IMPACT ASSESSMENT ON THE RELEASE OF X-RAY OR SIMILAR TREATED *WOLBACHIA-AEDES* MOSQUITOES FOR SUPPRESSION OF THE URBAN *AEDES AEGYPTI* MOSQUITO POPULATION

Summary

The impact assessment process involved critical reviews of existing knowledge and research, consultations with domain experts from the International Atomic Energy Agency (IAEA), and laboratory experiments carried out at the Environmental Health Institute (EHI), of the National Environment Agency (NEA). No ecological risk has been identified. A potential risk identified is the reduction in competitiveness of the X-ray or similarly treated male *Wolbachia-Aedes* mosquitoes, leading to ineffective suppression of the urban *Aedes aegypti* mosquito population at the study sites, thus necessitating the need to release larger numbers of such male mosquitoes.

Background

Project *Wolbachia* – Singapore takes advantage of the cytoplasmic incompatibility (CI) attribute of the naturally occurring *Wolbachia* bacterium, to help suppress the urban *Aedes aegypti* mosquito population in Singapore, for further reduction of the dengue risk. Impact assessment and laboratory studies conducted since 2012 at the Environmental Health Institute (EHI), of the National Environment Agency (NEA), have shown that the *Wolbachia* technology is safe and poses no harm to humans or the environment. From 2016 to 2017, NEA carried out the Project *Wolbachia* Phase 1 field study, to gain an understanding of the behaviour of male *Wolbachia-Aedes* mosquitoes, by releasing these mosquitoes in our urban environment. The Phase 1 field study – carried out at three study sites at Braddell Heights, Tampines West and Nee Soon East – yielded valuable data on the bionomics of the released male *Wolbachia-Aedes* mosquitoes, such as their longevity in the field, and their vertical and horizontal dispersal ranges. The data also indicated the ability of male *Wolbachia-Aedes* mosquitoes to compete with urban *Aedes aegypti* males to mate with urban *Aedes aegypti* females, leading to a 50 per cent suppression of the urban *Aedes aegypti* mosquito population at the study sites.

The Phase 1 field study also surfaced ecological challenges posed by Singapore's high-density and high-rise housing. The impact of the releases was found to be limited by the movement of *Aedes aegypti* mosquitoes from the surrounding areas into the release sites; as well as the high *Aedes aegypti* mosquito densities at higher floors of some blocks, where insufficient numbers of male *Wolbachia-Aedes* mosquitoes reached. In addition, a very small percentage of fertile *Wolbachia-Aedes* females being released alongside the males could also have limited the impact of the releases.

As highlighted in our earlier impact assessment on the *Wolbachia-Aedes* technology, a very small number of female *Wolbachia-Aedes* mosquitoes may be released along with the male *Wolbachia-Aedes* mosquitoes, due to imperfect sex-sorting. The current sex-sorting method uses a mechanical size-separation device to separate the smaller male pupae from the larger female pupae. Whilst the device has consistently achieved a high accuracy of separating male from female pupae (over 99.7% accuracy), a very small number of the smaller-sized females may be segregated with the males. Though the small number of females will have negligible potential for disease transmission, as viral replication is blocked by the *Wolbachia* present, long-term releases of female *Wolbachia-Aedes* mosquitoes may potentially hamper the continued use of *Wolbachia-Aedes* technology to suppress the urban *Aedes aegypti* mosquito population

in those areas. This is due to the biological mating compatibility between female *Wolbachia-Aedes* mosquitoes and males with or without *Wolbachia*. These matings could lead to the propagation of offspring with *Wolbachia*, and as these compatible matings increase over time and produce more progeny with *Wolbachia*, *Wolbachia Aedes aegypti* population may dominate in the field. The female *Wolbachia Aedes* will be biologically compatible with the released *Wolbachia-Aedes* males and will thus hamper the continued use of the same strain of male *Wolbachia-Aedes* to suppress the mosquito population.

Use of a combined SIT/IIT approach for the suppression of *Aedes* mosquitoes

One approach to address the potential establishment of *Wolbachia-Aedes* mosquitoes in the community is to subject the sex-sorted pupae to X-ray or similar treatment, to sterilise any female *Wolbachia-Aedes* mosquitoes that may have slipped through the sorting process. X-ray or similar treatment affects the fertility of female mosquitoes more than the virility of male mosquitoes, and laboratory studies have shown that a low dosage X-ray or similar treatment can sterilise the females without diminishing the virility of the males.

The potential of using radiation for the control of mosquito populations was first demonstrated in the late 1950s, when eggs from matings between female *Anopheles quadrimaculatus* and treated males failed to hatch. However, earlier field studies had shown that male mosquitoes exposed to the treatment dose required for sterility (over 100Gy) had rendered them unfit and uncompetitive due to somatic cell damage. Further scientific research later showed that lower dosage treatment could render the females infertile without diminishing the virility of the males.

Coupling the *Wolbachia*-based Insect Incompatibility Technique (IIT) with the classical irradiation-based Sterile Insect Technique (SIT) thus has the potential to produce competitive male *Wolbachia-Aedes* mosquitoes, whilst rendering the small number of female *Wolbachia-Aedes* mosquitoes infertile. Laboratory studies have shown the potential of this approach for *Aedes albopictus*, *Aedes polynesiensis* and *Culex pipiens* mosquito species, with promising results. Encouraging findings from an ongoing field study in Guangzhou, China, have also shown the feasibility of this approach for the *Aedes albopictus* mosquito (Prof. Xi Zhiyong, personal communication). In 2017, the World Health Organization's Vector Control Advisory Group (VCAG) assessed and concluded that the combined SIT/IIT technology has the potential for long-term control of *Aedes aegypti* and *Aedes albopictus* mosquito vectors. VCAG strongly recommended further entomological and epidemiological field trials to validate the use of this intervention.

Impact assessment

Impact of X-ray or similarly treated *Wolbachia-Aedes* mosquitoes on the environment

Does treatment of Wolbachia-Aedes mosquitoes render them radioactive or cause them to emit radiation?

No. Treated mosquitoes are not radioactive, as the mosquitoes do not come into physical contact with the radioactive source. X-ray or similar treatment (less than 30 Gy) applied to the *Wolbachia-Aedes* mosquitoes does not render them radioactive – just like X-ray screening of baggage at airport and seaport checkpoints, does not cause the baggage to become radioactive or emit radiation. The use of such treatment for commercial sterilisation of medical devices is also widely implemented throughout the world. The reference dose for sterilisation of medical devices is 2500 Gy.

Could there be an adverse effect when predators eat, or when other animals come into contact with, the treated Wolbachia-Aedes *mosquitoes?*

For the same reason as above, predators that eat, or other animals that come into contact with, the treated mosquitoes will not be affected by the treatment. X-ray or similar treatment is commonly applied to our food to improve food safety, quality and prolong shelf-life; and to agricultural products as part of phytosanitation measures for removing and reducing pests that may be harmful or destructive to these products. The doses applied under these circumstances are more than 100 times higher than that required to sterilise insects to control pest populations.

Impact of X-ray or similarly treated *Wolbachia-Aedes* mosquitoes on public health

Will treatment increase the biting frequency of female Wolbachia-Aedes mosquitoes?

No. Studies carried out at EHI, have shown that treated female *Wolbachia-Aedes* mosquitoes have similar biting behaviour when compared to non-treated female mosquitoes.

Will treatment cause female Wolbachia-Aedes mosquitoes to lay more eggs?

No. Studies carried out at EHI have shown that treated female *Wolbachia-Aedes* mosquitoes have reduced fecundity (over 99.9% reduced) when compared to non-treated female *Wolbachia-Aedes* mosquitoes.

Will treatment affect the level of cytoplasmic incompatibility (CI) induced by treated male Wolbachia-Aedes *mosquitoes, such that mating with urban* Aedes aegypti *females will result in viable offspring?*

No. Studies conducted at EHI have shown that treatment has no impact on cytoplasmic incompatibility (CI) between the male *Wolbachia-Aedes* mosquitoes and urban female *Aedes* mosquitoes. All eggs laid by urban *Aedes aegypti* females that had mated with treated *Wolbachia-Aedes* males did not hatch.

Will treatment lower the ability of male Wolbachia-Aedes *mosquitoes to compete with urban* Aedes aegypti *males to mate with urban* Aedes aegypti *females?*

Studies conducted at EHI and at other international research laboratories have shown that treatment reduced the mating competitiveness of male mosquitoes. To compensate for this reduction in mating competitiveness, a larger number of male *Wolbachia-Aedes* mosquitoes may need to be released. The Project *Wolbachia* Phase 2 field study will aim to understand the impact of X-ray or similar treatment on the fitness of the male *Wolbachia-Aedes* mosquitoes in the urban setting.

Despite the reduced competitiveness, mass release of irradiated insects to control or eradicate various agricultural pest species has been successful and well documented since the 1950s, The New World screw worm fly (*Cochliomyia hominivorax*) – an important veterinary parasite affecting livestock – was eradicated from the US and all Central American countries in the 1950s. Since then, SIT has also successfully eradicated or suppressed: the Mediterranean fruit fly (*Ceratitis capitate*) in many parts of Central and South America, South Africa and Australia; the melon fly (*Bactrocera cucurbitae*) in Okinawa and all of Japan's south-eastern islands; the codling moth (*Cydia pomonella*) in British Columbia, Canada; and a tsetse fly species that transmits African animal trypanosomiasis in Zanzibar, Tanzania.

Will the increase in the number of treated male Wolbachia-Aedes *mosquitoes to be released lead to an increase in the number of* Wolbachia-Aedes *females being unintentionally released?*

The number of *Wolbachia-Aedes* females unintentionally released will increase proportionally with the increase in the number of *Wolbachia-Aedes* males released. However, the X-ray or similar treatment will render any released females infertile. The *Wolbachia* in the females will also help block the transmission of dengue virus. So even though this small number of released *Wolbachia-Aedes* female mosquitoes may still bite, they would neither be able to transmit disease nor reproduce. Furthermore, the number released would be only be a negligible fraction of the population of urban *Aedes aegypti* females at the release sites. It would therefore not add significantly to any mosquito bite encounters that is already present at these release sites due to the presence of the urban *Aedes* mosquitoes.