Project Title	**Enhance Agency's capacity to provide support to Member States to control Aedes mosquitoes as vectors of human pathogens, particularly Zika virus, using integrated vector management approaches with a Sterile Insect Technique component** *Subprogramme 2.1.4 Nuclear Techniques for Sustainable Control of Major Insect Pests* *Project 2.1.4.3: Development of the SIT for the control of disease transmitting mosquitoes (2000023)*
Rationale and Project Description	Mosquitoes (Diptera: Culicidae) are bloodsucking insects that may carry pathogenic micro- organisms which cause infectious diseases resulting in severe morbidity or lethality. In the absence of vaccines and efficient, safe and inexpensive drugs to control dengue, chikungunya and the Zika virus disease, many consider integrated population control of the insect vector, to which the sterile insect technique (SIT) can be included, as the most effective way to manage these diseases. The Agency, through the Insect Pest Control (IPC) Section and Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, has been implementing under its Major Programme 2 a project (2.1.4.4) "*Development of the SIT for the control of disease transmitting mosquitoes*." The project has carried out research and development (R&D) to further develop all aspects of the SIT package for some major disease vectors, in particular *Anopheles arabiensis*, *Aedes aegypti* and *Aedes albopictus*. The Joint Division is also providing crucial backstop support to a number of national, regional and interregional technical cooperation (TC) projects related to the SIT for mosquitoes. Responding to recent outbreaks of Zika virus and requests from affected Member States for urgent assistance, the Agency is developing an off-cycle regional TC Project "*Strengthening Regional Capacity in Latin America and the Caribbean for Integrated Vector Management Approaches with a Sterile Insect Technique Component, to Control Aedes Mosquitoes as Vectors of Human Pathogens, particularly Zika Virus*" (GOV/2016/12). While a growing interest by Member States in the project is foreseen and an urgent implementation of the project is expected, the Agency is facing challenges of in-house capacity in providing timely backstop support for activities envisaged in this off-cycle regional project: namely, expert support, coordination meetings, training courses and the procurement of equipment for Member States necessary to carry out mosquito rearing and related SIT activities. With the current budget for the mosquito project, the Agency has limited manpower and infrastructure at the Insect Pest Control Laboratory (IPCL) at the FAO/IAEA Agriculture and Biotechnology Laboratories to carry out all the activities required to service the above large off-cycle regional TC project. Most importantly, several mosquito strains of *Aedes aegypti* and *Aedes albopictus* to apply the integrated vector management SIT/IIT (sterile insect technique/incompatible insect technique) approach need to be developed for Member States. In addition, before transferring these strains to Member States, they need to be introgressed into the genomic background of different local target populations. Furthermore they need to be evaluated in respect to their mass-rearing stability and suitability, as well as to assess their biosecurity and quality under large-scale rearing conditions for use in SIT-based population suppression pilot projects. Furthermore, in view of the rapidly growing demand, ongoing efforts need to be accelerated to develop genetic sexing strains and to further improve and refine mosquito rearing efficiency, handling, shipping and air releasing procedures.

	Against this background, this project proposes to enhance the Agency's in-house scientific and technical capacity to provide backstop support to ongoing and future TC projects using the SIT for mosquitos, in particular to the aforementioned TC regional project for Latin America and the Caribbean region, and will focus on (1) development of strains required for the SIT/IIT integrated vector management approach; (2) development of genetic sexing lines; (3) mosquito rearing efficiency, handling, shipping and aerial release methods; (4) introgression and quality control analysis of strains to be sent to Member States.
Project Technical Background	The SIT consists of (1) the mass rearing of a target species, (2) the separation of males and females, (3) the use of ionizing irradiation to sterilize males, (4) the handling of the irradiated males and their release in the field where they should compete with wild males for mating with wild females; (5) matings between irradiated sterile males with wild females result in no offspring. The continuous and systematic release of sterile males over a town or city will ultimately result in the suppression of the target mosquito population. The critical component in the mosquito SIT package is sex separation to avoid the release of females, which are the blood-sucking gender and the ones transmitting the pathogens such as Zika to humans. Currently, the sex separation in *Aedes* mosquitoes (*Aedes aegypti* and *Aedes albopictus*) is being done with a manual / mechanical procedure which is tedious and labor intensive. This is not completely effective and results in sexing errors. It is clear that this method will be inefficient in medium to large-scale operational programmes. The way to overcome this problem requires the development of genetic sexing strains (GSS) similar to the strains currently used in all large-scale area-wide integrated pest management programmes with a SIT component to control the Mediterranean fruit fly worldwide. The development of a GSS is based on the availability of a selectable genetic marker and an irradiation-induced translocation which links this genetic marker with the sex-determining chromosomal region. Males carry the wild-type (normal) phenotype while females carry the marker, thus facilitating their separation from males. The IPCL has initiated genetic screening to isolate such genetic markers in both *Aedes aegypti* and *Aedes albopictus*. An initial set of morphological markers is available and now requires evaluation in respect to their rearing efficiency and male mating competitiveness before proceeding to the induction of irradiation-induced translocations. In addition, the CRP D4.40.01 - *Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes* (2013-2018) is ongoing with the participation of 19 scientists from 13 Member States to develop such GSS. In the meantime, until an efficient and robust sex separation method can be made available, the IPCL has proposed the integration of the SIT with the incompatible insect technique (IIT) which can eliminate two major risks: (a) the risk of pathogen transmission associated with the release of any females while applying the SIT (even if they are irradiated, the released sterile females can still bite and transmit pathogens); and (b) the risk of mosquito population replacement associated with the IIT (the release of fertile females will ultimately result in population replacement instead of population suppression with all the accompanying biosafety and biosecurity issues). It has been shown in *Aedes* (and *Anopheles*) mosquitoes that the symbiotic bacterium

	Wolbachia, which is responsible for IIT, can also provide protection against human pathogens such as Dengue, Chikungunya and Zika. In other words, females carrying this symbiont cannot transmit these pathogens. It is understood that, in most insect species, females can be fully sterilized with low irradiation doses. Hence, by applying low irradiation doses to a mass-reared *Aedes* mosquito strain carrying this symbiont, both risks can be eliminated because even if some females are released, these females will be fully sterile and unable to transmit pathogens. The IPCL has provided the proof of concept for this integrated vector management SIT/IIT approach against *Aedes albopictus* and is transferring it to *Aedes aegypti* too. It is important to note that every single strain which is developed, either GSS or for the integrated SIT/IIT approach, needs to be evaluated for its rearing efficiency and male mating competitiveness. In addition, before its release in the field it will have to be introgressed into the genomic background of the local targeted mosquito population. For example, if a mosquito strain is developed on an "Italian" genomic background, it might not be suitable for release in Brazil if it is not first transferred to a Brazilian genomic background. Such introgression steps are often necessary for biosecurity reasons. Another critical component for *Aedes* mosquito SIT is the handling of sterile mosquitoes, their transfer to the release sites and their actual release. *Aedes* mosquito SIT aims to suppress populations at the level of a village, small town or eventually large city. For small pilot studies, mosquito releases are currently being done from the ground, which will however not be effective and cost-efficient for larger-scale programmes, for which aerial releases will be considered. CRP D4.40.02 - *Mosquito Handling, Transport, Release and Male Trapping Methods* (2015-2020) is ongoing with the participation of 22 scientists from 19 Member States to develop such procedures. The IPCL has also been working towards the further improvement of the mass rearing, the handling and shipping methods for sterile *Aedes* mosquitoes, including their quality control analysis. However, it should be mentioned that every single strain which is developed for *Aedes* mosquito SIT pilot projects in Member States should undergo such a quality control analysis in confined environment (such as the IPCL greenhouse) prior to any field release.
Planned Activities	**1. Development of strains required for an integrated vector management SIT/IIT approach for *Aedes aegypti* and *Aedes albopictus* (in the absence of efficient sex separation methods)** a) Develop strains for the integrated vector management SIT/IIT approach for *Aedes albopictus* and *Aedes aegypti*. One *Wolbachia*-infected *Aedes albopictus* strain has already been developed and was used to provide the proof of concept that this approach can suppress populations of this species in field cage studies performed at the IPCL. The same strain is currently being tested in pilot field trials in China. More than one strain of each vector species need to be developed in order to select the best ones after evaluation and quality control analysis. b) Determine / confirm the minimum irradiation dose required for this integrated approach. **2. Development of genetic sexing lines in *Aedes aegypti* and *Aedes albopictus* mosquitoes**

	a) Screen natural and laboratory populations of *Aedes aegypti* and *Aedes albopictus* for the presence of morphological or conditional temperature lethal genetic markers. b) Induce morphological or conditional temperature lethal genetic markers in *Aedes aegypti* and *Aedes albopictus* by chemical mutagens (EMS). c) Establish lines with the isolated markers, characterize them genetically and evaluate their rearing efficiency and male mating competitiveness under small scale rearing conditions. d) Use the stable and evaluated lines carrying the markers for the construction of irradiation-induced translocation lines and GSS. **3. Mosquito rearing efficiency, handling, shipping and air releasing:** a) Refine rearing protocols and equipment, e.g. the development of a more cost-effective larval diet; different ingredients and probiotics will be assessed for the development of a higher quality and more cost-effective diet for both *Aedes aegypti* and *Aedes albopictus*. b) Optimize handling and shipping methods that need to be compatible with the release of sterile mosquitoes by air. c) Assess different air release strategies ,be coupled with quality control analysis of the released sterile mosquitoes. **4. Introgression and quality control analysis of strains to be sent to Member States:** a) Evaluate mosquito strains developed for the integrated vector management SIT/IIT approach in respect to their rearing stability and suitability. b) Evaluate the constructed GSS in respect to their stability and suitability for use in SIT-based population suppression programs under small scale rearing conditions. c) Introgress the developed strains into the genomic background of different local target populations and assess their biosecurity and quality under large-scale rearing conditions for use in SIT-based population suppression pilot projects. **5. Technical backstopping the off-cycle regional project activities, including:** a) Manage technical coordination meetings. b) Design and lecture at regional training courses. c) Carry out technical advisory missions to Member States and prepare job descriptions for other specialist experts to carry out supportive missions. d) Provide specifications for mass-rearing and related equipment and materials for their procurement. e) Ship specialized supplies and strains to Member States participating in the off-cycle regional project.

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<tr><td>Impact</td><td>This project will result in the development and transfer to Member States, in particular those participating in the aforementioned regional TC project for Latin America and the Caribbean region, of evaluated strains of Aedes aegypti and Aedes albopictus mosquitoes. These strains will be used in integrated vector management approaches with a SIT component for the population control of mosquitoes transmitting Dengue, Chikungunya and particularly Zika. The procedures developed and the information generated will be made available for Member States of IAEA interested in embarking on similar integrated vector management activities with a SIT component.</td></tr>
<tr><td>Proposal Period</td><td>Four years (April 2016 - April 2020)</td></tr>
<tr><td>Total Estimated Budget</td><td>Timeline for activities and expenses:
<table>
<tr><th>Year</th><th>Activity</th><th>Cost</th></tr>
<tr><td>April 2016-April 2017</td><td>Materials and equipment required for maintenance of all the different the mosquito strains.</td><td>€89 000</td></tr>
<tr><td></td><td>Materials and equipment required for the development of strains for the integrated vector management SIT/IIT approach.</td><td>€101 000</td></tr>
<tr><td></td><td>Materials and equipment required for the genetic work for the construction of GSS.</td><td>€88 000</td></tr>
<tr><td></td><td>Materials and equipment required for refining the rearing efficiency, handling, shipping and air releasing procedures.</td><td>€113 000</td></tr>
<tr><td></td><td>Materials and equipment required for the introgression and quality control analysis of strains which will be sent to Member States for pilot releases.</td><td>€105 000</td></tr>
<tr><td></td><td>Human resources – 1 P-3 and 3 G-3 TA positions</td><td>€201 000</td></tr>
<tr><td></td><td>Containers to provide laboratory and office space to implement the significantly enlarged activities of the mosquito project. These containers need to be bio-secure with netting systems and double doors.
Note: The containers are a temporary solution pending construction of required additional space under ReNuAL and ReNuAL Plus. The containers may also address problems related to space limitations in the available IPCL greenhouse.</td><td>€140,000</td></tr>
<tr><td></td><td>Total cost</td><td>€837 000</td></tr>
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	Programme Support Cost (7%)	€58 590	
	Total cost for 2016-17 (including 7% PSC)	**€895 590**	
April 2017-April 2018	Materials and equipment required for maintenance of all the different the mosquito strains.	€64 000	
	Materials and equipment required for the development of strains for the integrated vector management SIT/IIT approach.	€75 000	
	Materials and equipment required for the genetic work for the construction of GSS.	€75 000	
	Materials and equipment required for refining the rearing efficiency, handling, shipping and air releasing procedures.	€105 000	
	Materials and equipment required for the introgression and quality control analysis of strains which will be sent to Member States for pilot releases.	€79 000	
	Human resources – 1 P-3 and 3 G-3 TA positions	€201 000	
	Total cost	€599 000	
	Programme Support Cost (7%)	€41 930	
	Total cost for 2017-18 (including 7% PSC)	**€640 930**	
April 2018-April 2019	Materials and equipment required for maintenance of all the different the mosquito strains.	€49 000	
	Materials and equipment required for the development of strains for the integrated vector management SIT/IIT approach.	€65 000	
	Materials and equipment required for the genetic work for the construction of GSS.	€65 000	
	Materials and equipment required for refining the rearing efficiency, handling, shipping and air releasing procedures.	€105 000	
	Materials and equipment required for the introgression and quality control analysis of strains which will be sent to Member States for pilot releases.	€69 000	

		Human resources – 1 P-3 and 3 G-3 TA positions	€201 000
		Total cost	€554 000
		Programme Support Cost (7%)	€38 780
		Total cost for 2018-19 (including 7% PSC)	**€592 780**
	April 2019-April 2020	Materials and equipment required for maintenance of the all the different mosquito strains.	€49 000
		Materials and equipment required for the development of strains for the integrated vector management SIT/IIT approach.	€65 000
		Materials and equipment required for the genetic work for the construction of GSS.	€65 000
	2	Materials and equipment required for refining the rearing efficiency, handling, shipping and air releasing procedures.	€105 000
		Materials and equipment required for the introgression and quality control analysis of strains which will be sent to Member States for pilot releases.	€69 000
		Human resources – 1 P-3 and 3 G-3 TA positions	€201 000
		Total	€554 000
		Programme Support Cost (7%)	€38 780
		Total cost for 2019-20 (including 7% PSC)	**€ 592 780**
	Grand Total		**€ 2 722 080**
Beneficiary Member States	All Member States, in particular the ones which are facing problems with *Aedes* mosquitoes and the pathogens they transmit (Zika, Dengue and Chikungunya). The immediate focus will be given to those States in Latin America and the Caribbean region which participate in the aforementioned regional TC project.		