Risk assessment of GM insects: key issues

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Overview

- Current situation and pipeline
- Example: Oxitec's GM mosquitoes
- How should decisions be made?
- Summary

GM insects: pipeline

- Current expected commercial applications: **reduction of disease** (malaria/dengue) using GM mosquitoes; **reduction of crop losses** using GM agricultural pests (bollworms, olive & fruit flies)
- Engineered to reduce insect populations (e.g. Oxitec's RIDL) or to reduce disease transmission
- Potential for use in association with GM Bt crops to tackle resistant pests (especially pink bollworm in cotton)
- Production traits: e.g. 'spider silk' and pharmaceutical proteins from GM silkworms (may be contained use)
- Experiments on improving fitness of beneficial insects
- Experiments to use GM mosquitoes to vaccinate people (trigger antibodies)
- Range of species could expand: "5000 Insect and Other Arthropod Genome Initiative" (i5k)

Oxitec's GM technology

- RIDL technology: a conditional lethality trait (offspring die as pupae in absence of tetracycline), plus a fluorescent marker
- **A*edes aegypti* (Yellow Fever) mosquitoes OX153A (bisex RIDL) strain: open release experiments in Cayman, Malaysia, Brazil**
- Female-flightless strains (fsRIDL): *Aedes aegypti* OX3604C, *Aedes albopictus* (Asian Tiger Mosquito) OX3688 and Mediterranean fruit fly OX3647
- OX1138 pink bollworms with fluorescent marker gene were released for 3 years in US (2006-08) in open field trials. OX3402 (RIDL) pink bollworm was given a positive Environmental Impact Assessment by the USDA in 2009.
- R&D: Mexican fruit fly, olive fly, diamondback moth.

Relevant international instruments

- **Cartegena Protocol** to the Convention on Biological Diversity (CPB): covers Biosafety laws; plus export notification requirements for Living Modified Organisms (LMOs)
- **Regulation (EC) No. 1946/2003** specifies the information required for exports of LMOs from EU, including “a previous and existing risk assessment report consistent with Annex II of **Directive 2001/18/EC**”. Copies must be sent to the exporting authority and the EC and be available to the public.
- **Aarhus Convention** (access to environmental information, public participation, access to justice)
- **Helsinki Declaration** (ethical requirements for informed consent to medical experiments)

Cayman Islands

- A British Overseas Territory (one of the last non-self-governing territories)
- Elected legislative assembly (covers domestic affairs): Cabinet selected from members of assembly
- Premier currently under criminal investigation
- A tax haven, but the Cayman Islands Govt is bankrupt
- UK Govt loans: $217m (Oct 2009); $155m (June 2010)
- Governor represents Queen; Deputy Governor runs civil service; UK Foreign and Commonwealth Office responsible for extension of treaties to British Overseas Territories
- **Not covered by the CPB: no biosafety law**
- **Not covered by the Aarhus Convention: no FoI law**
- First open trials of OX513A with Cayman MRCU in late 2009 (mating competitiveness)
- 3 million mosquitoes from May to Oct 2010 (population suppression)
- Inhabited area, not dengue endemic

Cayman: public engagement

- No published risk assessment for consultation (no legal requirement)
- Cayman Mosquito Research and Control Unit (MRCU) YouTube video: http://www.youtube.com/watch?v=_nY_AlWe5kM Describes mosquitoes as sterile, does not mention GM. Put on the Cayman Islands Government Information Service (GIS) website on 4th October 2010 (after the experiments).
- Cayman press reported the idea was being considered (in October 2009), but not that experiments had gone ahead
- Many people did not know about the experiments until after Oxitec and MRCU press release 4th November 2010.
- GM Mosquito Trial Strains Ties in Gates-Funded Project. *Science Insider*. 16 November 2010. http://news.sciencemag.org/scienceinsider/2010/11/gm-mosquito-trial-strains-ties.html?ref=hp

Cayman: export of eggs from UK

- A transboundary movement of a living modified organism (LMO) under the CBD
- Oxitec notified UK Department of Environment (DEFRA) on 1st December 2010 (after UK parliamentary questions, after the trials); RA (dated October 2009) released to UK parliament 13th Jan 2011.
- The EC has no register of transboundary notifications and initially could not decide which department was responsible. It was not notified until after DEFRA.
- The RA plus other docs were supplied to GeneWatch UK by the EC on 3rd Feb 2011.
- The import was approved by Cayman in Aug 2009: before the RA was written.

Standard of the RA

- The RA is the one many of you looked at on the course
- It is supposed to be “a previous and existing risk assessment report consistent with Annex II of Directive 2001/18/EC” (which covers deliberate releases of GMOs in the EU).
- There is no guidance on RA for GM insects in the EU
- There is no independent oversight process for whether or not Oxitec met the standard

Response of DEFRA to complaint about the Cayman egg shipments

- Not appropriate to take enforcement action
- The company did supply the documents, even if they were late
- The key point is that they did liase with the Cayman Authorities
- The company and others have been reminded about the requirements
- (Letter to GeneWatch 9th April 2011)

Malaysia

- Party to the CPB but not the Aarhus Convention
- Biosafety Act 2007
- DEFRA told UK parliament transboundary notification was not needed because import of OX513A strain was for contained use. A new strain OX513(My1) was developed in Malaysia.
- Malaysia published summary RA on website and Biosafety Clearing House of CBD (not a requirement for non-food/non-feed applications)
- Consultation process (including responses to comments)
- Limited 3-day MMR trial in December 2010 (6,000 mosquitoes), uninhabited area; trapping, fogging, post-trial monitoring
- Concerns: process insufficiently publicised; pressure to rush process; trial reported as postponed when it was conducted; full RA not published.
- Unclear how trial feeds into the future assessment process
- Liability issues unclear

Brazil

- Party to the CPB but not the Aarhus Convention
- OX513A field trials approved by Brazil's National Biosecurity Technical Commission (CTNBio) in December 2010 (following public notification in July).
- (Brazil has also imported OX3604C *Aedes aegypti* eggs)
- Partnership with the University of Sao Paulo and Moscamed (funded to develop radiation-induced SIT)
- Releases in state of Bahia, near the city of Juazeiro (northern Brazil).
- Initial releases (10,000 mosquitoes) in February 2011.
- 2nd phase: six weeks of releases at 3,000 males per hectare per week.
- **Inhabited area, dengue endemic**
- House visits, media and pamphlets: but unclear whether this amounts to "fully informed consent" (Helsinki Declaration), including from children.

Brazil transboundary notification documents

- On 13th Jan 2011, DEFRA told UK parliament it had only one export notification from Oxitec (for Cayman): notification for Brazil was not required because all shipments were for contained use.
- Brazil trials started 24th February 2011.
- On 9th April DEFRA informed GeneWatch that a notification was required after all.
- (Partially redacted) documents were finally supplied to us on 4th August 2011 (the initial response withheld the RA as commercially confidential)
- The shipping invoice (5,000 eggs) is dated 9th February, notification is dated February. The RA is a copy of the request to conduct the trials by the University of San Paulo (October 2010, in Portuguese) which is largely a description of the technology & the trials. Plus documents on mating competitiveness, rearing, life cycle, tetracycline contamination.

Summary of process issues

- Use of Cayman (no biosafety law)
- Transboundary notification process not correctly followed; lack of clarity about in-country developed strains
- No register of transboundary movements in EC
- Poor risk assessment standards for Cayman and Brazil: no guidance or oversight, limited content
- Malaysia has recognised more issues should be addressed if there is a larger trial (including consent)
- Issues about transparency and public engagement
- No informed consent
- No requirement under CBD to provide information to Biosafety Clearing House (although Malaysia provided its summary RA)

How should decisions be made?

- Policy/regulatory framework (what are the rules?)
- Role of transparency/consultation/public involvement (deciding who decides)
- (1) Investment decisions (R&D)
- (2) Decisions on open releases (experimental and commercial releases)
- (3) Post-market decisions: monitoring, payments, withdrawal, reversibility, liability

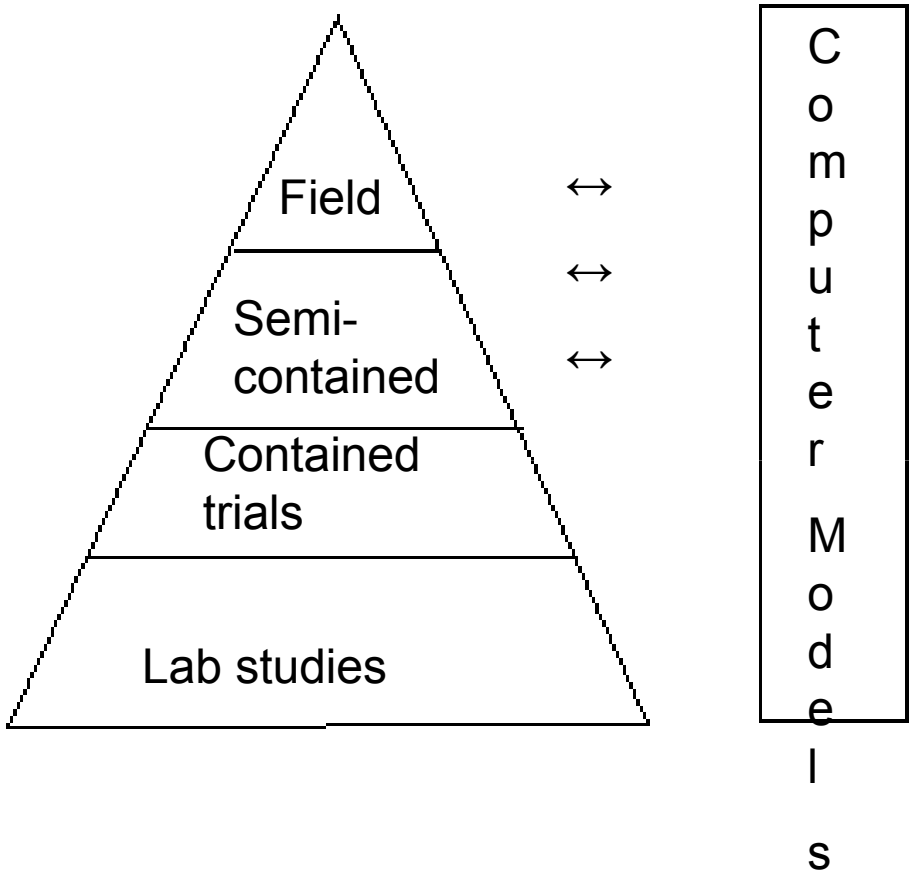
GM crops (for comparison)

- Commercialised GM crops: intended to have a direct effect on pests (Bt crops) or herbicide applications (HT crops). May have unintended (incl. unexpected) effects on biodiversity or human health (e.g. via Bt toxins or herbicide residues). Also have indirect (harmful) effects: resistant weeds and pests; growth in secondary (chewing) pests.
- Intended to be contained in the field. Debate about preserving non-GM/consumer choice.
- Biosafety (incl. unintended health risks) decided by regulators ("acceptable" risk)
- Use decided by farmers (cost/benefit)
- Initially implemented in US, later North-South transfer

GM mosquitoes (in comparison)

- GM mosquitoes: intended to have an indirect effect on disease incidence. Benefit or harm to health depends on interactions with wild species and resulting disease incidence in humans.
- Does mosquito population (or disease transmission) reduce; does this lead to less (or less severe) disease; is this sustained?
- In addition: unintended (incl. unexpected) effects on health or ecosystems (e.g. effects on predators/prey, gene transfer etc.)
- Intended to spread and mate with wild population: no individual opt-out
- Regulators decide 'biosafety' (what is this?)
- Use decided by governments (incl. costs/benefits)
- Initial North-South technology transfer

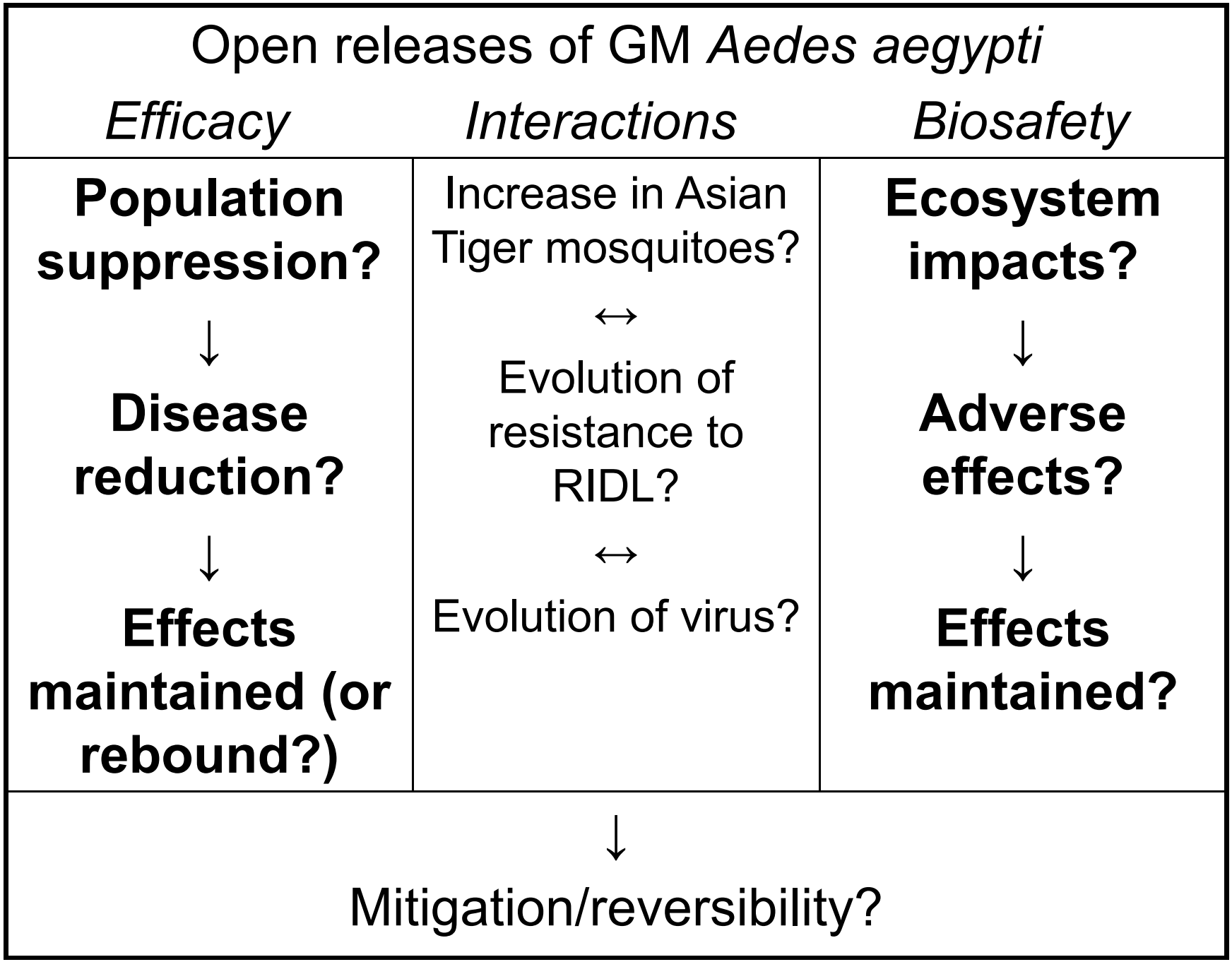
Quantifying risks



From Pew Institute (2004)

The diagram does not show what is really going on

- The trials are designed to demonstrate (short-term) efficacy (or relevant parameters): mating effectiveness, population suppression, dispersal distance.
- They are not designed to answer biosafety questions, or long-term efficacy
- But regulation relates to the biosafety questions and does not cover efficacy!
- A regulatory gap and a data gap


Open releases of GM *Aedes aegypti*
Efficacy
Interactions
Biosafety
Population suppression?
↓
Disease reduction?
↓
Effects maintained (or rebound?)
Increase in Asian Tiger mosquitoes?
↔
Evolution of resistance to RIDL?
↔
Evolution of virus?
Ecosystem impacts?
↓
Adverse effects?
↓
Effects maintained?
↓
Mitigation/reversibility?

What are the biosafety issues?

- Scientific/Technical report submitted to EFSA "**Defining Environmental Risk Assessment Criteria for Genetically Modified Insects to be placed on the EU Market**": includes descriptions of relevant species and transgenic strains; areas of potential risk, methods to investigate adverse effects and key parameters, baseline information (habitats, ecology), surrogate and modelling approaches, expertise, institutes and scientists.
- This is not yet EFSA draft guidance, it is a report to EFSA. It will feed into draft guidance that will then be subject to consultation.

Report to EFSA: areas of potential risk

- Adverse effects associated with gene flow
  - Vertical gene flow to populations of the same or sexually compatible species
  - Horizontal gene transfer
- Interactions of the GM-arthropod with the target organisms
  - Triggering adaptive processes in the target population
  - Host range
- Interactions of the GM-arthropod with non-target organisms
  - Effects on predators and parasitoids
  - Biodiversity
  - Pollination
- Impact on specific agricultural management practices and management measures to control arthropods vectoring diseases
- Effects on biogeochemical processes
- Effects on human health
  - Allergies and irritation
  - Presence of viable female GM mosquitoes
  - Potential changes in vector competence (transmission of diseases)
  - Accidental ingestion (e.g. of larvae, eggs)

CBD guidance

- CBD focus on biodiversity “taking into account human health”: but health assessment is a central part of assessing risks/benefits of releases.
- CBD guidance (currently) does not include effects such as evolution of virus or a rebound in cases of disease.

‘Density-dependent’ effects

- SIT eradication of New World screwworm in US and central America. Very large numbers of irradiated insects needed (billions): typically 10 to 1 wild male, or more.
- SIT is not currently used for mosquitoes
- The problem is ‘density dependent’ effects on population (population growth varying with the density of population): usually caused by a shortage of food or breeding sites
- GM lethality traits are intended to be more effective than SIT: fitness reduced less than with irradiation and larvae compete for food before dying, reducing population further
- But, density-dependence introduces a new level of complexity…

Density-dependence means…

- Effect of GM mosquito releases may vary in different places and at different times, depending on the extent to which other factors (such as shortage of food or breeding sites) restrict the population
- Populations may fluctuate and even increase, rather than reducing
- Oxitec found this effect in a model of SIT, but the model found RIDL was more effective and populations could not increase (Yakob et al. 2008)
- For SIT, they concluded this problem meant that more theoretical assessment is needed involving the disease's transmission dynamics i.e. what happens to disease cases?
- This is a prelimary (unvalidated) model: is it right that this problem cannot occur with RIDL?

Dengue fever

- Dengue fever: 50 million cases/year; 12,500+ fatal; increasing/spreading
- Varying severity: fever, hemorrhagic fever, shock syndrome
- There are four serotypes of the dengue virus. An infected person gains lifelong immunity to that serotype and temporary immunity to the others, but later infection with another serotype appears to enhance severity. Different genotypes in each serotype vary in risk of severity (Medlock et al., 2009)
- *Aedes Aegypti* (Yellow Fever mosquito) also transmits Yellow Fever (200,000 cases/year, 30,000 fatal) and Chikungunya viruses
- *Aedes albopictus* (Asian tiger mosquito) also transmits dengue (more invasive but less effective vector) and Chikungunya

Interaction with human immunity

- "*Dengue researchers do not have a simple and reliable entomological measure for assessing disease risk. ..The currently proposed indices for Ae. aegypti density at best weakly correlate with human dengue infection, and their relationship to disease is understudied. Ae. aegypti mosquitoes persist and effectively transmit dengue virus even at very low population densities because they preferentially and frequently bite humans.* ***A successful GMM dengue control program that falls short of vector eradication will result in a reduction in human herd immunity and a corresponding decrease in already low transmission threshold levels****. Because there is no commercially available vaccine or clinical cure for dengue, predicting and testing transmission thresholds is among the most important unanswered questions in dengue epidemiology and GMM-based control approaches*." (Scott, 2002)

The “rebound effect”

- Is better studied in Malaria
- A reduction in cases leads to a reduction in immunity. If the control measure becomes less effective, there can be a rebound in cases.
- This was confirmed recently in a study of bednets in Senegal (Trape et al.,Lancet, 18th August 2011)

Evolution of the dengue virus

- "*Dengue virulence in mosquitoes can be selected on by transgenic strategies of blocking transmission, decreased mosquito biting, increased mosquito background mortality, and increased mosquito infection-induced mortality. Our results suggest that dengue control strategies that raise mosquito background mortality…pose less risk*…" (Medlock et al. 2009)
- Only one dengue serotype is included in this theoretical computer model

Asian Tiger mosquito, *Aedes albopictus*

- Vector of dengue, chikungunya
- Less effective vector of dengue, but more invasive
- Overlap in habitats
- Possibility that *Aedes albopictus* populations increase if *Aedes aegypti* decrease

Role of computer models

- Computer models are used to predict impacts of GM mosquito releases on wild mosquito populations, and on disease transmission
- Computer models depend on: assumptions in the equations (the conceptual model); knowledge and data from past experiments (e.g. releases of irradiated insects); input parameters (e.g. numbers of matings)
- Computer models of complex models normally give poor predictions. They require calibration with existing data and validation (checking whether their predictions are 'fit for purpose')
- There are uncertainties, unknowns and 'unknown unknowns' and conceptual errors: "There is a lot more than imprecision" (Brian Wynne)
- Bad models lead to bad decisions: e.g. the financial crisis!
- None of the models yet include more than one species of mosquito or more than one serotype of virus

Who predicts/assesses benefit?

- "*Participants wanted to see evidence that GM mosquitoes can reduce malaria prevalence without negative consequences for human health and the environment*." (Marshall et al. 2010 Perspectives of people in Mali toward genetically modified mosquitoes for Malaria control. Malaria Journal, **8**: 128)
- Main concern: that the strategy would not work
- 2nd concern: health effects (e.g. transmission of other diseases)
- 62 participants said they would support a release that satisfied their conditions, 14 said they would not support a release under any circumstances, and four were unsure.

US regulation

- FDA regulates medicines and GM animals
- EPA regulates pesticides (including biopesticides)
- USDA APHIS (Animal Health Protection Act) regulates plant pests
- Who regulates GM mosquitoes? Oxitec favours APHIS co-ordinating with EPA and the Centres for Disease Control (NIH)
- But the FDA's role may be critical to assess some risks e.g. the "rebound effect"

Pew Initative Reports 2004

- Reports critical of lack of a coherent regulatory framework in US
- In US, the FDA would be likely to regulate GM mosquitoes as a drug
- "*An agency like APHIS may have strong authority and expertise to deal with animal and plant health issues but they may not be as well focused on public health*," [US lawyer] Olsen said. "*Vice versa, FDA might have strong authority on public health but they may not be able to adequately assess and manage environmental risks*." EPA might also have a role.

Yes, no or maybe?

- The decision on open releases could be yes, no or maybe (i.e. with safeguards) at any stage in the step up to commercial releases: the process must be able to say no.
- None of these options is ‘irrational’ but they involve different values and interpretations
- Does a public health intervention require universal support?
- What about consent from children?
- Can “sufficient” confidence in the predictions of effects be obtained before each step is taken?
- Who decides what is sufficient? When?
- Are problems reversible? At what point?
- Who is liable if anything goes wrong?

Alternatives

- “Scientifically-informed ethical decisions” require consideration of alternatives;
- All interventions have pros and cons (which can be serious e.g. pesticide poisoning)
- Improved larvicides & pesticides, impregnated curtains, better water containers, other biological controls (e.g. fungi); tackling poverty and water supply in city slums; vaccine development
- Social investment e.g. Cuba island-wide eradication, includes family “self-control” of areas
- Availability of better alternatives depends on investment decisions and approach to innovation
- Diversity of R&D is needed (examples in Cuba: Guzman & Kouri 2009 Lancet, 374, 1660-1)
- Economic choices e.g. money to community workers or scientists? Which scientists?

Malaria “population replacement”

- 300-500 million cases per year; 1 – 3 million fatal
- Complex transmission cycle. Parasite transmitted to uninfected mosquitoes by infected people.
- Several strains of malaria parasite. *Plasmodium falciparum* is the most deadly to humans
- Many attempts in the lab to create a “gene drive” system to replace wild mosquito populations (*Anopheles gambiae*) with GM mosquitoes with reduced malaria transmission
- Greater technical difficulties
- Greater uncertainties (GM mosquito population is intended to expand and permanently replace wild population)
- Quandary: release population must bite humans to survive
- Loss of function over time (by deactivation of genetic mechanism through mutation, or evolution of parasite) could lead to a serious rebound in disease cases
- *Anopheles gambiae* mosquitoes also transmit *lymphatic filariasis* (cause of elephantiasis, transmitted by parasitic worms): 120 million cases/year (non-fatal)

Conclusions

- Notification requirements under the Cartegena Protocol have not been fully met: changes are needed, especially to enforce transparency
- Experiments should not be conducted where there is no biosafety law
- Existing regulation is inadequate to address impacts of GM insects on human (or animal) diseases (and interactions with ecosystems)
- Computer models of mosquito populations are over-simplified and unvalidated
- How will data related to biosafety issues be collected?
- Focus of current assessments is on biodiversity: who will assess impacts on health? And costs/alternatives (technology assessment)?
- Open release experiments do not address most of the key issues and are premature
- Risk assessment guidance is needed
- Medical experiments require informed consent