Mosquito Irradiation, Sterilization and Quality Control

Report of the Second Research Coordination Meeting
of an FAO/IAEA Coordinated Research Project,
held in Vienna, Austria,
from July 18 to 22, 2022

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NOTE

TABLE OF CONTENTS

Summary:
The application of the Sterile Insect Technique (SIT) in area-wide integrated pest management (AW-IPM) programmes continues to increase in response to requests from Member States. These requests include the development and refinement of SIT packages for programmes to control populations of different insect pests of agricultural, veterinary and human health importance. The development and operational application of such programmes with an SIT component against human disease vectors depends on the efficiency of irradiated sterile males to induce sterility in the target populations. Irradiation, in combination with other steps in the workflow from production to release of the sterile males is a critical point ultimately affecting performance in the field. Quality control is critical at every step of the SIT workflow, both pre- and post-irradiation.
This CRP will be the opportunity to investigate the impact of endogenous factors such as stage, age and genetic background on irradiation efficacy, as well as physiological mechanisms associated with genetic variation in radiation responses. We will also explore the impact of exogenous factors on radio-sensitivity, such as temperature, density, oxygen availability, irradiation source, dose-rate and energy of the rays. Another important aspect to characterize is the impact of irradiation on vectorial capacity of females, as well as on the cytoplasmic incompatibility and pathogen interference conferred by *Wolbachia* infection.
Finally, it will be important to develop and validate new quality control tools to monitor the product quality along the production chain and compare it between production and release centres.

Background

Scientific situation and problems to be researched: Insects are the most abundant, speciose and diverse animal group on this planet. Although most insect species are beneficial or harmless, there are a select few which are vectors of human diseases and their populations need to be managed. Conventional control methods are primarily based on insecticides. However, there are increasing concerns about their negative impact on human health and environment, as well as the inevitable selection of insecticide resistance due to their extensive use. The Sterile Insect Technique (SIT) represents a species-specific, non-polluting and environment-friendly approach that has been extensively used over the last 60 years to control populations of plant and animal pests, and animal disease vectors as a component of AW-IPM programmes. Due to its successful use against different target species, the requests for the application of the SIT against human disease vectors continues to increase from FAO and IAEA Member States (MS). Programme efficiency and cost-effectiveness depend on the efficiency of irradiated sterile males to induce sterility in the target populations. Irradiation, in combination with other steps in the workflow from production to release of the sterile males, is a critical point ultimately affecting performance in the field. Quality control is also critical at every step of the SIT workflow.

Targeted species: Currently,the following species of human health importance are considered potential targets for the SIT: *Aedes aegypti, Ae. albopictus, Ae. polynesiensis, Anopheles albimanus, An. arabiensis, An. darlingi, An. gambiae* complex*, An. stephensi, Culex pipiens* complex.

Importance of irradiation and quality control in SIT programmes

Historically, a variety of chemosterilants were used to sexually sterilize male mosquitoes with varying success and suitability for larger scale SIT programmes, and the evaluation of sterilizing male mosquitoes by irradiation has suggested that this is, to date, the most practical, safe and environment-friendly way to induce sterility, especially at large scale [Helinski et al., 2006]. The use of isotopic sources for gamma radiation (usually cobalt-60 or caesium-137), has been most commonly used for AW-IPM programmes with an SIT component. However, X-ray and high energy electrons (in this case "high" refers to a minimum of 1-5MeV) are now becoming viable and practical alternatives (Gómez-Simuta et al., 2021). In irradiation processes, the key factor is the absorbed dose, which needs to be accurately controlled to ensure that treated insects are rendered sufficiently sterile but are still able to compete for wild females upon release. Therefore, accurate dosimetry (measurement of absorbed dose) is critical. Factors such as insect age and stage, handling methods, oxygen level, ambient temperature, dose-rate and many others prior to- and during irradiation, influence both the radio-sensitivity and biological viability of the irradiated mosquito. Because of these sources of variation, there is presently a lack of consensus on the irradiation protocols to produce high quality sterile male mosquitoes for field release, especially at a large scale. Thus, exploring the impact of these different factors, and their impact on the quality of the resulting sterile males, in interaction with other handling steps before release, is essential. A careful evaluation of these factors in the design of irradiation protocols can help to find a balance between the sterility and competitiveness of the irradiated males destined for field releases.

Many SIT programmes apply higher doses than required as a "precautionary" measure to ensure full sterility. However, this is likely to decrease the overall competitiveness of the sterile males which could compromise their effectiveness in the field. Therefore, the studies in this CRP proposal aim to understand the various factors affecting dose-response in mosquitoes to standardize the irradiation processes to optimize male sterility and quality.

Moreover, the quality of the mosquitoes should be monitored with standard quality control (QC) procedures throughout the production process of the sterile males. The QC is necessary to both assess the efficacy and efficiency of mass-rearing and to predict the performance of those insects. There is a need to validate existing QC tests for both mass-rearing and field performance. Therefore, the studies in this CRP proposal aim to develop two types of QC indicators:

- Quick and efficient parameters that will be monitored routinely;
- More labour-intensive parameters that will be measured periodically over longer time intervals.

CO-ORDINATED RESEARCH PROJECT (CRP)

This Coordinated Research Project (CRP) is based on an expert group meeting on "Harmonization of Irradiation and Dosimetry Protocols for *Aedes* Invasive Mosquitoes" conducted in February 2018 and May 2019, Vienna, Austria.

The overall objective of this new **CRP D44004-CR-1**, approved for the **period 2020-2025**, is to understand irradiation induced effects, endogenous and exogenous factors that affect or improve dose-response, irradiation dosimetry procedures, impact of irradiation on vectorial capacity, impact of irradiation on cytoplasmic incompatibility and pathogen interference by *Wolbachia*, and to develop tools to assess quality of sterile male mosquitoes.

SECOND RESEARCH CO-ORDINATION MEETING (RCM)

16 scientists from 15 countries travelled to IAEA Headquarters to present their results from the past year, and 2 additional participants presented virtually, for the 2nd RCM in Vienna, Austria from July 18-22, 2022. The list of participants, which included CRP contract and agreement holders is given in **Annex 5** The agenda for the meeting is attached in **Annex 6**.

During the first two days of the RCM meeting, participants presented research relevant to the CRP, as well as their research plans for the following 18 months of the CRP.

During the last three days of the meeting, general discussions were held to define and review the thematic areas of the CRP, the review of the general and specific R&D objectives to be addressed in the next 18 months of the CRP. The Logical Framework was also reviewed to discuss the previously planned outputs of the CRP.

Abstracts of the presentations are compiled in **Annex 7** and a copy of all presentations were made available to all participants at the end of the RCM.

Workshop on irradiation methods and dosimetry

A workshop for both theory and hands-on training on irradiation methods and dosimetry took place for contract holders at the FAO/IAEA Laboratories in Seibersdorf on July 14 and 15. Presentations on irradiation and dosimetry basics, how to design an irradiation set-up according to the experiments, how to obtain a dose response curve, how to irradiate adults vs pupae, and the importance of dosimetry and how to calibrate and use Gafchromic films were accompanied by practical group sessions. The participants were familiarized with the gamma irradiators Foss Model 812, Gammacell220, and X-ray irradiators Raycell MK2, and Radsource RS2400, what the main differences are between the devices regarding dose rates and chamber geometry, and were shown how to use dosimetric films to verify absorbed dose, and to create a dose map. Participants learned how to use the Gafchromic system to read the films exposed on the previous day, and to obtain the absorbed dose values. The agenda is provided in **Annex 4**.

Participants of the Workshop on Irradiation methods and dosimetry, and the IPCL in Seibersdorf, Austria.

1. Irradiation & Sterilization

1.1 Background

Irradiation and dosimetry procedures

Standard dosimetry procedures using Gafchromic® film dosimeters for SIT are presented in **(IAEA, 2004)** and ISO standards are available for the use of radiochromic films (ISO/ASTM 2011) and dosimetry for SIT programmes (ISO/ASTM 2013). However, there is a need to develop standardized protocols for both irradiation and dosimetry procedures for human disease vectors.

Endogenous factors on irradiation efficacy

Life stage and age within each life stage are known to impact radiation sensitivity in many insect taxa, with radiation sensitivity decreasing with increasing developmental stages i.e. adults are less susceptible to radiation than larvae **(Cogburn, Tilton and Brower, 1973; Dey and Manna, 1983; Dongre *et al.*, 1997; Hallman and Thomas, 2010; Williamson, Mitchell and Seo, 1985)**. Similarly, it is well known that some insect taxa are more susceptible to sterilization by radiation than others **(Bakri *et al.*, 2005; Bakri, Mehta and Lance, 2005)**. For example, it takes much higher doses of radiation to sterilize moths than mosquitoes. It is not surprising that highly divergent insect taxa vary in their radiation sensitivities, but there is

some limited evidence for radiation susceptibility differing between populations **(Azizyan and Ter-Hovhannesyan, 2010)**. Terzian and Stahler **(Terzian and Stahler, 1966)** showed that radiation tolerance increases in eggs of the mosquito *Aedes aegypti*. Specifically, they showed substantially greater survival of mosquitoes after egg irradiation in a line selected over 35 generations compared to the unselected parental colony that had been in the laboratory for more than 20 years. Thus, we know there can be heritable variation for radiation susceptibility segregating even within long-standing laboratory colonies. Similarly, there is a substantial body of literature that shows naturally segregating genetic variation in radiation susceptibility within populations of the fly Drosophila melanogaster **(Enfield, North and Erickson, 1981; Westerman and Parsons, 1973)**.
In addition to genetic variation, there may be other types of heritable effects on radiation sensitivity in insects due to epigenetic changes or vertically transmitted microbes. Beyond intracellular bacteria that are clearly vertically inherited (see specific paragraph on *Wolbachia* below), other microbiota may impact radiosensitivity by modulating the immune system which warrants more research.

Exogenous factors on irradiation efficacy

Mass-rearing and diet (nutritional state) affects mosquito overall biological quality, and may also affect dose-response, or more likely the propensity to repair cellular/DNA damage during and following irradiation procedures. Handling procedures prior to- and during irradiation may also have an effect on the resulting sterile adult male biology. Handling procedures include variable factors such as irradiation medium (in water or air), or pupal densities inside the sample irradiated **(Yamada *et al.*, 2019)**.
It has been suggested that lowering the ambient temperature during irradiation treatments reduces radiosensitivity, by reducing the insects' metabolic rate **(Rananavare, Harwalkar and Rahalkar, 1991)**. This has yet to be assessed and confirmed for mosquito pupae, however maintaining consistency in all irradiation experiments in terms of temperature is good practice- not only for sterilizing pupae, but also for consistency and reliability in the dosimetry applied.
The oxygen levels, i.e. the atmospheric condition in which mosquitoes are subjected to before, and during radiation exposure can greatly influence the resulting induced sterility following irradiation, as is seen in other insects. Radiation effects are generally reduced in oxygen-poor environments (hypoxia) as compared to relatively oxygen-rich environments, or normoxia. Normoxia is defined as having roughly the same atmospheric oxygen content as would be expected in the local atmosphere at normal pressure and temperature, where normal temperature is 20°C and pressure is 1 atmosphere. Gaseous oxygen levels are often estimated as the partial pressure of oxygen relative to the other gases in the atmosphere, but for insects in regular air the field often approximates this as a percentage of oxygen relative to other gases in that air sample if at near normal pressure and temperature conditions. For example, the oxygen content of atmospheric air at normal pressure is often approximated as 21%. While a hypoxic condition is technically any atmosphere that is less than 21% oxygen, insects do not typically react to hypoxic conditions until oxygen levels reach less the 16% and hypoxia does not generally acutely affect insect growth, performance, and reproduction until oxygen content falls below 6% - termed severe hypoxia **(Harrison, Greenlee and Verberk, 2018)**. There is a substantial literature showing that exposure of insects to severe hypoxia prior to and during irradiation affects the insects response to radiation, including lower levels of induced sterility or mortality for a given dose

in severe hypoxia as well as occasionally improved post-irradiation performance of sterile males. Thus, the availability of oxygen to mosquito pupae and adults prior to and during the irradiation process should be carefully considered as a parameter that could affect the outcomes of the radiation treatment.
The effects of irradiation source, dose-rate and energy on dose-response in insects are not well understood. Their effects are often difficult to assess as researchers seldom have multiple irradiation devices to directly compare their sterilizing efficiency while controlling all (or most) other internal and external factors. Therefore, little data exists regarding this variable

Impact of male residual fertility on target populations
The impact of the potential introduction of colony genotypes and irradiation induced mutations into targeted populations through male residual fertility (e.g. possible effect on vectorial capacity) has not been studied. While it is known that optimal irradiation doses, designed to achieve the best combination of competitiveness and sterility, usually confer sub-sterility, no information is available on the traits of the produced progeny, which is relevant in the case of mosquitoes.

Impact of irradiation on vector capacity
When considering a mosquito release programme, one of the primary issues to be addressed is to the elimination of the females since females are blood feeders and therefore are potential disease vectors. However, current sex sorting techniques are not 100% efficient, and a small number of females are dispersed with males during releases. For this reason, the impact of irradiation on vectorial capacity needs to be assessed to determine at which level irradiated female mosquitoes can still transmit disease pathogens.
It has been shown that irradiated females of several species of mosquitoes did not produce eggs at doses lower than those required for male sterilization (for *Anopheles arabiensis*, **(Poda *et al.*, 2017)**; **(Dandalo *et al.*, 2017)**, *Aedes albopictus*, **(Balestrino *et al.*, 2010; Bond *et al.*, 2019; Yamada *et al.*, 2014**; Damiens personal communication), and *Ae. aegypti*, **(Bond *et al.*, 2019)** but no effect on blood feeding behaviour or survival in the laboratory has been observed for *Anopheles arabiensis* **(Dandalo, 2017)** and for *Aedes albopictus* **(Moretti *et al.*, 2021;** Damiens personal communication). However, for *Aedes aegypti*, a reduction of blood feeding and longevity has been observed **(Aldridge *et al.*, 2020)**. Further experiments are needed to study this potential impact of irradiation. A modification of blood feeding or survival will have a strong effect on vectorial capacity.
Moreover, direct effects of irradiation on vector competence should also be assessed to determine if irradiated female mosquitoes can transmit disease pathogens. In a trial conducted in Burkina Faso on *Anopheles arabiensis*, oocyst prevalence of *Plasmodium falciparum* was significantly reduced in irradiated females but oocyst intensity (mean number of oocyst in the midgut of infected females) was not affected by irradiation **(Guissou *et al.*, 2020)**.

Impact of irradiation and mass-rearing on cytoplasmic incompatibility and pathogen interference conferred by *Wolbachia*
The combined IIT/SIT approach has been proposed to minimize the possibility of unintended population replacement, in which low-dose irradiation is used to sterilize any residual females not removed from released male mosquitoes without affecting the latter's fitness

or mating performance **(Zheng *et al.*, 2019)**. In these combined IIT/SIT strategies, cytoplasmic incompatibility between Wolbachia-infected males from the colony with wild females not infected with the same Wolbachia strain plays the primary role in population suppression in the release program **(Zhang *et al.*, 2015)**. While some have suggested that IIT induced by *Wolbachia* or other vertically transferred microbes is sufficient as a mechanism for suppressing mosquito populations, the potential for accidental release of *Wolbachia*-infected females that could in turn cause the novel *Wolbachia* to be established in the environment is substantial when considering the release of millions of sterile male insects **(Zhang *et al.*, 2015)**. Thus, radiation treatments are often combined with *Wolbachia*-based IIT to sterilize the few females that are released during a large-scale operational program. In this case of IIT/SIT combined technique, the goal is to select doses that will completely sterilize females, but not necessarily sterilize males – although the additional partial sterilizing effects of radiation on males may also be useful for population suppression. Intracellular endosymbiotic bacteria in the genus *Wolbachia* are well known for their many physiological effects on insects, from inducing reproductive incompatibilities to altering vector competence for a number of viruses that can cause human diseases, among others **(Dutra *et al.*, 2016; van den Hurk *et al.*, 2012; Pan *et al.*, 2017**, etc.). To our knowledge, there are no published studies evaluating the extent to which infection with *Wolbachia* or the particular strain of *Wolbachia* used for infection may affect the dose of radiation needed to sterilize females. It is possible that some *Wolbachia* strains may have greater effects on radiation sensitivity than other strains due to the physiological mechanisms that they may induce. Furthermore, it is also possible that the effects of any particular *Wolbachia* strain on mosquito physiology may be dependent on the genetic background of the mosquito strain involved. *Wolbachia* infection has been clearly shown to affect mosquito physiology with respect to the immune system and vector competence for a wide range of viruses, thus there may be additional interactions between radiation dose responses, *Wolbachia* genetic background, and the genetic background of the mosquito colony that require further investigation.

The *Aedes albopictus* line ”HC” is superinfected with three different strains of *Wolbachia*: the original native double-infection involving wAlbA and wAlbB, and the transinfected wPip **(Zheng *et al.*, 2019)**. The wPip strain originated from the mosquito *Culex pipiens* and induces complete unidirectional cytoplasmic incompatibility (CI) when the triple-*Wolbachia*-infected HC males mate with wild-type *Ae. albopictus* females carrying only the native wAlbA and wAlbB double-infection. Given that IIT is based on CI conferred by *Wolbachia*, it is necessary to assess the impact of irradiation or mass-rearing on cytoplasmic incompatibility. Longitudinal monitoring every three months indicated no impact of mass-rearing on cytoplasmic incompatibility in *Aedes albopictus* HC line on CI (Y. Wu, pers. com.). wPip in HC mosquitoes inhibited replication of both Zika and dengue virus and blocked both horizontal and vertical transmission of Zika virus, indicating its ability to generate *Wolbachia*-mediated viral blocking. Data of annual monitoring indicate no impact of mass-rearing on pathogen interference in the *Aedes albopictus* HC line after 4 years of monitoring (Y. Wu, pers. com.). However, there is evidence of an impact of radiation on the density of *Wolbachia* in tsetse **(Demirbas-Uzel *et al.*, 2018)**. On the contrary, it did not modify *Wolbachia* density or pathogen interference in the *Aedes albopictus* line ”HC”**(Li *et al.*, 2021)**. Moreover, there is evidence that wMel density in the adults of the *Ae. aegypti* line declined when eggs were held at 26-36°C or above with complete loss at 30-40°C **(Ross *et al.*, 2019)** and no pathogen

interference against Zika & Dengue 2 was observed in the *Ae. aegypti* line used in Singapore (C.H. Tan, pers. Com.). There is therefore a need to better characterize the impact of irradiation and mass-rearing on pathogen interference and CI conferred by *Wolbachia*.

1.1 Preliminary results

In the last years, and due to the improvement of X-ray tubes, more and more X-ray irradiators, in particular those designed for blood irradiation are becoming attractive options as alternatives to self-contained gamma irradiators, as dose rates, dose uniformity, and processing capacity are meeting the technical requirements for mosquito sterilization. Following the characterization of the Raycell MK2 blood X-ray irradiator, and the use of the Cegelec blood X-ray machine in an active SIT project, other blood X-ray devices are being considered such as the Gilardoni Radgil2. This device will be assessed for its suitability for mosquito sterilization in the frame of this CRP.

Finding alternative solutions for panoramic irradiators is also of interest for large operational SIT programmes with high insect throughput, especially those programmes with automated, conveyor belt systems for efficient irradiation processing. Here, e-beam technology may offer a good solution. However, the use of e-beams for insect sterilization is still in its R&D stage and many parameters still need thorough assessment for the development of standardized protocols. E-beam energy and related penetration depth as well as dose-rates and handling of the various e-beam devices need consideration when processing mosquitoes. An addition, the biological effects of energy (independent of dose rates) still need to be elucidated. First studies using a 10MeV industrial e-beam device has shown to successfully irradiate up to 500 million fruit flies per week **(Plá *et al.*, 2021)**. and has shown promising results and application potential for mosquito sterilization. Electrons are highly efficient in terms of power efficiency, whereas X-ray conversion will cause a loss of more than 90% of heating power in cooling water, which is an important aspect in terms of sustainability. Once sample thickness and density is configured, lower energy e-beam may become a more suitable and economical option.

Following the series of experiments to identify factors in mosquito irradiation that may affect dose-response in terms of sterility **(Yamada *et al.*, 2022, 2019)**, additional variables have been investigated to further standardize irradiation procedures such as pupae density dependent hypoxia, where it was found that increasing pupal load size decreased the overall average sterility levels, indicating the increased hypoxia levels occur within the load during radiation exposure. Contrarily, water temperature during irradiation did not affect the dose response in pupae. Preparation methods of pupa samples and irradiation media are important for achieving target sterility, as irradiation in water does not produce the same results as irradiation in air. The age of adult mosquitoes and the geographic origin of mosquito strains seem to have little or no effect in the studies performed thus far. Although a recent publication reported that Drosophila strains that are reared in underground laboratories, may develop different inherent radiosensitivity, possibly due to the lack/presence of background environmental and cosmic radiation levels **(Porrazzo *et al.*, 2022)**. It is now known that dose and dose-rate have a complex interaction and can affect dose response in insects. It has also been shown that irradiating mosquitoes with Gamma-rays and X-rays can result in different sterility levels although the same dose is applied. Further tests are needed to assess whether this results from different dose rates or energies.

The effect of irradiation on female mosquitoes is important as changes in physiology could affect their host seeking, blood feeding, and flight behaviour, and additionally, it may affect vector competence. As no perfect sexing system exists for mosquitoes to date, the release of some females together with the sterile males is inevitable. These females will have been irradiated at the male-sterilizing dose, which significantly exceeds female sterilizing doses. Two recent studies from Italy and France (La reunion) have shown that irradiation did not increase the transmission of CHIKV and DENV in both Ae. aegypti and Ae. albopictus females but did increase dissemination of CHIKV in the mosquito body in both species. Additional experiments from Thailand, Singapore and China have confirmed that irradiation did not increase vector competence in irradiated females. Other studies investigating the effects of irradiation on female vector capacity have presented varying results, depending on the protocols used. However, irradiation was generally found to alter blood feeding rates, longevity and flight ability in contradictory ways. Further experiments with harmonized protocols are still needed to fully understand these effects in sterile females, and to evaluate the implications in the field.
In SIT/IIT combined strategy, the impact of irradiation on Wolbachia densities is an important topic. Recent studies have found that doses up to 45Gy did not reduce Wolbachia densities in *Ae. albopictus*.

1.1 Individual Plans according to topics

Sub-topics:	Researchers	main interests
Irradiator qualification	**Florent Kuntz**	**dosimetry; irradiators**
	Patricio Ponce	***mosquito organ depth, sperm production/age***
	Hadian Sasmita	***dosimetry; SOP e-beam***
	Carlos Tur Lahiguera	***dosimetry; e-beam***
	Romeo Bellini	*evaluation of Radgil2 (X-ray)*
Factors affecting dose-response ***of X-ray, gamma-ray & ebeam***	**Dan Hahn**	***hypoxia; stress factors***
	Carlos Tur Lahiguera	*various factors*
	Hadian Sasmita	*dose-rate; geographical origin*
	Glenda Obra	***various factors, comparison***
	Romeo Bellini	***pupae densities***
	Diana Iyaloo	*strain origin; Cs^{137} vs Co^{60}, pupae density & water temp*
	Louis Clement Gouagna	*endo. exo. factors*

	Jair Virginio	***pupae densities (atm); adult/pupae***
	Kajla Seheli	***dose rate, water temp, anoxia***

Effects of irradiation on vector competence

	Ariane Dor	*transm. risk released females*
	Simon Sawadogo	*irrad. An. arab. & coluzzi*
	Pattamaporn Kittayapong	*Ae. aegypti*

Effect of irradiation on symbionts	**Pattamaporn Kittayapong**	***Ae. aegypti***

2. Quality Control

2.1 Background

Quality control methods for mosquito SIT

The term “Quality Control” (QC) in insect mass-rearing refers to the methods to assess and ensure the ability of produced sterile males to compete successfully with wild conspecifics to mate fertile females **(Boller *et al.*, 1981)**. The main objective of quality measurement is to detect any significant change in the produced insects, providing a rapid diagnosis so that the production process can be amended and ensure that sterile males will perform successfully after release.

Quality control in a mass-rearing facility needs to be divided into three basic processes: 1) quality control of the production, 2) quality control of the process and 3) quality control of the product. It is thus necessary to determine the correct size of the samples of eggs, larvae, pupae and adults, as well the number of repetitions (FAO/IAEA/USDA, 2014). The mass-rearing process must be monitored continuously to verify that each development stage meets the established requirements **(Calkins and Parker, 2005; Hernandez *et al.*, 2010**, see Figure 1).

As mosquitoes are maintained in artificial rearing conditions and are submitted to strong selection during colonization to increase yields, behavioral modifications may produce some mating incompatibility between mass-reared males and wild females **(Rull, Brunel and Mendez. M.E, 2005)**.

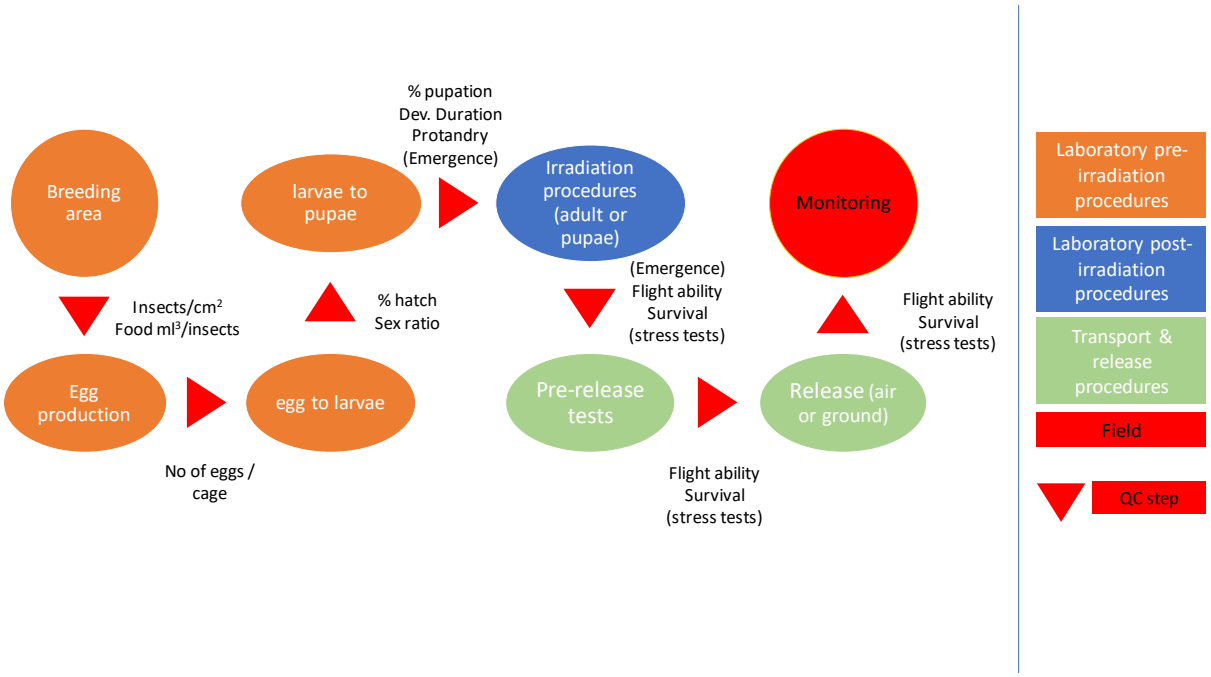

Fig. 1 Examples of quality control monitoring along the production chain of sterile male mosquitoes (Adapted from (FAO/IAEA/USDA, 2014))

Several quality-control tests have been developed and are regularly used along the production-release process **(Balestrino *et al.*, 2017; Culbert *et al.*, 2018**; **FAO/IAEA/USDA. 2014**). Most of these methods identified problems during rearing, irradiation, transport, holding, chilling, and release of the sterile males. Also, some field cage quality-control tests have been developed to evaluate male mating performance **(Rull *et al.*, 2012)**.
The procedures for releasing sterile males will generally be performed using the chilled adult technique, which immobilize the insects using low temperatures (6 to 10 ° C), which can also have a significant effect on the quality of sterile insects. For this reason, every batch of sterile males need to be monitored at each step of the production process, in order to detect any problem related with the final quality of the released insects **(Arredondo *et al.*, 2016)**.
Moreover, some more complex tests which are costly and time consuming need to be conducted at regular intervals, e.g. competitiveness tests in semi-field settings (Bellini et al. 2013).

2.2 Preliminary results

Unlike SIT programmes against other insect pests with a long history of implementation and success, the SIT package for mosquitoes has yet to develop a variety of QC tests to assess the produced sterile males before their release, and their performance once in the field. The few available methods to assess competitiveness and field performance are tedious and time consuming such as field cage competitiveness tests to determine Fried's competitiveness index, or MRR studies in the open field. Prototypes of the FAO/IAEA flight test device (FTD) were developed several years ago, however it was not until recently that the FTD design, as well as the protocols for its use were improved and standardized. A link between escape rates and competitiveness is being investigated.
Other QC tests based on escape rates from various containers have been developed independently and have shown to be good indicators for general quality, such as the adult release cup (in Greece, Brazil and La Reunion) and the stress-escape cup (Spain), where sterile males are subjected to additional stress (deprivation of sugar) before allowed to attempt escape.
Pupal size (or weight) has been an indicator of product quality in mosquitoes, as well as other insects. Large pupae indicate good rearing practices and homogeneity of larval development and the storage of teneral reserves, which in turn suggests good wing length, good survivorship and energy for flight ability, and high fecundity in females. Pupal color is also an indicator for age, and these two indicators may be evaluated as process QC parameters in SIT programmes.

Temperature in general is an important factor that can influence a variety of mosquito QC parameters. Temperature is relevant during egg hatching, larval rearing, pupation time, pupation rates and emergence to adulthood, swarming/mating activities etc. Exposures to cold temperatures can stress or protect from stress depending on the temperature and exposure duration, and is an important part of handling, irradiation, packing, transportation, release strategies. It has been shown that chilling (7C) can be slightly radioprotective, probably due to the reduced metabolic rates in the mosquito while being subjected to greater stress factors. A greater radioprotectant that has significant effects in dose response is hypoxia (in pupae irradiated in water, and anoxia in adults irradiated in nitrogen). Three

species of mosquitoes (*An. arabiensis, Ae. albopictus* and *Ae. aegypti*) showed up to 30% reduction in induced sterility when irradiated in low oxygen environments as compared to irradiation in air with the same dose.
Another stress factor during packing, transportation and release is the density at which the adults are packed. Marking methods, for example when excessive quantities of commonly used fluorescent dusts are applied, and some colors (e.g. blue) has also been shown to reduce flight ability and survival, and thus likely the overall performance in the field. Outdoor, or simulated outdoor conditions can allow lab reared males to acclimate to field climate conditions and achieve similar cold hardiness to wild mosquito populations and thus improve their performance once released, in particular in colder seasons. This data has important implications for application in the SIT against mosquitoes, especially in regions with cooler seasons.

Another instance in which preconditioning mosquitoes to a stress factor can induce hormesis, and maintain quality when re-exposed to the same stressor is dose fractionation. Adults exposed to an acute, fully sterilizing dose showed reductions in longevity, and mating competitiveness compared to those irradiated with 2 fractionated doses, despite the double handling needed for 2 exposures. However, the savings in quality reductions may not justify the costs in process efficiency.

2.3 Individual Plans according to topics

Sub-topics:	Researchers	main interests
Product Quality control	Carlos Tur Lahiguera	*product QC*
	Ariane Dor	***estab. baseline QC param.; novel tools***
	Thierno Bakhoum	***QC test validation, product QC***
	Diana Iyaloo	***QC test validation***
	Antonios Michailakis	***novel QC tests, product QC***
	Glenda Obra	*QC test validation*
	Simon Sawadogo	***An. arab. Swarming dynamics; optimize use of rhod.B for mating tests***
	Zhiyong Xi	***product QC***
	Louis Clement Gouagna	***product QC (MRR)***
	Romeo Bellini	*product QC (MRR)*
	Patricio Ponce	*product QC*
	Jair Virginio	*product QC*

Process quality control

Carlos Tur Lahiguera	*automated systems; process e-beam*
Ariane Dor	*novel tools*
Hadian Sasmita	*process e-beam irrad*
Michael Samuel	***irrad en masse Anoph.***
Kajla Seheli	*irrad. en masse; UPSCALING PROCESS*
Louis Clement Gouagna	*irrad protocols*

Factors affecting downstream sterile male quality

Ariane Dor	*effects of chilling*
Michael Samuel	*handling/irrad. methods*
Dan Hahn	*cold tolerance*
Heath MacMillan	***cold tolerance***
Simon Sawadogo	*temp, dose on dispersal & comp; swarming*
Glenda Obra	*irrad effects on dispersal*
Zhiyong Xi	*factors improve irrad. outcome for females*

3. Logical Framework

Logical Framework (table):

Narrative Summary	*Objective Verifiable Indicators*	*Means of Verification*	*Important Assumptions*
Overall Objective **develop and evaluate irradiation and quality control procedures to be used for sterile insect technique (SIT) applications, as part of AW-IPM programmes, to control populations of mosquitoes, vectors of human diseases**	N/A	N/A	Requests by Member States in the area of mosquito control using the SIT are increasing. To transfer this nuclear technology to Member States, the availability of irradiation and quality control procedures at large scale is an essential precondition. Biological material is available.

Specific Objectives			
1. Understand the factors that affect sterilization by irradiation and downstream performance of the sterile male mosquitoes	At least three specific factors affecting irradiation described	Reports and published papers.	Specific factors affecting irradiation can be identified.
2. Design and validate irradiation and dosimetry protocols for large numbers of mosquitoes, appropriate for operational programmes	Protocol for irradiation and dosimetry developed.	Reports and protocols.	Irradiating large numbers of mosquitoes without impacting their quality is possible.
3. **Develop and validate standard product QC procedures for sterile male mosquitoes**	At least three QC tests developed and validated.	Reports and or published papers.	QC tests allowing fast, reproducible and cheap evaluation can be developed and adopted by MSs.

Outcomes **1. Major drivers of variation in the efficacy of sterilization by irradiation identified and integrated into MSs protocols**	Protocols adopted	Data collected	Protocols can be integrated into MS production systems.
2. Guidelines for large-scale irradiation adopted and implemented	Guidelines developed	Data collected	Guidelines can be adopted by MSs.
3. Guidelines for QC adopted and implemented within operational SIT mosquito programmes	Guidelines developed	Data collected	Guidelines can be adopted by MSs.

Outputs **1. Data on the impact of endogenous factors on irradiation efficacy including physiological and cellular processes available**	At least two endogenous factors impacting irradiation efficacy identified	Reports and or published papers	Biological material is available. Methods are available or can be applied to mosquitoes.
2. Data on the impact of exogenous factors on irradiation efficacy available characterized	At least two exogenous factors impacting irradiation efficacy identified	Reports and or published papers	Biological material is available. Methods are available or can be developed
3. Impact of the potential introduction of colony genotypes and irradiation induced mutations into targeted populations through residual fertility quantified	Frequency of transfer of mutations quantified	Reports and or published papers	Methods are available or can be developed
4. Impact of irradiation on vectorial capacity including female behaviour, vector competence and longevity evaluated	At least two traits related to vectorial capacity characterized	Reports and or published papers	Laboratory with appropriate level of biosafety for vector competence studies available.
5. Impact of irradiation on cytoplasmic incompatibility and pathogen interference conferred by Wolbachia quantified	At least two Wolbachia strains assessed One procedure developed	Reports and or published papers	Biological material available. Laboratory with appropriate level of biosafety for vector competence studies available.
6. Irradiation and dosimetry procedures for SIT applications harmonized	Impact of irradiation compared to at least two production steps	Protocols published	MSs will adopt irradiation and dosimetry protocols
7. Impact of irradiation relative to other production steps on the quality of male mosquitoes evaluated	(measure impact of production steps & irradiation on mosquito quality)	Reports and published papers	QC protocols are available or can be developed
8. Quality control methods	At least two quality control tests for the	Reports and published papers	QC protocols can be developed

to be applied to monitor the mosquito production process developed and validated **9. Quality control methods to be applied to monitor sterile male performance developed and validated** **10. Results published in a peer reviewed journal**	production process validated At least two quality control tests for sterile males performance validated Papers drafted and submitted.	Reports and published papers Journal issue with published scientific papers.	QC protocols can be developed Data for publication available

Activities			
1. Announce project amongst established entomologists working on vectors and establish CRP	Proposals evaluated and 10 Research Contracts, 10 Research Agreements and 1 Technical Contract awarded.	Signed contract and agreements.	Suitable proposals submitted, funding available and approval of Contract and Agreements by CCRA-NA committee.
2. Organize first RCM to refine the logical framework and plan the overall activities of the CRP (3Q 2020)	1st RCM held 2021.	Participants' activities and logical framework revised.	Contracts and Agreements signed by counterpart organizations.
3. Conduct Research and Development	New knowledge created on irradiation and QC of mosquitoes	Scientific papers and reports from the participants	Methods and resources available.
4. Organize second RCM to analyse progress in delivering research outputs and plan the next phase of the project (1Q, 2022).	2nd RCM held 2022. (Q3, in Vienna)	Participants and RCM Progress Reports.	Progress satisfactory.
5. Organize a training on irradiation and quality control in conjunction with the second RCM	Training held in 2022 (2-3 day workshop, before the 2.RCM)	Training report	Suitable location to held the training and training material available
6. Conduct Research and Development	New knowledge created on irradiation and QC of mosquitoes	Scientific papers and reports from the participants	Methods and resources available.
7. Review the CRP after its third year	Satisfactory progress of research agreements and technical contract	Report	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
8. Organize third RCM to analyse progress in delivering the research outputs and plan the final phase of the project. (3Q, 2023)	3rd RCM to be held 2023.	Participants and RCM Progress Reports.	Progress satisfactory and mid-CRP evaluation approved by CCRA-NA committee.

9. Conduct Research and Development	New knowledge created on irradiation and QC of mosquitoes	Scientific papers and reports from the participants	Methods and resources available.
10. Organize final RCM to assess the success of the CRP in reaching its objectives and review the final publication. (1Q, 2025)	4th RCM to be held 2025.	Participants and RCM Final Reports	Final reports are submitted to the Agency.
11. Evaluate the CRP and submit evaluation report.	Satisfactory completion of research agreements and technical contract	Report	Contracts and Agreements properly managed by counterpart organizations. Methods and resources available.
12. Publish the results of the CRP in a special issue of a peer reviewed journal.	At least 20 publications accepted.	Scientific publications.	Consensus can be found on appropriate peer review journal and acceptance by journal obtained.

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ANNEX 1: List of participants of the 1st RCM

D44004-CR-1
First Research Coordination Meeting on Mosquito Irradiation, Sterilization and Quality Control
Seibersdorf, Austria
31 May to 4 June 2021

List of Participants
(as of 2021-05-06)

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ANNEX 2: Agenda of the 1st RCM

First FAO/IAEA Research Co-ordination Meeting
"Mosquito Irradiation, Sterilization and Quality Control"
May 31 – June 4, 2021

Vienna, Austria – Virtual

Monday May 31, 2021

Day 1: The Americas (time zone)

Webex session open at 14:00 CET (Vienna time)

CET (local time for presenter)

14.30 – 15.00 OPENING REMARKS

15.00 – 15:30 **Hanano Yamada & Hamidou Maiga**

Irradiation studies & Quality Control methods and tools at the IPCL

15.30 – 16.00 (10.30 – 11.00) **Jair Virginio** (Brazil, -5)

Adult mosquito irradiation at Moscamed Brasil

16.00 – 16.30 (10.00 – 11.30) **Dan Hahn** (USA, FL, -6)

Thinking about hypoxia and temperature treatments for improving sterile male performance in the field.

16.30 – 17.00 (10.30– 11.00) **Heath MacMillan** (Canada, (Ottawa) -6)

Can we improve the field performance of SIT Aedes aegypti through assisted thermal acclimation?

17.00 – 17.30 (10.00 – 10.30) **Patricio Ponce** (Ecuador, -7)

Irradiation assays for Aedes aegypti from Ecuador

17.30 – 18.00 (10.30 – 11.00) **Ariane Dor** (Mexico, -7)

Development and evaluation of quality control methods for the application of the SIT in Aedes aegypti

BREAK 10 min

1 hour Discussion

Tuesday June 1, 2021

Day 2: **Europe & Africa (time zones)**

Webex session open at 12:00 CET (Vienna time)

CET (local time for presenter)

12.45 – 13.00 OPENING REMARKS

13.00 – 13.30 (15.00 – 15.30) **Ambicadutt Bheecarry** (Mauritius, +2)

Optimizing the quality of sterile Ae. albopictus males released as part of a Sterile Insect Technique (SIT) feasibility study in Mauritius

13.30 – 14.00 (13.30 – 14.00) **Louis Clement Gouagna** (France)

Standardization of irradiation process of Aedes albopictus males under mass-rearing conditions in support of SIT program in La Reunion Island

14.00 – 14.30 (15.00 – 15.30) **Antonios Michailakis** (Greece, +1)

Testing quality control methods for irradiated Aedes albopictus males

14.30 – 15.00 **Florent Kuntz** (France)

Dosimetry challenges and irradiation modality effect on mosquito

15.00 – 15.30 **David Almenar** (Spain)

Development of protocols and materials for medium-large scale sterilisation by ionizing irradiation and electron-beam technology, and Quality Control of Aedes mosquitoes

15.30 – 16.00 **Romeo Bellini** (Italy)

Scenarios for the application of SIT on Aedes invasive species in Europe

BREAK

16.30 – 17.00 **Michael Samuel** (South Africa)

Optimizing bulk irradiation of mass-reared Anopheles arabiensis males in prospective of a pilot sterile male release programme in South Africa

17.00 – 17.30 (14.30 – 15.00) **Mame Thierno Bakhoum** (Senegal, -2)

Quality control procedures of Aedes aegypti sterile males to ensure effective area-wide integrated management programmes

17.30 – 18.00 (15.30 – 16.00) **Simon Sawadogo** (Burkina Faso, -2)

Evaluating the influence of environmental factors on the swarming and mating competitiveness of sterile males of Anopheles arabiensis and the irradiation dosage on the vector competence of sterile females under semi-field and field conditions

1 hour Discussion

Wednesday June 2, 2021

Day 3: Asia (time zones)

Webex session open at 08:00 CET

CET (local time for presenter)

08.45 – 09.00 OPENING REMARKS

09.00 – 09.30 (15.00 – 15.30) **Glenda Obra** (Philippines, +6)

Irradiation, Sterilization and Quality Control of Dengue Mosquito, Aedes aegypti in the Philippines

09.30 – 10.00 (15.30 – 16.00) **Yongjun Li** (China, +6).

Quality control of long-term mass-reared Aedes albopictus for suppression

10.00 – 10.30 (15.00 – 15.30) **Hadian Sasmita** (Indonesia, +5)

Exploring the critical factors influencing the radiation sensitivity in irradiated males Aedes aegypti

10.30 – 11.00 (15.30 – 16.00) ~~**Pattamaporn Kittayapong**~~ (Thailand, +5)

Title to be provided

11.00 – 11.30 (15.00 – 15.30) **Kajla Seheli** (Bangladesh, +4)

Optimization of irradiation dose and quality assurance of sterile male Aedes aegypti: A laboratory study

BREAK

1 hour Discussion

Thursday, 3 June 2021

Day 4**:**

Group discussions: (re)defining R&D workplan and goals: **open sessions** (*use the same link*)

09.00-10.00 Vienna time: Matching & optimizing irradiator and dosimetry systems. Discussion and Q&A time with Florent on getting our dosimetry right!

10.00-12.00 Vienna time (see table on page 5 for your local time) Europe, Africa & Asia

15:00-17.00 Vienna time (see table on page 5 for your local time) The Americas (& Europe)

Revision of the Logical matrix framework (to be edited on the document)

ANY TIME Other sub-group discussion, coordination & collaborations on specific activities, or One-on-one discussions on data/protocols/planned activities/etc (let me know by email if you wish to meet and when)

Final report- completion of all sections

Friday, 4 June 2021

Day 5**:**

Open sessions

10.00-12.00 Vienna time (see table on page 5 for your local time) Europe, Africa & Asia

15:00-17.00 Vienna time (see table on page 5 for your local time) The Americas (& Europe)

Final report compiled and completed

ANNEX 3: Abstracts of the 1st RCM

Optimizing the quality of sterile *Ae. albopictus* males released as part of a Sterile Insect Technique (SIT) feasibility study in Mauritius

Ambicadutt Bheecarry, Diana P. Iyaloo, Khouaildi B. Elahee, Varina Ramdonee-Mosawa, Nabiihah R. Munglee, Nilesh Latchooman, Srutee Ramprosand, Surendra Puryag

Vector Biology and Control Division, Ministry of Health and Wellness, Mauritius

When the national project MAR 5019 (aiming to investigate the use of the Sterile Insect Technique to control *Aedes albopictus* in Mauritius) was implemented; several areas for improvements were identified. Some of those areas are enumerated below and will be addressed during this CRP in Mauritius.

(1) Although the sterility of released males were assessed throughout MAR 5019, a quality control system to accurately monitor male fitness was lacking. Hence, building from the work started at the IPCL, male performance will be assessed in the laboratory, semi-field and field conditions and the results compared and evaluated with the aim of developing a quick and effective quality control system for sterile males.

(2) During irradiation studies conducted in a cesium-137 irradiator, the density of pupae at irradiation significantly impacted their level of sterility and pupae had to be irradiated at a density not exceeding 1500 per petri dish for an effective sterilization. Since gamma rays can only be effectively attenuated by very dense materials such as lead, it is unclear how pupal density could have impacted the level of radiation dose received. The main hypothesis is an increasing level of hypoxia with density, thus conferring increasing resistance to irradiation. This will be investigated during this CRP.

(3) In an operational sterile release programme, it is essential that a compaction system for sterile *Ae. albopictus* males be developed to ensure that males are transported in a practical, cost-effective way with reduced effects on their competitiveness. Preliminary works have started at the VBCD to develop optimized protocols for the irradiation of *Ae. albopictus* males in the pupal and adult stages and to compare male fitness using both protocols. Furthermore, the effect of dose-rate and energy of the rays on the sterility and performance of the males using two different type of gamma irradiators (Cobalt-60 and Cesium-137) will also be investigated.

(4) Finally, if travel restriction becomes more relaxed, the impact of irradiation on different strains of *Ae. albopictus* (originating from two dependencies of Mauritius), will be investigated.

Quality control procedures of *Aedes aegypti* sterile males to ensure effective area-wide integrated management programmes

M. T. Bakhoum[1], G. Diouf[1], A. G. Fall[1] and M.T. Seck[1]

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Although the sterile insect technique (SIT) remains the most effective strategy used to control insect pests, its application in area-wide integrated pest management (AW-IPM) programmes continues to increase in response to requests from the Member States. These requests include the development and refinement of the SIT packages for programmes to control mosquito populations. The successful implementation of area-wide integrated pest management, with an SIT component against these vectors, depends on the efficiency of irradiated sterile males to induce sterility in the target populations. Many factors from the sterile male production of mosquitoes to their release in the field determine the quality of released males and ultimately affect their performance in the field. The challenge is thus to perform adequate quality control procedures to monitor the quality of the sterile males of mosquito along the production chain and release, to ensure the effectiveness of implementing area-wide integrated pest management (AW-IPM) programmes. The main objective is to develop and evaluate adequate quality control procedures to be used for sterile insect technique (SIT) applications to control populations of *Aedes aegypti*. In this project, based in Senegal, we propose to assess the impact of the irradiation on the evolutionary response of *Aedes aegypti* populations in testing the flight ability and the competitiveness of *Aedes aegypti* males irradiated in the different stages (adult and pupae) under semi-field cages and field trial, including their survival and dispersal.
For the next 18 months, we will (1) assess the impact of dose responses in *Aedes aegypti* males irradiated as pupae and adult on the flight ability of irradiated males, and (2) evaluate the competitiveness of *Aedes aegypti* males irradiated in adult and pupae stages using doses giving induced sterility (IS) >99% under semi-field conditions.

Testing quality control methods for irradiated *Aedes albopictus* males

G. Mastronikolos[1], G. Balatsos[2], V. Karras[2], D. Papachristos[2], A. Kapranas[2], C.S. Ioannou[1], P. Milonas[2], Romeo Bellini[3], Arianna Puggioli[3], N. Papadopoulos[1] and A. Michaelakis[2]

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The evaluation of this sterile insect technique (SIT) for Greece has already begun since the autumn 2018 in the area of Vravrona (Municipality of Markopoulo) against the *Aedes albopictus.* For the SIT trials irradiated males are transported from the CAA facility (Italy) to the Benaki Phytopathological Institute in Athens by air express carrier. In order to conduct the SIT pilot program, adults were sent every week in chilled foam boxes. Under the frame of the current CRP program (CRP code: D44004), we assessed several experimental procedures trying to investigate different parameters that might indicate the quality of the transported sterile males. Initially, the residual fertility of Greek *Aedes albopictus* strain (Vravrona colony) was evaluated and compared with other European strains. The quality characteristics of the sterile males of *Ae. albopictus* Vravrona colony in comparison with those of the wildish males of a recently established laboratory colony. Specifically, we evaluated the effect of mating, food and water stress on male survival, Effect of mating on male longevity and the male mating performance (mating propensity and male mating competitiveness). The results showed that the quality importance of the transported sterile males was very good. As a next step we are planning to implement a Mark-Release-Recapture studies on the dispersal capacity and post-capture longevity of irradiated of the *Ae. albopictus*. We will also try to include in MRR studies non-irradiated males of the Asian tiger mosquito aiming to record the (remaining) lifespan of the recaptured individuals maintained under laboratory conditions to clarify how their exposure to natural settings shape their longevity.

Standardization of irradiation process of *Aedes albopictus* males under mass-rearing conditions in support of SIT program in La Reunion Island

Lucie MARQUEREAU, Brice DEREPAS, Antonin LECLERCQ, Louis Clement GOUAGNA

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Abstract. Under the phase 2 of the R&D program to study the feasibility of the Sterile Insect Technique (SIT) for the control of *Aedes albopictus* on Reunion Island, one of the main challenge is to upscale the mass rearing as well as to optimize the irradiation system for large number of mosquitoes. The factors influencing the sterility level of male *Aedes albopictus* exposed to ionising irradiation are complex and include both endogenous parameters, i.e. developmental stages, duration of rearing in the lab, geographic origin of mosquito strains, and exogenous factors such as the bulk number of mosquitoes to be irradiated, holding and exposure conditions. Early experiments suggested that 35-40Gy from gamma and X-ray sources is the most appropriate dose for implementing SIT for the control of *Aedes albopictus* on the Island. This presentation will provide an update of further radio-sterilization studies carried out in an ongoing SIT feasibility program, where we used varying number of *Aedes albopictus* male pupae (c.a. 2000 – 14000 pupae per irradiation cycle) derived from a mass-rearing system, and test different conditioning atmospheres in order to establish optimal conditions for the mass-production of sterile males, beside reducing the level of residual fertility and maintaining sterile male quality. Though our objectives have been to identify the dose of X-ray radiation and irradiation conditions that would fully sterilize male pupae under mass-rearing conditions, the accidental release of sub-sterile male mosquitoes and residual females in an SIT program is possible, and likely has some associated potential ecological and health risks. Further studies are planned (1) to examine the fitness of male and female offspring originating from sub-sterile male parents x fertile female crosses, (2) to document the effects of releasing semi-sterile irradiated male *Aedes albopictus* on the target population gene pool, and (3) to investigate the response of sterile female *Aedes albopictus* to arbovirus infection. The implication of such studies for mass release of sterile mosquitoes in SIT program will be discussed.

Exploring the critical factors influencing the radiation sensitivity in irradiated males *Aedes aegypti*

Hadian I. Sasmita[1], Beni Ernawan[1] and Intan Ahmad[2]

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Several factors within the radiation sterilization process are linked to the radiation sensitivity of males *Aedes aegypti*. Those factors could come from the environmental conditions and physics during the irradiation process, such as atmosphere, dose rate, and temperature; and/or biological conditions including stage, age, size & weight, diapause, and nutritional state. Exploring those factors is critically important to improve or, at least, to maintain the quality of irradiated males, leveling their competitors in the field. The studies exploring the listed factors should receive more attention to develop consistent, reproducible, and reliable irradiation procedures as one of determining factors in successful SIT application against *Ae. aegypti*. Among the above-mentioned factors, we have been investigating life stage, age and temperature factors on irradiation process conducted in Gamma-cell 220 applying varied doses. Biological parameters related to males' quality have been characterized for each dose and factor. Briefly, males' longevity, induced sterility level and mating competitiveness index were affected by dose received, life stage and age. Higher temperature applied prior to the irradiation resulted in higher induced sterility level and lower longevity. Further works within the scopes of CRP will be investigating acute and chronic irradiation using different type of gamma irradiators; different levels of oxygen atmosphere before and during irradiation; and different origins of the species. In order to do those works, we have been preparing the two irradiators with appropriate dosimetry system; mastering the technique to create hypoxia and normoxia conditions; as well as collecting *Ae. aegypti* field strains from the different origins of Indonesia.

Simultaneous application of MAT and SIT for management of B*actrocera dorsalis* in South Africa: environmental and physiological considerations

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Males of many *Bactrocera* Macquart (Diptera: Tephritidae) species are attracted to one of a number of semiochemical compounds referred to as phenylpropanoids. Due to their attractiveness, some of these chemicals, including methyl eugenol, are highly effective lures in traps that are also used for "male annihilation technique" (MAT). MAT can be used prior to sterile insect technique (SIT) programmes targeting *Bactrocera* species to reduce the abundance of wild males, thereby improving the sterile:wild male ratio. This sequential application of MAT followed by SIT was believed necessary to prevent attraction of released sterile males to MAT baits. However, it has been proposed that MAT and SIT may be applied simultaneously because prior exposure to a phenylpropanoid reduces subsequent male attraction to the same or a different chemical. An issue that needs to be addressed is whether pre-release nutritional treatments supported by joint FAO/IAEA-funded research are also compatible with simultaneous application of MAT and SIT. This is because sterile male *Bactrocera* fed yeast hydrolysate to improve their survival and mating performance respond more strongly to phenylpropanoid lures. This project, based in South Africa, will investigate some of the environmental and physiological variables that may interact with semiochemical pre-treatment to influence response of *Bactrocera dorsalis* (Hendel) to methyl eugenol. Specifically, we will: (1) Identify alternative, affordable and readily available semiochemicals for pre-release treatment of *B. dorsalis* that reduce responsiveness to traps baited with methyl eugenol; (2) Establish the responsiveness of *B. dorsalis* to traps baited with methyl eugenol when pre-treated with selected semiochemicals and nutritional supplements under varying semi-field conditions; (3) Determine whether pre-release treatment of *B. dorsalis* with selected semiochemicals and nutritional supplements affects flight, dispersal and metabolic rate; and (4) Document the field response to methyl eugenol by sterile *B. dorsalis* receiving pre-release treatments relative to untreated and wild males.

Development and evaluation of quality control methods for the application of the SIT in *Aedes aegypti*

Ariane Dor[1], Génesis Sánchez[2], Carlos F. Marina[3], J. Guillermo Bond[3] and Pablo Liedo[3]

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In southern Mexico, a pilot project on *Aedes aegypti* Sterile Insect Technique is currently performed with a Genetically Diverse Strain (GDS). Initially, mosquitoes were chilled and manually released by ground; after that, they were released by air using a drone, involving an additional chilling step in the process. Because of the COVID-19 pandemic, releases have been stopped since March 2020, as well as the production of the mosquito's strain, making impossible to achieve any planned research. Nevertheless, in 2021, part of the specific objective 1 (to establish the baseline of the quality parameters of the mass-reared GDS) and the whole objective 3 (to assess the sexual performance of the mass-reared males) are attended. We are testing whether the chilling processes affects sterile males' survival, flight ability and sexual competitiveness. First, we will determine the effect of different chilling times on the survival and flying abilities of male mosquitoes under laboratory conditions. For that, four treatments plus a control (mosquitoes without chilling) will be performed. The first treatment consists of the manual release protocol, that is a chilling at 4 ° C for 25 minutes. The second, third and fourth treatment correspond to the air release protocol, consisting of a second chilling for 25, 50 or 100 minutes. For the survival test, the daily mortality of 100 males from each treatment will be evaluated until the last adult dies (three repetitions/treatment and control). For the flight ability test, 50 mosquitoes will be tested in a flight device for 2 hours (ten repetitions/treatment and control). To test the sexual competitiveness of irradiated males, the irradiated males will be chilled under two treatments: ground (on chilling at 4°C for 25 min) and air release (two chillings at 4ºC for 25 minutes each). The control will consist of unchilled irradiated mosquitoes. The experiment will be carried out according to the protocol established by the IAEA and the Fried (1971) competitiveness index will be calculated.

Optimization of irradiation dose and quality assurance of sterile male *Aedes aegypti*: A laboratory study

Kajla Seheli, Md. Forhad Hossain, Ananna Ghosh, Mahfuza Momen, Nahida Sultana and Md. Aftab Hossain

Insect Biotechnology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Dhaka 1349, Bangladesh.

A country of some 160 million people, Bangladesh had struggled against its worst outbreak of dengue fever causing 164 deaths in the year 2019. Just two years back in 2017 chikungunya, another *Aedes* borne disease created significant concern in the country as more than 13000 people were infected. When it comes to transmitting diseases among people, mosquitoes are unsurpassed in the economic and health burdens they impose. In the absence of efficient drugs or vaccines and given the need to reduce the use of insecticides, international efforts are required to develop and implement new, complementary control techniques for mosquito species. The nuclear Sterile Insect Technique (SIT) is one such technique. Therefore, logical and phase conditional approach for mosquito population management through SIT could be considered very promising for future dengue mosquito management programme in Bangladesh. Insect Biotechnology Division of Institute of Food and Radiation Biology under the IAEA CRP grants, previously had established a mosquito insectary and has accomplished some basic researches on mass rearing, male sterility dose, adult male longevity and egg hatching rate etc. As radiation is deleterious to mosquito, careful studies are needed to select an optimal irradiation dose that will ensure the quality of sterile males for field release. Hence, currently we are focusing to determine the appropriate radiation dose that may satisfy the fitness qualities of irradiated male mosquito of *Aedes aegypti* for SIT applications. This study was conducted in laboratory conditions. We determined the effects of different doses of gamma radiation on flight ability, adult longevity and wing size of sterile males. The work plan for next two years are as follows: 1) Mating competiveness analysis of the irradiated males of *A. aegypti*. 2) Sex separation process will be further improved. 3) Up-scaling the production of sterile *A. aegypti* males up to 50 thousand per week. 4) The irradiation process for large volume of male pupae will be established.

Quality control of long-term mass-reared *Aedes albopictus* for suppression field trial in China

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The endosymbiotic bacterium *Wolbachia* is being developed as a tool to suppress mosquito populations and their transmitted pathogens, with successful field trials in multiple countries having resulted in efforts to scale-up the capacity to mass-produce mosquitoes for release. However, major challenges exist to achieving this goal, including concerns that mass-reared mosquitoes will adapt to laboratory conditions during long-term maintenance and experience inbreeding depression, resulting in poor performance of the released mosquitoes in the field. Here, we assessed the performance of the *Aedes albopictus* HC line infected with a triple-strain *Wolbachia* after mass-rearing at scaled-up densities of up to 15 million mosquitoes per week for over 50 generations. In comparison to the wild-type GUA line, the HC mosquitoes had desirable characteristics for mass-rearing and release, including robust male mating competitiveness, high female reproductive capacity, reduced vector competence for dengue virus, and increased *Wolbachia* density. Although the larval survival rate of the HC and GUA lines was similar, the HC larvae developed significantly faster, possibly because of up-regulation of the molting hormone 20-hydroxyecdysone-related gene *E75* in the HC larvae. Our results indicate that over many generations mass-reared mosquito lines can retain their quality if large effective population sizes with sufficient genetic heterogeneity are maintained under optimized rearing conditions, and demonstrate the long-term feasibility of deploying *Wolbachia*-based approaches for area-wide management of mosquito vectors for disease control.

Irradiation assays for *Aedes aegypti* from Ecuador

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Dengue, zika and chikungunya diseases are present in Ecuador and affect mainly the population on the Pacific coastal zones, where dengue is considered endemic. Every year there are thousands of confirmed dengue cases. Chikungunya and Zika cases, since the first locally acquired cases reached high incidences in the coastal provinces and in the Amazon basin region.

The National Institute of Research in Public Health (INSPI) with IAEA support engaged in the SIT (Sterile Insect Technique) program to control *Aedes aegypti* populations in continental Ecuador and the Galapagos Islands.

We are monitoring the population of *Aedes aegypti* in the continent (Lita and Cachaco) and the Galapagos Islands. A colony of the insect from Lita has been reared in the laboratory to implement the SIT essays.

A critical part of the technique is to determine the radiation doses to sterilize the insects maintaining the competitiveness of males to mate with wild females when released in the field.

We have started a study to determine the doses to sterilize male mosquito pupae and measure the effects on mosquito male performance.

The irradiation is done in a semiautomatic irradiator JLShepard, Model 109 Cobalt-60, with 157.75 Gy /y dose rate. A dose map of the canister was done using Alanine dosimeters (Bruker, pellet type) to obtain dose rate and the calibration curve.

A canister was designed to hold seven plastic containers (30 ml) in five levels. Three pupae were placed in each container, 21 for each dose. The doses used were 40, 55, 60, 70 and 80 Gy. Radiation time varies from 14 (40 Gy) to 28 minutes (80 Gy). Preliminary results show high mortality rates (pupae and adults) as radiation doses increase. Mortality in the control group was 19%, with 40 Gy 38.1%, 55 Gy 61.9%, 60 Gy 57.1, 70 Gy 85.7%, 80 Gy 81%). Fertility rates (mean number of eggs per non-irradiated female) varied from 4 (control), 11 (40 Gy), 0 (55 Gy), 5 (55 Gy) 4 (60 Gy), and 0 (80 Gy). Viable eggs from irradiated males x non-irradiated females were produced only at 40 Gy (20%).

The next 18 months we plan to increase the number of irradiated pupae and measure the effects of radiation in the laboratory and field cages.

Optimizing bulk irradiation of mass-reared *Anopheles arabiensis* males in prospective of a pilot sterile male release programme in South Africa

Michael Samuel[1,2,3], Nonhlanhla Ntoyi[1,2,3] and Givemore Munhenga[1,2,3]

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Anopheles arabiensis is a dominant mosquito vector of malaria in sub-Saharan Africa and the primary vector of the disease in South Africa. While the country has made great strides in malaria control through the use of indoor residual spraying of insecticide – namely DDT, supplemental measures are necessary to achieve the target of eliminating local transmission of the disease by 2023. The sterile insect technique (SIT) has shown potential to suppress and possibly eliminate target populations of non-vector pest species as well as *Aedes* arbovirus vector species. As such, it may be a feasible option for the country's vector control arsenal, as well as have implications for other sub-Saharan African countries aiming to control this malaria vector.

To date, we have collected several years of surveillance data at a target site in Mamfene, KwaZulu Natal and have established a wild type strain of the predominant *An. arabiensis* vector at the NICD, Johannesburg. A genetic sexing strain was crossed with the wild strain to provide us with a suitable strain for mass rearing, designated as GMK. We have also investigated alternative sexing methods, and produced irradiation data as a platform for the prospective bulk irradiation – including dosimetry. A customized irradiation canister was also designed to contain mosquito pupae for baseline irradiation investigation. Finally, we have a newly established mass rearing facility where we are currently upsizing the colony for large scale experiments, including a pilot release of sterile males planned for 2021. This follows multiple mark-release-recapture exercises conducted between 2016-2018.

Our aim in the immediate future is to optimize a process for the bulk irradiation of *An. arabiensis* mosquitoes and to develop appropriate quality measures. This will primarily require further investigation into a suitable irradiation surface/canister for large quantities of mosquitoes and assessment of the impacts of pupal density, the absence and presence of water, and, the absence and presence of oxygen on sterilization and fitness. We will also investigate the potential for irradiating adults of the species and develop a standardized method for conducting this, if feasible. The current target is to consistently be able to produce 50,000 sterile male mosquitoes per week by the end of 2021 and henceforth expand capacity and production.

Irradiation, Sterilization and Quality Control of Dengue Mosquito, *Aedes aegypti* in the Philippines

Glenda B. Obra [1], Abigaile Mia J. Hila, [1] Eleanor A. Rebua [1], Haydee M. Solomon[2]

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The implementation of this CRP Project started only in the last quarter of 2020. For the first year, the objective was to determine the sterilizing dose for *Aedes aegypti* and determine the effect of irradiation on survival and mating of Ae. *aegypti*. Dose mapping was initially done using alanine dosimeters to determine the position of the minimum and maximum dose absorbed by the sample using the container designed for the test. Irradiation of male *Ae. aegypti* pupae was done using the new PNRI Gamma Irradiator (Ob-Servo Sanguis) procured through the IAEA TC project PHI5033, which became operational June 2020. Preliminary trials included only two doses (15 and 90 Gy) and a control lot. Survival did not differ significantly between 15 and 90 Gy, including the control. For the subsequent trials, pupae were irradiated using six doses ranging from 15 – 90 Gy at 100% shielding. Unirradiated pupae served as the control. The longevity of adult males, in general, decreased with an increase in dose. Based on the results of the sterility tests, fertile eggs were observed even at the highest dose (90 Gy) in Trial 1, but not in Trials 2 and 3, where complete sterility was observed at 75 Gy. For the next 18 months, we will be continuing with the maintenance of our stock colony, radiosensitivity studies using 50 and 25%shielding, and quality control of irradiated and control *Ae. aegypti.* We will also try to test on supplements for irradiated mosquitoes. If the situation will permit, we will visit another potential site for SIT.

Development of protocols and materials for medium-large scale sterilisation by ionizing irradiation and electron-beam technology, and Quality Control of Aedes mosquitoes

CarlosTur[1], Ignacio Pla[1], David Almenar[1]

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The Sterile Insect Technique (SIT) has proven to be effective to suppress the wild mosquito populations at a small scale. However, the actual feasibility of SIT as a valid area-wide vector control method has to be tested at large scales. Most of the methods currently used in pilot projects will not be directly applicable to large scales considering a cost-effectiveness point of view. The Spanish public company TRAGSA is currently involved in the research of large-scale methods in Aedes SIT. This research includes the mass sterilization of males and the application of quality control (QC) systems for the mass production of mosquitoes.
Our recent experience in the pilot project against *A.albopictus* combined with new research in the area of sterilization by ionizing radiation in the genus Aedes shows that there is an unexpected inconsistence concerning the outcome of sterilization, even when similar methodologies are in use. The identification of the key factors involved in this lack of uniformity is currently a subject of major importance for the Aedes-SIT pilot projects. Although traditionally the sterilization at pupal stage has been preferred in SIT projects, there is an increasing interest in the sterilization in adult stage, since there may be advantages that can exceed the inconveniences of their difficult handling. On the other hand, the electron beam technology is currently used for the sterilization of millions of Medflies in Valencia. Our goal is the development of protocols for the sterilization of male mosquitoes with beam electron systems. We will explore the possibility of the irradiation in pupae and adult stages. Concerning QC, our goal is the incorporation of quality control tests in a global quality system that includes the routine performance of quality tests and an integrated system of quality information management. This protocol of QC will also include the routine irradiation dosimetry.
For the next 18 months we plan to (1) Analyse the collected data on sterilization during the pilot project; (2) Analyse the data on QC during the pilot project and stablishment of QC parameters and thresholds; (3) Research on the factors associated to heterogeneity in the induced sterility to pupae with Gammacell 220, including geometry of dose, environment, and pupae condition; (4) Validation of containers specifically designed for the irradiation and transport of pupae and adults in Gammacell 220 and electron-beam (5) Research on the sterilization by electron-beam; (6) Development of protocols for the routine dosimetry for Gammacell 220; (7) Evaluation and research on the application of image analysis to CQ methods.

Evaluating the influence of environmental factors on the swarming and mating competitiveness of sterile males of *Anopheles arabiensis* and the irradiation dosage on the vector competence of sterile females under semi-field and field conditions

Simon P. Sawadogo[1], Jean Jacques B Tioye[1], Serge Poda[1] and Roch K. Dabiré[1]

[1] *Institut de Recherche en Sciences de la Santé/Centre Muraz, Bobo-Dioulasso BP 545, Burkina Faso.*

Due to the insecticide resistance developed by several malaria vector species and also to the lack of compliance of malaria control tools in the community level, the only use of insecticide- based control tools become challenging to reach the elimination level of malaria in the endemic African countries. The development of complementary methods became a crucial need. Among them the use of biological or genetic control of vectors being developed in lab level began promising. However, prior to implementing such tools or strategy in public health level, many steps remain to be rabbeted. In this perspective IAEA supports all countries aiming to develop biological control of both arboviruses or malaria vectors using the release of sterile males by irradiation alone or in combination with Wolbachia. But to better implement these techniques basic studies are needed to refine, standardise and sharing protocols. The mean objective of this study through this CRP is to test the competitiveness and the vector capacity and competence of respectively irradiated males and females of *An. arabiensis* under the influence of external factors such as temperature, seasonality, irradiation dosage. Specifically, we'll work on: (1) the kinetic of swarming and mating patterns of irradiated males in the lab using 3D system according to two irradiation dosages and temperature points, (2) the competitiveness of irradiated males in malaria sphere in dry and wet seasons (3) the density/dispersion and survival of marked/released and recaptured of irradiated males in the field around the river sides (4) the vector competence and capacity of irradiated females according two irradiation dosages.

Thinking about hypoxia and temperature treatments for improving sterile male performance in the field.

Daniel A. Hahn

Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA

An important facet of sterile insect technique (SIT) is to consistently deliver high-quality, sexually competitive sterile males to the field that will be able to perform well. Yet, many steps in the sterile insect production and distribution pipeline can have negative effects on male performance: from mass rearing and handling to sterilization, shipping, and release protocols. Here I will give two case studies about how our lab and collaborators have used a variety of techniques in evolutionary physiology research to ameliorate the stresses incurred by insects at various stages in the sterile male pipeline. One important consideration for irradiation of large groups of insects is whether insects have access to adequate oxygen. Due to either accidental crowding or intentional packaging of insects in modified or controlled atmospheres, experiencing hypoxia prior to and during irradiation can affect both the dose needed to provide adequate sterility and post-irradiation male performance. I will discuss work in our lab that has shown irradiating *Aedes aegypti* pupae in low oxygen atmospheres increases the radiation dose needed to achieve 99% sterility and potentially improve post-irradiation male performance. Then, I will outline work currently underway that explores the effects of irradiation of compacted adult males under low-oxygen atmospheres on sterilizing doses and post-irradiation male performance. A second important consideration for sterile insect programs is to begin releases early in the season before wild populations grow large enough to be difficult to affect. Yet, males raised at warm temperatures in the lab may not perform well at seasonally low temperatures in the field. I will discuss work from our lab and collaborators showing substantial changes in thermotolerances of wild field populations across an entire mosquito community in North Florida, data that suggest wild males may be better prepared to perform in cool springtime conditions than lab-reared males. Then, I will outline work in progress to characterize the timescales over which lab-reared *Ae. aegypti* adults may be acclimating under field conditions to try to improve sterile male performance.

Can we improve the field performance of SIT *Aedes aegypti* through assisted thermal acclimation?

Heath A. MacMillan

Department of Biology, Carleton University, Ottawa, Canada, K1S 5B6

The majority of insects, including *Aedes aegypti*, are chill susceptible, meaning they suffer from performance deficits, sustain injury, and die at temperatures well above those that cause them to freeze. *Ae. aegypti* have historically been restricted to tropical and subtropical regions, but adults have been recently and repeatedly found in more poleward climates. This suggests that adult *Ae. aegypti* are capable of overwintering, but very little is known about their capacity for, and plasticity of, thermal performance and whether/how this plasticity could be used to improve performance of sterile males released in the field when the climate is suboptimal for release. We are working to characterize how thermal environments influence male *Aedes* performance in the laboratory and identify the physiological mechanisms that underlie plasticity in thermal performance observed. Our early work on this topic confirmed that *Ae. aegypti* are indeed capable of cold acclimation that mitigates cold-induced injury. In the coming months, we will extend this understanding with a focus on how chilling specifically impairs reproduction in this species and whether and how specific thermal treatments can mitigate these effects. We plan to do this using a custom-built laboratory system capable of producing 10-20 dynamic thermal environments for different animals simultaneously. Ultimately, we hope to test promising pre-treatments in a field setting using SIT males in order to develop best practice recommendations in matching thermal pre-treatments of sterilized insects to the prevailing climate.

Dosimetry challenges and irradiation modality effect on mosquito

Florent Kuntz and Abbas Nasreddine

Aerial CRT 250 rue Laurent Fries, 67400 Illkirch

X-ray irradiation of pupae and larvae becomes more and more attractive compared to gamma irradiation due to environmental and economic reasons. However, the usage of low to medium energy X radiation (lower than 300 keV) for low dose applications such as SIT treatments implies to control this process with sensitive, well characterized, and calibrated dosimetry systems.
Dosimetry for this kind of radiation energy is not trivial and if Cobalt60 calibrated dosimeters are used, one may have up to 30 % deviation due to the radiation spectrum differences.
In the past years, Alanine/EPR dosimetry system's response have been evaluated at Aerial. The first objective of this project is to set up a protocol which helps estimating the X radiation energy spectrum characteristics of partner's X- ray generators.
Traceable primary dosimetry with ionization chambers needs to be setup for this purpose and will make it possible to calibrate the Alanine /EPR dosimetry system in this particular radiation field. This would ensure consistency of irradiation doses between this CRP partners whatever the radiation type and energy in use.
The second work package of the proposed project is dedicated to radiation modality and dose rate impact on irradiated larvae and pupae. Samples from partners will be irradiated at same dose with low (up to100 kV), medium and high (starting from 0.8 MV up to 7 MV) energy X-rays as well as medium (2.2 MeV) and high (10 MeV) energy electron beams.
Dose rates (Gy/min) will be selected to obtain at least 1 decade difference between experiments. Thus, after post irradiation characterizations performed by the partners, irradiation modality and dose rate effect will be assessed. (Selection of larvae and pupae type is left to the competent partners.)

ANNEX 4: Agenda of the Workshop on irradiation methods and dosimetry

IAEA
International Atomic Energy Agency
Atoms For Peace

"Workshop on irradiation methods and dosimetry"

Seibersdorf, Austria

14-15 July 2022

DRAFT AGENDA

Thursday, 14 July, 2022 (Room TC18)		
11:00	**Opening Address** • **Objective of the workshop and targeted outputs** • **Introduction of participants** • **Review and adoption of Agenda**	**Jeremy Bouyer & Hanano Yamada**
11:30-12:30	**Presentation "Irradiation & Dosimetry Basics"**	**Hanano Yamada**
12:30-13:30	**LUNCH at IPCL cafeteria**	**(Vegetarian or meat option)**
13:30-14:15	**Presentation "Irradiation: How to"** **Explanation of group exercise**	**Hanano Yamada**
14:15-16:45	**Group practical exercise (Room GF70, IPCL)** • Introduction to the Foss812, GC220, MK2, RS2400 • Irradiation set-up (container + sample + dosimeters + cold packs (opt)) • X-ray & Gamma ray exposures	**Group exercise**

	(MK2 and GC220) • Dose distribution map (simplified)	

Friday 15 July 2022 (Room TC18)		
9:30-12:30	**Presentation & discussion** • Irradiating adults vs. pupae (dose-response curves) • Variables to consider in irradiation & dosimetry • Troubleshooting & common issues --- • Mass-irradiation (chilling, packing & transport) --- • Group discussion on various protocols used by participants	**Hanano Yamada** --- **Hamidou Maiga** --- **Group discussion**
12:30-13:30	**LUNCH at IPCL cafeteria**	**Vegetarian or meat option**
13:30-14:30	**Presentation: Calibration of Gafchromic films (how to use the excel spreadsheet)** **Q&A**	**Hanano Yamada**
14:30-16:45	**Group practical exercise (TC18)** • Reading and analysis of the Gafchromic films • Dose distribution map • Discussion of results	**Group exercise**

ANNEX 5: List of Participants of the 2nd RCM

S. No.	Authority	Personal Details
1	Bangladesh (WS/RCM)	Ms Kajla SEHELI Insect Biotechnology Division Institute of Food and Radiation Biology AERE Bangladesh Atomic Energy Commission Ganakbari 1349 SAVAR BANGLADESH Tel:+880 27790527 Email:kseheli@gmail.com
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3	Burkina Faso (WS/RCM)	Mr Simon SAWADOGO Institut de recherche en sciences de la santé - IRSS 399, Avenue de la Liberté P.O. Box 545 BOBO-DIOULASSO BURKINA FASO Tel: Email:sawsimp2005@yahoo.fr

S. No.	Authority	Personal Details
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5	Ecuador (WS/RCM)	**Mr William PONCE YAULEMA** **Instituto Nacional de Investigación en Salud Publica (INSPI)** **Coordinador INSPI- Quito Zona 9.** **Iquique No14-285 y Yaguachi** **170136 QUITO** **ECUADOR** **Tel:+593 (6299)(5939)99584167** **Email:wpponcey@yahoo.com** **wponce@inspi.gob.ec**
6	France (RCM)	**Mr Florent KUNTZ** **Aerial CRT - Parc d'innovation** **250, rue Laurent Fries** **67412 ILLKIRCH** **FRANCE** **Tel:+33 3 88 19 15 17** **Email:florent.kuntz@aerial-crt.com**
7	Greece (WS/RCM)	**Mr Antonios MICHAILAKIS** **Laboratory of Insects & Parasites of Medical Importance** **Benaki Phytopathological Institute** **8, Stefanou Delta street** **ATHENS** **GREECE** **Tel:00306977484842** **Email:a.michaelakis@bpi.gr**

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S. No.	Authority	Personal Details
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ANNEX 6: Agenda of the 2nd RCM

Second FAO/IAEA Research Co-ordination Meeting

"Mosquito Irradiation, Sterilization and Quality Control"

July 18 – 22, 2022

Vienna, Austria

Vienna International Centre (IAEA Headquarters), MOE100 (main room), MOE58 (side room if needed), MOE59 (side room if needed)

AGENDA

Monday June 18, 2022

08.00 - 09.00 Registration at Gate 1 (by underground U1 station)

09.00 – 09.30 **Jeremy Bouyer & Rui Cardoso Pereira:** Welcome & opening remarks; Administrative details

Session I: Irradiation / Sterilization (Chairperson: Florent Kuntz)

09.30 – 10.00 **Hanano Yamada** *Characterization and dose-mapping of an X-ray blood irradiator for mosquitoes*

BREAK 30 min

10.30 – 11.00 **Hadian Sasmita** *Investigation of Endo- and Exogenous Factors Influencing Dose-Response and Male Quality: High vs. Low Dose Rate Irradiators and Strain Geographical Origin*

11.00 – 11.30 **Patricio Ponce** *Sterilization of* Aedes aegypti *pupae and adults from Ecuador using a gamma irradiator.*

11.30-12.00 **Diana Iyaloo** *Challenges with the use of the Cesium-137 irradiator (Gammacell 1000) and next steps*

LUNCH 1 hr

Session I: Irradiation / Sterilization (continued, Chairperson: Hadian Sasmita)

13.00 – 13.30 **David Almenar** *Recent achievements in the sterilisation methods, process and quality control at a medium-scale in the SIT-based project against* Aedes albopictus *in the Valencian Community (Spain)*

13.30-14.00 **Ariane Dor** *Effect of chilling in flight ability and sexual competitiveness of the Tapachula* Aedes aegypti *Genetically Diverse Strain*

14.00 – 14.30 **Aline Taiane** *Impact of the age on induced sterility and flight ability during adult irradiation of* Aedes aegypti

BREAK 30 min

15.00 – 15.30 **Glenda Obra** *Establishment of Sterilization Dose for Dengue Mosquito,* Aedes aegypti, *using Gamma and X-ray Irradiation.*

15.30 – 16.00 **Fabrizio Balestrino** *Scaling up mass irradiation of* Aedes albopictus *at pupal stage*

Tuesday July 19, 2022

Session I: Irradiation / Sterilization (continued, Chairperson: Clement Gouagna)

09.00 – 09.30 **Yongjun Li (visio-conference)** *The value of combining incompatible and sterile insect technique to control mosquitoes*

09.30 – 10.00 **Simon Sawadogo** *Effect of different irradiation doses on* Anopheles coluzzii *males' survival and fertility*

BREAK 30 min

10.30 – 11.00 **Uruyakorn Chansang** *Effect of different irradiation dosages on sterility and quality of* Aedes aegypti *mosquitoes with and without Wolbachia symbionts*

Session II: Quality Control (Chairperson: Clement Gouagna)

11.00 – 11.30 **Hamidou Maiga** *Towards the standardisation of the IAEA flight test device in* Aedes mosquitoes

11:30 – 12.00 **Louis Clément Gouagna** *Recent developments in mass irradiation of* Aedes albopictus *pupae in Reunion Island and field performance of sterile males*

LUNCH 1 hr

Session II: Quality Control (continued, Chairperson: Antonios Michailakis)

13.00 – 13.30 **Kajla Seheli** *Effect of some exogenous factors (nutritional state, water quality and oviposition medium) on quality parameters of laboratory reared* Aedes aegypti

13.30 – 14.00 **Antonios Michaelakis** *Quality Control tests for transported irradiated* Aedes albopictus *males*

14.00 – 14.30 **Michael Samuel** *Quality management in the mass production of the malaria vector* Anopheles arabiensis *for the development of a functional and sustainable Sterile Insect Technique (SIT)*

BREAK 30 min

15.00 – 15.30 **Assane Gueye Fall** *Impact of long-distance transport on the quality of* Aedes aegypti *sterile males*

Wednesday July 20, 2022

Session IV: Review of individual proposals (Chairperson: Jeremy Bouyer and Group Leaders)

09:00 – 12.00 Working Groups: revising individual plans for the 5 year of the CRP and for the next 18 months

Lunch

13.00 – 15.00 Working Groups: Preparing individual plans

Coffee break

15.30 – 17.00 Working Groups: Preparing individual plans

Thursday July 21, 2022

09:00 – 09:30 **Dan Hahn (virtual)**

Lab-reared Aedes aegypti adults can quickly achieve similar cold hardiness to wild populations with 3 days of outdoor acclimation

Session III: Inter-lab studies. (Chairperson: Hanano Yamada / Hamidou Maiga)

09.30 – 10.00 **Florent Kuntz** *Intercomparison of dosimetry systems*

Coffee break

10.30 – 11.00 **Maiga Hamidou** *Inter-lab comparison of flight ability and semi-field competitiveness*

11.00 – 12.00 Review of the protocols by groups of participants

Lunch

SESSION IV: RCM report (Chairperson: Jeremy Bouyer and Group Leaders)

13.00 – 14:30 Review of the logical framework

Coffee break

15:00 – 16.00 Review and agreement on content of RCM report (main achievements and recommendations)

Friday July 22, 2022

SESSION IV: RCM report cont. (Chairperson: Jeremy Bouyer and Group Leaders)

09:00- 10:00 Review and agreement on content of RCM report (main achievements and recommendations)

Coffee break

10.30 – 12.00 Review and agreement on content of RCM report (main achievements and recommendations).

Lunch

13.00 – 15.00 Presentation of the final RCM report (main achievements and future work)

ANNEX 7: Abstracts of the 2nd RCM

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Scaling up mass irradiation of *Aedes albopictus* at pupal stage

AUTHOR (S): Balestrino F., Puggioli, A., Malfacini M., Carrieri M., Bellini R.

ORGANIZATION: Centro Agricoltura Ambiente "G. Nicoli" srl, Crevalcore, ITALY

SHORT SUMMARY OF PAPER

Abstract:

Sterilization by irradiation remains the most practical way to induce sterility in insects for area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT). At CAA we used a self-shielded gamma irradiator (Cs137; IBL 437 CIS Bio international), with a current central dose rate of about 2 Gy/min to sustain SIT campaigns against *Aedes albopictus*. The current adopted SOP indicates to irradiate male pupae in a thin layer of water when they are 24-36 hours old at lab temperature (20-22 °C), using the dose of 35 Gy. Past studies have confirmed that this procedure induces a sterility level of about 99% in adult males, without evidence of significant impact on male longevity, mating capacity and competitiveness in semi-field and field setting. Moreover, comparative studies on the sensitivity to radiation of several *Aedes albopictus* European populations shown no significant differences between populations of different origins. To maximise irradiation procedures, we investigated the effect of different maturation, transportation and irradiation methods on the sterility levels of males irradiated at pupal stage in water with 35 and 50 Gy at 30 h from collection (age 30-40 h). The pupae were maturated, transported and irradiated while maintained at low (6 pupae per ml) or high density (100 pupae per ml) in order to identify the radiation dose and method capable to induce effective sterility. Results indicated that transportation and irradiation methods significantly reduce the radiosensitivity of *Ae. albopictus* at pupal stage. As previously observed, we assumed that pupae can completely deplete the dissolved oxygen in the water and an additional concurrent strong reduction of the air available to the pupae can have a potential synergistic radioprotective effects during irradiation. Moreover, in this study, we observed a decreased radiosensitivity at pupal stage at 35 Gy with the standard irradiation procedures in comparison with our data records from different European strains. The possible radioprotective effect of some components of the larval and adult diets are currently being studied to understand and calibrate the mass irradiation procedures to obtain males with adequate sterility and sexual competitiveness using available gamma and X-ray radiators recently purchased.

In this presentation we will also report on the effect of radiation on the dissemination and transmission rate of dengue and chikungunya viruses in Ae. aegypti and Ae. albopictus females exposed at pupal stage at the radiation dose of 40 Gy.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Lab-reared *Aedes aegypti* adults can quickly achieve similar cold hardiness to wild populations with 3 days of outdoor acclimation

AUTHOR (S): Dylan Tussey & Dan Hahn

ORGANIZATION: University of Florida

SHORT SUMMARY OF PAPER

Abstract:

SIT programs are often most effective when they begin releases well before pest populations rise, typically in the spring when night-time and early morning temperatures are low. The problem is that males reared under high and stable laboratory temperatures may not perform well in the field under temperatures that fluctuate daily between warm in the afternoon and cold in the early morning. One potential solution to this problem is to acclimate sterile males under fluctuating thermal regimes prior to their release into the field. The extent to which mass-reared males of a tropical mosquito species can benefit from cold acclimation for use in SIT programs has not yet been determined. A faster recovery time from chill coma in mass-reared and acclimated sterile males could potentially allow for increased survival, greater dispersal capacity, and better mating competitiveness in released sterile males during early season releases when nightly temperatures may induce chill coma. The objectives of this study were to determine the extent to which lab reared *Aedes aegypti* can acclimate to field environmental temperatures over 3 or 7 days across seasons, compare the CT_{min} values of outdoor acclimated lab-reared mosquito to wild mosquitoes in an equivalent time period, and identify which environmental temperature metrics are most closely associated with changes in CT_{min}.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Effect of different irradiation dosages on sterility and quality of Aedes aegypti mosquitoes with and without Wolbachia symbionts

AUTHOR (S): Pattamaporn Kittayapong[1,2,3], Jiraporn Yongyai[2], Parinda Thayanukul[1,2], Suwannapa Ninphanomchai[1,3], Wanich Limohpasmanee[4] and Uruyakorn Chansang[5]

ORGANIZATION: [1]Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Thailand, [2]Department of Biology, Faculty of Science, Mahidol University, Thailand, [3]Go Green Co., Ltd., [4]Thailand Institute of Nuclear Technology, Ministry of Higher Education, Science, Research and Innovation, Thailand, [5]Department of Medical Sciences, Ministry of Public Health, Thailand,

SHORT SUMMARY OF PAPER

Abstract:

In this paper, we evaluated the effect of X-ray irradiation on sterility and quality of *Aedes aegypti* with and without *Wolbachia* endosymbionts when they were irradiated with different dosages, i.e., 30 Gy, 50 Gy and 70 Gy, at the pupal stage. In addition, we also investigated the effect of the same different X-ray irradiation dosages on density of *Wolbachia* endosymbionts infecting *Ae. aegypti*. In the experiment, male and female pupae of the wild type and *w*AlbB *Wolbachia* trans-infected *Ae. aegypti* were reared, sex separated and irradiated with the above dosages. They were then subjected to assess adult emergence, survival rate, longevity and induced sterility. Result showed that irradiation had no impact on adult emergence of wild-type *Ae. aegypti*. However, increase of irradiation dosage dramatically decreased survival rate and longevity of both wild-type males and females. Irradiation dosage of 30 Gy was sufficient to induce complete sterility in wild-type females whereas higher irradiation dosage of 50 Gy was required to induce near to complete sterility in wild-type males and complete male sterility was achieved when irradiation dose was at 70 Gy. In *Wolbachia* trans-infected *Ae. aegypti*, a significant reduction of 71-87% of male emergence was observed at 30 and 50 Gy and decreased to 34% at 70Gy, while a significant reduction of 85-88% of female emergence was observed at 50 and 70 Gy. In terms of survival rate and longevity, irradiated *Wolbachia* trans-infected males had lower survival rate and longevity. Surprisingly, this was not the case for irradiated *Wolbachia* trans-infected females. Some females irradiated at 30 and 50 Gy showed higher survival rate, but their longevity was not different when compared to non-irradiated females. In term of induced sterility, irradiation dosage of 70 Gy induced complete sterility in males, while lower irradiation dosage of 30 Gy could induce complete sterility in females. To assess the effect of different X-ray irradiation dosages on density of *Wolbachia* endosymbiont in *Wolbachia* trans-infected *Ae. aegypti*, irradiated male and female adults aged 7, 14 and 21 days were sampled for detection of *Wolbachia* density focusing on the *w*AlbB strain by using qPCR analysis. Results showed that X-ray irradiation significantly reduced density of *Wolbachia* in irradiated *Wolbachia* trans-infected males when compared to those of non-irradiated ones. Irradiation dosage of 30 Gy showed no impact on the density of *Wolbachia* in older irradiated males when compared to those of younger irradiated ones. In contrast, when irradiation dosage was equal to 50 Gy, significant reduction of the density of *Wolbachia* in older irradiated males was observed. However, when

irradiation dosage was at 70 Gy, irradiated males were very weak and assessment on the effect of irradiation on the density of *Wolbachia* was difficult and unclear. When focused on *Wolbachia* trans-infected females, it was found that X-ray irradiation reduced the density of *Wolbachia* in irradiated females when compared to those of non-irradiated ones when females aged 7 days only. The density of *Wolbachia* was proportionally reduced in relation to age in non-irradiated females when the older females showed lower density of *Wolbachia*. However, they were very weak after irradiation and did not live long when compared to those of non-irradiated ones. In addition, irradiated females could not lay any eggs and they were completely sterile when irradiation was at 30 Gy. Therefore, it was possible that irradiation had some impacts on *Wolbachia* trans-infected females resulting in reducing *Wolbachia* density. Therefore, further studies are needed to investigate the effect of irradiation on *Wolbachia* density in *Wolbachia* trans-infected males and females.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Effect of different irradiation doses on *Anopheles coluzzii* males' survival and fertility

AUTHOR (S): Simon P. Sawadogo[1], Jean Jacques B Tioye[1], Serge Poda[1] and Roch K. Dabiré[1]

ORGANIZATION: [1] *1Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso BP 545, Burkina Faso.*

SHORT SUMMARY OF PAPER

Abstract:

The definition of the appropriate radiation dose-response for an insect species such as disease-transmitting mosquitoes is very important for male sterility and the application of the sterile insect technique. This technique can only work if sterile males can successfully mate in the field despite significant stressors such as artificial rearing conditions, handling, and radiation exposure. Previous work has indicated that radiation sensitivity varies considerably between species, which justifies the need to define an appropriate dose-sterility and dose rate for *Anopheles coluzzii* males. Here, we performed experiments to observe the interaction between rhodamine B and irradiation to assess their effects on males and induced sterility in females. Males' mosquitoes were exposed in pupal stage with 70, 80 and 90 Gy radiation dose. After irradiation males' have been separate in two groups. A group was fed glucose and the

other glucose coupled with rhodamine B to measure parameters such as survival and mating success of mating over six (6) treatments. Irradiation had no significant adverse effects on the male's survival. The measurement of longevity was respectively: control with glucose: 23 D; control with glucose + Rho B: 23 D; 70 Gy: 23 D; 70 Gy + Rho B: 23 D; 80 Gy: 23 D; 80 Gy + Rho B: 20 D; 90 Gy: 22 D; 90 Gy + Rho: 21 D. At the mating, 1:1 sex ratio (30♂: 30♀), the number of females inseminated according to dose was: control with glucose: 21; control with glucose + Rho B: 19♀; 70 Gy: 19♀; 70 Gy + Rho B: 20♀; 80 Gy: 14♀; 80 Gy + Rho B: 16♀; 90 Gy: 15♀; 90 Gy + Rho B: 15♀. Eggs of control had hatched, no eggs of different doses hatched in each treatment. Residual fertility was the same for all six (6) treatments, equal to zero (0). The induced sterility for all the treatments gives the same value, it is 100%. Thus, we observed induced sterility in females following mating with irradiated males for all treatments.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Establishment of Sterilization Dose for Dengue Mosquito, *Aedes aegypti* using Gamma and X-ray Irradiation

AUTHOR (S): G. Obra, A.M. Hila, Z. Loberiza, H. Solomon, F. Pares, C. Feliciano, S. Tatu, J.P. Cabahug

ORGANIZATION: Philippine Nuclear Research Institute (PNRI), Diliman, Quezon City, PHILIPPINES

SHORT SUMMARY OF PAPER

Abstract:

The sterilizing dose for *Aedes aegypti* was established using the OB Servo Sanguis Gamma Irradiator at the PNRI. Male pupae of *Ae. aegypti* (26-41 hour-old) were irradiated with doses ranging from 15 to 90 Gy. Unirradiated lot served as the control. A dosimetry system was used to determine the absorbed dose using the alanine dosimeter. Based on the results of the seven trials, induced sterility of 99.29 ± 0.29% was achieved at 60 Gy. Mean pupal mortality ranged from 0 to 2 % (0 to 90 Gy), indicating that irradiation did not significantly affect the pupae. In addition, the mean longevity was not significantly different at 60 Gy (18.1±1.08 days) and 75 Gy (16.8 ±1.16 days).

Two trials were conducted using X-ray irradiation. Results of the first trial showed that a 99% induced sterility could be achieved at a lower dose of 45 Gy. The induced sterility for the first trial was 99.51% at 45Gy and 99.88% at 60Gy. There were no significant differences among treatments in % pupal mortality (0.33 to 1.67%) and % adult emergence (97.0 to 99.3%). Male longevity at 45 and 60 Gy was not significantly different from each other, with 18.9 ±1.18 days at 40 Gy and 18.5 ± 0.39 days at 60 Gy. The difference in results may be due to differences in dose rate and temperature inside the irradiation chamber of gamma and X-ray irradiators. Data collection for Trial 2 is ongoing.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Effect of some exogenous factors (nutritional state, water quality and oviposition medium) on quality parameters of laboratory reared *A. aegypti*

AUTHOR (S): **Kajla Seheli, Md. Forhad Hossain, Ananna Ghosh, Mahfuza Momen and Md. Aftab Hossain**

ORGANIZATION: *Insect Biotechnology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Dhaka 1349, Bangladesh.*

SHORT SUMMARY OF PAPER

Abstract:

Dengue – a leading cause of serious illness and death in some Asian and Latin American countries – was first reported in Bangladesh in 2000 and claimed 93 lives. When it comes to transmitting diseases among people, mosquitoes are unsurpassed in the economic and health burdens they impose. In 2022, the Directorate General of Health Services (DGHS) has so far recorded 969 dengue cases and 854 recoveries in Bangladesh. The first death from dengue this year was reported on June 21, 2022. A total of 105 dengue patients including 95 in Dhaka division alone died in 2021. Our team under IAEA RAS 5082 has performed a dose response test to obtain the appropriate radiation dose that may satisfy the fitness qualities of irradiated male mosquito, *Aedes aegypti* for SIT applications with ~99% sterility. The results of this study showed that the radiation dose of 60 Gy can be considered as the effective dose for achieving male sterility while keeping the male performances to a satisfactory level. The irradiator used in these experiments is a dry storage irradiator (BRIT, India) with a 90 kCi *Cobalt-60* (^{60}Co) gamma source located in the Institute of Food and Radiation Biology (IFRB). Doses were determined by using Fricke dosimetry system. 17.328 Gy/minute dose rate was used which was obtained from the source with current activity of 38.62 kCi. However, there is no or little study on impact of environmental/ exogenous factors (diet, low temperature, water quality, oxygen depletion) on insect quality and dose response to standardise the irradiation process.

Nutritional state is the most important component of insect rearing. A balance is essential between large scale insect production and performance of the adults. To assess the performance of three diets namely, two IAEA standard diets (50% TM + 15% BY + 35% BSF and 50% TM + 50% BSF) with our laboratory diet (fish feed), the diets were ground to fine powder and 0.7g of each diet was sprinkled on the water of the rearing trays daily depending on treatment. Experiments regarding the comparison have been completed and a manuscript is under preparation.

Water is a crucial element in mosquito's life cycle. To optimize mass rearing conditions for SIT application, a comparative laboratory study was done to evaluate the respective responses of *Aedes aegypti* life stages to water of three different qualities (in terms of electric conductivity, total dissolve solids, dissolve oxygen, and pH). Deionized, distilled and tap water were used for larval rearing. The total larval development time, pupation rate, adult emergence, body size, and longevity were determined. Preliminary results showed that rearing water with different quality have some effect on of life cycle of *A. aegypti* mosquito.

Investigations on oviposition behaviour of dengue vectors are critical for effective controlling of vector breeding. Oviposition responses with respect to the texture of the ovistrips, water type and presence/absence of larvae in the water were evaluated by enumerating the number of eggs laid by the gravid female. Seed germination papers are convenient to use in comparison to the normal offset paper and filter paper because of their creped structure and wet strength as ovistrips. Preliminary findings showed *A. aegypti* mosquitoes also prefer seed germination paper lining for oviposition compared to plain regular papers. For oviposition preference, different water types were used and it was observed that the maximum number of

eggs were laid in ovitraps containing water holding larvae of the same species than ovitraps containing normal tap water.

The work plan for next eighteen months are as follows: 1) Impact of chilling temperature and rearing water temperature on the development of *Aedes aegypti*. 2) The role of oxygen depletion and subsequent radio-protective effects during irradiation of mosquito pupae in water.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Investigation of Endo- and Exogenous Factors Influencing Dose-Response and Male Quality: High vs. Low Dose Rate Irradiators and Strain Geographical Origin

AUTHOR (S): Hadian I. Sasmita, Beni Ernawan, Nurfitrianto, Bimo Saputro, Muhamad Yasin Yunus.

ORGANIZATION: Research Center for Radiation Process Technology, Research Organization for Nuclear Energy, National Research and Innovation Technology.

Abstract:

We investigated two factors that may significantly affect the dose-response and male quality, which were high versus low dose rate irradiators and strain geographical origin. In the dose rate irradiators study, we compared high dose rate irradiators (Gammacell-220, Co-60: 4575.69 Ci, dose rate: 3327.70 Gy/hour) and low dose rate irradiators (Gamma Chamber 4000A, Co-60: 222.67 Ci, dose rate: 175.36 Gy/hour). Results indicate that induced sterility was differ significantly as the high dose rate irradiator induced 69.61±7.35% sterility, which was much lower than low dose rate irradiator with 85.43±5.91% (P = 0.001). Significant lower rate was also observed in flight ability (high: 95.71±3.35% vs. low: 99.42±0.98%, P = 0.005), but not in emergence rate (high: 99.42±1.51% vs. low: 98.85±1.95%, P = 0.346), and water supplied longevity (both median = 6 day). For the geographical origin factor, we examined six local strains from four different islands in Indonesia. As the results, significant differences were found in induced sterility (P = 0.006), flight ability (P < 0.001), emergence rate (P < 0.001), sugar supplied longevity (P < 0.001), and wing length (P < 0.001). Under laboratory conditions, these results indicate that irradiated males with larger wings do not necessarily have a better dose-response and quality. This demonstrates the need for further investigation on physical or genetic features influencing dose-response and male quality. A trial to explore the possibility of irradiating non-chilled adults inside a rearing cage measuring 1 x 0.2 x 1 m using panoramic irradiator resulted in large interval between the minimum and maximum dose received, which was initially targeting 70 Gy. From 24 Gafchromic films (HD-V2) installed in every 20 cm interval (front and back vertical surface), the minimum and maximum dose received were 23.3 Gy and 93,5 Gy at lower-left corner and center of the front surface, respectively. Further trials are needed to explore different sides of irradiator and lower dose rates in order to make adjustments in the dimension and shape of the cage.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Recent achievements in the sterilisation methods, process and quality control at a medium-scale in the SIT-based project against Aedes albopictus in the Valencian Community (Spain)

AUTHOR (S): Tur, Carles; Almenar, David; Benlloch, Sandra; Pla, Ignacio.

ORGANIZATION: TRAGSA (Empresa de Transformación Agraria, S.A). Spain

SHORT SUMMARY OF PAPER

Abstract:

The SIT-based control project against *Aedes albopictus* in the Comunitat Valenciana (Spain) has been releasing sterilised males since 2018. The sterilisation protocol is based on the application of Gamma radiation to males in the pupal stage. The scaling-up of the project entails the need to implement new and more efficient sterilisation protocols. Promising results are presented on the sterilisation of adult mosquitoes using an industrially operational electron beam irradiator. The results indicate that it is possible to obtain correctly sterilised males (about 1% residual fertility) with minimal reduction in quality. Different combinations of operation, the possible residual fertility in females and the long-term preservation of sterility in males have also been explored. All results indicate that this application is feasible and operationally convenient. The results of the current quality control methodology and process control routinely applied to gamma sterilised males are also presented, including a discussion of possible factors associated with variation in dose response.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Impact of long-distance transport on the quality of *Aedes aegypti* sterile males

AUTHOR (S): Mame Thierno BAKHOUM, Gorgui DIOUF and Assane Gueye FALL

ORGANIZATION: Institut Sénégalais de Recherches Agricoles (ISRA)

SHORT SUMMARY OF PAPER

Abstract:

Mosquito-borne viruses, such as Zika and dengue fever, are of global importance. The mitigation strategies of mosquito-borne arbovirus infections continue to rely on vector control. The sterile insect technique (SIT) is the most effective strategy for vector control. However, its success depends on the ability of released sterile males to survive, disperse, compete with wild males and inseminate the wild females. Hence the need to develop and validate sterile male quality control procedures along the production chain and release. This is how we propose in the CRP "Mosquito Irradiation, Sterilization, and Quality Control" to develop and evaluate adequate quality control procedures to be used for SIT applications to control *Aedes aegypti*. Thus, we will evaluate the flight ability and the competitiveness of *Aedes aegypti* sterile males and wild males under semi-field cages and field trials, including their survival and dispersal, in order to achieve our objectives under this Research Project which are (i) to link the flight ability tool[i] developed by the Insect Pest Control Laboratories

(IPCL) of IAEA to male competitiveness of *Aedes aegypti* in semi-field cages as well as in the field; (ii) to link the flight ability of sterile males to their dispersal; and (iii) to develop of an SOP of the quality control procedures.
In the absence of an irradiator in Senegal, *Aedes aegypti* males from the AeISRA-LNERV_2019 colony (Senegalese origin) were mass-reared at ICPL, compacted, and transported from Austria to Senegal with chilling. For this year, the work performed under this CRP was to assess the impact of long-distance shipments of irradiated Aedes aegypti males. The survival rate of sterile males of Aedes aegypti after long-distance transport (2 to 4 days) was assessed, as well as their flight ability, competitiveness in semi-field cages, and dispersal.
The survival rate was 75.68±17.25 % with a percentage of flight ability of 73.85±7.04 % and a competitiveness index of 0.73±0.30.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: STERILIZATION OF *Aedes aegypti* PUPAE AND ADULTS FROM ECUADOR USING A GAMMA IRRADIATOR.

AUTHOR (S): Patricio Ponce, Ximena Galarza, Carolina Molina, Edison Vera, Carlos Cherrez, Varsovia Cevallos.

ORGANIZATION: Instituto Nacional de Investigación en Salud Pública-INSPI, Quito-ECUADOR, Escuela Politécnica Nacional, EPN, Ministerio de Energía y Minas.

SHORT SUMMARY OF PAPER

Abstract:

Colonies of *Aedes aegypti* were maintained under controlled conditions at 27 ± 2°C, 70 ± 5% relative humidity (RH), with a photoperiod of 12:12 h (light:dark). Larvae were reared at a density of 1 larvae/ml in 32x26x10 cm plastic trays containing 1500 ml rested tap water and were fed daily a liquid diet of water with a 4% suspension of powdered rat pellets feed (Coprobalan, Ecuador). We determined the dose rate of a semi-automatic irradiator J.L. Shepherd Model 109 using alanine dosimeter to irradiate the insects. A device with containers for specimen's irradiation was designed to map irradiations. The dose rate was 157.95 Gy/ h in the central reference position. Male pupae were irradiated with 40, 55, 60, 70 and 80 Gy, control groups were handled under the same conditions. Groups of 100 pupae were irradiated for each dose with three replicates. After irradiation male pupae were placed in screened cages to emerge and adult females were introduced in the cage in 1:1 male/female proportion. Fertility was 2.8% at 40 Gy, 1.5% at 55 Gy, 1.1% at 60 Gy, 0.5% at 70 Gy and 0 at

80 Gy. Pupae mortality after irradiation was low, between 0.9% in the control up to 3.2% in pupae irradiated at 80 Gy. Adult survival was indirectly proportional to the irradiation dose received. Number of eggs per female decreased as irradiation dose increased.

We also tested the effect of irradiation in recently hatched adults up to 36 hours old. Batches of 40 mosquitoes were irradiated for each dose with three replicates. Mosquitoes were cooled at -11°C for two minutes and placed into small plastic containers of 30ml in volume (diameter 4 cm, height 3 cm) to be irradiated. Mosquitoes were transported for two hours at about 12 °C from the laboratory to the irradiator site. Adult mosquitoes were irradiated at 55, 60, 70, 80 and 90 Gy. Control groups were handled under the same conditions. After irradiation male pupae were placed in screened cages to emerge and adult females were placed in 1:1 male/female proportion. Fertility was 4.3% at 55 Gy, 3.2% at 60 Gy, 1.8% at 70 Gy and 0 at 80 and 90 Gy. Number of eggs per female decreased as irradiation dose increased.

The data showed that recently hatched adult mosquitoes need higher doses to be sterilized than pupae. This may be due to germinal cells development stage at the moment of irradiation.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: *Recent developments in mass irradiation of* Aedes albopictus *pupae in Reunion Island and field performance of sterile males*

AUTHOR (S): Louis C Gouagna

ORGANIZATION: IRD

SHORT SUMMARY OF PAPER

Abstract:

The SIT pilot program against *Aedes albopictus* in Reunion Island is now undergoing a final round of field release and evaluation. Since the start, the implementation rationale required further elaboration, both under laboratory and field conditions, to ensure that all aspect from mass-rearing, sexing, irradiation necessary for the production of sterile, conditioning, transport and release, are improved and standardized. Previous studies have determined the desired irradiation procedures by testing a range of radiation conditions, including the effect of varying pupae densities (500, 1000, 2000, 4000 per petri dish), holding conditions (air versus water) and radiation dose (35Gy vs. 40Gy) on the level of male sterility. We determined that X-ray radiation at the dose of 40Gy reduced egg hatch by 99% when applied on a

maximum density of 2000 male pupae /petri dish held in water compared to pupae in air. Furthermore, early studies also (1) examined the fitness of male and female offspring originating from sub-sterile male parents x fertile female crosses, (2) documented the effects of releasing semi-sterile irradiated male *Aedes albopictus* on the target population gene pool, (3) investigated the response of sterile female *Aedes albopictus* to arbovirus infection, and (4) assessed field dispersal, survival and competiveness of sterile males marked with Rhodamin B and fluorescent dust. This presentation will include summaries of the steps taken from these previous investigations to progress in optimizing and standardizing the mass-rearing conditions and irradiation procedures for the production of sexually competitive sterile *Ae. albopictus* males intended for field releases. This consisted of improving our weekly production schedule and irradiation of large number of male pupae per single irradiation cycle, and assessing the various fitness characteristics of sterile males, and finally establishing the crucial steps to demonstrate the efficacy of SIT in an urban setting in La Reunion Island.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Quality management in the mass production of the malaria vector *Anopheles arabiensis* for the development of a functional and sustainable Sterile Insect Technique (SIT)

AUTHOR (S): M. Samuel, N. Ntoyi, L. Lobb, T. Mashatola, P. Mphaki and G. Munhenga

ORGANIZATION: The National Institute for Communicable Diseases, Wits Research Institute for Malaria

SHORT SUMMARY OF PAPER

Abstract:

Anopheles arabiensis is a dominant malaria vector species in the WHO Afrotropical region, transmitting the disease widely across sub-Saharan Africa. A Sterile Insect Technique may serve as a bio-control alternative to the recommended approaches which namely involve pesticides that may select for resistance over time. The functional mass-production of *An. arabiensis* for release is novel and as such requires a distinct quality management approach, which can be practically applied at our facility in Johannesburg, but can be implemented elsewhere to good effect. In order for this approach to be successfully implemented, however, baseline data is required to establish acceptable control limits applicable to our mosquito strain. This paper describes the collection of such data during preparation for the first ever pilot release of sterilised *An. arabiensis* mosquitoes. In addition, we discuss challenges in data

collection, as well as data processing, and finally, persisting and newly identified gaps in the knowledge of the mosquito product and production processes.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Quality Control tests for transported irradiated *Aedes albopictus* males

AUTHOR (S): Antonios Michaelakis[1], Nikolaos Papadopoulos[2] and Romeo Bellini[3]

ORGANIZATION: [1] Scient. Directorate of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, Kifissia, Greece; [2] Dept. of Agriculture, Crop Production and Rural Environment, University of Thessaly, Magnisias, Greece; [3] Centro Agricoltura Ambiente "G. Nicoli", Crevalcore, Italy (IAEA Collaborating Centre)

SHORT SUMMARY OF PAPER

Abstract:

In 2018 the first sterile insect technique (SIT) pilot trial against *Ae. albopictus* in Greece showed encouraging results justifying the continuation with larger scale trials. The main scope of this trial was to investigate the efficacy of SIT on wild population egg hatch rate in Greece using mass-produced sterile male mosquitoes from another country (Italy). Assessing the quality of mass reared, sterilized males that are transported to release sites is of outmost importance for the success of the SIT programs. The current study presents a series of quality control (QC) tests that have been conducted at delivery points in Greece to assess the impact of shipping on survival during transportation, longevity, flight ability and mating performance. Furthermore, a Mark-Release-Recapture (MRR) study on the post-capture longevity of irradiated and non-irradiated males was conducted aiming to record the (remaining) lifespan of the recaptured individuals maintained under laboratory conditions.

Our results for QC tests revealed detrimental effects of long transportation period on the sterile male *Ae. albopictus* while the flight ability of sterilized males following one day transportation time was satisfactory (over 60%). Response of sterile males to food and water starvation was comparable and slightly lower than that of wild non-transported males. Longevity of sterile males was shorter than that of wild counterparts and it seems it was not affected by mating to wild females. Regarding the MRR study, results revealed that both sterile and non-sterile males that were not released and maintained under laboratory

conditions had almost the same lifespan which is almost 20 days. This finding is very important since it's highlighting that irradiation is not affecting the lifespan of the transported males. Similar average lifespan was also recorded for the non-irradiated males which were released and recaptured. Another interesting finding is that when irradiated males are released and recaptured, the lifespan was 30-35 days slightly longer than non-irradiated released males.

Overall, our results clearly demonstrate that transported irradiated males are of good quality suggesting a series of reliable QC tests and MRR study on the post-capture longevity to be considered in future SIT operations employing transportation.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: The value of combining incompatible and sterile insect technique to control mosquitoes

AUTHOR (S): Yongjun Li, Luke Anthony Baton, Dongjing Zhang, Jérémy Bouyer, Andrew G. Parker, Ary A. Hoffmann, Lee Ching Ng, Cheong Huat Tan & Zhiyong Xi

ORGANIZATION:

SHORT SUMMARY OF PAPER

Abstract:

We have demonstrated the feasibility of applying combined incompatible insect technique (IIT) and sterile insect technique (SIT), or known as combined IIT-SIT, on a large scale to suppress or even eliminate the populations of vector mosquitoes. The classical SIT uses radiation to sterilize male mosquitoes, while in combined IIT-SIT, sterile matings are induced by *Wolbachia*, and the role of radiation is to sterilize Wolbachia-infected females that are mixed in the males (due to the imperfect sex-separation), so as to avoid the occurrence of population replacement. As female mosquitoes are more sensitive to radiation, combined IIT-SIT uses lower dose, thereby reducing the adverse effects of either the radiation itself or the handling process of radiation on the fitness of male mosquitoes. Under small-scale laboratory experiments, the dose required to sterilize the female pupae of *Aedes albopictus* HC line (infected with *Wolbachia w*AlbA, *w*AlbB and *w*Pip) is about 30 Gy, whereas we found that, during the large-scale pupae radiation process with more than fifty thousands of pupae loaded in a canister, the dose required for fully sterilizing female HC pupae is as high as 60 Gy. The main reason for the need of higher dose to sterilize female mosquitoes in large-scale is

the rapid consumption of oxygen, which results in hypoxia and thereby providing strong radiological protection in insects as it boosts antioxidant defence mechanisms. Therefore, when using radiation to sterilize mosquito pupae on a large scale, we should fully consider the density dependent issues.

In order to balance the irradiation effect on sterilization and male fitness, we chose 45 Gy (rather than 60 Gy) in the combined IIT-SIT. Therefore, HC females released into the wild were not completely sterilized. In fact, we did find *w*Pip positive *Ae. albopictus* larvae during mosquito releases, indicating that the accidentally released HC females had progenies in the field. However, during the two-year field monitoring, we observed that the offspring of HC mosquitoes temporarily and spatially isolated; in addition, after mosquito release activities stopped, we continued to monitor the *w*Pip infection status in *Ae. albopictus* population for a year and none of *w*Pip-infected individuals was detected. The above results indicate that combined IIT-SIT prevents population replacement occurring, which may be caused by the below reasons: (1) In combined IIT-SIT, HC male mosquitoes are partially sterilized (the sterilization rate was as high as 86.4%), so a large number of HC male mosquitoes released into the field also produces high sterilization effect after mating with HC females that may exist in the field. (2) HC mosquitoes, as compared to the wild-type Ae. albopictus, have relatively higher fitness costs in the field, as indicated by its lower larvae survival rate under malnutrition, shorter adult life span under stressed condition, and poor ability of females to locate the host.

Finally, in combined IIT-SIT, even if a small number of female mosquitoes are released, they have lower probability to transmit pathogens due to *Wolbachia*-mediated pathogen blocking. We have found that the *Wolbachia* density in ovaries and somatic tissues of irradiated HC female mosquitoes did not change, and they maintained resistance to dengue virus serotype 2. Furthermore, the greater radiosensitivity of female mosquitoes will further reduce their vectorial capacity by decreasing longevity.

In conclusion, our results show that combined IIT–SIT provides a practical way to solve current challenges due to a lack of perfect sex separation, through: (1) preventing population replacement; (2) potentially minimizing male fitness costs through the use of lower radiation doses than required by SIT (although we note that the mating competitiveness of male mosquitos can be reduced by up to half, which can be caused by several factors during scale-up for mass production); (3) reducing vectorial capacity of any accidently released females; and (4) facilitating scaling-up of mass production by removing the need for manual inspection after mechanical sex separation.

SECOND RESEARCH COORDINATION MEETING

On "Mosquito Irradiation, Sterilization and Quality Control"

Vienna, Austria

18-22 July 2022

TITLE OF WORKING PAPER: Effect of chilling in flight ability and sexual competitiveness of the Tapachula *Aedes aegypti* Genetically Diverse Strain

AUTHOR (S): Ariane Dor, Génesis Sánchez, Guillermo Bond, Carlos F. Marina and Pablo Liedo

ORGANIZATION: El Colegio de la Frontera Sur

SHORT SUMMARY OF PAPER

Abstract:

A pilot project on *Aedes aegypti* Sterile Insect Technique was initiated in Southern Mexico in 2018 with a Genetically Diverse Strain (GDS). Initially, mosquitoes were chilled and manually released by ground; after that, they were released by air using a drone, involving an additional chilling step in the process. Because of the COVID-19 pandemic and lack of government support, releases have been stopped since March 2020. Nevertheless, until 2022, part of the specific objective 1 (to establish the baseline of the quality parameters of the mass-reared GDS) and the whole objective 3 (to assess the sexual performance of the mass-reared males) were assessed. We tested whether the chilling processes affects sterile males' survival, flight ability and sexual competitiveness. First, we determined the effect of different chilling times on the survival and flying abilities of fertile male mosquitoes under laboratory conditions in four treatments plus a control (treatment 1: ground release protocol, with a chilling at 4 ° C for 25 minutes; treatments 2, 3 and 4: air release protocol, with a second chilling for 25, 50 or 100 minutes, respectively). To test the sexual competitiveness of irradiated males, the irradiated males were chilled under two treatments: ground (one chilling at 4°C for 25 min) and air release (two chillings at 4ºC for 25 minutes each). The experiment was carried out according to the protocol established by the IAEA and the Fried (1971) competitiveness index was calculated. Results show that chilling process affect the survival and the flight abilities of the mass-reared males. Flight abilities decreased even more with the two chilling processes and their time length. Sexual competitiveness is also affected by ground chilling process and even more by the air release process. These results show that it is necessary to meet compromises regarding the chilling processes to maintain the quality of the GDS male mosquitoes.

ANNEX 8: Intercomparison of dosimetry systems

PROFICIENCY TESTING BY INTERCOMPARISON OF DOSIMETRY SYSTEMS IRRADIATED WITH GAMMA (COBALT 60 SOURCE), 10 MEV ELECTRONS OR HIGH ENERGY X-RAYS

Issued on: August 22, 2016
By: Florent KUNTZ

INTRODUCTION

The Proficiency Testing Programs by interlaboratory comparisons have proven to be a useful tool to give opportunity for participants to demonstrate their technical competence and ensure the quality and traceability of their measurements. It may also help to identify problems (if any) related to the performance of personnel, equipment calibration and adequacy of methods.

This exercise is developed to evaluate two aspects:

Part 1: The ability of participants to meet preset dose values (targeted doses).

Part 2: The ability of the participants to measure doses actually applied.

REFERENCES

ISO/IEC 13528:2005, Statistical methods for use in proficiency testing by interlaboratory comparisons.

NPL Report CIRM 29 “Guidelines for the Calibration of Routine Dosimetry Systems for use in Radiation Processing”; September 2009

ISO 14470-11 Food irradiation -- Requirements for the development, validation and routine control of the process of irradiation using ionizing radiation for the treatment of food

ISO/ASTM 51204-12 Standard Practice or Dosimetry in Gamma Irradiation Facilities for Food Processing

ISO/ASTM 51431-11 Standard Practice for Dosimetry in Electron and Bremsstrahlung Irradiation Facilities for Food Processing

ISO/ASTM 51607:2013 “Practice for Use of an Alanine-EPR Dosimetry System”

ISO/ASTM51275-13 Standard Practice for Use of a Radiochromic Film Dosimetry System

ISO/ASTM51650-13 Standard Practice for Use of a Cellulose Triacetate Dosimetry System

ISO/ASTM 51276-12 “Practice for Use of a Polymethylmethacrylate Dosimetry System”

ISO/ASTM 51401-13 Standard Practice for Use of a Dichromate Dosimetry

ISO/ASTM 51702-13 Standard Practice for Dosimetry in Gamma Radiation Facilities for Radiation Processing.

PROFICIENCY TESTING MANAGEMENT

Testing dosimeters

Proficiency test dosimeters: 4 alanine dosimeters (containing 4 pellets each) for each participant (1 is identified as control (Control / Do not irradiate) provided by Aérial.
For each target dose value, only one provided alanine dosimeter and if necessary one dosimeter from the participant dosimetry system is irradiated.

Confidentiality

To ensure confidentiality performance of the participants, Aérial will safeguard the results issued by each participant so as to maintain the confidentiality of the data and results for the performance of participants.

Schedule

Date of reception of dosimeters by participants: October 2022
Deadline for reception of irradiated dosimeters by Aérial: December 2022
Date of report delivery: March 2022

Parameter to be evaluated

The parameter to determine is the delivered dose to the dosimeters and the ability to target a given dose.
Dosimeters should be irradiated to doses (to water) of 35 Gy, 70 Gy and 150 Gy.
At this stage, irradiation with gamma (Cobalt 60 source), 10 MeV electrons or with X-rays (1 MV accelerating voltage minimum) is requested.

Proficiency testing registration

For registration, each DEXAFI participant will be sent an invitation email. Individual acknowledgement for participation is required to get the dosimeters.
Aerial will send to the participants, via the IAEA IPCL a package containing:
a) 3 alanine dosimeters (each dosimeter contains 4 pellets)
b) 1 alanine dosimeter to preserve as control, which should not be irradiated.

Proficiency testing progress

After registration, Alanine dosimeters will be sent to each participant along with an irradiation form, which must be completed by the participant after irradiation and sent with rapid mail to Aerial along with the irradiated dosimeters at the following address:

Florent KUNTZ
DEXAFI Intercomparison
Aerial-CRT
250 rue Laurent Fries
67412 Illkirch
France
@: florent.kuntz@aerial-crt.com
Phone: +33 3 88 19 15 17

Each dosimeter referred to in paragraph a) should be irradiated only with one of the indicated doses, (35Gy, 70 Gy, and 150 Gy)
The control dosimeter referred to in paragraph b) must remain with the other 3 dosimeters mentioned in a), except at the time of irradiation. The control dosimeter should never be irradiated.
The minimum dosimeter temperature has to be measured at the start of irradiation process, using a thermometer.
The maximum dosimeter temperature during irradiation has to be measured/evaluated throughout the irradiation process, using a thermometer/GEX thermolabel/experience. (Alternatively, measure the temperature near the dosimeter before and after irradiation and calculate effective temperature).

For each dose point, only one alanine dosimeter described in paragraph a) should be used, plus routine dosimeters belonging to the participant dosimetry system.

To ensure electronic balance, alanine and participant´s dosimeters must be enclosed with polystyrene or PMMA of 3 to 5 mm thickness.

The design and manufacture of container phantom of dosimeters is in charge of the participant. It must ensure that Alanine dosimeter and routine dosimeter from participant gets the same dose. The dimensions of alanine dosimeters are found in Annex.
Measurements of dose from dosimeters belonging to the participant´s dosimetry system must be made using the technique and equipment used by the participant in their normal routine work.
Participants must return to Aerial the irradiated Alanine dosimeters and the control dosimeter, along with the irradiation form right after irradiation is completed.
Irradiation form should be completed with the results of the participant’s dosimeters dose, temperature of alanine dosimeter before irradiation and maximum temperature during irradiation, irradiation date, irradiation duration, radiation type, participants details, ...

STATISTICAL EVALUATION

Performance evaluator

xi dose value of the dosimeter irradiated by the participant measured by Aerial
X dose value given by the participant (close to the assigned 35Gy, 70 Gy, and 150 Gy)
u(xi) uncertainty (k=1) on dose measured by Aerial
u(X) uncertainty (k=1) on dose given by participant

The evaluator will be the Z performance parameter:

$$Z = \frac{xi - X}{\sqrt{u(xi)^2 + u(X)^2}}$$

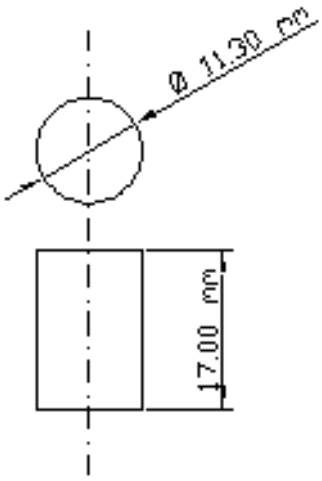
The acceptability criterion is:
If -2 ≤ Z ≤ 2 the result is considered acceptable.
If $\mathbf{2} < |\boldsymbol{z}| < \mathbf{3}$ the result is considered questionable.
If $|\boldsymbol{z}| \geq \mathbf{3}$ the result is not acceptable.

NOTE: The statistical treatment of the data may vary from that described according to the feedback of the participant's results.

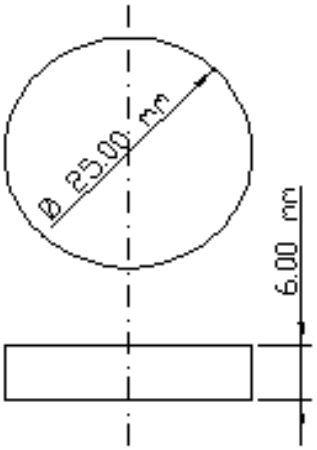
REPORT
Once the results are obtained from the participants, a preliminary report with the confidential results will be made and presented to IAEA.
Final results will be presented during the second RCM of the CRP 'Mosquito Irradiation, Sterilization and Quality Control'.

ANNEX

Dosimeter holder dimensions for gamma irradiation



Dosimeter holder dimensions for electron beam irradiation



Aérial	IRRADIATION FORM	ID FE/I 01 - --- / -------

IRRADIATION FORM TO BE COMPLETED BY DEXAFI PARTICIPANT

Participant details:	Contact person:
Full postal Address :	Phone : Fax :
	E-mail :

Irradiation information

Dosimeter ID	Irradiation date	Irradiation duration	Temperature before irradiation (°C)	Maximum temperature during irradiation (°C)	Radiation type (XRay, ɣ, EBeam)	Energy (MeV)	Assigned dose (kGy)

Control	**Do not irradiate**						

Absorbed dose readings with participant's dosimetry system(s)

Dosimeter ID	**Participant dosimetry system**	**Measured dose (kGy)**	**Uncertainty (k=2) (kGy)**

Date:

Visa:
Please return this form with irradiated dosimeters to:
Florent KUNTZ
DEXAFI Intercomparaison
Aérial-crt
250 rue Laurent Fries
F 67412 Illkirch
France

E mail :

ANNEX 9: Inter-lab comparison of flight ability and semi-field competitiveness

How is male *Aedes* mosquito flight ability data related to semi-field competitiveness index?

Objective: To know whether flight ability scores are correlated to male mating competitiveness.

Biological material: *Aedes aegypti* and *Aedes albopictus*

Rearing, irradiation and experimental conditions:

- Rear male mosquitoes following standard validated protocols,
- Provide 10 % sucrose solution *ad libitum* to adults
- Make sure that the rearing schedule gives males of desired and synchronized age groups

Pupae sampling: Aliquot 110 pupae/sex in 100mL- cups (males and females separate). Collect enough for each experiment and the number of replicates needed (see below for flight tests and competitiveness designs). Make sure that all females are virgin.

Adult-compaction: after emergence, chill adult cages (15×15×15cm) into the cold room (4°C for 10min) to knock down the mosquitoes; use a mouth aspirator to collect mosquitoes from each cage and transfer to 1×1×1 cm compaction boxes (100 males/cm^3). Close the box with a piece of netting (5×5cm) and a plastic rubber band (1.5cm=diameter) (**Figure 1)**

Figure 1. Mosquito compaction (100males/cm^3) using 1×1×1 cm boxes for adults (2-3-day-old)

Irradiation: Irradiate *en masse* (100males/cm^3) using 1×1×1 cm compaction boxes for adults (2-3-day-old) or pupae (24h-30h-old) following standard irradiation procedures (See Guidelines for Irradiation of Mosquito Pupae in Sterile Insect Technique Programmes | IAEA , Appendix I and III) with the dose inducing 99% sterility for your strain/irradiator. A higher dose will be used (100 Gy more for the effect of high dose irradiation on mating competitiveness experiment). For instance, 150Gy for *Ae. aegypti* and 100Gy for *Ae. albopictus*.

Laboratory room test conditions for flight test: 26 ±2 C, RH%= 70±10, light: 500-1000 lux; time= 8-10 am (morning).

Semi-field large cage size: 175×175×175 cm, 5.36 m^3 (Live Monarch. Boca Raton. USA)

Experiment. Effect of high irradiation dose

Irradiation: Adult males of 2-3 day-old will be irradiated with low irradiation (inducing more than 99% sterility) and high irradiation (100Gy more than the low dose) doses.

A_ Flight ability

- Three treatments (low, high doses and non-irradiated)
- Run the flight test on irradiated (with low and high doses) and non-irradiated

mosquitoes

- Run the test 24h after irradiation
- Three technical replicates for each treatment

B_ Competitiveness in large cages

The following design will be used for the competitiveness (**Figure 2A)**. The test will be performed 1 day after irradiation with 3-4-day-old males.

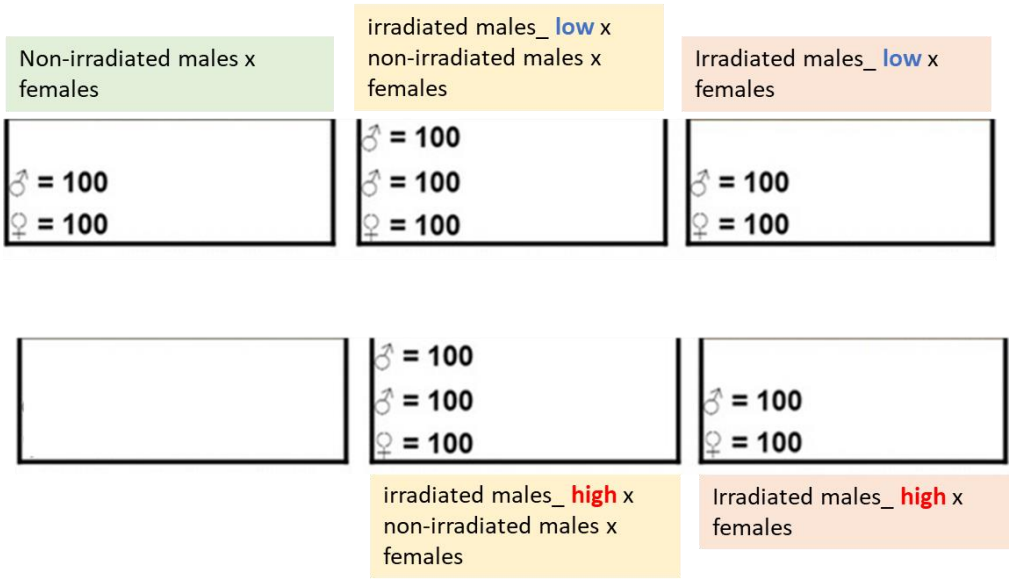


Figure 2A. Experimental design in semi-field conditions. Three control cages (irradiated (low, high doses) and non-irradiated males only) and competition cages, each containing 100 males (irradiated and/or non-irradiated) and 100 virgin females. Low and high stand for low irradiation (inducing more than 99% sterility) and high irradiation dose (100Gy more that the low dose)

Figure 2B. Example of a semi-field cage experiment setting: Nine 30×30×30cm control cages (irradiated (low, high doses) and non-irradiated males only) and six large competition cages, each containing 100 males (irradiated and/or non-irradiated) and 100 virgin females. Low and high stand for low irradiation (inducing more than 99% sterility) and high irradiation dose (100Gy more that the low dose)

Procedures as per the Figure 2B:

- Only competition cages will be used in large cages in the semi-field settings
- Control mating cages will be 30 × 30 × 30 cm but placed in the semi-field conditions.

Mosquitoes (males and females) from control groups (low, high, non-irradiated) will be chilled to ease the transfer into the cages (Figure 2C).

- 

Figure 2C. Plastic cups containing chilled adult to transfer into control (low, high, non-irradiated) cages

- Three cages for each competition treatment (6 large cages in total) will be placed in semi-field conditions
- Males (irradiated and non-irradiated) will be released first and will be allowed to acclimate for 60 min prior to releasing females (3-4-day-old).
- After the mating period of 24 hours, females will be collected using mechanical aspirators, chilled to remove potential remaining males and cages.
- Females will be brought back to the laboratory
- Collected number of females will be recorded.
- Females will be offered a blood meal for 2 consecutive days (30-60min/feeding event).
- Daily survival will be monitored and egg cups for egg collection offered. One egg batch will be collected. Dead females will be kept in the freezer for dissection.
- Competitiveness index will be calculated following Fried formula (see below parameters to be measured)
- A sample of 15 females/cage will be dissected to assess insemination rates (number of spermathecae filled with sperm) after egg collection.

PS: the use of rhodamine will allow true competitiveness

Parameters to be measured:

Flight test:

Number of escaped (flyers) and non-escaped (non-flyers) males

Competitiveness:

- **Number of eggs per cage** (based on females recaptured from semi-field cages)
- **Fecundity** for each treatment by dividing the number of eggs laid by the number of females still alive (before egg collection)
- **Insemination rate**s to estimate the average number of females that laid eggs and the average number of eggs laid per female; to assess mating propensity/treatment
- **Egg hatch rates** (fertility) by dividing the number of hatched eggs counted by the number of laid eggs (hatched + non-hatched). Eggs will be dried (1 week) prior to hatching (See Guidelines for Irradiation of Mosquito Pupae in Sterile Insect Technique Programmes | IAEA, pages 20-24)).
- **The competitiveness index** (C) (Fried index): using egg hatch rates from the non-irradiated control (Ha), irradiated control (Hs) and competitiveness treatments (Ho) as follows: $C = ((Ha–Ho)/(Ho–Hs)) \times (N/S)$; where N is the number of non-irradiated males and S the number of irradiated males.

Each experiment will be repeated three times.

Reference of flight ability test and video:

Frontiers | Standardization of the FAO/IAEA Flight Test for Quality Control of Sterile Mosquitoes (frontiersin.org)

QR code_ video of the flight test operation:

Or Link :
https://www.dropbox.com/s/22tks0hhp31qokq/Flight%20test%20device%20instructional%20video%20cut2%20with%20sub%20and%20music.mp4?dl=0

Script of the Flight ability test video:

0:00-0:06 “The flight ability of mosquitoes can be measured using a Flight Test Device, as developed by the Insect Pest Control Subprogramme.”

0:19-0:28 “The Flight Test Device consists of six separate parts:

- a base plate;
- a transparent containment box with an open top and a circular netted opening;
- a top cover;
- the flight tube itself, composed of 40 individual tube’s each 25 cm high with an inside diameter of 8 mm placed inside a large containment tube;
- a 12V fan with an air flow of 0.218m^3/min, am acoustic noise of 20.6dB and a rated speed of 6000rpm;
- and finally, a rubber base ring with a square of netting.”

0:29-0:39 “To assemble the Flight Test Device, begin by placing the base plate inside the rectangular containment box. The base should be placed with the metal rods facing downwards so that there is a space between the bottom of the containment box and the base plate.

0:39-0:51 “Next, cover the rubber base ring with the square of netting and fit it to the bottom of the large containment tube. Make sure the rubber ring fits the containment tube tightly, but do not to cover the small, 1 cm hole at the bottom of the tube yet.”

0:52-0:57 “Move the tube inside the rectangular containment box and cover the latter with the top cover.”

0:59-1:06 “Put 2-3 small pellets of BG lure from Biogents, Regensburg, Germany in the decoy cap on the top cover.”

1:07-1:13 “Place the 12V fan directly on the decoy cap facing downwards so that air is blown into the containment box.”

1:17-1:24 “Switch on the fan for all Flight Test Devices. Gently aspirate 100 male mosquitoes of the same age with a manual aspirator.”

1:28-1:37 “Release them via the small hole at the bottom into the large containment tube of the Flight Test Device. Use the containment box’s netted opening to ensure mosquitoes do not escape the Flight Test Device.”

1:37-1:45 “Once all the mosquitoes are inside the containment tube, push the rubber base ring and netting upwards to cover the small hole through which mosquitoes were introduced by attaching the containment tube to the base plate. Repeat this procedure for at least 5 replicates per treatment that can be run in parallel.”

1:48-2:14 “Confined within a small volume, the instinct of mosquitoes is to fly upwards, through one of the 40 flight tubes, and out into the large, containment tube.”

2:19-2:25 “After two hours, cover the top of the containment tube with a petri dish of 9 cm of diameter and turn off all fans. At this point, the experiment is considered as complete.”

2:26-2:32 “Slowly remove the containment tube through the net of the large circular opening in the containment box.”

2:32-2:36 “When the tube is half-way out, be careful to hold the petri dish covering the top of the tube in place, to avoid any escapees.”

2:36-2:41 “The mosquitoes remaining in the tube are considered as ‘Non-fliers’ whereas the ones remaining in the containment box are considered as ‘fliers” to calculate the flight rate.”

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[i] Culbert NJ, Balestrino F, Dor A, et al. (2018) A rapid quality control test to foster the development of genetic control in mosquitoes. Sci Rep 8, 16179.

Hamidou Maïga, Deng Lu, Wadaka Mamai, Nanwintoum Séverin Bimbilé Somda, Thomas Wallner, Mame Thierno Bakhoum, Odet Bueno Masso, Claudia Martina, Simran Singh Kotla, Hanano Yamada, Gustavo Salvador Herranz, Rafael Argiles Herrero, Chee Seng Chong, Cheong Huat Tan and Jeremy Bouyer. Standardization of the FAO/IAEA Flight Test for Quality Control of Sterile Mosquitoes. Front. Bioeng. Biotechnol., 18 July 2022 Sec. Biosafety and Biosecurity. https://doi.org/10.3389/fbioe.2022.876675