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Monitoring of Temephos Fluctuation in Bathwater and Its Toxicity Towards HaCaT Cells

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Dengue virus is the most prevalent mosquito-borne Flavivirus and is known to cause dengue haemorrhagic fever. Currently, there are no specific anti-dengue treatments or any licensed vaccine available to treat or prevent the disease, causing dengue incidence to rise up to 30-fold in the past few decades. To stop the increasing incidence, dengue eradication programs have leaned towards vector control using the larvicide Temephos. Temephos can be used to eradicate mosquito larvae in household water bodies such as drinking water containers and bathwater containers. While the World Health Organization has claimed that Temephos-treated drinking water is safe for consumption, the toxicity of Temephos towards human skin from repeated bathwater exposure has not yet been analysed. Therefore, this study aims to investigate the effect of daily Temephos-treated bathwater exposure towards immortalized human keratinocytes (HaCaT cells). We monitored how Temephos concentration would fluctuate by simulating daily bathwater cycles. We found that the concentration of Temephos quickly diminished from the original application of 1 mg/L to almost undetectable using HPLC after 5 days of Temephos application. Furthermore, our toxicity assay results have demonstrated that Temephos could not significantly reduce the viability of HaCaT cells and neither could it induce more cell death compared to negative control. However, further expression studies on apoptosis-related-proteins are needed to elucidate the full extent of Temephos toxicity at the cellular level. Aside from the toxicity, Temephos rapid decline within water reservoir after water usage is a rather huge problem in vector control. A new policy on Temephos application should be reviewed.

The duration of temephos residual effects in household-owned water-holding tanks (the most productive container type and main target for control) was estimated prospectively in two trials. Temephos was applied using spoons or inside perforated small zip-lock bags. Water samples from the study tanks (including positive and negative controls) were collected weekly and subjected to larval mortality bioassays. Water turnover was estimated quantitatively by adding sodium chloride to the study tanks and measuring its dilution 48 hs later. The median duration of residual effects of temephos applied using spoons (2.4 weeks) was significantly lower than with zip-lock bags (3.4 weeks), and widely heterogeneous between tanks. Generalized estimating equations models showed that bioassay larval mortality was strongly affected by water type and type of temephos application depending on water type. Water type and water turnover were highly significantly associated. Tanks filled with piped water had high turnover rates and short-lasting residual effects, whereas tanks filled with rain water showed the opposite pattern. On average, larval infestations reappeared nine weeks post-treatment and seven weeks after estimated loss of residuality.

Dengue is currently the most important viral disease of humans transmitted by arthropods worldwide. Aedes aegypti, a human-biting mosquito dwelling in artificial domestic containers, is the main vector of dengue. Ae. aegypti larval control programs are frequently based on the application of the insecticide temephos. A five-year larval control program in northeastern Argentina significantly reduced infestations but could not maintain them below target levels, especially during summer. Identifying the underlying processes responsible for such shortcomings is important for improving dengue prevention strategies. Large water-holding containers were the most productive container type and the main targets for control. We found that the duration of temephos residual effects in household-owned large tanks was much shorter than expected and allowed early reinfestation post-treatment. The main factor limiting temephos residuality was fast water turnover, caused by householders' practice of refilling tanks overnight to counteract the intermittence of the local water supply.

The duration of the residual effects of a given treatment (i.e. the amount of time the treatment is effective for vector control after its application) is a very important metric needed to estimate the frequency of treatment applications required to achieve control objectives. Under field conditions, treatments are affected by site-specific processes that modify the duration of residual effects relative to what is measured in controlled experiments under more artificial conditions. Therefore, the ultimate evaluation of treatment effectiveness is under field conditions.

Temephos, an organophosphate insecticide not toxic for humans at recommended doses, has been extensively used as a larvicide against Ae. aegypti during the past 40 years. It is generally applied in granular formulation and delivered into containers using spoons, and more recently, inside permeable bags for slow release and reintroduction after householders clean treated containers. Reference publications traditionally considered that temephos residual effects lasted between 8 and 12 weeks or about 5 weeks. A recently published guide for dengue vector control indicated: "Two or three application rounds carried out annually in a timely manner with proper monitoring of efficacy may suffice, especially in areas where the main transmission season is short. Recent studies using different temephos formulations, application procedures and experimental conditions have shown widely variable durations of residual effects ranging from 1 to 6 months. The actual residuality of temephos under field conditions has rarely been documented. Infestation was detected within 7 days post-

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treatment with temephos in Brazil and Nicaragua, but in the former study the number of experimental units was very limited (<18) whereas in the latter containers from 1,903 study houses were treated without the supervision of the investigators and observed only once post-treatment. In Peru, the larvicidal effect of temephos started to decline 7 weeks post-treatment but the field study only included eight experimental units. None of these studies sought to identify the processes that caused such limited, widely variable effectiveness of temephos.

Our field-based study conducted in Clorinda had four objectives: (i) Estimate the duration of the residual effects of temephos in large water-storage tanks by means of larval mortality bioassays; (ii) Compare the effectiveness of temephos applied with spoons or inside permeable zip-lock bags; (iii) Identify factors and processes associated with the eventual decay of temephos residuality, and (iv) Describe the temporal pattern of Ae. aegypti immature infestation in containers treated with temephos. During each trial, lots with selected containers were visited and the proposed activity was explained to the head of each household who was asked to give oral consent for temephos treatment, following customary practices of the ongoing larval control program since 2003. If permission was granted, consent was recorded in a form and each tank was treated with temephos at the recommended dose of 1 ppm (1% granular formulation, Fersol) by experienced FMS field personnel who regularly conduct vector control operations in the area. Following treatment, water samples from each study tank were collected weekly into 500 ml glass jars. Prior to collection, the water of each tank was stirred. Each jar was placed in expanded polystyrene thermic boxes and transported to the local FMS laboratory. This procedure is very similar to the one described by Palomino and others. Two control tanks, one positive (treated with temephos) and one negative (untreated), were prepared in 300 liter-fibrocement tanks filled with piped water and then kept fully lidded and protected from rain and direct sunlight at the backyard of the laboratory.

Immediately after the arrival of water samples to the laboratory, mortality bioassays were performed by exposing 20 third- or fourth-instar larvae of Ae. aegypti to each water sample and recording mortality 24 hs later. The glass jars were left unlidded during the bioassays. Similar methodologies have been used previously. The larvae used were the second generation of larvae collected in a randomly selected block of Primero de Mayo in 2007 and were reared at the local FMS laboratory. A temephos-treated container was considered to have lost larvicidal effects when bioassay larval mortality was <70%.

Conclusions

Temephos residuality in the field was much shorter and more variable than expected. The main factor limiting temephos residuality was fast water turnover, caused by householders' practice of refilling tanks overnight to counteract the intermittence of the local water supply. Limited field residuality of temephos accounts in part for the inability of the larval control program to further reduce infestation levels with a treatment cycle period of 3 or 4 months

Biography

Wibi Sindjaja has completed his Biomedicine (infectious disease stream) Bachelor degree on 2019 from Indonesia International Institute for Life-sciences (i3L).

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