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REVIEW ARTICLE

Toxicity of the Organophosphorus Pesticide Temephos

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ABSTRACT

Dengue is a major public health problem in tropical urban areas, not only because it can quickly progress from the mild dengue fever to the deadly dengue hemorrhagic fever, but also because there is no single cure or licensed vaccine available to this day. To control the disease, the World Health Organization has recommended insecticides to control the number of mosquito vectors *Aedes aegypti* and *Aedes albopictus*. One of the main insecticides used is temephos, which inhibits the progression of the mosquito life cycle at the larvae stadium. Temephos is a member of the organophosphorus group of insecticides which is known to exhibit neurotoxicity through a common cholinergic pathway to insects and mammals. Despite its possible toxicity towards humans and other non-target organisms, temephos has been used widely to treat household water, including drinking water and bathwater. Although clinical studies have yet shown any detrimental effects due to chronic consumption of temephos, studies on animal models have shown neurodevelopmental toxicity, while at the molecular level, exposure to temephos has demonstrated genotoxic effects. Temephos is also considered an environmental contaminant and accumulation in soil and water have caused toxicity towards water organisms. Considering the extensive and repeated usage of temephos in public health, understanding and confirming the safety of temephos towards human health is crucial. Therefore, the objective of this paper is to review the current body of work available on the toxicity of temephos as a common dengue vector control.

Keywords: *Aedes; Dengue; Organophosphate Insecticides; Temephos; Toxicity*

INTRODUCTION

Dengue virus is transmitted through the mosquito *Aedes aegypti* and *Aedes albopictus*. The virus comes from the genus *Flavivirus* of the family *Flaviviridae,* and it is believed that dengue infections come from four antigenically distinct serotypes which are dengue virus (DENV)-1, DENV-2, DENV-3, and DENV-4. Recently in 2013, the fifth variant DENV-5 was isolated and reported (Mustafa, 2015). This disease presents with a wide spectrum of clinical findings that range from asymptomatic illness, dengue fever (DF), dengue hemorrhagic fever (DHF), and ultimately dengue shock syndrome (DSS) (Rajapakse, 2011). The mild manifestations include febrile fever, headache, joint pain, and low levels of thrombocytes in a

blood examination. However, in the more severe cases, plasma leakage, respiratory distress, bleeding, and organ impairment may occur (Simmons *et al*, 2012; World Health Organization, 2014). While the exact mechanism that triggers severe dengue is currently unknown, it is known that a sequential secondary infection of dengue from different dengue strains and having a symptomatic infection at a younger age of 3-18 month increases the chance of getting a severe manifestation (Guzman, Alvarez, & Halstead, 2013; Trung & Wills, 2014). Systemic prolonged plasma leakage lowers circulating blood volume which leads to hypovolemic shock while plasma leakage in the lungs causes pulmonary edema which leads to respiratory failure (Ranjit & Kissoon, 2011; Simmons *et al*, 2012). Death by severe dengue is caused by organ failure and plasma leakage (Sam *et al*, 2013).

Dengue causes about 96 million clinical cases annually, in which 500,000 cases result in severe dengue that leads to 20,000 deaths (Bhatt *et al*, 2013; World Health Organization, 2012). Since a decade ago, dengue infection has risen by 30-fold in 40 years (Olivieira, 2017). This upsurge has been driven by many factors such as population growth, global warming, and inefficient vector control. The World Health Organization (WHO) has thus deemed dengue a major public health challenge, especially in tropical and subtropical countries (Bhatt, 2013). Even so, till now, there is no specific anti-dengue treatment or any licensed vaccine effective enough to treat or prevent the disease spread (World Health Organization, 2012). Therefore, to reduce the incidence number of this arthropod-borne disease, it is preferable to target the vector's life cycle as a cost-effective and efficient way to cut the chain of infection (Lemon *et al*, 2008).

Dengue virus enters and infects a host organism through the skin following an infected mosquito bite. Female *Aedes* mosquitoes are particularly notorious for being infected and transmitting these viruses to humans. Although not the only vector, *Aedes aegypti* is the main vector of dengue and it also causes other diseases such as Zika fever and Chikungunya fever, which are endemic in many countries in the world. It is a domestic species with a preference for human blood as the female mosquitoes rely on blood for reproduction. Other than through biting an infected host, virus transmission can also occur vertically, i.e., when an infected female transmits the virus to its offspring (Olivieira, 2017). It is also observed that *Aedes aegypti* favor water containers as a breeding site, while *Aedes albopictus* favors wet trash. Even so, both prefer still water to breed (Dom, Ahmad, & Ismail, 2013). Moreover, both adult *Aedes aegypti* and *Aedes albopictus* mosquitoes often hide within human residences or around the perimeter to breed (de Moura Rodrigues et al, 2015). The habitat preference of both the adult and larvae serve as a basis for vector control.

One of the most widely practiced means in the effort for sustainable and integrated eradication of not only dengue but also other arthropod-borne diseases is through vector control using organic or inorganic insecticides (Rose, 2001). Knowing the preferred habitat of mosquito vectors, governments around the world have tried controlling adult mosquitoes through fogging methods and eliminating larvae through the distribution of larvicides (World Health Organization, 2012). Insect control practices have actually been used for centuries to keep insects away from human settlements and facilities with insecticides mostly used in agriculture to protect crops against harmful insects (Matthews, 2015). Although insect

control is beneficial in agriculture and public health, it is also a source of both direct and indirect insecticide exposure for workers and the general population, respectively. Daily exposure of insecticides, for example through contaminated food products (Franklin & Worgan (Eds.), 2005), can be detrimental for human health since most insecticides, for example, organophosphates, often have neurotoxic effects.

Organophosphates are the most commonly used class of insecticides in agriculture but are also the most common cause of pesticide poisoning (Peter & Cherian, 2000). Principally, organophosphates work by interfering with neuronal transmission regulation, causing cholinergic overactivation that leads to neuronal death (Prahlow & Kincaid, 2013). Despite the danger of neurotoxicity, there is one organophosphate that is widely recommended by the WHO for dengue control purposes, which is temephos. Temephos is used to control mosquito larvae in potable water and drinking water sources (World Health Organization, 2012). Considering the widespread usage of temephos as a vector control for *Aedes* and the well-known harmful effects of organophosphates on human health, this review aims to discuss the safety of temephos for dengue control and the toxic effect of temephos towards humans.

Dengue Vector Control

In Indonesia and many other nations where dengue is endemic, fogging is the government-driven, widely implemented, method of vector control, especially in urban areas and during the rainy season (Oki *et al*, 2011). Fogging refers to the application of insecticides in the form of aerosol droplets, which can be achieved either through heat (thermal fogger) or air pressure (cold fogger) (Matthews, 2008). The thermal fogger utilizes oil-based insecticides heated using a fogger machine which turns it into very small fog-like droplets (Hoffmann *et al*, 2008). While, the cold fogger disperses insecticides into small droplets in the air, allowing the insecticides to spread to small spaces where insects hide while only using a small amount of insecticide (Farooq, Salyani, & Walker, 2013).

The fogging method targets adult mosquitoes and the commonly used adulticides are malathion, piperonyl butoxide, pyrethrins, and pyrethrum extract (Hazra *et al*, 2017). The application of adulticides has been shown to have significant effects to inhibit the prevalence of dengue when applied during the rainy season, while the application during other times has been found to have no significant effects toward dengue prevalence (Oki *et al*, 2011). This might be caused by the increase of mosquito breeding sites, as the main vectors for dengue, *Aedes aegypti* and *Aedes albopictus*, are known to lay eggs on still water in urban areas (Barrera, Amador, & MacKay, 2011; Gratz, 2004; Rapley *et al*, 2009). Although the use of adulticides to control dengue vector is effective enough to affect the prevalence of the disease, it is only effective as a temporary solution. Moreover, the insecticides used for fogging such as malathion have negative environmental effects including organ malfunction in fish, death of beneficial insects, and the possibility of polluting ground-water (Newhart, 2006).

Other than fogging, the most common method of dengue vector control is mosquito larvae control using larvicides. Temephos is a larvicide that is commonly used under the recommendation of WHO, specifically for *Aedes aegypti* and *Aedes albopictus* mosquito larvae (Gratz, 2004; World Health Organization, 2012). The suggested application of temephos is by adding one gram of temephos per ten litres of water into a water container once in every

month. This application is considered effective as the residual larvicidal effect of temephos may persist for more than a month depending on water turnover rate and water salinity (Garelli *et al*, 2011; Pinheiro & Tadei, 2002). Therefore, using temephos is preferred to hold the progression of dengue vector all year long. Temephos is easy to obtain and apply, and it provides at least a month of protection against mosquito larvae on still water surfaces.

Pharmacokinetics of Temephos

Temephos is a non-systemic organophosphorus pesticide mainly used in public health for vector control (World Health Organization, 2012). It has a low water solubility of 30 µg/L at 25^{o}C giving it a slow-release property, hence, making the residual presence and residual larvicidal effect possible for a longer period of time (Thavara *et al*, 2005). It also has a low melting point of 30-30.5^{o}C (Milne, 2018; Thavara *et al*, 2005). Temephos is also soluble in other solvents such as acetonitrile, carbon tetrachloride, diethyl ether, dichloromethane, and toluene (Milne, 2018).

Temephos works similarly to any other organophosphate insecticide, which is by binding to the serine residue on the acetylcholinesterase enzyme (AChE) active site within the neural synapse of insects (Figure 1) (Marsillach, Costa, & Furlong, 2013; Pontual *et al*, 2012). AChE has a role in cleaving acetylcholine which is responsible to convey neuronal signals through the synaptic cholinergic pathway, stopping neural stimulation (Clementi (Ed.), 2012; Gutzeit & Ludwig-Müller, 2014). The loss of AchE activity causes accumulation of acetylcholine which then activates the nicotinic and muscarinic acetylcholine receptors (Jokanović & Kosanović, 2010). The nicotinic acetylcholine receptors are responsible to increase cellular influx of calcium ions while the muscarinic acetylcholine receptor is responsible to release glutamate for signaling (Alkondon *et al*, 2000; Araque *et al*, 2002; Grasshoff *et al*, 2003; Martin & Alger, 1999; Rathouz, Vijayaraghavan, & Berg, 1996; Shin *et al*,2015). Glutamate released by muscarinic acetylcholine receptors will activate N-methyl-D-aspartic acid (NMDA) receptors which act as an ion channel to increase calcium influx (Rothstein, 1996). The high increase of cellular calcium influx then will cause osmotic disbalance, causing the cell to swell and eventually rupture (Beck *et al*, 2003). On the other hand, calcium will also cause mitochondrial stress and increased metabolism, which will trigger higher production of reactive oxygen species and the release of intracellular apoptotic signals (Roman, Clark, & Swanson, 1981; Szalai, Krishnamurthy, & Hajnóczky, 1999).

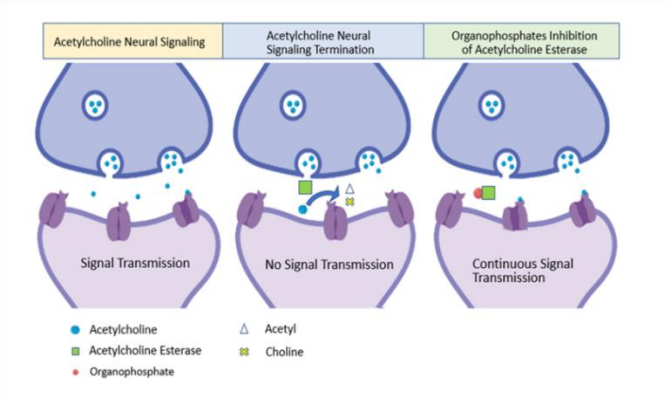

Figure 1. The acetylcholine pathway and its interaction with organophosphates. Acetylcholine neural signaling starts when the axon terminal of presynaptic neurons release vesicles of acetylcholine that will attach to acetylcholine receptors on the dendrites of postsynaptic neurons. This cascade terminates when acetylcholinesterase (AchE) binds to free acetylcholine and cleaves it into acetyl and choline. However, in the case of organophosphate exposure, organophosphates will competitively bind to AchE and inhibit acetylcholine from being cleaved by acetylcholine esterase, thus, maintaining constant acetylcholine signaling (Jokanović and Kosanović, 2010).

A hypothesis exists that states the toxicity of temephos relies not only on temephos itself but also on its transformation products. It is found

that transformation products of organophosphates are generally more toxic than its unaltered compound (Chambers, 1993; Sparling & Fellers, 2007). Temephos in nature can transform into temephos sulfoxide, temephos oxon, temephos sulfoxide isomer, and temephos oxon isomer (Figure 2) (Lacorte, Ehresmann, & Barceló, 1996). The temephos transformation products are able to persist in the environment, mostly as temephos sulfoxide (Kamel *et al*, 2009; Lacorte, Ehresmann, & Barceló, 1996). More importantly, temephos transformation products are also found to be produced quickly in the rat body and the transformation products are known to be metabolically stable as they are not further transformed before excreted through feces and urine (Blinn, 1969). However, the past studies are insufficient to elucidate the exact mechanism of action of temephos and its transformation products.

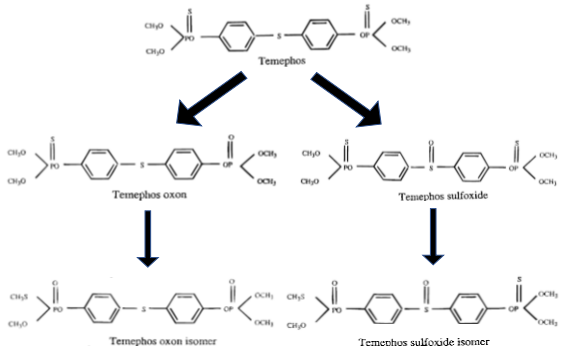

Figure 2. Temephos transformation products. In nature, temephos can transform into temephos oxon, temephos sulfoxide, and their isomers (Lacorte *et al*, 1996).

Toxicity of Temephos

Similar to target organisms of temephos, humans share the cholinergic pathway and neuronal communication mechanism. Therefore, organophosphates may also harm humans if the cholinergic pathway is disrupted (Fest & Schmidt, 2012). Furthermore, organophosphates are also known to target other pathways with or without serine residues (Casida & Quistad, 2004; Lockridge & Schopfer, 2010; Marsillach, Costa, & Furlong, 2013; Pope, 1999; Terry Jr, 2012). The fact that organophosphates can interact with many other proteins allows organophosphates to exhibit different kinds of toxicity aside from cholinergic toxicity. However, for the case of temephos, non-cholinergic toxicity has yet to be investigated.

Temephos has been long deemed to be safe, even for drinking water treatment. For example, a toxicity study done by Laws Jr *et al* have shown that in clinical trials, temephos does not cause any toxicity upon acute ingestion of either 256 mg/person/day for 5 days or sub-chronic ingestion of 64 mg/person/day for four weeks (Laws Jr *et al*, 1967). Laws Jr also found that there were no symptoms reported by the participants in the short period of time that the study was done. On the following year, Laws Jr published another study on temephos application as a drinking water treatment. In the study Laws Jr gave water treated with 1 ppm temephos to 20 participants for 19 months and found that there was neither lethal side effects nor toxicity symptoms that could be linked directly with temephos consumption (Laws Jr *et al*, 1968).

The latest study done in Brazil took a different approach in which Magalhães and Caldas took medical records of patients suffering from insecticide poisoning (Magalhães & Caldas, 2019). They found that adult pesticide poisoning cases that happen in the Brazilian Federal District are mostly occupational, meaning the majority of the patients were either farmers or environmental monitoring agents. The study also found that cases of temephos poisoning happened to patients who were working as environmental monitoring agents and made about more than 70 % of the

total pesticide poisoning cases in that occupation. The study also found that the most reported symptoms of temephos poisoning was a headache and no mortality happened.

Put together, the earlier study done by Laws Jr *et al* showed that the subjects without any occupational exposure risks did not have any poisoning upon either acute or chronic consumption of temephos, while his newest study showed that occupational exposure may have played a great role in temephos poisoning. The environmental monitoring agents might be exposed to temephos contaminated water to gather water samples and other environmental data (Gan & Bondarenko, 2008; Lacorte, Ehresmann, & Barceló, 1996). However, the amount of contamination and period of exposure that is required to exhibit any symptoms is not known. From previous studies, it seems that temephos does not cause lethal side effects in cases of human consumption, while, temephos occupational exposure exhibits more danger.

While temephos does not exhibit any toxicity in clinical trials and was only suggested to exhibit mild neuronal symptoms on occupational exposure, the mechanism of toxicity of temephos in mammals and their cells has not been exactly elucidated. Aiub *et al* tried to find non-neuronal toxicity of temephos and found that temephos might have mild genotoxic effects fragmenting DNA in blood cells (Aiub *et al*, 2002). The genotoxicity of temephos was measured using comet assay to see individual blood cell DNA strand breaks using single-cell electrophoresis. The result of the study was that higher doses of temephos corresponded to the increase of the severity of DNA strand breaks. However, the limitation of the method used in this study to classify DNA strand break severity was that the classification was conducted non-quantitatively by observing the comet tail length.

The fact that treatment with higher concentrations of temephos corresponded to longer tails in comet assay using lymphocytes, was confirmed in a more recent study by Benitez-Trinidad *et al* (2015). Within the study, the length of DNA was quantified to confirm the actual genotoxic effect and it was found that the genotoxic effect does correspond positively with temephos concentration. Even so, the genotoxicity of 10 µM of temephos is still inferior to that of 0.05% dimethyl sulfoxide (DMSO), a commonly used solvent in biochemistry and cell biology. Coupled by the fact that DMSO is known to not exhibit any significant genotoxic effects means that temephos has very slight genotoxic effects (Aye *et al*, 2010; Valencia-Quintana *et al*, 2012).

Demonstrating that temephos has the ability to cause genotoxic effects *in vitro,* leads to the thought that temephos exposure might be dangerous in early life stages such as embryo development. That is why a recent study conducted by Vani *et al*, tried to elucidate any toxic effects of temephos towards pregnant Swiss mice and their embryos. In the study, Vani *et al* discovered that administration of temephos at 10 times (0.0043 mg/Kg) the commercially recommended concentration for 18 days of the gestational period does not correlate to any physiological malformation of the mice fetus even though temephos was also discovered inside the placenta (Vani *et al*, 2018). Furthermore, the study also measured the genotoxicity of temephos during the 16^{th}, 17^{th}, and 18^{th} day of gestation using micronucleus assay. They found that temephos does exhibit a concentration-dependent genotoxic effect towards the female mice on the 16^{th} and 17^{th} day of gestation. However, the cause of micronuclei decline in female mice was

not known and the direct genotoxicity of temephos towards the fetus was not measured either. The study concludes that temephos exhibits genotoxicity at a very low level.

Knowing that temephos exhibits genotoxic effects towards embryos and that it primarily targets nerves cells, it raises a great question if temephos exposure during the gestational period might have detrimental effects on fetus development. To answer the question, Laurentino *et al* further looked into the behavioral effect of temephos consumption during the gestational period (Laurentino *et al*, 2019). However, in contrast to the study done by Vani, Laurentino used a higher dose of 50 mg/kg during the 6^{th} to 13^{th} day of gestational period. The study showed that pups with mothers that had been exposed to temephos during the gestational period, showed hyperactivity during open a field test while having less social interaction during the reciprocal social interaction (RSI) test. Through this study, it is clear that temephos exposure causes a detrimental effect to mice fetus. The hyperactivity and the impaired social interaction of mice with prenatal temephos exposure might have been caused by impairment of the hippocampus. On the other hand, it happens that the hippocampus is one of the brain regions that utilize acetylcholine signaling heavily to regulate motoric function, spatial learning, and social behavior (Calandreau *et al*, 2006; Fadda, Cocco, & Stancampiano, 2000; Gold, 2003; Rubin *et al*, 2014). Previous studies have not shown the effect of temephos towards individual areas of the brain that corresponds to different tasks, so future research is important to assess the effect of ingested temephos towards individual regions of the brain.

With evidence from the aforementioned behavioral studies, there is indeed an indication that temephos might possess a teratogenic effect. Temephos contamination to fertile mallard egg on LC50 concentration is found to cause general growth reduction as a form of complication, but do not cause any organ-specific growth reduction or deformities as other pesticides do (Hoffman & Albers, 1984). In another study on mallard hens, the consumption of 100 PPM temephos also causes a reduction in the weight of the ducklings while having an increased indicator of liver damage (Franson, 1983). Both studies found that temephos consumption is not enough to cause miscarriage or fetal abnormalities, but there might be some unobservable damages happening in the fetus that cause growth and weight reduction. Aside from the teratogenic effect, temephos is also known to have higher toxicity towards young invertebrates. A study by Fleming and colleagues (1985) found that drinking water with 100 PPM temephos causes higher mortality and depression of AChE activity while drinking water with 10 PPM temephos does not cause an increase in the mortality of the ducklings. Another study also found that temephos is able to increase mortality in frog tadpoles alongside a decrease in AChE activity.

From these studies, it is demonstrated that temephos consumption in early age and maternal consumption within the gestational period is able to cause growth reduction and even mortality if consumed in high amount. Although temephos usage in drinking water containers is considerably low compared to the amount taken by the animal models, the gestational period of a human is far longer than those of animal models. So far, there are no accurate models of low dose chronic exposure of temephos towards the human fetus. Studies in higher-level animals with similar gestational periods might be needed to actually mimic

chronic temephos exposure during the gestational period.

In comparison to temephos, prenatal exposure of organophosphates in general is known to be associated with hyperactivity, reduced brain development and volume, as well as reduced cognitive performance shown in adolescence. A study on the organophosphate prenatal exposure by Whyatt and colleagues (2004) found that the concentration of organophosphate found in umbilical cord such as diazinon and chlorpyrifos was correlated with lower birth weight and length. In some other studies, prenatal organophosphate exposure marked by a common metabolic marker called dialkyl phosphate (DAP) was associated with a slight decline in intellectual quotient and working memory (Bouchard, 2011; Rauh, 2011). Furthermore, Rauh and colleagues (2012) compared the brain structure of babies exposed with different level of chlorpyrifos based on umbilical cord blood analysis to discover that higher level of chlorpyrifos exposure results in reduced frontal and parietal cortical thickness. The evidence of organophosphate developmental toxicity in further confirmed by a finding that points out that reduced maternal organophosphate enzymatic breakdown also contributes to the severity of the cognitive decline in prenatal organophosphate exposure (Engel, 2011).

Looking at the evidence, general prenatal exposure of organophosphates have a detrimental effect of brain and cognitive development, even when it does not cause any major deformities. Although temephos is an organophosphate pesticide, there are no human studies on the brain and cognitive development after temephos prenatal exposure. However, the effect of prenatal exposure in animal studies has a similar outcome to human population studies on other types of organophosphates. Both other types of organophosphates and temephos prenatal exposure caused a decrease in birth weight and a slight growth inhibition.

CONCLUSION

Temephos is indispensable for dengue control as it remains the most cost-effective, relatively safe, and the only method that provides long term effect against dengue vector. Clinical trials have provided data that temephos has little to no observable effect on general human health. However, it has been proven that temephos possesses a mild genotoxic effect, thus, it could be possible that temephos would exhibit detrimental effects in the long run and towards fetal development. Behavioral studies have also shed light on the neurodevelopmental effect of temephos exposure towards the mouse brain and cognitive function. However, based on the available data, we can conclude that more studies on the toxicity of temephos, especially regarding prenatal exposure must be conducted. Lastly, essential policy reforms must be reviewed regarding the safety of temephos usage and environmental exposure to children and pregnant women.

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