forecasting breeding hotspots would be beneficial for identifying targets for GMM deployment.

Risk 2b: Migration of mosquitoes affects efficacy of GMMs

HIGH RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

India has favourable weather for mosquito breeding. Expanding city limits provide additional breeding sites. Thus, it is likely that deployment of GMMs may be hampered by migration of mosquitoes from adjoining areas. This is particularly true in population suppression strategy where the availability of food resources may act as an added attractant.

Risk Evidence

Mosquitoes generally live in the vicinity of their breeding sites – travelling roughly 50 – 100 meters. However, they can travel further in search of food resources. Some mosquito species have been known to travel as far as 100-150 km.[13]

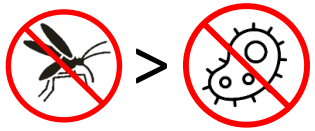
Potential Mitigation

Stronger reporting systems would facilitate data-based deployment of GMMs. Migration may be combated by increasing the deployment of GMMs. Interstate cooperation would be required, particularly in the border areas to ensure efficient use of GMMs.

Who will be affected?

Who is responsible?

Risk comparison



Recommendations

- Constant monitoring of mosquito populations to identify any changes.
- For any significant change in population of any particular species, measures should be put in place for population control.
- For state border areas, interstate bodies should agree on a single action plan for deploying GMMs.

Risk 2c: Spread of mosquitoes/pathogens across the country would affect the efficacy of GMMs

MEDIUM RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

India is home to a wide spread of disease-causing mosquitoes and pathogens. Even for a single pathogen there are various existing vectors. Hence, even if one mosquito species is targeted, the pathogen may just proliferate in another vector species. Thus, for GMM strategy to work, all vector species for a target pathogen species would need to be modified.

Risk Evidence

For example, there are six primary vectors (*Anopheles culicifacies*, *An. dirus*, *An. fluviatilis*, *An. minimus*, *An. sundaicus* and *An. stephensi*) for malaria in India and five of them operate in species complexes.[14] Their distribution across India is variable and needs to be studied locally to define the GMM strategy.[15]

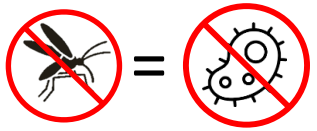
Potential Mitigation

A targeted approach identifying the disease and vector spectrum to be used for GMM has to be identified.

Who will be affected?

Who is responsible?

Risk comparison



Recommendations

- Mosquito populations must be constantly monitored to identify changes.
- GMM deployment should be done concomitant with ongoing measures for mosquito control.
- Government should take expert opinion before approving field trials to prevent overlap of GMMs from different sources.

Deploy - Recommendations

For Deploying Agency:

- Target areas for deployment/field trials should be based on evidence of mosquito breeding hotspots. Mosquito population/disease incidence should be measured for at least 3 years before deployment and 3 years post deployment.
- Sampling of mosquito populations/disease incidence should be performed according to government prescribed standards.
- Any changes in mosquito populations or disease incidence should be immediately reported to the designated governmental authority and laboratory.

For Government:

- Guidelines and best practices for measuring mosquito populations/disease incidence should be prescribed.
- Field trial/deployment approval should be given by a committee of scientists, ecologists and citizens.
- Awareness about GMMs and public engagement over their possible effects should be created before field trials. Representatives from both the laboratory and deploying agency should be present for such engagement.

Stage 3: Monitor

Post deployment of mosquitoes, the ecosystem should be continuously monitored for changes in disease incidence, mosquito populations and food chain impacts.

Risk 3a: GMM have a negative impact on the food chain

MEDIUM RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

Mosquitoes are a part of the food web and reduction in their population may impact other species. For example, reduced pollination because of absence of male mosquitoes may affect plants and thus, other species. Frogs and lizards which eat mosquitoes may be affected, though in a limited manner. This risk is higher for the population suppression strategy.

Risk Evidence

There is considerable debate on how important mosquitoes are in the food chain. Male mosquitoes are not primary pollinators of crops required for human consumption. Predators may also quickly find other insects to fill up their diet. However, specialised predators like the mosquitofish may be severely impacted.[16]

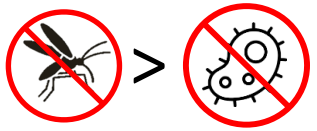
Potential Mitigation

Mathematical and field testing should be done to simulate the impact of population loss on the food chain. Gene drive strategies which inhibit the pathogen instead of reducing mosquito populations may be preferred.

Who will be affected?

Who is responsible?

Risk comparison



Recommendations

- Mathematical modelling and field testing should be done to simulate the impact of population loss on the food chain.
- Identification of species dependent on mosquitoes in the area of deployment should be made and their populations should be monitored for changes.

Risk 3b: Transfer of gene drive to other species

LOW RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

Gene drives may be passed onto other mosquito species or in rare occasions unrelated species. Most known cases of such horizontal gene transfers have occurred as a result of evolutionary dynamics and hence, it is a likely risk with the population replacement strategy where the gene modification will remain in the environment forever.

Risk Evidence

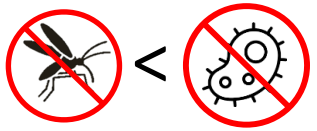
Horizontal gene transfer is the transmission of genomic fragments between organisms other than that from parent to offspring. Horizontal gene transfer has been widely studied as the mechanism by which antibiotic resistance spreads in bacteria.[17] However, such gene transfers have been reported across eukaryotes.[18]

Potential Mitigation

The chances of this occurring are low; however if such a transfer does happen, it could be potentially devastating for other species. Random sampling of other species would need to be performed to identify if such a transfer has occurred.

Who will be affected? 

Who is responsible? 

Risk comparison 

Recommendations

- Random sampling of other species should be performed to identify if there has been any transfer of gene drives.

Risk 3c: Emergence of new species/increased population of another species

MEDIUM RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

Development of resistance or change in ecological balance may lead to the emergence of a new mosquito species or increased population of another species. This may consequently cause an increase in the incidence of other vector-borne diseases. The probability of risk is higher in population suppression because of resource availability.

Risk Evidence

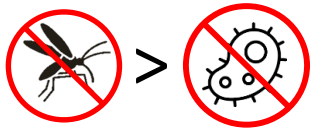
Mosquito populations are known to compete with each other over food resources.[19] It is thus likely, if the population of one species is reduced, other species population may increase. Further, adaptation of other species to exploit the available food resources may also occur.

Potential Mitigation

Gene drive strategies which inhibit the pathogen instead of reducing mosquito populations may be preferred.

Who will be affected? 

Who is responsible? 

Risk comparison 

Recommendations

- Mosquito populations should be closely monitored.
- For any significant change in population of any particular species, measures should be in place for population control.
- For state border areas, interstate bodies should agree on a single action plan for deploying GMMs.

Risk 3d: Transfer of infectious pathogen to another vector

LOW RISK PROBABILITY

CREATE | DEPLOY | MONITOR

Risk Description

In the absence of the existing vector, the pathogen may move into another vector. If this happens, the GMM will have no effect on the disease incidence. The risk probability for this happening is higher in population suppression strategy because the absence of the vector might force the pathogen to find another vector.

Risk Evidence

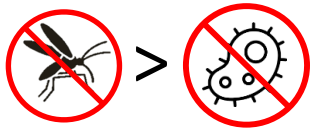
Cross-species transmission of pathogens is a well-studied phenomenon. Both bacteria and viruses have been known to find new hosts.[20] Examples for cross-species transmission include HIV, SARS, and influenza. It is therefore reasonable to conclude that it is probable for such a cross-species vector transmission to occur over time.

Potential Mitigation

There is no way to prevent the transfer of pathogen from happening; but if it does occur, GMMs would have to be created against the new vector.

Who will be affected? 

Who is responsible? 

Risk comparison 

Recommendations

- Monitoring of disease incidence and mosquito population should be performed.
- If a decrease in mosquito population is not correlated by decrease in disease incidence, the possibility of a new vector-host relationship should be researched.

MONITOR - Recommendations

For Monitoring Agencies:

Monitoring agencies should be in place for the following parameters:

- GMM population
- Target mosquito population
- Population of other mosquito species
- Disease incidence
- Impacts on food chain
- Transfer of gene drive to other species

For Government:

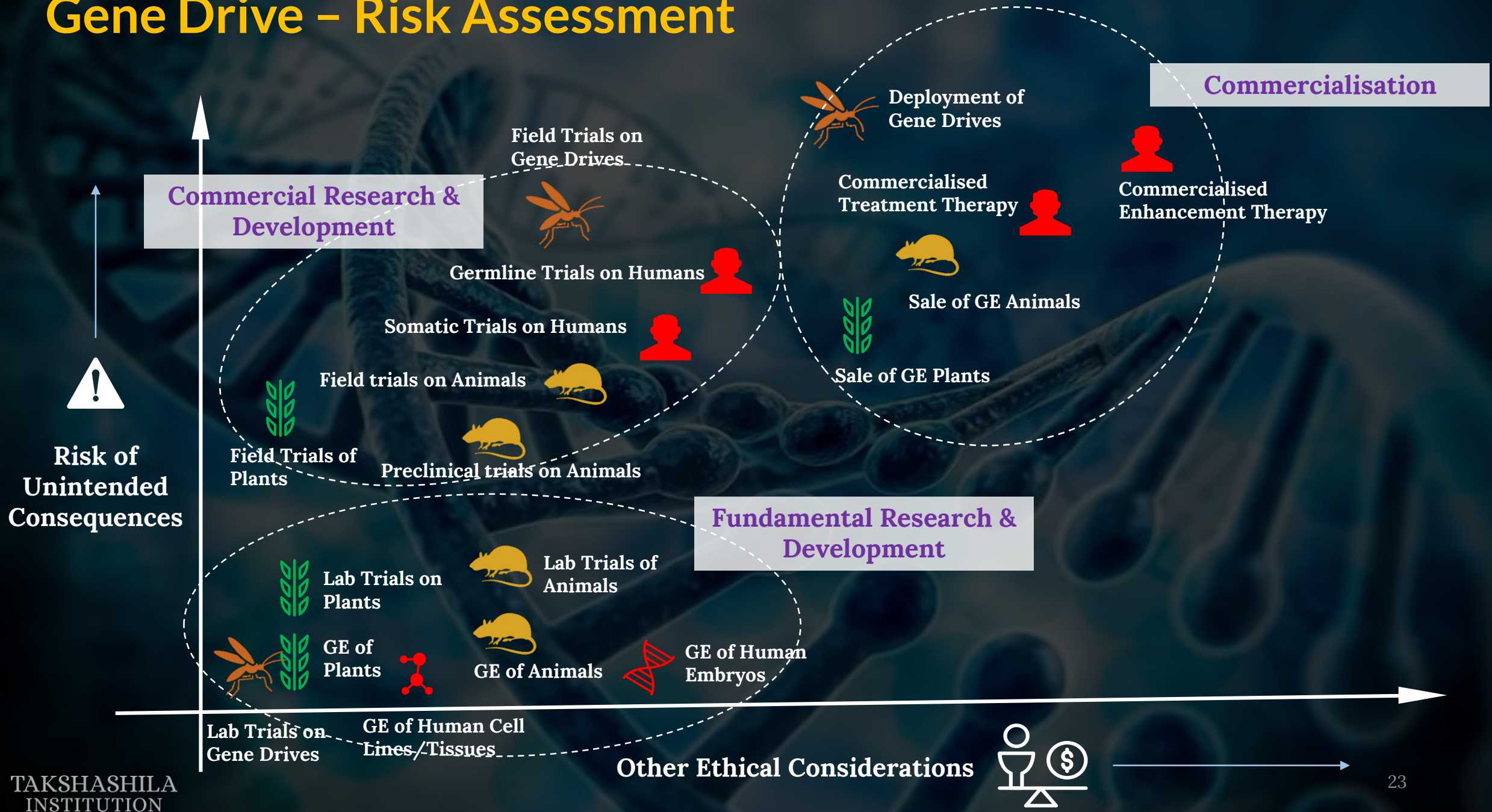
Government should form monitoring agencies and prescribe standards to be followed by them.

Any changes in ecology or adverse events should be reported to the government authority.

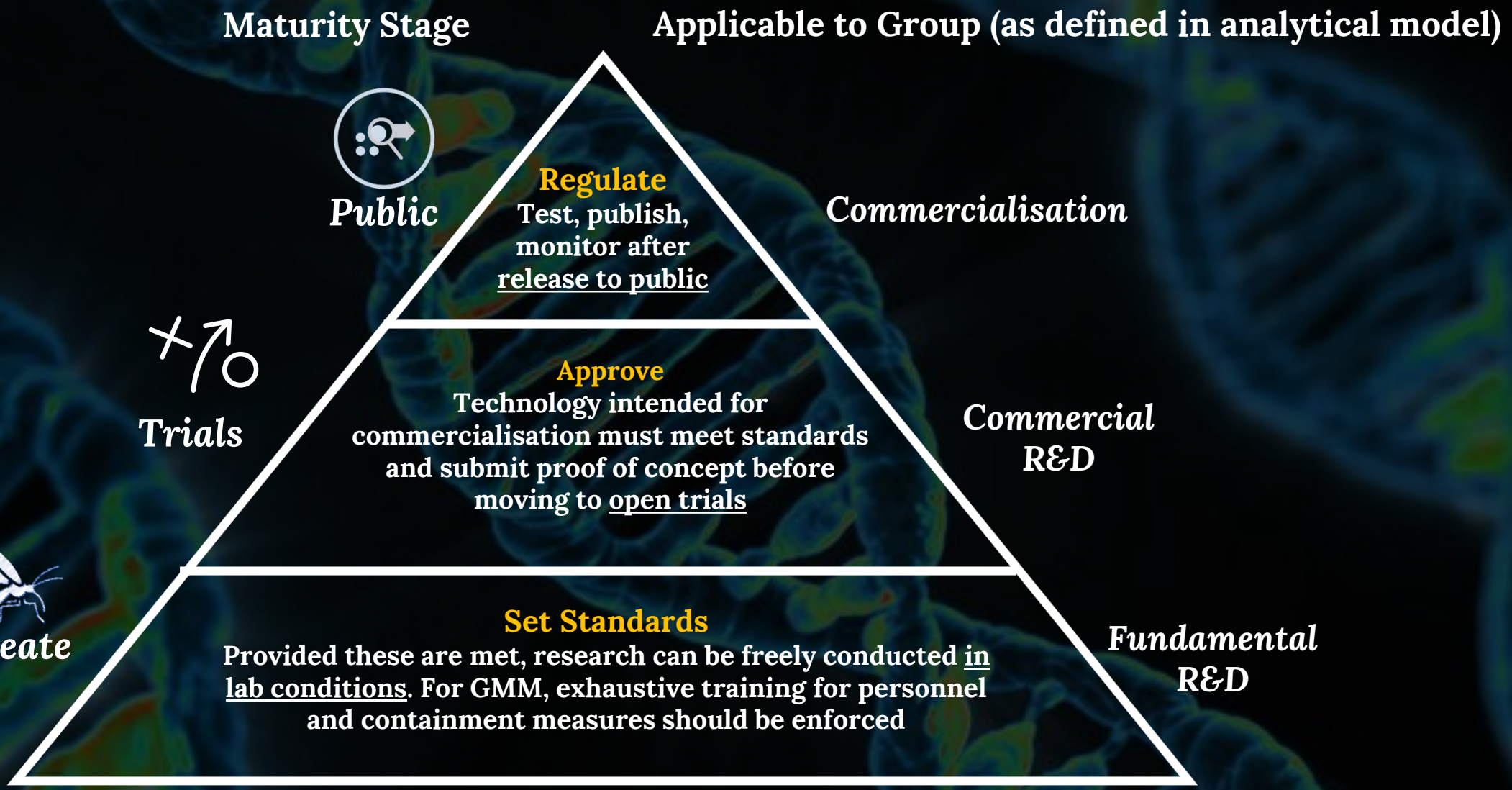
A redressal cell should be set up for addressing concerns arising out of adverse events following GMM use.

Liability of adverse events should be decided between laboratory, deploying agency, monitoring agency and governmental bodies before deployment.

Gene Drive – Risk Assessment



Pyramidal Regulation



Summary of Recommendations

Stage 1: Create GMM

Research into GMMs should be allowed in laboratories with appropriate infrastructure and containment facilities for mosquitoes and pathogen.

Personnel with access to mosquitoes/pathogens should have adequate training and adhere to GLP guidelines.

Stage 2: Deploy

Deployment of mosquitoes should be contingent on absence of any significant non-target mutations and efficacy of GMM in laboratory trials.

Selection of target areas for trials or release should be dependent on accurate data about disease burden and mosquito breeding hotspots.

Stage 3: Monitor

Defined monitoring agencies should be set up to determine mosquito populations, disease incidence and impacts on food chain.

Public engagement and dialogue should precede any trials or deployment of GMMs.

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