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# Risk Analysis on the Australian release of *Aedes aegypti* (L.) (Diptera: Culicidae) containing *Wolbachia*

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## 1. ACKNOWLEDGEMENTS

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## 2. EXECUTIVE SUMMARY

**The Bill and Melinda Gates Foundation fund a number of projects under the Grand Challenges in Global Health (GCGH) initiative designed to achieve breakthroughs in global health issues. This includes using novel approaches to control human diseases transmitted by mosquitoes. An international project led by the University of Queensland is responsible for the development of a new biological approach for the control of dengue fever. Dengue is a viral disease which affects 50 - 100 million people annually and is primarily spread by the mosquito *Aedes aegypti* (L.). The proposed approach is to introduce strains of an intracellular endosymbiotic bacterium called *Wolbachia* into *Ae. aegypti*. Different strains of *Wolbachia* produce different effects in the mosquito including a direct blocking of virus transmission as well as reductions in the expected lifespan of the mosquito. Since only old mosquitoes transmit dengue viruses this reduction in lifespan is predicted to reduce dengue transmission. *Wolbachia* are also able to actively spread into insect populations without being infectious. They are transmitted between generations inside the eggs of the mosquito and invade mosquito populations by inhibiting reproduction of females that do not carry *Wolbachia* when mated by *Wolbachia* infected males by a phenomenon known as cytoplasmic incompatibility (CI). This is viewed as a biological control programme with the expectation that the *Wolbachia Ae. aegypti* will be self sustaining after the inoculative release and the beneficial characteristics will be driven into the Australia *Ae. aegypti* populations by the CI mechanism.**

**A trial Australian release of *Wolbachia* infected *Ae. aegypti* has been proposed for the 2010 wet season prior to releases in Thailand and Vietnam. Because of the novel nature of this project, the CSIRO was requested to undertake an independent risk analysis to evaluate the hazards associated with the release. The risk analysis was evaluated against the end point (adverse hazard which we do not want to occur) that the proposed release would result in more harm than that expected to be caused by naturally occurring *Ae. aegypti* ('Cause More Harm'). The risk of this event occurring was estimated with a time frame of 30 years.**

**Because of the novelty and complexity of the study there was a lack of knowledge or actual data on the possible hazards that could occur in the project and allow estimation of risk (the likelihood of an event x consequence of the event). Expert solicitation on risk is appropriate under these circumstances to attain an informed set of *priors* (first assigned scores which can then be tested or updated with new information and forms the basis of this risk analysis.**

**The risk analysis consisted of five stages. Stage one was hazard mapping and fault tree analysis. Hazards were solicited from mosquito experts at a GCGH workshop (17$^{th}$-22$^{nd}$ May 2009) and by email to augment the hazards, social issues and concerns previously identified by the community engagement programme. Fault tree analysis provides a logical structure describing the relationships between hazards. Fifty hazards were identified through the solicitation exercises and construction of the fault tree. The fault tree showed a cut set (shortest possible route to endpoint failure) of some form of ecological harm resulting from the release. As a result Stage two was a one day expert workshop in Cairns (September 18th 2009) on this question of ecological interactions of *Ae. aegypti* and possible impacts that could result from a decline in populations following release. Hazard mapping and fault tree exercises showed that reduced populations could reduce ecosystems services (such as a food source for predators or pollination services) and lead to reduced competition to invasions by mosquitoes inhabiting a similar niche. However the experts concluded that ecological interactions are unlikely as *Ae. aegypti* is an alien invasive species, is highly anthropophilic (needs to lives near humans and requires artificial containers holding water), and has such a low biomass that it would not represent an important food source. Resulting consequences**

**were negligible and it was noted that currently *Ae. aegypti* populations are continually reduced as a strategy to prevent disease transmission by insecticide treatments and habitat removal that have numerous non-target impacts.**

**Stage three was a one day workshop in Cairns on the 17th September 2009. The goals of this workshop were firstly to combine mosquito and community experts to first model the relationships between the end point hazards and then to assign failure likelihoods for each hazard as a set of *priors* (first set of estimates). A Bayesian Belief Net (BBN) was used as the tool to model the risk analysis as it uses a graphical interface to allow users to explore how probabilities assigned to hazards affect the hazards they are linked with. Following review of a draft model where the experts were allowed to modify, remove or add nodes the resulting BBN for 'Cause More Harm' contained 30 nodes representing key hazards including the adverse endpoint. The hazard themes in the BBN described ecological impacts, a degradation in the effectiveness of mosquito control, changes to public behaviour, the reduction in standards of public health, and economic harm.**

**A set of likelihoods was elicited for the summary (child) nodes through breakout groups although full workshop consensus was not achieved. This provided an estimated likelihood of failure of 97.9% for 'Cause More Harm'. Stage four was the by email solicitation of expert scores on the remaining 23 parent nodes (nodes that feed into the child nodes but do not themselves have inputs from other nodes) that had not been scored during the workshop. Twenty experts responded but the results were notable for divergence and outliers, with low agreement amongst experts indicating high uncertainty from a number of sources including linguistic interpretation of the hazard definitions without the context of the previous workshop. As a result the modal score was used to populate each hazard as the mean values were not considered representative of group scoring behaviour. The resulting BBN provided an estimated failure likelihood of 77.8% for 'Cause More Harm'.**

**Stage Five was a two day workshop in Brisbane over 28$^{th}$-29$^{th}$ January 2010 to address a number of issues including the high uncertainty and lack of a full consensus score for any parts of the BBN. The high uncertainty in the email response and lack of a consensus expert scores suggested that the existing *priors* were unlikely to reflect the expert opinion of risk associated with this project. Estimates for both likelihood and consequence were solicited to allow a calculation of risk (likelihood of an event x consequence of the event). After review and populating using consensus likelihoods, the final BBN for 'Cause More Harm' contained 30 nodes, 38 links and 363 conditional probabilities. The 'Tourism' (reduction in tourism) and 'Dengue evolution' (dengue evolves to overcome inhibition by *Wolbachia*) hazards were noted as having a significant contribution to the endpoint estimate and had original risk estimates of 10% failure. These results were re-solicited from the workshop experts by email using a 100 point rather than a 10 point scale to attain more accuracy (n = 6 returns )and had final failure estimates of 2% for tourism and 3% for dengue evolution. This resulted in the approximate halving of the end point failure likelihood of 'Cause More Harm' from 25.4 to 12.5% likelihood that some form of harm could eventuate over the 30 year time frame from the release. It is important to note that likelihood does not equate to risk, as risk is the product of likelihood x consequence. Sensitivity analysis suggests that the most important individual node that could contribute to reduction of the endpoint likelihood was a hazard of a decline in 'Mosquito Management Efficacy'. 'Mosquito Management Efficacy' was defined as management and control of *Ae. aegypti* and took into account factors such as need for control, emergence of insecticide resistance and household control practices. This hazard was most influenced by the hazard that there would be a perception that the release had solved the dengue problem and hence less mosquito control effort would occur.**

**Risk was calculated for the final 30 hazards using the consensus likelihood and consequence scores. The highest estimated risk was for ‘Perceptions’ (defined as the likelihood of the belief that the threat of dengue had been eliminated) which scored as low risk. Four hazards were scored as very low risk,**

> **‘Avoidance Strategies’ (defined as the likelihood of change in normal mosquito avoidance strategies because of the presence of *Wolbachia Ae. aegypti*),**
>
> **'Household Control’ (defined as the likelihood that households in areas containing *Wolbachia Ae. aegypti* will change their expenditure and effort to control mosquitoes because of perceptions about the *Wolbachia Ae. aegypti* mosquito),**
>
> **‘*Wolbachia* Fitness’ (defined as the likelihood that a genetic change in *Wolbachia* will cause a fitness change in *Ae. aegypti*),**
>
> **’Mosquito Density’ (defined as the likelihood that the average density of *Wolbachia Ae. aegypti* (e.g. average numbers per household) will be higher than would occur for the naturally occurring *Ae. aegypti*)**

**The remaining 25 hazards were all scored as having negligible risk through 8 different combinations of likelihood and consequence. This included the endpoint hazard of ‘Cause More Harm’.**

**This set of *priors* represents the opinion of the involved experts under current knowledge and should be treated as a baseline estimate against which the effects of introducing new data or information can be evaluated. Within the constraints of a risk analysis process incorporating expert opinion and the noted presence of uncertainty at various stages, these *priors* provide an estimate that there was a negligible risk of the release of *Wolbachia Ae. aegypti* resulting in more harm than that currently caused by naturally occurring *Ae. aegypti* over a 30 year timeframe.**

## 3. STRUCTURE

With the exception of risk mitigation or communication components which are outside the scope of this analysis, this report is based on the Risk Analysis Framework developed by the Office of the Gene Technology Regulator (OGTR)[2]. This documents the Australian risk analysis process on the release of genetically modified organisms (GMO). The OGTR has already ruled that mosquitoes containing *Wolbachia* are not considered to be GMOs. However, the format required by the OGTR provides an appropriate structure for a proposal to release an organism with a novel modification into the environment. This report provides a basic project background and summary of the three organisms pertinent to the risk analysis; the host organism *Ae. aegypti*, the dengue virus, and the *Wolbachia* bacteria responsible for the modification. The risk analysis section describes problem formulation, hazard mapping and fault tree analysis, and construction of a Bayesian belief net used to capture expert opinion of the likelihood and consequence of the project hazards.

## 4. EXPERT COMPOSITION

Appendix 1 details the participation of experts against each stage. For transparency we have additionally flagged participating experts considered directly associated with the Grand Challenges in Global Health (GCGH) project.

## 5. GLOSSARY

| | |
|---|---|
| *Ae. aegypti* | abbreviated name of *Aedes aegypti*. |
| Arbovirus | (arthropod-borne-virus) viruses that are transmitted by insects such as mosquitoes. |
| BBN | Bayesian Belief Network. |
| BGS trap | a commercially produced passive trap used to capture mosquitoes |
| Child node | A node in a BBN that receives input from other nodes |
| CI | cytoplasmic incompatibility |
| CPT | conditional probability table |
| CSIRO | Commonwealth Scientific and Industrial Research Organisation |
| DART | Dengue Action response team |
| DCG | Dengue Consultation Group (Appendix 1) comprising mosquito experts or GCGH project members asked to provide expert opinion |
| EIP | Extrinsic Incubation Period, the time period between an arthropod vector taking up a pathogen and being able to transmit that pathogen to a host |
| GMO | Genetically modified organism |
| Horizontal transfer | The transfer of *Wolbachia* from one organism such as *Ae. aegypti* to another species |
| Fitness | describes the ability of an organism to survive and pass on its genes |
| Mb | Megabase, a measurement of the length of DNA or RNA, representing one million nucleotide bases |
| *Wolbachia Ae. aegypti* | *Ae. aegypti* containing *Wolbachia* |
| mtDNA | abbreviated form of mitochondrial DNA |
| MRF | mosquito research facility based at James Cook University, Cairns |
| OGTR | Office of the Gene Technology Regulator |
| Parent node | A node in a BBN that feeds into but does not receive links from other nodes |
| PCR | Polymerase Chain Reaction, a molecular method that amplifies specific sections of DNA |
| *Prior* | A first set of estimates used in a BBN |
| Naturally occurring *Ae.* | Naturally occurring *Ae. aegypti* that does not contain *Wolbachia* |

[2] (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1).

| | |
|---|---|
| ***aegypti*** | |
| ***Wolbachia*** | **a genus of bacteria that naturally occur in more than 20% of all insect species** |

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## 10. BACKGROUND

**The Bill and Melinda Gates Foundation together with the Foundation for the National Institutes of Health fund and manage the Grand Challenges in Global Health (GCGH) initiative to solve global health issues. An international collaboration led by the University of Queensland is responsible for the project "Modifying Mosquito Population Age Structure to Eliminate Dengue Transmission" which falls under Grand Challenge 7 (Develop a Genetic Strategy to Deplete or Incapacitate a Disease-transmitting Insect Population) of the Foundation Goal 3 (Control Insect Vectors). This project is evaluating the possibility of modifying the transmission of mosquito borne diseases such as dengue by either preventing the virus's development in the vector, or by shortening the vector's lifespan. Because dengue has an extrinsic incubation period (EIP) of between 8-12 days in the host before it can be transmitted, virus transmission is by older mosquitoes. Consequently, life span reduction of vectors could theoretically prevent pathogen transmission (Cook *et al.* 2007; Rasgon *et al.* 2003; Brownstin *et al.* 2003). Further, some *Wolbachia* have the ability to suppress the reproduction of RNA viruses including dengue (Hedges *et al.* 2008; Teixeira *et al.* 2008; Moreira *et al.* 2009; Osborne *et al.* 2009) in the host and this offers an additional capacity to suppress the transmission of dengue.**

**The target is the yellow fever mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). This is the primary vector of dengue that affects approximately 50-100 million people worldwide annually (Gubler 1998). *Aedes aegypti* was modified by the stable introduction of the obligate symbiotic bacteria *Wolbachia pipientis* (McMeniman *et al.* 2009). *Wolbachia* can confer a range of beneficial, neutral and pathogenic phenotypic characteristics on hosts (McGraw & O'Neill 2004) including cytoplasmic incompatibility (CI) where matings between infected males and uninfected females are generally sterile. In contrast infected females can successfully reproduce with both infected and uninfected males, and this reproductive advantage facilitates the spread of *Wolbachia* in populations. The *Wolbachia* strain was identified as a consequence of its capacity to shorten the life span of *Drosophila melanogaster* through over-replication in host tissues (Min & Benzer 1997; Reynolds *et al.* 2003). Both the CI and life shortening phenotypes were evident after transfer to *Ae. aegypti* (McMeniman *et al.* 2009) as was the suppression of dengue replication in the vector (Moreira *et al*. 2009).**

**Release of *Ae. aegypti* containing *Wolbachia* into Australia has been proposed with similar releases planned for Thailand and Vietnam if this is successful. The proposed release is proposed for December 2010 in the Cairns region in Far North Queensland. Preparations for the release include an extensive community engagement programme and the opening of a dedicated mosquito research facility (MRF) in Cairns to evaluate and mass rear the *Wolbachia* mosquitoes. Because of the novel nature of this proposal, the CSIRO was requested to undertake an independent risk analysis on the proposed release via engagement with community and mosquito experts using a series of risk analysis tools, and the results of this process are reported here.**

## SECTION ONE: BACKGROUND ON ORGANISMS

### 1. *AEDES AEGYPTI* (L.)

#### 1.1. Taxonomy

The genus *Aedes* (Diptera: Culicidae) contains at least 700 species and is divided into a number of sub-genera including *Aedes* and *Stegomyia*. Two *Ae. aegypti* strains are recognised, *Ae. aegypti aegypti* and *Ae. aegypti formosus*. *Ae. aegypti formosus* is African in distribution, prefers natural breeding sites (is partially sylvatic) and is less anthropophilic than the globally widespread *Ae. aegypti aegypti* which is associated with the transmission of dengue viruses (Mousson *et al.* 2005).

#### 1.2. Biology

*Aedes aegypti* is highly domesticated, living exclusively in the presence of human habitation (Vezzani *et al.* 2005). Breeding is tied exclusively to artificial containers holding water such as pot plant holders, discarded tyres, water tanks, drains and roof guttering where developing larvae feed on detritus (Chadee 2004; Montgomery & Ritchie 2002; Montgomery *et al.* 2004). Hence, breeding site removal is an important component of population control (Vezzani & Albicocco 2009; Vezzani *et al.* 2004). Adults typically rest indoors, and females tend to blood feed during light hours with peaks in the early morning for several hours and again in the late afternoon (Gubler & Meltzer 1999). Feeding is nearly exclusively on human blood (Harrington *et al.* 2001; Scott *et al.* 2000) and multiple blood meals may be taken from different hosts during each oviposition cycle (Michael *et al.* 2001; Scott *et al.* 1993; Yasuno & Tonn 1970). A small fraction of avian and bovine feeding events have been detected, but these appear to be exceptional events (Tandon & Ray 2000).

*Aedes aegypti* is a small mosquito (3-4 mm long) and holometabolous, undergoing complete metamorphosis from egg through four larvae stages, pupation and adulthood, with larval development taking about 2 weeks depending on environmental conditions (Hopp & Foley 2001). Males have a lifespan of around two weeks compared to the females which can live for several months. Fecundity varies with ranges from 47.1 ± 14.2 to 307.4 ± 86.4 eggs per female in Argentinean populations (Tejerina *et al.* 2009) and >300 eggs/female reported in two Indonesian populations (Wahyuningsih *et al.* 2006).

#### 1.3. Distribution and Dispersal

*Aedes aegypti* originates from Africa, but is now distributed globally in tropical and subtropical regions (Figure 1). Global redistribution was assisted by mass human migrations, first to the New World associated with the slave trade between the 15$^{th}$ to 19$^{th}$ centuries and secondly to Asia as a result of trade during the 18$^{th}$ to 19$^{th}$ centuries. A third, more thorough worldwide redistribution occurred after the Second World War (Mousson *et al.* 2005).

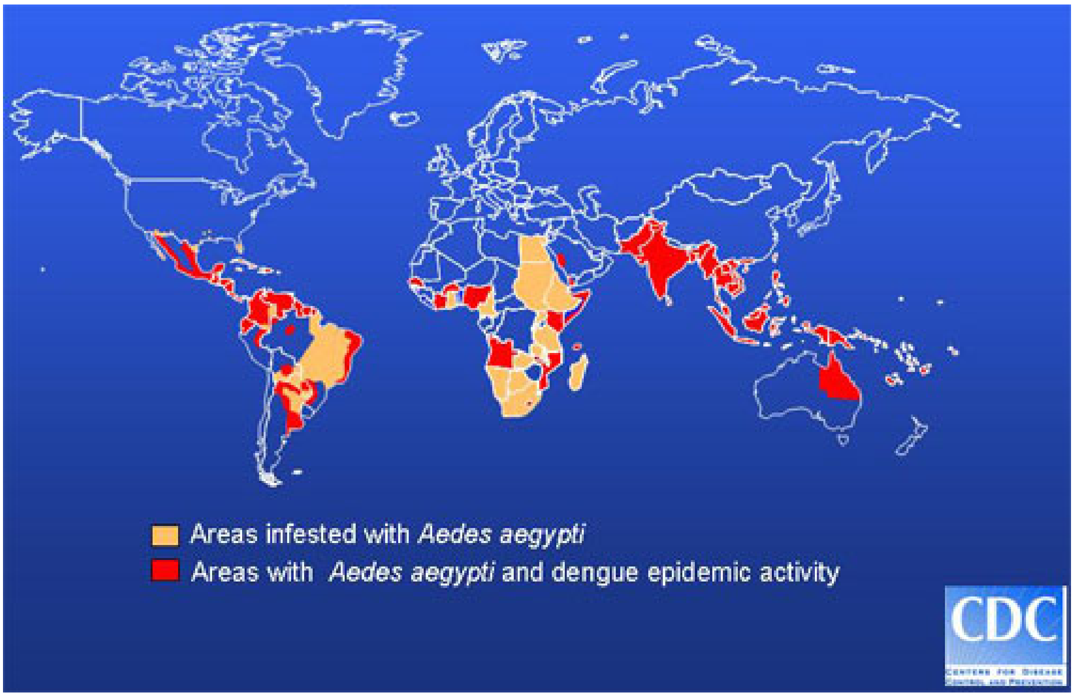


**Figure 1. Distribution of dengue fever and host *Ae. aegypti* as at 2005 (Source: Centre for Disease Control and Prevention[3]).**

**The current Australian distribution of *Ae. aegypti* is primarily restricted to northern areas of Queensland. However, Figure 2 from Kearney *et al.* (2009) shows that the distribution spanned the Northern Territory through to New South Wales and included Western Australia in the early 1900s. This range reduction is believed to be partially the result of the reduction in suitable breeding habitats associated with increased use of reticulated water (Beebe *et al.* 2009).**

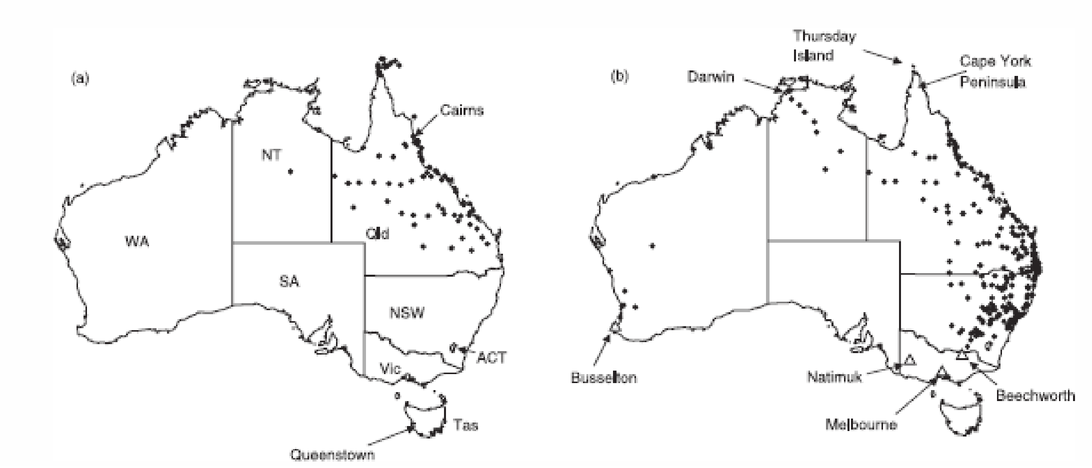


**Figure 2. Current and historic (early 1900s) distributions of *Ae. aegypti* in Australia (From Kearney *et al.* 2009).**

**Takahashi *et al.* (2005) explained *Ae. aegypti* dispersal as occurring in three modes. Winged flight by females searching for human blood meals or oviposition sites result in limited spread, whereas strong wind currents may allow more passive and sudden movements of populations into new areas. However, longer distance dispersals tend to be the result of anthropogenic activities, particularly by**

**[3] (http://www.cdc.gov/ncidod/dvbid/dengue/map-distribution-2005.htm)**

**transport systems that circumvent natural barriers, potentially moving different life stages thousands of kilometres in days (Harrington *et al.* 2005; Merrill *et al.* 2005; Maciel-De-Freitas *et al.* 2007; 2004; Reiter 2007).**

**In Australia, Muir and Kay (1998) released fluorescently marked male and female *Ae. aegypti* in North eastern Australia and recorded a maximum dispersion of 160m for both males and females, with mean recapture distances of 56 m for females and 35 m for males. Russell *et al.* (2005) monitored the dispersal of 1948 fluorescently marked *Ae. aegypti* in Cairns over 15 days within a 200m radius of the release site and for the 67 females recaptured (3.4%), the mean dispersal was 78m, with 23% of captures made outside the 100m radius. A catch made at the 200m limit suggests the mosquito could have spread beyond this distance and *Ae. aegypti* has been reported dispersing 800m in 6 days (Honorio *et al.* 2003) and 690m with a mean distance of 288m in nine days (Maciel-De-Freitas & Lourenco-De-Oliveira 2009) in Rio de Janeiro. Reiter (2007) evaluated available mark and release data for *Ae. aegypti* and concluded that initial daily flight capability estimates ranging from 25-30m underestimated dispersal ability, as mature adults could average over 11 km daily in laboratory flight mills. A key factor in dispersal is the 'need' to disperse and so may be influenced by the size of the pool of available feeding hosts and oviposition (egg laying) sites such as water containers, a deficiency of either could trigger longer distance dispersal.**

## 1.4. *Aedes aegypti* as a vector of dengue viruses

***Aedes aegypti* is medically important as the primary vector of arboviruses (arthropod borne viruses) such as Chikungunya, yellow fever and in particular dengue (Pialoux *et al.* 2007; Ponlanwat & Harrington 2005). Dengue is a positive sense single stranded RNA [(+)ssRNA] virus of approximately 10-11 kb in size in the genus *Flavivirus* (Henchal & Putnak 1990; Russell & Dwyer 2000; Zanotto *et al.* 1996). Molecular studies have shown the Flavivirus arboviruses have undergone an explosive radiation in the last 200 years, attributed to the world wide intermixing of hosts, vectors and virus as a result of ever increasing and dispersing human populations (Zanotto *et al.* 1996).**

**Globally, the number of annual dengue cases exceeds 50 million (Rigau-Perez *et al.* 1998). Dengue presents a range of clinical symptoms responsible for its synonym 'break bone fever' including headaches, muscle pain, nausea etc (Gubler 1998). Dengue viruses consist of four related serotypes (DENV-1, DENV-2, DENV-3 & DENV-4) and it is not uncommon to have several serotypes circulating simultaneously in a region. Exposure to one serotype provides immunity, but not cross immunity to other serotypes, exposure to which can increase risk of contracting the more serious Dengue Haemorrhagic Fever (DHF) which has resulted in over 70 000 deaths since the 1950s worldwide (Deen 2004). Thailand, where all four serotypes circulate, had over 850 000 DHF cases between 1983 and 1997 (Cummings *et al.* 2004).**

**Dengue incubation in human hosts following biting averages 4-7 days with a 3-14 day range. The febrile phase which lasts an average of five days results in the virus entering peripheral blood supplies and potential exposure to blood feeding vectors. The extrinsic incubation period (EIP) describes the developmental time required for a pathogen in an arthropod vector before it can be transmitted and is about 8-12 days for dengue in *Ae. aegypti* (Gubler & Meltzer 1999; Brownstin *et al.* 2003). This lag between uptake and transmission explains why older females are more important in transmitting the virus. Infected mosquitoes can also vertically transmit dengue to their offspring via the eggs (Joshi *et al.* 1996) although the importance of transovarial transmission is controversial.**

**Dengue is considered endemic to the Americas, Southeast Asia, western Pacific, Africa and the eastern Mediterranean, but is not currently endemic to Australia (Russell & Dwyer 2000). According to Esteva and Vargas (1998), dengue is only endemic in tropical regions where a combination of**

**suitable climate and weather allows continuous mosquito breeding. In Australia and temperate and subtropical and tropical regions where it is not endemic, dengue needs to be continually reintroduced by infected travellers (Gould & Solomon 2008). Dengue was considered absent from Australia for 25 years until it reappeared in 1981 (Dwyer 2002). Queensland Health declared the North Queensland dengue epidemic during the 2008/9 wet season to be the largest for over 50 years. Of the 1025 cases confirmed by September 2009, 931 were DENV-3 with the remaining 94 representing the three other serotypes (Anon 2009a). This is substantially smaller than the Townsville outbreak of 1953-55 estimated to involve ~15 000 cases (Anon 2009b).**

## 2. *WOLBACHIA*

***Wolbachia* are obligate intracellular endosymbiotic bacteria belonging to the order *Rickettsiales* and are classified as strains of one species (*W. pipientis*) (Perlman *et al.* 2006). Rickettsiales appear to have a more dynamic genome with more repeats and labile genetic components than found in other eukaryote inhabiting bacteria (Wernegreen 2005) and include a unique bacteriophage designated as WO phage (Sanogo *et al.* 2005) which may be associated with CI (Bordenstein & Reznikoff 2005). *Wolbachia* genomes range in size from about 1 to 1.6 Mb, and the *Wolbachia* strain introduced into *Ae. aegypti* has a genome of ~1.36 Mb (Sun *et al.* 2001) as opposed to 1.27 Mb for wMel (Wu *et al.* 2004). The major genetic difference between *w*MelPop and *w*Mel is a single genomic inversion (Sun *et al.* 2003). *w*Mel was thought to contain large amounts of repeated DNA mobile genetic elements (Wu *et al.* 2004) although subsequently it was found to have a smaller genome and less mobile elements than in *w*Pip (Klasson *et al.* 2008). Because both mtDNA and *Wolbachia* are maternally inherited, *Wolbachia* have been associated with decreases in mtDNA diversity (Hale & Hoffmann 1990; Hurst & Jiggins 2005). Riegler *et al.* (2005) have found evidence of a global *w*Mel sweep in *D. melanogaster* that carried a particular mtDNA haplotype into high frequency and Turelli *et al.* (1992) observed a *Wolbachia* infection of *D. melanogaster* in California where all infected flies had the same mtDNA haplotype.**

**The bacteria has been observed in a wide range of invertebrates including crabs, mites and filarial nematodes (Sun *et al.* 2003), and generally behave as parasites in arthropod hosts and as mutualists in nematodes (Fenn & Blaxter 2006; Werren *et al.* 2008; Mercot & Poinsot 2009). There is currently some controversy as to whether the *Wolbachia* that infect filarial nematodes should be classified as a separate species as their biology is quite distinct to the *Wolbachia* that infect insects (Pfarr *et al*. 2007). The presence of *Wolbachia* can be detected in the host by PCR using primers specific to *Wolbachia* such as the *Wolbachia* outer surface proteins (*wsp*) (Braig *et al.* 1998; Dobson *et al.* 1999) or the *fts*Z gene (Lo *et al.* 2002). An estimated 20% of all insect species contain *Wolbachia* (Cook & Butcher 1999; Werren 1997), but this is likely an underestimate because of low prevalence infections, inadequate sampling and false negatives as a result of PCR primer sets not being able to amplify all *Wolbachia* (Jeyaprakash & Hoy 2000; Weinert *et al.* 2007) and Stevens *et al.* (2001) suggests that a more likely figure is that 75% of all arthropod species are infected. *Aedes aegypti* is not known to naturally harbour *Wolbachia* (Ruang-areerate & Kittayapong 2006) although many other species of mosquito are known to be infected (Tsai *et al.* 2004; Rasgon & Scott 2004).**

***Wolbachia* are maternally transmitted, and infect reproductive tissues and manipulate the host reproductive cycle to increase their spread (Stevens *et al.* 2001; Tram *et al.* 2003; Werren 1997). Reproductive strategies associated with *Wolbachia* infection include parthenogenesis, male killing or feminisation, sex ratio distortions (Cook & Butcher 1999; Dyson *et al.* 2002; Hurst *et al.* 2002) and cytoplasmic incompatibility (CI) (McGraw & O'Neill 2004; Turelli 1994). Although exactly how CI is achieved has not yet been resolved, cytoplasmic incompatibility offers the potential to control arthropod transmission of disease agents by providing a drive mechanism by which *Wolbachia* could invade a target host species and confer a desirable trait such as virus blocking (Kent & Norris**

**2005; Poinsot *et al.* 2003). CI provides an asymmetric mating advantage to infected females who can mate successfully with either infected or uninfected males, whereas matings between uninfected females and infected males are sterile. Multiple *Wolbachia* strains may circulate within a host species, leading to super-infections and bi-directional incompatibility within populations (Hoffmann & Turelli 1988). *Wolbachia* induced CI potentially allows a relatively small number of propagules to drive through an uninfected population. This has been observed in both the field (e.g. Hoffmann *et al.* 1986; Turelli & Hoffmann 1991) and laboratory, e.g. after Xi *et al.* (2005) successfully introduced the *w*AlbB strain into an *Ae. aegypti* culture they were able to fix it in a caged *Ae. aegypti* population within seven generations. Brownstein *et al.* (2003) used models to show that low initial frequencies of *Wolbachia* modified *Ae. aegypti* (0.2 – 0.4) could drive into a population and substantially reduce dengue transmission, but this was limited by the rate of CI achieved and any reduction on host fecundity.**

***Wolbachia* may induce a range of beneficial, neutral or pathogenic phenotypes in hosts (Stouthamer *et al.* 1999; Weeks *et al.* 2002), including life extension under dietary restriction (Mair *et al.* 2005) or life shortening (Min & Benzer 1997), increased immune response to filarial nematodes (Kambris *et al.* 2009), increased fecundity (Vavre *et al.* 1999; Wade & Chang 1995) or reduced fecundity (Hoffmann *et al.* 1990; Min & Benzer 1997; Silva *et al.* 2000; Wenseleers *et al.* 2002; Wright & Barr 1980), reduced ability to disperse (Silva *et al.* 2000) and reduced adult survival and locomotor performance (Evans *et al.* 2009; Fleury *et al.* 2000; Peng *et al.* 2008). *Wolbachia* is believed to have originated in laboratory *D. melanogaster* cultures because it has not been detected in natural populations. It was first noted for its effect in reducing *Drosophila melanogaster* fitness and lifespan by prolific replication causing tissue damage (Min & Benzer 1997). In addition to life shortening, *Wolbachia* causes a 'bendy proboscis' phenomenon in ageing *Ae. aegypti* females where they cannot penetrate human skin to blood feed (Turley *et al.* 2009). *Wolbachia* have also been implicated in providing resistance to RNA viruses in their hosts by delaying accumulation of the virus. This was demonstrated by Teixeira *et al.* (2008) and Hedges *et al.* (2008) where *Wolbachia* infected *Drosophila melanogaster* lived significantly longer than uninfected flies challenged by RNA infection. This resistance did not apply to a DNA virus. Subsequently it has been shown that *Wolbachia* can interfere with a range of pathogens infecting *Ae. aegypti* including filarial nematodes, bacterial pathogens, dengue and Chikungunya viruses as well as *Plasmodium* (Kambris *et al.* 2009; Moreira *et al*. 2009).**

**The close association between *Wolbachia* and host reproductive tissues is expected to increase the possibility of horizontal gene transfer events. Genomic comparisons of arthropods and *Wolbachia* indicate this has repeatedly occurred, but the majority of exchanged material is non-functional (Woolfit *et al.* 2009). Hotopp *et al.* (2007) found evidence of *Wolbachia* transfer in the genomes of four insect and four nematode species, and Nikoh *et al.* (2008) estimated that 30% of *w*Mel genes had been integrated into the genome of the beetle *Callosobruchus chinensis* in an event occurring about one million years ago, but they were currently inactive. Klasson *et al.* (2009) identified a functional gene in *Ae. aegypti* that they associated with an ancient *Wolbachia* horizontal gene transfer event, and Woolfit *et al.* (2009) found the genes coding for salivary gland surface (SGS) proteins unique to mosquitoes (including *Ae. aegypti*) had putative homologs in *Wolbachia*, with genetic evidence suggesting transfer from the eukaryote to the bacteria rather than the other direction. In addition to genetic exchange, *Wolbachia* may also interact directly with host genomes as Xi *et al.* (2008) observed *Wolbachia* activating genes in *Drosophila* hosts that facilitated *Wolbachia* movement into reproductive tissues.**

### 2.1. Method of Modification

***Wolbachia* are routinely cultured in insect cell lines (Dobson *et al.* 2002; Furukawa *et al.* 2008; Jin *et al.* 2009; O'Neill *et al.* 1997; Xi & Dobson 2005) although transfer to novel hosts by microinjection can**

**be technically challenging and success unpredictable (McMeniman *et al.* 2008). Ruang-areerate and Kittayapong (2006) were able to introduce a double infection of the *w*AlbA and *w*AlbB strains into *Ae. aegypti* by microinjecting adults. The *Wolbachia* used to transinfect *Ae. aegypti* was sourced from Australian laboratory cultures of *D. melanogaster*, maintained in an *Ae. albopictus* cell line for ~240 passages (about 2.5 years) then transferred to an *Ae. aegypti* cell line and cultured for another 60 passages. Stable infection in live *Ae. aegypti* was achieved by embryonic microinjection (McMeniman *et al.* 2008; McMeniman *et al.* 2009).**

***Wolbachia* infections can be removed from arthropods by exposure to antibiotics such as tetracycline and rifampicin or by heat treatment (Breeuwer & Werren 1993; Dobson & Rattanadechakul 2001; Dutton & Sinkins 2005; Glover *et al.* 1990; Hermans *et al.* 2001; Min & Benzer 1997). Van Opijnen and Breeuwer (1999) found 71% of the two-spotted spider mite (*Tetranychus urticae*) lost their infection after rearing at 32°C for four generations, and after six generations infection was completely removed. They suspect that temperature may be important in determining the frequency of *Wolbachia* infections in field populations. Kyei-Poku *et al.* (2003) found that tetracycline treatment eliminated *Wolbachia* from the wasp *Urolepis rufipes* in four generations whereas heat treatment (34°C) required six generations, and significantly lower densities of *Wolbachia* were found in *Ae. albopictus* reared at 37°C compared to 25°C (Wiwatanaratanabutr & Kittayapong 2006).**

**3. SUMMARY OF ORGANISM BACKGROUNDS**

- ***Aedes aegypti*** **is the primary vector of dengue which affects over 50 million individuals each year. Although not endemic in Australia, last year the disease affected over 1000 individuals in Queensland. Dengue is a positive sense single stranded RNA virus classified into four serotypes.**

- ***Aedes aegypti*** **is highly anthropophilic, lives exclusively with human habitation and relies on artificial containers for breeding. Flight dispersal is limited, whereas longer distance spread is achieved through wind assisted or human assisted transportation.**

- **Female** ***Ae. aegypti*** **acquires dengue when taking a blood meal from a person vireamic for dengue. The extrinsic incubation period (EIP) between acquiring and being able to transmit the virus is between 8 to 12 days. Life shortening that removes female mosquitoes from a population before they reach this stage could theoretically result in the prevention of dengue transmission.**

- **Life shortening was achieved in** ***Ae. aegypti*** **by stable introduction of the** ***Wolbachia*** **strain of the obligate intracellular bacteria** ***Wolbachia pipientis*** **by embryonic microinjection.** ***Wolbachia*** **occur widely in arthropods, but have not been detected in** ***Ae. a*****egypti.** ***Wolbachia*** **sourced from Australian cultures of** ***Drosophila melanogaster*** **was cultured in** ***Ae. albopictus*** **cell lines for 2.5 years and 6 months in an** ***Ae. aegypti*** **cell line before being successfully transferred to** ***Ae. aegypti*** **laboratory cultures by embryonic microinjection. It has subsequently been determined that** ***Wolbachia*** **infection in** ***Ae. aegypti*** **directly interferes with dengue transmission in a mechanism independent of life-shortening.** ***Wolbachia*** **also induces a 'bendy proboscis' phenotype in some older females where they cannot penetrate host skin to blood feed.**

- ***Wolbachia*** **causes cytoplasmic incompatibility (CI) in** ***Ae. aegypti*****. CI results in asymmetric mating success because naturally occurring female** ***Ae. aegypti*** **have unsuccessful matings with** ***Wolbachia*** **males, whereas** ***Wolbachia*** **females can successfully mate with both** ***Wolbachia*** **and naturally occurring males. This could allow a relatively small number of propagules to drive into and replace naturally occurring** ***Ae. aegypti*** **population.**

- ***Wolbachia*** **and associated phenotypes can be removed from arthropod hosts by both antibiotic and heat treatments.**

## SECTION TWO: RISK ANALYSIS USING EXPERT SOLICITATION

### 1. PROBLEM FORMULATION

**The risk analysis was restricted to one end point. An end point is the adverse event that we do not wish to occur as a risk analysis only evaluates the occurrence of adverse events, not beneficial ones. The dengue risk analysis end point was defined as:**

**1) The release of *Wolbachia Ae. aegypti* will cause more harm than that currently provided by naturally occurring *Ae. aegypti*, referred to as 'Cause More Harm'.**

## 2. STAGE ONE: HAZARD MAPPING AND FAULT TREE ANALYSIS (FTA)

### 2.1. INTRODUCTION

Hazard mapping is a process used to identify the hazards associated with the nominated risk analysis end points and is a recommended tool of the risk analysis OGTR framework. An ideal set would contain all possible hazards, so to maximise our ability to achieve this Hayes *et al.* (2007) recommend that a number of elicitation tools be used and that opinions should be solicited from a wide range of relevant experts and stakeholders. At this stage the list of hazards obtained has little organisation, so in order to extract information regarding the relevance, level of risk posed by each hazard and the relationships between hazards, more structured tools are required.

Logic trees and similar variants allow the cognitive and graphical conversion of loose sets of hazards into a hierarchal and sequential hazard framework (Bobbio *et al.* 2001; Siu 1994). They have been used frequently in engineering systems and more recently applied to ecological risk assessment (e.g. Hayes 2002a; 2002b). Fault Tree Analysis (FTA) itself was developed by Bell Telephone Laboratories in the late 1960s for hazard identification and analysis in missile systems (Lee *et al.* 1985). The tree starts at one nominated failure of interest or 'top event', which is interchangeable with the term end point used here. The series of hazards that need to fail to cause the undesired event are boxes linked by logic gates (AND/OR), allowing a properly constructed tree to graphically describe where sequential or parallel series of hazard failures are required.

We constructed a fault tree for the end point with the purpose of organising the solicited hazards into a logical framework, and to help identify within the potentially numerous hazards those that were of most importance for further evaluation. Fault trees also allow identification of cut sets which describe the shortest combination of hazard failures that could lead to the top event and are therefore of importance in risk analysis (Long *et al.* 2000; Vatn 1992). It was also expected that the fault tree process would uncover additional hazards and identify those outside the analysis scope.

During the processes of hazard mapping and fault tree development, the lack of knowledge about the ecological role of *Ae. aegypti* was identified as an area of concern. Assessment of the interconnectedness of ecosystems and the possible ecosystem roles serviced by an organism are considered important components in evaluating the release of both biological control agents (Simberloff & Stiling 1996) and transgenic organisms (Ervin *et al.* 2001). Ecosystem services can be divided into ecosystem goods (such as food) and ecosystem services such as pollination (Costanza *et al.* 1997). The concern was that *Ae. aegypti* potentially provides some of these services so the release of *Wolbachia Ae. aegypti* could interrupt these systems if the organism behaved differently or had different biological characteristics. We therefore convened a workshop to explore and evaluate the ecological hazards and consequences that could result from the reduction or loss of *Ae. aegypti* populations.

### 2.2. METHODS

#### 2.2.1. Hazard Solicitation

Hazards were identified by expert solicitation through four discrete phases. The initial phase solicited expert judgement from researchers associated with the GCGH project during a two hour hazard mapping session on May 20$^{th}$ 2009 (Eliminate Dengue Workshop, Thala Beach Resort, Port Douglas). Participants were provided with the two end points and asked to identify the types of hazard categories they thought relevant and the hazards under each category. Secondly, hazards of concern to the Gordonvale/Cairns communities were solicited though the GCGH's public engagement programme.

The hazard set was refined by combining synonymous or redundant hazards (i.e. those that were essentially the same) and breaking broad hazards into discrete hazard components. A brief description of each hazard was written. The resulting list of hazards was emailed to the Dengue Consultation Group (DCG) comprised of researchers nominated by both the University of Queensland and researchers not attached to the project to provide expert opinion from a range of backgrounds with a request to add any additional hazards. The fourth phase occurred during the process of fault tree construction in which missing hazards were identified and added to the tree. Some additional hazards were identified in the Stage Three ecological hazards workshop and are not discussed here.

### 2.2.2. Fault Tree Construction

A draft fault tree was constructed for the top event (end point) based on the identified hazards and sent to the DCG with background information on fault trees. The DCG was asked to determine the relevance of the hazards and identify any that had been missed. Experts were also asked to evaluate tree topology (structure). Feedback was incorporated, and the numbers of hazards, undeveloped events and logic gates were recorded and minimal cut sets identified.

## 2.3. RESULTS

### 2.3.1. Hazard Solicitation

A total of 52 possible hazards associated with the release of *Wolbachia Ae. aegypti* were initially mapped at the Cairns GCGH meeting spanning thirteen themes (Appendix 2). Theses hazards spanned the regulatory compliance, community acceptance and capacity to cause harm beyond that already caused by *Ae. aegypti* and dengue. In the construction of the fault tree some of the hazards (see table 1) which were later moved to the 'Don't Achieve Release' analysis were included. The 'Don't Achieve Release' analysis deals with the logistical and compliance related hazards for the project and does not form part of this report.

Some of these hazards were aggregations of hazards that were subsequently broken into discrete hazards (Appendix 3) and resulting set screened to identify hazards that fell outside the scope of the analysis end point (Appendix 4). The remaining raw hazard set was refined by grouping similar hazards into themes resulting in 27 hazards (Appendix 5). The fault tree building exercise helped identify additional hazards. The final 50 hazards used in the FTA, the source of the hazard identification (workshop, DCG consultation, community engagement, or FTA) and a brief description of each hazard are shown in Table 1.

Table 1. Final 50 hazards identified by Stage one workshop (W) and email solicitation from the dengue consultation group (DCG), community engagement (CE), through the process of fault tree analysis (FTA) or and the number of occurrences in the fault tree for Cause More Harm (CMH).
*H = Hazard, UE = Undeveloped Event.

| Name | *Type | Source | Repeats | Description |
|---|---|---|---|---|
| Adverse media | H | CE, W | 5 | • Adverse media coverage (reduces community and/or regulatory and/or institutional support by raising controversy or spectre of GMO). |
| *Ae. aegypti* population crash | H | CE, W | 2 | • *Ae. aegypti* population density crashes, possible local extinction. |
| *Ae. aegypti* vectors other arboviruses | H | CE | | • *Ae. aegypti* gains ability from *Wolbachia* to vector arboviruses that it otherwise would not be able to vector. |

| | | | | |
|---|---|---|---|---|
| All serotypes in circulation | H | FTA | | • All four dengue serotypes in circulation in same geographic area at the same time. |
| Change in behaviour | H | W | | • People's behaviour changes to reduce interaction with *Wolbachia Ae. aegypti.* Includes avoidance, household insecticide use and removal of breeding sites. |
| Changes in *Ae. aegypti* behaviour | H | FTA | 2 | • *Ae. aegypti* behaviour deviates from naturally occurring *Ae. aegypti* as a result of *Wolbachia*. |
| Changes in herd immunity | H | W | | • Changes in disease epidemiology that adversely affect herd immunity, e.g. result of circulating serotypes which could lead to increased incidence of DHF etc. |
| Community knowledge | H | CE, DCG | | • Community have insufficient technical or incorrect knowledge of dengue, *Wolbachia* and *Ae. aegypti* to make informed decisions. |
| Dengue carrier(s) present | H | FTA | 2 | • As dengue is not endemic in Australia, outbreaks require infected individuals returning from overseas (or possibly other infected area in Australia). |
| Dengue evolves in response to *Wolbachia* | H | W, DCG | | • Dengue fitness evolves in response to non- or limited transmission by *Ae. aegypti* to increase its transmission rates. |
| Dengue vector | UE | FTA | | • A dengue vector needs to be present |
| Ecosystem change | H | CE, DCG | | • Release of *Wolbachia Ae. aegypti* results in ecological change. |
| Fitter *Ae. aegypti* | UE | FTA | | • *Wolbachia Ae. aegypti* is more likely to pass on genes than naturally occurring *Ae. aegypti*. |
| Horizontal *Wolbachia* transfer | H | CE, W, DCG | | • Transfer of *Wolbachia* to other species (vertebrate or invertebrate) via predation or host feeding events. |
| Host biting | H | FTA | | • Host biting needs to occur. |
| Increased biting | H | FTA | 3 | • Increased biting or number of blood meals required by *Wolbachia Ae. aegypti.* |
| Increased control costs | H | FTA | 3 | • *Wolbachia Ae. aegypti* populations will require increased or more intensive treatments. |
| Increased emigration | H | FTA | 2 | • The rate of emigration from release area increases because of fear of *Wolbachia Ae. aegypti.* |
| Increased exposure to dengue | H | DCG | | • Individuals are increasing exposed to potential dengue transmission events. |
| Increased fear of *Ae. aegypti* | H | CE, DCG | | • Community fear of mosquito increases due to factors such as adverse media and poor knowledge of mosquito system. |
| Increased geographic range | UE | W, FTA | 2 | • *Ae. aegypti* increases geographic distribution beyond predicted limits or at a faster than expected rate. |
| Increased insecticide use | H | FTA | | • More insecticide use is necessary to achieve same control. |
| Increased medical care | H | DCG | | • Cost of community medical care increases above expected values as a result of the release. |
| Insecticide resistance | UE. | W | | • *Wolbachia* provides increased *Ae. aegypti* insecticide resistance. |
| Larger *Ae. aegypti* | H | W | 2 | • *Ae. aegypti* population density per unit |

| | | | | | |
|---|---|---|---|---|---|
| population | | | | | area increases permanently above current mean. |
| Lost income | H | W | | • | Individual and businesses lose income through reduced real estate, tourism or employment. |
| Lost productivity | H | W | | • | Adverse effects on economy as less seasonal or permanent workers available in region. |
| Metabolic costs of *Wolbachia* | UE | FTA | | • | Metabolic costs of *Wolbachia* on host. |
| Natural increase | H | FTA | | • | *Wolbachia Ae. aegypti* populations undergo a natural; increase in size because of optimal conditions. |
| New exotic mosquito species | H | W | 2 | • | New species is able to establish. |
| New mosquito species arrives | UE | W | | • | New species arrives (but not established). |
| New serotype | H | W | | • | New dengue serotype evolves. |
| Other arboviruses present | H | FTA | | • | Other arboviruses in circulation at the time. |
| Perception *Wolbachia* solves problem | H | DCG | 2 | • | Perception that *Wolbachia* will solve *Ae. aegypti* dengue problem. |
| Predation | UE | CE, W | | • | Horizontal transfer of *Wolbachia* to predator species could occur when they feed on the mosquito. |
| Reduced *Ae. aegypti* fitness | H | FTA | | • | *Wolbachia* incurs changes in *Ae. aegypti* biology that render it less competitive against naturally occurring conspecifics and/or other mosquito species. |
| Reduced control | H | FTA | 2 | • | Conflict of interest or assumption that *Wolbachia Ae. aegypti* will reduce Dengue problem, so less investment in control development or control effort. |
| Reduced Immigration | UE | DCG | | • | The rate of immigration into release area decreases because of fear of *Wolbachia Ae. aegypti*. |
| Reduced real estate values | H | W | | • | Real estate values declines because of fear of *Wolbachia Ae. aegypti* in region. |
| Reduced seasonal workers | H | W, DCG | | • | Reduced numbers of seasonal workers (e.g. backpackers) available because of fear of *Wolbachia Ae. aegypti.* |
| Reduced tourism | H | W | | • | Tourism declines because of fear of *Wolbachia Ae. aegypti* in region. |
| Release site damaged | H | W | | • | Risk of proposed release sites (e.g. Gordonvale) being damaged or unable to sustain *Ae. aegypti* population d.t. extreme event, e.g. flooding, drought, absence of people or habitat. |
| Social behaviour changes | H | FTA | | • | Social behaviours change directly as a result of the release of *Wolbachia Ae. aegypti.* |
| Social vilification | H | W | | • | Community divided over release of *Ae. aegypti* and may target those associated with supporting release. |
| Vacant niche | H | W, DCG | | • | *Ae. aegypti* vacates niche for other species or is uncompetitive against new species. |
| *Wolbachia* failure | H | CE, W | | • | Risk that *Wolbachia* does not provide expected reduction in dengue vectoring or provides some other adverse effect. |
| Worse community | H | DCG | 3 | • | A decline in overall community health |

| | | | | |
|---|---|---|---|---|
| **health** | | | | **from dengue events.** |
| **Worse dengue** | **H** | **CE, DCG** | • | **The overall effects of dengue (prevalence, transmission rate, severity) increase as a result of the release.** |
| **Worse ecological impacts** | **H** | **FTA** | • | **The release of *Wolbachia Ae. aegypti* results in worse ecological harm than would be expected by naturally occurring *Ae. aegypti*.** |
| **Worse economic impacts** | **H** | **FTA** | • | **The release of *Wolbachia Ae. aegypti* results in economic losses.** |

**The Fault Tree for 'Cause More Harm' (Figure 3) contains two major themes, 'Worse ecological impacts' describing the hazards associated with the release contributing to negative impact on ecosystems and 'Worse social impacts' describing potential hazards leading to a loss of social conditions. 'Worse social impacts' contains three hazard themes, a decline in community health, changes to social behaviour and economic losses. The tree has a high ratio of AND to OR gates, suggesting that the majority of hazard failures are not conditional on simultaneous failures. Within the fault tree a total of 13 hazards were repeated. The most repeats were found for 'Adverse media' (5), with 'Increased biting', 'Increased control costs' and 'Worse community health' all occurring three times. The hazards '*Ae. aegypti* population crash', 'Changes in *Ae. aegypti* behaviour', 'Dengue carriers(s) present', 'Increased emigration', 'Increased geographic range', 'Larger *Ae. aegypti* population', 'New exotic mosquito species', 'Perception *Wolbachia* solves problem' and 'Reduced control' all appeared twice. The number of 'Adverse media' repeats indicates it is a critical hazard with the potential to negatively influence the hazard chain at multiple locations.**

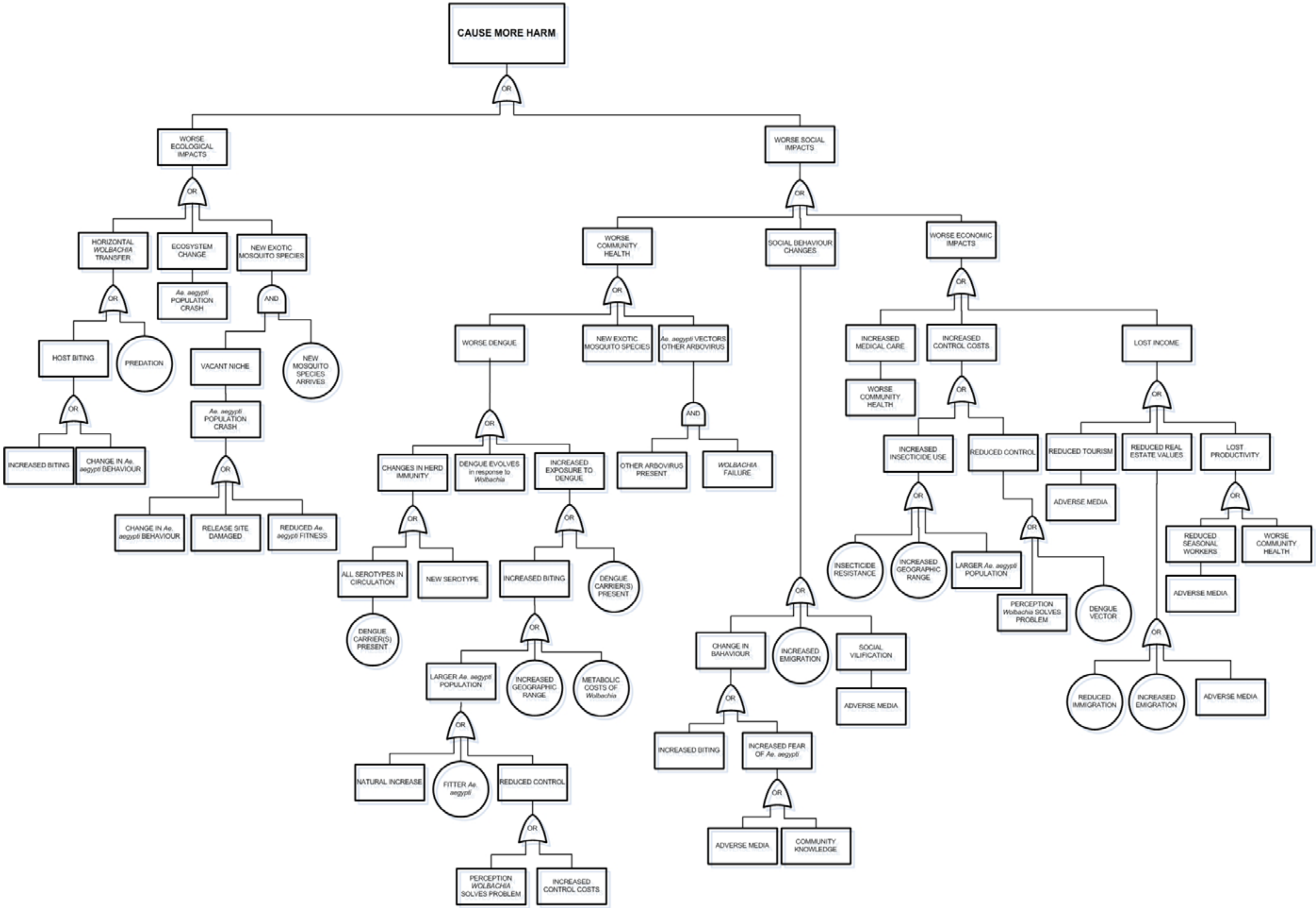


**Figure 3. Fault Tree for 'Cause More Harm'.**

### 2.3.2. Worse ecological impacts Sub-Tree ('Cause More Harm')

**The 'Worse ecological impacts' sub-tree (Figure 4) of the 'Cause More Harm' fault tree contains 13 events and two undeveloped events ('Predation' and 'New mosquito species arrives') with the hazards of an '*Ae. aegypti* population crash' and 'Change in *Ae. aegypti* behaviour' repeating (Table 2). This sub-tree incorporates 'Horizontal *Wolbachia* transfer', 'Ecosystem change' (evaluated separately below) and 'New exotic mosquito species' hazards. A hazard exists where the release could facilitate the establishment of a 'New exotic mosquito species' currently absent from the region. A candidate species for invasion is *Ae. albopictus* due to its physical proximity to mainland Australia (Ritchie *et al.* 2006) and because it has previously outcompeted and displaced *Ae. aegypti* in the Americas (Mousson *et al.* 2005). Reduced resistance to invasion ('Vacant niche') could eventuate from an '*Ae. aegypti* population crash' triggered by an adverse 'Change in *Ae. aegypti* behaviour' (e.g. non-optimal host selection for blood meals), or if the 'Release site damaged' hazard occurred, or as a result of 'Reduced *Ae. aegypti* fitness'. The risk of 'Horizontal *Wolbachia* transfer' could result from two paths, either directly via 'Host biting' or indirect transfer to a predacious species via feeding on the *Wolbachia Ae. aegypti.***

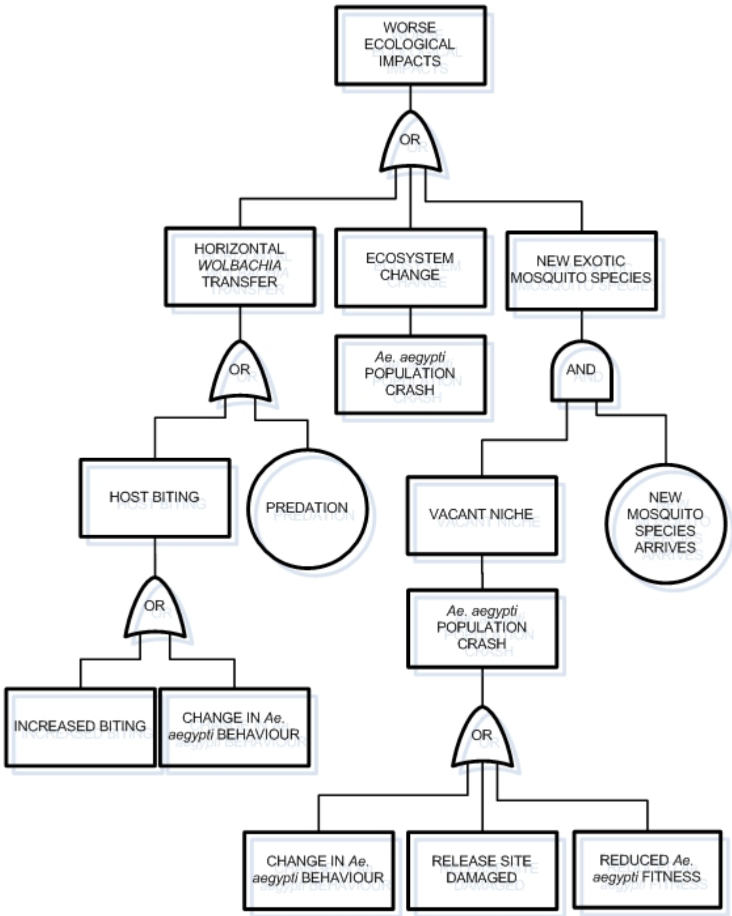


**Figure 4. 'Worse ecological impacts 'component of 'Cause More Harm' fault tree.**

**Table 2. Hazard definitions for 'Worse ecological impacts' sub-tree in 'Cause More Harm'**

| Name | Type | Description |
|---|---|---|
| *Ae. aegypti* population crash (x2) | Hazard | • *Ae. aegypti* population density crashes, possible local extinction. |
| Changes in *Ae. aegypti* behaviour (x2) | Hazard | • *Ae. aegypti* behaviour changes as result of *Wolbachia* effects. |
| Ecosystem change | Hazard | • The release leads to ecosystem change or removal of ecosystem services. |
| Horizontal *Wolbachia* transfer | Hazard | • Transfer of *Wolbachia* to other species (vertebrate or invertebrate) via predation or host feeding events. |
| Host biting | Hazard | • Host biting needs to occur. |
| Increased biting | Hazard | • Increased biting or number of blood meals required by *Wolbachia Ae. aegypti.* |
| New exotic mosquito species | Hazard | • New species is able to establish. |
| New mosquito species arrives | Und. Event | • New species arrives (but not established). |
| Predation | Und. Event | • Horizontal transfer of *Wolbachia* to predator species could occur when they feed on the mosquito. |
| Reduced *Ae. aegypti* fitness | Hazard | • *Wolbachia Ae. aegypti* incurs changes in biology that render it less competitive against naturally occurring conspecifics and/or other mosquito species. |
| Release site damaged | Hazard | • Risk of proposed release sites (e.g. Gordonvale) being damaged or unable to sustain *Ae. aegypti* population d.t. extreme event, e.g. flooding, drought, absence of people or habitat. |
| Vacant niche | Hazard | • *Ae. aegypti* vacates niche for other species or is uncompetitive against new species. |
| Worse ecological impacts | Hazard | • The release of *Wolbachia Ae. aegypti* results in worse ecological harm than would be expected by naturally occurring *Ae. aegypti.* |

### 2.3.3. 'Social behaviour changes' Sub-Tree ('Cause More Harm')

**The 'Social behaviour changes' sub-tree (Figure 5) of the 'Cause More Harm' fault tree has eight hazards and one undeveloped event ('Increased emigration') with the 'Adverse media' hazard repeated twice (Table 3) and describes events that could cause changes in the normal behavioural pattern of people (e.g. lifestyle).**

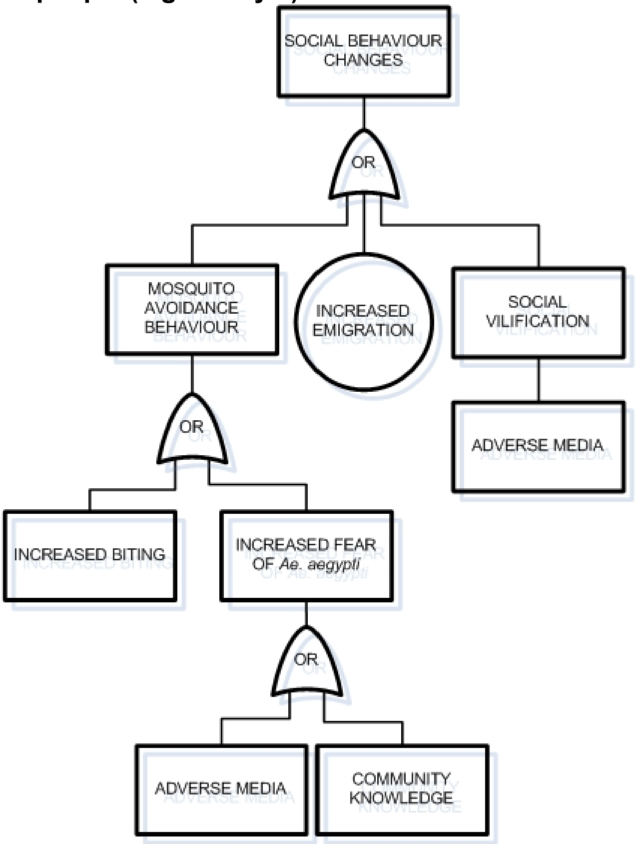


**Figure 5. 'Social behaviour changes' component of 'Cause More Harm' fault tree.**

**Table 3. Hazard definitions for 'Social behaviour changes' sub tree in 'Cause More Harm'.**

| Name | Type | Description |
|---|---|---|
| **Adverse media (x2)** | **Hazard** | • **Adverse media coverage (reduces community and/or regulatory and/or institutional support by raising controversy or spectre of GMO).** |
| **Change in behaviour** | **Hazard** | • **People's behaviour changes to reduce interaction with *Wolbachia Ae. aegypti.* Includes avoidance, household insecticide use and removal of breeding sites.** |
| **Community knowledge** | **Hazard** | • **Community have insufficient technical or incorrect knowledge of dengue, *Wolbachia* and *Ae. aegypti* to make informed decisions.** |
| **Increased biting** | **Hazard** | • **Increased biting or number of blood meals required by *Wolbachia Ae. aegypti.*** |
| **Increased emigration** | **Und. Event** | • **Emigration rate of population around release site increases because of fear of release and *Wolbachia* mosquito.** |
| **Increased fear of *Ae. aegypti*** | **Hazard** | • **Community fear of mosquito increases due to factors such as adverse media and poo knowledge of mosquito system.** |
| **Social behaviour changes** | **Hazard** | • **Social behaviours change directly as a result of the release of *Wolbachia Ae. aegypti*** |
| **Social vilification** | **Hazard** | • **Community divided over release of *Ae. aegypti* and may target those associated with supporting release.** |

**The three components that contribute to social behaviour change are 'Social vilification', 'Increased emigration' or a 'Change in behaviour'. An 'Increased fear of *Ae. aegypti'* could occur and result in negative perceptions arising from 'Adverse media' coverage or lack of 'Community knowledge' surrounding the mosquito. 'Increased fear of *Ae. aegypti'* and 'Increased biting events' could lead to the hazard of changes in individual behaviours to avoid contact with the *Wolbachia* containing mosquito. This is considered adverse because it directly results from the release, and would not otherwise occur with naturally occurring *Ae. aegypti*. 'Social vilification' of community individuals or groups who were associated with the release could result from disaffected parties following the release. 'Increased emigration' from the release site because of the presence of *Wolbachia Ae. aegypti* may also occur. The potential for adverse social impacts resulting from hazards such as 'Adverse media' coverage and the state of 'Community knowledge' is why the community engagement programme is valuable both to inform the community and identify their concerns.**

### 2.3.4. 'Adverse economic impacts' Sub-Tree ('Cause More Harm')

**'Adverse economic impacts' sub-tree (Figure 6) of the 'Cause More Harm' fault tree has 17 hazards (including three 'Adverse media' repeats) and five undeveloped events (Table 4). The hazard of 'Worse community health' occurs twice in this sub-tree and is expanded in Section 2.3.5 below. 'Adverse economic impacts' would occur where the release directly resulted in an increase in 'Increased medical care', a requirement for increased expenditure on mosquito control ('Increased control costs'), or by resulting in fiscal and economic loss ('Lost income'). 'Increased control costs' could occur if more insecticide was required to combat increased 'Insecticide resistance', if *Ae. aegypti* populations were now more geographically dispersed ('Increased geographic range') requiring greater coverage of control, or populations were at higher densities ('Larger *Ae. aegypti* populations') and required more control effort to reduce. Larger *Ae. aegypti* populations could result from a 'Perception *Wolbachia* solves problem' and 'Reduced control' effort is allocated to suppressing mosquito populations.**

**The adverse social effect of 'Lost income' could result from hazards associated with 'Reduced tourism', 'Reduced real estate values' or 'Lost productivity' and all could result from 'Adverse media'. 'Reduced tourism' would be of particular concern in Cairns where tourism constitutes the primary regional industry. Real estate values could be reduced by imbalances in emigration and immigration rates and 'Lost productivity' would result from the hazard of 'Reduced seasonal workers' visiting the region (or higher wages were necessary) as a result of 'Adverse media' or 'Worse community health' conditions.**

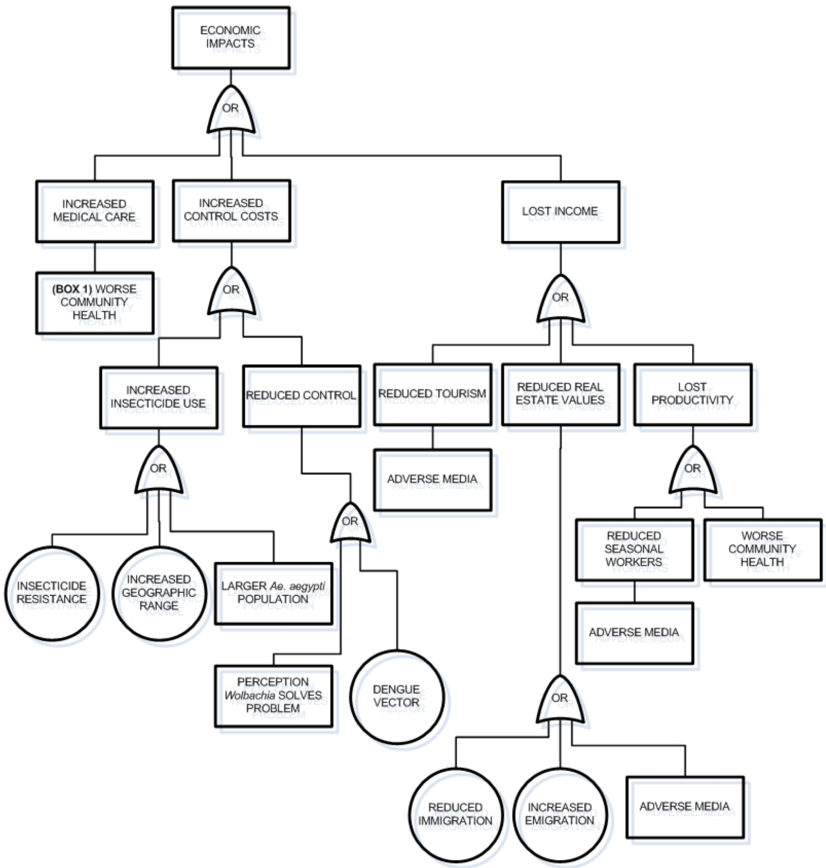


**Figure 6. ‘Economic impact’ component of ‘Cause More Harm’ fault tree.**

**Table 4. Hazard definitions for ‘Adverse Economic Impacts’ sub-tree in ‘Cause More Harm’**

| Name | Type | Description |
|---|---|---|
| Adverse media (x 3) | Hazard | • *Wolbachia Ae. aegypti* populations will require increased or more intensive treatments. |
| Dengue vector | Und. Event | • A dengue vector needs to be present. |
| Increased control costs | Hazard | • *Wolbachia Ae. aegypti* populations will require increased or more intensive treatments. |
| Increased geographic range | Und. Event | • *Ae. aegypti* increases geographic distribution beyond predicted limits or at a faster than expected rate. |
| Increased emigration | Hazard | • The rate of emigration from release area increases because of fear of *Wolbachia Ae. aegypti.* |
| Increased insecticide use | Hazard | • More insecticide use is necessary to achieve same control. |
| Increased medical care | Hazard | • Cost of community medical care increases above expected values as a result of the release. |
| Insecticide resistance | Und. Event | • *Wolbachia* provides increased *Ae. aegypti* insecticide resistance. |
| Larger *Ae. aegypti* population | Hazard | • *Ae. aegypti* population density per unit area increases permanently above current mean. |
| Lost income | Hazard | • Individual and Business lose income through reduced real estate, tourism or employment. |
| Lost productivity | Hazard | • Adverse effects on economy as less seasonal or permanent workers available in region. |
| Perception *Wolbachia* solves problem | Hazard | • Perception that *Wolbachia* will solve dengue problem. |
| Reduced control | Hazard | • Conflict of interest or assumption that *Wolbachia Ae. aegypti* will reduce *dengue* problem, so less investment in control development or control effort. |

| | | |
|---|---|---|
| **Reduced immigration** | **Und. Event** | • **The rate of immigration into release area decreases because of fear of *Wolbachia Ae. aegypti.*** |
| **Reduced real estate values** | **Hazard** | • **Real estate values declines because of fear of *Wolbachia Ae. aegypti* in region.** |
| **Reduced seasonal workers** | **Hazard** | • **Reduced numbers of seasonal workers (e.g. backpackers) available because of fear of *Wolbachia Ae. aegypti.*** |
| **Reduced tourism** | **Hazard** | • **Tourism declines because of fear of *Wolbachia Ae. aegypti* in region.** |
| **Worse community health (x2)** | **Hazard** | • **A decline in overall community health from dengue events.** |
| **Worse economic impacts** | **Hazard** | • **The release of *Wolbachia Ae. aegypti* results in economic losses.** |

### 2.3.5. ‘Worse community health’ Sub-Tree (‘Cause More Harm’)

**The ‘Worse community health’ sub-tree (Figure 7) of the ‘Cause More Harm’ fault tree has 17 hazards and five undeveloped events (Table 5). ‘Worse community health’ could result from hazard failures that lead to ‘Worse dengue’, establishment of a ‘New exotic mosquito species’, or if ‘*Ae. aegypti* vectors other arboviruses’.**

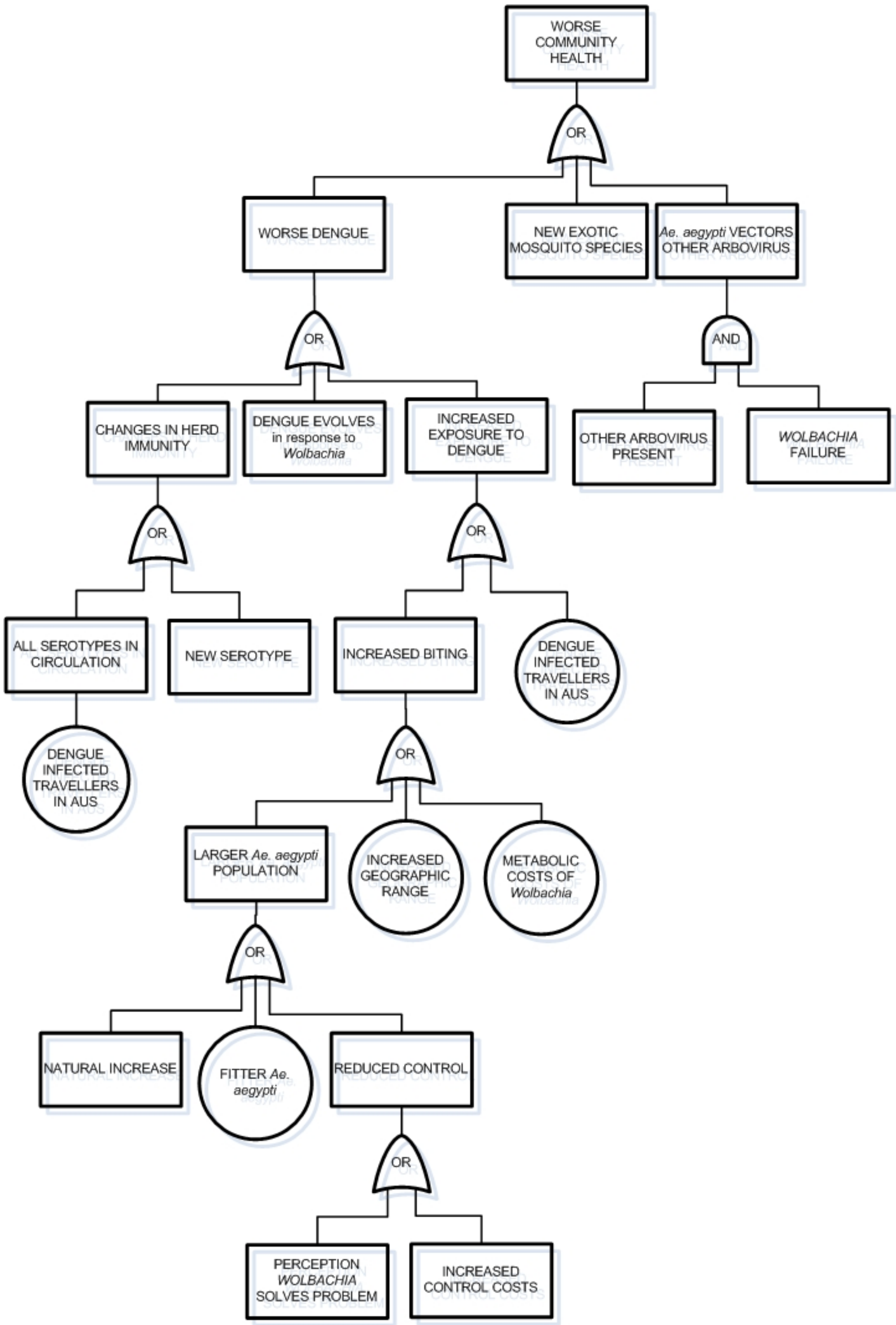


**Figure 7. 'Worse community health' component of 'Cause More Harm' fault tree.**

Table 5. Hazard definitions for 'Worse community health' sub-tree in 'Cause More Harm'

| Name | Type | Description |
|---|---|---|
| *Ae. aegypti* vectors other arboviruses | Hazard | • *Ae. aegypti* gains ability from *Wolbachia* to vector arboviruses that it others would not be able to vector. |
| All serotypes in circulation | Hazard | • All four dengue serotypes in circulation in same geographic area at the same time. |
| Changes in herd immunity | Hazard | • Changes in disease epidemiology that adversely affect herd immunity, e.g. result of circulating serotypes which could lead to increased incidence of DHF etc. |
| Dengue evolves in response to *Wolbachia* | Hazard | • Dengue fitness evolves in response to non- or limited transmission by *Ae. aegypti* to increase its transmission rates. |
| Dengue carriers present (x2) | Und. Event | • Infected travellers arrive in Australia and could act as potential sources of dengue outbreak. |
| Fitter *Ae. aegypti* | Und. Event | • *Wolbachia Ae. aegypti* is more likely to pass on genes than naturally occurring *Ae. aegypti*. |
| Increased geographic range | Und. Event | • *Ae. aegypti* increases geographic distribution beyond predicted limits or at a faster than expected rate. |
| Increased biting | Hazard | • Increased biting or number of blood meals required by *Wolbachia Ae. aegypti.* |
| Increased control costs | Hazard | • *Wolbachia Ae. aegypti* populations will require increased or more intensive treatments. |
| Increased exposure to dengue | Hazard | • Individuals are increasing exposed to potential dengue transmission events. |
| Larger *Ae. aegypti* population | Hazard | • *Ae. aegypti* population density per unit area increases permanently above current mean. |
| Metabolic costs of *Wolbachia* | Und. Event | • Metabolic costs of *Wolbachia* on host |
| Natural increase | Hazard | • *Wolbachia Ae. aegypti* populations undergo a natural; increase in size because of optimal conditions. |
| New exotic mosquito species | Hazard | • New species is able to establish. |
| New serotype | Hazard | • New dengue serotype evolves. |
| Other arboviruses present | Hazard | • Other arboviruses in circulation at the time. |
| Perception *Wolbachia* solves problem | Hazard | • Perception that *Wolbachia* will solve *Ae. aegypti* dengue problem. |
| Reduced control | Hazard | • Conflict of interest or assumption that *Wolbachia Ae. aegypti* will reduce *dengue* problem, so less investment in control development or control effort. |
| *Wolbachia* failure | Hazard | • Risk that *Wolbachia* does not provide expected reduction in dengue vectoring or provides some other adverse effect. |
| Worse dengue | Hazard | • The overall effects of dengue (prevalence, transmission rate, severity) increase as a result of the release. |
| Worse community health | Hazard | • A decline in overall community health from dengue events. |

The left branch of the tree describes how 'Worse dengue' could occur as a result from the hazards of 'Changes in herd immunity', 'Dengue evolves in response to *Wolbachia'* and 'Increased exposure to dengue'. 'Changes in herd immunity' could result from 'All serotypes in circulation' or a 'New serotype' evolving. A more complex series of hazards is responsible for the hazard of 'Increased exposure to dengue' with a primary hazard being that of 'Increased biting' events. This could result if there are 'Metabolic costs of *Wolbachia'* so that the host requires more feeding events, an 'Increased geographic range' so that more people are potentially exposed to dengue, or as a result of a 'Larger *Ae. aegypti* population'. This could occur via a 'Fitter *Ae. aegypti'* phenotype as a result of *Wolbachia*, or if there was 'Reduced control' over mosquitoes as a result of 'Perception *Wolbachia*

**solves problem' or due to 'Increased control costs'. 'Worse community health' could also result if '*Ae. aegypti* vectors other arboviruses' which could result from a '*Wolbachia* failure' in reducing the ability of *Ae. aegypti* to transmit RNA arboviruses such as dengue.**

### 2.3.6. Cut Set

**Cut sets are identified by the fewest hazards required for the top event to manifest (Figure 8). The cut set for 'Cause More Harm' is the causation of adverse ecological effects as a result of the long term reduction in *Ae. aegypti* population size. This hazard was further evaluated in the Stage two workshop (following section).**

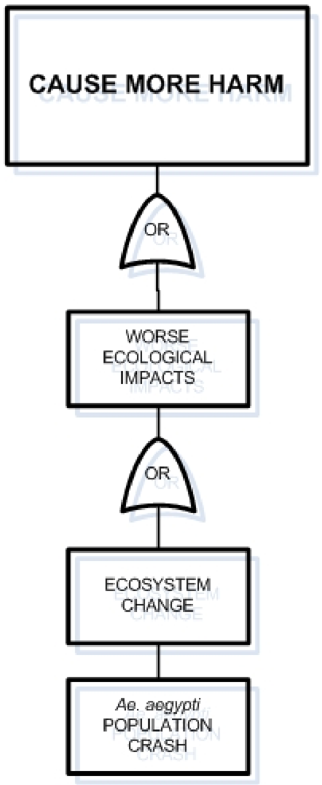


**Figure 8. 'Worse Ecological Impacts' Cut set for 'Cause More Harm'**

### 2.4. SUMMARY OF STAGE ONE: HAZARD MAPPING AND FAULT TREE ANALYSIS (FTA)

- **The risk analysis end point identified for the risk analysis was that the release of *Wolbachia Ae. aegypti* would result in more harm than would be expected to be caused by the naturally occurring *Ae. aegypti* ('Cause More Harm') over a 30 year timeframe from the release occurring.**

- **A final total of 50 hazards relevant to the end point were described soliciting expert opinion by workshop, email and community engagement formats and the construction a fault tree that graphically and logically describes the relationship between the hazards.**

- **The fault tree for 'Cause More Harm' contained two main sub-trees describing hazards representing worse social or ecological impacts. 'Worse social impacts' could result from a decrease in community health, changes to social structure and behaviour, and 'Worse economic impacts' would eventuate from impacts on tourism, real estate and the local economy. Worse ecological impacts could result from changes in *Wolbachia Ae. aegypti* behaviour, horizontal *Wolbachia* transfer or direct impacts via reduced ecological interactions.**

- **13 hazards had multiple appearances in the fault tree which indicates their potential to influence other hazards and could lead to failure cascades. 'Adverse media' had the most repeats at five and has the potential to influence many social hazards. 'Increased biting', 'Increased control costs' and 'Worse community health' all occurred three times and nine other hazards were repeated twice.**

- **The cut set (shortest hazard route to failure of the endpoint hazard) for 'Cause More Harm' was that reduced *Ae. aegypti* populations would lead to ecological changes.**

## 3. STAGE TWO: EXPERT WORKSHOP ON ECOSYSTEM CHANGE (CAUSE MORE HARM ENDPOINT)

### 3.1. Introduction

**The Stage one process identified ecological harm caused by the release of *Wolbachia Ae. aegypti* as the cut set, or shortest route to the failure of 'Cause More Harm'. The importance of this hazard was also identified by the community and independent observers (K. Hayes *pers. comm.*) particularly as there seemed to be a paucity of knowledge on the ecological interactions of this species in Australia. Because of the lack of knowledge, uncertainty about ecological interactions and any possible adverse outcomes would be high. To address this issue it was necessary to convene a technical expert workshop to evaluate the likelihood that the release would result in the adverse outcome of ecological change (where any change is considered adverse). Technical experts would have knowledge of the both peer-reviewed and 'grey' literature (unpublished data or results) and exchange of this information in a structured setting (workshop with facilitators) was considered the best way to approach this issue.**

### 3.2. Methods

**A one day workshop was held on 18th September 2009 in Cairns to elicit expert opinion from mosquito researchers on the potential ecological impacts of removing or reducing *Ae. aegypti* populations, two outcomes which may result from the release. The participants were asked to discuss and identify the known ecological roles of *Ae. aegypti* and any possible ecological consequences of the loss or reduction in numbers of *Ae. aegypti* and then a fault tree was constructed to explore these interactions. At this stage the purpose was to conceptual exploration on this issue without assigning likelihoods or possible consequences to these events.**

### 3.3. Results

**The expert researchers including those not part of the GCGH (e.g. AQIS) are shown in Appendix 1. Through facilitated discussion the experts provided a number of key reasons why they considered it unlikely that the release of *Wolbachia Ae. aegypti* would not result in adverse ecological change. Firstly, *Ae. a*egypti is one of the most studied invertebrate species in the world because of its global impacts on human health, but despite this no ecological interactions with the environment outside of human domestication have been detected. The reliance on human domestication to provide suitable breeding habitat restricts the species to this ecological niche. So highly tied is the species to its human habitat that the removal of artificial breeding containers is the primary means of population control along with insecticide treatments. Combined, two practices were considered to inflict greater non-target adverse ecological effects than the replacement of *Ae. aegypti* with *Wolbachia Ae. aegypti*.**

**Even though the experts considered the likely impacts to be low they were able to describe putative ecological hazards that could result from changes in *Ae. aegypti* population density The resulting fault tree based on possible *Ae. aegypti* ecosystem services is shown in Figure 9. Table 6 shows the 16 hazards and one undeveloped event ('Reduced *Ae. aegypti* fecundity or longevity') in this tree. Both diminished fecundity or longevity provide the same outcome of smaller *Ae. aegypti* populations as a result of 'Fewer eggs', 'Fewer larvae' and 'Fewer adults'. This would flow through to providing less food to predators and 'Reduced predator fitness' (where predator populations may decline in number and allow increases in prey species), 'Reduced pollination' services which could lead to a change in vegetation composition, and 'Reduced detritus feeding' would free resources for**

**competing species. Smaller population sizes would also lead to 'Reduced vectoring of diseases to other species'. The experts noted here that under existing legislation *Ae. aegypti* is considered an exotic species and treatment of populations is mandatory, meaning that populations are continually suppressed and present in low density. Because of the low density (and to some extent because it is an exotic species that has not co-evolved in evolutionary timeframes with the native ecosystem) and extensive association with human rather than natural ecosystems, the experts considered that no plants species would be reliant on *Ae. aegypti* pollination services and no predators would be reliant upon this mosquito as a prey source because it would represent an ephemeral intermittent resource.**

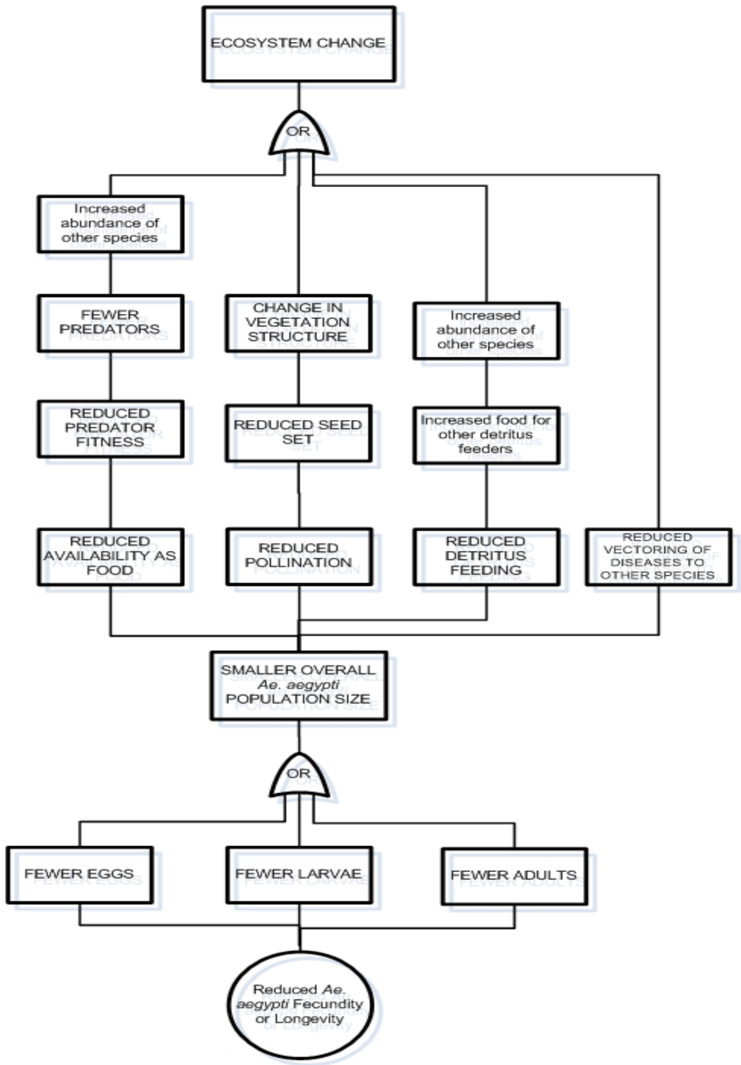


**Figure 9. Expert derived fault tree describing the ecological implications of reduced *Ae. aegypti* life span or fecundity.**

**Table 6. Hazard definitions for 'Ecosystem Change' fault tree.**

| Name | Type | Description |
|---|---|---|
| Change in vegetation Structure | Hazard | • The vegetative composition of an area changes because of changes (decline) in plant fecundity. |
| Ecosystem change | Hazard | • The release leads to ecosystem change or removal of ecosystem services. |
| Fewer adults | Hazard | • Fewer *Ae. aegypti* adults present in environment. |
| Fewer eggs | Hazard | • *Wolbachia Ae. aegypti* has lower fecundity than naturally occurring *Ae. aegypti.* |
| Fewer larvae | Hazard | • Fewer *Ae. aegypti* larvae present in environment. |
| Fewer predators | Hazard | • Fewer predators occur in ecosystem because of reduced prey. |
| Increased abundance of other species (x2) | Hazard | • Some species increase in numbers because of a reduction in predation or increased availability of food (e.g. detritus) and reduced competition because of smaller *Ae. aegypti* populations. |
| Increased food for other detritus feeders | Hazard | • Reduced cycling of detritus as a result of lower numbers of *Ae. aegypti* larvae leaves more food for other detritus feeders. |
| Reduced *Ae. aegypti* fecundity or longevity | Und. Event | • *Wolbachia Ae. aegypti* has lower fecundity or shorter life span than naturally occurring *Ae. aegypti.* |
| Reduced availability as food | Hazard | • Reduced numbers of eggs, larvae and adult *Ae. aegypti* reduces their availability to predators. |
| Reduced detritus feeding | Hazard | • Lower numbers of *Ae. aegypti* larvae provide reduced cycling of detritus. |
| Reduced pollination | Hazard | • Plant species receive less pollination by *Ae. aegypti* males as a result of fewer *Ae. aegypti* being present to feed on flowers. |
| Reduced predator fitness | Hazard | • Predators have reduced fitness because of reduced *Ae. aegypti* prey. |
| Reduced seed set | Hazard | • Plant species have lower fecundity because of reduced pollination services. |
| Reduced vectoring of disease to other species | Hazard | • A smaller *Ae. aegypti* population leads to lower transmission of diseases/arboviruses. |
| Smaller overall *Ae. aegypti* population size | Hazard | • Mean density of *Wolbachia Ae. aegypti* is lower than that of naturally occurring *Ae. aegypti.* |

*Aedes aegypti* is considered 'uniquely domestic among mosquito vectors' by Morrison *et al*. (2008) and this is the primary reason why the experts did not consider it likely that adverse ecological change would result from the release. The expert consensus was that *Ae. aegypti* is an exotic species that was probably introduced with the First Fleet to Australia (supported by Canyon et al. 2008), has a limited distribution in Australia (Beebe et al. 2009), occurs at low densities, is closely tied to domestic dwellings and has limited interactions with ecological systems outside this domestic setting.

Because of its low biomass, *Ae. aegypti* was not considered to represent an important component of food webs. The container-inhabiting mosquito simulation (CIMSiM) can rapidly estimate population sizes using data from sample containers (Williams *et al.* 2008). Using life stage weight data from Christophers (2009) and Cairns survey data, the mean biomass of *Ae. aegypti* was estimated at 6.7g $ha^{-1}$ (range of 2.1 to 13.2 g $ha^{-1}$) (S. Ritchie *pers. comm*.). Mean life stage numbers per hectare were 131 pupae, 187 females and 131 males. (Garelli *et al.* 2009) report a survey of approximately 2500

**properties in an Argentinean city that recovered nearly 8400 *Ae. aegypti* larva, less than 3 per property. This is substantially lower than the reported density of the congener *Ae. communis* in Russia where densities frequently exceed 1000 individual larvae m$^2$ (Nekrasova 2004) although this species lives in natural water bodies rather than artificial containers. The experts concluded that no predator species would be reliant on *Ae. aegypti* and removal or reduction in population density would not lead to ecological change.**

**The continual suppression of populations to levels which cannot sustain an epidemic is a fundamental approach to control of viruses such as dengue (Morrison et al. 2008). As the presence of suitable breeding habitat strongly influences populations, this is the focus of control methods. This reduces the carrying capacity of the environment; Abe *et al.* (2005) calculated that a Venezuelan cemetery with approximately 39 *Aedes* infested containers per ha could generate over 4185 pupae/ha per 48 hours and approximately 3000 females daily. Current dengue control practices in Cairns deliberately reduce or remove *Ae. aegypti* populations and reduce the carrying capacity of the environment. For example, Queensland Health estimates that the response to the 2008/09 dengue outbreak in Cairns involved removal of over 518 000 breeding sites and 8300 interior insecticide sprays across the ~75,000 properties visited had a considerable impact on these populations. It has been estimated that annual control costs possibly reach $400 000 AU in the Cairns region (cited in Canyon 2008) In effect, current control practices and measures provide the same result that is being considered a hazard, that of severely reduced *Ae. aegypti* population sizes.**

**In addition, the chemical control treatments used for *Ae. aegypti* control were considered to provide a greater ecological impact than would result from the removal of *Ae. aegypti*. Material Safety Data Sheets (MSDS) for the insecticides used to treat mosquito breeding sites, house interiors and exteriors under current control programmes by Queensland Health[4] indicate their potential to affect non-target organisms. For example, Cislin[5] and Demand[6] are both pyrethroid based residual surface treatments, with Cislin considered hazardous to both fish and aquatic organisms such as *Daphnia*, and Demand considered very toxic to fish, moderately toxic to algae, and has a high potential for bioaccumulation. Biflex Aqua[7] (also used indoors) is considered toxic to aquatic organisms and Baygon[8] surface spray is considered toxic to humans, birds, fish and other wildlife. The mosquito growth regulator Prolink[9] used for treating breeding containers such as gutters and rainwater tanks also has some toxicity and non-target issues.**

**In summary, because *Ae. aegypti* appears unlikely to have any interactions with natural systems and occurs at very low densities, it was considered unlikely that other species would rely heavily or even moderately on this mosquito as either a food item or as a provider of ecosystem services such as pollination. It is likely that the major ecological role of *Ae. aegypti* in the urban context is that of vectoring diseases such as dengue. The hazard of reduced *Ae. aegypti* populations is regularly achieved as a result of current mosquito control practices which in all likelihood cause more harm to a range of non-target organisms than any reduction in *Ae. aegypti* numbers.**

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[4] (http://www.health.qld.gov.au/dengue/managing_outbreaks/mosquito.asp)
[5] (http://www.bayeres.com.au/es/products/productdetail.asp?id=33)
[6] (http://www.syngenta.com.au/Start.aspx?PageID=10101&ProductID=538586&menuId=2053)
[7] (http://www.micropest.com/chemical-data/biflex-aqua-msds.pdf).
[8] (http://www.sentinelpestcontrol.com/msds/Baygon70WPMSDS.pdf).
[9] (http://www.garrards.com.au/zone_files/msds/m_prolink__pellets_v2.pdf)

**The experts were aware that the absence of evidence of ecological interactions does not constitute evidence of absence. But it is known that evidence of these putative interactions has been previously sought and not encountered or reported ('non-findings' do not generally appear in the literature) provides some confidence in this conclusion. It is also noted that the fault tree process identifies areas where further experiments or post-release monitoring should be targeted, e.g. monitoring for pollination services or predator-prey relationships.**

### 3.4. SUMMARY OF STAGE TWO: EXPERT WORKSHOP ON ECOSYSTEM CHANGE

- **The hazard of adverse ecological impacts resulting from the release of *Wolbachia Ae. aegypti* was evaluated at a workshop with mosquito experts because of the concern that this was a poorly explored hazard.**

- **A fault tree was constructed indicating that a reduction in *Ae. aegypti* populations could lead to ecosystem changes resulting from a reduction as a source of prey, loss of pollination services if they occur, and reduced competition to invasion by other mosquitoes with a similar niche.**

- **However, as *Ae. aegypti* represents a low overall biomass and is unlikely to be a reliable food source for predators, is obligately tied to human habitation and few if any ecological interactions can be identified despite international effort to detect such interactions, it was considered unlikely to have any meaningful ecological interactions or play a role in overall ecosystem health.**

- **Furthermore, *Ae. aegypti* is regularly subject to a range of mosquito control methods such as habitat destruction and application of pesticides. Not only do these practices continually decrease *Ae. aegypti* density but they cause a range of non-target effects. These impacts were considered to have a greater potential for impact than a reduction in *Ae. aegypti* numbers caused by *Wolbachia*.**

- **The absence of evidence of ecological interactions should not be construed to be evidence of absence of these interactions. The workshop identified a number of key areas where more robust conclusions can be arrived at following experimentation or monitoring such as for pollination services. However, it was considered that these findings were highly unlikely to change the overall conclusion regarding the broader ecological role of *Ae. aegypti*.**

## 4. STAGE THREE: EXPERT SOLICITATION ON BAYESIAN BELIEF NETWORK (BBN) STRUCTURE AND LIKELIHOODS

**The previous risk analysis steps of hazard identification and fault tree mapping identified and logically organised the hazards. In order to determine the probability of each hazard occurring as a preliminary step towards calculating risk, likelihoods would have to be estimated. Because of the novel nature of this modification, data is often not available or either incomplete or insufficient to describe possible effects and their interactions. In the absence of actual data the use of expert solicited values can be valuable but introduces its own set of issues including uncertainty. This can be apportioned to the natural *variability* or complexity of systems (particularly ecosystems) which is particularly relevant here, *incertitude* or *epistemic uncertainty* which is the lack of knowledge about models and parameters, and *linguistic uncertainty* which is a communication problem such as ambiguity or context dependence which arise from scientific language (Regan *et al.* 2002; Carey & Burgman 2008). Carey and Burgman (2008) suggest that linguistic uncertainty can be pervasive in forums such as workshops, leading to different interpretations of key words and phrases.**

**Bayesian Belief Nets (BBN) was used as a tool to elicit expert opinion on the interactions between hazards and the probability of each hazard failing. BBNs are acyclic graphs or models that explore the causal and correlative relationships between variables, allowing an intuitive representation of systems (McCann *et al.* 2006). Each node in the net represents a variable which could variously represent data or a hypothesis or in this case a hazard that we do not want to occur. Nodes are allocated states or a set of probable values which can be continuous or discrete. Connections (edges) are defined between nodes with the direction indicating causality (by definition an acyclic graph cannot have feedback loops). Parent nodes have no incoming connections, but feed into child nodes that can be considered as summary nodes that merge the input from their parents (Nyberg *et al.* 2006). This organisation of sometimes complex combinations of different data inputs into summary nodes is considered a strength of this approach by Marcot *et al.* (2006).**

**Each node contains a *conditional probability* table (CPT) based on the parent nodes that feed into it. This determines the probability of each state in the node depending on values assigned in the parent nodes (Marcot *et al.* 2001). BBNs explore the causal and correlative relationships between variables both graphically and with probabilities and can allow an intuitive representation of ecological systems. BBNs can be driven forward (conditional probabilities flowing through the model) or backwards to infer the most likely set of casual conditions for a given outcome (McCann *et al.* 2006). Although Bayes theorem was developed in the late 1700s, only recently has it enjoyed increased popularity because modern computation power is no longer limiting and because the method can calculate probabilities for large numbers of variables with different states as evidence or belief changes.**

**A BBN is first populated with *priors* that represent our current hypothesis or available information. *Posterior* probabilities are then added when more evidence or knowledge is available and the effects of this information on the probabilities can be evaluated. Qualitative or quantitative states can be assigned to each node. Where data is missing to assign *priors*, likelihoods can be elicited using expert opinion making this approach useful for systems with incomplete, uncertain or contrasting data or where we do not know the most important data to collect. Because of this flexibility and intuitive graphical portrayal of a system, BBN are being used increasingly to model systems with high uncertainty such as ecological systems and webs. For example Marcot *et al.* (2001) used BBNs to identify key environmental variables influencing the effects of land management on wildlife populations, Park *et al.* (2007) trained a BBN to recognise urban land use patterns from satellite images with a small data set, and Aitkenhead and Aalders (2009) used a BBN to predict land cover change from existing maps.**

As the hazards spanned a range of science and community related themes (i.e. some were technical, others had an anthropological component), it was decided to include both mosquito workers and members of the community, representing the people who would be directly affected by hazards eventuating following the release. Ideally a workshop of this nature would span several days to engage expert opinion directly on the sequential processes of hazard mapping, fault tree development and BBN design. However, we were advised that it would be difficult to retain community engagement for more than a day because of the technical nature of the information and competing responsibilities of community members.
Because of this constraint a draft BBN was constructed prior to the workshop. This was presented to the workshop participants for review and to make consensus modifications to its content, structure and definitions before likelihoods were solicited.

## 4.1. METHODS

### 4.1.1. Pre-Workshop Draft BBN

The draft BBN was constructed by CSIRO Entomology staff (Appendix 1) either associated with the risk analysis or experienced with BBNs. Nodes and their relationships were based on the information collected in Step One. The resulting model was built using the software package Netica© (Norsys Software Corp.) which is a graphical front end to support Bayesian modelling. A definition was drafted for each node describing the adverse hazard, factors to take into account when assessing this hazard, and the possible scoring states (generally same/worse).

### 4.1.2. Workshop Composition

A one day workshop was held on the 17th September in Cairns. The workshop design called for twelve experts that could be split evenly into three breakout groups of four people. Australian mosquito experts were identified by multi-media searches and through lists provided by the GCGH team with a bias towards including where possible experts not directly associated with the GCGH project. This was not always possible due to the small size of the available pool and the fact that in some areas all available researchers were linked to the project. In addition, to representing different and diverse organisations (e.g. medical, government, research), a broad range of criteria were scoped because of the range of hazards identified including entomologists, ecologists, vector ecologists, regulators, mosquito rearing, epidemiologists etc.

Community experts who had at least a rudimentary background knowledge of the project through he GCGH community engagement programme were approached for their availability. The key requirement for the community experts were that they were local residents, and ideally would represent a broad cross section relevant to the hazards such as media, political, indigenous, local or environmental NGOs.

### 4.1.3. Expert BBN Review

The attendees were given an introductory session of presentations on the project background, biology of *Ae. aegypti*, dengue and *Wolbachia*, the risk analysis process, and an introduction to Bayesian methods. The experts were asked to organise themselves into three breakout groups with equal numbers of mosquito and community experts. Each breakout group was provided with an A3 printout of the draft BBN and definitions. They were asked to evaluate this material and achieve consensus on any changes to the BBN structure, hazards (nodes), links and definitions they felt necessary required to adequately describe the hazards and their relationships. Each breakout group presented their suggested changes to the workshop for discussion and these were incorporated into the draft BBN where consensus was reached.

### 4.1.4. Expert Likelihoods

**The experts were asked to separate into new groups and were then assigned different submodels of the BBN and a workshop convener. A conditional probability table (CPT) calculator was used to capture the possible scoring combinations for each summary (child) node that reflect the linked hazards and their states. The groups were asked to discuss and reach consensus on each conditional probability. These values were used to populate the relevant BBN nodes in Netica.**

## 4.2. RESULTS

### 4.2.1. Pre-Workshop Draft BBN

**Figure 10 shows the draft BBN for 'Cause More Harm' which consisted of 28 nodes, 32 links and 33 conditional probabilities and consisted of four submodels. 'Ecology' contained hazards of horizontal transfer of *Wolbachia* to other organisms and changes in the geographical range, niche and density of *Wolbachia Ae. aegypti.* 'Mosquito Management Efficacy' represents the hazard that the effectiveness of current treatments strategies would decline because of a need for more control treatments, increased resistance to insecticides used to suppress populations, and a decline in future research and household control of mosquitoes because of perceptions that the dengue problem is resolved by the release. 'Economic effects' captures the hazards of reduction in tourism, real estate values and the availability of labour in areas containing *Wolbachia Ae. aegypti.* The 'Standard of public health' submodel describes hazards that could reduce community health because of various factors such as changes in host preference or biting rates and the increased transmission of dengue and other pathogens.**

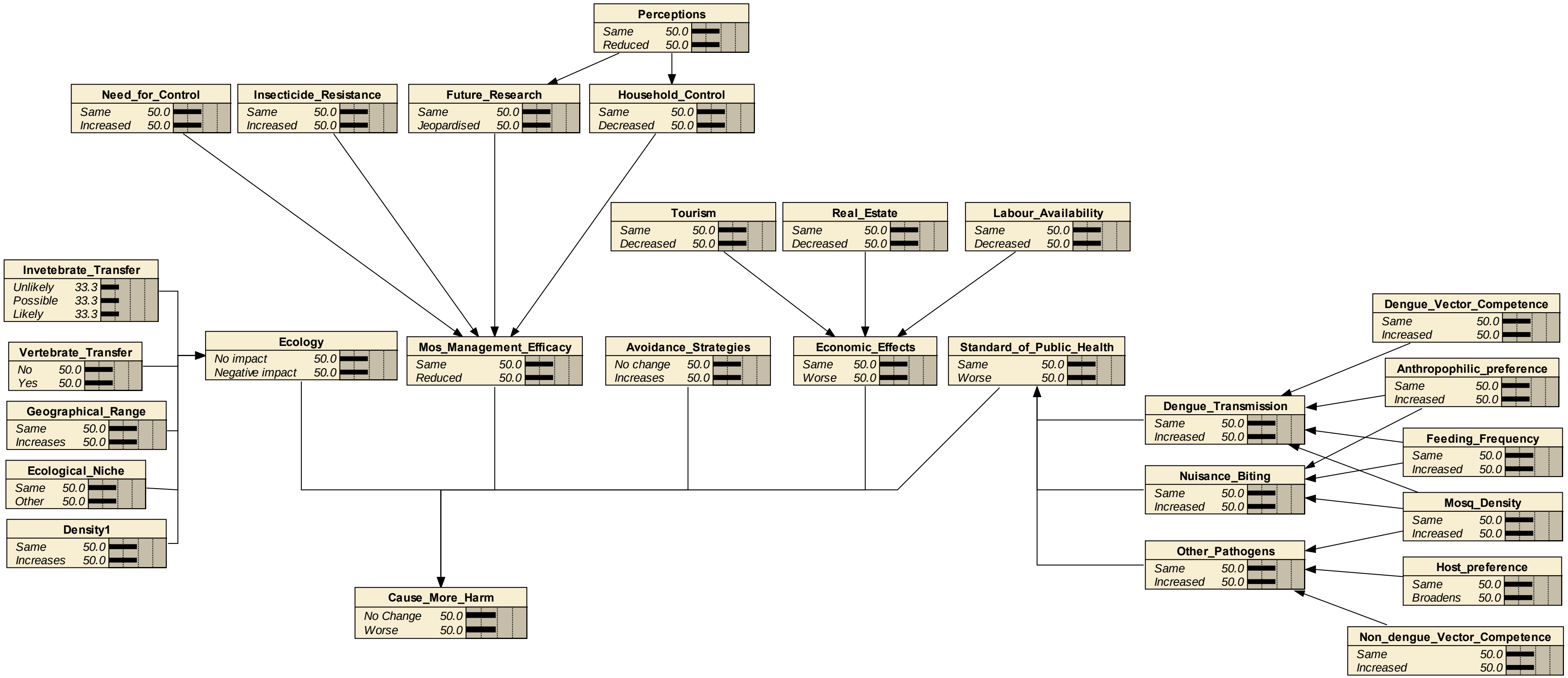
### 4.2.2. Workshop Participation

**The four CSIRO workshop conveners, two observers, six mosquito and eight community representatives that attended the workshop are detailed in Appendix 1.**

### 4.2.3. Expert BBN Review

**Three nodes were added ('*Wolbachia* fitness', 'Monitoring', 'Health care'), one was renamed, ('Future research' → 'Future mosquito management') and the nodes 'Anthropophilic preferences' & 'Non-Anthropophilic preferences' were amalgamated into a node called 'Host preference'. Definitions were provided for the new nodes. The resulting BBN for 'Cause More Harm' contained 30 nodes, 40 links and 397 conditional probabilities. A 30 year timeframe over which to estimate the likelihood of a hazard occurring was agreed at.**

**Figure 10. Draft BBN for 'Cause More Harm' developed prior to workshop**



### 4.2.4. Expert Likelihoods

**The results of the expert review and workshop solicited likelihoods assigned at the workshop to the 'Cause More Harm' BBN is seen in Figure 11. Note that the topology of 'Cause More Harm' was changed after the workshop for clarity but this was a cosmetic change only.**

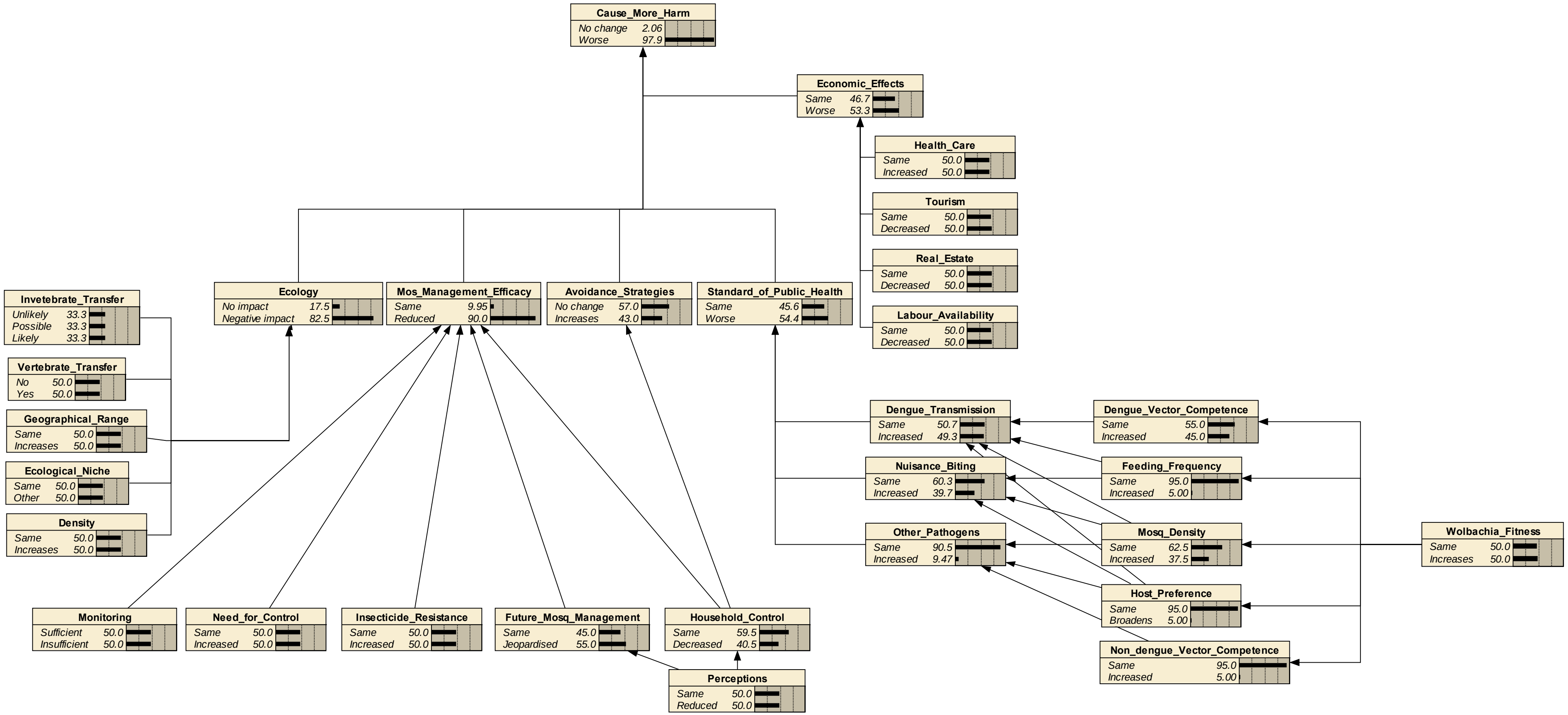
**Cause More Harm**
**What is the likelihood that the release of *Wolbachia Ae. aegypti* will cause more harm than that currently provided by naturally occurring *Ae. aegypti*? Take into account:**

- **Ecology**
- **Mosquito management efficacy**
- **Avoidance strategies**
- **Economic effects**
- **Standard of public health**

**No Change (no more harm is achieved)**
**Worse (more harm results from the release of *Wolbachia Ae. aegypti* )**

**The solicited likelihoods provided an estimated failure likelihood 97.9% for 'Cause More Harm'. Likelihoods of the 'Mosquito management efficacy' (90%) and negative 'Ecological impacts' (82.5%) hazards failing were considered more likely than for a decline in the 'Standard of public health' (54.4%), 'Economic effects' (53.3%) or 'Avoidance strategies' (43%).**

**Figure 11. 'Cause More Harm' BBN after expert review and likelihood scoring for child nodes. Note also that the topology has changed from the draft BBN, but this is cosmetic only).**



## 4.3. DISCUSSION

**Results from the hazard identification methods used in Stage One provided the basis for the draft BBN. For example, a number of major themes identified in the Stage One (Appendices 2-5) are represented as submodels (e.g. ecological, economic). The Fault Trees components were used to help identify possible relationship between different hazards. For example the 'Worse Community Health' sub-tree describes a set of hypothetical relationships captured in the 'Standard of Public Health' submodel of the 'Cause More Harm' BBN. We acknowledge that the draft BBN could have influenced the experts and that they would not necessarily have arrived at the same conclusions if the model was built from scratch at the workshop, but it was considered a necessary step to allow more workshop time for likelihood scoring, and there were no constraints to the changing of the model other than workshop consensus. Workshop consensus was achieved on a number of changes including addition, subtraction (via amalgamation of two nodes) and modification.**

**Constraints to creating an exact replica of the fault trees and using all hazards include the fact that BBNs are acyclic and therefore cannot incorporate feedback loops, and a known weakness of BBNs (and fault trees) is that they cannot handle time dependant interactions (Siu 1994; Bobbio *et al.* 2001; Nyberg *et al.* 2006). Large BBNs also become cumbersome to populate because they generate proportionately large numbers of conditional probabilities that need to be populated. Even though the CPT calculator reduces the work load associated with scoring likelihoods by extrapolating the full table from a subset of responses, there was insufficient time after this exercise to allow a full workshop consensus on the scores. The resulting child node likelihoods were a combination of group scores, with consensus within but not between groups, and no parent node likelihoods that feed into the child nodes were obtained.**

**Because of their intuitive approach and graphical and causal features, and to some extent avoidance of statistical and technical jargon, McCann *et al.* (2006) considered BBNs more valuable than complex models in workshop situations where participants may lack formal technical training. They note the flexibility of Bayesian nets in that they can draw *priors* both from empirical data or experts, but warn that use of expert judgement requires careful documentation and justification. The participation of community representatives was an attempt to incorporate community views into the analysis, particularly as a number of hazards involved community attitudes and behaviours. In these areas they were able to present their expert view to help inform group consensus scoring, but the majority found the scientific terminology and methods unfamiliar and intimidating. As a result, some questioned the value of their contribution in the presence of technical experts who tended to dominate discussion over the more science related nodes. It is likely under these circumstances that they accepted the status quo of opinion in group situations rather than presenting a viewpoint or asking for additional information where they were unsure.**

**Because of these issues the resulting end point assessment score of 97.9% for 'Cause More Harm' probably does not accurately reflect the values that would have been obtained had a full workshop consensus been achieved.**

## 4.4. SUMMARY OF STAGE THREE: EXPERT SOLICITATION ON BAYESIAN BELIEF NETWORK STRUCTURE AND LIKELIHOODS

- **A one day workshop was held in Cairns on 17th September 2009 to solicit expert opinion from both community and research experts on the hazards associated with the release of *Wolbachia Ae. aegypti.***

- **A draft Bayesian Belief Net (BBN) and definitions representing the possible casual relationships between hazards associated with the assessment endpoint of 'Cause More Harm' was prepared before the workshop and subjected to review by the workshop participants. The draft BBN for 'Cause More Harm' consisted of 28 nodes, 32 links and 33 conditional probabilities.**

- **The review of the BBN added three new nodes ('*Wolbachia* fitness', 'Monitoring', 'Health care') and the 'Anthropophilic preferences' & 'Non-Anthropophilic preferences' nodes were amalgamated into a 'Host preference' node.**

- **The modified BBN for 'Cause More Harm' contained 30 nodes, 40 links and 397 conditional probabilities.**

- **Preliminary *priors* were undertaken by soliciting group consensus values for the child (summary) hazard nodes, but workshop consensus was not achieved. The combined group consensus scores provide a likelihood for the adverse endpoints occurring of 97.9% for 'Cause More Harm'.**

- **This initial set of *priors* was incomplete and additional expert opinion was required to allow a more robust assessment of hazard likelihoods.**

## 5. STAGE FOUR: EMAIL EXPERT SOLICITATION ON BBN PARENT NODE LIKELIHOODS

**Consensus likelihoods for the parent node hazards were required to complete the *prior* likelihood estimates of hazard failure. The most rapid means to achieve this was by soliciting expert opinion via an email questionnaire. This provides an opportunity to rapidly access expert opinion from a wider community including overseas experts but the downside is that only a small proportion might respond. This also raises the possibility of linguistic uncertainty, particularly where respondents lacked the contextual experience of the prior workshop. Uncertainty can also result from a lack of knowledge or knowledge which can be reduced by additional research or other information generating processes (McMann *et al.* 2006), but as each expert has different experience, knowledge and biases to draw upon some variation would be expected.**

**As the amount of variation expressed in expert solicited values can be indicative of the level of certainty, which is high when experts are in agreement, but decreases as experts diverge (Martin *et al.* 2004) we evaluated the variation in individual responses to test how robust this approach was for soliciting likelihoods.**

### 5.1. METHODS

#### 5.1.1. Expert Solicitation on Likelihoods

**Likelihoods for the parent nodes (nodes without any other nodes feeding into them) were solicited from both the DCG and the non-DCG workshop mosquito experts who attended the Stage two workshop on ecology hazards (Appendix 1). The experts were emailed definitions of the 14 relevant nodes and asked to assign likelihoods to each state in a spreadsheet.**

#### 5.1.2. Variation Analysis

**The variability in the responses to each state was examined by calculating the mean, mode, standard deviation and range (difference between highest and lowest assigned likelihoods). Histograms were built to examine the distribution of likelihoods in each node before deciding which summary statistic would be used to populate the BBN.**

### 5.2. RESULTS

#### 5.2.1. Expert Solicitation on Likelihoods

**A total of 20 replies were received although not all respondents scored all of the likelihoods**

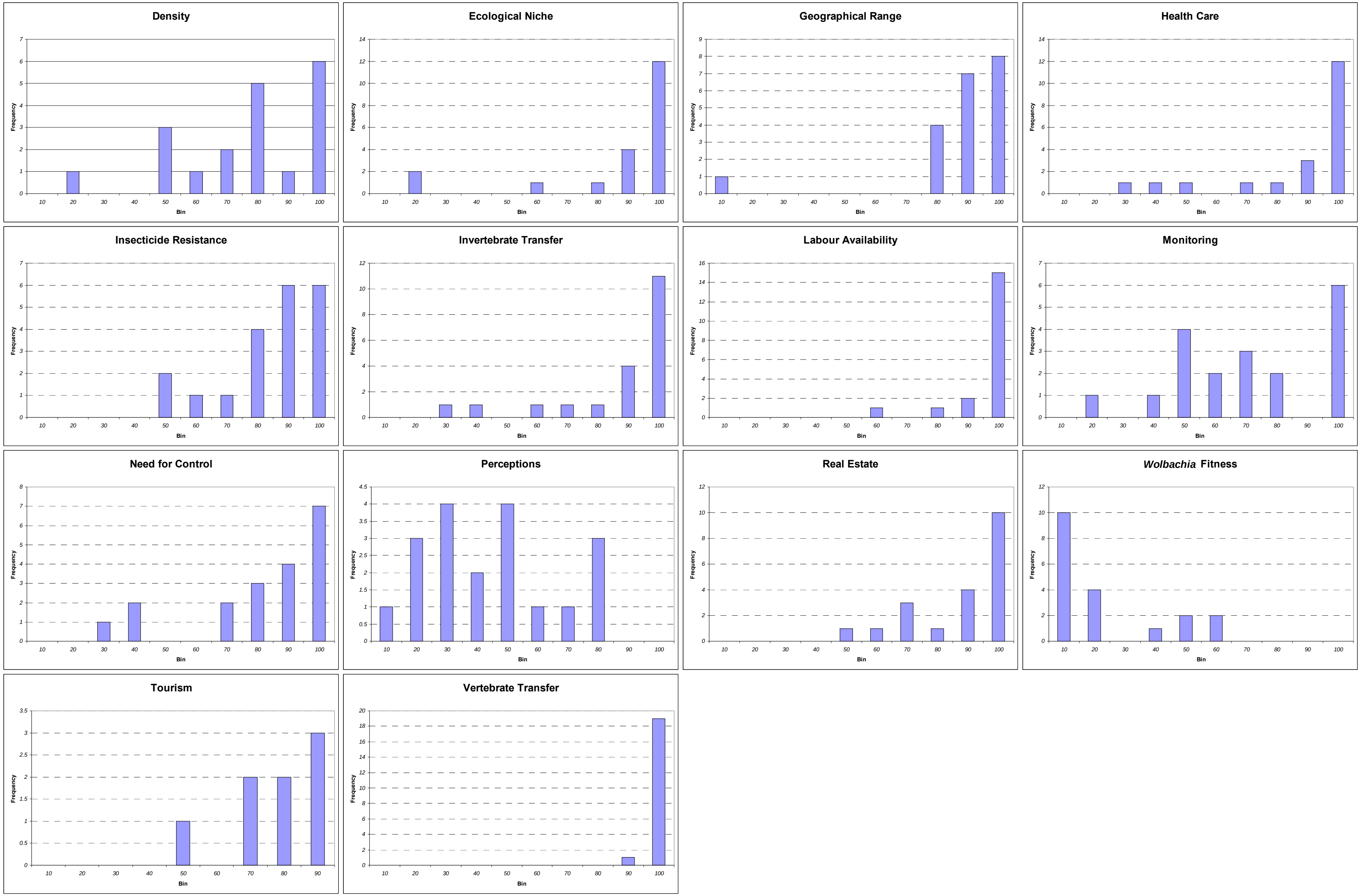
#### 5.2.2. Variation Analysis

**Table 7 shows the summary of 20 responses statistics of mean, median, modal (most frequent score) and range of expert likelihoods assigned to each of the 14 parent nodes (although note in some cases the experts chose not to score a particular node e.g. density). The data is notable for the generally high ranges in likelihoods. Only 2 of 14 hazards had a range of estimated likelihoods less than 50%, 'Vertebrate transfer' (10 point range) and 'Labour availability' (40 point range). Although the 'Invertebrate transfer' node had two states with a range between 30 and 40, this is a three-state (unlikely/possible/likely) node so not directly comparable. Histograms of the likelihoods assigned for each parent node (Figure 12) show the presence of outliers (e.g. 'Ecological Niche') and diverging scoring (e.g. 'Perceptions'). This is indicative of high uncertainty in comparison to 'Vertebrate transfer' which show high similarity amongst the experts. Because of these outliers and scoring spread, the mean values do not reflect the group voting trend, so the modal (most frequent score) was used to populated the BBN. Results of the mean values on the child nodes and end points are presented for comparison with the modal values.**

Table 7. Summary table of expert likelihood mean, median, mode and range for parent nodes.

| Node | State | Mean | Median | Mode | Range | Count |
|---|---|---|---|---|---|---|
| Density | Same | 75.7 | 80 | 80 | 80 | 19 |
| | Increases | 24.3 | | | | |
| Ecological niche | Same | 84.7 | 95 | 95 | 80 | 20 |
| | Other | 15.3 | | | | |
| Geographical range | Same | 86.1 | 90 | 90 | 90 | 20 |
| | Increases | 13.9 | | | | |
| Health care | Same | 85.3 | 100 | 100 | 70 | 20 |
| | Increased | 14.7 | | | | |
| Insecticide resistance | Same | 85.0 | 80 | 80 | 50 | 20 |
| | Increased | 15.0 | | | | |
| Invertebrate transfer | Unlikely | 84.0 | 95 | 95 | 69.99 | 20 |
| | Possible | 11.0 | 5 | 1.0 | 39.99 | |
| | Likely | 5.0 | 0.5 | 0.0 | 33 | |
| Labour availability | Same | 94.2 | 98 | 100 | 40 | 20 |
| | Decreased | 5.8 | | | | |
| Monitoring | Sufficient | 67.9 | 70 | 50 | 79 | 20 |
| | Insufficient | 32.1 | | | | |
| Need for control | Same | 79.2 | 90 | 80 | 75 | 19 |
| | Increased | 20.8 | | | | |
| Perceptions | Same | 43.6 | 40 | 30 | 75 | 19 |
| | Reduced | 56.4 | | | | |
| Real estate | Same | 86.4 | 92.5 | 90 | 50 | 20 |
| | Decreased | 13.6 | | | | |
| *Wolbachia* fitness | Likely | 22.0 | 10 | 10 | 59.99 | 19 |
| | Unlikely | 78.0 | | | | |
| Tourism | Same | 89.0 | 95 | 95 | 50 | 20 |
| | Decreased | 11.0 | | | | |
| Vertebrate transfer | No | 98.3 | 100 | 100 | 10 | 19 |
| | Yes | 1.7 | | | | |

**Figure 12. Histograms of expert assigned likelihoods to the 14 parent nodes of 'Cause More Harm' BBN.**



### 5.2.3. Mean and Modal Likelihood BBN

**The results of populating the BBN parent nodes with the mean expert derived likelihoods can be seen in Figure 13. In comparison to the BBN with only child node priors from the Stage three workshop, the likelihood of decreased 7.1% for 'Cause More Harm' (90.8%). The modal parent node expert likelihoods reduced the likelihood of 'Cause More Harm' to 77.8% (down 21.1%). The full 'Cause More Harm' BBN is shown in Figure 14.**

**Figure 13. Effects of using mean (top) and modal (bottom) expert likelihoods assigned to parent nodes on likelihoods of 'Cause More Harm'**

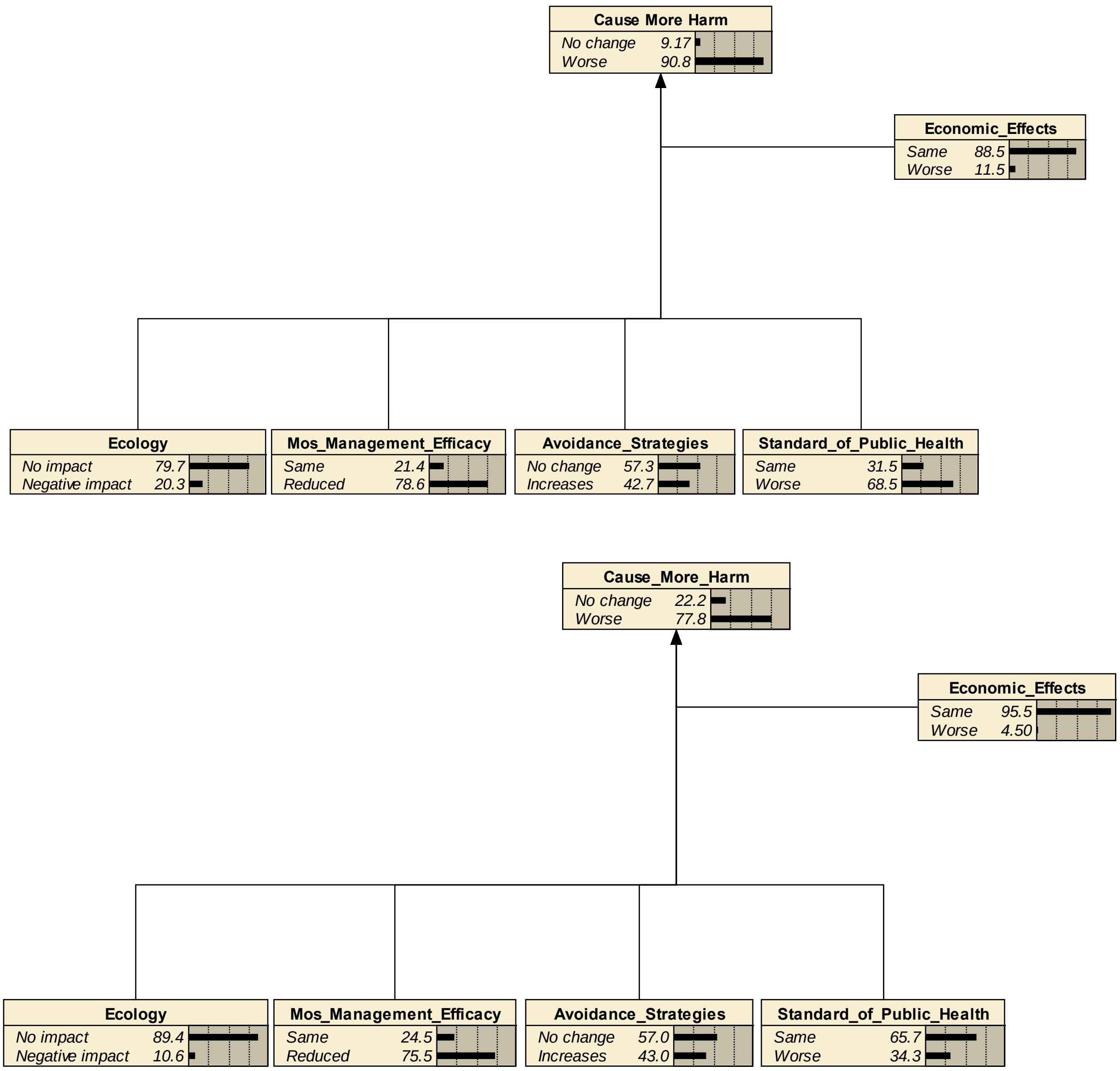
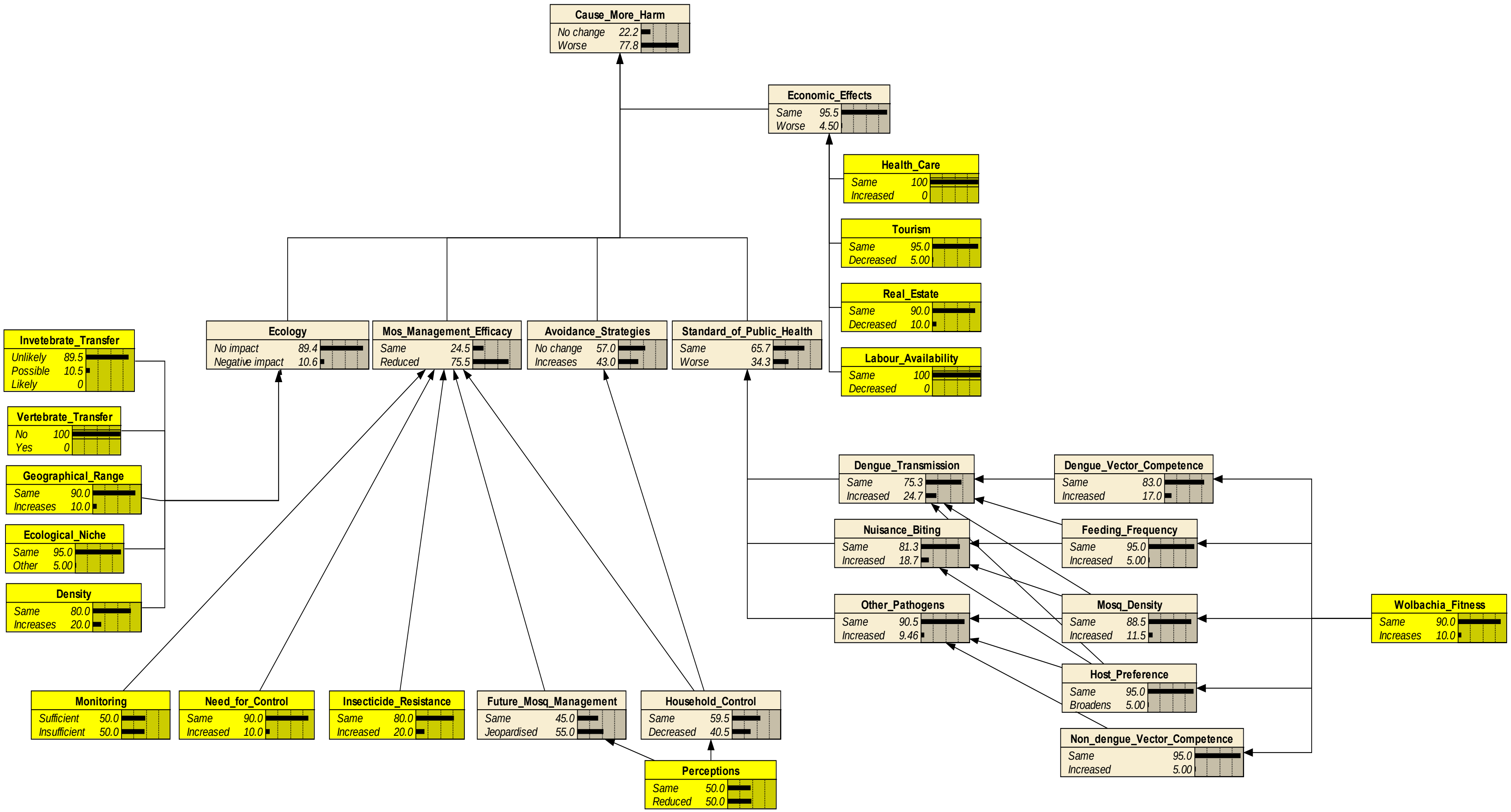


**Figure 14. 'Cause More Harm' BBN after email solicitation and use of modal parent node likelihoods (Note: yellow indicates parent nodes)**



## 5.3. DISCUSSION

**Likelihood estimates for the failure of parent nodes were solicited by email from the community of experts familiar with the project. This was done both as a matter of expediency to attain a fuller set of *priors* and to also evaluate the effectiveness of this approach for future consideration. Although a few nodes such as 'Vertebrate transfer' had high agreement, others contained outliers, scoring was highly disparate. This high variation indicated the mean response scores were unlikely to represent how the majority of the experts were scoring, and the modal or most frequent scores were used to populate the BBNs.**

**The full set of *priors* achieved with the modal values for the parent nodes led to a reduction in the endpoint likelihoods, although clearly this does not constitute an expert consensus score. The likelihood of 'Cause More Harm' by 21.1% to 77.8%. This is 13% less than the model output of 90.8% using the mean of the expert scores. Despite issues with email solicitation as a means of communicating hazard likelihoods, the data was useful in clarifying that the endpoint risks were not as high as initially indicated with the child nodes populated alone.**

**The major issue with the email approach was the amount of variation in the scoring which indicated high uncertainty. In particular *epistemic* and *linguistic* uncertainties were likely with some of the variation resulting from different expert knowledge and experience, and some from the hazard definitions. Feedback from respondents who lacked the context of participating in the development of the BBN models and the hazard definitions indicated there was difficulty in their interpretation. These were intended to be succinct statements accessible to both science and community representatives, had been vetted by a workshop, and included a glossary of key terms to help avoid vagueness. This suggest that communication of the intent of some definitions is lost by email, whereas group discussion in workshop formats has an advantage in reducing uncertainty because consensus can be reached on definition meanings, new information such as experimental results can be exchanged, and there is the possibility of consensus likelihood scoring.**

**The different sources of noise evident in both the child node (not consensus) and parent node (high variation and uncertainty) likelihoods led to low confidence that the amalgamated set of *priors* reflected expert opinion.**

### 5.4. SUMMARY OF STAGE FOUR: EMAIL EXPERT SOLICITATION ON BBN PARENT NODE LIKELIHOODS

- **Likelihoods for the 14 parent nodes for 'Cause More Harm' were solicited from experts by email in order to complete a first set of *priors* for the BBN.**

- **20 responses were received although not all hazards were scored by all respondents. For many hazards the experts values diverged, were not normally distributed or outliers were present in some cases.**

- **The divergence in expert likelihoods indicates the presence of uncertainty. This is likely to arise from a number of sources including linguistic and incertitude because of the differences in experience, knowledge and particularly interpretation the definitions where the respondent had not participated in the Stage three workshop where they were finalised.**

- **Some experts indicated that they found difficulty in assigning a solitary value to a likelihood eventuating, and that they would be more comfortable if they could provide a range that they believed captured their estimate.**

- **The mean scores were not considered a robust indication of the group scoring so modal likelihoods were used to populate each parent node.**

- **The combined *priors* collected from both the Stage three workshop (child nodes) and Stage Four email solicitation exercise (parent nodes) provided a likelihood of 77.8% for 'Cause More Harm' being realised which was a decrease of 20.1% from the workshop scores alone. This is lower than the 90.8% produced with the expert means which do not appear to be reflective of group scoring.**

- **There was concern that the current amalgamated set of *priors* was affected by various sources of uncertainty and as it was not a consensus set of hazard likelihoods it still did not adequately reflect the state of expert opinion.**

## 6. STAGE FIVE: EXPERT WORKSHOP TO REDUCE UNCERTAINTY AND CALCULATE RISK

### 6.1. BACKGROUND

Stage Four of the risk analysis indicated there was considerable uncertainty in expert scoring of hazard likelihoods and opportunities for group consensus scoring (e.g. consensus achieved by discussion and agreement) had been limited. As a result we were not confident in the *prior* likelihoods for the risk analysis endpoint. A two day workshop was undertaken at CSIRO Entomology (Longpocket Laboratories) in Brisbane to resolve some of the sources of uncertainty and obtain a set of both likelihood and consequence estimates that could then be used to calculate expert derived risk estimates.

The aims of the workshop were to convene a small (<10) group of experts familiar with *Aedes aegypti* biology, ecology, interaction with *Wolbachia*, management and control and:

- provide experts with summary of risk analysis background (provide context)
- provide experts with updates on the latest relevant research (new information to reduce incertitude)
- allow discussion of definitions (reduce linguistic uncertainty)
- agree on scale for likelihood and consequence (reduce linguistic uncertainty)
- allow review of the BBN structure to ensure it captured the key hazards
- use a four point scoring scale (lower bound, upper bound, median, confidence) to better capture expert values (reduces over-confident scoring)
- solicit individual and group consensus scores for both likelihood and consequence to allow calculation of risk
- populate the BBN (including any changes) with new *priors*

Both individual and group consensus scores were solicited to allow comparison of these approaches for future use. However, this report only includes the group consensus scores in the final BBN and calculation of risk.

### 6.2. METHODS

#### 6.2.1. Expert Composition

The expert team was selected from available mosquito researchers the majority, but not all were familiar with the GCGH project (Appendix 1) to ensure that the participants were at a minimum familiar with the project and able to provide up to date information for discussion. There is an acknowledged trade off between incorporating bias by involving project related members and the advantage of having experts with fundamental knowledge of the project and research.

#### 6.2.2. New Information

The experts were requested to bring and discuss any new information or research which might be useful during the workshop.

#### 6.2.3. Scale

The participants were asked to define what the scale represented both qualitatively and quantitatively so that they were using the same context

#### 6.2.4. BBN and definition modifications

The existing BBN (with blank probability scores) was reviewed by the participants to determine if any changes (node and link additions or removals) were required.

### 6.2.5. Risk (Likelihood x Consequence) Scoring

Individual scoring was undertaken before group consensus to allow a subsequent comparison of the two approaches. The consensus values were used to populate the final BBN in preference to the individual score results because they are a consensus, and the individual results are not discussed further. A four point scoring approach was used after feedback from the Stage Two Workshop and Stage Three email exercise indicated that the experts sometimes felt uncomfortable scoring a single likelihood and were more comfortable indicating a range. The four point method achieves this by asking the expert to first score a lower then upper estimate, their best guess and then a confidence estimate that their range of values has captured the 'real' value. This approach has been shown to reduce the overconfidence of expert scoring compared to methods without asking for a confidence score (Speirs-Bridge *et al*. 2009).

During individual scoring each node definition was discussed to clarify meaning before scoring on a 10 point scale for both likelihood and consequence. Consensus group scoring consisted of plotting individual scores (bounds and median) on a whiteboard against the 10 point scale. The experts were then asked in a group setting to explain and discuss the reasoning for this scoring until a group consensus for all four scoring points were agreed for both likelihood and consequence. Consensus CPT scores for the child nodes were solicited using the same methods for the Stage Three workshop and the BBN repopulated with the new data.

### 6.2.6. Sensitivity analysis

A sensitivity analysis is a study of how much variation in a model output can be attributed to variations in the model input. This was undertaken to examine the contribution of each individual node or combinations of child nodes on the likelihood of the relevant end point. After populating the BBN with the expert solicited likelihoods, the positive state for each node was set at 100% and the change in the likelihood of the relevant end point noted. This was repeated iteratively using the possible combinations of child nodes.

### 6.2.7. Email Re-solicitation of key node likelihoods

After populating the BBN two nodes were identified as requiring more attention as the analysis endpoint of ‘Cause More Harm’ was sensitive to their values. These were ‘Dengue Evolution’ and ‘Tourism’ which were both scored at 10% probability of failing. We asked the workshop participants to re-score these two nodes using a 100 point scale as we observed a disconnect between the likelihood scores and the group discussion where the discussion indicated a very low to negligible risk yet the likelihood score approached the low range. We concluded therefore that the 10 point scale provided insufficient accuracy when scoring sensitive nodes. The resulting mean scores were used to populate these two nodes.

### 6.2.8. Calculation of Risk

A risk matrix was set up to combine the estimates of likelihood and consequence as a risk matrix. Risk was estimated for each hazard using the group consensus scores and plotted in the matrix.

## 6.3. RESULTS

### 6.3.1. Expert Composition

Nine experts and three CSIRO Entomology workshop conveners attended the workshop (Appendix 1).

### 6.3.2. New Information

The following additional new information was provided:

- **Back up rearing colony in Brisbane and Melbourne had been or were about to be established in case the MRF in Cairns was rendered unable to operate.**
- **An alternative release site had been identified in case the incumbent site (Gordonvale) was unsuitable at time of release.**
- **Funding was available from other sources such as the National health and Medical Research Council (NHMRC) if the Gates Foundation withdrew**
- **Insecticide resistance trials for two chemicals which indicated no significant increase in *Wolbachia Ae. aegypti* insecticide resistance over naturally occurring *Ae. aegypti*.**
- **Data from trials testing for horizontal transmission of *Wolbachia* found no evidence of it occurring. This data is as yet unpublished. There were two studies (1) explored transmission of *Wolbachia* via the consumption of wMelPop *Ae. aegypti* by mosquito predators *Poecilia reticulate* (guppy), *Pseudomugil signifier* (Pacific blue-eyes), *Menemerus bivittatus* (jumping spider), *Pholcus phalangoides* (daddy long legs), *Hemidactylus frenatus* (Asian house gecko) and copepods; (2) explored the possibility of dissemination of *Wolbachia* into the environment. Here the presence of *w*MelPop was searched for in soil, mulch, plant tissues and invertebrates (millipedes) feeding on mulch.**
- **Data indicating that dengue replication was significantly and considerably reduced in *Wolbachia Ae. aegypti.* This research showed that inhibition of dengue virus replication by *Wolbachia* was in addition to the life shortening effect (Moreira *et al*. 2009).**
- **Approximately 1000 mosquitoes would be released each week following suppression of naturally occurring populations, but mosquito density would not be increased above long term average numbers.**

**Group discussion focused on the issue of horizontal *Wolbachia* transfer from *Wolbachia Ae. aegypti* and a number of scientific papers produced on this topic. It was agreed that there is accumulating genetic evidence that many insect and nematode species carried genetic fragments of *Wolbachia* as a result of inter-specific horizontal transfer (Hotopp *et al*. 2007). For example, a large genetic transfer of up to 31 *Wolbachia* genes was found in the beetle *Monochamus alternatus* (Aikawa *et al*. 2009), and evidence was presented that naturally occurring *Ae. aegypti* also carries the genetic signature of a *Wolbachia* HGT event that occurred in the evolutionary past of the organisms (Klasson *et al*. 2007). It was noted that the majority of these transfers are non-functional and evidence indicates that considerable periods of time have passed since their occurrence (e.g. Klasson *et al*. 2007; Woolfit *et al*. 2009).**

**Heath *et al*. (1999) showed horizontal *Wolbachia* infection between an egg parasitoid and its infected *Drosophila simulans* host, but this was gradually lost during vertical transmission (transmission to offspring), and Huigens *et al*. (2004) found a similar pattern in *Trichogramma* egg parasitoids, where *Wolbachia* can transfer from infected larvae of one *Trichogramma* species to uninfected larvae of another *Trichogramma* species. Again the infection was lost within several generations. The issue of the artificial and stable transfer of *Wolbachia* to a mammalian cell line (e.g. Noda *et al*. 2002; Fenollar *et al*. 2003) was also discussed and noted that artificial *Wolbachia* transfers are difficult to achieve, they require exhaustive effort and many attempts have failed. Noda *et al*. (2002) also acknowledge that *Wolbachia* have a broader host range in vitro than nature.**

**The experts were provided with presentations by the workshop conveners describing the risk analysis process. The BBN from Stage three was demonstrated, but was set with blank values to reduce the possibility of this influencing their scoring although we acknowledge that two participants (O'Neil and Sutton) had previous access to these results.**

### 6.3.3. BBN and definition modifications

**The experts included a new node 'Dengue Evolution' which is linked to 'Dengue Transmission' and describes the hazard of the dengue virus overcoming *Wolbachia* inhibition. After discussion the 'Future Mosquito Management' was removed because whether future investment in mosquito was negatively affected by a release should not be considered a reason for not proceeding.**

**The experts also agreed to make the following assumptions about the definitions:**

- **the time frame of 30 years was set for estimating likelihoods as extrapolating beyond this increases uncertainty substantially.**
- **previous release dates benchmarks has been set for November 2010, but as substantial time had elapsed since the formation of the definition this was modified to the 2010 wet season (although note that this is part of the 'Logistical Constraints' submodel which has subsequently been removed from the BBN).**

### 6.3.4. Scale

**The experts agreed on a scale to score likelihood and consequence as seen in Table 8.**

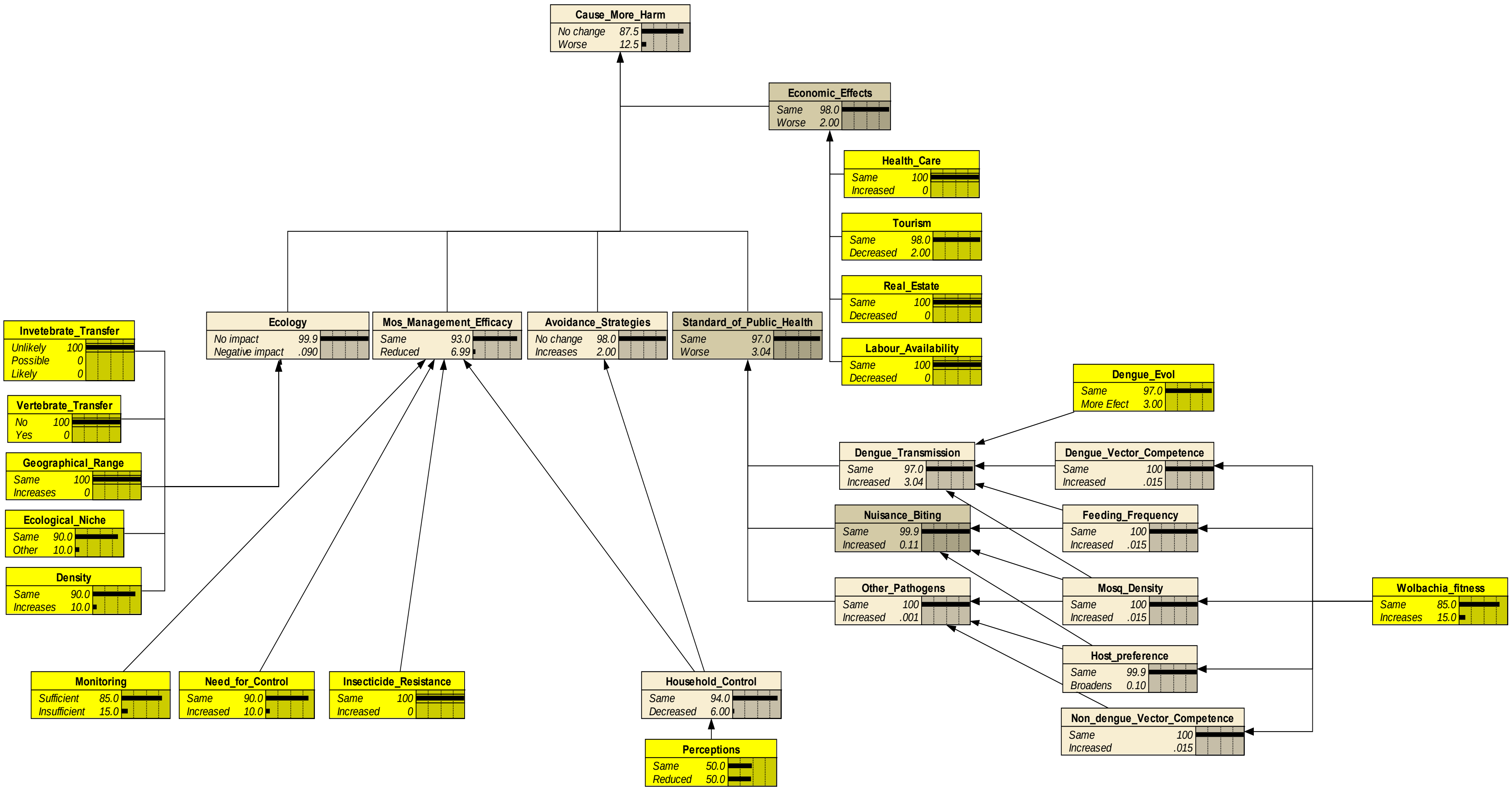
**Table 8. Scale for likelihood and consequence used for the 2nd dengue Workshop**

| Scale | Negligible | Very Low | Low | Moderate | High | Very High |
|---|---|---|---|---|---|---|
| Probability | 0 – 0.01 | 0.02 – 0.10 | 0.11 – 0.30 | 0.31 – 0.74 | 0.75 – 0.94 | 0.95 – 1.0 |

### 6.3.5. Final 'Cause More Harm' BBN

**Figure 15 shows the final BBN for 'Cause More Harm' after removal of 'Future Mosquito Management', addition of 'Dengue Evolution' node and resolicited scores for 'Dengue Evolution' and 'Tourism' nodes. This provides an assessment endpoint failure likelihood of 12.5%. The BBN contains 30 nodes, 38 links and 363 conditional probabilities.**

**Figure 15. Consensus BBN for ‘Cause More Harm’ after removal of ‘Future Mosquito Management’, addition of ‘Dengue Evolution’ node and resolicited scores for ‘Dengue Evolution’ and ‘Tourism’ nodes (Note: yellow indicates parent nodes)**



### 6.3.6. 'Ecology' Submodel ('Cause More Harm')

**The 'Ecology' child node in Figure 16 captures the hazard that ecological harm will result from the release of *Wolbachia Ae. aegypti* and is a summary node linked by five discrete ecological hazards. The likelihood of ecological harm was considered negligible at 0.09%. The hazard of the horizontal gene transfer (HGT) of *Wolbachia* was captured for both vertebrate and invertebrates hosts but for both hazards a zero likelihood of harm was indicated. An expansion of the 'Geographical range' as a result of *Wolbachia* was also considered negligible (0%). There was a very low risk that *Wolbachia Ae. aegypti* would increase its 'Ecological niche' (10%) or that there would be a subsequent increase in *Ae. aegypti* 'Density' (10%).**

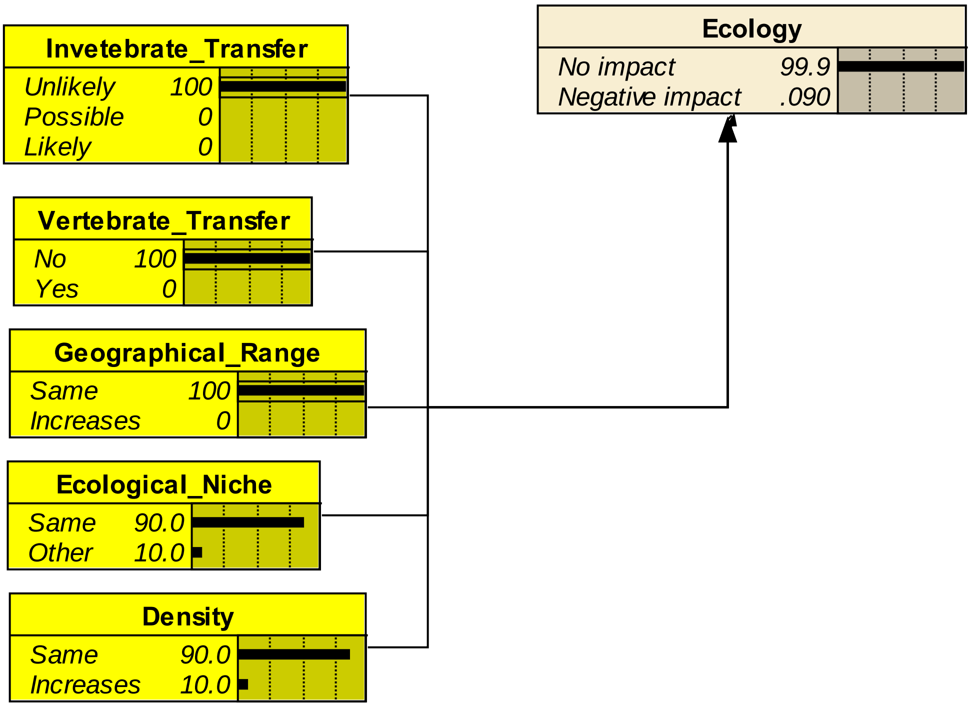



**Figure 16. Nodes and final likelihoods feeding into the 'Ecology' submodel for 'Cause More Harm'.**

**There is no evidence for *Wolbachia* transfer from invertebrates to vertebrates despite this being a topic of interest to researchers. There is accumulating molecular data to suggest *Wolbachia* has historically moved between invertebrate species by horizontal transfer and exchange of genetic material (e.g. Cook & Butcher 1999; Hotopp *et al.* 2007; Woolfit *et al.* 2009; Baldo *et al.* 2008). Although *Ae. aegypti* is not known to contain *Wolbachia,* there is evidence it contains a functional *Wolbachia* gene presumably from an ancient horizontal gene transfer event (Klasson *et al.* 2009). The experts could not conceive how *Wolbachia* would move from this system into a vertebrate host given that there is no evidence of this having occurred previously. Yet to be published data was presented at the workshop on horizontal transfer trails and this is likely to have informed the scoring. The possibility of *Wolbachia* being transmitted to other invertebrates could exist, but the likelihood of this occurring over a 30 year projection was considered negligible by the experts based on their knowledge and the evidence presented to the workshop.**

**The expansion of *Ae. aegypti* 'Geographical range' as a result of introducing *Wolbachia* above what would occur with naturally occurring *Ae. aegypti* was considered negligible. The current Australian distribution of *Ae. aegypti* is actually a fraction of its known former distribution over the past 100 years (Kearney *et al.* 2009). The decline in its range has been attributed to changes in water reticulation, particularly a reduction in water tanks which serve as stable reservoirs (Beebe *et al.***

**2009), but much of this is speculative. Water tanks have again proliferated in Australia under current water shortages and it would be hard to disentangle any increase in distribution between spread due to *Wolbachia* and spread due to other causes.**

**Both an increase in mean *Ae. aegypti* 'Density' and the possibility of a change in 'Ecological niche' were considered very low likelihood events (10%) because the experts did not anticipate any biological change that could increase density because the environmental holding capacity is a major limiting factor, and movement from a primarily domestic to alternate niche unlikely due to the highly anthropophilic behaviour of the species. This reflects the outcomes from the Stage Two workshop on ecological harm.**

**The hazard definitions and their likelihood states for the 'Ecology' submodel were:**

**Density**
**What is the likelihood that the average density of *Wolbachia Ae. aegypti* (e.g. average numbers per household) will be higher than would occur for naturally occurring *Ae. aegypti*? This could be a result of changes in factors such as:**
- **Fecundity**
- **Longevity**
- **Population dynamics**

**Same (average density remains the same)**
**Increases (average density increases)**

**Ecological Niche**
**What is the likelihood that *Wolbachia Ae. aegypti* will change its ecological niche from being a predominantly domestic species to a broader or alternative niche? Niche changes could be the result of physical, biological, genetic or behavioural changes induced by *Wolbachia*.**
**Same (niche remains unchanged)**
**Other (change or broadening of niche)**

**Ecology**
**What is the likelihood that the release of *Wolbachia Ae. aegypti* leads to adverse ecological impacts? Possible issues include, but are not limited to:**
- **Reduction of larval and adult *Ae. aegypti* as a food source to predators**
- **Reduction in detritus removal by larval feeding**
- **Reduced pollination services by males feeding on flowers for energy**
- **Horizontal transfer of *Wolbachia***

**No Impact (no ecological impacts)**
**Negative Impact (adverse ecological impacts occur)**

**Geographic Range**
**What is the likelihood that *Wolbachia Ae. aegypti* will achieve a greater potential geographic range than that of naturally occurring *Ae. aegypti*? Consider the historic range of *Ae. aegypti*, possible constraints on geographic distribution, and whether we could discriminate between random geographic dispersal or actual adaptation. Possible drivers for this include:**
- **Better climatic tolerance, e.g. drought or desiccation tolerance**
- **Changes in host range**

**Same (potential geographic range would not be greater than expected)**
**Increases (potential geographic range would be greater than expected)**

**Invertebrate Transfer**
**What is the likelihood of horizontal transfer of *Wolbachia* from *Wolbachia Ae. aegypti* to another invertebrate species? Possible routes of transmission may include predation on the mosquito, co-feeding on a plant or animal host, parasitism of mosquito.**
**Unlikely (little or no chance of horizontal transfer to an invertebrate)**
**Possible (low to moderate chance of horizontal transfer to an invertebrate)**
**Likely (High chance of horizontal transfer to an invertebrate)**

**Vertebrate Transfer**

| What is the likelihood of horizontal transfer of *Wolbachia* from *Wolbachia Ae. aegypti* to a vertebrate species? |
|---|
| No (no chance of horizontal transfer of *Wolbachia* to a vertebrate) |
| Yes (chance of horizontal transfer of *Wolbachia* to a vertebrate) |

### 6.3.7. 'Mosquito Management Efficacy' and 'Avoidance Strategies' Submodel ('Cause More Harm')

Figure 17 shows two submodels in the BBN for 'Cause More Harm'. 'Mosquito management efficacy' is the hazard that the release will result in the reduction of the effectiveness or increased requirement for mosquito control. This had a low (~6%) likelihood of occurring. This node is linked by four parent nodes. 'Monitoring' has an estimate of 15% that surveillance would be insufficient or incapable of detect change or adverse outcomes. The 'Need for Control' (10%) is the hazard that *Wolbachia Ae. aegypti* would require increased control effort in comparison to naturally occurring *Ae. a*egypti. The likelihood that 'Insecticide Resistance' would result from *Wolbachia* was considered negligible (0%) based of data presented at the workshop.

'Avoidance strategies' is a hazard that the public would change their normal behaviours to avoid contact with *Wolbachia Ae. aegypti*, but had a very low (2%) estimate of eventuating. 'Perceptions' that the release of *Wolbachia Ae. aegypti* will solve the dengue problem influences whether the public reduce their 'Household Control' effort had the highest likelihood in the risk analysis of 50%. A reduction in Household Control' contributes to both 'Mosquito management efficacy' and 'Avoidance strategies' but this outcome estimated to have a very low probability (6%).

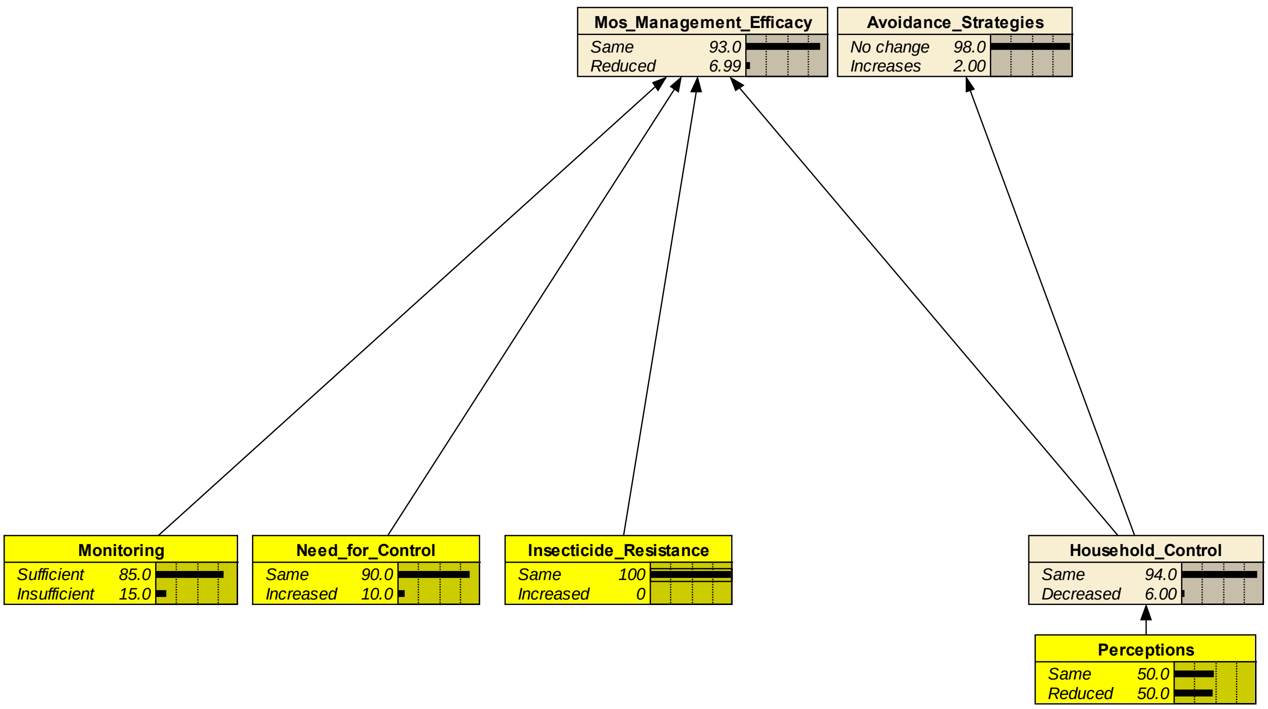



Figure 17. Nodes and final likelihoods feeding into the 'Mosquito Management Efficacy' and 'Avoidance Strategies' submodels in 'Cause More Harm'.

Monitoring had a low estimate of failing, but this may be an optimistic score. Ervin *et al.* (2003) suggest that there has been insufficient and ineffective monitoring associated with transgenic crop

**releases to reliably detect impacts in ecosystems. Both positive and negative unanticipated events can occur where a 'complex system' such as a GMO or microorganisms (e.g. Krimsky *et al.* 1995) interact with complex ecological systems. The BBN describes numerous hazards that need to be monitored and the scale of effort required may be underestimated. This hazard could be rescored when there is new information available describing a proposed monitoring strategy.**

**'Insecticide resistance' has been detected in *Ae. aegypti* as a result of frequent heavy exposure in vector control programmes (e.g. Cui *et al.* 2006; Ponlawet *et al.* 2005; Luz *et al.* 2009). Although there is an association between *Wolbachia* and insecticide resistance, a direct influence on resistance has not been observed and the development of resistance in *Ae. aegypti* is not due to *Wolbachia* as the species is not infected. Duron *et al.* (2006) evaluated the interactions between *Wolbachia* infection and insecticide resistance that had evolved in *Culex pipiens*. *Wolbachia* was found to impose a metabolic cost on the host and to increase in density with insecticide resistance, but was not directly responsible for the resistance. Evidence was also presented at the workshop that indicated no significant increase in resistance occurring in *Wolbachia Ae. aegypti* which is likely to have driven the negligible likelihood score.**

**The hazard definitions and their likelihood states for the 'Mosquito Management Efficacy' and 'Avoidance Strategies' submodels were:**

| |
|---|
| **Avoidance Strategies**<br>**What is the likelihood that people will change their normal mosquito avoidance strategies because of the presence of *Wolbachia Ae. aegypti*? Possible behavioural changes include:**<br>• **Increased social avoidance behaviour, e.g. reduced outdoor activities**<br>• **Increased household insecticide use**<br>• **Removal of breeding sites around household**<br>**No Change (avoidance strategies do not change)**<br>**Increases (increased rate of avoidance)** |

| |
|---|
| **Household Control**<br>**What is the likelihood that households in areas containing *Wolbachia Ae. aegypti* will change their expenditure and effort to control mosquitoes because of perceptions about the *Wolbachia Ae. aegypti* mosquito?**<br>**Same (no change in household expenditure and control effort)**<br>**Decreased (decreased household expenditure and control effort)** |

| |
|---|
| **Insecticide Resistance**<br>**What is the likelihood that *Wolbachia Ae. aegypti* will have increased insecticide resistance above the expected level for naturally occurring *Ae. aegypti*?**<br>**Same (same levels of insecticide resistance)**<br>**Increased (increased levels of insecticide resistance)** |

| |
|---|
| **Monitoring**<br>**What is the probability of having a sufficient system in place to monitor *Wolbachia Ae. aegypti* to ensure that the concept of no harm is measurable?**<br>**Sufficient (sufficient monitoring will occur to measure)**<br>**Insufficient (monitoring will be insufficient to detect change)** |

| |
|---|
| **Mosquito Management Efficacy**<br>**What is the likelihood that the efficacy of mosquito management and control efforts will be compromised due to the introduction of *Wolbachia Ae. aegypti*? Consider changes in:**<br>• **Need for control**<br>• **Emergence of insecticide resistance**<br>• **Investment in future research**<br>• **Household control practices**<br>**Same (no changes in efficacy of control measures)**<br>**Reduced (reduced efficacy of control measures)** |

Need for Control
What is the likelihood that the release of *Wolbachia Ae. aegypti* will result in the need for increased levels of mosquito control? Take into account whether there may be a need to apply more or greater diversity of treatments.
Same (control effort remains the same)
Increased (greater control effort required)

Perceptions
What is the likelihood that the release of *Wolbachia Ae. aegypti* will lead to a widespread perception that the threat of dengue has been eliminated permanently?
Same (perception of dengue threat unchanged)
Reduced (perception of dengue threat is reduced)

### 6.3.8. 'Economic Effects' Submodel ('Cause More Harm')

The 'Economic effects' submodel (Figure 18) provides a 2% (negligible) likelihood that the release will result in economic harm. The four parent nodes that describe possible economic hazards are increases in the costs of 'Health care' (0%), a reduction in 'Tourism' (2%), decreases in the value of 'Real estate' (0%) and a reduced supply or increased cost of 'Labour availability' (0%). The final likelihood for 'Tourism' is the resolicited score after initially been set at 10%. There is no evidence that the 2009 dengue outbreak in Cairns led to declines in real estate value, tourism or labour availability. Further, Australian tourist frequently visit countries affected to a greater extent by mosquito-borne diseases and the risk of being infected does not appear to be regarded as a disincentive to travel.

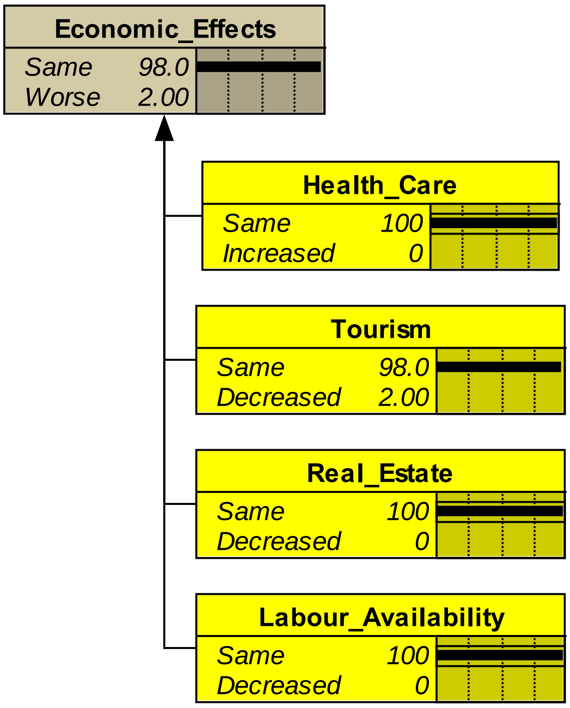



Figure 18. Nodes and final likelihoods feeding into the 'Economic Effects' submodel for 'Cause More Harm'.

The hazard definitions and their likelihood states for the 'Economic effects' submodel were:

Economic Effects

What is the likelihood that the introduction of *Wolbachia Ae. aegypti* will result in adverse economic impacts? Take into account:

- Tourism
- Real estate
- Labour availability

Same (no adverse economic effects)
Worse (adverse economic effects occur)

Health Care
What is the likelihood that the cost of general community health care will increase over time as a result of the release of *Wolbachia Ae. aegypti*?
Same (community health costs remain unchanged)
Increased (community health costs increase)

Labour Availability
What is the likelihood that there is change in supply of labour (permanent and/or seasonal) in areas containing *Wolbachia Ae. aegypti* due to its presence? This includes the possibility of increased labour costs as result of labour shortage.
Same (no changes in availability and cost of labour)
Decreased (decreased labour availability and increased cost of labour)

Real Estate
What is the likelihood that real estate or property values will be affected in areas where *Wolbachia Ae. aegypti* is present because of its introduction?
Same (no changes in real estate values)
Decreased (real estate values decline)

Tourism
What is the likelihood that international and local tourism will be affected in areas containing *Wolbachia Ae. aegypti* because of its introduction?
Same (no changes in tourism)
Decreased (reduced tourism occurs)

### 6.3.9. ‘Standard of Public Health’ Submodel (‘Cause More Harm’)

The submodel contributing to the 3% likelihood of a decline in the ‘Standard of public health’ as a result of the release contains eleven nodes and is link intense (Figure 19). The likelihood (15%) that *Wolbachia* will confer a fitness advantage to *Ae. aegypti* is the parent node to negligible likelihoods of increased ‘Dengue vector competence’ (0.015%) or other arboviruses and parasites (‘Non-Dengue vector competence’ – 0.015%), a tendency to take more blood meals (‘Feeding frequency’ – 0.015%), an increase in average ‘Mosquito density’ (0.015%) and the inclusion of novel hosts into the feeding range (‘Host preference’ – 0.1%). These provide likelihoods for an overall increase in ‘Dengue transmission’ of 3.04%, ‘Nuisance biting’ (0.11%) and a 0.001% likelihood of an increased propensity for *Ae. aegypti* to transmit ‘Other pathogens’.

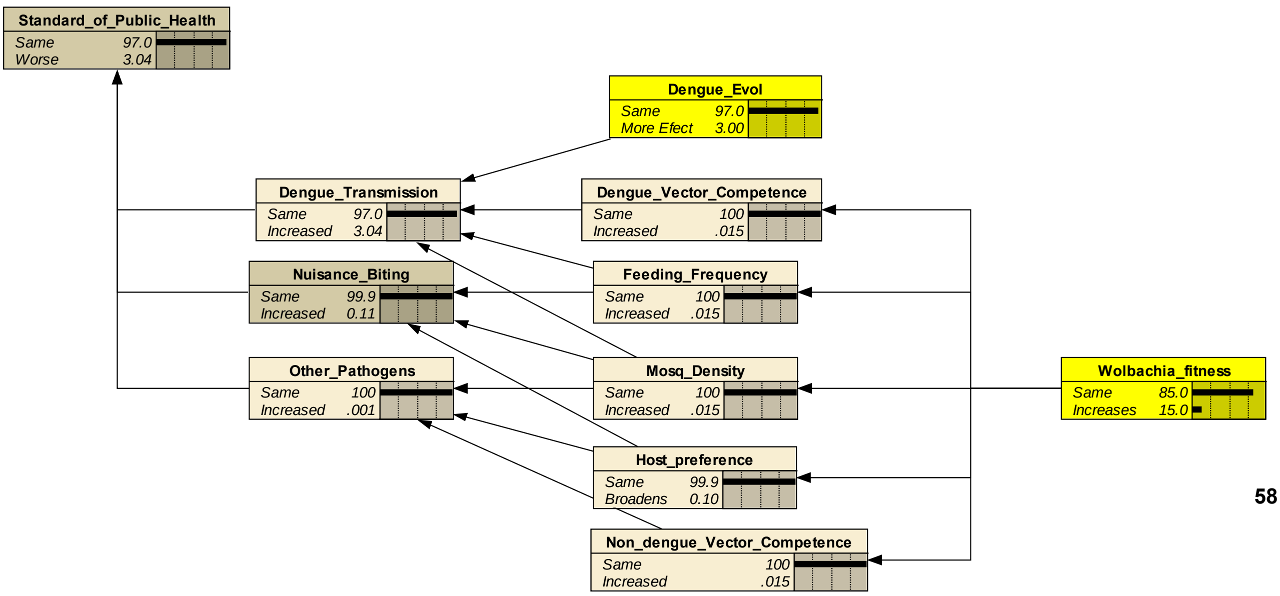


**Figure 19. Nodes and final likelihoods feeding into the ‘Standard of public health’ submodel for ‘Cause More Harm’.**

Results for overall ‘Standard of public health’ submodel indicate that increased health issues were considered of a very low likelihood to occur as a result of the release. With the exception of ‘*Wolbachia* fitness’ the remaining nodes were estimated as having negligible to very low likelihoods of eventuating. A key factor here was the evidence that the replication of dengue and other pathogens in the vector is substantially reduced (Moreira *et al*. 2009).

The likelihood of *Wolbachia* providing beneficial fitness characteristics to *Ae. aegypti* was the highest rated likelihood at 15%. The phenotypic effects of *Wolbachia* have been shown to be malleable under selection pressure from both environment and host background. McMeniman *et al.* (2008) evaluated the effects of *Wolbachia* host adaptation after transplant through several mosquito species by re-introduction into *D. melanogaster* and found weakened CI and life shortening effects. Carrington *et al.* (2009) also demonstrated that intense selection pressure could modify the effects of life shortening, but concluded that selection pressure in natural conditions would be less intense. Because of the concerns with *Wolbachia* being present in different host tissues and potentially manipulating a diverse range of biological host processes, including locomotion behaviours which may be related to host finding, mate selection and choice of resting or oviposition sites, Evans *et al.* (2009) evaluated these effects in ageing *Ae. aegypti* containing *Wolbachia*. The increase in diurnal locomotion of both males and increased female $CO_2$ production (this was not maintained as long in males) could be interpreted as resulting from *Wolbachia* pathogenicity or increased metabolic costs as *Wolbachia* can synthesise relatively few metabolites and the remainder are drawn from the host. Metabolic costs of the bacteria have been implicated with reduced fecundity (Reynolds *et al.* 2003) so adverse changes in *Ae. aegypti* fitness may also be possible.

The hazard of dengue evolving to overcome transmission inhibition was included during the BBN review indicating it was considered an important omission. Dengue is an RNA virus and capable of rapid evolution as evidenced by three of the dengue strains having evolved separately in the last 2000 years by host shifting from primates to humans and shifting host vector species (Weaver & Barrett 2004). A molecular study by Zanotto *et al.* (1996) on arboviruses in the *Flavivirus* genus containing dengue showed an explosive radiation in the last 200 years as a result of the global expansion of the vector and virus. Change can occur rapidly as indicated by Aaskov *et al.* (2006) who detected a stop-codon mutation in the DENV-1 in Myanmar in 2001 that appeared to fix in the population over 18 months. Medlock *et al.* (2009) modelled how dengue would behave under proposed transgenic manipulation of mosquito vectors and cautioned that dengue may evolve to be more virulent under strategies that seek to block its transmission or reduce biting. The initial consensus estimate for ‘Dengue evolution’ was 10% but this was reduced to 3% by asking experts score the hazard of a 100 rather than ten point scale. This was undertaken to provide more accuracy to an important node.

An increase in nuisance biting is feasible as Turley *et al.* (2009) found that *Wolbachia* infected *Ae. aegypti* were less successful at taking blood meals, and although aging females did not rest on the

**host longer than normal, they attempted more biting attempts and took fewer and smaller blood meals than naturally occurring *Ae. aegypti*. However the likelihood of this occurring was considered negligible (0.11%).**

**The hazard definitions and their likelihood states for the 'Standard of Public Health' submodel were:**

| |
|---|
| **Host Preference**<br>**What is the likelihood that *Wolbachia Ae. aegypti* takes a higher proportion of blood meals from humans than naturally occurring *Ae. aegypti*?**<br>**Same (percentage of meals from humans remains the same)**<br>**Increased (percentage of meals from humans increases)** |

| |
|---|
| **Feeding Frequency**<br>**What is the likelihood that *Wolbachia Ae. aegypti* takes blood meals more frequently than naturally occurring *Ae. aegypti* due to physiological, behavioural or other changes?**<br>**Same (feeding rates remain the same)**<br>**Increased (feeding rates increase)** |

| |
|---|
| **Dengue Evolution**<br>**What is the likelihood that the dengue virus will evolve to be transmitted more effectively?**<br><br>**Same (Dengue virus does not evolve to be more effective at transmission)**<br>**More Effective (Dengue virus evolves to be more effective at transmission)** |

| |
|---|
| **Dengue Transmission**<br>**What is the likelihood that the rate of dengue transmission will be increased by *Wolbachia Ae. aegypti* compared with naturally occurring *Ae. aegypti*? Possible issues include:**<br>• ***Ae. aegypti* gains increased vectorial capacity for dengue**<br>• **Changes in *Ae. aegypti* feeding habits**<br>• **Dengue virus mutation under selection pressure from *Wolbachia***<br>**Same (dengue transmission rates remain the same)**<br>**Increased (dengue transmission rates increase)** |

| |
|---|
| **Dengue Vector Competence**<br>**What is the likelihood that *Wolbachia Ae. aegypti* becomes a more capable vector of dengue viruses than naturally occurring *Ae. aegypti*? Possible factors include:**<br>• **Reduced infection barriers to dengue virus**<br>• **Increased dengue virus growth in mosquitoes**<br>• **Faster development of dengue virus in *Wolbachia Ae. aegypti***<br>• **More effective transmission during feeding**<br>**Same (vector competence for dengue viruses remains the same)**<br>**Increased (vector competence for dengue viruses increases)** |

| |
|---|
| **Increased Biting**<br>**What is the likelihood that *Wolbachia Ae. aegypti* takes blood meals more frequently than naturally occurring *Ae. aegypti* due to physiological, behavioural or other changes?**<br>**Same (feeding rates remain the same)**<br>**Increased (feeding rates increase)** |

| |
|---|
| **Host Preference**<br>**What is the likelihood that *Wolbachia Ae. aegypti* will feed on a greater variety of host animals than naturally occurring *Ae. aegypti*?**<br>**Same (host species do not change)**<br>**Broadens (greater variety of host species)** |

| |
|---|
| **Mosquito Density**<br>**What is the likelihood that the average density of *Wolbachia Ae. aegypti* (e.g. average numbers per household) will be higher than would occur for the naturally occurring *Ae. aegypti*? This could be a result of changes in factors such as:**<br>• **Fecundity** |

| |
|---|
| • Longevity<br>• Population dynamics<br>Same (average density remains the same)<br>Increased (average density increases) |

| |
|---|
| **Nuisance Biting**<br>**What is the likelihood that *Wolbachia Ae. aegypti* will result in an increased pest status of this species due to an increase in human biting events compared to naturally occurring *Ae. aegypti*? Contributing factors may include:**<br>• Increased tendency to associate with people<br>• Higher mosquito densities than for naturally occurring *Ae. aegypti*<br>• Increased biting behaviour (require more frequent blood feeding events)<br>• Increased tendency to inhabit houses<br>Same (nuisance status remains the same)<br>Increased (nuisance status increases) |

| |
|---|
| **Non-Dengue Vector Competence**<br>**What is the likelihood that *Wolbachia Ae. aegypti* will become a better vector of pathogens other than dengue (including viruses, bacteria, parasites) in comparison to naturally occurring *Ae. aegypti*?**<br>Same (vector competence for non-dengue pathogens remains the same)<br>Increased (vector competence for non-dengue pathogens increases) |

| |
|---|
| **Other Pathogens**<br>**What is the likelihood that the transmission rate of pathogens other than dengue virus (virus, bacteria, parasite) is increased by *Wolbachia Ae. aegypti*. This may arise due to changes in:**<br>• Mosquito density<br>• Host preference<br>• Increased vector competence for these pathogens<br>Same (transmission rate of other pathogens remains the same)<br>Increased (transmission rate of other pathogens increases) |

| |
|---|
| **Standard of Public Health**<br>**What is the likelihood that the standard of public health overall will be worse as a result of the release of *Wolbachia Ae. aegypti*? Consider:**<br>• Arbovirus transmission rates<br>• Nuisance biting<br>• Any other factor affecting public health standards<br>Same (standard of public health does not change)<br>Worse (standard of public health declines) |

| |
|---|
| ***Wolbachia* Fitness**<br>**What is the likelihood that a genetic change in *Wolbachia* will cause a fitness change in *Ae. aegypti*? Consider fitness to describe the ability of an organism to survive and pass on its genes, but ignore the selective mating advantage provided by Cytoplasmic Incompatibility (CI).**<br>Same ( *Wolbachia* will have no effect on *Ae. aegypti* fitness)<br>Increases ( *Wolbachia* will increase *Ae. aegypti* fitness) |

### 6.3.10. Sensitivity Analysis

Table 9 shows that the child node for 'Mosquito management efficacy' could contribute 5.9% to the reduction of the endpoint likelihood of 'Cause More Harm'. The next best result was for the parent node of household control (4%) and both 'Standard of public health' and 'Dengue transmission' reduced it by ~2.8%. Eleven nodes offered no additional reduction of the endpoint likelihood.

Although 'Tourism' (1.8%) and 'Dengue evolution' (2.75%) do not add much to the reduction of the adverse endpoint likelihood at this point, these were the values that were re-solicited because their influence on the endpoint score was noticeable. In comparison to the final 'Cause More Harm' score of 12.5%, at their original consensus scores of 10% failure, individually 'Tourism' would have resulted in a final endpoint score of 19.6%, and 'Dengue evolution' would have led to 18.8%. With both hazards set at 10% failure simultaneously this increased to 25.4% so there was an approximate halving the adverse endpoint likelihoods following this improvement in scoring accuracy.

**Table 9. Sensitivity analysis of individual node contribution to 'Cause More Harm' from base figure of 12.5% (child nodes in bold).**

| Hazard | Resulting Likelihood (%) | Change (%) |
|---|---|---|
| Mosquito management efficacy | 6.59 | 5.91 |
| Household control | 8.52 | 4.00 |
| Standard of public health | 9.71 | 2.79 |
| Dengue transmission | 9.71 | 2.79 |
| Dengue evolution | 9.75 | 2.75 |
| Perceptions | 9.83 | 2.67 |
| Economic effects | 10.7 | 1.80 |
| Tourism | 10.7 | 1.80 |
| Need for control | 10.9 | 1.60 |
| Avoidance strategies | 11.0 | 1.50 |
| Monitoring | 12.2 | 0.30 |
| Density | 12.4 | 0.10 |
| Ecology | 12.4 | 0.10 |
| Nuisance biting | 12.4 | 0.10 |
| Dengue vector competence | 12.4 | 0.10 |
| Feeding frequency | 12.4 | 0.10 |
| Mosquito density | 12.4 | 0.10 |
| *Wolbachia* fitness | 12.4 | 0.10 |
| Invertebrate transfer | 12.5 | 0 |
| Vertebrate transfer | 12.5 | 0 |
| Geographical range | 12.5 | 0 |
| Ecological niche | 12.5 | 0 |
| Insecticide resistance | 12.5 | 0 |
| Health care | 12.5 | 0 |
| Real estate | 12.5 | 0 |
| Labour availability | 12.5 | 0 |
| Other pathogens | 12.5 | 0 |
| Host preference | 12.5 | 0 |
| Non dengue competence | 12.5 | 0 |

Table 10 shows the contribution of different child nodes combinations to the 'Cause More Harm' end point. The combination of all five child nodes reduced the end point likelihood to 0% and the best combination of four nodes (without 'Avoidance strategies') differed by only 0.09%. The best combination of three nodes ('Mosquito management efficacy', 'Standard of public health' and 'Economic effects') contributed a 10.9% reduction. The highest ranked duo was 'Mosquito management efficacy' and 'Standard of public health' which is consistent with their high rankings as individual nodes.

Table 10. Contribution of child node combinations to likelihood of 'Cause More Harm' from base figure of 12.5%.

| Ecology | Mosquito Man. Efficacy | Avoidance Strategies | Std. of Public Health | Economic Effects | Resulting Likelihood | Contribution (%) |
|---|---|---|---|---|---|---|
| + | + | + | + | + | 0.00 | 12.50 |
| | + | + | + | + | 0.09 | 12.41 |
| + | + | | + | + | 1.60 | 10.90 |
| | + | | + | + | 1.69 | 10.81 |
| + | + | + | + | | 2.00 | 10.50 |
| | + | + | + | | 2.09 | 10.41 |
| + | + | + | | + | 3.04 | 9.46 |
| | + | + | | + | 3.13 | 9.37 |
| + | + | | + | | 3.60 | 8.90 |
| | + | | + | | 3.65 | 8.85 |
| + | + | | | + | 4.60 | 7.90 |
| | + | | | + | 4.68 | 7.82 |
| + | + | + | | | 4.98 | 7.52 |
| | + | + | | | 5.07 | 7.43 |
| + | | + | + | + | 6.29 | 6.21 |
| | | + | + | + | 6.37 | 6.13 |
| + | + | | | | 6.50 | 6.00 |
| + | | | + | + | 7.79 | 4.71 |
| | | | + | + | 7.87 | 4.63 |
| + | | + | + | | 8.16 | 4.34 |
| | | + | + | | 8.24 | 4.26 |
| + | | + | | + | 9.14 | 3.36 |
| | | + | | + | 9.22 | 3.28 |
| + | | | + | | 9.63 | 2.87 |
| + | | | | + | 10.60 | 1.90 |
| + | | + | | | 11.00 | 1.50 |

### 6.3.11. Calculation of Risk

The risk estimation matrix (Figure 20) shows the possible combinations of likelihood x consequence. Note that the risk matrix introduces new scale components (e.g. very high likelihood x very high consequence = Extreme risk). The matrix is also slightly asymmetrical and weighted towards impacts rather than likelihood. For example, very high likelihood x negligible consequence = negligible risk.

**In contrast a negligible likelihood x very high consequence event has a risk of very low, not negligible.**

**Figure 20. Risk estimation matrix where risk is the product of likelihood x consequence**

| | | CONSEQUENCE | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Negligible** | **Very Low** | **Low** | **Moderate** | **High** | **Very High** |
| LIKELIHOOD | **Negligible** | Negligible Risk | Negligible Risk | Negligible Risk | Negligible Risk | Negligible Risk | Very Low Risk |
| | **Very Low** | Negligible Risk | Negligible Risk | Negligible Risk | Negligible Risk | Very Low Risk | Low Risk |
| | **Low** | Negligible Risk | Negligible Risk | Negligible Risk | Very Low Risk | Low Risk | Moderate Risk |
| | **Moderate** | Negligible Risk | Negligible Risk | Very Low Risk | Low Risk | Moderate Risk | High Risk |
| | **High** | Negligible Risk | Very Low Risk | Low Risk | Moderate Risk | High Risk | Extreme Risk |
| | **Very High** | Negligible Risk | Very Low Risk | Low Risk | Moderate Risk | High Risk | Extreme Risk |

**Table 11 shows a summary of the workshop consensus likelihood estimates for likelihood and consequence and the resulting estimated risk. Figure 21 shows the risk matrix after populating with the consensus scores, by plotting the hazard likelihood and then the consequence for each hazard. Of the 30 hazards the highest estimated risk was for 'Perceptions' which scored as low risk as a result of being scored as moderate for both likelihood and consequence. This hazard had the highest assigned likelihood of moderate whereas 20 hazards were scored as having moderate consequence. 'Labour Availability' had the highest assigned consequence score of (high). Four hazards were scored as very low risk ('Avoidance Strategies'/'Household Control'/'*Wolbachia* Fitness'/'Mosquito Density'). The remaining 25 hazards were all scored as having Negligible Risk through 8 different combinations of likelihood and consequence including the endpoint hazard of 'Cause More Harm'.**

**Eleven hazards were assigned zero likelihood of occurring ('Labour Availability'/'Invertebrate Transfer'/'Host Preference'/'Health Care'/'Ecology'/'Insecticide Resistance'/'Standard of Public health'/'Real Estate'/'Vertebrate Transfer' and 'Dengue vector competence') and two of these also had zero consequence estimates ('Vertebrate transfer' and 'Dengue vector competence').**

**Table 11. Summary of 30 consensus estimates for likelihood, consequence and risk (ranked by risk) for 'Cause More Harm' endpoint**

| Hazard | Consensus Likelihood | Likelihood Scale | Consensus Consequence | Consequence Scale | Consensus RISK | Risk Matrix State |
|---|---|---|---|---|---|---|
| Perceptions | 0.50 | Moderate | 0.40 | Moderate | 0.20 | Low Risk |
| Avoidance Strategies | 0.30 | Low | 0.50 | Moderate | 0.15 | Very Low Risk |
| Household Control | 0.20 | Low | 0.60 | Moderate | 0.12 | Very Low Risk |
| *Wolbachia* Fitness | 0.15 | Low | 0.45 | Moderate | 0.0675 | Very Low Risk |
| Mosquito Density | 0.15 | Low | 0.45 | Moderate | 0.0675 | Very Low Risk |
| Need for Control | 0.10 | Very Low | 0.40 | Moderate | 0.04 | Negligible Risk |
| Feeding Freq | 0.10 | Very Low | 0.40 | Moderate | 0.04 | Negligible Risk |
| Ecological Niche | 0.10 | Very Low | 0.40 | Moderate | 0.04 | Negligible Risk |
| Density | 0.10 | Very Low | 0.40 | Moderate | 0.04 | Negligible Risk |
| Mosq_Man_Eff | 0.10 | Very Low | 0.35 | Moderate | 0.035 | Negligible Risk |
| Nuisance Biting | 0.10 | Very Low | 0.30 | Low | 0.03 | Negligible Risk |
| Monitoring | 0.15 | Low | 0.15 | Low | 0.0225 | Negligible Risk |
| Economic Effects | 0.10 | Very Low | 0.20 | Low | 0.02 | Negligible Risk |
| Other Pathogens | 0.10 | Very Low | 0.10 | Very Low | 0.01 | Negligible Risk |
| Non_Deng_Vect_Comp | 0.10 | Very Low | 0.10 | Very Low | 0.01 | Negligible Risk |
| Dengue Transmission | 0.10 | Very Low | 0.10 | Very Low | 0.01 | Negligible Risk |
| Cause More Harm | 0.10 | Very Low | 0.10 | Very Low | 0.01 | Negligible Risk |
| Dengue Evolution | 0.03 | Very Low | 0.15 | Low | 0.0045 | Negligible Risk |
| Tourism | 0.02 | Very Low | 0.10 | Very Low | 0.002 | Negligible Risk |
| Labour Availability | 0.00 | Negligible | 0.80 | High | 0.00 | Negligible Risk |
| Invertebrate Transfer | 0.00 | Negligible | 0.70 | Moderate | 0.00 | Negligible Risk |
| Host Preference | 0.00 | Negligible | 0.60 | Moderate | 0.00 | Negligible Risk |
| Health Care | 0.00 | Negligible | 0.60 | Moderate | 0.00 | Negligible Risk |
| Ecology | 0.00 | Negligible | 0.55 | Moderate | 0.00 | Negligible Risk |
| Insecticide Resistance | 0.00 | Negligible | 0.35 | Moderate | 0.00 | Negligible Risk |
| Geographic Range | 0.00 | Negligible | 0.35 | Moderate | 0.00 | Negligible Risk |
| Std_Pub_Health | 0.00 | Negligible | 0.10 | Very Low | 0.00 | Negligible Risk |
| Real Estate | 0.00 | Negligible | 0.10 | Very Low | 0.00 | Negligible Risk |
| Vertebrate Transfer | 0.00 | Negligible | 0.00 | Negligible | 0.00 | Negligible Risk |
| Deng_Vec_Comp | 0.00 | Negligible | 0.00 | Negligible | 0.00 | Negligible Risk |

**Figure 21. Risk estimation matrix populated with final 30 hazards based on group consensus scores for likelihood and consequence**

| | | **CONSEQUENCE** | | | | | |
|---|---|---|---|---|---|---|---|
| **LIKELIHOOD** | | **Negligible** | **Very Low** | **Low** | **Moderate** | **High** | **Very High** |
| | **Negligible** | Negligible Risk<br>Dengue V. Comp.<br>Vertebrate Tran. | Negligible Risk<br>Real Estate<br>Std. Public health | Negligible Risk | Negligible Risk<br>Ecology<br>Geographical range<br>Health Care<br>Host Preference<br>Insecticide resistance<br>Invertebrate Transfer | Negligible Risk<br>Labour Avail. | Very Low Risk |
| | **Very Low** | Negligible Risk | Negligible Risk<br>Cause More Harm<br>Dengue Trans.<br>Non-Deng. Vector<br>Other Pathogens<br>Tourism | Negligible Risk<br>Dengue Evol.<br>Economic Eff.<br>Nuisance Biting | Negligible Risk<br>Density<br>Ecological Niche<br>Feeding Freq<br>Mosq. Man. Eff.<br>Need for Control | Very Low Risk | Low Risk |
| | **Low** | Negligible Risk | Negligible Risk | Negligible Risk<br>Monitoring | Very Low Risk<br>Avoidance Strat.<br>Household Control<br>Mosquito Density<br>*Wolbachia* Fitness | Low Risk | Moderate Risk |
| | **Moderate** | Negligible Risk | Negligible Risk | Very Low Risk | Low Risk<br>Perceptions | Moderate Risk | High Risk |
| | **High** | Negligible Risk | Very Low Risk | Low Risk | Moderate Risk | High Risk | Extreme Risk |
| | **Very High** | Negligible Risk | Very Low Risk | Low Risk | Moderate Risk | High Risk | Extreme Risk |

## 6.4. DISCUSSION

**The Stage five workshop met the objectives of generating a full set of consensus likelihoods and consequence estimates that would allow an estimation of project risk. Attempts made to reduce uncertainty included introduction of new information during the workshop, agreement on scales, definitions and timeframes for measurement, and the use of a four point scoring scale so that the experts were more comfortable in scoring likelihoods. We cannot measure directly how much uncertainty was reduced, but the end result is a full set of consensus priors provided by a panel of knowledgeable experts. This of course does not indicate that all hazard scores are necessarily a 'correct' estimate, but an initial sets of *priors* now exists which can be updated when new information becomes available.**

**'Cause More Harm' had an estimated BBN failure likelihood of 12.5% (low likelihood) and the equivalent workshop consensus score (i.e. when asked to score the likelihood of the endpoint failure) was 10%, or very low likelihood. Of the major submodels, 'Ecology' (0.09%) had a negligible likelihood, and 'Mosquito Management Efficacy' (6.99%), 'Avoidance Strategies' (2.0%), the 'Standard of Public Health' (3.04%) and Economic effects (2.0%) were all considered very low likelihood hazards. The highest individual node likelihoods were 50% for 'Perceptions' (moderate), 15% for 'Monitoring' and '*Wolbachia* fitness' (low), and 10% for 'Ecological Niche', 'Density', and 'Need for Control' (very low).**

**The highest estimated risk for all of the final 30 hazards was for 'Perceptions' which reflects the hazard that the community and organisations responsible for mosquito control which change behaviours as a result of assuming that the dengue problem has been solved. This scored as low risk as a result of being scored as moderate for both likelihood and consequence. Four hazards had a very low risk resulting from a low likelihood x moderate consequence ('Avoidance Strategies, 'Household control' and 'Mosquito density' and '*Wolbachia* fitness'). The remaining 25 hazards were considered of negligible risk including the endpoint of 'Cause More Harm'. There were eight different combinations of likelihood and consequence providing this outcome (i.e. the same risk value can be attained through a number of different likelihood and consequence combinations in the risk matrix). The sensitivity analysis showed that 'Mosquito management efficacy' could contribute the highest individual reduction (5.9%) and was a consistent presence in the highest ranking child node combinations.**

**It is notable that the experts considered 11 of these hazards to have no likelihood of occurring, and of these two hazards also had consensus estimates of no consequence ('Vertebrate transfer' and 'Dengue vector competence'). This suggest the experts were either very confident that there was no possibility of failure, or it may be an artefact of using a 10 point scale where there is little resolution, but it is easy to score. The 100 point scale used for the re-solicitation of the 'Dengue evolution' and 'Tourism' hazards showed that this allows the experts to be more specific in scoring and had the effect of approximately halving of the 'Cause More Harm' likelihood from 25.4% to the final *prior* estimate of 12.5%. Rescoring of some of these nodes on a 100 point scale might indicate where there is a small but non-zero likelihood or consequence.**

**6.5. SUMMARY OF STAGE FIVE: EXPERT WORKSHOP TO REDUCE UNCERTAINTY AND CALCULATE RISK**

- **A two day workshop was held in Brisbane on the 28th – 29th January 2010 with nine experts in attendance.**

- **The goals of the workshop were to attain a full set of consensus *priors* for the BBNs, reduce uncertainty by providing relevant new information, and provide an expert derived estimate of risk for each hazard.**

- **A review of the existing BBNs resulted in the inclusion of a 'Dengue evolution' node which captures the hazard that the dengue virus will evolve to overcome the inhibition properties of *Wolbachia*. This linked to a node capturing the hazard that rates of dengue transmission will increase following the release.**

- **The hazard of 'Future Mosquito Management' was removed as it was felt that the hazard of reduced investment in dengue control research as a result of a successful release was not an appropriate hazard or an adverse outcome. An equivalent example would be to not release a proven dengue vaccine because investment in *Wolbachia Ae. aegypti* may diminish.**

- **A 10 point scale was agreed upon to help provide common understanding of likelihoods both quantitatively and qualitatively. The experts used a 30 year time frame when considering hazard likelihoods**

- **The final 'Cause More Harm' BBN contains 30 nodes, 38 links and 363 conditional probabilities. Using workshop consensus scores for both summary and parent nodes, there was an estimated 12.5% likelihood that some form of harm could eventuate over the 30 year time frame from the release.**

- **Sensitivity analysis suggests that the most important individual node that could contribute to reduction of the endpoint likelihoods was 'Mosquito management efficacy' (reduced 'Cause More Harm' by 5.9%) and was also present in all the best combinations of summary (child) nodes. The next largest in sequence that contributed a >2.0% change were 'Household control' (4.0%), 'Standard of public health' (2.79%), 'Dengue transmission' (2.79%) and 'Dengue evolution' (2.75%).**

- **The 'Tourism' and 'Dengue evolution' hazards were noted as having a significant contribution to 'Cause More Harm'. The original failure estimates of 10% were re-solicited from experts on a 100 point scale to attain more accuracy and resulted in halving of the final likelihood. The original 'Tourism' score would have resulted in a final 'Cause More Harm' score of 19.6%, and 'Dengue evolution' would have led to 18.8%. Combined, the endpoint value would have been 25.4% so there was an approximate halving the adverse endpoint likelihoods following this improvement in scoring accuracy.**

- **Risk was calculated for the final 30 hazards using the consensus likelihood and consequence scores. The highest estimated risk was for 'Perceptions' which scored as low risk. Four hazards had estimated very low risk ('Avoidance Strategies'/'Household Control'/'Mosquito Density'/'*Wolbachia* Fitness') and the remaining 26 nodes had an estimated negligible risk.**

- **The overall adverse endpoint 'Cause More Harm' was calculated to have negligible risk.**

- **11 hazards had a zero consensus likelihood of failing, and two ('Vertebrate transfer' and 'Dengue vector competence') also had a zero consequence score. Future use of a scale that provides**

**higher resolution than the 10 point scale used here might generate small but non-zero estimates which would provide for more accurate risk estimates.**

## 7. OVERALL DISCUSSION

### 7.1. Summary

**A risk analysis was undertaken on the proposal to release *Wolbachia Ae. aegypti* in Far North Queensland, Australia, to prevent the transmission of dengue. The modification was introduction of the endosymbiotic bacteria *Wolbachia*, which induces a number of traits in *Ae. aegypti* which would limit its ability to transmit dengue, including shortening the life span of *Ae. aegypti* (McMeniman *et al.* 2009) that potentially could reduce dengue transmission by killing females before they can transmit the virus (Cook *et al.* 2007; Rasgon *et al.* 2003) as well as directly inhibiting the ability of the dengue virus to replicate in the mosquito (Moreira *et al.* 2009).**

**The risk analysis covered the adverse end point that the release would lead to adverse impacts above that predicted for naturally occurring *Ae. aegypti* in the next 30 years ('Cause More Harm').**

**The analysis used five stages of expert solicitation. After defining the risk analysis end points, the first stage was the opportunity to elicit expert opinion from mosquito researchers at a GCGH workshop on possible hazards associated with the project. A total of 50 hazards were identified from this and the subsequent Fault Tree Analysis exercise for each endpoint which was an attempt to define the relationships between hazards in a logical structure. A key concern was that ecological interactions of naturally occurring *Ae. aegypti* were poorly understood and potentially represented a considerable hazard gap in the analysis. Stage two consisted of a one day workshop in Cairns with relevant experts to consider this issue and explore the possible hazards using fault tree analysis. This determined that the reduction or removal of *Ae. aegypti* populations would not represent a threat to ecosystem health as ecological interactions were extremely limited.**

**Stage three was a one day workshop in Cairns which combined both mosquito experts and community representatives and explored the structure and relationships of different hazards in the form of a Bayesian Belief Net. A consensus net and set of definitions was achieved and the summary nodes in the model then populated with an initial set of *prior* likelihoods representing a combination of workshop group consensus on different model components, although workshop consensus was not achieved. Additional likelihoods for the failure of the parent hazard nodes were solicited by email from mosquito researchers in Stage four. The high disparity on expert scoring for a number of hazards indicated high uncertainty amongst the experts and a number of sources of this noise were suspected. Stage five used a two day workshop in Brisbane to convene a small group of mosquito experts knowledgeable on the project, address some of the sources of uncertainty that had been identified, and attain a full set of consensus *priors* to populate the BBN. Expert estimates of hazard consequences were also obtained to allow a calculation of risk.**

**The results from the final BBNs populated with consensus likelihoods provide an estimate 12.5% that 'Cause More Harm' of some magnitude may be realised within 30 years of the release. This harm may arise from one or a number of the hazards. The expert consensus scores for both likelihood and consequence were then used to estimate risk. Using these *priors*, there was no indication of high risk hazards. Of the final 30 hazards captured in the analysis the highest ranking was scored as low risk ('Perception'). Four were considered very low risk and the remaining 25 hazards were of negligible risk. It was notable that 11 hazards received a zero likelihood of failure estimate. This could be because the experts were certain or overconfident that this was the case or because of the low resolution (10 point) scale used. Future solicitation of expert scores could use the 10 point scale in a first pass screening of likelihoods and consequence as it is quicker to achieve consensus with less values, and the 100 point scale applied to any hazards identified in this process as requiring more accuracy (e.g. any hazard assigned a 100% or 0% value).**

**In this assessment expert judgement was used to provide likelihoods of hazard failures as a surrogate for incomplete or absent data, but this approach has limitations. For example, expert judgement is based on observation and experience (Regan *et al.* 2002) which would have varied both between and within the research and community representatives. How individuals perceive and quantify numerical risk also varies (Peters 2008). Uncertainty was definitely present in different forms (at least variability, incertitude and linguistic) and although steps were taken to minimise its effect, in some cases this may have enhanced it. For example the hazard definitions were intended to be succinct and accessible to both science and community representatives and included a glossary of key terms to avoid vagueness. But the Stage four email solicitation exercise was notable for the linguistic difficulty some respondents had with the definitions particularly when they had not participated in their development. This manifested as highly divergent hazard scoring and in some cases a lack of confidence to assign a likelihood to a hazard. The inability to easily discuss aspects such as definitions and reach consensus on interpretation, and the sometimes low response rates are failings of this individual-focused approach. But the value lies in the fact that a set of *priors* with some known issues can be rapidly obtained. The Stage five workshop was a response to these identified issues with an aim to reduce uncertainty and obtain a consensus set of *priors* which adequately reflected expert opinion.**

**Another issue is that all stages of solicitation involved experts associated with the project, and hence the *priors* include the possibility of biased scoring approaches. Their inclusion was necessary because there is a limited pool of mosquito researchers in Australia, few of whom could be considered to be fully independent or not have had at some stage, some degree of interaction with the project or key staff. The need for international experts was considered. The reality is that those considered international experts are already involved in the project and were consulted during one or more stages of the risk analysis. Experts familiar with the project have the advantage over relatively naive experts in that they require less background before they can start scoring hazards and are a valuable source of relevant up to date research findings. To further counter the issue of bias, an independent panel of external reviewers was convened as part of the project (Table 12). The panel reviewed the first draft report and their recommendations were used to revise the report and guide Stage 5 of the risk analysis. The final reports from the Independent Panel will be appended to the report.**

**The *priors* represent a starting point for examining the likelihoods of hazard failure and the consequences of any failure. These *priors* can be updated when new information is made available, and actual data can be used in place of expert values when available. For example where robust scientific data is available for example on horizontal transfer rates it may be more valuable than the equivalent expert opinion.**

**Table 12. Composition of Independent Panel assigned to review the risk analysis process**

| Panel Member | Organisation | Email Contact | Experience |
|---|---|---|---|
| Dr Mikael Hirsch | CSIRO Corporate | Mikael.Hirsch@csiro.au | Australian regulatory processes surrounding the release of new organisms |
| Dr Tim Heard | CSIRO Entomology | Tim.Heard@csiro.au | CSIRO reviewer of biocontrol agent release applications |
| Prof Kerrie Mengersen | QUT | k.mengersen@qut.edu.au | Risk analysis, in particular elicitation of expert opinion |
| Prof Dave Andow | Uni of MN | dandow@umn.edu | Analysis of risks associated with release of living modified organisms |
| Dr Jenny Firman | NAMAC (Office of Health Protection, | Jenny.Firman@health.gov.au | Chair National Arbovirus and Malaria Advisory |

| | |
|---|---|
| **DOHA)** | **Committee. Knowledge and understanding of the regulatory and related jurisdictional processes that are involved with mosquitoes and the viral diseases that they transmit** |

## 7.2. Conclusions

**This risk analysis has evaluated the hazards associated with the release of *Wolbachia Ae. aegypti* in Australia for the purposes of preventing the transmission of dengue. The adverse endpoint (target hazard that we do not wish to occur) evaluated was that the release of *Wolbachia Ae. aegypti* would result in more harm than that provided by naturally occurring *Ae. aegypti* ('Cause More Harm') over a 30 year timeframe. The final results are the culmination of five discrete stages of soliciting expert opinion to identify hazards, model their relationships and estimate risk. Each stage that involved expert scoring in either workshop or email forum introduced types of uncertainty and the final stage consisted of a workshop designed to help reduce this uncertainty and collate a set of consensus *prior* estimates that truly reflected expert opinion.**

**The estimated risk for the endpoint hazard of 'Cause More Harm' was considered negligible.**

## 8. RISK ANALYSIS SUMMARY

- **Five stages of expert solicitation were undertaken to estimate the risk associated with the release of *Wolbachia Ae. aegypti* in Far North Queensland, Australia, to prevent the transmission of dengue.**

- **The risk analysis was carried out against the adverse endpoint that the release would cause additional harm beyond that provided by naturally occurring *Ae. aegypti* within a 30 year time frame from release ('Cause More Harm').**

- **Stage one included hazard identification by workshop and email solicitation resulting in a total of 50 discrete hazards. The relationship between the hazards was explored using Fault Tree Analysis (FTA). This process identified the cut set (shortest possible route to endpoint failure) of 'Worse Ecological Impacts' resulting from the release and the prevalence of 'Adverse media' as an influential hazard.**

- **Stage two was a one day expert workshop on the question of the ecological interactions of *Ae. aegypti* and possible impacts that could results from a decline in populations following release. FTA showed that reduced populations could reduce ecosystems services such as acting as a food source and incidental pollination services, and provide reduced competition to invasions by mosquitoes inhabiting a similar niche. However the experts concluded that *Ae. aegypti* is an exotic and highly anthropophilic species, and occurs at such low biomass that ecological interactions are unlikely and no parts of the system would likely endure impacts from a reduction in populations. It was also noted that population reduction, considered to be a risk, is in fact is the backbone of current dengue control strategies that include use of chemicals with numerous non-target impacts.**

- **Stage three was a one day workshop in Cairns on September 17 2009. The goals of this workshop were to combine mosquito and community experts and have them model the relationships between hazards and assign likelihoods of their failure in a Bayesian Belief Net (BBN). Following review of a draft model, the resulting BBN for 'Cause More Harm' contained 30 nodes (hazards). The model contained components dealing with possible sources of ecological, social, mosquito control, health and economic harm.**

- **A set of likelihoods was elicited for the summary (child nodes) although full workshop consensus was not achieved. This provided an estimate of 97.9% that 'Cause More Harm' would occur.**

- **Stage four was to solicit expert scores by email on the remaining 14 parent nodes that had not been scored. 20 experts responded but the results were notable for divergence and outliers, with low agreement amongst experts indicating high uncertainty from at least linguistic difficulties in interpreting definitions. The modal score was used to populate each hazard as the mean values were not considered representative of group scoring behaviour. This provided an estimated failure likelihood of 77.8% for 'Cause More Harm'.**

- **Stage Five was a two day workshop in Brisbane over 28th-29th January 2010 to address a number of issues including the high uncertainty and lack of a full consensus score for any parts of the BBNs. These issues indicated that previous *priors* were unlikely to reflect the real expert estimate of risk associated with this project. The final 'Cause More Harm' BBN contains 30 nodes, 38 links and 363 conditional probabilities after the hazard of 'Future mosquito management' was removed and a new hazard of 'Dengue evolution' incorporated. Likelihoods**

**for two hazards ('Dengue evolution' and 'Tourism') were resolicited to get a more accurate estimate and this reduced the final model o**

- **After populating the model with group consensus scores and resolicited values for two hazards, the *priors* result in an estimate of 12.5% likelihood that some form of harm could eventuate over a 30 year time frame from the date of release.**

- **Sensitivity analysis suggests that the 'Tourism' and 'Dengue evolution' hazards were noted as having a significant contribution to 'Cause More Harm'. The original risk estimates of 10% were re-solicited from experts on a 100 point scale to attain more accuracy and resulted in halving of the final likelihood. Without this re-solicitation of scores, the final *prior* estimate for 'Cause More Harm' would have been 25.4%**

- **Risk was calculated for the final 30 hazards as the product of the group consensus likelihood and consequence scores. The highest estimated risk was for 'Perceptions' which scored as low risk. Four hazards had estimated risk of very low ('Avoidance Strategies'/'Household Control'/'*Wolbachia* Fitness'/'Mosquito Density') and the remaining 25 nodes had an estimated negligible risk including the adverse endpoint of 'Cause More Harm'.**

- **Eleven hazards were assigned zero likelihood and two of these also had a zero consequence score. It is probable that some of these hazards have a small but non-zero likelihood (or consequence) and these could be elucidated by re-soliciting expert scores on a higher resolution scale.**

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# 10. APPENDICES

**Appendix 1. Summary of expert participation in the dengue risk analysis by analysis stage including reviewers of daft reports.**

[1] **member of the Dengue Consultation Group (DCG)**

[2] **community representative at the Stage three workshop.**

| | | Risk Analysis Solicitation Stage | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stage One | | | Stage two | Stage three | | Stage Four | | Stage Five | | |
| **Name** | **Affiliation** | FNQ Workshop | Email Hazard Solicitation | Email with Fault Trees | Ecological Role Workshop | Draft BBN and definitions | Cairns BBN Workshop | Email Solicitation of BBN Likelihoods | 1st Draft Report Review | Brisbane BBN Workshop | Email Re-Solicitation of BBN Likelihoods | 2nd Draft Report Review |
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## Appendix 2. The 52 hazards and hazard themes identified at Cairns Workshop 20th May by expert solicitation

| Theme | Number | Hazard |
|---|---|---|
| Behavioural | 1.1 | • *Ae. aegypti* becomes a more aggressive biter |
| | 1.2 | • Bendy proboscis changes behaviour |
| Biological | 2.1 | • Change in mating behaviour |
| | 2.2 | • Do not achieve cytoplasmic incompatibility |
| | 2.3 | • Do not achieve life shortening |
| | 2.4 | • Do not achieve viral interference |
| | 2.5 | • Population of *Ae. aegypti* increases after release |
| | 2.6 | • *Wolbachia* transmits to humans |
| | 2.7 | • *Wolbachia* induces insecticide resistance |
| | 2.8 | • Resident *Ae. aegypti* population collapses |
| Ecological | 3.1 | • New species emerges to fill niche |
| | 3.2 | • Horizontal transfer |
| | 3.3 | • Dengue host shifts to another species of mosquito |
| | 3.4 | • Failure to have sufficient baseline data to support release |
| Economic | 4.1 | • Reduction in availability of fruit pickers |
| | 4.2 | • Tourism declines |
| | 4.3 | • House prices fall |
| Environmental | 5.1 | • Cyclone damages production infrastructure or release site |
| | 5.2 | • Severe drought removes breeding sites |
| Epidemiological | 6.1 | • Dengue outbreak at time of release |
| | 6.2 | • Better vector for dengue emerges |
| | 6.3 | • Better vector for other viral diseases emerges |
| | 6.4 | • Release leads to decreased immunity in human population |
| | 6.5 | • Different disease/pest outbreak stops release |
| | 6.6 | • Dengue vaccine released |
| | 6.7 | • Dengue changes |
| Institutional | 7.1 | • Risk to UQ's reputation |
| | 7.2 | • Risk to project team/individual reputation |
| | 7.3 | • Risk to funders' reputation e.g. Gates Foundation |
| | 7.4 | • Loss of key IP |
| Media | 8.1 | • Antagonistic journalist takes an interest in project |
| | 8.2 | • Internet communication media lead to loss of support |
| Political | 9.1 | • Change of government |
| | 9.2 | • NGOs unsupportive and move to block release |
| | 9.3 | • Community group(s) unsupportive |
| | 9.4 | • Jurisdictions (local, state, federal) unsupportive |
| | 9.5 | • WHO unsupportive |
| | 9.6 | • Gates Foundation unsupportive |
| Regulatory | 10.1 | • Premature release/escape of *Ae. aegypti* |
| | 10.2 | • OGTR claims domain |
| | 10.3 | • No one claims domain |
| | 10.4 | • Disagreement over mandate |
| | 10.5 | • Legislation emerges to stop release |
| | 10.6 | • Injunction taken out to prevent release |
| Social | 11.1 | • Change in household behaviour |
| | 11.2 | • Decline/loss in stakeholder acceptance |
| | 11.3 | • No stakeholder acceptance |
| | 11.4 | • Social vilification of local collaborators |
| Technological | 12.1 | • Other competing technologies are preferred |
| | 12.2 | • Cage results not predictive |
| | 12.3 | • Release models fail to show benefit |
| Tourism | 13.1 | • Tourism declines |

## Appendix 3: Hazards that were broken into discrete hazard components [the numbering system was based on the full hazard set of which only 'Cause More Harm' hazards are shown]

| Old # | Old hazard | New Hazards |
|---|---|---|
| 2.2 | Unanticipated effects on target mosquito causing detrimental changes, e.g. increased resistance to insecticides or other control measures, increased biting/nuisance activity, increased tendency to breed in areas of human activity, increased host seeking for valued animals (e.g. dogs, cats, cows, sheep) and transmission of animal pathogens). | 2.2a increased competence for other pathogens<br>2.2b increased resistance to insecticides or other control measures<br>2.2c increased biting/nuisance activity,<br>2.2d increased tendency to breed in areas of human activity<br>2.2e Host seeking on non-human targets (e.g. dogs, cats, cows, sheep)<br>2.2f Transmission of animal pathogens |
| 2.3 | *Wolbachia* will become a more competent vector for Dengue or other mosquito borne viruses) | 2.3a More competent vector for Dengue<br>2.3b More competent vector for other mosquito borne viruses |
| 2.16 | Unanticipated evolutionary effects, e.g. development of "super" Dengue transmitter through changes in mosquito or virus, selection of mosquito with faster reproductive rate. | 2.16a development of "super" Dengue transmitter through changes in mosquito or virus<br>2.16b selection of mosquito with faster reproductive rate |
| 4.1 | Changes in the efficacy of Dengue control/suppression over time. | 4.10a Changes in the efficacy of Dengue control/suppression over time<br>4.10b Changes in investment of alternate control technologies for mosquito suppression |

## Appendix 4: Hazards that were removed (with justification)

| Hazard # | Hazard | Reason |
|---|---|---|
| 2.2d | Increased tendency to breed in areas of human activity. | • This is natural behaviour of *Ae. aegypti* |
| 2.7 | Is *Wolbachia* humane (community concerns)? | • Arthropods are generally excluded from ethic approval<br>• *Wolbachia* naturally occurs in many insect species/mosquitoes<br>• Is in a biological control programme |
| 7.3 | Cost-benefit analyses indicate that *Wolbachia* is not cost effective. | • Outside scope |

## Appendix 5. 27 remaining project hazards following refinement

| New Hazard # | Top Event # | New Theme | Description | Contributing Hazards/Synonyms |
|---|---|---|---|---|
| 4 | 2 | *Wolbachia* failure | Risk that *Wolbachia* does not provide expected reduction in Dengue vectoring or provides some other adverse effect. | 2.8 *Wolbachia* does not achieve life shortening<br>2.9 *Wolbachia* does not achieve viral interference<br>2.4 *Wolbachia* does not achieve cytoplasmic incompatibility (CI)<br>2.3a More competent vector for Dengue<br>2.16a development of "super" Dengue transmitter through changes in mosquito or virus<br>2.3b *Wolbachia* will become a more competent vector for other mosquito borne viruses<br>2.2a Increased competence for other pathogens<br>2.2f transmission of animal pathogens<br>2.5 *Wolbachia* becomes less effective or ineffective in the long term<br>2.19 *Ae. aegypti* loses *Wolbachia* upon release<br>*Wolbachia* not equally effective on all Dengue serotypes |
| 5 | 2 | Insecticide resistance | *Wolbachia* provides increased *Ae. aegypti* insecticide resistance. | 2.14 *Wolbachia* induces increased insecticide resistance<br>2.2b Increased resistance to insecticides or other control measures |
| 6 | 2 | Increased control costs | *Wolbachia Ae. aegypti* populations will require increased or more intensive treatments. | 4.10b Changes in investment of alternate control technologies for mosquito suppression |
| 8 | 1, 2 | Changes in *Ae. aegypti* behaviour | *Ae. aegypti* behaviour changes as result of *Wolbachia* effects. | 2.2e Host seeking on non-human targets (e.g. dogs, cats, cows, sheep)<br>3.3 Bendy proboscis phenotype changes behaviour<br>3.1 *Wolbachia* changes *Ae. aegypti* mating behaviour |
| 9 | 2 | Increased *Ae. aegypti* biting | Increased biting or number of blood meals required by *Wolbachia Ae. aegypti*. | 3.2 *Ae. aegypti* becomes a more aggressive biter<br>2.2c increased biting/nuisance activity<br>3.4 *Ae. aegypti* requires more host feeding events |
| 10 | 2 | Reduced ecosystem services | Lower density of *Ae. aegypti* populations reduce source of food to predators or other ecosystem services (if any) provided. | 2.6 Effects of a decrease in the size of the *Ae. aegypti* populations (*Wolbachia* and naturally occurring) on local ecology (i.e. on mosquito predators).<br>2.18 Reduction of food supply available to animals that eat mosquitoes |
| 11 | 2 | Larger *Ae. aegypti* population | *Ae. aegypti* population density per unit area increases permanently above current mean. | 2.10 Overall *Ae. aegypti* population size increases after release<br>2.16b Selection of mosquito with faster reproductive rate |
| 12 | 2 | *Ae. aegypti* crash | *Ae. aegypti* population density crashes, possible local extinction. | 2.11 Resident *Ae. aegypti* population collapses |
| 13 | 2 | Increased *Ae. aegypti* geographic range | *Ae. aegypti* increases geographic distribution beyond predicted limits or at a faster than expected/modelled rate. | 2.12 Overall *Ae. aegypti* geographic distribution increases after release |
| 14 | 2 | Vacant niche | *Ae. aegypti* vacates niche for other species or is uncompetitive against new species. | 6.1 New mosquito species fills niche |
| 15 | 2 | Horizontal transfer | Transfer of *Wolbachia* to other species (vertebrate or invertebrate) via predation or host feeding events. | 2.17 Horizontal transfer of *Wolbachia* causes detrimental effects on "valued" non-target organisms such as bees, butterflies, etc. |

| | | | | |
|---|---|---|---|---|
| | | | | **2.13 *Wolbachia* transmitted to humans and/or other vertebrate species as a result of biting**<br>**6.2 Horizontal transfer of *Wolbachia* to other species** |
| **16** | **2** | **Dengue evolves** | **Dengue evolves to be more pathogenic in response to *Wolbachia*.** | **2.15 Changes/mutations in Dengue virus biology e.g. selection pressure under reduced host life span**<br>**4.7 Changes in Dengue occur**<br>**4.10a Changes in the efficacy of Dengue control/suppression over time** |
| **18** | **1, 2** | **Dengue vector** | **Other (more effective?) Dengue vectors establish in Australia, control of *Ae. aegypti* becomes lower priority.** | **4.4 Better vector for other viral diseases emerges**<br>**6.3 Dengue host shifts to another species of mosquito**<br>**4.3 Better vectors for Dengue arrive or emerge in Australia** |
| **19** | **2** | **Changes in herd immunity** | **Changes in disease epidemiology that adversely affect herd immunity.** | **4.5 Release influences immunity in human population** |
| **28** | **1, 2** | **Reduced control** | **Conflict of interest or assumption that *Wolbachia Ae. aegypti* will reduce Dengue problem, so less investment in control development or control effort.** | **10.9 Competition of Ae. albopictus and DART (Dengue Action Response Team) programs in terms of operational management**<br>**11.10 Failure to invest and develop alternative control strategies due to reliance upon the *Wolbachia*-infected *Ae. aegypti*.** |
| **30** | **1, 2** | **Community knowledge** | **Community have insufficient technical or incorrect knowledge of Dengue, *Wolbachia* and *Ae. aegypti* to make informed decisions.** | **9.7 Community do not accept release because of incorrect assumptions/ knowledge of mosquitoes and Dengue issue**<br>**9.8 People are unable to distinguish between *Ae. aegypti* and other mosquitoes so may attribute outbreaks by other mosquito species to *Wolbachia Ae. aegypti***<br>**9.10 Local people (or Australians in general) do not perceive Dengue fever as a significant risk to their health**<br>**Community are not informed of risk or clearly understand issues** |
| **32** | **2** | **Economic impact** | **Adverse economic impacts in release area occur as result of proposed release.** | **7.1 Reduction in availability of seasonal workers (e.g. fruit pickers)**<br>**7.2 Tourism declines**<br>**9.12 Visitor numbers decline**<br>**Real estate or other values decline as result of release*** |
| **35** | **2** | **Mosquito avoidance behaviour** | **People's behaviour changes to reduce biting with modified *Ae. aegypti*. Includes avoidance, household insecticide use and removal of breeding sites.**<br>**Behavioural changes in release area to reduce interactions with *Ae. aegypti.*** | **Increased household insecticide use***<br>**Removal of breeding sites***<br>**Less outside socialising***<br>**9.9 Those working outdoors or at home during the day (notably women/carers and children) at greater risk of being bitten**<br>**9.1 Changes in household behaviour occur** |
| **36** | **2** | **New mosquito species** | **New species is able to establish because of modified *Ae. aegypti*.** | |
| **37** | **2** | **New serotype** | **New dengue serotype emerges.** | |
| **38** | **2** | **Perception *Wolbachia* solves problem*** | **Perception that *Wolbachia* will solve dengue problem.** | |

**Appendix 6. Conditional probability tables (CPT) used in the BBN as provided by experts at the Stage five workshop.**

| Host Preference | | |
|---|---|---|
| *Wolbachia* Fitness | Same | Broadens |
| Same | 0.999 | 0.001 |
| Increases | 0.999 | 0.001 |

| Avoidance Strategies | | |
|---|---|---|
| Household Control | No Change | Increase |
| Same | 0.98 | 0.2 |
| Decreased | 0.98 | 0.2 |

| Dengue Vector Competence | | |
|---|---|---|
| *Wolbachia* Fitness | Same | Increased |
| Same | 1.0 | 0 |
| Increases | 0.999 | 0.001 |

| Feeding Frequency | | |
|---|---|---|
| *Wolbachia* Fitness | Same | Increased |
| Same | 1.0 | 0 |
| Increases | 0.999 | 0.001 |

| Household Control | | |
|---|---|---|
| Perceptions | Same | Decreased |
| Same | 0.98 | 0.02 |
| Reduced | 0.9 | 0.1 |

| Mosquito Density | | |
|---|---|---|
| *Wolbachia* Fitness | Same | Increased |
| Same | 1 | 0 |
| Increases | 0.999 | 0.001 |

| Non-Dengue Vector Competence | | |
|---|---|---|
| *Wolbachia* Fitness | Same | Increased |
| Same | 1.0 | 0 |
| Increases | 0.999 | 0.001 |

| Other Pathogens | | | | |
|---|---|---|---|---|
| Mosq. Dens | Host Pref | Non_Dengue_Comp | Same | Increased |
| Same | Same | Same | 1.0 | 0 |
| Same | Same | Increased | 0.98 | 0.02 |
| Same | Broadens | Same | 0.99 | 0.01 |
| Same | Broadens | Increased | 0.97 | 0.03 |
| Increased | Same | Same | 0.99 | 0.01 |
| Increased | Same | Increased | 0.97 | 0.03 |
| Increased | Broadens | Same | 0.98 | 0.02 |
| Increased | Broadens | Increased | 0.5 | 0.5 |

| Nuisance Biting | | | | |
|---|---|---|---|---|
| Host_Pref | Feed_Freq | Mosq_Density | Same | Increased |
| Broadens | Same | Same | 1.0 | 0 |
| Broadens | Same | Increased | 0 | 1.0 |
| Broadens | Increased | Same | 0 | 1.0 |
| Broadens | Increased | Increased | 0 | 1.0 |
| Same | Same | Same | 1.0 | 0 |
| Same | Same | Increased | 0 | 1.0 |
| Same | Increased | Same | 0 | 1.0 |
| Same | Increased | Increased | 0 | 1.0 |

| Dengue Transmission | | | | | |
|---|---|---|---|---|---|
| Deng_Vect_Comp | Feed_Freq | Mosq_Dens | Deng_Evol | Same | Increased |
| Same | Same | Same | Same | 1.0 | 0 |
| Same | Same | Same | More Effective | 0 | 1.0 |
| Same | Same | Increased | Same | 0.05 | 0.95 |
| Same | Same | Increased | More Effective | 0 | 1.0 |
| Same | Increased | Same | Same | 0 | 1.0 |
| Same | Increased | Same | More Effective | 0 | 1.0 |
| Same | Increased | Increased | Same | 0 | 1.0 |
| Same | Increased | Increased | More Effective | 0 | 1.0 |
| Increased | Same | Same | Same | 0 | 1.0 |
| Increased | Same | Same | More Effective | 0 | 1.0 |
| Increased | Same | Increased | Same | 0 | 1.0 |
| Increased | Same | Increased | More Effective | 0 | 1.0 |
| Increased | Increased | Same | Same | 0 | 1.0 |
| Increased | Increased | Same | More Effective | 0 | 1.0 |
| Increased | Increased | Increased | Same | 0 | 1.0 |
| Increased | Increased | Increased | More Effective | 0 | 1.0 |

| Economic Effects | | | | | |
|---|---|---|---|---|---|
| Tourism | Labour_Avail | Health Care | Real Estate | Same | Worse |
| Same | Same | Same | Same | 1.0 | 0 |
| Same | Same | Same | Decreased | 0 | 1.0 |
| Same | Same | Increased | Same | 0 | 1.0 |
| Same | Same | Increased | Decreased | 0 | 1.0 |
| Same | Decreased | Same | Same | 0 | 1.0 |
| Same | Decreased | Same | Decreased | 0 | 1.0 |
| Same | Decreased | Increased | Same | 0 | 1.0 |
| Same | Decreased | Increased | Decreased | 0 | 1.0 |
| Decreased | Same | Same | Same | 0 | 1.0 |
| Decreased | Same | Same | Decreased | 0 | 1.0 |
| Decreased | Same | Increased | Same | 0 | 1.0 |
| Decreased | Same | Increased | Decreased | 0 | 1.0 |
| Decreased | Decreased | Same | Same | 0 | 1.0 |
| Decreased | Decreased | Same | Decreased | 0 | 1.0 |
| Decreased | Decreased | Increased | Same | 0 | 1.0 |

| | | | | | |
|---|---|---|---|---|---|
| Decreased | Decreased | Increased | Decreased | 0 | 1.0 |

| | | | Ecology | | | |
|---|---|---|---|---|---|---|
| Invert_Trans | Vert_Trans | Geo_Range | Ecol_Niche | Density | No Impact | Neg Impact |
| Unlikely | No | Same | Same | Same | 1.0 | 0 |
| Unlikely | No | Same | Same | Increase | 1.0 | 0 |
| Unlikely | No | Same | Other | Same | 1.0 | 0 |
| Unlikely | No | Same | Other | Increase | 1.0 | 0 |
| Unlikely | No | Increase | Same | Same | 1.0 | 0 |
| Unlikely | No | Increase | Same | Increase | 1.0 | 0 |
| Unlikely | No | Increase | Other | Same | 1.0 | 0 |
| Unlikely | No | Increase | Other | Increase | 1.0 | 0 |
| Unlikely | Yes | Same | Same | Same | 0.99 | 0.01 |
| Unlikely | Yes | Same | Same | Increase | 0.99 | 0.01 |
| Unlikely | Yes | Same | Other | Same | 0.99 | 0.01 |
| Unlikely | Yes | Same | Other | Increase | 0.99 | 0.01 |
| Unlikely | Yes | Increase | Same | Same | 0.99 | 0.01 |
| Unlikely | Yes | Increase | Same | Increase | 0.99 | 0.01 |
| Unlikely | Yes | Increase | Other | Same | 0.99 | 0.01 |
| Unlikely | Yes | Increase | Other | Increase | 0.99 | 0.01 |
| Possible | No | Same | Same | Same | 1.0 | 0 |
| Possible | No | Same | Same | Increase | 1.0 | 0 |
| Possible | No | Same | Other | Same | 1.0 | 0 |
| Possible | No | Same | Other | Increase | 1.0 | 0 |
| Possible | No | Increase | Same | Same | 1.0 | 0 |
| Possible | No | Increase | Same | Increase | 1.0 | 0 |
| Possible | No | Increase | Other | Same | 1.0 | 0 |
| Possible | No | Increase | Other | Increase | 1.0 | 0 |
| Possible | Yes | Same | Same | Same | 0.99 | 0.01 |
| Possible | Yes | Same | Same | Increase | 0.99 | 0.01 |
| Possible | Yes | Same | Other | Same | 0.99 | 0.01 |
| Possible | Yes | Same | Other | Increase | 0.99 | 0.01 |
| Possible | Yes | Increase | Same | Same | 0.99 | 0.01 |
| Possible | Yes | Increase | Same | Increase | 0.99 | 0.01 |
| Possible | Yes | Increase | Other | Same | 0.99 | 0.01 |
| Possible | Yes | Increase | Other | Increase | 0.99 | 0.01 |
| Likely | No | Same | Same | Same | 1.0 | 0 |
| Likely | No | Same | Same | Increase | 1.0 | 0 |
| Likely | No | Same | Other | Same | 1.0 | 0 |
| Likely | No | Same | Other | Increase | 1.0 | 0 |
| Likely | No | Increase | Same | Same | 1.0 | 0 |
| Likely | No | Increase | Same | Increase | 1.0 | 0 |
| Likely | No | Increase | Other | Same | 1.0 | 0 |
| Likely | No | Increase | Other | Increase | 1.0 | 0 |
| Likely | Yes | Same | Same | Same | 0.99 | 0.01 |
| Likely | Yes | Same | Same | Increase | 0.99 | 0.01 |
| Likely | Yes | Same | Other | Same | 0.99 | 0.01 |
| Likely | Yes | Same | Other | Increase | 0.99 | 0.01 |
| Likely | Yes | Increase | Same | Same | 0.99 | 0.01 |
| Likely | Yes | Increase | Same | Increase | 0.99 | 0.01 |
| Likely | Yes | Increase | Other | Same | 0.99 | 0.01 |
| Likely | Yes | Increase | Other | Increase | 0.5 | 0.5 |

| Mosquito Management Efficacy | | | | | |
|---|---|---|---|---|---|
| Need_Control | Insect_Resist | House_Cont | Monitoring | Same | Reduced |
| Same | Same | Same | Sufficient | 1.0 | 0 |
| Same | Same | Same | Insufficient | 0.98 | 0.02 |
| Same | Same | Decreased | Sufficient | 0.2 | 0.8 |
| Same | Same | Decreased | Insufficient | 0.2 | 0.8 |
| Same | Increased | Same | Sufficient | 0.3 | 0.7 |
| Same | Increased | Same | Insufficient | 0.29 | 0.71 |
| Same | Increased | Decreased | Sufficient | 0.06 | 0.94 |
| Same | Increased | Decreased | Insufficient | 0.06 | 0.94 |
| Increased | Same | Same | Sufficient | 0.8 | 0.2 |
| Increased | Same | Same | Insufficient | 0.78 | 0.22 |
| Increased | Same | Decreased | Sufficient | 0.16 | 0.84 |
| Increased | Same | Decreased | Insufficient | 0.16 | 0.84 |
| Increased | Increased | Same | Sufficient | 0.24 | 0.76 |
| Increased | Increased | Same | Insufficient | 0.24 | 0.76 |
| Increased | Increased | Decreased | Sufficient | 0.05 | 0.95 |
| Increased | Increased | Decreased | Insufficient | 0.05 | 0.95 |

| Standard of Public Health | | | | |
|---|---|---|---|---|
| Dengue_Trans | Nuisan_Bite | Other_Path | Same | Worse |
| Same | Same | Same | 1.0 | 0 |
| Same | Same | Increase | 0 | 1.0 |
| Same | Increased | Same | 1.0 | 0 |
| Same | Increased | Increase | 0 | 1.0 |
| Worse | Same | Same | 0 | 1.0 |
| Worse | Same | Increase | 0 | 1.0 |
| Worse | Increased | Same | 0 | 1.0 |
| Worse | Increased | Increase | 0 | 1.0 |

| Cause More Harm | | | | | | |
|---|---|---|---|---|---|---|
| Ecology | Mosquito_Man Efficacy | Avoidance Strategies | Economic Effects | Std_Public Health | No Change | Worse |
| No Impact | Same | No Change | Same | Same | 1.0 | 0 |
| No Impact | Same | No Change | Same | Worse | 0 | 1.0 |
| No Impact | Same | No Change | Worse | Same | 0 | 1.0 |
| No Impact | Same | No Change | Worse | Worse | 0 | 1.0 |
| No Impact | Same | Increase | Same | Same | 0.2 | 0.8 |
| No Impact | Same | Increase | Same | Worse | 0 | 1.0 |
| No Impact | Same | Increase | Worse | Same | 0 | 1.0 |
| No Impact | Same | Increase | Worse | Worse | 0 | 1.0 |
| No Impact | Reduced | No Change | Same | Same | 0.1 | 0.9 |
| No Impact | Reduced | No Change | Same | Worse | 0 | 1.0 |
| No Impact | Reduced | No Change | Worse | Same | 0 | 1.0 |
| No Impact | Reduced | No Change | Worse | Worse | 0 | 1.0 |
| No Impact | Reduced | Increase | Same | Same | 0.02 | 0.98 |
| No Impact | Reduced | Increase | Same | Worse | 0 | 1.0 |
| No Impact | Reduced | Increase | Worse | Same | 0 | 1.0 |
| No Impact | Reduced | Increase | Worse | Worse | 0 | 1.0 |
| Neg Impact | Same | No Change | Same | Same | 0 | 1.0 |
| Neg Impact | Same | No Change | Same | Worse | 0 | 1.0 |
| Neg Impact | Same | No Change | Worse | Same | 0 | 1.0 |
| Neg Impact | Same | No Change | Worse | Worse | 0 | 1.0 |
| Neg Impact | Same | Increase | Same | Same | 0 | 1.0 |
| Neg Impact | Same | Increase | Same | Worse | 0 | 1.0 |
| Neg Impact | Same | Increase | Worse | Same | 0 | 1.0 |
| Neg Impact | Same | Increase | Worse | Worse | 0 | 1.0 |
| Neg Impact | Reduced | No Change | Same | Same | 0 | 1.0 |
| Neg Impact | Reduced | No Change | Same | Worse | 0 | 1.0 |
| Neg Impact | Reduced | No Change | Worse | Same | 0 | 1.0 |
| Neg Impact | Reduced | No Change | Worse | Worse | 0 | 1.0 |
| Neg Impact | Reduced | Increase | Same | Same | 0 | 1.0 |
| Neg Impact | Reduced | Increase | Same | Worse | 0 | 1.0 |
| Neg Impact | Reduced | Increase | Worse | Same | 0 | 1.0 |
| Neg Impact | Reduced | Increase | Worse | Worse | 0 | 1.0 |