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Persistence of Organophosphorus and Pyrethrinoid Insecticides, used for Malaria Control, in Soil and Water: Case of the District of Vohipeno, South-Eastern Madagascar

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Abstract

Malaria still persists in underdeveloped countries, including Madagascar, where it is the eighth leading cause of morbidity in Madagascar (SSS, 2011). Thanks to the application of the two insecticide-impregnated mosquito nets (ITN) and the Intra-Home Spraying Campaign (IDAC) systems, this rate has risen from 21.57% in 2003 to 5% in 2011 for children under 5 years of age and from 17.57% to 2.3% in 2011 for children over 5 years of age. The malaria mortality rate increased from 25.92% to 19% for children under 5 years of age and from 13.5% to 5% for those over 5 years of age (SSS, 2011). However, these control methods are based on the use of pesticides and require great caution. Indeed, due to poor practice, organophosphorus (insecticide used in CAID) and pyrethrinoid (insecticide impregnated on ITNs) were detected after analysis The instrument used was an Infinity 1290 Agilent Technologies HPLC Infinity 1290 system (Santa Clara, CA, USA) coupled with an Agilent Technologies 6460 triple quadrupole mass spectrometer in soil, surface water and groundwater from the three villages (Tanandava, Vohitramba and Savagna) in the south-eastern region of Madagascar where sampling takes place.

Keywords: Insecticides; Surface water; Soil; Groundwater; CAID; MID; Vohipeno

Introduction

Globally, 86% of deaths due to malaria concern both children under five years' old and pregnant women. Ten to 20% of children who survive from severe malaria have serious and disabling neurological sequels [1]. In 2013, Madagascar presented the peaks of malaria especially during the periods of rains in the east and the south-east regions of the Island. The most serious and alarming cases were observed in March and April 2013: the number was multiplied by 8 to 10 Infants (with a prevalence of 6% for the whole country) and pregnant women are the most vulnerable. The solid intensification of the fight against malaria interventions between 2001 and 2013, contributed to 47% to reduce rates of mortality due to malaria, avoiding an estimated 4.3 million deaths [2].

This rate currently shows a decline thanks to different systems of struggles, including the Intra-Domestic Spray Campaign (CAID) and the distribution of Mosquito Nets impregnated with insecticides (MID). These control strategies are largely based on chemical pesticides. However, safety and hygiene after each campaign must be reinforced in order to avoid the risks to the environment, including the impact on non-target organisms and the persistence of the product. This study focuses on safety, hygiene and the follow-ups of the methods of struggles after their application (post-campaign of intra domestic spray and after the distribution of MID), carried out in order to maintain environmental and human health. Due to the Eco toxicity and the application of these insecticides every year, the presence of these molecules in the environment will present a risk to the environment in general.

Thus, in the context of the persistence of insecticides during the fight against malaria in water and environmental risk assessments, the general objective of this study is to detect the presence or absence of insecticides in water (surface water and groundwater), in soils within villages where CAID and MID distributions occur in order to protect non-target organisms and ecosystems. The specific objectives of the research are to: (1) take surface water, groundwater and soil samples from the villages where the two systems (of struggles) have been applied, (2) analyse the water and soil samples (Surface and groundwater) in the targeted villages from upstream to downstream.

So, we have made hypothesis that insecticides used during intra-domestic spraying and in bed nets impregnated during malaria fight can permanently degrade the quality of surface water or the river of Matitanana, groundwater (water pumps) for consumption and soil.

Study Area

The south-eastern region of Madagascar is among the regions where malaria epidemic persists [3]. Despite major results recorded on the progress and performance of the interventions, a dramatic resurgence of malaria cases occurred in late 2011 and the first quarter of 2012 in the endemic south-east coast of Madagascar. From 2008 to 2010, an increase of 2.5 to 10 times of the reported cases number was observed and this affects all the age groups with inconstant RTD rates of positivity. These results suggest that a usual endemic perennial transmission zone is in the phase of mutation to an epidemic with an epidemic tendency. Preliminary findings of the survey show rates of coverage and lower MID use than expected, 2 years after a universal campaign. This could be due to climate change and/or due to the consequences of various strategies of the fight against malaria in the events of behaviour changes of vectors or human immunological status

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changes. The official launch of CAID took place on August 5, 2016 in the south-east of Madagascar. MID have also been distributed at the same time, in order to fight more effectively to the vectors of malaria. After a year, 90% reduction in mortality due to malaria and 50% of cases were found, thanks to two additional preventive measures used in the fight against malaria (Figure 1).

The next CAID for the year 2017 will be on July 24. Before the campaign; samples were collected from sprayed villages in the district of Vohipeno, region of Vatovavy Fitovinany on July 3, 2017 (Table 1). These areas were chosen because the Intra-Domestic Spay Campaign (CAID) took place there and these are places where MID have been distributed. This study can help documenting on the good practice of CAID, MID spray and mosquito nets distribution so that insecticides do not harm the environment.

Materials and Methods

Data collection

Water sampling: During this study, three water sampling were performed in the section of the river Matitanana close to each of the three studied village. Water samples were collected at about 10 cm from the river edge using a container. Collected water were poured into a half-litre sterile glass bottles, previously rinsed with distilled water and washed three times with the water from the river. The top of each bottle was closed tightly with an aluminium foil. Each bottle was labelled and stored in a cooler until the lab analyses [4].

Ground water: In the three villages 3 groundwater samples were taken from the zip pumps. The water was bottled and washed with water from the pump before use. Then they were sealed with aluminium foil and kept in a glacier until they reached the analysis laboratory [4].

Soil sampling: A hole of about 60 cm of depth was dug in the ground. A soil sample was taken in at 5 cm depth in the vertical edge of the hole. Soil samples were taken with a shovel for the underlying horizons, stored in a cooler at 4°C until the lab analyses. For Vohitramba and Tanandava, ten samples per village were taken near the holes where wastewater was discharged. The holes were spaced 1 m apart [4]. While for Savagna the soil samples were taken near the sump where the insecticides residues spilled, the used materials washed and the sprinklers took their bath. The sample No. 1 was taken from the soils near the sump for the spill of the insecticides residues, washing of the materials used in spraying and the showering place for the decontamination of the sprinklers. Whereas samples No. 2 to 10 were taken away gradually from the sump. It is worth noting that this sump is a rectangle 1 m in wide, 2 m in long and 1 m in high. This sump is closed and present in both sides of the fence of 1 m^2 of side and 1 m of height. This sump contains from the depth of the materials preventing the infiltration of residues to the soil (gravel, gravillonnette ...). Then at the end of the campaign, the sump has been covered with concrete so that it won't be reused. In the next campaign, another well will be

Samples	Description
Cultivated Plot	-
0-30 cm	laboured horizon, presence of crops
30-60 cm	Underlying horizon Horizon not taken up by labour
More than 60 cm deep	Pesticide leaching horizon

Table 1: Delimitation of horizons in the profiles and morphological used criteria.

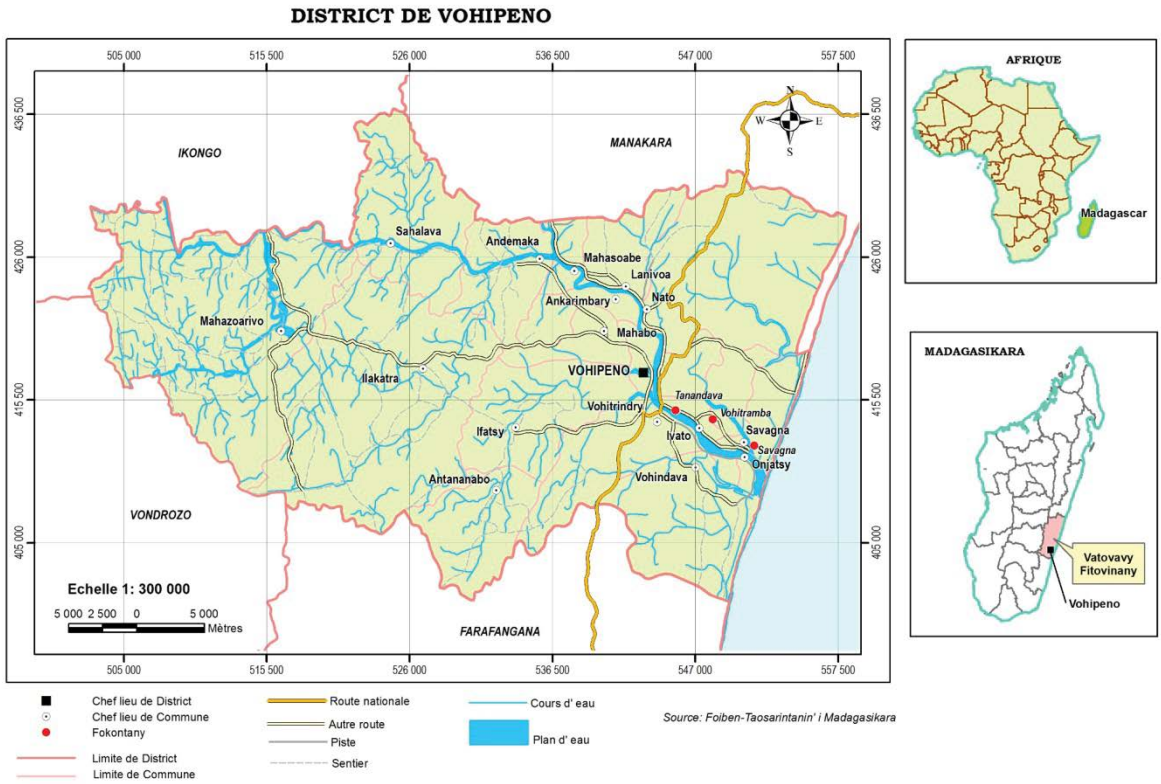

Figure 1: Study area and sampling sites in south-eastern Madagascar.

built in another village. Soil samples are carefully examined to remove stones, leaves or other plant material found there.

Extraction of pesticide residues from soil samples

Extraction and analysis method

Calibration: The principle is based on the addition of a reference compound to the sample. This reference compound, called a standard, must be as close as possible in terms of physico-chemical properties to the one being analyzed and must not be present in the initial sample. The isotopically labelled molecule is chosen. The standards are added at the beginning of sample preparation, and will have the same behavior as the molecules to be analyzed with respect to the different stages of extraction and analysis.

Standard pyrethrinoid standard solution of 100 mg/l*: We dissolved 0.0100 g of pyrethrinoids in approximately 80 ml ethyl acetate in an ultrasonic bath for 2 minutes and added 100 ml with ethyl acetate.

Organophosphorus standard solution of 100 mg/l*: We dissolved 0.0100 g organophosphate in approximately 80 ml ethyl acetate in an ultrasonic bath for 2 minutes and make up to 100 ml with ethyl acetate.

Extraction technique

Soil sample extraction technique: We added 10 g of Na_2SO_4 to the wet soil sample and then 50 µl of the standard solution, this mixture was stirred to homogenize. Then we added 60 ml ethyl acetate to the sample and stir for 1 hour on the mechanical stirrer. Then, the filtration mountain was prepared by depositing a layer of Na_2SO_4 on a Gelman filter type A/E, the supernatant was decanted in an Erlenmeyer. The extracts were then transferred to a 250 ml flask of which we added 2 ml of isooctane and evaporated to a volume of 2-3 ml using a rotary evaporator. The bath temperature must not exceed 30°C. The extract was transferred into a 15 ml tube previously calibrated to 5 ml, rinsing the flask with isooctane.

Water sample extraction technique (surface and ground water): A control solution is prepared by measuring 500 ml of demineralized water. An enriched solution is prepared in introduction with a 250 µl of the 500 ml standard solution of demineralized water. Then we added 30 ml of saturated Na_2SO_4 and 50 µl of the extraction standard solution to the samples, control and enriched solution, followed by 250 ml of dichloromethane in each sample bottle (300 ml for control and enriched solution) and stir for about 30 seconds and release the gas. The bottles are placed on the mechanical stirrer and shake vigorously for 20 minutes, taking care to place foil under the caps. The sample is transferred to a 2-litre ampoule and the organic phase was recovered in a 500 ml flask by passing it through an anhydrous bed of Na_2SO_4. The ampoule and bottle are rinsed with a little dichloromethane and added to the 500 ml flask. In each 500 ml flask containing the extracts, 1 ml of isooctane was added and evaporated to about 2-3 ml with a rotary evaporator. The temperature of the bath should be approximately 30°C. The centrifugation tubes are calibrated from 10 ml to 0.5 ml. The extract is transferred to the 10 ml tube. The flask is rinsed with dichloromethane. Then concentrated in a small volume under argon jet at about 400 µl in a bath between 25-30°C. For each tube of isooctane was added and calibrated to 500 µl. The extract is transferred to a glass micro flask for analysis.

Analysis technique: The instrument used was an Infinity 1290 Agilent Technologies HPLC Infinity 1290 system (Santa Clara, CA, USA) coupled with an Agilent Technologies 6460 triple quadrupole mass spectrometer. After injection of 5 ml of sample, the compounds are separated by a Kinetex column (100 × 2.1 mm; 1.7 m, Phenomenex, Torrance CA, USA) in C18 reverse phase grafted, maintained at 35°C with a moving phase gradient (0.5 ml min^{-1}) composed of ultrapure water acidified with 0.1% acetic acid buffered with ammonium acetate (5 mM) and MeOH. The gradient begins at 100% aqueous phase and changes to 100% organic phase in 14 minutes, then reverts to 100% aqueous phase to condition the system (during 4 minutes) for a new injection. After separation, the compounds are ionized with an electrospray source and analyzed in tandem mass spectrometry. The injection of each sample was followed by the injection of a rinsing methanol blank and a methanol blank to determine the state of the system before a new sample was injected. In addition, maintenance (cleaning) at the source was regularly carried out between each series of analyses to ensure proper ionization of the molecules and thus not lose sensitivity.

Data Processing

The acquired data have been reprocessed with software SpectrAA.

Results

The soil, surface water and groundwater contamination activities for the three villages were carried out with variable non-parametric univariate statistical test. Kruskal-Wallis analysis of variance for independent samples. All calculations were done at the threshold α=0.05 with the software [5].

Persistence of organophosphorus and pyrethrinoid in water

We carried out 30 analyses on soil samples near the insecticide tailings storage sump (fokontany Savagna) and near waste water weirs (fokontany Tanandava and Vohitramba). Pyrethrinoids were detected in 73% of the analyses carried out, while organophosphates were detected in 77% of the analyses carried out. In the 10 analyses of surface water samples, pyrethrinoids were detected in 70% of the analyses carried out, while organophosphates were detected in 80% of the analyses carried out. Finally, in the 10 analyses of groundwater samples, pyrethrinoids were detected in 70% of the analyses carried out, while organophosphorus was detected in 80% of the analyses carried out.

In the village of Savagna, pyrethrinoids concentrations vary between 0.008 mg. l^{-1} and 0.128 mg. l^{-1} in groundwater (Table 2) while

Village	Type	Pyrethrinoid (mg.l^{-1})		Organophosphorus (mg.l^{-1})	
		Min-max	Mean ± SD	Min-max	Mean ± SD
Savagna	Groundwater	0.008-0.128	0.079 ± 0.063	0.018-1.126	0.743 ± 0.628
	Surface water	0.000-0.025	0.008 ± 0.014	0.000-0.045	0.022 ± 0.022
Tanandava	Groundwater	0.000-0.034	0.012 ± 0.016	0.000-0.267	0.072 ± 0.131
	Surface water	0.000-0.002	0.001 ± 0.001	0.000-0.019	0.005 ± 0.010
Vohitramba	Groundwater	0.000-0.003	0.001 ± 0.001	0.000-0.015	0.008 ± 0.008
	Surface water	0.035-0.103	0.080 ± 0.039	0.085-0.875	0.598 ± 0.445
Note: Savagna (N=3), Tanandava (N=4), Vohitramba (N=3), min=minimum; max=maximum; SD=standard deviation					

Table 2: Rate of organophosphorus and pyrethrinoid in water.

Village	Pyrethrinoid (mg.kg^{-1})		Organophosphorus (mg.kg^{-1})	
	Min-max	Mean ± SD	Min-max	Mean ± SD
Savagna	0.000-0.122	0.069 ± 0.048	0.000-1.248	0.721 ± 0.491
Tanandava	0.000-0.021	0.003 ± 0.007	0.000-0.034	0.009 ± 0.013
Vohitramba	0.000-0.125	0.024 ± 0.037	0.000-0.585	0.167 ± 0.190

Table 3: Rate of organophosphorus and pyrethrinoid in soil N=10.

in surface water concentrations of pyrethrinoids vary from 0.000 mg. l^{-1} to 0.025 mg. l^{-1}. Then in the village of Tanandava the concentrations of pyrethrinoids in groundwater vary from 0.000 mg. l^{-1} and 0.034 mg. l^{-1} while in surface waters the concentrations of pyrethrinoids vary from 0.000 mg. l^{-1} and 0.002 mg. l^{-1}. Finally, in the village of Vohitramba, concentrations of pyrethrinoids in groundwater vary from 0.000 mg. l^{-1} and 0.003 mg. l^{-1} while in surface waters, concentrations of pyrethrinoids vary from 0.035 mg. l^{-1} and 0.103 mg. l^{-1}. The lowest concentrations were 0 mg. l^{-1}. We can see in the Table 3 that the pyrethrinoids reaches the maximum concentration in the groundwater of the village of Savagna (0.128 mg. l^{-1}). For surface water, the concentration of pyrethrinoid is present in large quantities in the village of Vohitramba (0.103 mg. l^{-1}).

The concentrations of organophosphorus in the ground water of Savagna village vary from 0.018 mg. l^{-1} and 1.126 mg. l^{-1}, whereas in surface water concentrations vary from 0.000 mg. l^{-1} and 0.045 mg. l^{-1}. Then in the village of Tanandava, the variations in concentrations of organophosphorus in groundwater were 0.000 mg. l^{-1} and 0.267 mg. l^{-1} and in surface waters this variation in concentrations was 0.000 mg. l^{-1} and 0.019 mg. l^{-1}. Finally, in the concentrations of organophosphorus in the village of Vohitramba vary from 0.000 mg. l^{-1} and 0.015 mg. l^{-1} in groundwater and surface water, this variation was 0.085 mg. l^{-1} and 0.875 mg. l^{-1}. The lowest concentration of organophosphorus was 0 mg. l^{-1} and the highest concentration was 1.126 mg. l^{-1} in Savagna groundwater. For surface water, the highest concentration of organophosphate in the village of Vohitramba was 0.085 mg. l^{-1}.

Persistence of organophosphorus and pyrethrinoid in soil

The concentration of organophosphorus and pyrethrinoid decreased as the sampling place were distant form the pollution. For all samples, Pearson's correlations between the concentration of organophosphorus and pyrethrinoid were respectively r=-0.51, p=0.004 and r=-0.41, p=0.025).

Soils in Savagna village have pyrethrinoid concentrations of 0.000 mg. kg^{-1} and 0.122 mg. kg-^{1}. In Tanandava soils the variation of these concentrations was 0.000 mg. kg^{-1} and 0.021 mg. kg^{-1}. Finally, in Vohitramba we observed that these concentrations were 0.000 mg. kg^{-1} and 0.125 mg. kg^{-1}. The concentration of pyrethrinoid in the lowest soil was 0.000 mg. kg^{-1} or the highest was 0.125 mg. kg^{-1}. Variations in organophosphate concentrations in these villages were 0.000 mg. kg^{-1} and 1.248 mg. kg^{-1} in Savagna village while in Tanandava the variation was 0.000 mg. kg^{-1} and 0.034 mg. kg^{-1}. Finally, in the village of Vohitramba this variation was 0.000 mg. kg^{-1} and 0.585 mg. kg^{-1}. The lowest concentration of organophosphorus was 0.000 mg. kg^{-1} and the highest concentration was 1.248 mg. kg^{-1} in the village of Savagna.

Discussion

Variations in concentrations of pyrethrinoids and organophosphates were detected even at low concentrations during analysis of the Matitanana River (surface water), drinking water (groundwater) and soils.

Pyrethrinoids that have been detected in the samples analyzed are effective at very low doses compared to carbamates or organophosphates and are generally safer to use for humans or insects auxiliary to agriculture [6]. However, degradation by ultraviolet rays is easy and persistence on treated plants and soil rarely exceeds one week [6]. However, Mosquitos Nets (MID) have been used as fish fillets and their persistence in river waters (Vohitramba village above the Matitanana River) has been observed.

The amount of organophosphorus was very high in Savagna soil where the storage sump for insecticide residues was constructed. According to the WHO (Minton et al.), however, the incidence of organophosphate in 19 Asian countries was estimated in 1972 at 50,000 per year with an estimated mortality rate of 5,000 (1%), in 1981 at 750,000, and in 1983 at 2,000,000 with fatal changes in 40,000 cases. In the United States, of the 36,541 insecticide/pesticide poisonings reported to the Poison Control Centers in 1986, 12,142 were due to organophosphate insecticides [7]. In this country, organophosphate insecticides account for 3% of all poisoning reported to poisons centers [7]. In developing countries, organophosphate insecticide poisonings are particularly frequent, often voluntary. In Sri Lanka, for example, they are estimated at 10,000 deaths per year with 10% mortality [8].

Most organophosphate insecticides are poorly soluble in water, not very volatile, but very fat-soluble. Organophosphates have been widely used since 1935 as insecticides to replace organochlorines. They are responsible for high mortality from acute poisoning [9]. Indeed, it is estimated that of the 2,000,000 pesticide poisonings resulting in more than 220,000 deaths per year, half are thought to be caused by organophosphorus insecticides [9]. Organophosphates used as insecticides are responsible for high mortality from poisoning. Organophosphorus that is not very soluble in water, not very volatile, is very fat-soluble and binds to cholinesterases. The oral Lethal Dose 50 (LD50) does not depend exclusively on their own toxicity, but involves other factors, in particular their ease of penetration into the body.

Professional exhibition

Accidental poisonings occurring during the synthesis of organophosphates are exceptional. This is done in a closed environment with protected personnel. However, conditioning, storage and transport can be the cause of accidental contamination, by dermal, digestive or respiratory routes. Transdermal contamination appears to be the most widespread route of contamination [10], with the potential toxic risk by inhalation being less significant than transdermal [11,12]. Dilution with solvents and emulsifiers reduces vapour pressure and minimizes the risk of inhalation, but facilitates skin absorption. Powder formulations have the best dermal absorption [11]. Employees handling organophosphate require medical surveillance, specific health education and regular monitoring of their serum cholinesterase levels. There is a significant decrease in plasma cholinesterase activity, an exposure indicator that is more sensitive than intraerythocytic cholinesterase activity in chronic exposure in subjects exposed to plasma cholinesterase [13].

Domestic exposure

The general population is exposed accidentally either by inhalation or percutaneous exposure. Accidental ingestion can be observed because groundwater intended for human consumption is contaminated. The incidence of voluntary intoxication by ingestion is not negligible, particularly in developing countries [14].

Symptomatology, which can persist for one month [15], usually ends in about 10 days [16]. Behavioural disorders can occur after chronic or acute poisoning, even minor. They are associated with a significant decrease in plasma and globular cholinesterases. They result

in a decline in intellectual performance, with electroencephalographic changes considered by some to be specific [17]. CT scan studies have found aspects of diffuse cerebral atrophy [18,19].

Conclusion

The fate of the remains of insecticides after an in-home sprinkler campaign and the use of mosquito nets for other circumstances (fishing), washing them in rivers and near wells for drinking water is bad practices of these malaria control materials. These insecticides used in malaria control become persistent in soils and water (wells, surface, groundwater). People are at risk of exposure to insecticides due to accidental ingestion of insecticide, inhalation of solvent vapors from emulsifiable concentrate formulations.

The risk to the public will depend on opportunities for contact with treated or contaminated items. This can be done by destroying the remains of insecticides, contaminating drinking water and soil contamination. All necessary information, education and communication measures should be taken to ensure that the public can take the necessary precautions to avoid dangerous contamination. Consideration should also be given to other specific local problems, such as potential contamination or adverse effects on crops essential to the local economy, such as bees or possible contamination of crops stored in homes for long periods of time, such as drying tobacco leaves or vanilla. Periodic control of the use of MID after distribution, ecotoxicity control, recycling of unsprayed insecticide residues in appropriate laboratories should be prioritized.

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