UNIVERSITE DE LIEGE

FACULTE DE MEDECINE VETERINAIRE

DEPARTEMENT DES SCIENCES DES DENREES ALIMENTAIRES

SERVICE D'ANALYSE DES DENREES ALIMENTAIRES

Use of pesticides in Fresh Water Aquaculture in the Mekong Delta, Vietnam, and impacts on environment and food safety

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THESE PRESENTEE EN VUE DE L'OBTENTION DU GRADE DE

Docteur en Sciences Vétérinaires

ANNEE ACADEMIQUE 2018-2019

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Acknowledgements

Foremost, I would like to express my gratitude to my supervisor Prof. Marie-Louise Scippo who has built my passions and directions for science as who I am and what I have today. Her guidance and enthusiasm have inspired and supported me moving forward and working by my best during my research and writing this thesis. I would also like to give my thanks to my co-supervisor, Prof. Nguyen Thanh Phuong, for always supporting, encouraging, and watching my steps during my study.

My sincere thanks also go to Caroline Douny for giving me special advices, writing correction, precious and initiative suggestions which contribute importantly in my thesis. Also, I acknowledge François Brose and Guy Degand who greatly supported on technique of analysis as well as spending time on talking together; it really helped to release a lot of stresses.

I would like to thank the staffs at the College of Aquaculture and Fisheries, Can Tho University (Vietnam), at the Department of Food Sciences, Laboratory of Food Analysis, FARAH – Veterinary Public Health, University of Liege, Liege, Belgium where my projects were carried out. Especially, I would like to thank Prof. Do Thi Thanh Huong, Dr. Tran Minh Phu, Mr. Nguyen Thanh Phong, Mr. Vo Hung Vuong, Mr. Nguyen Thanh Binh, Ms. Phan Thi Be Ngoan, Mr. Nguyen Van Qui and Ms. Nguyen Thi Bich Tuyen for helping me during my experiments.

I would also like to thank my fellow friends in CUD project for their friendship and all the funs we have during the time we worked together.

Finally, I would like to thank my family and my friends for their love and spiritually supporting me throughout my research, my writing, and my whole life.

My project was funded by Académie de Recherche et d'Enseignement Supérieur – Commission pour la Coopération au Développement (ARES-CCD).

Abbreviations

AChE	Acetyl choline esterase
ADI	Acceptable daily intake
AhR	Aryl hydrocarbon receptor
BNP	Bacillary Necrosis of *Pangasius*
BOF	Bio-concentration factor
CALUX	Chemical-Activated LUciferase gene eXpression
CAM	Chloramphenicol
DDT	Dichlorodiphenyltrichloroethane
DFI	Daily food intake
EC	European Commission
ELISA	Enzyme linked immunosorbent assay
FAO	Food Agricultural Organization
GC ECD	Gas Chromatography Electron Capture detector
GC MS	Gas Chromatography Mass Spectrometry
GSO	General Statistic Office
HCHs	Hexachlorocyclohexane isomers
HPLC	High performance liquid chromatography
IPM	Integrated Pest Management
LC MS	Liquid Chromatography Mass Spectrometry
LC50	Lethal concentration cause in 50% experiment animal die
LD50	Lethal dose cause in 50% experiment animal die
LLE	Liquid-liquid extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MD	Mekong Delta

MRL	Maximum residue level
OCP	Organochlorine pesticide
OPP	Organophosphate pesticide
PCB	Polychlorinated-biphenyl
PSA	primary or secondary amine
QC	Quality control
RASFF	Rapid Alert System for Feeds and Foods
RSD	Relative standard deviation
SIM	Selected ion monitoring
SLE	Solid liquid extraction
SPE	Solid phase extraction
TCDD	Tetrachlorodibenzo-p-dioxin
TL	Tolerance level
USEPA	United State Environment Protection Agency
VMARD	Ministry of Agriculture and Rural Development of Vietnam
WHO	World Health Organization

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Summary

Summary

The research "Use of pesticides in Fresh Water Aquaculture in the Mekong Delta, Vietnam, and impacts on environment and food safety" consisted of four sections.

To evaluate the pressure of drugs and chemicals in the environment, two surveys were performed in the Mekong Delta. The first was focused on rice and rice-fish system and was conducted in 2009. The second, an in depth survey, conducted in 2013, focused on rice-fish, striped catfish and red-tilapia systems. Results showed that, for the rice-fish system, most rice fish farmers grew 2 rice crops and 1 fish crop per year. Pesticides were applied generally 3 times per crop. Farmers normally applied pesticides based on the recommended doses of producers. Chess 50WG (containing 500g/kg pymetrozine, produced by Syngenta, Vietnam), Bassa 50EC (containing 500g/L fenobucard, produced by Việt Thắng Bắc Giang (Vithaco), Vietnam) and Kinalux (containing 250g/L quinalphos, produced by United Phosphorus Ltd., India) were the most common used commercial pesticides in rice crop, rice-fish crop and distributors as well. According to the majority of the distributors, the use of pesticides will increase in future. The in-depth survey showed that much more active compounds were used in 2013 compared to 2009, but, all of the active compounds belonged to the approved list of Vietnamese government. Few farmers used chemicals during fish crop. Farmers reported their awareness towards the use of agrochemicals in terms of health effects. The survey showed that the farmers select an agrochemical based on their experience. The study on red-tilapia demonstrated that many different types of disinfectants and antimicrobials are used. Further, the cost-effectiveness of such pesticide use, especially for feed supplement products, antimicrobials and disinfectants, is questionable and should be assessed. There is an urgent need to improve the farmer's knowledge and their access to advisory services on careful use of disinfectants and antimicrobials. All visited striped catfish farms applied drugs and chemicals with seven types of antibiotics during the fish production. Enrofloxacin, sulfamethoxazole and trimethoprim were reported to be the most used chemicals by farmers to treat Bacillary Necrosis of *Pangasius* (BNP).

The survey and practical situation demonstrated that quinalphos, trifluralin and dichlorvos were commonly used in rice fish system and, consequently, may contaminate aquaculture products. A Gas chromatography – mass spectrometry (GC-MS) analytical method was developed and validated according to European guidelines (SANTE/11945/2015) for the determination of residues of those pesticides in water. The developed method was then optimized using a gas chromatography – electron capture detector (GC ECD) technique to make the method more applicable in Vietnam. The developed method was used to analyze water samples collected from the aquaculture system in April 2013, at the beginning of the rainy season. Results showed that only 9 % of the total water samples analyzed contained residues of quinalphos, but only in water from rice fish systems. The other two pesticides, trifluralin and dichlorvos, were not detected. A comparison between GC-MS and GC-ECD indicated

that GC-ECD is less sensitive than GC-MS. However, for samples with concentrations detectable with both techniques, no significant difference was observed between the results obtained using both equipments GC-ECD and GC-MS.

The next step was to determine the distribution and elimination of quinalphos, the active substance of a popular insecticide used in the Mekong Delta, according to the first survey. An experiment was set up in a rice-fish integrated system in Can Tho City, Vietnam. Quinalphos was applied twice in a dose of 42.5 g per 1000 m^2, according to the producer recommendations. Samples (fish, water and sediment) were collected at time intervals and were analyzed by GC-ECD. The results showed that quinalphos residues in fish muscles were much higher than those in the water and the bioconcentration factor (log BCF) was above 2 for the fish. The half-lives, after the first and second quinalphos applications, were 12.2 and 11.1 days for sediment, 2.5 and 1.1 days for silver barb, 1.9 and 1.3 days for common carp, and 1.1 and 1.0 days for water, respectively.

Finally, as a case study including 3 commonly used pesticides (quinalphos, trifluralin and dichlorvos), dioxins and one forbidden antibiotic (chloramphenicol), the risk for the consumer, linked to the chemical contamination of the aquaculture related environment was evaluated. Sediments samples were collected including 10 samples collected from catfish ponds in An Giang Province and 12 samples randomly collected from rice-fish systems in Can Tho City. Analytical results showed that 3 from the 13 water samples collected from rice field were contaminated with low levels of quinalphos (with concentrations of 0.11, 0.08 and 0.04 µg/L). The other investigated pesticides were not detected in any sample. For chloramphenicol (CAM) residues in fish samples, analysis was performed on 36 fish samples of catfish (18 samples included 9 from small scale and 9 from large scale systems), snakehead (9 samples) and climbing perch (9 samples) collected at the beginning, middle and at the end of culture period. Results showed that one sample of climbing perch and one sample of snakehead were contaminated with traces of CAM (concentrations of 0.17 and 0.19 µg/kg, respectively). It appeared that CAM was not detected in catfish samples neither from the beginning to the end of the crop, nor from small and large scale systems. Dioxins were not detected in any of the collected sediments samples. In order to assess the general risk for the Vietnamese consumer of fish, a survey was performed in Can Tho City, using a questionnaire designed to collect information. A large part of interviewees (77%) stated that they like to eat fish. The number of days of eating fish was 3.4 days per week. In this study, the average amount of fish consumption ranged between 90 and 140 g per day. It was shown that the daily intake of trifluralin of interviewed people was 0.05 µg/kg body weight/day. This level of exposure was much lower than the acceptable daily intake (ADI) (15 µg/kg/day) (EFSA, 2015). However, trifluralin has not been approved in EU, so the presence of residues of trifluralin in aquatic product, even if they cause no problem for the consumer, would be a problem for aquatic product export.

Introduction

1. Generality in agriculture, aquaculture and chemical use

Vietnam is an agricultural country with 70% of the population contributing in the rural activity. The area used for agriculture and forest makes up to 77% of total area (GSO, 2011; GSO, 2012). Rice production in Vietnam has been intensified to meet the increasing food demand of rice. The intensity culture resulted in a change for Vietnam from a rice importer country in 1989 to a worldwide rice exporter in 1997. Regarding aquaculture, the national production reached 1.95 million tons in 2007, and increased to 2.7 million tons in 2010 from which marine shrimp (*Penaeus monodon*) and tra catfish (*Pangasianodon hypophthalmus*) were the predominant products. The Mekong Delta (Figure 1), with an area of 39,000 km^2 and 17 million inhabitants (Renaud and Kuenzer, 2012), is the biggest rice production region of Vietnam representing 50% of the national production and 90% of rice exportation. It is also the main region of fruit, vegetable and aquaculture production of Vietnam. In 2013, fish and shrimp production in the Mekong Delta accounted 72 and 79% of the total national production, respectively (GSO, 2014b). In recent years, the aquatic production of the Mekong Delta always shared a large portion of total national production (Figure 2), indicating the increase in aquaculture.

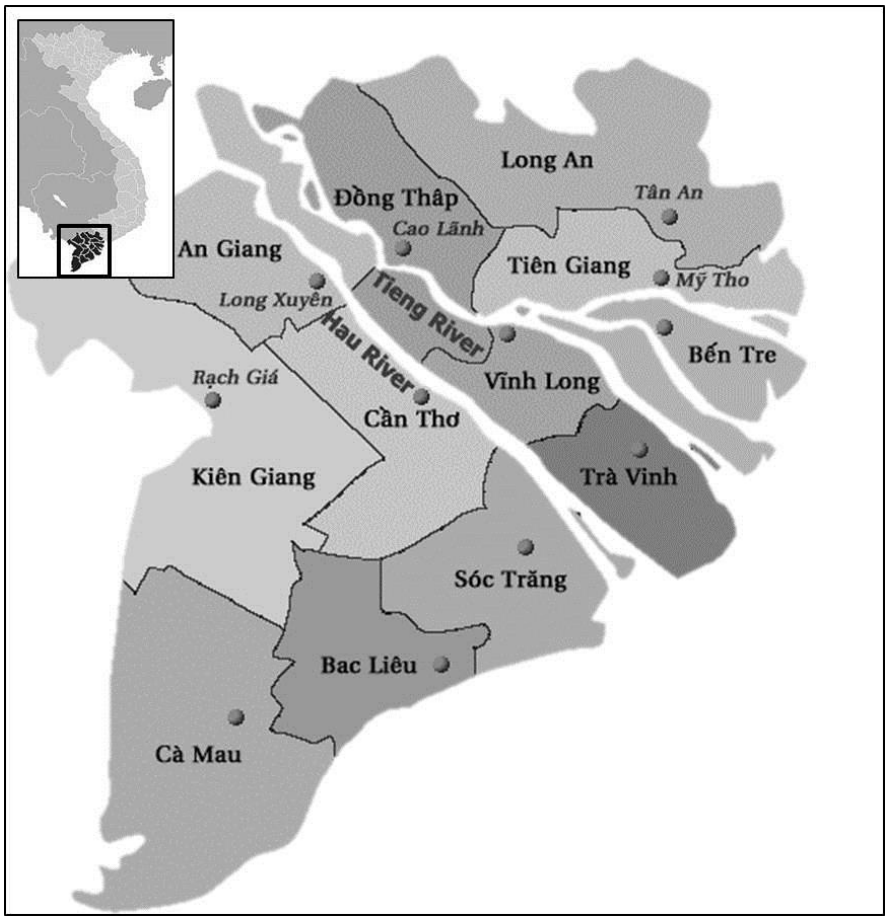

Figure 1. Geographical location of Mekong Delta, Vietnam

The increase in aquaculture production resulted from the intensification of many culture systems including shrimp and catfish. The increased intensification of culture systems (high stocking density, intensive feeding with dry pellets, etc.) has led to increased use of chemicals for controlling water environment and pathogens, and, consequently, increased pollution caused by the effluents from culture systems. Intensive culture of catfish in freshwater ponds is a typical example of the potential impacts of aquaculture on environment and food safety.

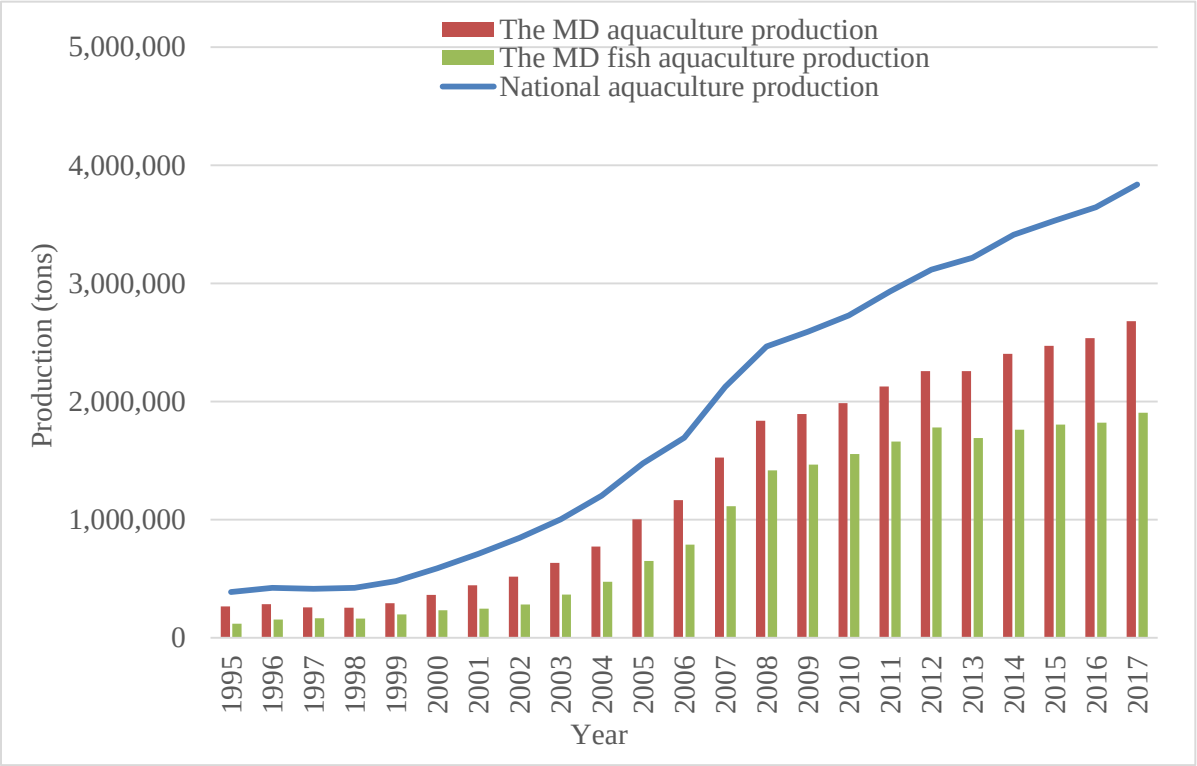

Figure 2. Total aquaculture production of Vietnam and the Mekong Delta (according to General statistical office of Vietnam) (GSO, 2014b).

Beside intensive culture of catfish, the Mekong delta has also many other intensive production systems such as integrated and alternative rice-cum-fish or giant freshwater prawn (*Macrobrachium rosenbergii*), black tiger or white leg shrimp culture (Figure 3 B). The systems were considered as a traditional and sustainable way of production of both animal protein (fish) and carbohydrate (rice), the basic component of Vietnamese food.

(A)

(B)

(C)

(D)

Figure 3. Examples of aquaculture systems in the Mekong Delta. (A) rice fish farm, (B) marine shrimp pond, (C) Catfish pond and (D) red tilapia cage.

The intensification of rice production (with the use of high yield variety) has led to an increase of pesticides application to cope with the damages caused by insects and weeds (Berg, 2001). According to Tin Hong (2017), the pesticide consumption has significantly increased in Vietnam during recent decades (Table 1).

Table 1. Amount of pesticide imported and applied in agriculture in Vietnam from 1981 to 2015 (Tin Hong, 2017).

Period	Pesticide import (tons)	Average dose applied in agriculture (kg active ingredient/ha)
1981 – 1986	6,500 to 9,000	0.3
1986 – 1990	13,000 to 15,000	0.4 to 0.5
1991 – 2000	20,000 to 30,000	0.67 to 1.0
2001 – 2010	33,000 to 75,000	2.54
2015	100,000	Not estimated

Along with the increase in quantity, the import value of pesticides has also progressively increased in Vietnam, going from 409 million euros in 2008, 466 in 2010 to about 607 million euros in 2015. In 2014, the total imported pesticides included 45% of herbicides, 27% of fungicides/bactericides, 23% of insecticides and 5% of others (Thuy Lien, 2015). The evolution of total import value of pesticides and raw material for pesticide production in Vietnam, from 1995 to 2017, according to the General Statistic Office of Vietnam are presented in Figure 4 (GSO, 2017b).

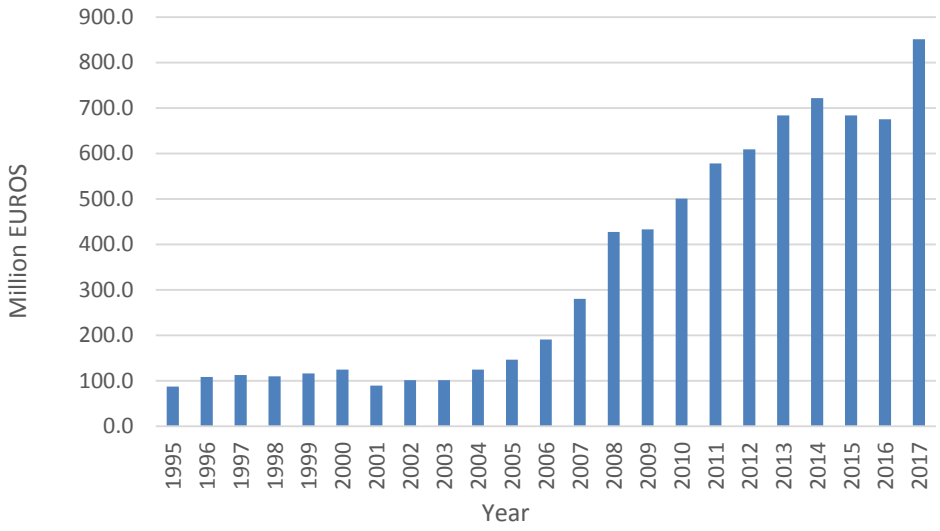

Figure 4. Import value of pesticide and raw material for pesticide production (GSO, 2018).

According to Heong and co-workers (Heong et al., 1998) the rice farmers in the Mekong Delta considered that the increased use of pesticides would result in a higher rice production, and this has led to a significant increase in the application of various types of pesticides. For instance, the average number of pesticide applications on a rice crop by farmers who did not follow the Integrated Pest Management (IPM) and by farmers who did follow the IPM program increased, respectively, from 5.7 and 3.5, in 1994, to 8.2 and 4, in 1999 (Berg, 2001). The pesticide application to rice may impact cultured animals of rice-cum-fish/prawn culture systems (inducing health adverse effects, mortality, and body contamination) environment (water and sediments contamination), and finally wild animals and humans through water and food utilization.

The increased use of chemicals in agriculture and aquaculture is now causing various problems, among that food safety being the most obvious because of export constraints and public health concern. Moreover, fish farmers are applying antibiotics and chemicals without a clear knowledge of the products used and, frequently, for disease prevention rather than for disease treatment. Therefore, the residues of chemicals used in intensive aquaculture systems (antibiotics, disinfectants) and rice fish system (pesticides) may contaminate food and water if the application is

not done properly, leading to deleterious effects on human population via the consumption of contaminated food or the use of water connected to aquaculture production systems for domestic purposes.

2. Pesticides and other contaminants overview

Food contamination is one of the problems of food safety, especially in the countries that have food products related to agriculture. In most countries, pesticides are widely used for the control of agricultural pests. In the last decades, pesticides were reported to seriously affect non-targeted organisms due to their use in large amount. Pesticides can affect non-targeted species at various levels from less to more acute. It can poison skin, liver, digestive track etc. Moreover, most pesticides can cause neurotoxicity because of their ability of crossing blood and brain barrier. Humans are mostly exposed to pesticides from food, especially the products originated from agriculture.

2.1. Pesticides overview

A pest refers to any insect, rodent, nematode, fungus, weed, or any other form of terrestrial or aquatic plant, animal, virus, bacteria, or other microorganisms that harm the garden plants, trees, foodstuffs, household articles, or is a vector of diseases. However, for farmers, pests include insects and mites that feed on crops; weeds in the fields; aquatic plants that clog irrigation and damage ditches; agents that cause plant diseases such as fungi, bacteria, viruses, nematodes, snails, slugs, and rodents that consume enormous quantities of plant seedlings and grains (Liu et al., 2010). According to United State Environment Protection Agency (USEPA), a pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. The term pesticide includes insecticide, herbicide, fungicide and various other substances used to control pests (USEPA, 2015).

History and market

History

The use of chemicals against harmful organisms has been realized for a very long time, but it can be separated into inorganic and organic eras. Around AD 70, Pliny, recommended that arsenic could be used to kill insects. The Chinese used arsenic sulfide as an insecticide in the late sixteenth century. The use of arsenical compounds has continued and, during the early part of the twentieth century, large quantities of these compounds such as lead arsenate were used to control insect pests (Liu et al., 2010).

Table 1. History of pesticide development (Erdoğan, 2002; Taylor et al., 2007; Unsworth, 2010).

Time	Event
2500 BC	Foul-smelling sulfur was believed to repel insects and mites by Sumerians
1500 BC	Egyptians produced insecticides against lice, fleas and wasps
1000 BC	The Greek poet Homer referred to a pest-averting sulphur. Mercury and arsenic compounds were used by Chinese to control body lice; predatory ants were also utilized to protect citrus, that might be the earliest form of IPM (Integrated Pest Management)
200 BC	The Roman writer Cato advises vineyard farmers to burn bitumen to remove insects
early 1700's	John Parkinson, author of 'Paradisus, The Ordering Of The Orchard' recommended a concoction of vinegar, cow dung and urine to be put on trees with canker
1711	In England, the foul smelling herb rue was boiled and sprayed on trees to remove cantharid flies
1763	In Marseille, a mixture of water, slaked lime and bad tobacco was a remedy for plant lice
1821	London Horticultural Society advised that sulphur is the remedy for mildew on peaches
1867	Beginning of modern pesticide use Colorado beetle invaded US potato crops and arsenic is applied Professor Millardet, a French professor, discovers a copper mixture to destroy mildew
Late 1800's	French vineyard growers have the idea of selective weed killers
1892	The first synthetic pesticide, potassium dinitro-2-cresylate, is marketed in Germany
1900's	Insecticides, fungicides and herbicides have all been discovered
1932	Products to control house hold pests are marketed
1939	The Second World War brings three discoveries: the insecticide DDT, the organophosphorus insecticides and the selective phenoxyacetic herbicides
1945	After the Second World War, farming intensity production
1950's	Geigy introduces the carbamates; herbicide atrazine, paraquat, and picloram were developed in 1958 and 1960
1962	"The Silent Spring" book of Rachel Carson was published and considered as the first warning of pesticide overuse
1970s and 1980s	Introduction of the herbicides glyphosate, sulfonylurea, imidazolinone, dinitroanilines. For insecticides, there was the synthesis of a 3rd generation of pyrethroids, the introduction of avermectins, benzoylureas and Bt (Bacillus thuringiensis) as a spray treatment. This period also saw the introduction of the

	fungicides. Many of the agrochemicals introduced at this time had a single mode of action, thus making them more selective. Problems with resistance occurred and management strategies were introduced to combat this negative effect.
1990s	Research activities concentrated on finding new members of existing families which have greater selectivity and better environmental and toxicological profiles.

In addition, between 1960 and 1970, the concept of integrated pest management (IPM) was re-introduced and some evidence about accumulation and effect of pesticide on non-target animal were shown, reducing the persistence of chemicals such as DDT in agriculture. Integrated pest management means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimize risks to human health and the environment. 'Integrated pest management' emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms (EC, 2018). Nowadays, pesticides are formulated to be safer and less persistent than those before (Taylor et al., 2007). In Vietnam, IPM trainings have been given to the farmers in 1990s. The year during IPM training, the ratio of chemical use was reduced. However, after a few years, the farmers turn back to rely on pesticide as a main mean of pest management (VMARD, 2011). A survey in 2015 performed in 5 rice cultivation districts of Dong Thap province, a province of the Mekong Delta, Vietnam, showed that only 16% of farmers applied IPM (Plant Protection Department, 2017).

The development of pesticides market

After 1950, the market of pesticides has significantly developed not only by the volume but also by the number of available chemicals. In 1979, the total number of chemicals used as active ingredients was approximately 550. Effective pesticides were produced in vast amount, the amount of DDT was estimated at 2.8 $x10^9$ kg in the period between 1943 and 1974 (Stenersen, 2004). The market of pesticides has increased rapidly from the 1970s: from 2.31 billion to more than 47.78 billion EURO in 2017 (Figure 5).

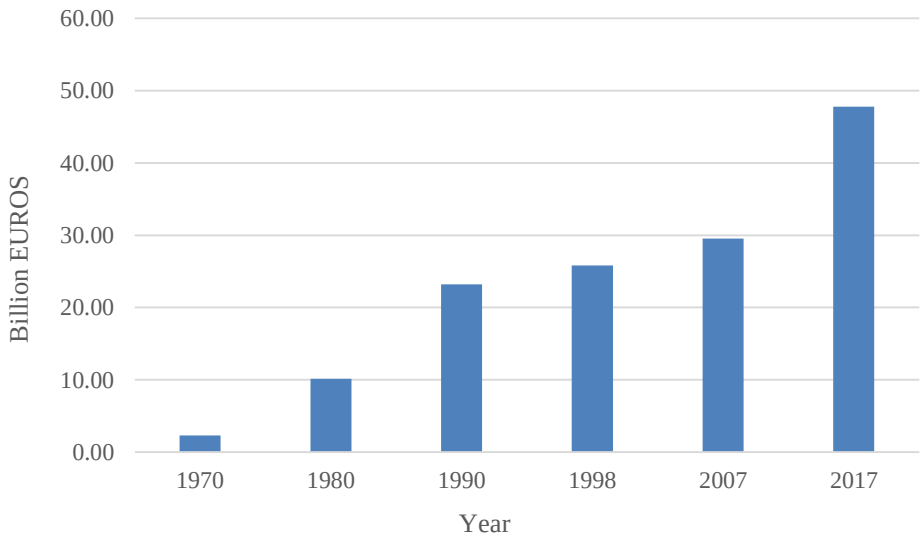

Figure 5. Global pesticides market (in billion EUROS) (Cabras, 2003; The Statistics Portal, 2017).

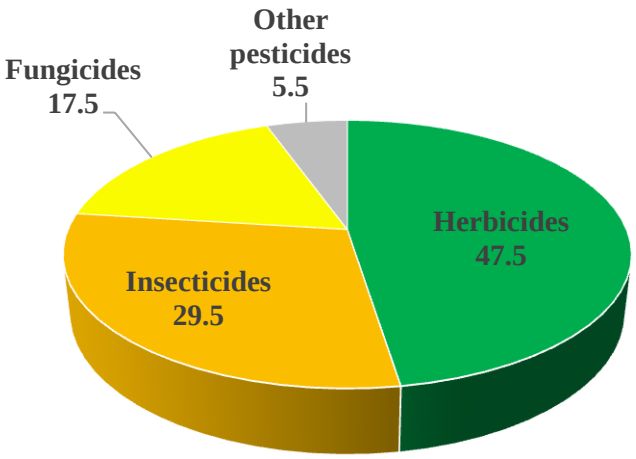

Figure 6. Contribution of different classes of pesticides (percentage) to the worldwide consumption of pesticides in 2014 (De et al., 2014b).

According to De et al. (2014b), the global amount of pesticide consumption was about 2 million tons per year. Herbicides shared the largest portion (47.5%), followed by insecticides (29.5%) and fungicides (17.5) (Figure 6).

2.2. Pesticides classification

Pesticides are chemical substances, but sometimes they can be biological agents like viruses or bacteria. Pesticides can be classified according to their use or chemical structure or they can be also classified based on their toxicities.

The classification according to their targets include: Insecticides (insect killers), Herbicides (plant killers), Fungicides (controlling fungi), Molluscicides (controlling mollusks), Nematicides (controlling nematodes), Rodenticides (controlling rodents), Bactericides (bacteria killers), Defoliants (removing plants leaves), Acaricides (killers of ticks and mites), Wood preservatives, Repellents (substances repelling pest), Attractants (substances attracting insects, rodents and other pests), Chemosterilants (substances inhibiting the reproduction of insects)

According to their chemical structures, pesticides can be divided into two main groups: inorganic and organic. As it can be seen in Table 1, inorganic compounds were very popular before World War II; after that, organic pesticides became more popular (Matolcsy et al., 1988). The organic pesticides consisted of different groups which are organochlorines, organophosphates, carbamates, pyrethroids (for insecticides), dithiocarbamates, benzimidazoles, dicarboxamides, triazoles, anilinopyrimidines, strobilutines (fungicides). For herbicides, the most common groups are phenoxy derivatives (phenoxyalkanoic acids), dipyridilic compounds, amides, dinitroanilines, ureas, triazines, sulphonylureas and amino acid derivatives.

Ecofriendly pesticides groups

Insect repellents is a group of chemicals in which compounds do not kill pest but prevent the damage to crop by carrying out a unattractive or offensive condition to pest. These compounds include dimethylphthalate, pyrethrum (used as mosquito repellent), naphthalene, p-dichlorobenzen or chemicals extracted from citronella plant (*Andropogon nardus*).

Insect attractants are chemicals that can be used to attract pest into traps or poison baits. The compounds can be divided into food and sexual attractants, food attractants being food products used to attract beneficial insects like ladybirds for instance.

Juvenile hormones are very important compounds implied in the development of insects and secreted from a part of the brain called *corpus allatum*. The hormones disturb the normal development of the insect and prevent its reproduction. These compounds do not kill the insect and do not harm human and warm-blood animals.

Pheromones are chemicals secreted by one sex and trigger behavior of another sex of the same species. The compounds were applied in small dose and attracted insects to insecticides.

Synergists are chemicals which are nontoxic to insects at the recommended dose. However, they increase the toxicity of pesticides, thus reducing the quantity of pesticides necessary and released in the environment.

Pesticides of plant origin are extracted from plant bearing insecticidal activities or repellant properties. The group possesses advantages such as low mammalian toxicity, least health hazard and is thus eco-friendly.

Modes of action

Pesticides are intended to disrupt a target, i.e. a specific protein that important in the pest living so the target is no longer working properly. The pesticide may bind to or interacts with a specific enzyme, receptor, protein, or membrane, initiating a series of events that is deactivated or lethal to the pest. Insecticides and herbicides have six primary targets that make up three-quarters of all mode of action, which are EPSP synthase, acetolactate synthase, photosystem II, fatty acid elongase, auxin receptor and acety-CoA carboxylase (Krieger, 2010). Most insecticides quickly disrupt neurotransmission to alter insect behavior or survival. Insecticides can be practical with only a limited biological range like aphids or caterpillars. On the other hand, herbicides generally inhibit specific pathways, blocking amino acid or fatty acid biosynthesis or photosynthesis to prevent the growth of the weed. Fungicides act on many basic cellular functions important to hyphal tip growth. Fungi are evolutionarily far more diverse than insects or weeds. They include not only the true fungi but also the Oomycetes having motile stages and controlled by oomyceticides. There are a broad variety of fungicide targets which vary in their importance for survival (Casida, 2009).

According to Stenersen (2004), the action of the pesticides in organism can be classified into seven types that are described below.

Enzyme inhibitor: the pesticides belonging to organophosphates and carbamates groups can kill the target by reaction with the enzymes or proteins and inhibit their functions. The pesticides have a similar structure to enzymes' substrates but have no biological function. Instead of processing a reaction, they stop the enzyme activity, e.g. chlorpyrifos and carbaryl.

Chemical signal system disturbance: two main types of substances act as disturbance agents called agonists and antagonists. The agonists imitate or replace the true signal and thus transmit it too strong, too long or at a wrong time. Some agonists act outside of the cell (nicotine) while some act

within the cell. The antagonists block the receptor site for the true signal, so prevent the contact between signal and target organs.

Reactive molecule generation: the most common reactive molecule is hydroxyl radical which is extremely aggressive and reacts with any first contact compound regardless of what it is.

-Membrane pH gradient change: some molecule can take a H^+ from cytoplast into mitochondria or chloroplasts, the difference of pH between the organelles and cytoplast is very important in energy generation. Therefore, the change of pH gradient may cause in severe disturbance in these pathways.

-Three other actions are 1) membrane malfunction: some substances which can dissolve into phospholipid layers cause malfunction of cell membrane, 2) electrolytic or osmotic balance disorder caused by substances like sodium chloride in a specific concentration, and 3) tissue of organisms destroyed by strong acid, strong base, bromine, chlorine and so on.

2.3. Pesticides and environment

When a pesticide is released in the environment, it may be dissolved in water, be absorbed in soil or sediment, bio-accumulate, be metabolized by an organism or be degraded by temperature or sunlight. In addition, pesticides can be transferred from site to site due to many processes such as volatilization, spray drift, runoff, leaching, absorption, organism movement and crop removal.

Volatilization is the process of a pesticide changing from the liquid into the gas phase. The movement results in pesticides transferring from the application site to others. This process is called vapor drift. Hot, dry, windy weather and small spray drops may increase volatilization.

Spray drift is the process of spray droplets moving from treatment site to another site. The movement depends on spray droplets size, wind speed and the distance between the nozzle to the target plan or soil.

Runoff is the movement of pesticides in water over a sloping surface; these pesticides can be either mixed in water or bound to soil. The amount of pesticide runoff depends on the slope, the texture of the soil, the soil moisture content, the amount and timing of a rain-event, and the type of pesticides used.

Leaching is the process that pesticides in water pass through the soil to ground water or side way. The movement depends on pesticide, soil type and rain event. Leaching can be increased when the pesticides are soluble, the soil is sandy or rain occurs shortly after application (Liu et al., 2010).

Affecting ecological system

Pesticides are designed to kill a certain group of organisms through biological effects, so some side effect cannot be completely eliminated. Although several regulations or decisions have been applied to limit the unwanted effects, pesticide use has resulted in many effects on ecological system (Tarazona and Dohmen, 2007).

Ecotoxicology or the study of adverse effects of toxic substances on ecosystems was proposed by Truhaut (1977). Ecotoxicology covers all effects of chemicals on organisms including exposure sources, ways of entry into body, individual or community influence at all effect levels such as molecular, organs or population.

Fate of pesticides

The fate is the process of pesticides disappearance after application. The process may take some hours to years and may involve the activities of microbe, chemical breakdown or photo-degradation.

Photo-degradation: all organic pesticides are susceptible to photo-degradation to some extent. The rate of breakdown depends on the pesticide properties, intensity of sunlight and time of exposure. The degradation of pesticides in plastic greenhouse is faster than in glass greenhouse due to the ultraviolet filtration properties of glass.

Bio-metabolite: some bacteria and fungi can degrade pesticides. The process is increased with warm temperature, optimal pH, soil moisture and good fertilizing.

Chemical breakdown is the breakdown of pesticides by chemical reaction and the degradation is influenced by pH level and temperature.

Residues of pesticides

Pesticides have been applied over the world for many years, so the residues of those chemicals can be found in every part of the environment (soil, air, ground water, surface water or biota…), even in areas where pesticides were never applied, such as the Antarctic for example (Fuoco and Ceccarini, 2001).

Exposure to pesticides

Pesticides are toxic chemicals, influencing any exposed organisms. The term pesticide exposure indicates the contact of the pesticide with the surface of an organism. For humans, it means

acquiring it in or on his body. The toxic effect resulting from a pesticide exposure depends on the amount, the duration and the organs which have been in contact with the pesticides. According to Srivastava et al. (2010), there are four main ways of human exposure to pesticides (Srivastava et al., 2010b).

Oral exposure includes eating, smoking or drinking after having handled pesticides without proper cleaning, or eating food contaminated with residues of pesticides.

Inhalation exposure is caused through the uptake of pesticides through breathing vapors from fumigant, contact with volatile pesticides in closed or poorly ventilated space, inhaling vapors coming from the pesticide application with a deficient respirator, etc.

Eye exposure is caused by splashing or spraying pesticide into eyes, rubbing eyes or forehead with contaminated gloves, hands or towel, applying pesticide under a windy weather without any eye protection.

Dermal exposure is caused by handling pesticides without appropriate protection, touching treated area, wearing contaminated clothes or the protective personal equipment.

Maximum residue levels determination (pesticides)

According to the European Commission, "A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Good Agricultural Practice)" (EC, 2018a). The MRLs are set based on the submitted information from producer of plant protection products, farmers, importers. That information includes the use of a pesticide on the crop (quantity, frequency and growth stage of plant) and experimental data on residue levels when the pesticide is applied "correctly". For each authorized pesticide, toxicological reference values are available, i.e. the acceptable daily intake (ADI) addresses the chronic toxicity and the acute reference dose (ARfD) addresses the acute toxicity. Based on the available information, the intake through all food that may be treated with the pesticide of interest is compared with the ADI and the ARfD for long and short-term exposure, respectively, for all consumer groups. In the case that the requested MRL is not safe, it is set at the lowest limit of analytical determination (LOD). By default, the LOD in EU regulation is 0.01 mg/kg (EC, 2018b).

According to Cabras (2003), toxicological studies include the studies of acute toxicity, short term toxicity (at least 90 days), long term toxicity (2 years), toxicity on reproduction and late neurotoxicity. These studies are carried out with all chemicals for which an authorization of use is asked. The results of the studies will allow determining the No Observed Effect Level (NOEL). The NOEL of long term studies of the most sensitive and similar to human species was used to determine

the ADI with the application of a correction factor between 10 and 1000, the factor of 100 being usually used to calculate ADI (mg/kg body weight (BW)/day).

2.4. Properties and toxicity of investigated chemicals

2.4.1. Pesticides

This study concerns three pesticides: dichlorvos, quinalphos and trifluralin. These pesticides were selected based on a survey realized in Vietnam in 2009 and on the practical situation of the aquaculture industry in Vietnam. According to Regulation 1107/2009/EC (EC, 2009), these pesticides are not approved in the EU. The MRL of the dichlorvos has been set under detection limit in products from vegetable origin (fruits, vegetables, tea, oils, etc.) and are ranging from 0.01 to 0.1 mg/kg. The MRLs of quinalphos and trifluralin are also under limit of detection with the range of 0.01 to 0.05 with the addition of animal origin products (EC, 2018). According to The Japan Food Chemical Research Foundation, MRL of trifluralin in fish has been set at 0.5 mg/kg, but MRLs of quinalphos in fish have not been found in fish and there is no information about MRLs of dichlorvos (JFCRF, 2018).

Quinalphos is an insecticide used in important crops in tropical and subtropical zones (Aizawa, 2001). It shows high efficacy on chewing, sucking, biting and leaf-mining pests thanks to its good penetration into plant tissues and insect cuticles and acts as contact and stomach insecticide (Wisson et al., 1980). In the Mekong Delta, this compound is used to treat rice panicle mite in rice fields under the brand name Kinalux™ (containing quinalphos) (Product of United Phosphorus Limited, India). Its use leads to a high probability of pesticide contamination in fish, especially in rice-fish production system.

Two other pesticides, trifluralin and dichlorvos, are often used in aquaculture. Trifluralin, a compound belonging to the dinitroaniline group, is an herbicide. It was introduced in 1963 as a pre-emergent herbicide and was reported to be a moderate to high toxic compound to aquatic animals and insects as well as to vertebrate animals (dogs or rabbits). This compound was banned by European Union in 2000 due to its persistence in soil and groundwater. Trifluralin can enter the body by absorption through the skin, by inhalation of contaminated air or from ingestion of contaminated food (Wallace, 2014). Although trifluralin is an herbicide, it has been found experimentally and in actual use to aid in the reduction of losses due to fungi in shrimp (Bland 1975; Lio-Po et al. 1982; and Aquacop 1977) reviewed by Williams et al. (1986). In Vietnam, trifluralin was first used for shrimp larvae to treat fungi diseases, then widely used in water treatment and for killing fish parasites (Truong, 2012).

Dichlorvos, a very effective organophosphate pesticide, is also a contact and stomach insecticide. Dichlorvos has been used globally since 1961 to protect stored product and crops from pests; it was also used in houses, buildings and in the hygiene sector, especially in controlling flies and mosquitos. As the compound volatilizes easily, it was also used as a fumigant agent and in greenhouse crops. In aquaculture, especially in intensive systems, dichlorvos was applied into water to control invertebrate fish parasites (Matolcsy, 1988; WHO, 1989). In Vietnam, dichlorvos was used in both agriculture and aquaculture to control pathogens; in fish culture, it was used to destroy parasites in shrimp pond preparation and to prevent external parasites during fish rearing periods (Tran and Do, 2011).

The physicochemical properties of these 3 pesticides are summarized in Table 2.

Table 2. General properties of investigated pesticides (PPDB, 2015).

	Dichlorvos	Quinalphos	Trifluralin
IUPAC name	2,2-dichlorovinyl dimethyl phosphate	O,O-Diethyl O-2-quinoxalinyl phosphorothioate	2,6-Dinitro-N,N-dipropyl-4-(trifluoromethyl)aniline
Group Type	Organophosphate Insecticide, acaricide	Organophosphate Insecticide, Acaricide	Dinitroaniline Herbicide
Mode of action	Respiratory, contact and stomach action, acetylcholinesterase (AChE) inhibitor	Contact and stomach action, Acetylcholinesterase (AChE) inhibitor	Selective, inhibition of mitosis and cell division
Origin	Synthetic	Synthetic	Synthetic
Formular	$C_4H_7C_{12}O_4P$	C12H15N2O3PS	$C_{13}H_{16}F_3N_3O_4$
MW (g/mol)	220.98	298.3	335.05
Apprearance	Pale yellow clear liquid	colorless liquid	Orange-yellow crystal solid
Solubility in water	18000 mg/L (high)	17.8 mg/L (low)	0.221 mg/L (low)
Solubility in solvent	Dichloromethane, v.s. (very soluble); 2-propanol, v.s.; toluene v.s.; ethanol s.(soluble); chloroform s.; acetone s.; kerosene s.	Hexane 250000 mg/L (at 20°C)	Hexane, toluene and acetone 250000mg/L , methanol 142000 mg/L (at 20°C)
Boiling point (°C)	Decomposition before boiling	N/A	Decomposition before boiling
Degradation (°C)	190	N/A	202
Log $P_{o/w}$	1.9 (low)	4.44 (high)	5.27 (high)
Vapour pressure (mPa)	2100	0.346 mPa (non-volatile)	9.5
ADI (mg/kg bw/day)	0.00008 (EC, 2018)	0.0005*(Meador and Ma, 2014)	0.015 (EC, 2018)

LC_{50} 96h in fish	2.51 mg/L (*Cyprinus carpio*) (Günde and Yerli, 2012) 0.2 -12 mg/L (fresh water and estuarine fish) (Das, 2013)	0.76 mg/L (*Cyprinus carpio*) (Tran et al., 2012) 0.86 mg/L (*Barbonymus gonionotus*) (Tran et al., 2012)	0.045 mg/L (*Cyprinus carpio*) (Poleksić and Karan, 1999)

Log $P_{o/w}$: logarithm of the octanol/water partition coefficient

Dichlorvos

Dichlorvos volatizes easily, so it was also used as a fumigant agent. It is used to apply as a main treatment for external parasites in fish culture (Wootten et al., 1982). In aquaculture, especially in intensive systems, dichlorvos was applied into water to control invertebrate fish parasites (Matolcsy, 1988; WHO, 1989). In Vietnam, dichlorvos was used in both agriculture and aquaculture to control pathogens in fish culture; it was used to prevent parasites in shrimp pond preparation and to prevent external parasites in fish rearing periods (Tran and Do, 2011). Widely used in the world for several year in agriculture, forestry and veterinary, it can produce bad effects on non-target species which have a habitat close to the agriculture area or event high level vertebrate animals.

Toxicological effects of dichlorvos

Acute toxicity of dichlorvos

Like other organophosphates, dichlorvos poisoning may cause cholinergic crisis including central apnea, pulmonary bronchoconstriction and recreation, seizures, muscle weakness, etc. (Gaspari and Paydarfar, 2007). The acute toxicity of dichlorvos was investigated in several organisms such as insects (Hoang and Rand, 2015), fish (Varó et al., 2008; Varó et al., 2007) and mammals (rodents, rats) (Gaspari and Paydarfar, 2007). According to Hoang and Rand (2015), the LD_{50} (oral) 24h of dichlorvos in caterpillars were 0.2 -2 depending on species. The LD_{50} (oral) of rat and mouse were 25-80 and 140-275 mg/kg, respectively. When applied as fumigant the LD_{50}(4h) would be 13 and 15 mg/m^3 for rat and mouse, respectively (Wilkinson et al., 1999a).

Chronic toxicity of dichlorvos

At a concentration lower than acute levels, dichlorvos causes many physiological problems. Rabbits having a diet with 0.31 to 2.5 mg dichlorvos/kg 5 days a week during 6 weeks showed humoral immune response and cell-mediated immunity inhibition. The rat, with the dose of 2 mg/L in

drinking water, showed an altered diurnal rhythm of pituitary/adrenal axis, a change in plasma adrenocorticotrophic hormone and adrenal cholesterol ester concentration (Wilkinson et al., 1999a).

Moreover, dichlorvos treated mammal (mouse) at the dose of 1/50 LD_{50} (1.22 mg/kg bw/day) and 1/10 LD_{50} (6.1 mg/kg bw/day) for 30 days showed no toxic clinical sign, histological change in liver and no abnormal activity or cholinergic overstimulation. However, oxidative markers and endogenous metabolites changes were found in liver and serum of investigated animals; in addition, glucose, fatty acids and proteins metabolism also changed significantly (Wang et al., 2014).

Reproductive and teratogenic effects of dichlorvos

At the concentration of 1/50 LD_{50} oral dose (1.6 mg/kg body weight), dichlorvos can cause a decrease in body and testis weights, sperm morphology, sexual hormone levels. In addition, necrosis, edema and cellular damages were also recorded after feeding the above dose for seven weeks. This study also indicated that antioxidant vitamins could not improve this serve situation (Dirican and Kalender, 2012).

Mutagenic and carcinogenic effects of dichlorvos

According to pesticide databases, dichlorvos was marked as mutagenic agent (PPDB, 2015a), and carcinogenic agent (Kegley et al., 2014a).

Ecological effects of dichlorvos

Toxicity of dichlorvos to aquatic animals

Dichlorvos can enter aquatic animal body through skin and gill. It was reported that dichlorvos reduced AChE (Acetyl choline esterase) activity in brain of fish and the RND/DNA ratio, that it increased lipid peroxidation in fish (Varó et al., 2007).

Environmental fate

Dichlorvos is a volatile compound so it can easily propagate into the air, that is why the chemical has to be used in enclosed area. In the air, it combines with water and is transformed into less harmful chemicals which are dimethyl phosphate and dichloroacetaldehyde. The more humidity in the air, the more degradation of dichlorvos (Richter and Corcoran, 1997).

Breakdown in soil and water

Dichlorvos can be hydrolyzed in water and the hydrolysis rate increases with the increasing of pH. In water, this compound degrades to dimethylphosphoric acid and dichloroacetaldehyde and finally to CO_2 and phosphate (AG, 2008).

Quinalphos

Quinalphos is popularly used in the Mekong Delta under the brand name Kinalus 25EC™ to treat *Steneotarsonemus spinki* and other pest in rice cultivation (Toan, 2014).

Toxicological effects

Acute toxicity

The toxicity of quinalphos (Figure 8) on rat is similar to dichlorvos with LD50 oral of 26-71 mg/kg bw. LD_{50} of intraperitoneal and subcutaneous application in rat were 34-39 and 55-56 mg/kg bw, respectively (Wilkinson et al., 1999).

Chronic toxicity

Quinalphos causes reduction in red blood cell and thrombocyte counts in chicken which were fed with the dose of 5 mg/kg bw/day for 20 days. It also showed damages in liver, lung and heart of the investigated animals. At the level of 0.5 mg/kg bw, quinalphos brought a decrease in the acetylcholinesterase activity and an increasing in superoxide radical and related enzyme activity in brain of observed rats (Wilkinson et al., 1999b).

Reproductive and teratogenic effects

Srivastava and Raizada (1999) studied the effects of quinalphos on pregnant rats and concluded that the "no observed effect level" on fetal and maternal toxicity of quinalphos is 2 mg/kg body weight. However, at higher levels (3 and 4.5 mg/kg bw), quinalphos induced significant changes in enzyme activities and changes in hepatocellular dams.

Mutagenic and carcinogenic effects

Apart from action on pest, quinalphos is also known to induce various toxic effects on non-target species. In the study on Swiss albino mice, quinalphos showed tumor-initiating potential at the dose of 10 mg/kg body weight, but quinalphos exposure failed to produce neoplasia and tumor promoting activity at all the test dose levels (Shukla et al., 2000).

Fate in human and animals

The fate of quinalphos in simulated gastric and intestine phases was investigated in rat after dosing with 5 mg/kg body weight by Gupta and co-workers (2012). The study used HPLC and GC-MS for detecting all metabolic derivatives. Results showed that quinalphos oxon, O-ethyl-O-quinaxalin-2-yl-phosphoric acid, 2-hydroxy quinoxaline and ethyl phosphoric acid are important metabolites identified both *in vitro* and *in vivo* conditions. In addition, 2-hydroxy quinoxaline and oxon, which are more toxic than quinalphos, persist for a longer time (Gupta et al., 2012).

Ecological effects

Toxic effects to aquatic animals

As other organophosphate pesticides, quinalphos is a neurotoxin and is an inhibited acetyl choline esterase (AChE) agent. Acetyl choline is a neurotransmitter and is the only transmitter compound which is inactivated by an hydrolysis enzyme, i.e.AChE , rather than re-uptake. Primary action of quinalphos and other OPs are inhibition AChE activity. Quinalphos decreased the activity of AChE in brain, muscle, gill and liver of fresh water teleost *Cyprinus carpio* (Chebbi and David, 2009). Quinalphos also effects testicular of *Clarias batrachus*, an air-breathing catfish species (Bagchi et al., 1990).

Environmental fate

In soil and water

According to Gupta and co-workers (2011), in water and soil conditions, the degradation of quinalphos increases with the increasing of temperature and pH (Gupta et al., 2011).

In the presence of humic acid, the decay of quinalphos also increases as it acts as a reducing agent, i.e. the higher the organic content, the lower quinalphos persistence (Gupta et al., 2011).

Breakdown in vegetation

In comparison with water and soil, the degradation of quinalphos in plant appears faster; for details, the half-life of quinalphos in tomato, radish leaf and root varies from 3 to 4 days comparing with 26 to 74 days in water and 9 to 53 days in soil in all conditions (Gupta et al., 2011).

The fast degradation of quinalphos also found in okra fruit when quinalphos was applied by spraying at the doses of 500 g and 1000 g per hectare revealed that the half-life of quinalphos in okra in such conditions is 1.25 to 1.43 days, and the safe waiting period are 5.3 and 6.7 days in lower and

higher doses (Aktar et al., 2008). In the case of cabbage, the half-life of quinalphos are 3.02 and 2.70 days for the doses of 500 g and 1000 g quinalphos application by spraying and the waiting period is 7 days for the application doses on cabbage (Chahil et al., 2011).

Trifluralin

Trifluralin is used to control annual broadleaf weeds since 1963. It acts as a germinating inhibitor based on prevention of root and shoot cell division. Trifluralin is listed in group C, possibly carcinogenic to human, by USEPA according to animal evidences (IRIS, 1987). Moreover, commercial trifluralin contains nitrosodipropylamine, a carcinogenic contaminant, which may induce mutation while reacting with O^6-guanin DNA (Fernandes et al., 2013).

Toxicological effects of trifluralin

Acute toxicity of trifluralin

According to data extracted by Fernandes (2013), toxicity of trifluralin varies between groups of animals. For mammals, trifluralin is not very toxic; for dogs and rabbits, the LD_{50} (oral) are higher than 200 mg/kg bw, while those values are higher (500 and 10,000 mg/kg bw) for laboratory mice (*Mus musculus* and *Ratus norvegicus*), respectively. Regarding to aquatic animals, the common carp (*Cyprinus carpio*) shows the highest tolerance to trifluralin, with a median lethal concentration (LC50) (48h) of 1000 µg/L, whereas bluegill (*Lepomis macrochirus*) and ocean sunfish (*Mola mola*) share a LC_{50}(48h) of 19 µg/L. Crustaceans can tolerate a high concentration of trifluralin, for instance, LC_{50} (96h) of lobster (*Procambarus clarkia*) and LC_{50} (48h) of a micro-crustacean (*Daphnia magma*) are 12,000 and 560 µg/L, respectively (Fernandes et al., 2013). For young rainbow trout, bluegill and ocean sunfish, the acute toxicity of trifluralin was different than in adults (Fernandes et al., 2013). The toxicity of trifluralin for a 3 cm length common carp was 45 µg/L (Poleksić and Karan, 1999).

Chronic toxicity

According to Ebert and co-workers (1992), the chronic and sub-chronic test showed that trifluralin was haematotoxic and slightly hepatotoxic. The author also stated that the NOELs of trifluralin on dogs and rats were 4.8 and 41.0 mg/kg body weight/day, respectively. In addition, ADI of trifluralin was suggested at 0.05 mg/kg body weight/day with the safety factor of 100 (Ebert et al., 1992).

Reproductive and teratogenic effects

There is no evidences of very high trifluralin concentration applied in animal which caused reproductive or teratogenic effect (Wallace, 2014).

Mutagenic and carcinogenic effects

Trifluralin was known as a tumor stimulant agent. At the dose of 441 mg/kg/day in two weeks, it induced the hypertrophy of thyroid gland through increasing the TSH (Thyroid-Stimulating Hormone) level in Fischer 344 rats (Saghir et al., 2008). Other studies of the chronic toxicity of trifluralin indicated hepatocellular carcinomas in animals (Rodriguez, 2014). However, according to Eastmond (2010), there is a limited evidence that trifluralin can cause cancer in animal; for human, there was inadequate evidence of carcinogenicity (Eastmond and Balakrishnan, 2010), while online databases indicated trifluralin as a possible carcinogen (Kegley et al., 2014b) and (PPDB, 2015b). However, the International Agency for Research on Cancer (IARC) has classified trifluralin in group 3, which means "not classifiable as carcinogenic to humans".

Ecological effects

Toxic effects to aquatic animals

In the study of Poleksić, the LC_{50} (96h) of trifluralin on fingerling common carp was 45 µg/L, and at sub-acute exposure (0.005, 0.01, and 0.02 mg/L), trifluralin decreased the growth rate of the fish in 14 days. Besides, the activity of enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase) and the gill and liver histology were also affected when the fish was exposed to sub-acute levels of trifluralin (Poleksić and Karan, 1999).

Toxic effects to other animals

Bioaccumulation of trifluralin was shown in invertebrates such as isopods or earth worms living in contaminated environments. The ratio of trifluralin and its metabolites in isopods were 6.7 to 18.6 higher than that in liter; the bioaccumulation in earth worms was about 7 times higher than that in isopods. However, trifluralin showed no toxic nor sub-toxic effects on this investigated organisms under recommended concentrations (Staak et al., 1998).

Environmental fate

Under sunlight exposure condition, trifluralin is readily degraded and showed a half time which varied from minutes to months depending on the matrix. As trifluralin has a high octanol/water

partition coefficient, it is poorly soluble in water and it strongly binds into soil components. Residues of trifluralin in soil are subjected to lose by runoff water and evaporation. The preferred pathway of trifluralin contamination to water environment is surface runoff from agriculture area (Boithias et al., 2011).

Breakdown in soil and water

In soil, trifluralin degrades through chemical and microbial pathways and photolysis. Chemical degradation pathway includes amino group dealkylation, amino group reduction and partial oxidation of trifluoromethyl to carboxyl group (Fernandes et al., 2013).

Under anaerobic conditions, trifluralin tends to be strongly degraded than in the aerobic condition with the ratio of 98% compared with 25%. The degradation of trifluralin was mainly caused by fungi, although *Pseudomonas sp* were also reported as microorganisms capable to degrade the compound (Fernandes et al., 2013).

The presence of trifluralin in water may be at a very low concentration due to its low mobility in soil and its low solubility in water, and only 0.5% of trifluralin applied in soil leaches to water. Although trifluralin is an herbicide, which is designed to inhibit the germination of broadleaf weeds, it is also used in aquaculture to prevent fungal disease in fish and surface fouling disease in shrimp (Truong, 2012). For this treatment, trifluralin is applied directly into water. The degradation of trifluralin, in natural water, was affected by many factors. Dissolve organic matters would slow down the rate to a constant value, whereas nitrate ions show higher degradation rate of trifluralin under sunlight exposure condition. The photodecomposition of trifluralin in water was mainly due to dealkylation, cyclization and reduction (Dimou et al., 2004).

2.4.2. Other groups of contaminants

Antibiotics

Among antibiotics, chloramphenicol was chosen for the screening, as it was banned in aquaculture (VMARD, 2009), but residues of CAM were found in aquaculture products exported from Vietnam to US in 2009 and 2013 (FDA, 2017). In the European Union, CAM is banned since the 90's, but during the period from 2002 to 2017, chloramphenicol residues in fish and fish products imported from Vietnam were frequently notified by the RASFF (rapid alert system for food and feed of the European Union) (Figure 7) (RASFF, 2018).

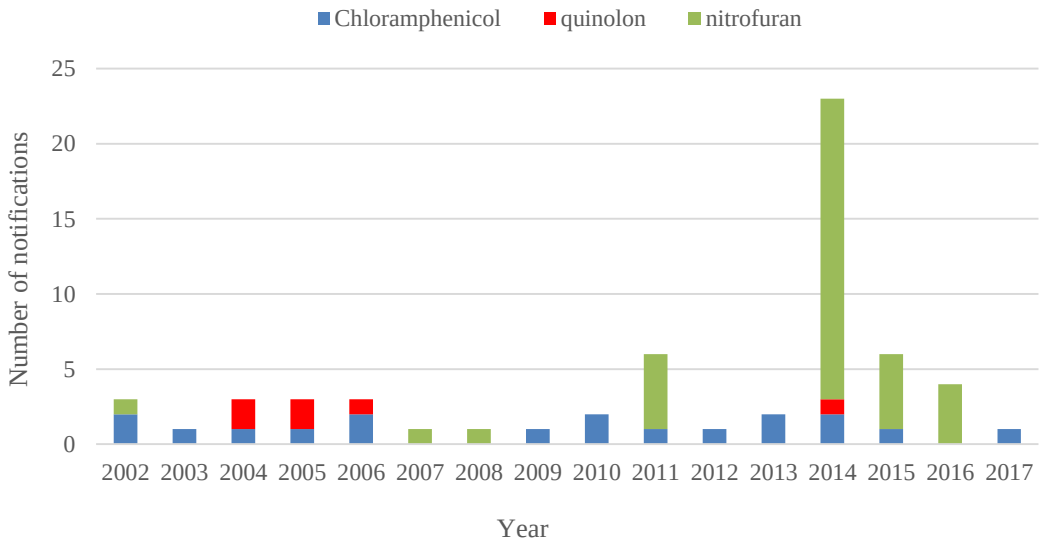

Figure 7. Number of notifications of residues of nitrofurans, quinolones and chloramphenicol in catfish and shrimp products imported from Vietnam, from 2002 to 2017. Note: after 2010, no residue of chloramphenicol in striped catfish has been noted, CAM have been found only in shrimp or frozen red mullets (RASFF, 2018).

Chloramphenicol was first isolated from cultures of *Streptomyces venezuelae* in 1947 but itis now produced synthetically. As the first discovered broad-spectrum antibiotic, it acts by interfering with bacterial protein synthesis. CAM is very effective to treat fish bacterial diseases (Dang et al., 2014; Reeves, 2012), but this compound was not approved by EU and US (reviewed by Dang et al., 2014). Indeed, this antibiotic shows some adverse effects in animals and humans and is listed as probable human carcinogen (Group 2A of IARC) (IARC, 1990). For ecotoxicology, chloramphenicol causes changes of leukocytes of amphibians, the phenomenon being similar to the one being caused by the carcinogen 7, 12-dimethylbenz(a)anthracene (Abdollahi and Mostafalou, 2014). In the years 2000, CAM was one of the commonly detected antibiotics in aquaculture products. Its residue was found in

many large import markets (i.e. Canada, US, EU and Japan) and in particular in aquaculture products exported from Vietnam (Love et al., 2011).

Dioxins and PCBs

The other investigated chemicals were dioxins (including furans and dioxin like PCBs). Dioxins and dioxin-like chemicals form a large group of compounds which are structurally related. They are environmentally and biologically persistent, induce a common spectrum of responses, and have a common mechanism of action (Van den Berg et al., 1998). These persistent organic pollutants are highly lipid soluble and can reach a toxic concentration in animal tissues (Franco et al., 2010). Dioxins are contaminants from many industrial processes, including incineration, chlorine bleaching of paper and pulp and the manufacture of some pesticides, herbicides, and fungicides (Lustenhouwer et al., 1980; Gilpin et al., 2005). According to Kulkarni et al. (2008), the source of dioxin includes four major categories (Figure 8). Dioxins in pure form are colorless solids and are formed as combustion products. Recent studies indicated that dioxins are still introduced into the aquatic environment, and dioxins concentration in some river sediment samples collected in the rivers of the South of Vietnam were higher than the standards of Canadian environment quality guideline (Minh et al., 2007).

Dioxin was a contaminant of the organochlorine herbicide “Agent Orange” used during the Vietnam War and possibly responsible for some of the adverse health effects associated with exposure to the defoliant (Young, 2014).

The mechanism of toxicity for dioxins is quite complex and still not understood clearly. In many studies, primarily with 2,3,7,8-TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin) affirms interaction with the Ah (aryl hydrocarbon) receptor especially with respect to alteration of gene expression. The dioxin-induced cellular effects such as hyperplasia, hypoplasia, metaplasia, and dysplasia are thought to be initiated via the Ah receptor (Young, 2014).

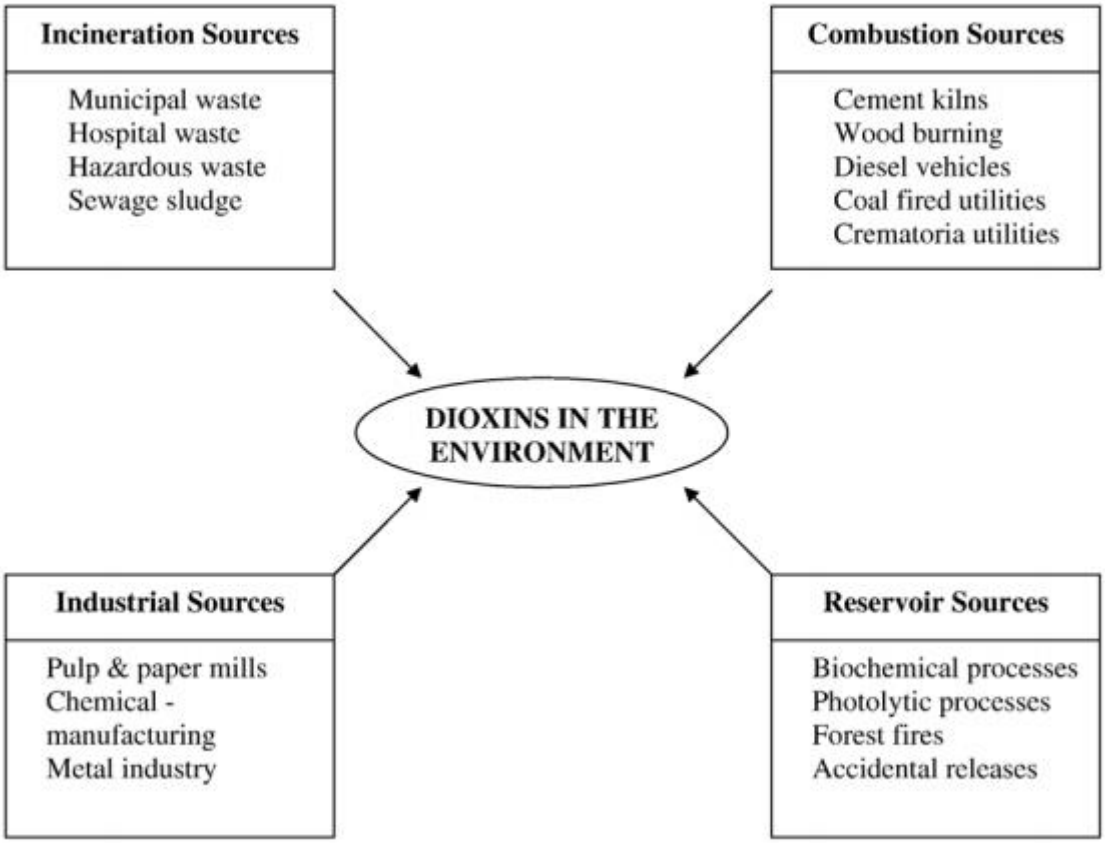

Figure 8. Dioxin release in the environment (Kulkarni et al., 2008).

3. Analytical method overview

According to Hubert and co-workers (2007), an analytical method could be divided into four phases:

1) Selection phase which allows defining objectives and initial conditions

2) Development phase

3) Validation

4) Application in routine.

3.1. Instrumental methods

Most of food or food related materials like raw products, ingredients, spices etc. need to be analyzed for several purposes related to satisfy the customer, quality control or regulation. Each commodity has its own problem of extraction and clean-up and needs to be applied with appropriate

procedures. The five common key factors used in the method selection are: speed, specificity, precision, accuracy and ruggedness, all these combined with the properties of the matrix to analyze. Moreover, method validation should be performed for each specific matrix to ensure the reliability of analytical results (Nielsen, 2010).

Regarding food safety, chemical residues and contamination analysis are mainly concerned by organic pesticides which have been used extensively in agriculture. To analyze pesticide residues, the procedure is quite similar to the scheme described above. Standards are firstly used for compound detection and extraction, which includes fluid phase partitioning method, adsorptive and membrane based extraction method. In pesticide analysis, after the extraction, a clean-up step should be made, especially for complex matrices like food. The final steps of pesticide analysis are estimating the quantity and confirmation.

Pesticide extraction step

This is one of the most important steps in pesticide residues analysis. The extraction step transfers the analytes from the biological matrix into the extraction support (liquid or solid), and allow to eliminate interferences and to increase the analytes concentration to a level which is higher than the detection limit of the analytical method. Traditional "old" methods were generally laborious, time consuming and used large amounts of solvent (Beyer and Biziuk, 2008).

According to Beyer and Biziuk (2008), regarding food or food related commodities, the most common extraction methods include (Beyer and Biziuk, 2008) :

- Liquid-liquid extraction (LLE): the technique is based on the partition of analytes between two immiscible liquid phases.

- Solid phase extraction (SPE): The principle of SPE is similar to that of liquid-liquid extraction (LLE), involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase (Żwir-Ferenc and Biziuk, 2006).

- Solvent extraction in a Soxhlet or Soxtec apparatus: a very common method in food analysis, which is also liquid extraction but allowing to extract pesticides.

- Focused microwave-assisted solvent extraction (FMASE): the method has the advantages of the Soxhlet technique and reduces environment pollution as less solvent is used.

- Ultrasonication extraction (USE): a conventional method applied in pesticide and PCB extraction from various commodities. The principle of the method is based on the ultrasonic to accelerate the pesticide washing from matrices.

- Some other extraction methods which have been widely used include matrix solid-phase dispersion (MSPD), microwave-accelerated extraction (MAE), accelerated solvent extraction (ASE; also known as PFE or PLE), supercritical fluid extraction (SFE) and membrane extraction techniques.

The technique applied for pesticides extraction is strongly depending on the matrix. The most widely used method for solid matrices like animal origin food is SPE. For liquid matrices like milk or water, LLE is preferred. Regarding to SPE, solid samples must be homogenized before extraction. The extraction procedure may use only one solvent or a mixture of solvents. The reason of combining solvents is extending the polarity range of extraction phase. A mixture of solvents is especially effective in multi-residues analysis, and in the case of degradation components of an analyte having a polarity different from the polarity of the parent compound (LeDoux, 2011). Recent methods were applied and standardized by different authors in several matrices such as meat and meat-products, fish and seafood, milk and milk products (Martins et al., 2013).

Traditional Soxhlet technique was mostly use for the extraction of organochlorine and organophosphate pesticides such as hexachlorocyclohexane isomers (HCHs), dichlorodiphenyltrichloroethane (DDT), polychlorinated-biphenyl (PCB) from pork, chicken or lamb organs (Covaci et al., 2004; Garrido Frenich et al., 2006), fish (Campos et al., 2005) and eggs (Tao et al., 2009). However, the method is time consuming and costly in term of solvent and energy use (LeDoux, 2011). In order to overcome the disadvantages of Soxhlet method, many modified techniques were developed such as Soxtec which reduces significantly the time consumption of Soxhlet. Ultra-sonication extraction commonly replaces Soxhlet method for PCBs extraction in environmental samples (Sporring et al., 2005). Two other alternative methods are supercritical fluid extraction and pressurized fluid extraction which are used for pesticide analysis in several solid matrices. These methods can also overcome the drawback of Soxhlet (solvent and time consuming), but the instruments and maintenance are expensive (LeDoux, 2011).

Matrix solid-phase dispersion (MSPD) is a method which combines homogenization, extraction, fractionation and clean-up in only one process. According to Barker (2007), this method was widely used with more than 250 publications citing the technique as extraction method since it was introduced in 1989. In this process, solid or semi-solid samples, like meat or seafood, which were ground with solid support bonded phase to form a new chromatography material. The mixture of blended sample and solid support bonded phase would then allow fractioning of analytes in appropriate solvents (Barker, 2007).

Pesticide clean-up step

Matrix components are usually co-extracted with pesticides during extraction step. The co-extracts then go through the analytical system and cause interferences on the results due to interfering target compounds in detectors. They also reduce the system lifetime. Depending on matrices, the co-extracts are different; for instance, in the case of baby foods, the co-extracted compounds may be proteins, lipids, pigments, carotenoids, melanoidines, benzopyranose (Przybylski and Segard, 2009). Regarding to fish or meat product, the co-extracts are proteins, saturated or unsaturated fatty acids, sterols, etc. The co-extracted components, especially lipids, may be retained in the analytical systems like in the injection port or on the column in chromatographic instruments. Those lead to reduce repeatability, sensitivity or shorten column lifetime. Several methods have been investigated to eliminate or reduce the co-extractants, the procedure is usually called the clean-up step. Many approaches can be applied in this step, such as, solvent partition, SPE and absorbent use. First of all, solvent partition is based on the solubility of chemicals in solvents and the coefficient partition of chemicals between solvents. The methods, however, need to be assessed carefully or the clean-up solvents will remove also the targets. According to Przybylski and Segard (2009), hexane can be used to eliminate fat present in acetonitrile extracts of samples from animal origin, resulting in the increasing of the sensitivity of the method and in the decreasing of the needs of instrument maintenance (Przybylski and Segard, 2009). The technique was sometimes combined with freezing to get the fat removing more efficiently (Khay et al., 2009). In this approach, the different melting points of extractants and co-extractants are exploited in the clean-up step. Under freezing conditions, co-extracted lipids are precipitated and separated from solvents while pesticides still remained dissolved in solvents. In the study of Argauer and co-workers (1997), when extracting pesticides from meat, filtered extraction solvent was frozen for several hours to freeze fat, and decanted solvent was used for analysis, but the method consumed a lot of solvents (Argauer et al., 1997).

SPE clean-up is applied to remove co-extracted interferences for most of the pesticides classes. Various sorbents types can be used such as, silica-gel, C18-bond silica, aluminum, primary or secondary amine (PSA), Florisil, graphite non-polar carbon (LeDoux, 2011). Beside the mentioned sorbents, most of the extraction and clean-up methods use anhydrous sodium sulfate to remove water from solvent (LeDoux, 2011). Practically, more than one cartridge may be combined to maximize interference elimination, e.g. a tandem of Extralut NT3, Sep-Pack C18 and Florisil cartridge were used in organochlorine pesticides (OCPs) and pyrethroids (PYRs) analysis (Stefanelli et al., 2009). Besides, two or three sorbents combined in one cartridge were also used (Shin and Shin, 2003). However, the SPE clean-up step sometimes did not show efficiency as it depends on the types of pesticides and absorbents (Khay et al., 2009). Moreover, the recovery of the clean-up step can be different for the various members of a same chemical group. For instance, in the study of Doong and Lee (1999): only

twelve OCPs out of fourteen were recovered after clean-up with C18 cartridges, but with Florisil and aluminum, all fourteen OCPs were recovered and Florisil showed higher recovery and repeatability, and worked better than the others for co-extractants elimination (Doong and Lee, 1999). In addition, co-extracted fat was better removed if Florisil was used in solid phase extraction (Hong et al., 2004).

Separation and detection instruments

Chromatography

Chromatography is a very effective technique for separation since it was first described in 1909. In this technique, the dissolved sample extract is introduced on the stationary phase (adsorbent material constituting the column) by a mobile phase. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. Chromatography can be classified based on mobile phase or stationary phase. The two most common chromatography techniques are gas chromatography (GC) and liquid chromatography (LC) (Moldoveanu and David, 2015).

Gas chromatography is applied to analyze compounds which are gases or can be volatized easily. Gases used as mobile phase can be helium, hydrogen or nitrogen. The separation principle of GC is based on the evaporation temperature of chemicals. The more volatile compounds will be eluted sooner than the other compounds which are less volatile. One other important factor affecting the separation in GC is the polarity of the stationary phase and of separated compounds. The column made by polydimethylsiloxane is considered as a nonpolar material while polyethylene glycol is a polar material (Moldoveanu and David, 2015). Gas chromatography is a largely used, versatile and sensitive method in pesticide residues analysis and the most common stationary phase material is organosilicon (Liu et al., 2010).

Detector

There are many kinds of detectors; some of them are non-selective, which are not specific for any compounds and the others are selective which are specific for one or a group of elements. The important features of a detector are sensitivity, stability and dynamic range. One of the most common detectors is the mass spectrometer (MS) detector. This detector allows to identify compounds (Moldoveanu and David, 2015) after their ionization based on their mass to charge ratio (m/z). There are different modes of MS operation; total ion chromatogram (TIC), which plots all ions detected, selected ion monitoring (SIM), which plots one selected ion and multiple reaction monitoring (MRM) or selected reaction monitoring (SRM), which is a method used in tandem mass spectrometry in which

an ion of a particular mass is selected in the first stage of a tandem mass spectrometer and an ion product of a fragmentation reaction of the precursor ion is selected in the second mass spectrometer stage for detection.

ECD (electron capture detector) is also widely used for pesticide detection. The detection is based on the capture of electrons emitted from radioactive beta-emitter by compounds eluted from GC column. This detector is especially sensitive to poly-halogenated compounds or electron withdrawing groups from those with conjugated carbonyl or with sulfur (Liu et al., 2010).

3.2. Bioassay application in chemical residues and contamination determination

The bioassay methods used in this study are the enzyme linked immunosorbent assay (ELISA) for antibiotic residues determination and the CALUX (Chemical-Activated LUciferase gene eXpression) bioassay, for dioxins determination.

ELISA is a simple and useful method in chemical detection. The method does not need modern and complicated instruments. There is direct and indirect form of ELISA. In the direct ELISA, both the target (unlabeled antigens) and labeled antigens compete each other to be recognized by the immobilized antibodies. The signals are directly evaluated. The latter assay is much more advanced as the antibodies competitively capture the dissolved targets and immobilized antigens. The labeled secondary antibodies bind to the compatible antibodies after the washing step. Following incubation and another subsequent washing step, the signals can be measured (Lee *et al.*, 2001). Development and application of enzyme-linked immunosorbent assay (ELISA) for analysis of antibiotics used in food producing animals have increased in the last decade. This method is rapid, sensitive, cost effective, requires little sample clean-up and lend it to routine testing of large numbers of samples. Moreover, ELISA can detect low level of residues. Therefore, it can be used for qualitative screening or quantitative analysis (Dixon-Holland, 1992). Many antibiotics are detected from fish using ELISA like neomycine with a LOD of 0.01 µg/kg (Wang, 2009), sulfonamides, tetracyclines (Cháfer-Pericás, 2011), chloramphenicol, gentamicin, fluoroquinolone-enrofloxacin. Metabolites of antibiotic can also be detected by ELISA, e.g. metabolites of furaltadone and furazolidone (Conti, 2015). The comparison of ELISA and other detection method was also made by some authors. For example, Cháfer-Pericás (2011) compared ELISA with LC MS-MS for sulfonamides and tetracyclines residue analysis in fish and feed samples. This author concluded that ELISA was correlated with LC MS-MS.

The CALUX bioassay is a method based on genetically modified cells which responds to compounds which are able to activate the aryl hydrocarbon receptor (AhR). The common pathway of

AhR activation is gene expression. The recombinant cells used in the CALUX bio assay contain a reporter gene (luciferase) which is expressed when the AhR is activated by dioxins or other similar compounds. The result of luciferase synthesis will be emission of light (which is the cell response), measurable using a luminometer. The level of cell response reflects the quantity of AhR activators to which cells are exposed (Windal et al., 2005). This method is used for dioxins and dioxin-like chemicals determination from various matrices, such as water (Addeck et al., 2014), sediments and soil (Baston and Denison, 2011), human milk (Croes et al., 2013), and animal origin commodities (Scippo et al., 2004; Vromman et al., 2012). The same kind of method, but using other intracellular receptors (steroid receptors) is also applied to detect steroid compounds in environment (Avberšek et al., 2011; Vandermarken et al., 2016).

3.3. Validation

The validation stage is considered as a step which is added after a new analytical method has been developed and validation performances will be assessed during the four stages of the analytical procedure (Hubert et al., 2007). Generally, validation parameters to be determined include: trueness, precision (repeatability and within laboratory reproducibility), specificity, detection limit, quantification limit, linearity and working range (ICH, 2005, SANCO/12495/2011, 2011).

The following definitions are those of the SANCO guidelines (SANCO, 2011):

-Accuracy is the closeness of agreement between a test result and the true, or the accepted reference value. When applied to a set of test results, it involves a combination of random error (estimated as precision) and a common systematic error (trueness or bias).

-Trueness is the closeness of agreement between the average value obtained from a series of test results (i.e. the mean recovery) an accepted reference or true value.

-Precision is the closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors which affect the results, the more precise the procedure. A measure of precision (or imprecision) is the standard deviation.

-Repeatability (r) is the precision (standard deviation) of measurement of an analyte (usually obtained from recovery or analysis of reference materials), obtained using the same method on the same sample(s) in a single laboratory over a short period of time, during which differences in the materials and equipment used and/or the analysts involved will not occur. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result.

-Reproducibility (R) is the precision (standard deviation) of measurement of an analyte (usually by means of recovery or analysis of reference materials), obtained using the same method in a number of laboratories, by different analysts, or over a period in which differences in the materials and equipment will occur. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result.

-Within-reproducibility (wR) is that produced in a single laboratory under these conditions.

-Specificity is the ability of the detector (supported by the selectivity of the extraction, cleanup, derivation or separation, if necessary) to provide signals that effectively identify the analyte.

-Limit of detection (LOD) of an analytical procedure is the lowest amount of an analyte which can be detected.

-Limit of quantification (LOQ) is the lowest amount of an analyte in a matrix which can be quantified with an acceptable accuracy and precision.

-Linearity is the ability of an analytical method to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

-Working range is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

According to the SANCO document guideline (2011), the accuracy of a method must vary in the range of 70 – 120% and precision, expressed as coefficient of variation, has to be lower or equal to 20% (SANCO/12495/2011, 2011).

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Objectives

General objectives

The general objective of the current project was to assess the chemical use situation in aquaculture and in aquaculture related agricultural systems, in the Mekong Delta region of Vietnam. Beside the use, of chemicals, the assessment of the chemical contamination of aquaculture products and environment were also included in the general objectives as well as its impact on the environment and food safety.

Specific objectives

1. To conduct a survey of the use on chemicals in fresh water aquaculture in the Mekong Delta.

2. To develop and validate analytical methods for selected pesticides (quinalphos, trifluralin, and dichlorvos) to be detected in various matrices (water, fish and sediment). Gas chromatography detection methods using both electron capture and mass spectrometry detectors will be developed, validated and compared to assess the possibility of analyzing the residues of common pesticides in various matrices.

3. To assess the current situation of chemical contamination of aquatic cultured products as well as the water of aquaculture production systems. The targeted pesticides (the most commonly used pesticides identified from the survey) residues will be determined using the developed methods above, while chloramphenicol (representative of antibiotics) and dioxins (representative as environmental contaminants) will be determined using ELISA, LC-MS and bioassay methods.

4. To study the elimination of the selected pesticides in practical situation, i.e. rice cum fish system

5. To assess the risk for the consumers linked to the chemical contamination of food, according to the levels of contamination in water and fish, and to diet habits of local population. This assessment study will collect information about food consumption which will be focused on the amount, kind of food as well as the health of consumers; this information will be combined with the chemical residue data to assess the risk for consumers.

6. To make recommendations for the control of chemical hazards in aquaculture products.

Experimental section

Experimental section

Study n°1: Survey of the use of chemicals in fresh water aquaculture in the Mekong Delta

Manuscript in preparation

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The Mekong Delta, Vietnam, contributes largely to the agricultural and the aquaculture production of Vietnam. However, many studies reported an overuse of agrochemicals in this area. Residues of pesticides and veterinary drugs in exported aquatic production which originated from the Mekong Delta have been detected and announced by import markets like Japan and countries of the European Union. Therefore, our first survey in 2009 was about the chemical use situation in rice and rice fish systems which consume a large amount of pesticides. A second survey was performed in 2013 about the chemical use in aquaculture from three common systems including catfish pond culture, red tilapia cage culture and rice fish integrated system. The purpose of the surveys was to assess the chemical situation, to figure out the reasons of chemical contamination in aquaculture products as well as the attitude of farmers on chemical use, environmental effect and human health.

Abstract

In order to assess the situation of chemical use in rice-fish, stripped catfish cultured in earth ponds and red tilapia cultured in cages systems, two survey were conducted in 2009 and 2013. The first survey was in Co Do, Thoi Lai and Vinh Thanh districts of Can Tho city. Forty-five rice-fish farms, 45 rice farms and 15 agrochemical stores in three selected districts were randomly selected for an interview using structured questionnaires. And, to evaluate the perspectives of the farmer about the pressure of pesticides on health and environment and to know the pesticide use in practice, a second survey was conducted in 2013. In total, 93 interviewees were involved in the second survey which included 30 rice fish farms, 22 red tilapia cages, 15 catfish ponds and 26 agrochemical stores. Among 26 stores, there were 16 aquaculture chemical stores and 10 stores which supported rice and other crop cultivation. The survey results showed that the frequency of application in rice and rice-fish farming was 2.9 times per crop and 2.7 times per crop on average, respectively. The most common application period was from the day 31 and 60 of the rice crop. Pymetrozine (Chess™, Product of Syngenta Vietnam) and fenobucarb (Basa™, Product of Vithaco, Bac Giang, Vietnam) were the commonly used pesticides in rice farming and rice-fish farming. Fenobucarb (Basa™) and quinalphos (Kinalux™, Product of United Phosphorus Limited, India) pesticides shared large sell portion of distributors. The average fertilizer quantity applied per hectare was 390 kg. According to the second survey, all systems used chemicals in culturing process. The number of chemicals used in rice-fish system was 37, with most of them (30 types) used for rice production. Nineteen chemicals were used in stripped catfish system; the chemicals included 10 types of water quality treatment and antiparasite agents, 7 types of antibiotics and 2 diet supplement elements. In red tilapia, 18 types of chemicals were used belonging to antibiotics, water treatment and antiparasite groups. Generally, the antibiotics used in these systems were similar, and all used chemicals were approved by the Government of Vietnam, but, most of antibiotics were listed for limited use by the Ministry of Fisheries. This may lead to the possible presence of residues of these compounds in aquatic products with concentrations higher than the maximum residue levels (MRLs) which may impact negatively the consumer health and exported activities.

Introduction

Pesticides, which are intentional added chemicals to improve the quality of environment for human, human's animals and plants (Srivastava et al., 2010), have been used over the world with an annual use of about two million tons, consisting of herbicides (47.5%), insecticides (29.5%), fungicides (17.5%) and 5.5% of others (De et al., 2014b). These chemicals show lots of benefits for plant and food protection from insect damages, but some negative side effects of pesticides are also recognized. Beside introducing risks to humans, animals and decreasing soil fertility, the excessive and continuous use of these chemicals results in a selective pressure giving the opportunity for resistant strains to survive and increase their population. Nowadays, there are more than 500 insect species, 270 weed species and 150 fungi strains which are pesticide resistant (De et al., 2014a). The Mekong Delta (MD) is the most intensive agriculture and important rice production area of Vietnam. The area account for 50 percent of agricultural products of Vietnam which includes fruits, vegetables, fishes and rice (Campbell, 2012). In the MD, the aquaculture consists of many levels of operation which vary from extensive where no feed is supplied to intensive systems, where feed is supplied frequently to maximize the growth rate. The aquaculture production of Vietnam is increasing gradually and the total production of aquaculture raised from 162 thousand tons in 1990 to 3,216 thousand tons in 2013 and 70 percent accounted by the MD with the amount of 2,263 thousand tons (GSO, 2014a). And, according to FAO (2014), Vietnam is one of the largest countries of aquaculture production and contributes with 4.6 percent to the word total aquaculture production. However, intensive aquaculture means also a lot of diseases which need to be treated with chemicals (Bondad-Reantaso et al., 2005). Besides, the use of fertilizers and pesticides also increased with the intensity of agriculture, affecting the biota of the Mekong Delta (Campbell, 2012). As a result, the drinking water sources in the MD become more and more polluted with pesticide residues (Chau et al., 2015). Pesticide use, residue and pollution in the Mekong Delta were investigated by some authors and in various systems and commodities, such as, rice and rice-fish systems (Berg, 2001), sediment, surface water and drinking water (Toan et al., 2013). Most of the studies, however, focused on agriculture. Generally, the upper and the lower area of the MD contain chemicals originating from both agricultural activities as well as human living activities. For instance, DDTs and PCBs concentration of urban surrounding sites were higher than in adjacent agricultural areas; and the high ratio of 4,4'-dichlorodiphenyltrichloroethane (4,4'-DDT) and 4,4'-DDE indicated the continuous contamination of aquatic environment by these compounds (Sudaryanto et al., 2011, Minh et al., 2007). However, pesticides are indispensable element to increase agricultural production. This statement is true for almost all countries in the world and pesticide is one important element in modern agriculture (Srivastava et al., 2010). In Vietnam, according to VMARD (2009), catfish and tilapia were two important economic species which have been culturing in the MD (VMARD, 2009). Cultured tilapia of Vietnam includes black tilapia (*Oreochromis niloticus*) and red tilapia (*Oreochromis sp*). The black

one is reared mostly in pond and for exporting; the latter is cultured in cage and for domestic consumption (Phan et al., 2011). After the decline of *Pangasius* catfish cage culture, red tilapia has dominated, developed and become the favorite and economic species. A range of chemicals and other compounds were used to prevent and control tilapia diseases and improve water quality. It resulted in the presence of chemical residues in harvested tilapia, e.g. antibiotics or other toxic compounds. However, limited information is available about the chemical use practices of red tilapia farmers. Therefore, this study aimed to evaluate the chemical use practices in red tilapia grow-out farms in the Mekong River Delta, Viet Nam.

Material and methods

Data collection

The first survey was conducted in 2009 in Co Do, Thoi Lai and Vinh Thanh districts of Can Tho City. Forty-five rice-fish farms, 45 rice farms and 15 agrochemical stores were randomly selected for interview using structured questionnaires (see questionnaires in annex).

To assess the chemical use situation as well as the knowledge of the farmers about the impact of the chemical use on health and environment, a second survey was conducted in 2013. In total, 93 interviewees were involved in the second survey; they included 30 rice fish farms, 22 farms of red tilapia cages, 15 catfish ponds, and 26 stores including 16 aquaculture chemical suppliers and 10 agrochemical suppliers.

Data analysis

Data were analyzed by descriptive statistics to identify key determinant of chemical use practices. Statistical analysis was made with the SPSS software, version 18.0.

Results and discussion

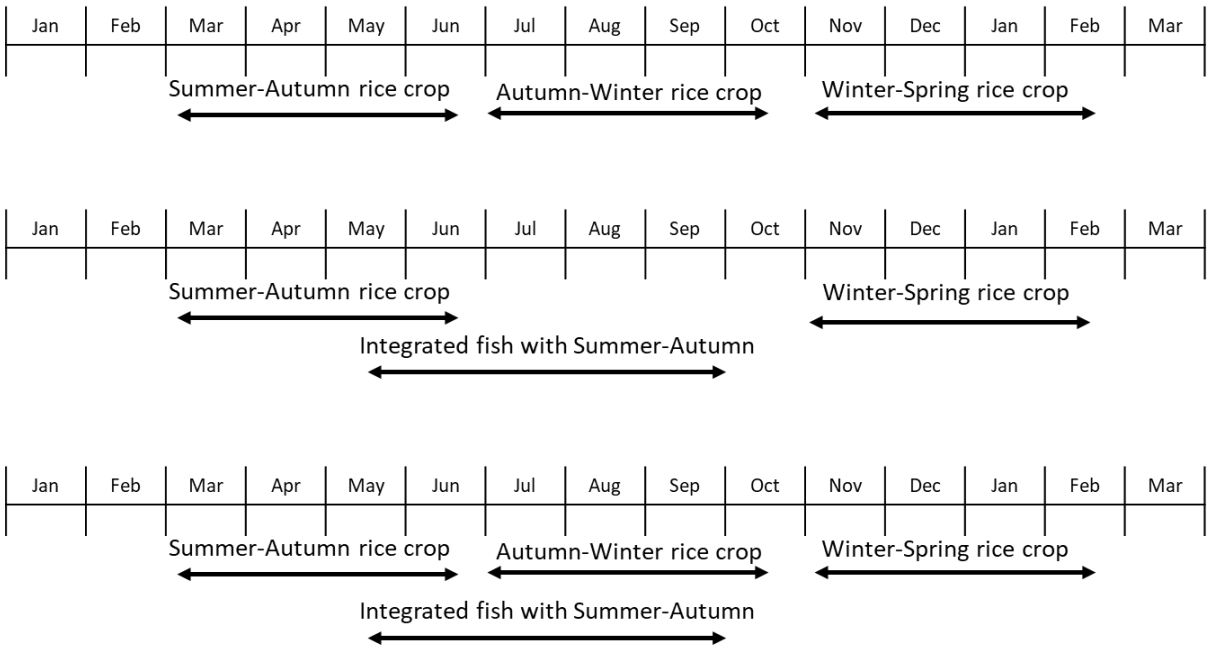
1. General information about rice and rice-fish farms of the Mekong Delta

There are two common types of rice and rice – fish integrated cultivation, the rice fish integrated consists of two operation models, i.e. two rice crops integrated with one fish crop, which more popular with 66.6 % of rice fish systems, and three rice crops integrated with 1 fish crop (sharing 33.3% of rice fish systems) (Figure 1).

Tables 1 to 4 show detailed results about general information obtained from the first survey performed in 2009 in 45 rice farms, 45 rice-fish farms of the Mekong Delta.

These general informations are educational level and ownership type, rice cropping in rice farms rice and fish cropping in rice-fish farms, and, economical aspects in both systems.

Figure 1. Schedule of rice only cultivation (upper), and rice fish integrated cultivation – two rice crops and one fish crop (middle) and three rice crops and 1 fish crop (lower).



Educational level and ownership type

Most farmers (62.2-64.5%) have obtained elementary education level (Table 1). Other farmers graduated from secondary or high schools (13.3-22.2%). Similarly, a study conducted in Long An Province, Heong et al. (1998) reported that most farmers interviewed had an educational level between 1 and 9 years. Only a small proportion (< 8%) did not attend school.

Table 1. General information on educational level and ownership type of farmers in the survey area (Mekong Delta), in 2009.

Items	Rice farmers (n=45)	Rice-cum-fish farmers (n=45)
Educational levels (%)		
Elementary school	62.2	64.5
Secondary school	17.8	22.2
High school	20.0	13.3
Ownership types		
Family ownership (%)	60	60
Enterprise ownership (%)	40	40
Total farm size (mean ± SD) (ha)	2.76 ± 1.93	2.71 ± 1.06
Rice production area (mean ± SD) (ha)	1.93 ± 0.91	2.02 ± 0.96

Family and enterprise ownership represented 60% and 40%, respectively. Total average farm size in rice and rice-fish farming was 2.76 ha and 2.71 ha, respectively, while area of rice production in rice and rice-fish farms were 1.93 ha and 2.02 ha, respectively (Table 1). These results differ slightly from those obtained by other authors who conducted surveys in another province of Mekong Delta. For example, 15 years earlier, Heong et al. (1998) showed that the farm sizes were generally less than 1 ha (study conducted in Long An province). Thanh (2008) reported that total farm area and rice area were 1.6 ha and 1.3 ha, respectively (study conducted in Can Tho City). In addition, Nga (2007) reported that farm size of rice production was 2.01 ha per household (study conducted in Can Tho City).

Rice variety, cropping calendar and fertilizer use in rice farms

Technical information about summer-autumn rice crop in rice farms is showed in Table 2. The most common rice variety was OM 2514 (26.7%) followed by OM 1490 and OM 4900 (17.8%). The cropping calendar was from March to July (88.9%). The average cropping duration was 95 days. Average fertilizer quantity was 390 kg per ha. The farmers used to apply many kinds of fertilizers at each application time. Urea-DAP-NPK-K were the most common fertilizers (55.6%).

Table 2. Technical information (rice varieties and cropping calendar and duration) of summer-autumn rice crop according to the practices of 45 rice farms in the Mekong Delta, in 2009.

Items	Unit	Rice farms (n=45)
Rice varieties		
OM 2514	% household	26.7
OM 1490	% household	17.8
OM 4900	% household	17.8
Cropping calendar		
March to July	% household	88.9
April to July	% household	11.1
Cropping duration (mean ± SD)	days	95.3 ± 5 .02
Fertilizers (mean ± SD)	kg/1,000 m^2	38.9 ±5 .7
No. of fertilizer application (mean ± SD)	time	3.7 ± 0.5
Kinds of fertilizers		
Urea-DAP-NPK-K	% household	55.6
Urea-DAP-NPK	% household	17.8
Urea-DAP-K	% household	11.1

Rice and fish cropping in rice-fish farms

In rice-fish farms (Table 3), most farmers (66.7%) applied two rice crops and one fish crop. A minority of farmers (13.3%) applied 3 rice crops and 1 fish crop. 100% of farmers stocked common carp *Cyprinus carpio* in rice field followed by silver barb *Puntius goniotus* (53.3%), bighead carp *Aristichthys nobilis* (51.1%), silver carp *Hypophthalmichthys molitrix* (46.7%) and tilapia *Oreochromis niloticus* (15.6%). According to Thanh (2008), the common farmed species were common carp, silver carp, bighead carp and silver barb and these species were cultured together in rice field. Among these species, the common carp and bighead carp are the most common cultured ones (by 100% of interviewed farmers) following by silver barb (25% of interviewed farmers) and silver carp (5% of interviewed farmers). Average stocking density was 0.4 fish per m^2. Farmers stocked low density in rice field because they didn't feed the fish. Natural food was the main feed source in the rice field. The result of the present study is similar to the study of Thanh (2008) reporting stocking density of 0.4 fish per m^2 or of Nga (2007) (0.5 fish per m^2). The majority of farmers applied population structure of common carp – bighead carp (33.3%) and common carp, silver barb and silver carp (31.1%). Average culture duration and productivity were 149 days and 577 kg/ha/crop, respectively. Thanh (2008) reported that culture duration of 2 rice crops and 1 fish crop was 5.7 months and 3 rice crops and 1 fish crop was 4.4 months. According to this author, the productivity was 745 kg/ha for 2

rice crops and 1 fish crop and 460 kg/ha for 3 rice crops and 1 fish crop. Total net income and net income from fish were approximately 4.8 million VND and 3.9 million VND per ha, respectively (Table 3).

Table 3. Information about rice-fish farming in the Mekong Delta, 2009.

Information	Unit	Rice-fish farms (n=45)
Farm areas (based on water surface) (mean ± SD)	ha	1.99 ± 0.98
Cropping structure		
2 rice crops and – one fish crop	%	66.7
3 rice crops and - 1 fish crop	%	33.3
Stocking species		
Common carp	%	100
Silver barb	%	53.3
Bighead carp	%	51.1
Silver carp	%	46.7
Tilapia	%	15.6
Stocking density (mean ± SD)	fish/m^2	0.42 ± 0.25
Fish population structure		
Common carp – Bighead carp	%	33.3
Common carp, Silver barb and Silver carp	%	31.1
Common carp, Silver barb and Bighead carp	%	11.1
Culture duration (mean ± SD)	Days	149 ± 31
Production (mean ± SD)	kg/ha/crop	577 ± 322
Total net income (mean ± SD)	VND/ha	4,785,570 ± 2,471,549
Net income from fish	VND/ha	3,945,679 ± 2,115,947

VND : Vietnam dong

The survey results in 2013 from the interview of 30 rice-fish farmers (data not shown) were similar to those of the first survey in 2009 in term of farming area (2.3 ha in average), fish stocking crop time (April to June, more than 80% of farmers) and fish harvest time (October-November, 90% of farmers). Stocking time and harvest time varied between farmers due to the fingerling size at the stocking and farm gate price at the time of harvest. It was also noted that the fish species composition was similar than in 2009, including common carp *Cyprinus carpio* (100% of farms), followed by silver barb *Puntius goniotus* (93.3% of farms), silver carp *Hypophthalmichthys molitrix* (63.3% of farms), except for bighead carp *Aristichthys nobilis* stocking, for which a reduction was observed (51.1% in 2009 and 3.33% in 2013). Also, in 2013, a new fish species appeared, the bronze featherback (*Notopterus notopterus*), used by one farmer out of the 30 interviewed farmers. The

common carp and silver barb were still the dominating species in rice-fish farming because they were highly disease resistant, with cheap price fingerlings, growing fast without feeding supplementation. Stocking density was increased, 0.8 ± 1.1 fish/m^2 in 2013 compared to 0.4 fish/m^2 in 2009. This could be explained by the fact that additional feeding was given to fish (36.7% of farmers in 2013).

Rice yield, cost and benefits from rice cropping in both rice and rice-fish farms

There was no significant difference ($p<0.05$) between average rice yield in Summer-Autumn crop in rice farms (4.5 tons per ha) and Summer-Autumn crop in rice-fish farms (4.4 tons per ha) (Table 4). Costs of pesticides ranged between 1.34 and 1.38 million VND per ha. It averagely made up about 11% of total of rice cost. Cost of rice production in Summer-Autumn crop (14.9 million VND/ha) was significantly higher ($p<0.05$) than in Summer-Autumn crop in rice-fish farms (10.7 million/ha). Gross income from rice ranged between 18.4 and 19.2 million VND/ha. Net income from rice in Summer-Autumn crop in rice-fish farms (7.7 million VND/ha) was significantly higher than in Summer-Autumn crop in rice farms (4.3 million VND/ha) ($p<0.05$). Heong et al. (1998) reported that average rice yield in Long An province in 1997 ranged between 4.1 and 5 tons per ha (29.2% farmers interviewed). Thanh (2008) showed that Autumn-Summer rice crop yield in Can Tho City was 5.1 tons per hectare.

Table 4. Cost and benefit analysis of summer-autumn rice crop (calculated for rice only) in rice farms and rice-fish farms, in the Mekong Delta, in 2009.

Information	Unit	Summer-Autumn (rice, n=45)	Summer-Autumn (rice-fish, n=45)
Rice yield	kg/ha	4,548 ± 965[a]	4.409 ± 1.095[a]
Cost of pesticides	VND/ha	1,378,301 ± 1,257,945[a]	1,340,121 ± 1,249,357[a]
Cost of rice production	VND/ha	14,874,395 ± 3,770,782[b]	10,710,252 ± 2,523,226[a]
Gross income from rice	VND/ha	19,194,186 ± 4,292,177[a]	18,434,552 ± 4,682,169[a]
Net income from rice	VND/ha	4,319,791 ± 5,236,047[a]	7,724,300 ± 5,104,351[b]

Data expressed as mean ± SD, the different letters (a, b) indicate a significant difference between rice-fish and rice only system ($p<0.05$).

2. Insecticides used to control rice pests, in rice and rice-fish farms, in 2009

The frequency and quantity of insecticide application to control rice pests, in rice and rice-fish farms, in 2009, is given in Table 5. The survey results showed that the number of applications in rice

and rice-fish farms were 2.9 times per crop and 2.7 times per crop on average, respectively. The study on the same system in Can Tho City and Tien Giang province of Berg (2001) showed that rice farmers applied insecticides 3.2 times per crop on an average and up to 8 times in extreme cases including applications during the first 40 days.

100% of farmers followed the recommended dose provided by the producers, which were 1-2 liters per hectare and 0.8-1.2 liters per hectare, for Kinalux ™ (quinalphos) (product of United Phosphorus Limited, India) and Basa™ (Fenobucarb) (Product of Vithaco, Bac Giang, Vietnam), respectively. The most common application time was from the 31st day (73.7% of interviewed farms) day to the 60th day (89.7% of interviewed farms) of rice crop. After the 90th day, only 2.6% of farmers applied insecticides. A majority of farmers (57.8 and 77.8%, in rice and rice-fish system, respectively) have learned to select pesticides from their own experience. This was followed by getting knowledge based on training (11.1 and 22.2%, in rice and rice-fish system, respectively).

Table 5. Information about insecticide application to control rice pests, in rice and rice-fish farms in the Mekong Delta, in 2009.

Information	**Unit**	**Rice (n=45)**	**Rice-fish (n=45)**
No. of application (mean ± SD)	time	2.9 ± 1.4	2.7 ± 1.4
Quantity used			
Based on recommended level of producers	%	100	100
in which			
Kinalux™ (quinalphos)	L/ha	1 - 2	1 - 2
Basa™ (fenobucarb)	L/ha	0.8 - 1.2	0.8 - 1.2
Application time			
Day 10 -30	% household	48.7	57.9
Day 31- 60	% household	89.7	73.7
Day 61- 90	% household	41.0	44.7
After day 90	% household	2.6	2.6
Pesticide selection			
Based on experience	% household	57.8	77.8
Based on training	% household	22.2	11.1
Based on recommendation of sellers	% household	11.1	8.9
Based on TV program	% household	22.2	
Based on others	% household		2.2

According to the answers of the farmers to the questionnaire, pymetrozine (Chess™, Product of Syngenta Vietnam) and fenobucarb (Basa™, Product of Vithaco, Bac Giang, Vietnam) appeared to be the most commonly used insecticides in rice farming and rice-fish farming (Table 6). Fenobucarb (Basa™) and quinalphos (Kinalux™) pesticides were the most sold by distributors, pymetrozine (Chess™) shared a smaller portion compared with Fenobucarb (Basa™) and quinalphos (Kinalux™) (Table 6). According to Heong et al. (1998), organophosphates, organochlorines and carbamates were the common used pesticides in Long An province from 1994 - 1997. Our results indicate that organochlorines were not popular any more in the Mekong Delta in 2009.

Table 6. Commonly used and sold pesticides in rice and rice-fish farming in the Mekong Delta, according to a survey performed in 2009.

Pesticides (trade name)	Active compound/ Producer	For rice farming (% of n=45)	For rice-fish farming (% of n=45)	Agrochemical stores (% of n=15)
Chess™	Pymetrozine Syngenta, Vietnam	48.9	35.6	46.7
Basa™	Fenobucarb Vithaco, Bac Giang, Vietnam	26.7	37.8	66.7
Kinalux™	Quinalphos United Phosphorus Limited, India	20.0	-	53.3
Abasuper™	Abamectin Phu Nong Co. Ltd, Vietnam	17.8	11.1	40.0
Dragon™	Cypermethrin 5.5% and Chlorpyriphos Ethyl 53% Saigon plant protection join stock company, Vietnam	11.1	15.6	26.7
Regent™	Fipronil Bayer, Vietnam		17.8	13.3
Tuncydan™	Chlorpyrifos Ethyl 25% and Cypermethrin 5% Ngoc Tung Join stock company, Vietnam			33.3

3. Chemical use in rice crop in rice-fish farms in 2013

The survey performed in 2013 indicated that there were 54 active ingredients which were used by rice-fish farmers and this number was much lower than that registered to Vietnamese Government. According to VMARD (2013), the total number of approved insecticide and herbicide active ingredients were 745 and 217, respectively.

In rice-fish farms, in 2013, 73.3 % of the farmers used propiconazole/tricyclazole, propiconazole/difenoconazole and isoprothiolane for the prevention and treatment of the rice blast disease. To control the brown planthopper caused by Nilaparvata lugens, farmers (63.3%) applied compounds as pymetrozine, azoxystrobin/ difenoconazole, fenobucarb, carbofuran, and fipronil whereas others used cartap, chlorantraniliprole/ thiamethoxam, flubendiamide (40%) and remaining others used quinalphos, tricyclazole, carbosulfan/ chlorfluazuron (30%) (Table 7). Compared to the first survey performed in 2009, it appeared that much more compounds were used to control rice blast disease and brown planthopper. However, all of the compounds and products found in this investigation belonged to the list of approved chemicals used for agriculture (VMARD, 2015). They are also included in the approved list of 607 active compounds in 1295 commercial products for disease control, and 769 active compounds (in 1690 commercial products) for insect control (VMARD, 2015). Berg (2001) reported that there were 64 different compounds used in rice crop in rice fish farming whereas only 26 different compounds were shown to be used in the 2013 survey of this study, which could be explained by the limited number of commercial products in the rice fish farm region. Moreover, Berg (2001) proposed that the application of Integrated Pest Management (IPM) during rice crop reduced by 2 to 3 times the pesticide application frequency and the amount of active ingredients. In this study, no farmers were found to apply IPM. Besides, rice-fish farmers used herbicides (36.5%) and other chemicals for rice quality improvement (43.3%). Among herbicides, 2,4 D dimethyl amine was still legally used which possibly caused negative environmental impacts to surrounding ecosystems (Carvalho et al., 2008, Lamers et al., 2011) (Table 7).

Table 7. Chemical use in rice-fish farms of the Mekong Delta according to a survey performed in 2013 (application only for rice crop).

Compound (product name)	**% (n=30)**
Rice blast disease prevention and treatment	
Propiconazole + Tricyclazole (Filia, Boom flower)	73.3
Propiconazole + Difenoconazole (Rocksai-Physan, Map super, Tilt super)	73.3
Isoprothiolane (Fuan)	73.3
Control brown planthopper caused by Nilaparvata lugens	
Pymetrozine (Chess)	63.3
Azoxystrobin + Difenoconazole (Amistar top)	63.3
Fenobucarb (Basa)	63.3
Carbofuran (Furadan)	63.3
Fipronil (Regent)	63.3
Cartap (Padan)	40.0
Chlorantraniliprole + Thiamethoxam (Virtako)	40.0

Flubendiamide (Takumi)	40.0
Quinalphos (Kinalux)	30.0
Tricyclazole (Beam)	30.0
Carbosulfan + Chlorfluazuron (Sulfaron)	30.0
Rice quality improvement	
Hexaconazole (Anvil)	43.3
Lychnis viscaria extract (Comcat)	43.3
Herbicide	
Pretilachlor (Dietmam, Sofit)	36.7
2,4 D Dimethyl amine (2,4D)	36.7
Pyazosufuron – Ethyl (Sirius)	36.7
Quinclorac + Bensulfuron methyl (Rocet)	36.7
Cyhalofop-butyl + Penoxsulam (Topshot)	36.7
Chemical mixing with seed to improve quality	
Thiamethoxam + Defenoconazole + Fludioxonil (Cruiser)	13.3
Bacterial disease control	
Validamycin A 3% (Validan)	3.3
Validamycin A 5% (Validancin)	3.3
Snail control	
Metaldehyde (Toxbait)	3.3
Total active compounds	

Regarding to pesticides available in the market, the survey on agrochemical stores in 2013 showed that there were 61 different active ingredients being used. These active compounds belonged to three groups, i.e. fungicides/bactericides, insecticides and herbicides. 2,4 D still appeared in agrochemical stores of the survey of 2013 with the proportion of 4/10 of stores. Among active ingredient of insecticide group, quinalphos and chlorpyrifos ethyl appeared with the same portion of 3/19 of the trade names (data not shown).

4. Chemical use in fish crop in rice-fish farms in 2013 and farmer perception

It is important to note that there was very few applications of chemicals during the fish farming. During fish rearing, only 10% of surveyed farmers reported to use lime (CaO) or chlorine to disinfect water. They also report to use rotenone under the form of raw material (root of *Derris elliptica*) to eliminate predator fish at the preparation stage before stocking fish into surrounding water area within rice field. A few farmers used oxytetracycline (10.0%) to treat bacterial disease e.g. hemorrhage, and BKC (benzalkonium chloride), Iodine, CuSO4 (6.7%) to control parasites. No

banned aquaculture chemicals, according Vietnamese regulation, were applied during fish crop. Compared to intensive catfish rearing, the limited chemical used during fish rearing in rice fish system is due to low stocking density and low or no feed input. However, the risk of cross contamination of fish with chemical used for rice treatment is not excluded, as well as contamination with environmental pollutants. For example, residues of PCBs and pesticides were found in the aquatic environment of the Mekong Delta, even if lower values were reported compared to other regions of Vietnam (Carvalho et al., 2008).

According to Ecobichon (2001), pesticide use has rapidly increased in developing countries, due to the change of the population structure. Indeed, a large portion of agricultural workers moved to urban area for searching better jobs, resulting in lack of labors and increase of food demand; this also led to an increase of pesticide reliance.

The median of farmers joined training was 2 times a year with the maxium of 10 time and minimum of 1 time. Media were not mentioned by farmers in terms of chemical use, even if a national mass media campaign was initiated, locally called “Ba Giam Ba Tang” or “Three Reductions, three inductions” (Heong et al., 1998; Huan et al., 1999).

More than half of farmers still stated that agrochemicals or pesticides have no negative effect on their own health, soil, surrounding water environment and air (Table 8). Less than a half of the farmers reported to know about legislation on agrochemical use, but only few of them can present or show what they are. Almost all the farmers reported to avoid direct contact with agrochemicals, by wearing protection during handling e.g. rain coat, glove, mask and store chemical separately with living space.

Table 8. Rice fish farmers' perspective on agrochemical use in the Mekong Delta, according to a survey performed in 2013, in 30 rice-fish farms.

	% (n=30)		
	Yes	**No**	**Do not know**
Perception on agrochemical impact			
Agrochemical negative effect on soil?	23.3	63.3	13.4
Agrochemical negative effect on surrounding water?	33.3	56.6	9.1
Agrochemical negative effect on air?	30.0	56.6	13.4
Agrochemical negative effect on farmer health?	43.4	56.6	-
Perception about health hazard and knowledge about chemical use			
Knowledge about chemical regulation	46.7	53.3	-
Record of drug and chemical use	6.7	93.3	-
Chemical storage separately from living area	93.3	6.7	-
Direct contact with chemical (do not apply any safe equipment)	23.3	76.7	-
Wearing protection during handling	76.7	23.3	-

5. Chemical use in red tilapia (*Oreochromis sp*) cage farms, in 2013

The results of the survey performed in 2013 in 22 farms of red tilapia cages showed that 86% of interviewed farmers used antimicrobials (i.e. antibiotics) with six types of products containing nine types of antimicrobials used as ingredient. Mixtures of antimicrobials were mainly used by farmers e.g. sulfadiazine and trimethoprim (55%), florfenicol alone or combined with doxycycline (23% and 14%), enrofloxacin, sulfadimidine and trimethoprim (23%) to treat bacterial diseases (Table 9). In 2012, a total of 28 antimicrobials were authorized for therapeutic use in Vietnamese aquaculture (Tai, 2012). Eight of these 28 antimicrobials were reported to be in use by farmers in our study. The dose applied to fish was mainly based on the mention on the label. It is emphasized that all of farmers used antimicrobials to treat bacterial diseases due to lacking of vaccination for red tilapia in Vietnam.

Table 9. Antimicrobial, disinfectants, and other compounds reported to be used in growth-out red tilapia farms in Mekong Delta, according to a survey performed in 2013.

Compounds	% of farmers (n=22)
Antimicrobials [(1)]	
Sulfadiazine and trimethoprim	54.5
Florfenicol + Doxycycline	13.6
Florfenicol	22.7
Enrofloxacin + Sulfadimidin + Trimethoprim	22.7
Kanamycin + Amoxicilin	9.1
Oxytetracycline	9.1
Disinfectants and external parasite control[(2)]	
Copper sulfate	40.9
Iodine	27.2
Salt	27.2
Potassium permanganate	22.7
Lime	22.7
Kurz, extract *Combretum dasystachyum*	4.5
Calcium hypochlorite	4.5
Yucca schidigera extract	4.5
Internal parasite control [(1)]	
Praziquantel	18.2
Nutritional supplementation products[(1)]	
Nutritional supplementation	86.4
Probiotics	27.2

[(1)] mixed with feed; [(2)] compounds applied into cage water for disinfection and parasite control.

There are only few studies on red tilapia diseases in Mekong Delta. *Streptococcus agalactiae* was firstly isolated and characterized from specimens with symptoms such as popeye and skin hemorrhage, in red tilapia cage culture in Mekong Delta (Oanh and Phuong, 2012). Oanh and Thy (2011) studied the histopathological change of red tilapia (*Oreochromis sp*) experimentally infected with *Streptococcus agalactiae* bacteria. Quan et al. (2013) also reported cases of hemorrhagic disease in tilapia caused by *S. agalactiae* in some northern provinces of Vietnam, and the isolated *S. agalactiae* was susceptible to several antibiotics including enrofloxacin and doxycycline. According to the survey of this study, the popular symptoms of bacterial diseases in red tilapia were described as swollen head (9%), swollen eyes (64%), body hemorrhage (59%) and red inflammation in mouth (27%) (data not shown). Previous studies identified *Streptococcus agalactiae* (cocci Gram-positive

bacteria) as being the main bacteria causing these symptoms, but the farmers did not know this. The ignorance of antimicrobial susceptibility in red tilapia disease may lead to a misuse of antimicrobials. Residue alerts for tilapia products reported in 2013 by the EU Rapid Alert System for Feed and Food (RASFF) mention two cases of contamination with trimethoprim (76 and 323 µg/kg) and one case of sulfadiazine contamination (199 µg/kg) (RASFF, 2014). This shows that withdrawal periods were not followed by tilapia farmers, especially since the elimination of some antimicrobial in tilapia (e.g. sulfamethoxazole and trimethoprim) is rapid (Kosoff et al., 2007). Moreover, most of farmers reported to sell red tilapia to local retailers who did not check for any residue in tilapia at the time of harvest. These retailers sold tilapia directly to local markets and this posed a risk of exposure to of antimicrobial residues from the consumption of red tilapia products. The findings of this study show that there is an urgent need to train farmers on the use of chemicals.

All farmers commonly used different disinfectants throughout the production cycle to disinfect water in cage and treat diseases in combination with antimicrobials. For prevention and treatment of the external parasites and gill damage diseases, farmers used copper sulfate (41%), iodine (27%), salt (27%) and potassium permanganate (23%) and lime (23%) (Table 10). Copper is highly toxic to living organisms, e.g. it has a negative effect on fish hematological parameters (Carvalho and Fernandes, 2006). The active compound, praziquantel, was also used periodically to prevent and control internal parasites. Moreover, most of farmers used nutritional feed supplement products containing mixtures of vitamins, minerals, sorbitol, amino acids, etc. to improve the fish health and feed digestibility; however, the efficacy of these products was not clear. Probiotic products were also used to improve the digestive tract function (27%). Shelby et al. (2006) set an experiment in Auburn, US with young Nile tilapia and concluded that commercial probiotic product containing *Bacillus spp*, did not provide beneficial effect to young Nile tilapia, *Oreochromis niloticus*. Limited evidence is also available on the cost-effectiveness of current use-practices. It is likely that farmers can substantially reduce both the amounts and costs of chemicals used without negatively impacting fish health and production yields

Most red tilapia grow-out farmers (86%) used their own previous experience and/or label instructions to decide on type and dosage of chemicals to be applied (Table 10). Few of the interviewed farmers reported that they got the support from veterinarians or other technical staff in diagnosing diseases and making decisions of chemical use. Nearly all of the farmers did not keep any written records on chemicals applied (Table 10). Thus, it was impossible to trace particular applications to specific batches of tilapia for traceability purposes.

During the handling of chemicals, very few farmers used protective measures (gloves, masks, etc.), with less farmers reporting knowledge on potential health hazards associated with the handling of chemicals (Table 10). Moreover, at the same time, farmers often reported that they mix antibiotics with pelleted feed with their bare hands as commercial medicated pelleted feed were not available.

Hands and arms had a particularly high risk of exposure due to the common practice of farmers preparing and mixing antimicrobial solutions with pelleted feeds using their bare hands and, during preparation and application of disinfectant solutions to water.

About a half of interviewed farmers reported to buy chemicals and store it until use (Table 10). From our observations, about a half of the farmers did not have a proper storage place, away from kitchen and living space. A few farmers reported accidents and direct health effects when handling chemicals (18%). The health effects included skin lesions and itching when handling chlorine compounds. Some of the antimicrobials commonly used by farmers (ampicillin, cotrimoxazole and quinolones) are also amongst the most common causes of antimicrobial allergies (Thong, 2010).

Table 10. Reported use of chemicals and perceptions of occupational health hazards by red tilapia farmers in the Mekong Delta, in 2013.

Chemicals administered according to	**% (n=22)**
Safety instructions on product packaging	5
Instructions by veterinarian/technician	9
Instructions by extension staff	0
Farmer experience	86
Record keeping of chemical use	13.6
Training course participation	50
Direct contact between skin and chemicals	32
Use protection during handling of chemicals	18
Chemical storage place nearby living place	41
Store chemical in cage before use	50
Farmers/workers were instructed how to handle chemicals safely	77
Knowledge about banned chemicals	9
Common clinical manifestations following use of chemicals (skin lesion, coughing)	18

6. Chemical use in striped catfish (*Pangasianodon hyphthalmus*) farms in 2013

According to the survey of this study performed in 2013 in 15 striped catfish farms (data not shown), the average area of catfish ponds was 3611 m^2 and each farm had more than two ponds. The production of catfish cultured was 12.41 kg per m^2. Regarding to cultural technical training, 50% of interviewed farmers responded that they attended training courses. According the farmers, these trainings were about cultural technique, disease management, drug and chemical application and the trainings were offered by aquaculture administrative office, veterinary companies or feed companies. The maximum and minimum training attendances of farmers were 5 times and 1 time per year, respectively. The duration of cultivation varied and strongly depended on market; in normal situation, it took about 4 months to get the market size (1 kg per fish). However, in the case of low price, the cultivation could take between 10 and 12 months. The survey results showed that the numbers of farms from which the cultural time were from 4 to 5 months and 5 to 6 months were 30% and 20%, respectively; 50% farmers respond that the cultural duration was between 7 and 12 months. According to many authors, the production of *Pangasius* catfish faced a decreasing trend between 2010 and 2013. This decrease was caused by the reduction of imported quantity from major markets such as EU, USA, China, Saudi Arabia and Egypt (Anh, 2014; Quang, 2013).

About chemical use (data not shown), 100% of visited farms applied drugs and chemicals during catfish culture. In total, seven types of antibiotics were utilized, and according to farmers, these compounds were used in bacterial disease treatment and prevention. Enrofloxacin, sulfamethoxazole and trimethoprim were reported to be mostly used by 40 – 50% of the farmers. According to farmers, these chemicals were used to treat BNP (Bacillary Necrosis of *Pangasius*), a disease was known to be caused by *Edwardsiella ictaluri* (Crumlish et al., 2002). According to our survey, the number of used chemicals in catfish pond culture was 19, which appears to be much lower than in Dong Thap province (56 chemicals including 28 chemicals for pond preparation, 14 nutrient supplement ingredients and 14 antibiotics) (Truong and Tran, 2012). That might result from the decreasing of striped catfish price, so the farmer reduced chemical utilization to decrease the input cost.

7. Information from chemical distributors, according to the survey performed in 2009

Information from pesticide distributors in the second survey (2009) is given in Table 11. Most of the pesticide distributors (46.6%) had knowledge on pesticides by attending training courses. A minority of distributors (6.7%) had a technical school degree or bachelor degree. Almost all types of pesticides distributors (93.3%) were wholesalers while wholesale and retail represent 6.7% of distributors. Most of the distributors (46.7%) thought that the amount of pesticides used in 2008 was more than 2009; other distributors (33.3%) believed that the amount of pesticide used in 2008 was less than 2009. The majority of pesticide distributors (53.3%) forecasted that the trend in the coming years

will be increased; while other distributors (33.3%) thought that pesticide use in coming years will be less than before; and 13.4% of the distributors thought that it will be stable in the future.

Table 11. Information from pesticide distributors/retailers in the Mekong Delta, in 2009.

Information	% surveyed retailers (n=15)
Level of knowledge about pesticides	
Attending training	46.6
Experiences and attending training	40.0
Technical school degree	6.7
Bachelor degree	6.7
Types of pesticide distribution	
Wholesale	93.3
Wholesale and retail	6.7
Pesticide used in 2008	
Less than in 2009	33.3
More than in 2009	46.7
No change	20.0
Trend of pesticide use in coming years	
Decrease	33.3
Increase	53.3
No change	13.4

Conclusions

Farmers in Mekong delta have been improving their life through rice and rice-fish farming. In rice-fish farms, most farmers applied 2 rice crops and 1 fish crop; common carp was the most common farmed species in rice field. The number of pesticide applications ranged from 2.7 to 2.9 per crop. Most farmers applied pesticides based on recommended levels of producers.

Pymetrozine (Chess™) (Product of Syngenta Vietnam), fenobucarb (Basa™) (Product of Vithaco, Bac Giang, Vietnam) and quinalphos (Kinalux™) (Product of United Phosphorus Limited, India) were the most common used pesticides in rice crop, rice-fish crop and distributors as well. The majority of distributors forecast an increase of the use of pesticides in future.

Few farmers used chemicals during fish crop. Almost all of the farmers reported awareness about agrochemical use in term of health effect, and declared to avoid direct contact with agrochemicals, by wearing protection during handling. However, they still decided about which types of agrochemical to use mainly based on their experience.

Our study highlighted that many different types of disinfectants and antimicrobials were used in red tilapia culture and often were applied with limited farmer knowledge and awareness of prudent use practices. Further, the cost-effectiveness of such use, especially for nutritional supplement products, antimicrobials and disinfectants, is questionable and should be assessed. There is an urgent need to improve the farmer's knowledge and their access to advisory services on prudent use of disinfectants and antimicrobials. It seems likely that farmers can maintain and even increase farm productivity with less, but correct, use of chemicals, and at the same time this might decrease environmental, food safety and occupational health hazards associated with chemical use.

Acknowledgements

The authors thank ARES-CCD (Académie de Recherche et d'Enseignement Supérieur – Commission pour la Coopération au Développement) for its financial support within the framework of the DELTAQUASAFE project and Mr. Nguyen Quang Trung, Mr. Huynh So Ni, Mr. Sébastien Quennery for helping to complete the surveys.

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Experimental section

Study n°2: Screening of quinalphos, trifluralin and dichlorvos residues in fresh water of aquaculture systems in Mekong Delta, Vietnam

Aquaculture Research https://doi.org/10.1111/are.13890

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The first study indicated that quinalphos, an organophosphate insecticide was commonly used by farmers as well as reported by agrochemical distributors. Moreover, practical situation and notifications of import markets showed that two pesticides i.e. dichlorvos and trifluralin may be a problem for the health of consumer because of the presence of their residues in aquaculture products. Among these three compounds, only dichlorvos was completely banned in both agriculture and aquaculture. Trifluralin, an herbicide, was found effective to treat fungal diseases and exoparasites in aquaculture, and trifluralin was used in aquaculture in Vietnam and other countries. Trifluralin was not approved in European and Japan due to its toxicity, in Vietnam trifluralin was banned only in aquaculture. The last compound, quinalphos, was used in rice and other crops to prevent insects. Residues of quinalphos were found in rivers and channels near rice cultivated area in some studies in the Mekong Delta. Quinalphos is a compound with high K_{ow} factor, which may lead to its accumulation in fish and other biota exposed to quinalphos contaminated water. This compound is also toxic for organisms living in water. Therefore, the three compounds were chosen for screening in this study. Before a screening was performed, in order to reduce time and chemical consumption, a reliable and easy method for quantifying the three mentioned pesticide in one extraction and injection was developed and validated according to the guidelines of SANCO 2015.

Abstract

To develop an easy and reliable method for detecting pesticides and their residues in the Mekong Delta, a GC-MS analytical method was developed and validated according to European guidelines (SANTE/11945/2015) for the determination of residues of three pesticides (quinalphos, trifluralin and dichlorvos) in water. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.002 and 0.007 µg/L, respectively, for quinalphos and trifluralin, and 0.016 and 0.053 µg/L, respectively, for dichlorvos. The repeatability, the within-laboratory reproducibility as well as the trueness met the European criteria. The recovery rate ranged between 72% (for dichlorvos and quinalphos) and 82% (for trifluralin). The developed method was then applied for the analysis of thirty three water samples, collected in April 2013, at the beginning of the rainy season in the Mekong Delta in Vietnam. Thirteen samples were from rice field, 10 were collected from catfish ponds and from red tilapia cages. Results showed that only 9 % of total water samples analyzed contained residues of pesticides, but only in water from rice fish systems. From the 13 samples taken in these systems, quinalphos was detected in 3 samples. The other two pesticides were not detected. A comparison between analytical results obtained from GC-MS and an alternative method, i.e. GC-ECD indicated that GC-ECD is less sensitive than GC-MS, with LOQ ranging from 0.37 to 1.18 (depending on the pesticide). However, for samples with concentrations above these LOQ, no significant difference was observed between the results obtained from the 2 analytical methodologies.

Introduction

In Vietnam Mekong Delta, beside intensive culture of shrimp and catfish, there are many other types of production systems such as integrated and alternative productions. These systems include rice and fish or rice and giant freshwater prawn (*Macrobrachium rosenbergii*) culture. According to Heong and co-workers (1998), the rice farmers in the Mekong Delta considered that an intensive use of pesticides will result in higher rice production. This has led to a significant increase in the application of various types of pesticides. Pesticide use on rice has shown negative impacts on fish and shrimp in integrated culture systems (physiological effect, mortality, muscle contamination) even at low or undetectable concentration (Nguyen et al., 2015; Tu et al., 2009). A wide range of pesticide residues were found in environment (water, soil and sediment) of Mekong Delta (Nguyen et al., 2013). These hazards may influence wild animals and human's health through environment exposure and food consumption, especially for the hydrophobic and persistent compounds, which bioaccumulate in individuals of the high trophic level in the food chain (Verhaert et al., 2013; Xu et al., 2014). Indeed, it was shown that chemicals with high Kow factor display also high bioconcentration factors (Katagi, 2010). According to both a survey realized in 2013 (Nguyen et al., 2014) and practical situation of the aquaculture industry in Vietnam, three pesticides appeared to be largely used in rice-cum fish systems, and have been chosen in this study: dichlorvos, quinalphos and trifluralin. These pesticides are not approved in the EU (European Commission, 2009), while only dichlorvos is forbidden in Vietnam since 2009 (VMARD, 2009 and 2010). However, it could continue to be used illegally and residues could be found. In order to control the residues of pesticides, analytical methods must be developed and validated. Several methods were involved in pesticide determination, such as bioassays, spectrophotometric determinations, chromatography or electrochemical techniques. Among these methods, gas chromatography has been widely used and the method is considered as the most sensitive method for pesticide investigation (Liu et al., 2010). Generally, results of analytical method can be used for many purposes: to assess whether a product complies with regulatory limits, to take decisions involving the control of the manufacturing process of a product, to take decisions about legal affairs, international trade, health problems or the environment (Boqué et al., 2002). In this paper, the method was first developed and validated for gas chromatography coupled to mass spectrometry (GC-MS), according to the SANCO guidelines (SANTE, 2014), and then it was optimized for gas chromatography coupled to electron capture detection (GC-ECD). Analytical results obtained with GC-ECD were compared with those obtained with GC-MS to evaluate the applicability of the two methods.

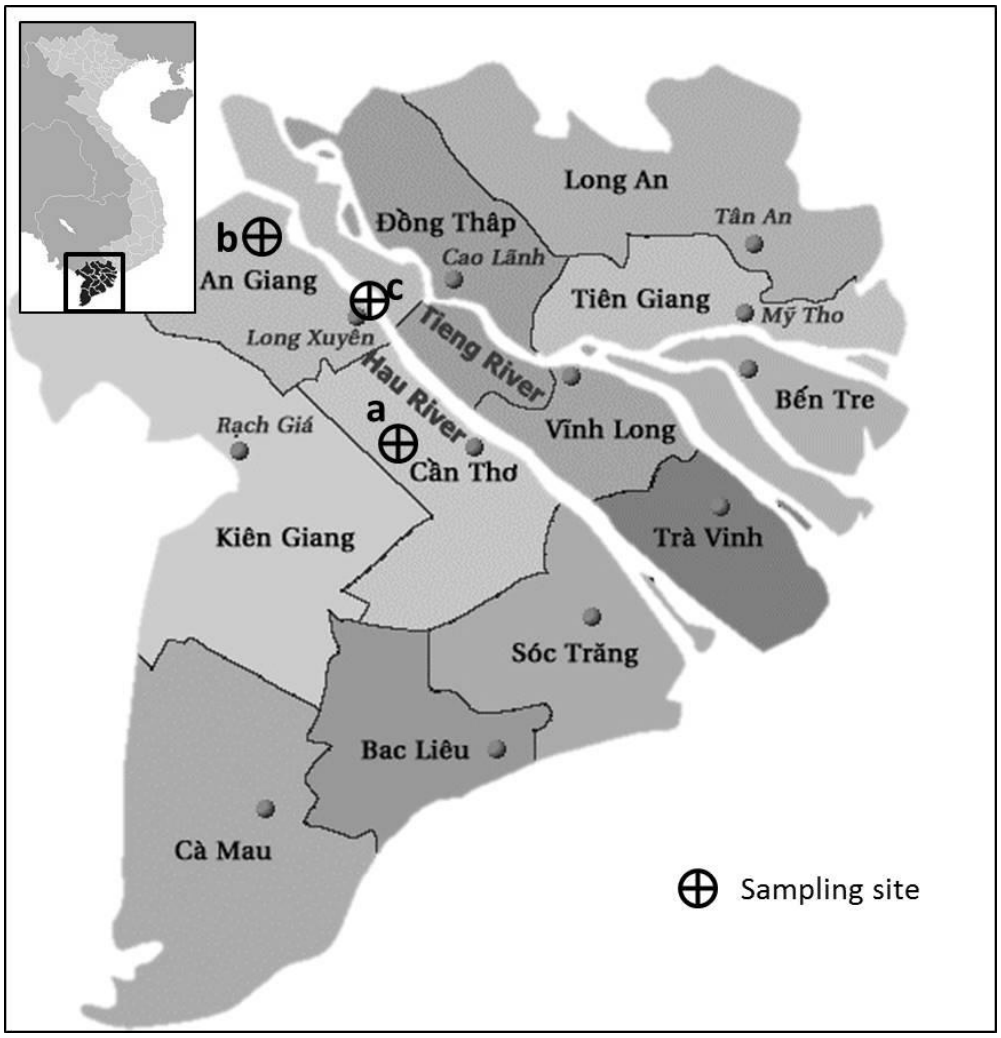

Figure 1. Geographical locations of the collection areas in the fresh-water region of the Mekong Delta, Vietnam. Three systems were selected: rice-fish area (a), intensive cat-fish area (b) and red-tilapia cage culture (c).

Material and methods

Reagents and instruments

Reagents, Chlorpyriphos-D10, dichlorvos-D6 and trifluralin-D14 were used as internal standard (IS) and purchased from Dr. Ehrenstorfer (Augsburg, Germany). Quinalphos (99.2%), Trifluralin (99.9%) and Dichlorvos (98.9%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Hexane was of Picograde quality and provided by Promochem (Wesel, Germany).

Individual stock solutions of each compound were prepared in acetone at a concentration of 1 mg/mL solvent. All solutions were kept at 4oC for up to six months.

Analytical instrument

For GC-MS analysis, pesticides were separated on a Focus GC gas chromatographer (Thermo Fisher Scientific) using an Equity 5 column (30 m x 0.25 mm x 0.25 µm) (Sulpelco, Bellefonte, PA, USA) and analyzed with an ion trap PolarisQ mass spectrometer (Thermo Fisher Scientific). Helium gas was applied as carrier gas. The temperature program was 50 °C for 1 min, followed by an increase of 20 °C per min to 100 °C and hold for 1 min, then 10 °C per min to 250 °C and hold for 1 min, then an increase of 20 °C per min to 300 °C and hold for 2 min; total run time was 42 minutes. The pesticides were detected using selected ion monitoring (SIM) mode in four segment windows. In each chromatographic run, different ions were monitored for each pesticide analysed, which allowed to perform detection and quantitative analysis (Table 1). Results were calculated using Xcalibur Software (Thermofinnigan).

The GC-ECD system was composed of a GC-2010 gas chromatographer (Shimadzu, Kyoto, Japan), an Equity 5 column (30 m x 0.25 mm x 0.25 µm) (Sulpelco, Bellefonte, PA, USA) and an electron capture detector (ECD, 63Ni, Shimadzu). The temperature program was quite similar to the GC-MS one, but the inlet was operated under split mode with the split ratio of 1:5. Nitrogen was used as carrier gas.

Field water samples

Thirty-three water samples were collected in April 2013, at the beginning of the rainy season in the Mekong Delta in Vietnam. The geographical locations of the collection areas are presented in Figure 1. The samples were composed of 13 samples from rice field, 10 samples collected from catfish ponds and 10 samples collected from red tilapia cages. At the collection time, most of farm had just finished the first rice crop and preparing for the second, fish had been stored in channel or separated pond for more than one month due to the overlapping of fish crop and second rice crop. Samples were collected into 1 liter plastic bottle, kept in ice and brought to the College of Aquaculture and Fisheries of Can Tho University (Vietnam), and then those samples were identified and stored at -20°C until analysis by GC-MS.

Extraction procedure

According to LeDoux (2011), the most widely used pesticide extraction technique from food of animal origin was direct solid–liquid extraction (SLE). This procedure has been applied to meat and meat products, fish, eggs etc. For extracting pesticide residues from liquid milk, liquid–liquid extraction (LLE) is still the preferred method (LeDoux, 2011). Similarly, in this study, because water samples have to be analysed, liquid–liquid extraction was selected.

GC-MS analysis

The applied method was developed based on Nguyen (2013) and Shin & Shin (2003) works. Twenty-five mL sample water was poured into a 60 mL glass tube with Teflon cap. Internal standards were added at the concentration of 1.2 µg/L for trifluralin-D14 and 2.4 µg/L for dichlorvos-D6 and chlopyrifos-D10. Note that, for quinalphos, chlorpyriphos-D10 was used as internal standard, because no commercial stable isotope labeled quinalphos was available. The pH of water sample was adjusted to four with HCl 0.1N, before extraction. Ten mL of ethyl-acetate:chloroform (1:1) were added into the tubes which were then shaken for 20 minutes at 300 rpm on a horizontal shaker. The organic layer was collected to a new tube and the water was extracted one more time. The extracts were combined and dried under nitrogen flow until the remaining volume was approximately 50 µL. The mixture was reconstituted to 300 µL with acetone. The solution was then filtrated through a 0.2 µm filter in an injection vial with an insert and injected to GC.

GC-ECD analysis

The same extraction procedure as for GC-MS was used, using ethyl acetate:chloroform (1:1) as solvent, except that no internal standard was added before extraction and that the final extract was reconstituted to 1 mL with acetone containing chlorpyrifos-D10 as injection standard.

Calibration curve preparation

Matrix matched calibration curves were prepared using eight samples of 25 mL blank HPLC water spiked with internal standards and with a mixture of the three pesticides to reach final concentrations of 0, 0.06, 0.12, 0.3, 0.6, 1.2, 1.8, 2.4 µg/L for dichlorvos and quinalphos and 0, 0.03, 0.06, 0.15, 0.3, 0.6, 0.9, 1.2 µg/L for trifluralin. To evaluate the matrix effect on calibration curves, the dry residue coming from the extraction of blank HPLC water was reconstituted with the mixture of the three pesticides, to reach the same eight corresponding concentrations. In parallel to these matrix matched calibration curves, solvent calibration curves were prepared. The test was realized in triplicate and data were plotted to assess the matrix effect.

For GC-ECD, matrix matched calibration curves were prepared using seven samples of 25 mL blank HPLC water spiked with a mixture of the three pesticides to reach final concentrations of 0, 0.2, 0.4, 2, 4, 6, 8 µg/L for dichlorvos and quinalphos and 0, 0.1, 0.2, 1, 2, 3, 4 µg/L for trifluralin. Chlorpyrifos D10 was used as injection standard.

For both techniques, the concentration range of the calibration curves was chosen to suit the range of concentrations observed for pesticides in water samples in Vietnam (Nguyen et al., 2013).

GC-MS and GC-ECD quantification

In GC-MS, the response (ratio between pesticides and their respective internal standard peak areas, considering the sum of all ions) was plotted against standard concentrations. A linear regression was used and no "fit weighting" was applied.

In GC-ECD, the ratio between the peak area of the analytes and the injection standard, chlorpyrifos D10, were used as responses, and plotted against concentration.

Validation of the GC-MS method

The GC-MS analytical method was validated according to the SANTE document (SANTE, 2015) and as described by other authors (Zainudin et al., 2015; Carneiro et al., 2013). The validation realized included the evaluation of the matrix effect on calibration, of the LOD and LOQ, the repeatability, the within laboratory reproducibility, the specificity, the recovery and the trueness. There are several approaches to calculate the LOD and LOQ, such as visual evaluation, the signal to noise approach, the procedure based on the standard deviation of the response and the slope of a calibration curve (ICH, 2005). In this study, for both GC-MS and GC-ECD methods, we used the last one where the limit of detection (or quantification) was calculated as 3.3 (or 10) times the standard deviation of the response divided by the slope of the calibration curve. According to the ICH guidelines (ICH, 2005), the standard deviation of the response was determined from the responses of five blank samples where the area of the chromatographic peak was integrated at the retention time corresponding to the expected compound.

Repeatability is the relative standard deviation of repeated measurements of an analyte using the same sample with the same method in a single laboratory over a short period of time, with no difference in instrument and materials. Within laboratory reproducibility is similar to repeatability, but obtained from different periods and analysts. Specificity is the ability of detecting an analyte from the background. Trueness is defined as the closeness of agreement between the average value obtained from a series of test results and a true value or accepted reference.

HPLC water fortified with standards of pesticides was used to assess the performance of the method. After determination of LOD and LOQ, 10 samples of blank HPLC water were fortified with pesticides at two different levels inside the calibration curve range to assess repeatability and reproducibility: five samples were fortified with the compounds at a concentration of 0.6, 0.3 and 0.045 µg/L for dichlorvos, quinalphos and trifluralin, respectively and 5 samples were fortified with the compounds at a concentration of 1.8 µg/L, 0.9 µg/L and 0.135 µg/L for dichlorvos, quinalphos and trifluralin, respectively. The fortified samples and a calibration curve were analysed in parallel and all procedure was repeated in two different days. Relative standard deviation and trueness were calculated based on the results obtained from the two fortified levels. Recovery rates of the target analytes were

measured by the analysis of spiked HPLC water as well as water extracts spiked after the extraction step with two levels of pesticides at the concentration of 0.6 and 1.8, 0.3 and 0.9, 0.045 and 0.135 µg/L for dichlorvos, quinalphos and trifluralin, respectively.

Comparison of both GC-MS and GC-ECD analytical methods

To compare the effectiveness of GC-MS and GC-ECD for pesticides analysis, water samples from an experiment containing only quinalphos were analysed. Water samples were obtained from an aquarium experiment realized at the College of Aquaculture and Fisheries of Can Tho University (Vietnam) in 2012, which investigated the effect of quinalphos on physiological parameters of silver barb fish (Barbonymus gonionotus Bleeker, 1849). Experiment included four treatments, which were control, 86, 172 and 430 µg/L quinalphos corresponding to 10%, 20% and 50% of the 96 hours lethal concentration (LC50-96hrs). This experiment was set to assess the changes in cholinesterase activity of silver barb fish. Samples were analysed with GC-ECD as triplicates and used to establish the kinetic of elimination of quinalphos in water. Among those samples, some were collected to be analysed by GC-MS to compare the results with those obtained by GC-ECD. The samples analysed by GC-MS corresponded to collection time of 5 minutes, 1 day and 28 days after application of 430 µg/L quinalphos and 28 days after the application of quinalphos at 172 µg/L.

Statistical analysis

Statistical analysis was made with the SPSS software, version 18.0. Independent sample T-test was applied to compare the means of two data groups. Significant difference was determined at $P < 0.05$.

Results and discussion

Method development and validation

The analytical parameters such as retention times and mass to charge ratios of the compounds analysed in GC-MS and GC-ECD are presented in Table 1.

Table 1. Mass to charge ratios and retention times for each compound analysed in GC-MS and GC-ECD.

Compounds	Retention time (min)		Ion mass to charge
	GC-ECD	GC-MS	ratio (Dalton)
Dichlorvos	11.22	10.62	109, 185
Dichlorvos-D_6	na	10.56	115, 191
Trifluralin	15.95	15.99	264, 306
Trifluralin-D_{14}	na	15.90	267, 315
Quinalphos	22.13	20.34	146, 156, 157, 298
Chlorpyrifos-D_{10}	20.52	19.32	260, 324

na = not applicable (standards not used in GC-ECD)

Calibration curves

The matrix matched and solvent calibration curves showed that matrix (water) only slightly affected the slope and the intercept of the calibration curves (data not shown). Indeed, there was no significant difference between the curves parameters due to very few co-extraction compounds. Compared with other fat containing matrices like milk, fish or cocoa, which may contain a large amount of fatty acids, alkaloids, esters or tocopherols (Zainudin et al., 2015), water sample is a simpler and cleaner matrix. Even if the impact of the matrix on the calibration curve was low, matrix matched calibration was used for quantification in this study. In the current method, the range of linearity of dichlorvos and quinalphos calibration curve was between 0.06 and 2.4 µg/L (with R2=0.967 for dichlorvos and R2=0.991 for quinalphos) and between 0.03 and 1.2 µg/L in the case of trifluralin (R2=0.996).

LOD and LOQ determination

LOD values were of 0.016, 0.002, 0.002 µg/L for GC-MS and 0.35, 0.36, 0.11 µg/L for GC-ECD for dichlorvos, quinalphos and trifluralin, respectively. LOQ values were of 0.053, 0.007, 0.007 µg/L for GC-MS (Table 2) and 1.15, 1.18, 0.37 µg/L for GC-ECD for dichlorvos, quinalphos and trifluralin, respectively.

Table 2. Validation parameters of the GC-MS method for the quantification of three pesticides in water samples. The two values for repeatability day 1 and day 2, within laboratory reproducibility, trueness and recovery rate are respective to the two mean introduced concentration values, which were 0.6 and 1.8, 0.3 and 0.9, 0.045 and 0.135 µg/L for dichlorvos, quinalphos and trifluralin, respectively (n=5).

	LOD (µg/L)	LOQ (µg/L)	Repeatability RSD (%) day 1	Repeatability RSD (%) day 2	Within laboratory reproducibility Overall RSD (%)	Trueness (%)	Recovery rate (%)
Dichlorvos	0.016	0.053	11.7 – 10.5	7.2 – 5.2	8.7 – 13.0	98.5 – 100.4	72 – 76
Quinalphos	0.002	0.007	2.3 – 2.5	5.5 – 3.3	6.4 – 5.6	101.0 – 101.0	82 – 81
Trifluralin	0.002	0.007	6.9 – 5.0	1.8 – 2.9	4.7 – 5.2	85.3 – 90.4	72 – 72

RSD = relative standard deviation

Selectivity and specificity

The absence of significant peaks was shown in the blanks and the presence of quantifiable peaks was seen in the fortified samples in both GC-MS (Fig. 2) and GC-ECD (Fig. 3). When a peak was detected in the blanks, it was shown that the relative retention times and/or the transition ratios (ratio between the peak area corresponding to the first transition and that of the second transition for a compound) did not correspond to those of the three pesticides analyzed here. For the fortified samples, it was also shown that the variations of relative retention times (RRTs) and of transition ratios corresponded to that of the calibration standard with a tolerance of ± 0.5% for the RRTs, and ± 30% for the relative of ion ratio (SANTE, 2015).

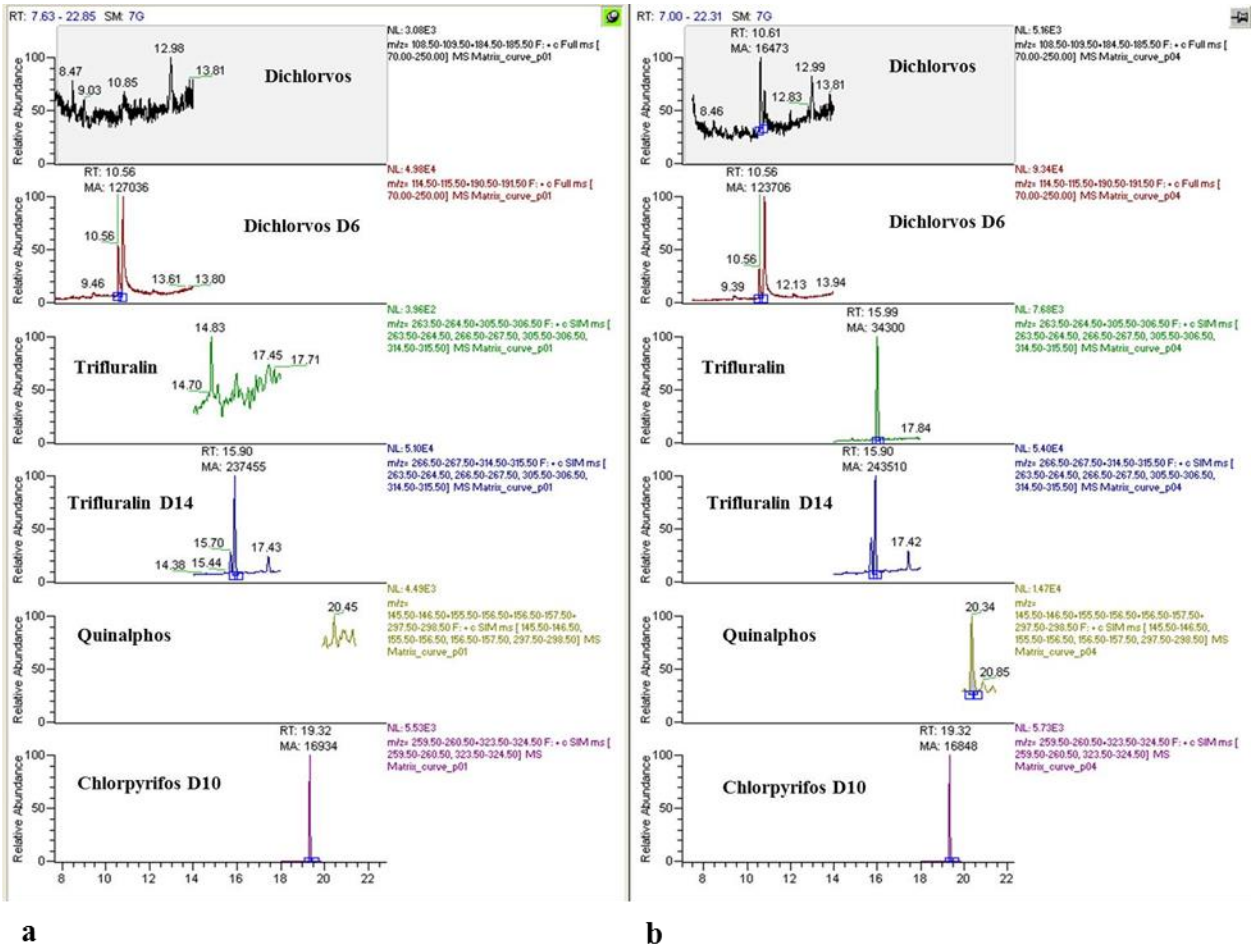

Figure 2. Chromatogram (GC-MS) resulting from the analysis of HPLC water taken as a blank sample (a) and the analysis of the same water sample spiked with the 3 target pesticides at a concentration of 0.3 µg/L for dichlorvos and quinalphos and 0.15 µg/L for trifluralin (b).

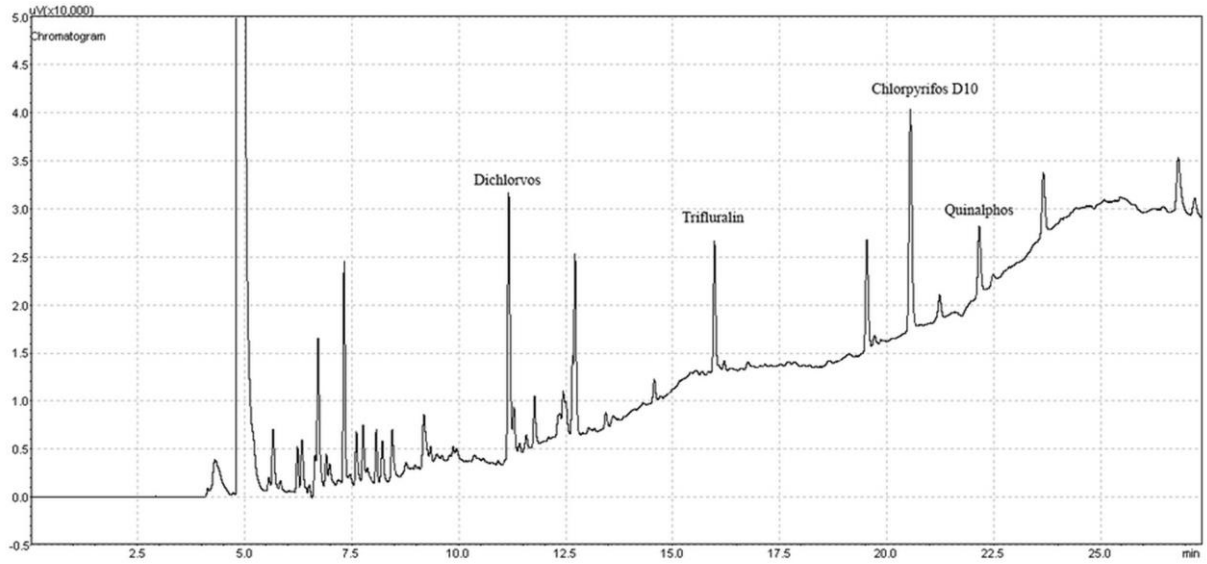

Figure 3. Chromatogram (GC-ECD) resulting from the analysis of HPLC water sample spiked with the 3 target pesticides at a concentration of 0.3 µg/L for Dichlorvos and Quinalphos and 0.15 µg/L for trifluralin

Trueness, repeatability, recovery rate

Results of trueness, repeatability and recovery rate of the GC-MS method are presented in Table 2. The coefficients of variation on the same day (repeatability) and on different days (reproducibility) were both lower than 20% at the two fortified levels, which means that the developed method is repeatable and reproducible according to the SANTE guidelines (2015). The trueness was also assessed with water samples fortified at two different levels and the calculated concentrations were compared with theoretical concentrations. Observed trueness ranged from 85.3 to 101.0 % and was satisfying the criteria of SANTE document which fixed trueness between 70 and 120% (SANTE, 2015). The recovery rates of the GC-MS method for the 3 target pesticides ranged between 72 and 82 % (Table 2).

In GC-ECD, a short validation was performed to assess the performances of the developed method. The linearity of dichlorvos, quinalphos and trifluralin matrix matched calibration curves ranged from 0 to 8 µg/L (dichlorvos and quinalphos) and 0 to 4 µg/L (trifluralin), with R-square values of 0.991, 0.995 and 0.994, respectively.

Analysis of field water samples

From the 33 water samples collected, from which 13 samples were from rice field, 10 were from catfish ponds and 10 from red tilapia cages, only 9 % contained residues of pesticides, but contaminated samples only came from rice fish farms. No pesticides residues were detectable in catfish ponds and water from red tilapia cages. From the 13 samples taken in rice fish systems, quinalphos was detected in three samples with the concentration of 0.11, 0.08 and 0.04 µg/L. The

other pesticides were not detected in any sample. The absence of dichlorvos and trifluralin can be explained by the fact that the use of dichlorvos is totally banned in Vietnam since 2009 while trifluralin has been banned in aquaculture only (VMARD, 2009 and 2010). These two toxic compounds were completely or partially banned for safety reasons. In particular for trifluralin, the ban in aquaculture resulted from the rejection of shrimps exported from Vietnam to Japan because of their trifluralin content (VASEP, 2010). These two pesticides were however kept in our study in order to check that the famers follow the ban, as it could be expected that banned chemicals are still used by farmers. For instance, even if banned since 2010, presence of trifluralin in *Pangasius* fillet imported from Vietnam was detected in 2011 (RASFF, 2011). According to our limited sampling in fresh water aquaculture system of the Mekong delta, the Vietnamese farmers seem to no longer use the banned dichlorvos and trifluralin chemicals in aquaculture. This has to be confirmed in a larger scale study.

Quinalphos, however, is still allowed to be used in agriculture (VMARD, 2015). In the field, farmers usually apply quinalphos one to two times per crop to prevent pest. Normally, quinalphos is applied 65 days after sowing to prevent rice panicle mite (Vien et al., 2012). In the area where samples were collected, the rice was sowed in December 2012 and raised for 90 to 100 days, so, the duration between quinalphos application and our water sampling time was estimated to be 1 to 1.5 months. That can explain why this compound was detected in 23% of the samples of water from the rice fish system (three out of 13 samples), at low levels. The low levels could be explained by the degradation of quinalphos. Gupta et al. (2011) showed that the rate of degradation of quinalphos is increased with the increasing of temperature, pH level, and the concentration of humic acid. The same authors showed that the half-life of quinalphos ranges from 40 to 27 days, at 30°C, at pH 6 to 8, in laboratory condition (Gupta et al., 2011). In field conditions, the degradation seems to be faster, as the half-life of quinalphos in soil (okra field at West Bengal, India) was shown to be only 1.07 to 1.2 days (Aktar et al., 2008).

Even if low levels of quinalphos were found in water, since quinalphos has a high partition coefficient octanol/water (4.4) (PPDB, 2014), its concentration may be very high in aquatic animals due to bio-accumulation through skin, gill or intestine tract (Xu et al., 2014). Moreover, that low concentration may be very harmful to crustacean, due to the very low lethal concentration of this compound for species belonging to this group of animals. According to Kegley et al. (2016), the LC50 48h of quinalphos on *Peneaus monodon* ranged between 0.12 to 0.55 µg/L and the LC50 24h of quinalphos was 2.7 µg L-1 for *Peneaus indicus*. This shows possible negative consequences on both cultured shrimp and wild shrimp, if quinalphos residues from the water of such rice fish system is released in shrimp cultured system or in the environment in general.

Comparison of GC-MS and GC-ECD analytical methods

For comparing the effectiveness of GC-MS and GC-ECD for the analysis of quinalphos, water samples from an aquarium experiment realized in Vietnam and concerning the physiological parameters of Silver barb fish exposed to quinalphos were used. This experiment included 4 different concentrations of quinalphos in water and the samples were analyzed as triplicates with GC-ECD in Vietnam. Some of the samples were also analyzed with GC-MS to establish a comparison of the two developed methods. The results obtained from the analysis of the samples with the two instrumental systems are presented in Table 3.

After 28 days of application of 172 µg/L of quinalphos in the aquarium, the pesticide concentrations measured were low. Indeed, the values were of 0.3 ± 0.01 µg/L with the GC-MS method and of 1.1 ± 0.5 with the GC-ECD method. After the application of 430 µg/L of quinalphos, it can be observed that pesticide levels were decreasing quickly in the water of the aquarium and detected at very low levels. After 5 minutes of pesticide application at that concentration, the levels in the water were of 295.9 ± 46.3 and 254.5 ± 31.0 µg/L measured with the GC-MS and the GC-ECD method, respectively while, after 1 day, the levels were of 93.2 ± 29.1 and 76 µg/L measured with the GC-MS and the GC-ECD method, respectively. Twenty eight days after the application of quinalphos at 430 µg/L, the concentration of quinalphos decreased to 1.53 ± 0.04 and 1.7 ± 0.2 µg/L, measured by GC-MS and GC-ECD respectively, corresponding to a level close to the one measured 28 days after the application of 172 µg/L of quinalphos.

Where applicable, independent samples t-test was applied to assess the difference between the two methods used to analyze the water samples. As shown in Table 3, statistical results demonstrated that the difference between methods was not statistically significant ($p>0.05$), which implies that the two developed methods give similar results and can be both used to analyze quinalphos in water samples, if residues levels are above the limit of quantification (LOQ) of the GC-ECD method. However, as mentioned above, the LOQ of the GC-ECD method is much higher than the one of the GC-MS method (see “LOQ and LOQ determination section”), so only the GC-MS method will be suitable to detect trace contamination of pesticides in water.

Table 3. GC-MS and GC-ECD analytical results obtained with aquarium water samples containing quinalphos.

Concentration of quinalphos applied	Collection time after application	Result obtained with GC-MS (µg/L)	Result obtained with GC-ECD (µg/L)	Sig. (2-tailed)
172 µg/L	28 days	0.3 ± 0.01	1.1 ± 0.5	0.106
430 µg/L	5 minutes	295.9 ± 46.3	254.5 ± 31.0	0.358
430 µg/L	1 day	93.2 ± 29.1	76	Not applied
430 µg/L	28 days	1.53 ± 0.04	1.7 ± 0.2	0.119

Samples were analyzed as triplicates, except for collection after 1 day after application of 430 µg/L where only one sample was analysed in GC-ECD. Sig.: Significant level which indicated no significant difference if the number is greater than test level (0.05)

Conclusion

The validation parameters of the GC-MS and GC-ECD methods developed in this study met the requirements of the SANTE guidelines (SANTE, 2015) but the GC-ECD method display higher LOQ than GC-MS.

No dichlorvos (banned in Vietnam since 2009), trifluralin or quinalphos residues were found in catfish ponds or water collected from red tilapia cages. In water samples from the rice fish system, dichlorvos and trifluralin were not detected while quinalphos was detected in 23% of samples. This shows that residues of quinalphos, a bioaccumulative pesticide, could be of concern in fish or prawn produced in rice integrated systems.

Acknowledgements

The authors thank CUD (Commission Universitaire pour le Développement) for its financial support (DELTAQUASAFE project).

The authors acknowledge also all staffs of Laboratory of Food Analysis, FARAH - Veterinary Public Health of the University of Liège as well as the staffs of the Department of Fish Nutrition and Aquatic Food Processing, CAF, Can Tho University for labours, knowledge and facilities supporting.

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Experimental section

Study n°3: Bioconcentration and half-life of quinalphos pesticide in rice-fish integration system in the Mekong Delta, Vietnam

Journal of Environmental Science and Health Part B, 2018, 53:1, 35-41

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The previous study showed that quinalphos residues were found in water samples collected from rice fish farms. These results agreed with the fact that we found in the survey study that quinalphos was one of the commonly pesticides used in rice and rice fish systems. Quinalphos is able to bioaccumulate in fat tissue of organisms exposed to its residues from water, so the residue of quinalphos may affect the fishes which are co-cultured in the rice field, and quinalphos residue in fish tissue may result in health problems for human who eat quinalphos contaminated fishes. Therefore, an experiment was set up in a rice fish system to evaluate the distribution and the elimination of quinalphos in this system. The experiment was performed in the same conditions than in normal rice fish culture i.e. two applications of quinalphos was applied to the rice crop which was integrated with fish crop. The experiment aimed to evaluate the elimination of quinalphos in a practical situation through quantifying the concentration of applied compound in water, fish and in sediment samples. After that, based on the residue levels of quinalphos in the matrix, half-life and bioconcentration factor of quinalphos in rice fish system were calculated.

Abstract

In order to determine the distribution and enable the elimination of quinalphos, a popular active pesticide compound used in the Mekong Delta, an experiment was set up in a rice-fish integration system in Can Tho City, Vietnam. Fish was stocked into the field when the rice was two-months old. Quinalphos was applied twice in doses of 42.5 g per 1000 m2. Water, fish and sediment samples were collected at time intervals and analyzed by a Gas Chromatography Electron Capture Detector system. The results show that quinalphos residues in fish muscles were much higher than those of the water and the bioconcentration factor (logBCF) was above 2 for the fish. The half-life of first and second quinalphos applications were 12.2 and 11.1 days for sediment, 2.5 and 1.1 days for silver barb, 1.9 and 1.3 days for common carp, and 1.1 and 1.0 days for water, respectively.

Keywords

Mekong Delta, aquaculture, rice-fish integration system, pesticide, quinalphos, bioconcentration

Introduction

The Mekong Delta (MD) is the most intensive agricultural area in Vietnam. In the MD there are several aquaculture systems, which include mono-and polyculture at various scales. Rice-fish integration systems are quite popular in other South and Southeast Asian countries. (Vromant et al., 2001) In Vietnam, polyculture is usually applied in rice-fish integration systems; (Vromant et al., 2001) the stocking species generally consist of silver barb (*Barbonymus gonionotus* Bleeker, 1849), common carp (*Cyprinus carpio* Linnaeus, 1758), and Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). The rice-fish integration system in the MD usually consists of two rice crops and one fish crop per year. The first rice crop is the main one, lasting from December to March without fish stocking. During the second rice crop, which is cultured from April to July, fish are normally stocked into the system after rice has grown for between one and two months. Fish are harvested between September and October and mostly fed by natural feed after the rice crop ends in July.

Quinalphos is a popular insecticide used to prevent rice panicle mites (*Steneotarsonemus spinki*) in rice fields, and is sold under the brand name Kinalux 25EC. (Nguyen et al., 2014) Quinalphos is an insecticide belonging to the organophosphorus group, sub-classified into the group of heteroaryl phosphorothioates because of its aromatic rings. (Matolcsy, 1988) Physiological effects of quinalphos alone were studied in many animal species (e.g., fish, (Bagchi et al., 1990; Chebbi and David, 2009; Chebbi and David, 2010; Das and Mukherjee, 2000) birds, (Anam and Maitra, 1995) and mammals (Dikshith et al., 1982; Dikshith et al., 1980)). The joint effects of quinalphos and other pesticides were also investigated in fish (Maske and Thosar, 2012). In humans, quinalphos can be metabolized and excreted through urine under the form of diethyl phosphate and diethyl phosphorothioate. Regarding to its toxicity, quinalphos can lower the cholinesterase concentration in serum and red blood cells of humans, and it takes more than 30 days to recover to normal concentration (Vasilić et al., 1992).

Residues and dissipation of quinalphos were studied a long time ago in cauliflower (Chawla et al. (1979) which indicated that 95% of this chemical degraded within eight days. Other studies regarding the elimination of quinalphos in okra fruit, (Aktar et al., 2008) tomato fruit and radishes, (Gupta et al., 2011) Kinnow Mandarin fruit, (Battu et al., 2008) cabbage and brinjal (Chahil et al., 2011; Pathan et al., 2012) were also conducted. However, limited information is available on the elimination of quinalphos in rice fields, which could represent a risk of quinalphos contamination in fish cultured in rice-fish integration systems. The main objective of this study was to investigate the bioconcentration and half-life of quinalphos in water, sediments and fish in an on-farm rice-fish integration system.

Materials and methods

Reagents and instruments

Chlorpyriphos-D10 was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Quinalphos standard (99.2%) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Kinalux 25EC, which contains 250 g L-1 of quinalphos, was purchased from United Phosphorus Ltd. (Worli, Bombay, India). The concentration of active ingredient quinalphos in Kinalux 25EC was confirmed by gas chromatography electron capture detector (GC – ECD) before use in this experiment.

Analytical instrument

The GC-ECD system was composed of a GC-2010 gas chromatographer (Shimadzu, Kyoto, Japan), an Equity 5 column (30 m × 0.25 mm × 0.25 µm) (Sulpelco, Bellefonte, PA, USA) and an electron capture detector (ECD, 63Ni, Shimadzu).

Field experiment and sample collection

Healthy fingerling silver barb (*Barbonymus gonionotus* Bleeker, 1849) and common carp (*Cyprinus carpio* Linnaeus, 1758) were purchased from a local hatchery (Can Tho, Vietnam).

The experiment was conducted in the Co Do District of Can Tho City, Vietnam. The experiment was triplicated and the experimental area was divided into three identical sections of 1000 m2 each, completely separated from the others and from outside areas by plastic barriers. The experiment was set up in the period from May to September 2013, corresponding to the second annual rice crop. Common carp (8.0 ± 1.5 g) and silver barb (5.0 ± 0.9 g) fingerlings were stocked at a density of three and two fish per m2, respectively. Fish were stocked after the rice was cultured for 50 days before first chemical application. Kinalux 25EC was applied over the rice at a dosage of 170 mL 1000 m-2, corresponding to 42.5 g of quinalphos per 1000 m2, as recommended by the producer. The pesticide was applied twice when the rice was 54 and 79 days, respectively. The trench water levels in the experimental field were adjusted following the normal farming practice, by 1.4 m for the first and 1.2 m for the second Kinalux 25EC application.

Water, fish and sediment samples were collected one day before quinalphos application and then after 30 minutes, 1 day, 3 days, 7 days, and 14 days of the first and second quinalphos applications. After 14 days of the second application, samples were collected every two weeks. At the sampling time of thirty minutes after the first and second applications of quinalphos, only water samples were collected. The analyses were processed until two consecutive samples fell below the detection limit.

Fish samples were collected by cast-net, the scales were removed and the muscle (with skin) from ten fish was homogenized and stored at -20°C until analysis. Water and sediment were collected following the method described by Lazartigues et al. (2011) Water samples were collected at a depth of 10 to 15 cm from the surface, and sediment was collected on a depth of up to 4 cm. All samples were kept at -20°C and thawed before analyzing. Temperature, pH and dissolved oxygen were recorded monthly. During the experiment, temperature, pH and dissolved oxygen were 30.8 ± 0.9°C, 7.3 ± 0.5 and 3.0 ± 0.5 mg L-1 (n = 4), respectively.

Extraction procedure

For water samples, to remove suspended matter, the sample was first centrifuged at 2500 g for 5 minutes, and then 30 mL sample was poured into another 50 mL centrifuge tube. The pH of the water sample was adjusted to 4 with 0.1N HCl before extraction. Ten mL n-hexane were added to the tube and then shaken for 20 minutes at 300 rpm on a horizontal shaker. The organic layer was collected into a new tube and the water was extracted one more time. The extracts were combined and evaporated to dryness under vacuum. The dried residue was reconstituted to 1 mL with internal standard (Chlorpyrifos D10) solution in acetone at the concentration of 40 µg L-1. The solution was then filtered through a 0.2 µm filter in an injection vial with an insert and 2 µL were injected into the GC-ECD.

For fish muscle, homogeneous grounded muscle (2 g) was weighed into a 50 mL centrifuge tube containing anhydrous sodium sulfate (2 g). Eight mL acetone: acetonitrile (1:1) was added. The tubes were then shaken for 20 minutes at 300 rpm by horizontal shaker. Supernatant was collected into new tube after centrifuge at 2500 g for 5 minutes. Extraction was repeated and supernatants were combined, evaporated, and reconstituted similar to the steps in water extraction method.

For sediment samples, the method described by Tse et al. (2004) was applied after modifications. Wet sediment (5 g; 62 ± 3% of dry matter) was weighed into a 50 mL conical flask. Ten mL n-hexane: acetone (9:1, v:v) was added to the flask, which was then shaken at 125 rpm overnight. Anhydrous sodium sulfate (2 g) were added to trap water; the samples were then filtered through paper filter and washed with 2 mL hexane: acetone (9:1, v:v). Solvents were then processed via steps similar to the water extraction method.

GC-ECD analysis

The temperature program of GC was first 50°C for 1 min, followed by an increase of 20°C per min to 100°C and holding for 1 min, then 10°C per min to 250°C and holding for 1 min, then an increase of 20°C per min to 300°C and holding for 2 min. Injection volume was 2 µL. Retention times of the quinalphos and chlorpyriphos D10 (IS) were 22.1 and 20.5 min, respectively.

Quinalphos quantification was done using matrix matched calibration curves. The linearity of quinalphos matrix matched calibration curves of water, fish and sediment ranged from 0-8 ng/mL, 0 to 200 ng/g and 0 to 80 ng/g with R-square values of 0.992 to 0.997, 0.990 to 0.998 and 0.996 to 0.999, respectively. The recoveries of quinalphos analysis in water, fish and sediment samples were 87.2 to 92.7%, 75.7 to 78.5% and 57.2 to 59.9%, respectively, limit of detection (LOD) were 0.4 ng/mL, 7.5 ng/g and 0.5 ng/g, respectively and limit of quantification (LOQ) were 1.2 ng/mL, 22.7 ng/g and 1.6 ng/g respectively. Examples of GC-ECD chromatograms of blank and contaminated water, sediment and fish samples are shown in figure 1.

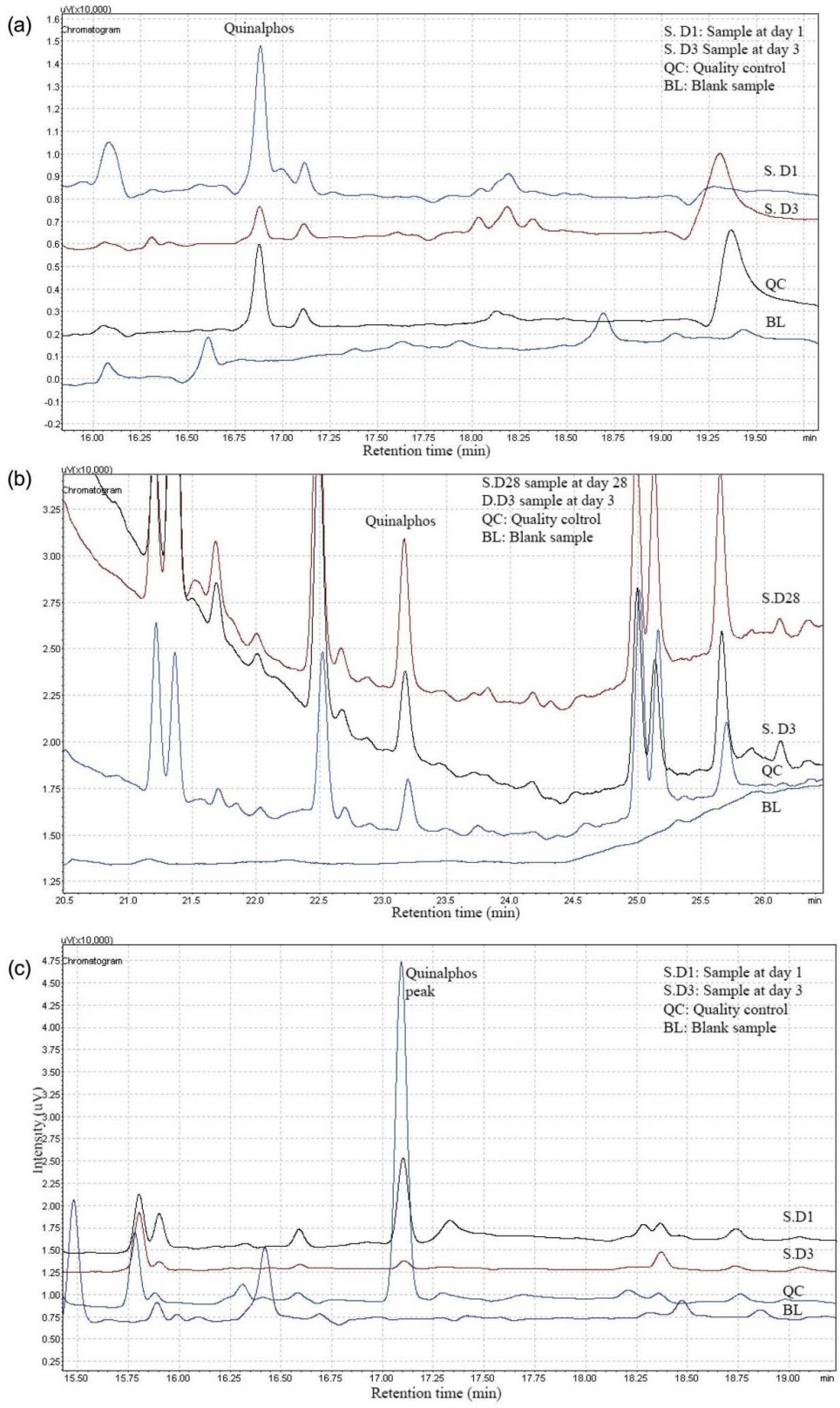

Figure 1. GC-ECD chromatograms of water (a), sediment (b) and fish (c) samples. Blank = blank sample. QC = blank matrix sample spiked with 2 ppb (water and sediment) or 2 ppm (fish) quinalphos.

Calculation of quinalphos half-life

The half-life of quinalphos was calculated in water, sediment and fish muscle, according to Lazartigues et al. (2013) based on the first order decay curve: ln (concentration) = a + bt, where t is the time (day), a is a constant, and b is the depuration rate or Kd (day-1). The half-life was calculated as t1/2 = ln(2)/Kd. The bioconcentration factor (BCF) in fish muscle was calculated according to Katagi (2010): BCF = Cpb/Cpw where Cpb is the chemical concentration in the organism and Cpw is the chemical concentration in water. BCF was calculated as the average between BCF calculated using concentrations in water and in fish measured one and three days after each quinalphos application when quinalphos was detectable in both fish tissue and water samples.

Results and discussion

Elimination of quinalphos in rice-fish system

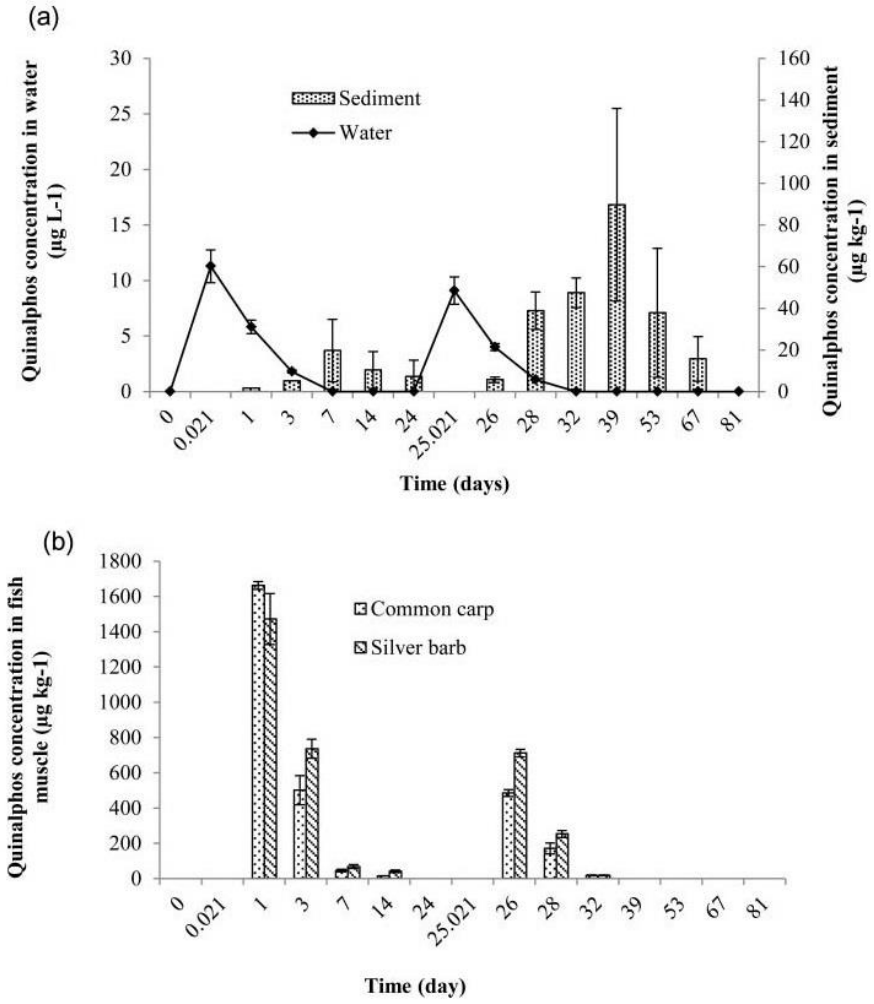

Figure 2. Elimination of quinalphos from water and sediment (a) and from fish muscle (b). The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were 0.4 and 1.2 mg L-1 for water, 7.5 and 22.7 mg kg-1 for fish and 0.5 and 1.6 mg kg-1 for sediment, respectively.

The analytical results showed that the highest concentrations of quinalphos in water after the first and the second application were respectively 11.3 ± 1.5 and 9.1 ± 1.2 µg L-1 (Fig. 2a). The lower concentration measured after the second quinalphos application might be caused by greater retention of pesticide in rice stalks, as the crop was older at the time of the second application. These concentrations were much lower than the quinalphos LC50-96 h of common carp (760 µg L-1) (Trung and Huong, 2012) and silver barb (856 µg L-1). (Tran et al., 2012) It is thus expected that quinalphos concentration in rice-fish field water might not affect the fish in this system. However, such concentrations may be toxic to other animals (e.g., crustaceans) to which quinalphos display a very low lethal concentration (LC). For example, LC50 after 48 h of quinalphos exposure in *Penaeus monodon* varied between 0.12 and 0.55 µg L-1, and for *Penaeus indicus*, the LC50 24 hours of quinalphos exposure was 2.7 µg L-1 (Kegley et al., 2014).

The shortest half-life of quinalphos (presented in Table 1) was found in water at 1.1 and 1.0 days after the first and the second application, respectively. In the current on-farm experiment, the half-life of quinalphos was much shorter than in a previous study (Gupta et al., 2011) where the half-life of quinalphos was 38.3 days under laboratory conditions with no sunlight and in pure (HPLC) water. However, under sunlight stimulation using lake water and groundwater, the half-life of quinalphos was shortened to 0.77 and 0.78 days, respectively; besides, the concentration of dissolved organic matter and nitrite ions affects the photolysis of quinalphos: nitrite ions accelerate the photolytic degradation while organic matter retards the process. (Gonçalves et al., 2006) This suggests that the degradation of quinalphos in water is strongly influenced by environmental parameters, such as sunlight, dissolved organic matter and biota in practical situations (i.e., in a rice field).

After the first application, the half-life of quinalphos in the muscle of common carp (1.9 days) and silver barb (2.5 days) was higher than in water (1.1 days). However, after the second application, the half-life of the compound in both fish species was shorter than the first application (1.3 and 1.1 days for common carp and silver barb, respectively). The decrease of the quinalphos half-life in fish muscle after the second application may be due to an up-regulation and an increased abundance of metabolizing enzymes resulting from the repeated chemical exposure. The up-regulation of a quinalphos metabolizing enzyme in fish is not described as for other enzymes, but the up-regulation of metabolic enzymes was observed when fish were exposed to other toxic chemicals, like nitrites. (Knudsen and Jensen, 1997) Moreover, the level of water in the field was lower at the time of the second chemical application because the rice was close to being harvested, subsequently, fish might prefer to move in the surrounding trenches. In addition, according to the study of Sancho et al. (1998) on fenitrothion, the lower chemical concentration in water would result in a higher rate of chemical metabolization or lower half-life of the chemical in fish. In mammals, the metabolism of quinalphos may be faster (e.g., in rat serum, the quinalphos half-life was 3.8 hours (Gupta et al., 2012)).

In sediment, the half-life of quinalphos was much longer than in fish and water after the first and the second pesticide application: 12.2 and 11.1 days, respectively (Table 1). The half-life of quinalphos in this situation was much longer than that in soil collected from an okra field, for which it was reported that 50% of quinalphos was degraded after 1 or 1.3 days depending on the original concentration (Aktar et al., 2008). The longer half-life of quinalphos in sediments may be explained by lower exposure of sediment to sunlight in the rice-fish integration system, as the photolytic pathway is one of dominant pathways of quinalphos degradation in soil (Gonçalves et al., 2006). However, the degradation of quinalphos in soil is influenced by the composition of the soil and soil pH variation, and the half-life of quinalphos increased from 9 to 53 days when the pH was changed from 5.1 to 8.1 (Gonçalves et al., 2006; Gupta et al., 2011). Moreover, the persistence of quinalphos in water and sediment is influenced by both biotic and abiotic degradation, including water pH, concentration of suspended matter, temperature, sunlight, and content of sediment (Warren et al., 2003).

Quinalphos distribution in rice field system

Figure 2a shows the results of quinalphos residue levels in the muscle of common carp and silver barb, while Figure 2b shows quinalphos levels in water and sediment during the experiment. The higher concentration of quinalphos in fish compared to water and sediment indicates the ability of quinalphos bioconcentration in fish. According to Gobas et al. (1999) the BCF is strongly dependent on the octanol/water partition factor ($K_{o/w}$) and on the fat content of organisms. Quinalphos is a pesticide that is highly soluble in organic solvents due to its high octanol/water partition coefficient (log $K_{o/w}$ = 4.44 at pH 7 and 20°C) (PPDB, 2015). Moreover, the fat content in common carp ranges between 5.7 and 7.8% in the case of fish fed natural feed (Urbanek et al., 2010) and around 4.4% in silver barb, (McGill, 2008) meaning that these fish belong to the medium to fatty fish group. (Sen, 2005) In the current study, BCFs (expressed as log BCF) were close to 2 in both common carp and silver barb after quinalphos application in the rice field. The BCF of 2 found in this study for quinalphos is quite high due to its high log $K_{o/w}$ and the relatively high lipid content in both fish species. These BCFs of quinalphos were quite similar to fenitrothion (with a log $K_{o/w}$ of 3.3), an insecticide used to prevent rice seed bugs. (VMARD, 2015) In the experiment of Sancho et al. (1998) European eel (*Anguilla anguilla*) were exposed to fenitrothion at 40 µg L-1 for 72 hours, and the result showed that the log BCF of this compound was 1.86. For common carp, the log BCF after 48 hours of quinalphos exposure was 1.6 to 2.2 (Tsuda et al., 1990).

Table 1. Bioconcentration factors (BCF) of quinalphos in fish and depuration rate of quinalphos in water, fish and sediment after quinalphos application in rice–fish field.

Sample types/quinalphos applications	Depuration rate K /R^2	$t_{1/2}$(day)	Bioconcentration Factor (log BCF)
Water			
First application	0.611 / R^2 = 0.999	1.1	
Second application	0.707 / R^2 = 0.996	1.0	
Sediment			
First application	0.057 / R^2 = 0.931	12.2	
Second application	0.062 / R^2 = 1	11.1	
Common carp			
First application	0.360 / R^2 = 0.906	1.9	2.45
Second application	0.553 / R^2 = 1	1.3	2.14
Silver barb			
First application	0.282 / R^2 = 0.846	2.5	2.52
Second application	0.624 / R^2 = 1	1.1	2.31

In the environment, chemicals can absorb into fish through gills, dermal pathways, and oral routes, mainly through diet. The uptake through dermal routes is dependent upon chemical polarity and lipid solubility (Schlenk, 2005). In this study, the concentration of quinalphos in fish muscle decreased following the fast elimination of quinalphos in water and an increase of quinalphos accumulation in sediment (Fig. 2a and 2b) suggested that the main chemical absorption into fish might be via dermal pathways (skin or gills). In field conditions, oral absorption may also play a role in chemical absorption, but the accumulation of pesticide in fish through oral routes varies depending on the chemical class of pesticides (Lazartigues et al., 2013). Chemicals firstly have to pass a diffusion membrane, (e.g., mucus) or biological layers before reaching the circulation system, and so the octanol/water partition coefficient and the molecular size of the chemical play an important role in bioconcentration. (Katagi, 2010) Also, Katagi (2010) and Lazartigues et al. (2013) showed a positive correlation between log BCF and log $K_{o/w}$, demonstrating that $K_{o/w}$ is an important factor contributing to the distribution of the chemical in the environment.

The residues of quinalphos in rice plants were not investigated in this study. However, according to Gupta et al. (2011) the degradation of quinalphos in plants was quite fast, with a half-life ranging from 3 to 4 days; thus, the rice stalks may also be a factor inducing quinalphos degradation from sediment and water as it could adsorb quinalphos through its roots and consequently metabolize the pesticide.

After two quinalphos applications, the concentration of the quinalphos in sediment increased, while its concentration in water went below a detectable level (Fig. 2a). It demonstrated an absorption of quinalphos from water to sediment, which is due to the high log $K_{o/w}$ factor of quinalphos (4.44). (PPDB, 2015) According to Katagi (2006) the pesticides with higher log $K_{o/w}$ will be more widely distributed in sediment rather than the pesticides with lower log $K_{o/w}$. After the second application, the concentration of quinalphos in sediment was much higher than after the first application (Fig. 2a). This could be due to the lower accumulation in fish muscle after the second application compared with the first one (Fig. 2b). As mentioned before, in the second application, the lower water level reduced the travel of the fish between channels within the rice area, and, subsequently, the fish would be less exposed and the concentration of quinalphos in sediment would be increased.

Conclusions

In rice-fish integration systems, beside the practical operation effects, the distribution of pesticide was influenced by various other factors. Indeed, sediment is a very complex matrix containing clay minerals, organic matter, and living organisms. In addition, interstitial pore water (portion of water located between small sediment particles) is different from the overlaying water, so it contributes greatly to pesticide distribution (Katagi, 2006). Also, elimination of the applied pesticide may be affected by climate conditions, such as wind or moisture. (Katagi, 2010).

Acknowledgements

The authors thank ARES-CCD (Académie de Recherche et d'Enseignement Supérieur – Commission pour la Coopération au Développement) for its financial support within the framework of the DELTAQUASAFE project.

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Experimental section

Study n°4: Chemical residues in environment and aquaculture products in the Mekong Delta and trifluralin exposure assessment through fish consumption

Manuscript in preparation

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The previous studies of this work showed that quinalphos, dichlorvos and trifluralin were pointed out in survey and practical situation. Furthermore, residues of quinalphos were found in water samples from rice farms and quinalphos was shown to bioaccumulate in fish coming from rice fish culture systems. Taking into account these results and the fact that import markets still show some non-compliancy with international regulation, it seemed relevant to assess the chemical residue situation related to aquaculture products of the Mekong Delta. In this fourth study, residues were thus measured in samples including muscle samples of common intensive fish culture systems (catfish, snakehead and climbing perch), as well as in fish of these species collected from markets. One forbidden antibiotic (chloramphenicol) was monitored as well in these samples and representative environmental contaminants (dioxins and PCBs) were determined in sediments from catfish ponds and rice fish systems. Finally, the exposure of the consumers to the only residue found in fish (trifluralin) was quickly estimated based on fish consumption surveys.

Abstract

The aim of this study was to evaluate the contamination of fish and environment from aquaculture in the Mekong Delta and to perform a survey of food consumption in order to assess the risk exposure of local consumer to chemicals through fish consumption.

Residues of some chemicals (pesticides such as dichlorvos, quinalphos and trifluralin and the antibiotic chloramphenicol) were screened in the flesh of striped catfish, snakehead and climbing perch sampled from the field. Besides, dioxins were also analyzed from aquaculture related sediments to evaluate the possibility of contamination of cultured fish with this compound.

Analytical results showed that climbing perch and snakehead were contaminated with traces of chloramphenicol (0.17 and 0.19 µg/kg, respectively), while it was not detected in catfish samples whatever the culture stage (from the beginning to the end of cultured crop), whatever the scale of the culture (small or large). Dioxins were not detected in sediments from the Mekong Delta in this screening study.

Trifluralin was the only detected pesticide in fish tissue which was cultured in the intensive system. However, all investigated pesticides were not detected in market samples.

Regarding the exposure assessment study, the survey results showed that 77% of interviewees stated that they like to eat fish and the number of days of eating fish was 3.4 days per week. The average amount of fish consumption varied between 90 to 140 g per person per day. Based on the trifluralin residue concentration in fish and average of body weight of interviewed people, it was shown that the daily intake of trifluralin of interviewed people was 0.05 µg/kg body weight/day. This level of intake was much lower than the acceptable daily Intake (ADI) of 15 µg/kg/day, and corresponds to 0.33% of this ADI. However, trifluralin has not been approved in EU and all maximum residue levels were set at the limit of quantification, so residues of trifluralin in aquatic product would be a problem for exportation to Europe.

Introduction

On the one hand, aquaculture products are important protein sources and are consumed every day in Vietnam and in 2012, Vietnam was the fourth largest exporter worldwide (reviewed by Uchida et al., 2016). On the other hand, aquaculture production is an important source of environmental pollution with veterinary medicines (Pham et al., 2015) and the risk of chemical contaminations in food is a concern. Also, food completely free of chemicals cannot be produced, according to Tennant (1997). Chemicals contained in food can belong to the following groups: food additives, contaminants, residues of pesticides or veterinary drugs, natural compounds, adulterant and malicious tampering.

Pesticides can be used directly or indirectly in intensive agriculture to protect crops or stored products from pest. These pesticides can be transferred to animals in various ways. Herbicides are mostly used at pre-harvest stage, while fungicides are employed at post-harvest storage stages and insecticides are applied at both stages of production. Consequently, the compounds can be transferred to animals via the food chain (Jones and Voogt, 1999), and can result in the chronic exposure of the population to pesticide residues. Pesticides and chemical contaminants may be harmful to animals and humans, especially for the hydrophobic and persistent compounds, which bioaccumulate in individuals of high trophic level organisms in the food chain (Verhaert et al., 2013; Xu et al., 2014). Thinh et al. (2018) conducted an experiment on application of quinalphos, an insecticide with 4.4 $K_{o/w}$, on rice fish system in Mekong Delta and found that the pesticide can accumulate in fish tissue with the log(BCF) of 2 (Thinh et al. 2018). The result of this study agrees with another author, Katagi (2010), who stated that the bioconcentration will be greater the higher the $K_{o/w}$ factor will be.

For rice rearing systems of the Mekong Delta, the average number of pesticide applications on rice crops by farmers was more than 8 times, in 1999 (Berg, 2001), but after that, in 2009 to 2013, the times of chemical application in rice cultivation dropped to 2 to 3 times per crop in the Mekong Delta (Study 1) However, pesticide use on rice has shown negative impacts on fishes and shrimps in integrated culture systems (physiological effect, mortality, muscle contamination) even at low concentration or after the concentration reached undetectable levels (Tam et al., 2015; Trung et al., unpublished results; Tu et al., 2009). Moreover, a wide range of their residue was found in environment (water, soil and sediment) of Mekong Delta (Toan et al., 2013). These hazards may influence wild animals and human health through environment exposure and food consumption.

According to both a survey realized in 2009 on rice (rice culturing only and rice integrated with fish) systems (Nguyen et al., 2014) and practical situation of the aquaculture industry in Vietnam, three pesticides appeared to be largely used, and have been chosen in this study: dichlorvos, quinalphos and trifluralin. Quinalphos is an important insecticide, which is used in important crops in tropical and subtropical zones (Aizawa, 2001). It shows high efficacy on chewing, sucking, biting and

leaf-mining pests thanks to its good penetration into plant tissue and insect cuticles and acts as contact and stomach insecticide (Wisson et al., 1980). In the Mekong Delta, this compound is used to treat rice panicle mite in rice fields under the brand name Kinalux^{TM}25EC. Its use leads to a high probability of pesticide contamination in fish, especially in rice-fish production systems. Two other pesticides, trifluralin and dichlorvos, are often used in aquaculture (Tran and Do, 2011; Truong, 2012). Trifluralin, a compound belonging to the dinitroaniline group, is an herbicide. It was introduced in 1963 as a pre-emergent herbicide and was reported to be a moderate to high toxic compound to aquatic animals and insects as well as to vertebrate animals, like dogs or rabbits. This compound was removed from the positive list of pesticides of the European Union in 2009 due to its persistence in soil and groundwater (EC, 2009). Trifluralin can enter the body by absorption through the skin, by inhalation of contaminated air or from ingestion of contaminated food (Wallace, 2014). The use of trifluralin in crab and shrimp hatchery to treat larval mycosis and grow up culture was already studied in the seventies and the eighties (Armstrong et al., 1976; Williams et al., 1986). According to Ruangpan et al. (2003), trifluralin was one of the compounds that have been screened for their efficacy against pathogenic aquatic fungi to replace malachite green, a potential carcinogen. This author found that the survival rate of the fungal contaminated shrimp *Penaeus merguiensis* PL5 (PL, post larvae) exposed to low concentrations of trifluralin (0.5–1 mg/L) was significantly higher than that of the non-exposed control group, which indicated that trifluralin is able to control pathogenic fungi (Ruangpan et al., 2003). In Vietnam, trifluralin was first used for shrimp larvae to treat fungi disease, then widely used in water treatment and for killing fish parasites (Truong, 2012). Trifluralin was banned in aquaculture by Vietnamese Government in 2010 (VMARD, 2010). Dichlorvos, a very effective organophosphate pesticide, is also a contact and stomach insecticide. Dichlorvos has been used globally since 1961 to protect stored product and crops from pests; it was also used in houses, building and in hygiene sector, especially in controlling flies and mosquitos. As the compound volatilizes easily, it was also used as a fumigant agent and in greenhouse crops. In aquaculture, especially in intensive systems, dichlorvos was applied into water to control invertebrate fish parasites (Matolcsy, 1988; WHO, 1989). In Vietnam, dichlorvos was used in both agriculture and aquaculture to control pathogens; in fish culture, it was used to destroy parasites in shrimp pond preparation and to prevent external parasite in fish rearing periods (Tran and Do, 2011). Similarly to trifluralin, dichorvos was also banned in 2009 (VMARD, 2009). According to Regulation 1107/2009/EC (EC, 2009), these pesticides are not approved in the EU. The Maximum Residue Limit (MRL) of the three compounds have been set in products from vegetable origin (fruits, vegetables, tea, oils) and are ranging from 0.01 to 0.1 mg/kg. Trifluralin is the only one among those three pesticides to have also an MRL in products of animal origin (terrestrial animals) of 0.01 mg/kg (EC, 2005).

Chloramphenicol (CAM) is a broad-spectrum antibiotic acting by interfering with bacterial protein synthesis. Chloramphenicol is very toxic and its adverse effects include aplastic anemia,

thrombocytopenia, or leucopenia. Newborns and young infants are particularly susceptible to a form of cardiovascular collapse known as "gray baby syndrome" (Dasgupta, 2012). The use of chloramphenicol in animals intended for food production has been prohibited in the EU since 1994 (EC, 1994). CAM is banned by the Vietnamese Government since 2001 (VMF, 2001), but according to the survey of Tran and co-workers (2017), this compound was still used in marine fish culture in some provinces in the North of Vietnam i.e. Hai Phong, Quang Ninh and Nghe An. In addition, between 2002 and 2017, the RASFF reported the presence of chloramphenicol residues in imported aquaculture products originating from Vietnam, every year except in 2007, 2013 and 2016 (RASFF, 2018). Because of the possible presence of CAM in aquaculture products, this compound was selected to be investigated in this study.

Besides, dioxins (including furans and dioxin like PCBs) were also investigated. These persistent organic pollutants are highly lipid soluble. In the human body, dioxins are in part metabolized and eliminated, and the rest is stored in body fat, dioxins are classified as known human carcinogens, but they also cause noncancerous effects like atherosclerosis, hypertension, and diabetes. Short-term exposure to high levels impairs the liver function and causes chloracne. Long-term exposures to dioxins cause disruption of the nervous, immune, reproductive, and endocrine system (Marinković et al., 2010). Moreover, according to some authors, cumulative risk is of concern because dioxins can have similar mode of action with pesticides and both dioxins and pesticides can possibly act synergically (Boobis et al., 2008; Moretto, 2008; Reffstrup et al., 2010).

Risk assessment, which is sometime called food safety assessment in the context of chemicals in food, is a process intended to calculate or estimate the risk to a given organism, system or (sub) population (Benford, 2013). Risk assessment is a separated component of risk analysis which includes three distinct but related parts: risk assessment, risk management and risk communication (Brimer, 2011 and FAO, 2005). Risk assessment includes the hazard identification, the hazard characterization, which is the investigation of the qualitative effects of the hazard and a quantitative study of the dose–effect relationship(s), the exposure assessment to the hazard, and finally the risk characterization, which is the comparison between the exposure level and a toxicological reference value (which is very often the acceptable daily intake or ADI). Risk management takes over, transforming the ADI to recommendations or legislation concerning MRLs of, for example, pesticides in different food commodities. Risk communication covers the activities to spread the knowledge about the risk management decisions and their background.

Material and methods

Reagents

Chlorpyriphos-D_{10}, dichlorvos-D_6 and trifluralin-D_{14} were used as internal standard (IS) and purchased from Dr. Ehrenstorfer (Augsburg, Germany). Solutions including the three internal standards at the concentration of 100 µg/L were prepared in acetone. Quinalphos (99.2%), trifluralin (99.9%) and dichlorvos (98.9%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Individual stock solutions of each compound were prepared in acetone at a concentration of 1 mg/mL solvent. All solutions were kept at 4°C for up to six months. Hexane was of Picograde quality and provided by Promochem (Wesel, Germany). Ethyl acetate, chloroform, water and dichloromethane were provided by VWR International (West Chester, Pennsylvania, USA) and were of Chromanorm quality for ethyl acetate, water and chloroform while dichloromethane was of AnalaR Normapur quality. Tert-butylmethylether was purchased from Riedel-de Haën (Seelze, Germany). Hydrochloric acid, 37 %, was from Merck (Darmstadt, Germany).

Sample collection

Fish and sediment were chosen for the screening of chemical residues. Samples were collected in 2010. Fish samples included 18 samples of striped catfish coming from 3 large scale and 3 small scale intensive farms, respectively, 9 snakehead samples coming from 3 intensive farms and 9 climbing perch samples coming from 3 intensive farms as well. Each farm was visited and samples were collected at three different periods of one cultural crop corresponding to after stocking (T1), middle of fish cultivation cycle (T2) and before harvest (T3).

All fish samples were analyzed to detect the residue of pesticides but due to the limited amount of sample, only 18 samples were used for antibiotic analysis (Table 1).

Sediment samples were used for dioxins analysis. These samples included 10 samples collected from 10 catfish ponds in An Giang province and 12 samples randomly collected from rice-fish systems in Can Tho City. Sediment samples were collected as described by Lazartigues et al. (2011) : 1 kg surface sediment at the depth up to 4 cm was collected into plastic bags, samples were kept in freezer at -20°C until analysis.

Three local markets and 3 supermarkets in Can Tho City were chosen for fish sampling. For each types of market, 3 striped catfish, 3 snakehead and 3 climbing perch samples were collected.

Analytical method

All the analyses were performed by Nguyen Quoc Thinh in the framework of his PhD. The residues of pesticides were analysis in the food safety laboratory of College of Aquaculture and Fisheries, Can Tho University, Can Tho, Vietnam. Chloramphenicol residues and dioxin contamination were determined in the Laboratory of Food Analysis, FARAH – Veterinary Public Health, University of Liège, Liège, Belgium. Sample codes and performed analyses per sample are given in Table 1.

Table 1. Sample list (after stocking (T1), middle of fish cultivation cycle (T2) and before harvest (T3)) and performed analyses per sample.

			Analytical application		
			Pesticides	Chloramphenicol	
		Code		ELISA	LC-MS^n
Catfish small scale	T1	111	X		
		121	X		
		131	X		
	T2	112	X		
		122	X		
		132	X		
	T3	113	X		
		123	X		
		133	X		
Catfish large scale	T1	211	X	X	X
		221	X		
		231	X		
	T2	212	X		
		222	X		
		232	X		
	T3	213	X		
		223	X		
		233	X	X	X
Snakehead	T1	311	X	X	X
		321	X	X	X
		331	X	X	X
	T2	312	X		
		322	X	X	X
		332	X	X	X
	T3	313	X		
		323	X	X	X
		333	X	X	X
Climbing perch	T1	411	X	X	X
		421	X	X	X
		431	X	X	X
	T2	412	X	X	X
		422	X	X	X
		432	X	X	X
	T3	413	X	X	X
		423	X	X	X
		433	X	X	X

Pesticide analysis

For the analysis of pesticide residues in fish tissue (muscle including skin), 2 g of homogeneous grounded tissue were weighed into a 50 mL centrifuge tube with 2 g sodium sulfate anhydrous. Eight mL acetone:acetonitrile (1:1) were added. The tubes were then shaken for 20 minutes at 300 rpm by horizontal shaker. Extraction was repeated a 2nd time under the same conditions. Centrifugation was used to separate solvents and fish tissue after shaking. Solvents were then evaporated to dryness under vacuum. The dried residue was reconstituted with 1 mL internal standard solution in acetone at the concentration of 40 µg/L. Solution was then filtered and injected to GC-ECD.

Chloramphenicol analysis

E.G.1. Chloramphenicol 2 hours EIA kit provided by the Laboratory of Hormonology (Marloie, Belgium) was used in analyzing CAM residues in fish samples. The results were then confirmed with HPLC-MSMS according to the description of Douny et al. (2013).

Dioxin analysis

The cell based assay named "Chemically activated luciferase gene expression" (CALUX) was applied for dioxins analysis. Ten grams of dry sediment were first extracted using toluene/methanol (80/20), in an ultrasonic bath during 10 minutes. After decantation, the liquid phase was transferred in a a clean tube. The sample was extracted a second time using 30 ml of toluene. Both liquid phases were pooled and filtered on paper before evaporation until 0.5 ml, and then very gently until dryness. The dry residue was immediately dissolved in 2 ml of hexane/diethylether (97/3). The dioxins were then purified on a column containing acidified silica, using hexane/diethylether as eluent. The solvent was evaporated until 50 µl, and the extracted was transferred to 25 µl dimethyl sulfoxide before evaporating the remaining hexane. The extract was then analyzed on the CALUX cell line as already described (Scippo et al., 2008). One soil sample collected in Belgium, previously analyzed in the lab using the CALUX assay, was used as internal quality control.

Consumption study

One hundred adult people, who lived in Can Tho City (none of them was farmer), were interviewed for eating habits related to aquatic products and other sources of protein. Daily chemical intake (per kg body weight) was calculated by multiplying the residue concentration of a compound by the daily estimated amount of food intake and divided by the averaged body weight.

Results and discussion

1. Chemical residues in fish and environment

1.1. Chloramphenicol residues in fish

Results from the ELISA method showed that all analyzed samples (striped catfish, snakehead and climbing perch) were suspected to be contaminated with low amounts of chloramphenicol residues (Figure 2). The LC-MSMS confirmatory method indicated that only one sample of climbing perch and one sample of snakehead were contaminated with chloramphenicol residues at concentrations of 0.17 and 0.19 µg/kg (LOQ = 0.1 µg/kg), respectively (Figure 1).

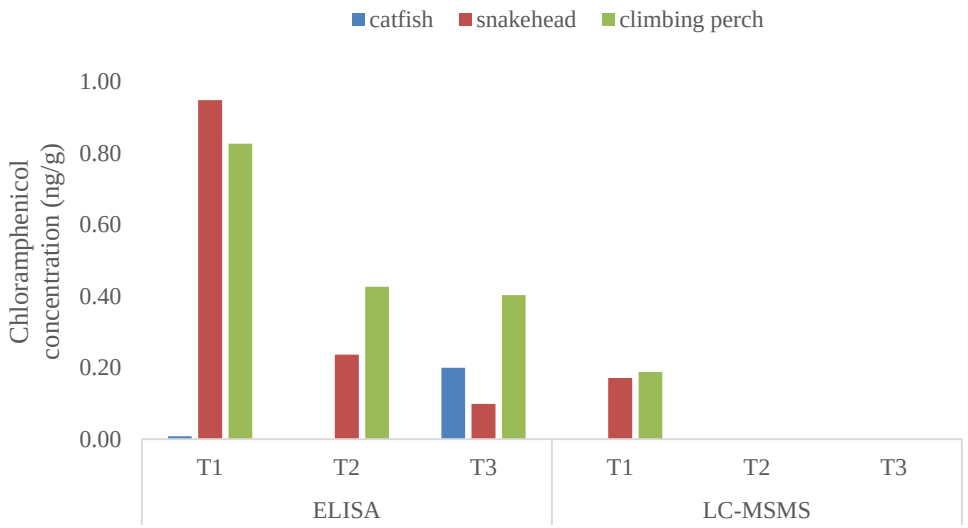

Figure 1. Residues of chloramphenicol in common cultured fish of Mekong Delta analyzed with ELISA and LC-MSMS methods (LOQ of ELISA and LC-MSMS are 0.025 and 0.1 µg/kg, respectively). Samples were collected at three different periods of one cultural crop corresponding to after stocking (T1), middle crop (T2) and before harvest (T3).

After confirmation with LC-MSMS (Figure 1), CAM was not detected from catfish samples neither from the beginning to the end of the cultured crop nor from small and large scale, indicating that positive results from the ELISA test were actually "false positive results". False positive results from CAM ELISA were already reported in the literature. For example, when screening residues of chloramphenicol in chicken meat, Yibar *et al.* (2011) found that only 2 out of 15 ELISA positive samples were confirmed after LC MS/MS analysis. The false positive may be caused by the presence of other food ingredients like in the case of breadcrumb coated prepared shrimps which showed CAM

ELISA false positives detection (Impens *et al.*, 2003). Another reason could be the presence of other phenicols such as thiamphenicol or florfenicol (Campbell et al., 1984). This could be plausible, as in Vietnam, after chloramphenicol was banned, farmers were recommended to use doxycycline or florfenicol, which is less toxic than chloramphenicol (reviewed by Sheu et al., 2013; Dung, 2014), to control bacterial diseases. However, the antibody used in the ELISA of this study cross-react very few with thiamphenicol and florfenicol (less than 1%). In contrast, the antibody is able to recognize the glucuronide metabolite of CAM (65 % of cross-reactivity), which could be the cause of the false positive, as the glucuronide metabolite was found in urine of trout with low ratio 1.8% (Cravedi et al., 1985).

If we exclude the presence of CAM glucuronide, the reason of no chloramphenicol detection in striped catfish could be the ban of this compound by the Vietnamese government combined with the fact that striped catfish is a main exported species which is strictly checked by export process companies. As a result of this regulation, after 2010, there was no notification anymore of chloramphenicol contamination in catfish imported from Vietnam (RASFF, 2018).

In contrast, in snakehead and climbing perch, residues of chloramphenicol were confirmed, at low levels. This may be due to the fact that the compound can be found in drug stores and the residue was detected from other livestocks like pig (Nguyen et al., 2016). In addition, those fish species are consumed locally and are not controlled regularly.

Beside the fact that this compound was banned by Vietnamese Government, low or null levels of CAM can be explained by the fact that its elimination in aquatic animal is quite quick. For example, the half-life of CAM reported in carp was 9.28 hours (Huang et al., 2006), and 10.04 hours for shrimp (Wang et al., 2004). Moreover, the elimination rate of CAM seems to vary according to organs. The elimination of CAM from liver, serum, gill, muscle and kidney of carp were reported to take 22.28, 15.47, 14.87, 9.28 and 5.32 hours, respectively (Huang et al., 2006). Other studies reported CAM residue concentrations of 0.7, 0.3 and 0.2 µg/ kg, respectively, in muscle of carp, chub and grass carp sampled from the same pond (Lu, 2009) and according to Bakar (2014), CAM concentration of 0.133 µg/ kg and 0.515 µg/ kg were determined in pungas fish and rui fish, respectively.

1.2. Pesticide residues in fish

According to the analytical results, only trifluralin was detected in samples collected from intensive systems in 2010 (Table 2). This result is not surprising as residues of trifluralin in frozen striped catfish fillet exported from Vietnam to Europe were found at high frequency in 2011, i.e. ten alerts by European countries through RASFF, but after 2011, no trifluralin residues have been notified through RASFF. However, according to the monitoring program for certain harmful substances done

by National agro forestry and fisheries quality assurance department of Vietnam in 2016, trifluralin was still detected with the ratio of 1/97 aquaculture samples (NAFIQAD, 2017). Regarding quinalphos, although this compound was popularly used in agriculture and could enter the aquaculture systems through water exchange, and residues of quinalphos were detected in river near rice cultivation areas (Toan et al. 2013). The absence of quinalphos residues in fish tissue can be explained by the fact that quinalphos is quickly degraded in practical situations (Pathan et al., 2012). Furthermore, the half-life of quinalphos in water in rice field system of Mekong delta was shown to be only one day and no residues were detected in water after 7 days of quinalphos application (Thinh et al., 2018). Although quinalphos has high K_{ow} and to be a bioaccumulation compound, quinalphos was not detected from samples water collected from intensive aquaculture systems (catfish ponds and red tilapia cages) (Study 2). In addition, quinalphos is not directly used in aquaculture and it is only introduced on rice and fruits. Whereas, trifluralin, an herbicide recommended to be used for preventing wild grass, was also used in aquaculture to remove fungi and external parasites (Truong, 2012). Although trifluralin was banned, the occasional use of trifluralin was shown in the North of Vietnam (Tran et al., 2017). That explains the presence of its residues found in aquaculture products in this study. Similarly to quinalphos, dichlorvos was neither detected in all samples. This compound was used to control agricultural insects and to control fish ectoparasites (Tran and Do, 2011). However, dichlorvos was banned by Vietnamese Government due to its high toxicity (VMARD, 2009), that might be the reason why there no residues of dichlorvos were found in all fish samples. In a previous study, neither trifluralin, nor dichlorvos were found in water collected from catfish ponds and red tilapia cage (Nguyen et al, 2018).

Fortunately, the analytical results showed no contamination with target pesticides in catfish, snakehead and climbing perch which were collected from the locals and supermarket in Can Tho City (18 samples/species, residues were measured by GC/ECD, LOQ of method were 8.0; 3.0 and 0.7 µg/kg for dichlorvos, quinalphos and trifluralin, respectively). Pesticide residues are depended on the areas where the survey was conducted. For instance, when taking a study on fisheries products comprised fish, bivalve, crustacean and cephalopod collected from different types of markets in Taiwan from 2001 to 2003, Sun et al. (2006) stated that there are only two kinds (organochlorine and organophosphate) of totally six pesticides (DDTs, dieldrin, chlorpyrifos, fenitrotion, fenthion and prothion) which have been detected from the fisheries products in this study.

Table 2. Residue of target pesticide (µg/kg) in samples collected from intensive systems (n=3).

Types of systems	Dichlorvos			Quinalphos			Trifluralin		
	After stocking	Mid of cycle	Going to harvest	After stocking	Mid of cycle	Going to harvest	After stocking	Mid of cycle	Going to harvest
Large scale catfish system	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ; 2.0; 4.4**	**<LOQ; 1.7; 78.6**	**10.6; 22.3; 55.0**
Small scale catfish system	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Snakehead system	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Climbing perch system	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ; <LOQ; 116.2**	<LOQ

LOQ of GC/ECD method were 8.0; 3.0 and 0.7 µg/kg for dichlorvos, quinalphos and trifluralin in fish, respectively.

1.3. Dioxins in sediments collected from the Mekong Delta

In Vietnam, dioxins are of concern as they were contaminating the Orange agent, a defoliant herbicide which was largely used by the US army during the Vietnam War between 1961 and 1971. It was estimated that an amount of 76 million liters were applied in the South of Vietnam (Lurker et al., 2014).

In this study, the sediments sampled in the Mekong Delta showed no detectable or very low levels (Table 3), compared to internal soil quality control samples, in which the level of dioxins (which were around 3 ng/kg dry weight, Table 3) was considered as a background level for industrialized countries. The low dioxin concentration could be explained by the fact that the Orange agent was not applied or was applied in very low amounts in the center area of the Mekong Delta during the war (Stellman et al., 2003). However, according to the results of Dwernychuk et al. (2002), the levels of dioxin in soil are very high in the Middle of Vietnam, especially in the area of a former military base, where the dioxin concentration in soil samples vary from more than 100 to about 900 ng/kg. The dioxin concentrations of other areas in the South of Vietnam are currently high. For example, around the airport in Tan Phong ward, Bien Hoa City, Dong Nai province, considered as a hot spot regarding dioxin contamination, dioxins levels are associated to a high risk, being up to 20 and 46 times higher than the considered safe level (Mai et al., 2007).

The analytical results of this study showed that the dioxin contamination of sediments in rice fish systems and aquaculture related systems were both lower than ISQG (Interim Sediment Quality Guideline) and PEL (Probable Effect Level) of 0.85 pg/g and 21.5 pg/g, respectively. These guidelines were established by the Canadian Council of Resource and Environment Minister (CCME) (CCME, 2002). Dwernychuk et al. (2002) stated that TCDD from contaminated soil can be transferred to cultured fish pond sediments to fish and duck tissues, then to humans. Therefore, the low concentration in sediment of collected samples means that the dioxin contamination in the sampled area is not of concern for the aquaculture products.

Table 3. Dioxin contamination in sediment samples coming from 10 catfish intensive culture ponds (CF) and 12 rice fish system (RF) in Mekong Delta (the data are expressed as 2,3,7,8 TCDD bioanalytical equivalents (BEQ)). One internal quality control of known soil (from Belgium) was analyzed in duplicate with each series of unknown samples (QC1 to QC4).

Sample	Dioxin concentration (pg BEQ/g)	Sample	Dioxin concentration (pg BEQ/g)
QC-1	2.06	QC-3	3.04
QC-2	1.99	QC-4	3.36
CF1	0.29	RF1	0.12
CF2	0.15	RF2	0.17
CF3	0.25	RF3	0.27
CF4	0.08	RF4	0.18
CF5	0.06	RF5	0.82
CF6	0.04	RF6	0.13
CF7	0.03	RF7	0.24
CF8	0.26	RF 13	0.27
CF9	0.01	RF 14	0.26
CF10	0.07	RF 15	0.22
		RF 16	0.26
		RF 17	0.20

2. Exposure assessment of the consumer to chemical residues through fresh water aquaculture products consumption

2.1. General information, knowledge and attitude of surveyed peoples about the risk linked to pesticide contamination

There were 51 men and 49 women involved in the survey. The median age was 28, the youngest being 18 and the oldest being 65 years old. The body weight of interviewed people was 55.2 ± 10 (Table 4 and appendix).

The survey showed that 74% of interviewed people know about pesticide risk and this knowledge comes from books, magazines or internet (data not shown). However, 27% of the investigated people applied no method to reduce the risk of pesticide from food consumption, the

remaining stated that they washed and cooked food carefully before consuming (data not shown). For market referring, 75% of interviewees declared that they shop in supermarkets. The local market is quite popular in Mekong Delta of Vietnam, but the origin of the products sold in this type of market is difficult to trace, contrarily to products sold in supermarkets. In addition, 82% of interviewed people declared liking to have fish in their meals (Table 4). Regarding to the source of pesticide contamination, 51% of interviewed people declared to think that the exposure to pesticides come from vegetables, while 36% said that contamination comes from meat and only 6% of people thought that consuming fish may result in exposure to pesticide residues. Moreover, 82% of interviewees stated that they like to eat fish and the number of days of eating fish was 3.4 days per week. The survey information demonstrated that fish is an important protein source for people in the Mekong Delta. This may result in health problems if fish or fish products are contaminated with chemicals.

Table 4. General information and diet habits about the surveyed population (n=100).

Item	Value			
	1	**2**	**3**	**4**
Age (1, mean ± SD)	28.8 ± 9			
Body weight (kg) (1*, mean ± SD)	55.2 ± 10			
Gender (1, % male, 2, % female)	49	51		
Aware about the risk of pesticide (1, % known about risk; 2, not known about risk)	74	26		
Aware about the risk of chemical (1, % known about risk; 2, not known about risk)	79	21		
Method applied to reduce the risk of chemical contamination (1, wash (%); 2, well cook (%); 3, wash and well cook (%); 4, no applied method (%))	30	8	35	27
Perspective on fish eating (1, like to eat (%); 2, do not like to eat (%))	82	18		

2.2 Exposure assessment to trifluralin through fish consumption

As only trifluralin residues were found in fish samples (Table 2), the exposure assessment for fish consumers was calculated for trifluralin.

About the amount of fish consumed by people living in the Mekong Delta, the survey results indicated that the average amount of fish consumption, in one meal, was 101.2 g, 140.0 g and 94.5 g per person per day for striped catfish, snakehead and climbing perch, respectively. More than 50% of consumers consumed snakehead, followed by climbing perch and catfish with 40% and 35%, respectively. The ratio of people consuming marine fish was also high (57%), while 20% of interviewed people stated that they consumed wild fish and only one percent consumed silver barb (Table 5).

Table 5: Percentage of customers consuming fish in their meals (n=100), and amount of fish consumed daily.

	Common consumed species							
	Catfish	**Snakehead**	**Climbing perch**	**Eel**	**Common carp**	**Silver barb**	**Marine fish**	**Wild fish**
Percentage of consumer % (n=100)	35	53	40	17	10	1	57	20
Mean amount* (g/person/day)	101.2	140.0	94.5	138.7	100.0	142.9	115.8	107.1
Median* (g/person/day)	71.4	71.4	71.4	142.9	71.4	142.9	71.4	71.4
Max* (g/person/day)	214.3	571.4	214.3	285.7	142.9	142.9	500.0	285.7
Min* (g/person/day)	35.7	28.6	28.6	71.4	71.4	142.9	28.6	71.4
P95* (g/person/day)	164.3	428.6	146.4	285.7	142.9	142.9	285.7	217.9

** Considering consumers only*

The trifluralin daily intake of interviewed people was calculated in two "worst case" scenarii i.e. considering the highest residue concentration of trifluralin found in striped catfish (55 µg/kg, Table 2) and both the median and the P95 daily consumption of catfish, which are respectively 71.4 g/person/day and 164.3 g/person/day. Based on an average body weight of interviewees of 55 kg (Table 4), it can be calculated that the daily intake of trifluralin of interviewed people was 0.07 µg/kg body weight/day for median consumption and 0.16 µg/kg body weight/day for the "high consumption" consumers (P95). These intakes were much lower than the acceptable daily intake (ADI) set by EFSA (15 µg/kg body weight/day) (EFSA, 2005), meaning that the daily intake of consumers was only 0.48% (median consumption) and 1.10% (P95 consumption) of the ADI. However, this result was calculated for catfish consumption only, but the consumer may intake trifluralin from other seafood like shrimp. Indeed, the presence of residue of trifluralin was the reason that caused rejection of shrimp product by Japan in 2010 (VASEP, 2010). In addition, trifluralin is used to control weeds in agriculture, causing possible residues in other crop production as the field half-life of trifluralin varies from 132 to 350 days (review by Vassilios, 2010). Unfortunately, studies about residues of trifluralin in other cultivated crops in the Mekong Delta are not available. Therefore, it is difficult to have a

precise assessment on the total exposure of consumer in the Mekong Delta. Currently, trifluralin is not approved anymore in EU (EC, 2015), so residues of trifluralin in aquatic product would be a problem in export.

Acknowledgements

The authors thank ARES-CCD (Académie de Recherche et d'Enseignement Supérieur – Commission pour la Coopération au Développement) for its financial support within the framework of the DELTAQUASAFE project and Mr. Vo Hung Vuong for helping to complete the surveys.

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Discussion

1. Pesticide use in the Mekong Delta

According to a recent report of GSO (2018), 64% of the total Vietnamese agricultural area is located in the Mekong Delta, but this region of Vietnam contributes to 55% of the total rice production and to 70% of the total aquaculture production. Besides, the Mekong Delta was known as a place where the pesticides were applied largely with low control of the public authorities (Toan et al., 2013).

In this study, a survey performed in 2009 found that farmers applied pesticides at the frequency of 3 times per crop during rice cultivation. This rate of pesticide application was similar to what was applied in 2001, according to the study of Berg (2001). However, the proportion of farmers applying chemicals based on their own experience was about 60% for farmers cultivating rice only, and 80% in operating rice fish farms. In addition, the IPM seemed to be effective not in the Mekong Delta. A survey performed in Dong Thap Province, Province located in the Mekong Delta, by the Plant Protection Department of Vietnam in 2015, showed that 84% of farmers did not apply IMP in rice cultivation, and used pesticides as the only mean to protect the crops. The lack of IPM implementation, the low level of knowledge of farmers about pesticide use and the pressure of agrochemical companies' advertisements resulted in a wasted use of pesticides. According to a report of the World Bank (cited in Tin Hong, 2017), the exceeding use of pesticides in the Mekong Delta is 7,470 tons of active ingredients per year. This exceed results in an increase of the total costs, cause negative effects on the environment and are harmful to people, especially the agricultural workers, who are easily exposed during their application (Tin Hong, 2017; Sunding and Zivin, 2000; Athukorala et al., 2012; Liu and Huang, 2013; Atreya, 2008; Soares and de Souza Porto, 2009). In rice cultivation, rice fish co-culture and aquaculture, the impact of pesticides on the environment is particularly important as spraying pesticides in such conditions increases the dispersion of the pollutants in environmental elements such as soil, air, water and the biota (Migheli, 2017). Therefore, the use of pesticide in the Mekong Delta should be improved through training about negative impacts of pesticides on environment and human health. Moreover, monitoring of pesticide use and pesticide residues should be performed periodically in the Mekong Delta, which is a main source of agricultural products in Vietnam.

2. Agrochemical residues and exposure of consumers in the Mekong Delta

Nowadays, food is globally distributed and food with chemical residues may be transported over the world. This has leaded to a stringent legislation and regulation about food quality and safety in order to protect consumers and to ensure fair trade. Despite these efforts, food safety incidents occasionally occur and originate from both microbial and chemical contamination. Pesticides and

veterinary drug residues, endocrine disruptors, food additives and packaging materials, environmental contaminants (including dioxins and heavy metals) and contaminants of natural origin (including mycotoxins and marine toxins) are of particular concern. In EU, food safety has been regulated under many regulations to deal with problems related to food safety which include pesticides, radioactive contaminations, residues of veterinary medical products, biological safety and packaging. In the same way, Vietnamese government has established many regulations, and in particular to control the use of pesticides. For example, regulations prohibiting chloramphenicol, dichlorvos and trifluralin in aquaculture were established in 2001, 2009 and 2010, respectively. However, in Vietnam, the reason to ban a chemical mostly resulted from the rules of the importation market but it was not based on its toxicity or to limit the exposure of Vietnamese farmers or consumers. Despite many regulation and inspections by national and international authorities of both aquaculture farms and aquatic based products, residues of banned chemicals still occur in Vietnam and around the world (Nielen and Marvin, 2008).

Regarding chemicals use in aquaculture, a significant source of chemicals in fisheries products is the large use of chemicals in intensive culture. Moreover, pesticides are wildly used in aquaculture and may be transferred to animals in various ways (LeDoux, 2011). These chemicals may include antibiotics and anti-parasites which can cause serious problems to humans. Beside the direct use of chemicals during aquaculture operations, the culture species may be contaminated from water used in aquaculture farms, which is directly coming from rivers.

According to this study, the water of the rice system may contain residues of pesticides in low concentration. However, even at low levels, they may have a negative impact on animals in aquatic environment. For example, low concentration of quinalphos may be very harmful to crustaceans. Indeed, according to Kegley et al. (2014c), the LC50 48h of quinalphos on *Penaus monodon* varied between 0.12 to 0.55 µg/L and the LC50 24h of quinalphos was 2.7 µg L-1 for *Peneaus indicus*.

In this study, analytical methods were developed to analyze the residues of commonly used pesticides in the Mekong Delta, using both GC-MS and GC-ECD. The GC-MS showed to be very effective in analysis but this system is quite expensive, while GC-ECD equipment is cheaper and more popular than the former. For the targeted pesticides, the effectivity of GC-ECD was comparable to GC-MS. Indeed, the validation parameters of both GC-MS and GC-ECD methods developed in this study met the requirements of the SANTE guidelines (SANTE/11945/2015, 2015) but the GC-ECD method displayed a higher LOQ than GC-MS.

In the field, after application, the pesticide is distributed to all organisms and environmental elements, in concentration depending on the properties of the pesticide and of the physiological characteristics of contacted organism. Among those, the octanol/water partition factor of the applied

chemical defines the route of absorption into the animal e.g. in fish. The routes of exposure may include gills, dermal or oral route (Schlenk, 2005).

In plants or vegetables, pesticide contamination may occur through absorption by roots or following a direct leaf pesticide application. Persistent pesticides may be transferred to other cultured animals resulting in contamination of meat, fish or milk (Holland and Sinclair, 2004).

In this study, in the case of quinalphos application in rice-fish system, the half-life of first and second quinalphos applications were more than 10 days in sediment, one to more than two days in fish, and around one day in water. This pesticide also showed bioconcentration properties with more than 2 log of BCF (fish quinalphos concentration/water quinalphos concentration). Quinalphos became undetectable in fish 2 weeks after application on rice. Many studies reviewed the metabolism of pesticides in fish (Edwards and Millburn 1985; Huckle and Millburn 1990; Schlenk 2005). Many kinds of enzymes are known to be involved in detoxifying pesticides and chemicals in fish (Schleck, 2005). However, study on metabolism of quinalphos in fish and other organism in rice-fish integrated systems should be carried out to know more about behavior of quinalphos in field system under tropical condition.

In water and sediment, the degradation of quinalphos was different from fish and depended on both biotic and abiotic degradation. The degradation of quinalphos in soil is influenced by the composition of the soil and soil pH, and the half-life of quinalphos increased from 9 to 53 days when the pH was changed from 5.1 to 8.1 (Gonçalves et al., 2006; Gupta et al., 2011). In addition, beside the biotic degradation, the persistence of quinalphos in water and sediment is governed by abiotic degradation, including water pH, concentration of suspended matter, temperature, sunlight, and content of sediment (Warren et al., 2003).

In this study, other contaminants susceptible to be found in the environment, such as dioxins, were monitored in sediments of catfish pond and rice fish fields. Residues of dioxins were not found or only at low levels in these kind of sediment samples, i.e. at lower levels than in soil samples collected in Belgium, which are considered as representative of background levels. These low levels can be explained by the fact that dioxin normally contaminate the surface of soil, but the depth of catfish pond is about 3 – 4 meters and the surface soil was removed when the pond was prepared. The other reason is that the organochlorine Orange agent herbicide (which was the cause of the large contamination of Vietnam with dioxin) was not applied or was applied in very low amounts in the center area of the MD during the war (Stellman et al., 2003). However, the concentration of dioxin was very high in the Middle of Vietnam (Dwernychuk et al., 2002). The dioxin concentrations in other areas of South of Vietnam are currently high. For example, the dioxin concentrations in sediment and soil samples in Bien Hoa, considered as a hot spot of dioxin contaminated area, were 20

to 46 times higher than the probable effect level set by the Canadian Environmental Quality Guideline which of 21.5 pg/g dry weight (Mai et al., 2007).

For antibiotics, the banned compound chloramphenicol (CAM) was chosen in this study to check if it was still used or not in the Mekong Delta. According to screening ELISA analytical results, chloramphenicol was detected in all samples of snakehead and climbing perch submitted to the analysis, but LC-MSMS analysis allowed to confirm the presence of CAM only in a limited number of samples (at stocking time). CAM is a very commonly detected antibiotic in large import markets (i.e. Canada, US, EU and Japan) (Love et al., 2011). In this study, after LC-MSMS confirmation, CAM was not detected from catfish samples neither from the beginning to the end of cultured crop nor from small and large scale farms. It may due to short elimination of CAM in fish. Indeed, the half-life of CAM in carp was 9.3 hours (Huang et al., 2006), for shrimp this value was 10.0 hours (Wang et al., 2004). The other reason may be that the farmers did not applied such antibiotic to striped catfish as this compound was on one hand, mainly exported and poorly consumed locally, and, on the other hand, banned by Vietnamese Government (VMARD, 2009a).

According to the food intake survey of this study, 77% of interviewees stated that they like to eat fish and the number of days of eating fish was 3.4 days per week. This indicated that fish is one important protein source for people in Mekong Delta of Vietnam, and this may result in health problems if fish or fish products are contaminated with chemicals. In this study, pesticides (dichlorvos, quinalphos and trifluralin) were not detected in fish samples from market or supermarket, while, the fish samples collected directly from growing farms were contaminated with trifluralin which may result in trifluralin exposure when consuming this fish. The average amount of fish consumption varied between 90 to 140 g per person per day. Based on the residue concentration and average of body weight of interviewed people, it was showed that the daily intake of trifluralin of interviewed people was 0.1 µg/kg/day. This number was much lower than the ADI set by EFSA (15 µg/kg/day) (EFSA, 2005), representing only 0.66% of this ADI.

Conclusions-Perspectives

Conclusions

Agricultural development in the Mekong Delta resulted in the increase of chemical use. In rice and rice fish systems, the farmers frequently use pesticides during cultivation. Much more active compounds were used in 2013 compared to 2009. Fortunately, all used active compounds belonged to the approved list of Vietnamese government. Farmers were aware that agrochemicals can affect their health. They claimed to avoid direct contact with agrochemicals, by wearing protection during handling. However, the farmers still decided of which types of agrochemical use mainly base on their experience.

Many different types of disinfectants and antimicrobials are used and often applied in aquaculture. In this study, all visited striped catfish farms applied drugs and chemicals during cultural operation to treat and prevent fish diseases. Enrofloxacin, sulfamethoxazole and trimethoprim were reported as the most used active substances by farmers to treat Bacillary Necrosis of *Pangasius*.

There is an urgent need to improve the farmer's knowledge and their access to advisory services on safe use of disinfectants and antimicrobials. Further, the cost-effectiveness of dietary supplement products, antimicrobials and disinfectants, is questionable and should be assessed.

It was shown that quinalphos was commonly used in rice fish system, as well as trifluralin and dichlorvos, two banned chemicals, which were also found in previous studies and in imported products. The developed method to detect these pesticides has shown to be efficient and applicable for screening and quantifying these pesticides residues. The methods can be applied in laboratories in the Mekong Delta to monitor the presence of these compounds in aquaculture products.

Regarding the distribution of quinalphos in the rice fish system in the Mekong Delta, it was shown that after its application, quinalphos is distributed to fish, water and sediment. The K_{ow} of quinalphos is 4.44 which lead to bioconcentration property of this compound and to high residue concentrations in fish muscles compared with those in water. Its bioconcentration factor (log BCF) was above 2 for the fish. The half-life of quinalphos in sediment, silver barb, common carp and water in first and second applications were 12.2 and 11.1 days; 2.5 and 1.1 days; 1.9 and 1.3 days, and 1.1 and 1.0 days, respectively.

Screening of the investigated pesticides in water from different aquaculture systems indicated that no residues of dichlorvos and trifluralin were detected. 23% (3/13) of the water samples collected from the rice field was contaminated with quinalphos at a low concentration, even if water samples were collected during periods of no pesticide application. Although none of the water samples was contaminated with trifluralin, the compound was found as a residue in the fish muscle. The estimated

daily intake of trifluralin of the interviewed people was 0.1 µg/kg/day and that number is much lower than the trifluralin ADI. However, trifluralin is not approved in EU anymore, so residues of trifluralin in aquatic products should be below the default limit of 10 mg/kg for aquatic product exportation.

For antibiotics, snakehead and climbing perch collected from culture system were contaminated with chloramphenicol (CAM) at low concentrations of 0.17 and 0.19 µg/kg, respectively. No CAM was detected from striped catfish sample neither from the beginning to the end of cultured crop nor from small and large scale. Dioxin was not detected in this screening study.

Perspectives

The study has given an overview of chemical use in aquaculture and rice fish system. The research on this situation should continue to evaluate the change in chemical use, as well as the attitude of farmers on this issue and awareness of farmer about the risk of chemicals to human health and to the environment. Surveys about chemicals use should be performed periodically with cultured farms and veterinary chemical stores to know the chemical use situation and to support information about the presence of toxic compounds in foodstuff.

The residues of pesticides in water collected from rice fish system should be of concern due to the detection of quinalphos in rice fish water systems, possibly leading to high concentration of this pesticide in the fish tissue because of its high bio-concentration potential. The use of pesticides showing properties of low bio-concentration and fast degradation is recommended. Studies on the fate of commonly used pesticides in rice fish systems should be conducted as well as the residues of pesticides used in aquaculture should be monitored in fish.

The residues of pesticides and antibiotics in fish collected from culturing system still exists and should be regularly investigated.

The GC-ECD developed method for trifluralin, dichlorvos and quinalphos detection can be used to monitor the use of these pesticides in water as well as in fish tissues, especially in small laboratories when a MS detector is to expensive and difficult to maintain.

Residues of trifluralin in cultivated crops should be studied to have sufficient data on exposure of this chemical on consumers.

Pesticide exposure assessments should be performed for all used pesticides for consumers as well as for people living in rural area. The risk assessment of chemical residue in aquaculture is still not popular in Vietnam and this should be considered by Vietnamese administrator in the future to reduce the risk to human health and improve the quality of aquaculture products.

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Appendices

Questionnaires in 2009

Appendix 1 : QUESTIONNAIRE FOR RICE SYSTEM

I. General information of farm

1. Farmer name: …… Age: ……..……

2 Address: Village:……. Commune:…….…….. District:…….……

4. Education (*1 = No education; 2 = Primary school; 3 = Secondary school; 4 = high school; 5 =Bachelor, post graduate*): .....

6. Kind of cooperation (*1 =Family ; 2 =Cooperative; 3 =Club; 5 =* Other):.....

7. Source of information for rice cultivation: (*1= experience; 2= books/magazines ; 3= television/radio; 4= training; 5= others……*): Number of year of rice cultivation

8. Other information:

Total area:.....

Owner (*1= own property; 2= hide; 3= belong to farm of government; 4= others……..*)

Rice cultivation area (ha): .....

II. Chemical use information

1. Current crop:.....

2. Crop duration: .....

3. Rice variety: .....

4. Type of fertilizer: .....

5. Fertilizer doser:.....

6. Number of application/crop: .....

7. Pesticide use (write into table)

Pesticide/ drug	Ingredient/ Active compound	Use purpose	Dose	When you use this chemical	Method of use (spray, throw…)	Is the chemical effective	Instructor	Fee/crop

8. Number of praying in a crop:.....

9. Method of spraying: .....

10. How to decide the name of chemical use (*1= experience; 2= other farmers; 3=booklet/training; 4=distributor; 5=others*): .....

11. Trend of pesticide use in future (*1= increase; 2= decrease*):.....

12. Variety cost: .....

13. Fertilizer cost:.....

14. Pesticide cost:.....

15. Labor cost:.....

16. Total income:.....

17. Net income:.....

Date:

Interviewer

Appendix 2 : QUESTIONNAIRE FOR RICE FISH SYSTEM

1. Farmer name: …… Age: ……..……

2 Address: Village:……. Commune:……..…….. District:…….……

4. Education (*1 = No education; 2 = Primary school; 3 = Secondary school; 4 = high school; 5 =Bachelor, post graduate*): .....

6. Kind of cooperation (*1 =Family ; 2 =Cooperative; 3 =Club; 5* = Other):.....

7. Source of information for rice cultivation: (*1= experience; 2= books/magazines ; 3= television /radio; 4= training; 5= others……*): Number of year of rice cultivation

8. Other information:

Total area:.....

Owner (*1= own property; 2= hide; 3= belong to farm of government; 4= others……..*)

Rice cultivation area (ha): .....

Water surface area (ha): .....

II. Chemical use information:

1. System operation: (*1= 01 rice - 01 fish ; 2 = 02 rice – 01 fish ; 3 = 03 rice – 01 fish):*.....

2. Fish stocking period (which month): .....

3. Fish species:.....

4. Portion of fish species: sp1.....%; sp2.....%; sp3.....%; sp4.....%

5. Size of fingerling (number of fish/kg):.....

6. Fish density (Id./m2): .....

7. Fish culture period: .....

7. Pesticide use (write into table)

Pesticide/ drug	Ingredient/ Active compound	Use purpose	Dose	When you use this chemical	Method of use (spray, throw…)	Is the chemical effective	Instructor	Fee/crop

8. Number of praying in a crop:.....

9. Method of spraying: .....

10. How to decide the name of chemical use (*1= experience; 2= other farmers; 3=booklet/training; 4=distributor; 5=others*):

12. Do you chose the pesticides which are less effect to the fish?

.....

13. How the fish culture affect to pest of rice (1= decrease; 2 = increase; 3=keep stable):

.....

14. How the pesticides use when operating rice fish system (1= decrease; 2 = increase; 3=keep stable):

.....

15. How effect of rice fish integrated on rice production (1= decrease; 2 = increase; 3=keep stable):

.....

16. Pesticide cost:..... :

17. Rice cultivation cost:.....

18. Fish culture cost: .....

19. Total income (rice + fish):.....

20 Total net income:.....

Date 2009

Interviewer

Appendix 3 : QUESTIONNAIRE FOR AGRICHEMICAL DISTRIBUTORS

I. General information

1. Agent name: .....Level of agent:.....

2. Address:..... Tel:.....

3. Knowledge from: Experience; Training

Intermedia ; Bachelor or higher ; Year of experience in selling ……years

4. Kind of distribution: Pesticide ; Others:.....

5. Scale of distribution: wholesale ; retail ; both wholesale and retail

6. Do you give advice/type of drug to the farmer:

Yes ; No

II. Trading chemicals

Number	Pesticide/ drug	Ingredient/ Active compound	Specification	Purpose of use	Yearly consumption

1. List of large consumption pesticides (Much to less)

.....

.....

.....

.....

.....

.....

.....

2. Status of pesticide consumption compared to previous years:

	Lower	Stable	More than
Three years ago			
Two years ago			
One year ago			
In near future			

3. How you update your knowledge/awareness in new regulation in agrochemical production/sell

- Through website of Ministry of Agriculture and Rural Development of Vietnam (or Department of

Plant protection): yes; no

- Through the documents of branch of Plant protection: yes; no

- Not update regularly:.....

- Update by other ways: .....

.....

Date 2009

Interviewer

Questionnaires in 2013

Appendix 4 : RICE CUM FISH CULTURE

1. General information

1. Name: …….....
2. Farm address: ……
3. Phone: ……
4. Do you take any training course? How many times per year? Who gave the course? Main content?

   ……
   ……
   ……
   …….

5. How many rice crops per year? .....
6. How many fish crops per year? ..... normally 1.....
7. Months of rice sowing? 1 2 3 4 5 6 7 8 9 10, 11 and 12 or not fix month.
8. Months of rice harvest? 1 2 3 4 5 6 7 8 9 10, 11 and 12 or not fix month.
9. Months of fish stocking? 1 2 3 4 5 6 7 8 9 10, 11 and 12 or not fix month.
10. Months of fish harvest? 1 2 3 4 5 6 7 8 9 10, 11 and 12 or not fix month.
11. Do you store fish before stocking into field? Yes/no. If yes
12. How many hapas in the systems to store fish before stocking into field?

    Cage1: Length……m, width……..m, depth……..

    Cage2: Length……m, width……..m, depth……..

    Cage2: Length……m, width……..m, depth……..

13. Stocking density…… fish per m^2 or m^3 in hapa, species……..
14. Stocking density…… fish per m^2 or m^3 in hapa, species……..
15. Stocking density…… fish per m^2 or m^3 in hapa, species……

    Common carp..... …….. kg,
    fish stocking size:…… fish/kg
    Silver barb……..kg
    fish stocking size: ……fish/kg
    ……..kg
    fish stocking size: ……fish/kg

16. How long for the culture period in hapa?
17. How long for the culture period in field to harvest?
18. Area of rice field? ……..ha

    Length ……m, width ……..m,

19. Surrounding channel in the rice field for fish culture? .....ha or ……..%

    Length……m, width……..m, depth ……..
20. How do you supply water to rice field? Pump or tide?
21. How many times you pump or exchange water?

    For rice:……in which month?

    For fish:……in which month?

22. Do you supply feed to fish in the initial stage? What types of feed use? Total amount? Kg
23. What is the approximate fish yield?

    Common carp..... ……..kg, fish size: ……. fish/kg

    Silver barb……..kg, fish size: …….. fish/kg

    ……..kg, fish size: ……. fish/kg
24. Do you treat water before throw out your water in the receiving water body/Mekong River?

    ☐ Yes

    ☐ No
25. List down the most common disease you deal with in the last crop?

    Rice…….

    Fish…….

2. Use of chemicals

1. Do you use chemicals (farm inputs) in rice culture?

   ☐ Yes

   ☐ No
2. When you apply fertilizer during rice crop? Describe when apply, what types, names for what and dose?

   ……

   ……

   ……

   ……

   ……
3. When do you apply pesticide during rice crop? Describe in details when apply, what types, for what and dose? **Take picture of available products and trace back the others from their memory or diary**

……
……
……
……
……
……
……
……
……..

4. Do you use any chemicals during the period for the culture of fish? ***Doses and application times/crop***?

5. Do you think that some products negatively influence :
   - 5.1 The soil quality? Yes/no/ Don't know
   - 5.2 The water quality? Yes/no/ Don't know
   - 5.3 The air quality? Yes/no/ Don't know
   - 5.4 The health (diseases, troubles, etc.) of consumers? Yes/no/ Don't know
   - 5.5 The health of producers? Yes/no/ Don't know

6. Have you ever participated in a project like DELTAQUASAFE (project trying to improve the use of chemicals)?
   - ☐ Yes
   - ☐ No

7. In your opinion, when you increase the quantity of chemicals,
   - 7.1 The **quantity** of fish will be higher. Yes/no/ Don't know
   - 7.2 The **quantity** of fish will be lower. Yes/no/ Don't know
   - 7.3 The **quality** of fish will be better. Yes/no/ Don't know
   - 7.4 The **quality** of fish will be worse. Yes/no/ Don't know

8. Do you think that a limitation (maximum amount) of use of chemicals could be beneficial for environment?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

9. Do you think that a limitation (maximum amount) of use of chemicals could be beneficial for health of consumers and producers?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know

10. Do you think there is a law/regulation to limit the use of chemicals?
    - ☐ Yes
    - ☐ No
    - ☐ Don't know
11. Do you know any kind of legislation on the use of chemical from government ? who provide it to you? ……..

3. Personal questions

12. Do you think you use too much chemicals?
    - ☐ Yes
    - ☐ No
    - ☐ Don't want to answer
13. In your <u>personal</u> opinion, is the use of chemicals a problem for your health and the quality of environment?
    - ☐ Yes
    - ☐ No
    - ☐ Don't want to answer
14. If someone provide you an opportunity to try new farming practices (via a project) to use the chemicals with environmental respect, would you be interested in the experience?
    - ☐ Yes
    - ☐ No
    - ☐ Don't want to answer
15. Do you have anything to add to this questionnaire?

4. Health aspect: circle the answer

16. Do the farmers keep records of amounts of chemicals used during operation? **Yes / No**
17. Are drugs/chemicals administered according to:
    a. safety instructions described on the package. Which ones:……..
    b. by veterinarian/technicians.
    c. extensionist.
    d. experiences.
18. Do you buy chemical and use it directly or buy it and store it to use later? ***observe the place and ask***
19. Do you separate the place of chemical storage and living/cooking place? ***Record by observe the place, do not ask them.***
20. Is the any direct contact between the skin of the workers and chemical use?

21. Is there any direct contact between the skin of the workers and the water used for treatment after chemicals apply?

22. Do farm workers use any protection during handling of pesticide and others? And which one?

……
…….

23. Do workers regularly clean their hands/take a shower after handling of chemicals or contact with water/feed containing chemicals?
24. Are workers/owner instructed to safety handling of chemicals?
25. Do you know about the banned chemical? Who showed you? Say some types if you know?

……..

26. Do some workers/owner have common signs of illness/poisons? List down here? Skin lesion, cough, vomit, pesticide poisonous

……..

Which are the main compounds causing these symptoms: ……

27. Is the water surrounding the used for any other purpose than rice cum fish?
28. Do you use the surrounding water to taking a bath, cooking, washing, drinking?

29. Is the water from the effluent recipient used by the local population?

Appendix 5 : CATFISH MONOCULTURE FARM

1. General information

1. Name:…….....
2. Farm address:……
3. Phone:……
4. Do you take any training course ? How many times per year? Who gave the course? Main content?

   ……
   ……
   ……
   …….

5. How many crops (yields) per year?.....
6. How many months per crop?.....
7. Months of stocking? 1 2 3 4 5 6 7 8 9 10, 11 and 12 or not fix month.
8. Stocking density…… fish per m^2
9. How many ponds in the systems? Total surface water: ……..ha
10. Number of grow-out ponds:……. Total area:……..
11. Number of input-water ponds: …… Total area: ……
12. Number of output-water ponds:……..Total area:……
13. Number of sediments/mud storage ponds:……Total area:…….
14. What types of feed use?
15. Feed name:…….. Avg FCR…….
16. Home-made:…….. Avg FCR…….
17. What is the approximated annual yield?.....tons/ha/crop
18. The farm is following any kind of standards/practices or certification scheme?
19. BMP, Global GAP, BAP-GAA/ACC, PAD/ASC, others/NO ……
20. How is the water supply into your farm?
    - ☐ By pumping
    - ☐ By rising water (tide)
21. Do you treat water before throw out your water in the receiving water body/Mekong River?
    - ☐ Yes
    - ☐ No
22. Where do you discharge sludge? Garden/channel/storage pond?
23. List down the most common disease you deal with in the last crop?

    …….

2. Use of chemicals

1. Do you use chemicals (farm inputs) in your culture?

☐ Yes

☐ No

2. What trademark of products do you use for the culture of fish? ***Doses and application times/crop***?

Take picture of available products and trace back the others from their memory or diary

☐ Antibiotic

☐ Disinfectant

☐ Probiotic

☐ Nutrition

3. Do you think that some products negatively influence :

5.6 The soil quality? Yes/no/ Don't know

5.7 The water quality? Yes/no/ Don't know

5.8 The air quality? Yes/no/ Don't know

5.9 The health (diseases, troubles, etc.) of consumers? Yes/no/ Don't know

5.10 The health of producers? Yes/no/ Don't know

4. Have you ever participated in a project like DELTAQUASAFE (project trying to improve the use of chemicals)?

☐ Yes

☐ No

5. In your opinion, when you increase the quantity of chemicals,

7.5 The **quantity** of fish will be higher. Yes/no/ Don't know

7.6 The **quantity** of fish will be lower. Yes/no/ Don't know

7.7 The **quality** of fish will be better. Yes/no/ Don't know

7.8 The **quality** of fish will be worse. Yes/no/ Don't know

6. Do you think that a limitation (maximum amount) of use of chemicals could be beneficial for environment?

☐ Yes

☐ No

☐ Don't know

7. Do you think that a limitation (maximum amount) of use of chemicals could be beneficial for health of consumers and producers?

☐ Yes

☐ No

☐ Don't know

8. Do you think there is a law/regulation to limit the use of chemicals?

☐ Yes

☐ No

☐ Don't know

9. Do you know any kind of legislation on the use of chemical from government ? who provide it to you?

……..

3. Personal questions

10. Do you think you use too much chemicals?

☐ Yes

☐ No

☐ Don't want to answer

11. In your personal opinion, is the use of chemicals a problem for your health and the quality of environment?

☐ Yes

☐ No

☐ Don't want to answer

12. If someone provide you an opportunity to try new farming practices (via a project) to use the chemicals with environmental respect, would you be interested in the experience?

☐ Yes

☐ No

☐ Don't want to answer

13. Do you have anything to add to this questionnaire?

4. Health aspect: circle the answer

14. Do the farmer keep records of amounts of chemicals used (including antibiotics, probiotic, pesticides, disinfectants) during operation? **Yes / No**

15. Are drugs/chemicals administered according to:

a. safety instructions described on the package. Which ones:……..

b. by veterinarian/technicians.

c. extensionist.

d. experiences.

16. Do you buy chemical and use it directly or buy it and store it to use later? ***observe the place and ask***

17. Do you separate the place of chemical storage and living/cooking place? ***Record by observe the place, do not ask them.***

18. Is the any direct contact between the skin of the workers and antibiotics, disinfectants and probiotics?
19. Is there any direct contact between the skin of the workers and the water used for treatment after chemicals apply?
20. Do farm workers use any protection during handling of antibiotics or disinfectants? And which one?

    ……
    …….
21. Do workers regularly clean their hands/take a shower after handling of chemicals or contact with water/feed containing chemicals?
22. Are workers instructed to safety handling of chemicals?
23. Do you know about the banned antibiotic? Who showed you? Say some types if you know?
24. Do some workers have common signs of illness/poisons? List down here? Skin lesion, cough,
    ……
    ……..

    Which are the main compounds causing these symptoms:…….

25. Is the water at the farm used for any other purpose than aquaculture

26. Do you use the surrounding water to taking a bath, cooking, washing, drinking?
27. Is the water from the effluent recipient used by the local population

28. Do you think the chemical use in the paddy rice field will effect to your cage culture? And how it is?

Appendix 6 : TILAPIA CAGE CULTURE

1. General information

1. Name:…….....
2. Farm address:……
3. Phone:……
4. Do you take any training course? How many times per year? Who gave the course? Main content?

   ……
   ……
   ……
   …….

5. How many crops (yields) per year?.....
6. Months of stocking? 1 2 3 4 5 6 7 8 9 10, 11 and 12 or not fix month.
7. How many hapas in the systems?

   Hapa1: Length……m, width……..m, depth,……..

   Hapa2: Length……m, width……..m, depth,……..

   Hapa3: Length……m, width……..m, depth,……..

8. Stocking density…… fish per m^2 or m^3 in hapa
9. How long for the culture period in hapa?
10. How long for the culture period in cage to harvest?
11. How many cages in the systems?

    Cage1: Length……m, width……..m, depth,……..

    Cage2: Length……m, width……..m, depth,……..

    Cage2: Length……m, width……..m, depth,……..

12. Stocking density…… fish per m^2 or m^3 in cage
13. How do you working with the sludge in the bottom of the cage? Is there sedimentation?

    Pumping to river or to garden or…….

14. What types of feed use?
15. Feed name:…….. Avg FCR…….
16. Home-made:…….. Avg FCR…….
17. What is the approximated annual yield?.....tons/ha/crop

18. The farm is following any kind of standards/practices or certification scheme?
19. BMP, GlobalGAP, BAP-GAA/ACC, PAD/ASC, others/NO……
20. How is the water supply into cage? Describe it……..***normally nothing to do because it was located in the river.***
21. Do you treat water before throw out your water in the receiving water body/Mekong River?
    - ☐ Yes
    - ☐ No
22. Where do you discharge sludge? Garden/channel/storage pond?
23. List down the most common disease you deal with in the last crop?

    …….

2. Use of chemicals

29. Do you use chemicals (farm inputs) in your culture?

☐ Yes

☐ No

30. What trademark of products do you use for the culture of fish? ***Doses and application times/crop***?

Take picture of available products and trace back the others from their memory or diary

☐ Antibiotic

☐ Disinfectant

☐ Probiotic

☐ Nutrition

31. Do you think that some products negatively influence :

5.11 The soil quality? Yes/no/ Don't know

5.12 The water quality? Yes/no/ Don't know

5.13 The air quality? Yes/no/ Don't know

5.14 The health (diseases, troubles, etc.) of consumers? Yes/no/ Don't know

5.15 The health of producers? Yes/no/ Don't know

32. Have you ever participated in a project like DELTAQUASAFE (project trying to improve the use of chemicals)?

☐ Yes

☐ No

33. In your opinion, when you increase the quantity of chemicals,

7.9 The **quantity** of fish will be higher. Yes/no/ Don't know

7.10 The **quantity** of fish will be lower. Yes/no/ Don't know

7.11 The **quality** of fish will be better. Yes/no/ Don't know

7.12 The **quality** of fish will be worse. Yes/no/ Don't know

34. Do you think that a limitation (maximum amount) of use of chemicals could be beneficial for environment?

☐ Yes

☐ No

☐ Don't know

35. Do you think that a limitation (maximum amount) of use of chemicals could be beneficial for health of consumers and producers?

☐ Yes

☐ No

☐ Don't know

36. Do you think there is a law/regulation to limit the use of chemicals?

☐ Yes

☐ No

☐ Don't know

37. Do you know any kind of legislation on the use of chemical from government? who provide it to you?

……..

3. Personal questions

38. Do you think you use too much chemicals?

☐ Yes

☐ No

☐ Don't want to answer

39. In your <u>personal</u> opinion, is the use of chemicals a problem for your health and the quality of environment?

☐ Yes

☐ No

☐ Don't want to answer

40. If someone provide you an opportunity to try new farming practices (via a project) to use the chemicals with environmental respect, would you be interested in the experience?

☐ Yes

☐ No

☐ Don't want to answer

41. Do you have anything to add to this questionnaire?

4. Health aspect: circle the answer

42. Do the farmers keep records of amounts of chemicals used (including antibiotics, probiotic, pesticides, disinfectants) during operation? **Yes / No**

43. Are drugs/chemicals administered according to:
    a. safety instructions described on the package. Which ones:……..
    b. by veterinarian/technicians.
    c. extensionist.
    d. experiences.
44. Do you buy chemical and use it directly or buy it and store it to use later? ***observe the place and ask***

45. Do you separate the place of chemical storage and living/cooking place? ***Record by observe the place, do not ask them.***

46. Is the any direct contact between the skin of the workers and antibiotics, disinfectants and probiotics?
47. Is there any direct contact between the skin of the workers and the water used for treatment after chemicals apply?
48. Do farm workers use any protection during handling of antibiotics or disinfectants? And which one?
    ……
    …….
49. Do workers regularly clean their hands/take a shower after handling of chemicals or contact with water/feed containing chemicals?
50. Are workers instructed to safety handling of chemicals?
51. Do you know about the banned antibiotic? Who showed you? Say some types if you know?
    ……..
52. Do some workers have common signs of illness/poisons? List down here? Skin lesion, cough,
    ……
    ……..

    Which are the main compounds causing these symptoms:…….

53. Is the water surrounding the cage used for any other purpose than aquaculture?
54. Do you use the surrounding water to taking a bath, cooking, washing, drinking?

55. Is the water from the effluent recipient used by the local population?

Appendix 7 : AGROCHEMICAL DISTRIBUTOR

1. Agent name: .....

2. Level of agent:.....

3. Name of owner

4. Address:

5. Kind of distribution

□ □ Aquaculture chemical
□ □ Veterinary and aquaculture chemical
□ □ other

6. Scale of distribution

□ wholesale □ retail □□ both wholesale and retail

7. Do you make any test before giving chemical?

□□ yes □□ No

8. Dose of giving chemical:

□□ Experience □ Instruction of producer

9. Trend of choosing chemical origin

□ Domestic chemical □□ Imported chemical □ Both

10. How many agrichemical companies that you are working with?

11. Type of agrochemical sell

Pesticide (4 common name)
Insecticide

	Name	Producer	active compound	proportion %
1				
2				

3

4

Fungal/bacterial disease chemicals

	Name	Producer	active compound	proportion %
1				
2				
3				
4				

Herbicide

	Name	Producer	active compound	proportion %
1				
2				
3				
4				

Aquaculture chemical (4 common types)

Antibiotic

	Name	Producer	active compound	proportion %
1				
2				
3				
4				

Environment treatment chemical

	Name	Producer	active compound	proportion %
1				
2				
3				
4				

Parasite treatment

	Name	Producer	active compound	proportion %
1				
2				
3				
4				

Mineral/vitamin

	Name	Producer	active compound	proportion %
1				
2				
3				
4				

12. Did you get any training on plant/aquatic disease?

How many times/year

Who organize the training?

13. Did you get any training on safety of agrochemical using?

□□ Yes □ No

14. Do you think agrochemical be able to residue in aquatic animal?

□□ Yes □ No

15. Do you think agrochemical can effect on:

a. Soil quality? □□ Yes □ No □ do not know
b. Water quality? □□ Yes □ No □ do not know
c. Air quality? □□ Yes □ No □ do not know
d. Health of consumer? □□ Yes □ No □ do not know
e. Your health/ worker health? □□ Yes □ No □ do not know

16. According to you, your customer come to buy agrochemical by their experience or follow your instruction (……%, ……)?

17. How many customers come your store in a day?

18. Which group of chemical is consumed more than the previous year?

For example: in this recent year, antibiotic or detergent are sold more than the other

Appendix 8 : RISK ASSESSMENT QUESTIONNAIRE

Assessment of Chemical Food Safety Related to Fresh Water Aquaculture in the Mekong Delta, Vietnam

Date: _____

General information

1. Name: _____
2. Address: _____
3. Jobs: _____
4. Household income: _____ VND
5. Health status: _____

   Body weight: _____

   Age: _____

   Gender: _____
6. Do you follow any diet according to physician?

   Yes □ No □

   If yes, please specific
7. Number of members in your family?
8. Are you and your family vegetarian?
9. Market refer

	Do not like	Like	Very like
Super Market			
Local Market			

10. Do you have any knowledge about the risk of pesticide residue in food

    Yes □ No □

    If yes, could you specific the source of information?
11. Do you think chemical residue is risk?

    Yes □ No □
12. Do you apply any method to reduce the risk?

    Yes □ No □

    If yes, please specific
13. What kind of food is the most risk of contamination of chemicals?

    Meat □ Fish □ Vegetable □ Other _____

Food uptake information for one person

14. How many days per week do you eat fish? _____ days
15. Do you like to eat fish?

    Do not like □ Like □ Very like □

16. What is the main protein source? How many kg/week? How interesting?

	kg/week	Do not like	Like	Very like
Fish				
Pork				
Beef				
Others				

17. Which kind of fish and how many kg/week?

Catfish	Snack head	Climbing perch	Eel	Common carp	Silver carp	Wild fish	Marine fish	Others

18. Where is the origin of fish?

Supermarket	Local market	Fishing (wild)	Cultured	Others

19. Other part of meal?

Vegetable	Fruit	Others

20. Fish consumption (kg) for a week

	Catfish	Sneak head	Climbing perch	Eel	Common carp	Silver barb	Wild fish	Marine fish	Others
Day 1									
Day 2									
Day 3									
Day 4									
Day 5									
Day 6									
Day 7									

21. The source of water that your family drinks?

    Tap water □ Well water □ Bottled water □

    Other_____

Appendix 9: Raw data about the fish consumption survey performed in Can Tho City, Mekong Delta

No.	Body weight (kg)	Age (years old)	Gender 1. Male 2. Female	Which kind of fish and how many (g/day) * ?							
				Catfish	Snakehead	Climbing perch	Eel	Common carp	Silver carp	Marine fish	Wild fish
1	60	22	1		71.43	71.43				142.86	
2	75	60	1	71.43	71.43					142.86	
3	57	28	1		142.86		142.86				
4	71	21	1			214.29					
5	80	31	1				71.43			35.71	
6	78	42	1	142.86		142.86				285.71	
7	56	30	1		571.43						
8	75	25	1	71.43	71.43					142.86	
9	58	24	1		71.43					71.43	
10	65	30	1	142.86	142.86		142.86			142.86	
11	51	23	2					71.43		71.43	
12	50	45	2					71.43		71.43	
13	40	22	2								
14	52	25	2	142.86	142.86						
15	46	24	2			142.86	71.43			71.43	285.71
16	60	25	1		571.43						
17	47	21	2		142.86						
18	55	22	1		428.57	142.86	285.71	142.86			
19	56	25	1		71.43		71.43			71.43	71.43
20	55	25	1	71.43	71.43	71.43				71.43	71.43
21	55	41	2							71.43	
22	58	54	2							142.86	
23	72	50	2		142.86						
24	51	65	1		71.43	71.43		71.43		71.43	
25	35	18	2		71.43	71.43		71.43			71.43
26	70	28	1	142.86	142.86	142.86	142.86		142.86		
27	54	23	1		428.57			142.86			
28	40	26	2		71.43					71.43	
29	43	26	2	71.43							
30	50	24	2	71.43		71.43	71.43			71.43	
31	74	35	1	142.86		142.86					
32	49	24	2		71.43						71.43
33	43	26	2								142.86
34	45	28	2							500.00	
35	51	40	2		214.29		214.29			214.29	
36	47	26	1								
37	44	26	2		28.57	42.86					
38	45	29	2								
39	50	24	2	71.43	71.43	71.43					
40	49	49	2		71.43					71.43	
41	57	27	1	214.29	214.29						
42	42	19	2	71.43		71.43				142.86	
43	46	25	2		71.43		71.43				
44	44	21	2		71.43	142.86					71.43
45	45	33	2	71.43							214.29
46	44	36	2	35.71	35.71	35.71				35.71	
47	55	32	1	142.86	71.43	71.43				71.43	71.43
48	48	30	2							71.43	
49	47	20	2			71.43					

** The question asked was how many kilos of fish do you eat per week. The response has been converted in daily consumption by diving the answered amount by 7 (and the daily consumption is expressed in g/day).*

No.	Body weight (kg)	Age (years old)	Gender 1. Male 2. Female	Which kind of fish and how many (g/day) * ?							
				Catfish	Snakehead	Climbing perch	Eel	Common carp	Silver carp	Marine fish	Wild fish
50	63	32	1	142.86	214.29	142.86		71.43		142.86	71.43
51	72	58	1		142.86					285.71	142.86
52	48	21	2	142.86							
53	41	24	2	71.43						71.43	
54	48	21	2							71.43	
55	50	32	2			71.43				71.43	
56	59	36	1	71.43	71.43	28.57				42.86	
57	40	26	2		71.43						
58	50	31	1		71.43	142.86					
59	54	29	2	71.43						142.86	
60	48	24	2		71.43	71.43					
61	46	21	2	71.43							214.29
62	44	27	2	71.43		71.43				142.86	
63	43	22	2		71.43	71.43	71.43			71.43	
64	52	23	2	71.43	71.43	71.43					
65	58	25	1		428.57	142.86	285.71	142.86			
66	65	30	1	142.86	142.86		142.86	0.00		142.86	
67	51	23	2					71.43		71.43	
68	56	23	1			42.86					
69	60	31	1	71.43						57.14	71.43
70	65	25	1	71.43		214.29					71.43
71	60	24	1								
72	54	24	2		71.43						
73	59	25	1							285.71	
74	69	32	1							285.71	
75	64	24	1								
76	42	20	2							214.29	
77	49	39	2							285.71	
78	75	35	1							71.43	
79	80	30	1							71.43	
80	65	20	1								142.86
81	75	21	1		71.43	71.43				71.43	71.43
82	52	25	2			28.57				28.57	
83	79	48	1		71.43	71.43				142.86	
84	51	43	2		142.86	142.86	71.43				
85	59	21	1	71.43	71.43	28.57				42.86	
86	64	22	1	71.43						142.86	
87	55	22	1	142.86	142.86		142.86			142.86	
88	57	27	1	214.29	214.29						
89	61	35	1	142.86	71.43	71.43				71.43	71.43
90	69	28	1							71.43	
91	52	27	2		71.43						71.43
92	48	23	2	71.43	71.43	142.86				71.43	
93	48	24	2							71.43	
94	50	32	1			71.43				71.43	
95	50	29	2	71.43	71.43	71.43					
96	55	26	1		428.57	142.86	285.71	142.86			
97	56	25	2		71.43		71.43			71.43	71.43
98	55	25	1	71.43	71.43	71.43				71.43	71.43
99	55	23	2							71.43	
100	58	24	1							71.43	

** The question asked was how many kilos of fish do you eat per week. The response has been converted in daily consumption by diving the answered amount by 7 (and the daily consumption is expressed in g/day).*

Appendix 10: Screening of quinalphos, trifluralin and dichlorvos residues in fresh water of aquaculture systems in Mekong Delta, Vietnam

Received: 5 July 2018 | Revised: 28 September 2018 | Accepted: 16 October 2018

DOI: 10.1111/are.13890

ORIGINAL ARTICLE

WILEY Aquaculture Research

Screening of quinalphos, trifluralin and dichlorvos residues in fresh water of aquaculture systems in Mekong Delta, Vietnam

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Funding information
Commission Universitaire pour le Développement; DELTAQUASAFE project

Abstract

To develop an easy and reliable method for detecting pesticides and their residues in the Mekong Delta, a GC-MS analytical method was developed and validated according to European guidelines (SANTE/11945/2015) for the determination of residues of three pesticides (quinalphos, trifluralin and dichlorvos) in water. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.002 and 0.007 μg/L, respectively, for quinalphos and trifluralin, and 0.016 and 0.053 μg/L, respectively, for dichlorvos and quinalphos. The repeatability, the within-laboratory reproducibility as well as the trueness met the European criteria. The recovery rate ranged between 72% (for dichlorvos and quinalphos) and 82% (for trifluralin). The developed method was then applied for the analysis of 33 water samples, collected in April 2013, at the beginning of the rainy season in the Mekong Delta in Vietnam. Thirteen samples were from rice field, 10 were collected from cat fish ponds and from red tilapia cages. Results showed that only 9% of total water samples analysed contained residues of pesticides, but only in water from rice fish systems. From the 13 samples taken in these systems, quinalphos was detected in three samples. The other two pesticides were not detected. A comparison between analytical results obtained from GC-MS and an alternative method, that is GC-ECD indicated that GC-ECD is less sensitive than GC-MS, with LOQ ranging from 0.37 to 1.18 (depending on the pesticide). However, for samples with concentrations above these LOQ, no significant difference was observed between the results obtained from the two analytical methodologies.

KEYWORDS
aquaculture, Mekong Delta, pesticides

1 | INTRODUCTION

In Vietnam Mekong Delta, besides intensive culture of shrimp and catfish, there are many other types of production systems such as integrated and alternative productions. These systems include rice and fish or rice and giant freshwater prawn (*Macrobrachium rosenbergii*) culture. According to Heong, Escalada, Huan, and Mai (1998), the rice farmers in the Mekong Delta considered that an intensive use of pesticides will result in higher rice production. This has led to a significant increase in the application of various types of pesticides. Pesticide use on rice has shown negative

impacts on fish and shrimp in integrated culture systems (physiological effect, mortality, muscle contamination) even at low or undetectable concentration (Nguyen, Berg, Nguyen, & Nguyen, 2015; Tu et al., 2009). A wide range of pesticide residues were found in environment (water, soil and sediment) of Mekong Delta (Nguyen, Sebesvari, Bläsing, Rosendahl, & Renaud, 2013). These hazards may influence wild animals and human's health through environment exposure and food consumption, especially for the hydrophobic and persistent compounds, which bioaccumulate in individuals of the high trophic level in the food chain (Verhaert et al., 2013; Xu, Guo, Zhang, & Meng, 2014). Indeed, it was shown that chemicals with high K_{ow} factor also display the high bioconcentration factors (Katagi, 2010). According to both the survey realized in 2013 (Nguyen et al., 2014) and practical situation of the aquaculture industry in Vietnam, three pesticides appeared to be largely used in rice-cum fish systems, and have been chosen in this study: dichlorvos, quinalphos and trifluralin. These pesticides are not approved in the EU (European Commission, 2009), while only dichlorvos is forbidden in Vietnam since 2009 (VMARD, 2009; 2010). However, it could continue to be used illegally and residues could be found. In order to control the residues of pesticides, analytical methods must be developed and validated. Several methods were involved in pesticide determination, such as bioassays, spectrophotometric determinations, chromatography or electrochemical techniques. Among these methods, gas chromatography has been widely used and the method is considered as the most sensitive method for pesticide investigation (Liu, Liu, & Lin, 2010). Generally, results of analytical methods can be used for many purposes: to assess whether a product complies with regulatory limits, to take decisions involving the control of the manufacturing process of a product, to take decisions about legal affairs, international trade, health problems or the environment (Boqué, Maroto, Riu, & Rius, 2002). In this paper, the method was first developed and validated for gas chromatography coupled to mass spectrometry (GC-MS), according to the SANCO guidelines (SANTE, 2015), and then it was optimized for gas chromatography coupled to electron capture detection (GC-ECD). Analytical results obtained with GC-ECD were compared with those obtained with GC-MS to evaluate the applicability of the two methods.

2 | MATERIALS AND METHODS

2.1 | Reagents and instruments

Reagents, Chlorpyrifos-D_{10}, dichlorvos-D_6 and trifluralin-D_{14} were used as internal standard (IS) and purchased from Dr. Ehrenstorfer (Augsburg, Germany). Quinalphos (99.2%), trifluralin (99.9%) and dichlorvos (98.9%) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Hexane was of picograde quality and provided by Promochem (Wesel, Germany).

Individual stock solutions of each compound were prepared in acetone at a concentration of 1 mg/ml solvent. All solutions were kept at 4°C for up to 6 months.

2.2 | Analytical instrument

For GC-MS analysis, pesticides were separated on a Focus GC gas chromatographer (Thermo Fisher Scientific) using an Equity 5 column (30 m × 0.25 mm × 0.25 µm) (Sulpelco, Bellefonte, PA, USA) and analysed with an ion trap PolarisQ mass spectrometer (Thermo Fisher Scientific). Helium gas was applied as carrier gas. The temperature program was 50°C for 1 min, followed by an increase in 20°C per min to 100°C and hold for 1 min, then 10°C per min to 250°C and hold for 1 min, then an increase in 20°C per min to 300°C and hold for 2 min; total run time was 42 min. The pesticides were detected using selected ion monitoring (SIM) mode in four segment windows. In each chromatographic run, different ions were monitored for each pesticide analysed, which allowed to perform detection and quantitative analysis (Table 1). Results were calculated using Xcalibur Software (Thermofinnigan).

The GC-ECD system was composed of a GC-2010 gas chromatographer (Shimadzu, Kyoto, Japan), an Equity 5 column (30 m × 0.25 mm × 0.25 µm) (Sulpelco, Bellefonte, PA, USA) and an electron capture detector (ECD, 63Ni, Shimadzu). The temperature program was quite similar to the GC-MS one, but the inlet was operated under split mode with the split ratio of 1:5. Nitrogen was used as carrier gas.

2.3 | Field water samples

Thirty-three water samples were collected in April 2013, at the beginning of the rainy season in the Mekong Delta in Vietnam. The geographical locations of the collection areas are presented in Figure 1. The samples were composed of 13 samples from rice field, 10 samples collected from cat fish ponds and 10 samples collected from red tilapia cages. At the collection time, most of the farm had just finished the first rice crop and preparing for the second, fish had been stored in channel or separated pond for more than 1 month due to the overlapping of fish crop and second rice crop. Samples were collected into 1 litre plastic bottle, kept in ice and brought to the College of Aquaculture and Fisheries of Can Tho University (Vietnam), and then those samples were identified and stored at −20°C until analysis by GC-MS.

TABLE 1 Mass to charge ratios and retention times for each compound analysed in GC-MS and GC-ECD

Compounds	Retention time (min)		Ion mass to charge ratio
	GC-ECD	GC-MS	
Dichlorvos	11.22	10.62	109, 185
Dichlorvos-D_6	na	10.56	115, 191
Trifluralin	15.95	15.99	264, 306
Trifluralin-D_{14}	na	15.90	267, 315
Quinalphos	22.13	20.34	146, 156, 157, 298
Chlorpyrifos-D_{10}	20.52	19.32	260, 324

Note. na = not applicable (standards not used in GC-ECD).

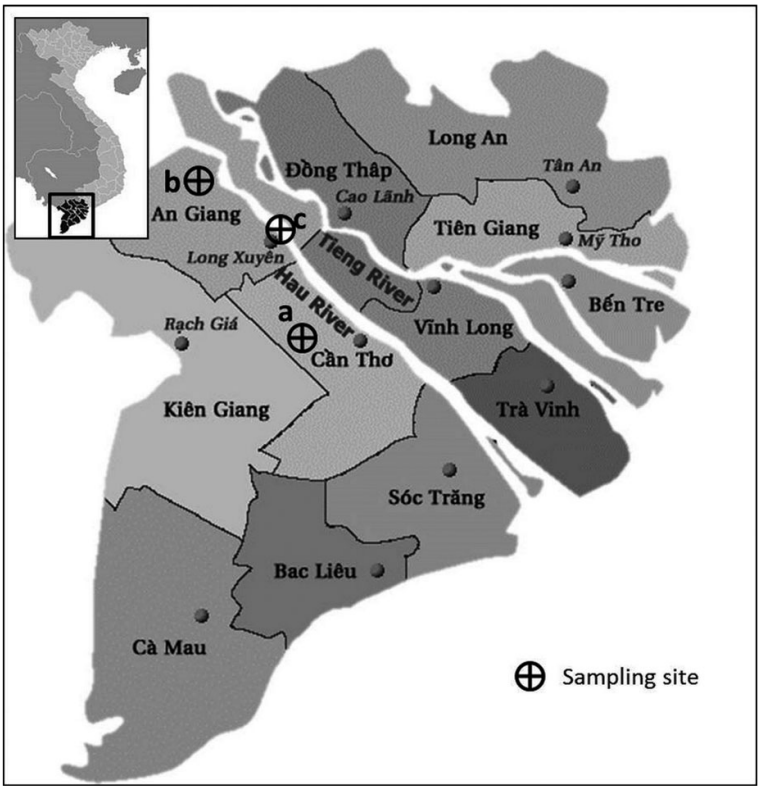

FIGURE 1 Geographical locations of the collection areas in the fresh-water region of the Mekong Delta, Vietnam. Three systems were selected, rice-fish area (a), intensive cat-fish area (b) and red tilapia cage culture (c)

2.4 | Extraction procedure

According to LeDoux (2011), the most widely used pesticide extraction technique from food of animal origin was direct solid–liquid extraction (SLE). This procedure has been applied to meat and meat products, fish, eggs etc. For extracting pesticide residues from liquid milk, liquid–liquid extraction (LLE) is still the preferred method (LeDoux, 2011). Similarly, in this study, because water samples have to be analysed, liquid–liquid extraction was selected.

2.5 | GC-MS analysis

The applied method was developed based on Nguyen (2013) and Shin and Shin (2003) works. A quantity of 25 mL sample water was poured into a 60 ml glass tube with Teflon cap. Internal standards were added at the concentration of 1.2 μg/L for trifluralin-D14 and 2.4 μg/L for dichlorvos-D6 and chlorpyrifos-D10. Note that, for quinalphos, chlorpyrifos-D10 was used as internal standard, because no commercial stable isotope-labelled quinalphos was available. The pH of water sample was adjusted to 4 with HCl 0.1 N, before extraction. Ten microlitres of ethyl-acetate:chloroform (1:1) was added into the tubes which were then shaken for 20 min at 300 *g* on a horizontal shaker. The organic layer was collected to a new tube and the water was extracted one more time. The extracts were combined and dried under nitrogen flow until the remaining volume was approximately 50 μl. The mixture was reconstituted to 300 μl with acetone. The solution was then filtrated through a 0.2 μm filter in an injection vial with an insert and injected to GC.

2.6 | GC-ECD analysis

The same extraction procedure as for GC-MS was used, using ethyl acetate:chloroform (1:1) as solvent, except that no internal standard was added before extraction and that the final extract was reconstituted to 1 ml with acetone-containing chlorpyrifos-D10 as injection standard.

2.7 | Calibration curve preparation

Matrix matched calibration curves were prepared using eight samples of 25 ml blank HPLC water spiked with internal standards and with a mixture of the three pesticides to reach final concentrations of 0, 0.06, 0.12, 0.3, 0.6, 1.2, 1.8, 2.4 μg/L for dichlorvos and quinalphos and 0, 0.03, 0.06, 0.15, 0.3, 0.6, 0.9, 1.2 μg/L for trifluralin. To evaluate the matrix effect on calibration curves, the dry residue obtained from the extraction of blank HPLC water was reconstituted with the mixture of the three pesticides, to reach the same eight corresponding concentrations. In parallel to these matrix matched calibration curves, solvent calibration curves were prepared. The test was realized in triplicate and data were plotted to assess the matrix effect.

For GC-ECD, matrix matched calibration curves were prepared using seven samples of 25 ml blank HPLC water spiked with a mixture of the three pesticides to reach final concentrations of 0, 0.2, 0.4, 2, 4, 6, 8 μg/L for dichlorvos and quinalphos and 0, 0.1, 0.2, 1, 2, 3, 4 μg/L for trifluralin. Chlorpyrifos-D10 was used as injection standard.

For both techniques, the concentration range of the calibration curves was chosen to suit the range of concentrations observed for pesticides in water samples in Vietnam (Nguyen et al., 2013).

2.8 | GC-MS and GC-ECD quantification

In GC-MS, the response (ratio between pesticides and their respective internal standard peak areas, considering the sum of all ions) was plotted against standard concentrations. A linear regression was used and no "fit weighting" was applied.

In GC-ECD, the ratio between the peak area of the analytes and the injection standard, chlorpyrifos-D10 were used as responses, and plotted against concentration.

2.9 | Validation of the GC-MS method

The GC-MS analytical method was validated according to the SANTE document (SANTE, 2015) and as described by other authors (Carneiro et al., 2013; Zainudin, Salleh, Mohamed, Yap, & Muhamad, 2015). The validation realized included the evaluation of the matrix effect on calibration, of the LOD and LOQ, the repeatability, the within laboratory reproducibility, the specificity, the recovery and the trueness. There are several approaches to calculate the LOD and LOQ, such as visual evaluation, the signal to noise approach, the procedure based on the standard deviation of the response and the slope of a calibration curve (ICH, 2005). In this study, for both GC-MS and GC-ECD methods, we used the latter where the limit of detection (or quantification) was calculated as 3.3 (or 10) times the standard deviation of the response divided by the slope of the calibration curve. According to the ICH guidelines (ICH, 2005), the standard deviation of the response was determined from the responses of five blank samples where the area of the chromatographic peak was integrated at the retention time corresponding to the expected compound.

Repeatability is the relative standard deviation of repeated measurements of an analyte using the same sample with the same method in a single laboratory over a short period of time, with no difference in instrument and materials. Within laboratory, reproducibility is similar to repeatability, but obtained from different periods and analysts. Specificity is the ability of detecting an analyte from the background. Trueness is defined as the closeness of agreement between the average value obtained from a series of test results and a true value or accepted reference.

High-performance liquid chromatography (HPLC) water fortified with standards of pesticides was used to assess the performance of the method. After determination of LOD and LOQ, 10 samples of blank HPLC water were fortified with pesticides at two different levels inside the calibration curve range to assess repeatability and reproducibility: five samples were fortified with the compounds at a concentration of 0.6, 0.3 and 0.045 μg/L for dichlorvos, quinalphos and trifluralin, respectively, and five samples were fortified with the compounds at a concentration of 1.8, 0.9 and 0.135 μg/L for dichlorvos, quinalphos and trifluralin respectively. The fortified samples and a calibration curve were analysed in parallel and all procedures were repeated in two different days. Relative standard deviation and trueness were calculated based on the results obtained from the two fortified levels. Recovery rates of the target analytes were measured by the analysis of spiked HPLC water as well as water extracts spiked after the extraction step with two levels of pesticides at the concentration of 0.6 and 1.8, 0.3 and 0.9, 0.045 and 0.135 μg/L for dichlorvos, quinalphos and trifluralin respectively.

2.10 | Comparison of both GC-MS and GC-ECD analytical methods

To compare the effectiveness of GC-MS and GC-ECD for pesticides analysis, water samples from an experiment containing only quinalphos were analysed. Water samples were obtained from an aquarium experiment realized at the College of Aquaculture and Fisheries of Can Tho University (Vietnam) in 2012, which investigated the effect of quinalphos on physiological parameters of silver barb fish (*Barbonymus gonionotus* Bleeker, 1849). Experiment included four treatments, which were control, 86, 172 and 430 μg/L quinalphos corresponding to 10%, 20% and 50% of the 96 hr lethal concentration (LC_{50}-96 hrs). This experiment was set to assess the changes in cholinesterase activity of silver barb fish. Samples were analysed with GC-ECD as triplicates and used to establish the kinetic of elimination of quinalphos in water. Among those samples, some were collected to be analysed by GC-MS to compare the results with those obtained by GC-ECD. The samples analysed by GC-MS corresponded to collection time of 5 min, 1 day and 28 days after application of 430 μg/L quinalphos and 28 days after the application of quinalphos at 172 μg/L.

2.11 | Statistical analysis

Statistical analysis was made with the SPSS software, version 18.0. Independent sample *t* test was applied to compare the means of two data groups; whereas, one way ANOVA with Duncan test was applied to evaluate the difference between mean values of data from more than two groups. Significant differences were determined at $p < 0.05$ and 0.1.

3 | RESULTS AND DISCUSSION

3.1 | Method development and validation

The analytical parameters such as retention times and mass to charge ratios of the compounds analysed in GC-MS and GC-ECD are presented in Table 1.

3.2 | Calibration curves

The matrix matched and solvent calibration curves showed that only matrix (water) slightly affected the slope and the intercept of the calibration curves (data not shown). Indeed, there was no significant difference between the curves parameters due to very few co-extraction compounds. Compared with other fat containing matrices like milk, fish or cocoa, which may contain a large amount of fatty acids, alkaloids, esters or tocopherols (Zainudin et al., 2015), water sample is a simpler and cleaner matrix. Even if the impact of the matrix on the calibration curve was low, matrix matched calibration was used for quantification in this study. In the current method, the range of linearity of dichlorvos and quinalphos calibration curve was between 0.06 and 2.4 μg/L (with R^2 = 0.967 for dichlorvos and R^2 = 0.991 for quinalphos) and between 0.03 and 1.2 μg/L in the case of trifluralin (R^2 = 0.996).

3.3 | LOD and LOQ determination

Limit of detection values were of 0.016, 0.002, 0.002 μg/L for GC-MS and 0.35, 0.36, 0.11 μg/L for GC-ECD for dichlorvos, quinalphos and trifluralin respectively. Limit of quantification values were of 0.053, 0.007, 0.007 μg/L for GC-MS (Table 2) and 1.15, 1.18, 0.37 μg/L for GC-ECD for dichlorvos, quinalphos and trifluralin respectively.

3.4 | Selectivity and specificity

The absence of significant peaks was shown in the blanks and the presence of quantifiable peaks was seen in the fortified samples in both GC-MS (Figure 2) and GC-ECD (Figure 3). When a peak was detected in the blanks, it was shown that the relative retention times and/or the transition ratios (ratio between the peak area corresponding to the first transition and that of the second transition for a compound) did not correspond to those of the three pesticides analysed here. For the fortified samples, it was also shown that the variations of relative retention times (RRTs) and of transition ratios corresponded to that of the calibration standard with a tolerance of ±0.5% for the RRTs, and ±30% for the relative of ion ratio (SANTE, 2015).

3.5 | Trueness, repeatability, recovery rate

Results of trueness, repeatability and recovery rate of the GC-MS method are presented in Table 2. The coefficients of variation on the same day (repeatability) and on different days (reproducibility) were both lower than 20% at the two fortified levels, which means that the developed method is repeatable and reproducible according to the SANTE guidelines (2015). The trueness was also assessed with water samples fortified at two different levels and the calculated concentrations were compared with theoretical concentrations. Observed trueness ranged from 85.3% to 101.0% and was satisfying the criteria of SANTE document which fixed trueness between 70% and 120% (SANTE, 2015). The recovery rates of the GC-MS method for the three target pesticides ranged between 72% and 82% (Table 2).

In GC-ECD, a short validation was performed to assess the performances of the developed method. The linearity of dichlorvos, quinalphos and trifluralin matrix matched calibration curves ranged from 0 to 8 μg/L (dichlorvos and quinalphos) and 0 to 4 μg/L (trifluralin) with R^2 values of 0.991, 0.995 and 0.994 respectively.

3.6 | Analysis of field water samples

From the 33 water samples collected, from which 13 samples were from rice field, 10 were from cat fish ponds and 10 from red tilapia cages, only 9% contained residues of pesticides, but contaminated samples only came from rice fish farms. No pesticides residues were detectable in cat fish ponds and water from red tilapia cages. From the 13 samples taken in rice fish systems, quinalphos was detected in three samples with the concentration of 0.11, 0.08 and 0.04 μg/L. The other pesticides were not detected in any sample. The absence of dichlorvos and trifluralin can be explained by the fact that the use of dichlorvos is totally banned in Vietnam since 2009 while trifluralin has been banned in aquaculture only (VMARD, 2009; 2010).These two toxic compounds were completely or partially banned for safety reasons. In particular for trifluralin, the ban in aquaculture resulted from the rejection of shrimps exported from Vietnam to Japan because of their trifluralin content (VASEP, 2010). These two pesticides were however kept in our study in order to check that the famers follow the ban, as it could be expected that banned chemicals are still used by farmers. For instance, even if banned since 2010, the presence of trifluralin in pangasius fillet imported from Vietnam was detected in 2011 (RASFF, 2011). According to our limited sampling in fresh water aquaculture system of the Mekong Delta, the Vietnamese farmers seem to no longer use the banned dichlorvos and trifluralin chemicals in aquaculture. This has to be confirmed in a larger scale study.

TABLE 2 Validation parameters of the GC-MS method for the quantification of three pesticides in water samples. The two values for repeatability day 1 and day 2, within laboratory reproducibility, trueness and recovery rate are respective to the two mean introduced concentration values, which were 0.6 and 1.8, 0.3 and 0.9, 0.045 and 0.135 μg/L for dichlorvos, quinalphos and trifluralin respectively (n = 5)

	LOD (μg/L)	LOQ (μg/L)	Repeatability RSD (%) day 1	Repeatability RSD (%) day 2	Within laboratory reproducibility Overall RSD (%)	Trueness (%)	Recovery rate (%)
Dichlorvos	0.016	0.053	11.7–10.5	7.2–5.2	8.7–13.0	98.5–100.4	72–76
Quinalphos	0.002	0.007	2.3–2.5	5.5–3.3	6.4–5.6	101.0–101.0	82–81
Trifluralin	0.002	0.007	6.9–5.0	1.8–2.9	4.7–5.2	85.3–90.4	72–72

Note. RSD = relative standard deviation

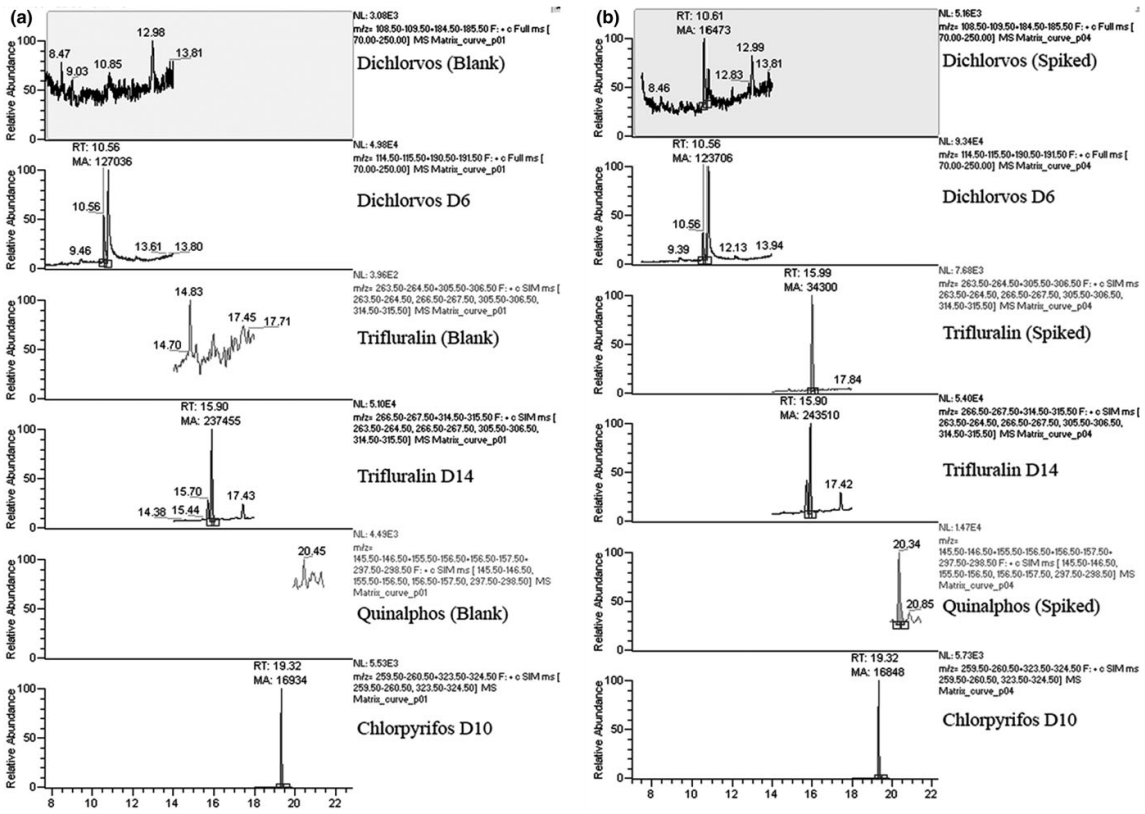

FIGURE 2 Chromatogram (GC-MS) resulting from the analysis of HPLC water taken as a blank sample (a) and the analysis of the same water sample spiked with the three target pesticides at a concentration of 0.3 μg/L for dichlorvos and quinalphos and 0.15 μg/L for trifluralin (b)

Quinalphos, however, is still allowed to be used in agriculture (VMARD, 2015). In the field, farmers usually apply quinalphos one to two times per crop to prevent pest. Normally, quinalphos is applied 65 days after sowing to prevent rice panicle mite (Vien, Loi, & Dinh, 2012). In the area where samples were collected, the rice was sowed in December 2012 and raised for 90 to 100 days, so, the duration between quinalphos application and our water sampling time was estimated to be 1–1.5 months. That can explain why this compound was detected in 23% of the samples of water from the rice fish system (three out of 13 samples), at low levels. The low levels could be explained by the degradation of quinalphos. Gupta, Rani, Kumar, and Dureja (2011) showed that the rate of degradation of quinalphos is increased with the increasing of temperature, pH level, and the concentration of humic acid. The same authors showed that the half-life of quinalphos ranges from 40 to 27 days, at 30°C, at pH 6–8, in laboratory condition (Gupta et al., 2011). In field conditions, the degradation seems to be faster, as the half-life of quinalphos in soil (okra field at West Bengal, India) was shown to be only 1.07–1.2 days (Aktar, Sengupta, & Chowdhury, 2008).

Even if low levels of quinalphos were found in water, since quinalphos has a high partition coefficient octanol/water (4.4) (PPDB, 2014), its concentration may be very high in aquatic animals due to bioaccumulation through skin, gill or intestine tract (Xu et al., 2014). Moreover, that low concentration may be very harmful to crustacean, due to the very low lethal concentration of this compound for species belonging to this group of animals. According to Kegley, Hill, Orme, and Choi (2016), the LC_{50} 48 h of quinalphos on *Peneaus monodon* ranged between 0.12 and 0.55 μg/L and the LC_{50} 24 h of quinalphos was 2.7 μg/L for *Peneaus indicus*. This shows possible negative consequences on both cultured shrimp and wild shrimp, if quinalphos residues from the water of such rice fish system is released in shrimp cultured system or in the environment in general.

3.7 | Comparison of GC-MS and GC-ECD analytical methods

For comparing the effectiveness of GC-MS and GC-ECD for the analysis of quinalphos, water samples from an aquarium experiment realized in Vietnam and concerning the physiological parameters of

silver barb fish exposed to quinalphos were used. This experiment included four different concentrations of quinalphos in water and the samples were analysed as triplicates with GC-ECD in Vietnam. Some of the samples were also analysed with GC-MS to establish a comparison of the two developed methods. The results obtained from the analysis of the samples with the two instrumental systems are presented in Table 3.

After 28 days of application of 172 μg/L of quinalphos in the aquarium, the pesticide concentrations measured were low. Indeed, the values were of 0.3 ± 0.01 μg/L with the GC-MS method and of 1.1 ± 0.5 with the GC-ECD method. After the application of 430 μg/L of quinalphos, it can be observed that pesticide levels were decreasing quickly in the water of the aquarium and detected at very low levels. After 5 min of pesticide application at that concentration, the levels in the water were of 295.9 ± 46.3 and 254.5 ± 31.0 μg/L measured with the GC-MS and the GC-ECD method, respectively, while, after 1 day, the levels were of 93.2 ± 29.1 and 76 μg/L measured with the GC-MS and the GC-ECD method respectively. Twenty-eight days after the application of quinalphos at 430 μg/L, the concentration of quinalphos decreased to 1.53 ± 0.04 and 1.7 ± 0.2 μg/L, measured by GC-MS and GC-ECD respectively, corresponding to a level close to the one measured 28 days after the application of 172 μg/L of quinalphos.

Where applicable, independent samples *t* test was applied to assess the difference between the two methods used to analyse the water samples. As shown in Table 3, statistical results demonstrated that the difference between methods was not statistically significant ($p > 0.05$), which implies that the two developed methods gave similar results and can be both used to analyse quinalphos in water samples, if residue levels are above the LOQ of the GC-ECD method. However, as mentioned above, the LOQ of the GC-ECD method is much higher than the one of the GC-MS method (LOQ and LOQ determination section), hence the GC-MS method will be suitable to detect trace contamination of pesticides in water.

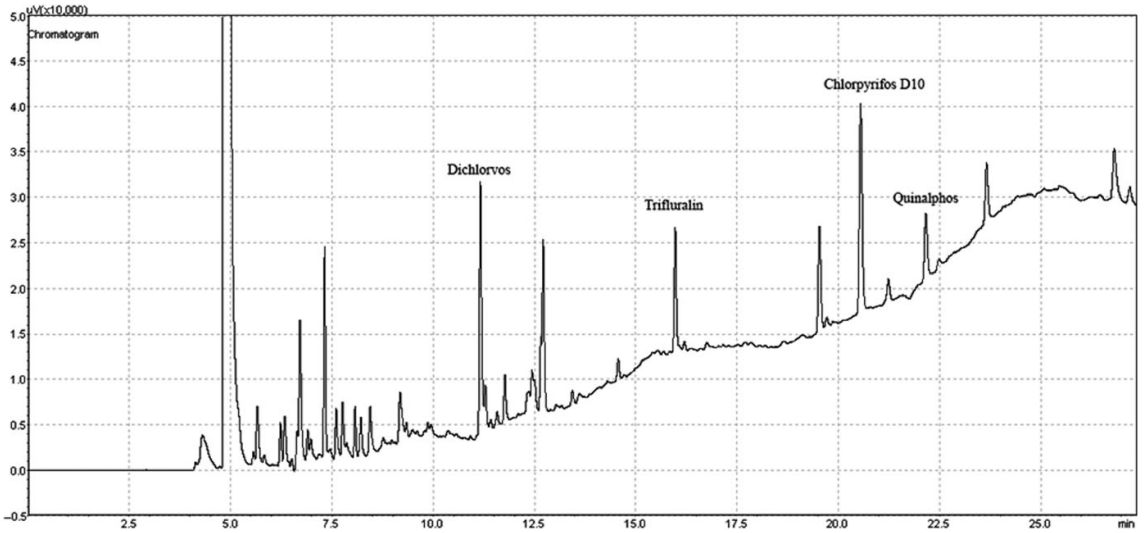

FIGURE 3 Chromatogram (GC-ECD) resulting from the analysis of HPLC water sample spiked with the three target pesticides at a concentration of 0.3 μg/L for dichlorvos and quinalphos and 0.15 μg/L for trifluralin

TABLE 3 GC-MS and GC-ECD analytical results obtained with aquarium water samples containing quinalphos

Concentration of quinalphos applied (μg/L)	Collection time after application	Result obtained with GC-MS (μg/L)	Result obtained with GC-ECD (μg/L)	Sig. (2-tailed)
172	28 days	0.3 ± 0.01	1.1 ± 0.5	0.106
430	5 min	295.9 ± 46.3	254.5 ± 31.0	0.358
430	1 day	93.2 ± 29.1	76	Not applied
430	28 days	1.53 ± 0.04	1.7 ± 0.2	0.119

Note. Samples were analysed as triplicates, except for collection after 1 day after application of 430 μg/L where only one sample was analysed in GC-ECD.

Sig.: Significant level which indicated no significant difference if the number is greater than test level (0.05).

4 | CONCLUSION

The validation parameters of the GC-MS and GC-ECD methods developed in this study met the requirements of the SANTE guidelines (SANTE, 2015) but the GC-ECD method displays higher LOQ than GC-MS.

No dichlorvos (banned in Vietnam since 2009) trifluralin or quinalphos residues were found in cat fish ponds or water collected from red tilapia cages. In water samples from the rice fish system, dichlorvos and trifluralin were not detected while quinalphos was detected in 23% of samples. This shows that residues of quinalphos, a bioaccumulative pesticide, could be of concern in fish or prawn produced in rice integrated systems.

ACKNOWLEDGMENTS

The authors thank CUD (Commission Universitaire pour le Développement) for its financial support (DELTAQUASAFE project). The authors also acknowledge all the staffs of Laboratory of Food Analysis, FARAH - Veterinary Public Health of the University of Liège as well as the staffs of the Department of Fish Nutrition and Aquatic Food Processing, CAF, Can Tho University for labours, knowledge and facilities supporting.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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How to cite this article: Nguyen QT, Douny C, Tran MP, et al. Screening of quinalphos, trifluralin and dichlorvos residues in fresh water of aquaculture systems in Mekong Delta, Vietnam. *Aquac Res*. 2018;00:1–9. https://doi.org/10.1111/are.13890

Appendix 11: Bioconcentration and half-life of quinalphos pesticide in rice-fish integration system in the Mekong Delta, Vietnam

JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART B
2018, VOL. 53, NO. 1, 35–41
https://doi.org/10.1080/03601234.2017.1371551

Bioconcentration and half-life of quinalphos pesticide in rice-fish integration system in the Mekong Delta, Vietnam

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ABSTRACT

In order to determine the distribution and enable the elimination of quinalphos, a popular active pesticide compound used in the Mekong Delta, an experiment was set up in a rice-fish integration system in Can Tho City, Vietnam. Fish was stocked into the field when the rice was two-months old. Quinalphos was applied twice in doses of 42.5 g per 1000 m^2. Water, fish and sediment samples were collected at time intervals and analyzed by a Gas Chromatography Electron Capture Detector system. The results show that quinalphos residues in fish muscles were much higher than those of the water and the bioconcentration factor (logBCF) was above 2 for the fish. The half-life of first and second quinalphos applications were 12.2 and 11.1 days for sediment, 2.5 and 1.1 days for silver barb, 1.9 and 1.3 days for common carp, and 1.1 and 1.0 days for water, respectively.

ARTICLE HISTORY
Received 5 April 2017
Accepted 20 August 2017

KEYWORDS
Mekong delta; aquaculture; rice-fish integration system; pesticide; quinalphos; bioconcentration

Introduction

The Mekong Delta (MD) is the most intensive agricultural area in Vietnam. In the MD, there are several aquaculture systems, which include mono-and polyculture at various scales. Rice–fish integration systems are quite popular in other South and Southeast Asian countries.[1] In Vietnam, polyculture is usually applied in rice–fish integration systems;[1] the stocking species generally consist of silver barb (*Barbonymus gonionotus* Bleeker, 1849), common carp (*Cyprinus carpio* Linnaeus, 1758), and Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). The rice–fish integration system in the MD usually consists of two rice crops and one fish crop per year. The first rice crop is the main one, lasting from December to March without fish stocking. During the second rice crop, which is cultured from April to July, fish are normally stocked into the system after rice has grown for between one and two months. Fish are harvested between September and October and mostly fed by natural feed after the rice crop ends in July.

Quinalphos is a popular insecticide used to prevent rice panicle mites (*Steneotarsonemus spinki*) in rice fields and is sold under the brand name *Kinalux 25EC*.[2] Quinalphos is an insecticide belonging to the organophosphorus group, sub-classified into the group of heteroaryl phosphorothioates because of its aromatic rings.[3] Physiological effects of quinalphos alone were studied in many animal species (e.g., fish,[4–7] birds,[8] and mammals[9,10]). The joint effects of quinalphos and other pesticides were also investigated in fish.[11] In humans, quinalphos can be metabolized and excreted through urine under the form of diethyl phosphate and diethyl phosphorothioate. Regarding to its toxicity, quinalphos can lower the cholinesterase concentration in serum and red blood cells of humans, and it takes more than 30 days to recover to normal concentration.[12]

Residues and dissipation of quinalphos were studied a long time ago in cauliflower (Chawla, Dhaliwal,[13] which indicated that 95% of this chemical degraded within eight days. Other studies regarding the elimination of quinalphos in okra fruit,[14] tomato fruit and radishes,[15] Kinnow Mandarin fruit,[16] cabbage and brinjal[17, 18] were also conducted. However, limited information is available on the elimination of quinalphos in rice fields, which could represent a risk of quinalphos contamination in fish cultured in rice–fish integration systems. The main objective of this study was to investigate the bioconcentration and half-life of quinalphos in water, sediments and fish in an on-farm rice–fish integration system.

Material and methods

Reagents and instruments

Chlorpyriphos-D_{10} was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Quinalphos standard (99.2%) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Kinalux 25EC, which contains 250 g L^{-1} of quinalphos, was purchased from United Phosphorus Ltd. (Worli, Bombay, India). The concentration of active ingredient quinalphos in Kinalux 25EC was confirmed by gas chromatography electron capture detector (GC-ECD) before use in this experiment.

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Analytical instrument

The GC-ECD system was composed of a GC-2010 gas chromatographer (Shimadzu, Kyoto, Japan), an Equity 5 column (30 m × 0.25 mm × 0.25 μm) (Sulpelco, Bellefonte, PA, USA) and an electron capture detector (ECD, 63Ni, Shimadzu).

Field experiment and sample collection

Healthy fingerling silver barb (*Barbonymus gonionotus* Bleeker, 1849) and common carp (*Cyprinus carpio* Linnaeus, 1758) were purchased from a local hatchery (Can Tho, Vietnam).

The experiment was conducted in the Co Do District of Can Tho City, Vietnam. The experiment was triplicated and the experimental area was divided into three identical sections of 1000 m^2 each, completely separated from the others and from outside areas by plastic barriers. The experiment was set up in the period from May to September 2013, corresponding to the second annual rice crop. Common carp (8.0 ± 1.5 g) and silver barb (5.0 ± 0.9 g) fingerlings were stocked at a density of three and two fish per m^2, respectively. Fish were stocked after the rice was cultured for 50 days before first chemical application. Kinalux 25EC was applied over the rice at a dosage of 170 mL 1000 m^{-2}, corresponding to 42.5 g of quinalphos per 1000 m^2, as recommended by the producer. The pesticide was applied twice when the rice was 54 and 79 days, respectively. The trench water levels in the experimental field were adjusted following the normal farming practice, by 1.4 m for the first and 1.2 m for the second Kinalux 25EC application.

Water, fish and sediment samples were collected one day before quinalphos application and then after 30 minutes, 1 day, 3 days, 7 days, and 14 days of the first and second quinalphos applications. After 14 days of the second application, samples were collected every two weeks. At the sampling time of 30 minutes after the first and second applications of quinalphos, only water samples were collected. The analyses were processed until two consecutive samples fell below the detection limit.

Fish samples were collected by cast-net, the scales were removed and the muscle (with skin) from ten fish was homogenized and stored at −20 °C until analysis. Water and sediment were collected following the method described by Lazartigues, Fratta.[19] Water samples were collected at a depth of 10–15 cm from the surface, and sediment was collected on a depth of up to 4 cm. All samples were kept at −20 °C and thawed before analyzing. Temperature, pH and dissolved oxygen were recorded monthly. During the experiment, temperature, pH and dissolved oxygen were 30.8 ± 0.9 °C, 7.3 ± 0.5 and 3.0 ± 0.5 mg L^{-1} ($n = 4$), respectively.

Extraction procedure

For water samples, to remove suspended matter, the sample was first centrifuged at 2500 g for 5 minutes, and then 30 mL sample was poured into another 50 mL centrifuge tube. The pH of the water sample was adjusted to 4 with 0.1 N HCl before extraction. Ten mL *n*-hexane were added to the tube and then shaken for 20 minutes at 300 rpm on a horizontal shaker. The organic layer was collected into a new tube and the water was extracted one more time. The extracts were combined and evaporated to dryness under vacuum. The dried residue was reconstituted to 1 mL with internal standard (Chlorpyrifos D10) solution in acetone at the concentration of 40 μg L^{-1}. The solution was then filtered through a 0.2 μm filter in an injection vial with an insert and 2 μL were injected into the GC-ECD.

For fish muscle, homogeneous grounded muscle (2 g) was weighed into a 50 mL centrifuge tube containing anhydrous sodium sulfate (2 g). Eight milliliter acetone: acetonitrile (1:1) was added. The tubes were then shaken for 20 minutes at 300 rpm by horizontal shaker. Supernatant was collected into new tube after centrifuge at 2500 g for 5 minutes. Extraction was repeated and supernatants were combined, evaporated, and reconstituted similar to the steps in the water extraction method.

For sediment samples, the method described by Tse[20] was applied after modifications. Wet sediment (5 g; 62 ± 3% of dry matter) was weighed into a 50 mL conical flask. Ten milliliter *n*-hexane: acetone (9:1, v:v) was added to the flask, which was then shaken at 125 rpm overnight. Anhydrous sodium sulfate (2 g) were added to trap water; the samples were then filtered through paper filter and washed with 2 mL hexane: acetone (9:1, v:v). Solvents were then processed via steps similar to the water extraction method.

GC-ECD analysis

The temperature program of GC was first 50 °C for 1 min, followed by an increase of 20 °C per min to 100 °C and holding for 1 min, then 10 °C per min to 250 °C and holding for 1 min, then an increase of 20 °C per min to 300 °C and holding for 2 min. Injection volume was 2 μL. Retention times of the quinalphos and chlorpyriphos D10 (IS) were 22.1 and 20.5 min, respectively.

Quinalphos quantification was done using matrix matched calibration curves. The linearity of quinalphos matrix matched calibration curves of water, fish and sediment ranged from 0 to 8 ng/mL, 0 to 200 ng/g and 0 to 80 ng/g with *R*-square values of 0.992–0.997, 0.990–0.998 and 0.996–0.999, respectively. The recoveries of quinalphos analysis in water, fish and sediment samples were 87.2–92.7%, 75.7–78.5% and 57.2–59.9%, respectively, limit of detection (LOD) were 0.4 ng/mL, 7.5 ng/g and 0.5 ng/g, respectively and limit of quantification (LOQ) were 1.2 ng/mL, 22.7 ng/g and 1.6 ng/g, respectively. Examples of GC-ECD chromatograms of blank and contaminated water, sediment and fish samples are shown in Fig. 1.

Calculation of quinalphos half-life

The half-life of quinalphos was calculated in water, sediment and fish muscle, according to Lazartigues,[21] based on the first order decay curve: ln (concentration) $= a + bt$, where t is the time (day), a is a constant, and b is the depuration rate or K_d (day^{-1}). The half-life was calculated as $t_{1/2} = \ln(2)/K_d$. The bioconcentration factor (BCF) in fish muscle was calculated according to Katagi:[22] $BCF = C_{pb}/C_{pw}$, where C_{pb} is the chemical concentration in the organism and C_{pw} is the chemical

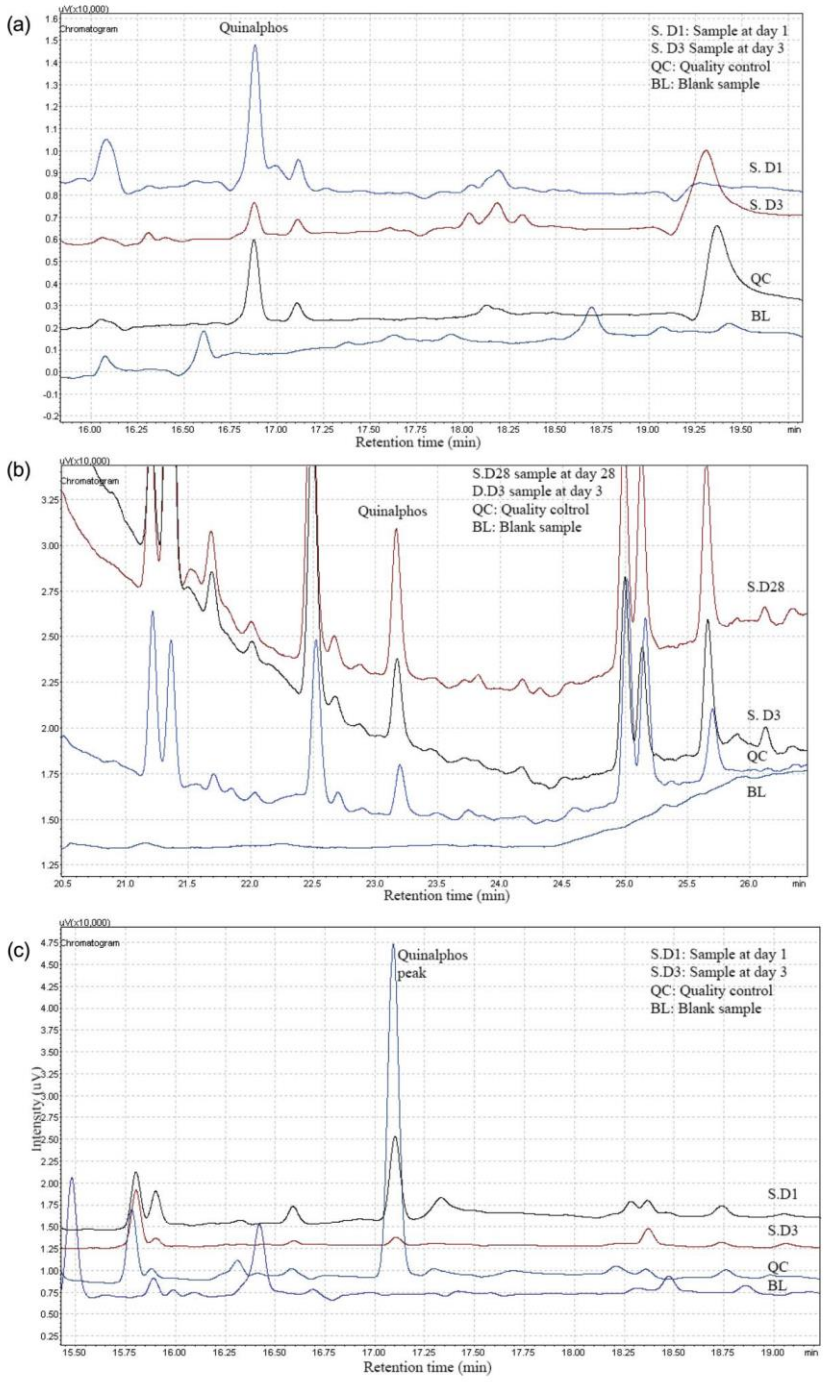

Figure 1. GC-ECD chromatograms of water (a), sediment (b) and fish (c) samples. Blank = blank sample. QC = blank matrix sample spiked with 2 ppb (water and sediment) or 2 ppm (fish) quinalphos.

concentration in water. BCF was calculated as the average between BCF calculated using concentrations in water and in fish measured one and three days after each quinalphos application when quinalphos was detectable in both fish tissue and water samples.

Results and discussion

Elimination of quinalphos in rice–fish system

The analytical results showed that the highest concentrations of quinalphos in water after the first and the second application

were respectively 11.3 ± 1.5 and 9.1 ± 1.2 $\mu g\ L^{-1}$ (Fig. 2a). The lower concentration measured after the second quinalphos application might be caused by greater retention of pesticide in rice stalks, as the crop was older at the time of the second application. These concentrations were much lower than the quinalphos LC_{50}-96 h of common carp (760 $\mu g\ L^{-1}$)[23] and silver barb (856 $\mu g\ L^{-1}$).[24] It is thus expected that quinalphos concentration in rice–fish field water might not affect the fish in this system. However, such concentrations may be toxic to other animals (e.g., crustaceans) to which quinalphos display a very low lethal concentration (LC). For example, LC_{50} after 48 h of quinalphos exposure in *Penaeus monodon* varied between 0.12 and 0.55 $\mu g\ L^{-1}$, and for *Penaeus indicus*, the LC_{50} 24 hours of quinalphos exposure was 2.7 $\mu g\ L^{-1}$.[25]

The shortest half-life of quinalphos (presented in Table 1) was found in water at 1.1 and 1.0 days after the first and the second application, respectively. In the current on-farm experiment, the half-life of quinalphos was much shorter than in a previous study[15] where the half-life of quinalphos was 38.3 days under laboratory conditions with no sunlight and in pure (HPLC) water. However, under sunlight stimulation using lake water and groundwater, the half-life of quinalphos was shortened to 0.77 and 0.78 days, respectively; besides, the concentration of dissolved organic matter and nitrite ions affects the photolysis of quinalphos: nitrite ions accelerate the photolytic degradation while organic matter retards the process.[26] This suggests that the degradation of quinalphos in water is strongly influenced by environmental parameters, such as sunlight, dissolved organic matter and biota in practical situations (i.e., in a rice field).

After the first application, the half-life of quinalphos in the muscle of common carp (1.9 days) and silver barb (2.5 days) was higher than in water (1.1 days). However, after the second application, the half-life of the compound in both fish species was shorter than the first application (1.3 and 1.1 days for common carp and silver barb, respectively). The decrease of the quinalphos half-life in fish muscle after the second application may be due to an up-regulation and an increased abundance of metabolizing enzymes resulting from the repeated chemical exposure. The up-regulation of a quinalphos metabolizing enzyme in fish is not described as for other enzymes, but the up-regulation of metabolic enzymes was observed when fish were exposed to other toxic chemicals, like nitrites.[27] Moreover, the level of water in the field was lower at the time of the second chemical application because the rice was close to being harvested, subsequently, fish might prefer to move in the

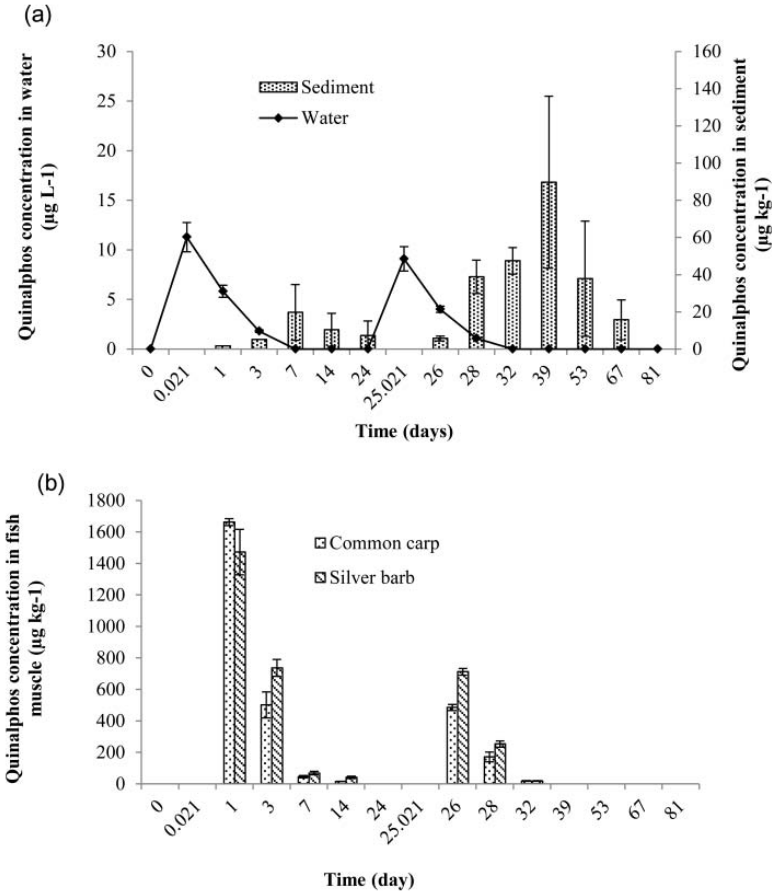

Figure 2. Elimination of quinalphos from water and sediment (a) and from fish muscle (b).

Table 1. Bioconcentration factors (BCF) of quinalphos in fish and depuration rate of quinalphos in water, fish and sediment after quinalphos application in rice–fish field.

Sample types/quinalphos applications	Depuration rate K/R^2	$t_{1/2}$(day)	Bioconcentration Factor (logBCF)
Water			
First application	$0.611/R^2 = 0.999$	1.1	
Second application	$0.707/R^2 = 0.996$	1.0	
Sediment			
First application	$0.057/R^2 = 0.931$	12.2	
Second application	$0.062/R^2 = 1$	11.1	
Common carp			
First application	$0.360/R^2 = 0.906$	1.9	2.45
Second application	$0.553/R^2 = 1$	1.3	2.14
Silver barb			
First application	$0.282/R^2 = 0.846$	2.5	2.52
Second application	$0.624/R^2 = 1$	1.1	2.31

The limit of detection (LOD) and limit of quantification (LOQ) of the analytical method were 0.4 and 1.2 μg L^{-1} for water, 7.5 and 22.7 μg kg^{-1} for fish and 0.5 and 1.6 μg kg^{-1} for sediment, respectively.

surrounding trenches. In addition, according to the study of Sancho[28] on fenitrothion, the lower chemical concentration in water would result in a higher rate of chemical metabolization or lower half-life of the chemical in fish. In mammals, the metabolism of quinalphos may be faster (e.g., in rat serum, the quinalphos half-life was 3.8 hours[29]).

In sediment, the half-life of quinalphos was much longer than in fish and water after the first and the second pesticide application: 12.2 and 11.1 days, respectively (Table 1). The half-life of quinalphos in this situation was much longer than that in soil collected from an okra field, for which it was reported that 50% of quinalphos was degraded after 1 or 1.3 days depending on the original concentration.[14] The longer half-life of quinalphos in sediments may be explained by lower exposure of sediment to sunlight in the rice–fish integration system, as the photolytic pathway is one of dominant pathways of quinalphos degradation in soil.[26] However, the degradation of quinalphos in soil is influenced by the composition of the soil and soil pH variation, and the half-life of quinalphos increased from 9 to 53 days when the pH was changed from 5.1 to 8.1.[15, 26] Moreover, the persistence of quinalphos in water and sediment is influenced by both biotic and abiotic degradation, including water pH, concentration of suspended matter, temperature, sunlight, and content of sediment.[30]

Quinalphos distribution in rice field system

Figure 2a shows the results of quinalphos residue levels in the muscle of common carp and silver barb, while **Fig. 2b** shows quinalphos levels in water and sediment during the experiment. The higher concentration of quinalphos in fish compared to water and sediment indicates the ability of quinalphos bioconcentration in fish. According to Gobas,[31] the BCF is strongly dependent on the octanol/water partition factor ($K_{o/w}$) and on the fat content of organisms. Quinalphos is a pesticide that is highly soluble in organic solvents due to its high octanol/water partition coefficient ($\log K_{o/w} = 4.44$ at pH 7 and 20 °C).[32] Moreover, the fat content in common carp ranges between 5.7 and 7.8% in the case of fish fed natural feed[33] and around 4.4% in silver barb,[34] meaning that these fish belong to the medium to fatty fish group.[35] In the current study, BCFs (expressed as logBCF) were close to 2 in both common carp and silver barb after quinalphos application in the rice field. The BCF of 2 found in this study for quinalphos is quite high due to its high $\log K_{o/w}$ and the relatively high lipid content in both fish species. These BCFs of quinalphos were quite similar to fenitrothion (with a $\log K_{o/w}$ of 3.3), an insecticide used to prevent rice seed bugs.[36] In the experiment of Sancho,[28] European eel (*Anguilla anguilla*) were exposed to fenitrothion at 40 μg L^{-1} for 72 hours, and the result showed that the logBCF of this compound was 1.86. For common carp, the logBCF after 48 hours of quinalphos exposure was 1.6–2.2.[37]

In the environment, chemicals can absorb into fish through gills, dermal pathways, and oral routes, mainly through diet. The uptake through dermal routes is dependent upon chemical polarity and lipid solubility.[38] In this study, the concentration of quinalphos in fish muscle decreased following the fast elimination of quinalphos in water and an increase of quinalphos accumulation in sediment (Figs. 2a and 2b) suggested that the main chemical absorption into fish might be via dermal pathways (skin or gills). In field conditions, oral absorption may also play a role in chemical absorption, but the accumulation of pesticide in fish through oral routes varies depending on the chemical class of pesticides.[21] Chemicals firstly have to pass a diffusion membrane, (e.g., mucus) or biological layers before reaching the circulation system, and so the octanol/water partition coefficient and the molecular size of the chemical play an important role in bioconcentration.[22] Also, Katagi[22] and Lazartigues[21] showed a positive correlation between logBCF and $\log K_{o/w}$, demonstrating that $K_{o/w}$ is an important factor contributing to the distribution of the chemical in the environment.

The residues of quinalphos in rice plants were not investigated in this study. However, according to Gupta,[15] the degradation of quinalphos in plants was quite fast, with a half-life ranging from 3 to 4 days; thus, the rice stalks may also be a factor inducing quinalphos degradation from sediment and water as it could adsorb quinalphos through its roots and consequently metabolize the pesticide.

After two quinalphos applications, the concentration of the quinalphos in sediment increased, while its concentration in water went below a detectable level (Fig. 2a). It demonstrated an absorption of quinalphos from water to sediment, which is due to the high $\log K_{o/w}$ factor of quinalphos (4.44).[32]

According to Katagi,[39] the pesticides with higher log$K_{o/w}$ will be more widely distributed in sediment rather than the pesticides with lower log$K_{o/w}$. After the second application, the concentration of quinalphos in sediment was much higher than after the first application (Fig. 2a). This could be due to the lower accumulation in fish muscle after the second application compared with the first one (Fig. 2b). As mentioned before, in the second application, the lower water level reduced the travel of the fish between channels within the rice area, and, subsequently, the fish would be less exposed and the concentration of quinalphos in sediment would be increased.

Conclusions

In rice–fish integration systems, beside the practical operation effects, the distribution of pesticide was influenced by various other factors. Indeed, sediment is a very complex matrix containing clay minerals, organic matter, and living organisms. In addition, interstitial pore water (portion of water located between small sediment particles) is different from the overlaying water, so it contributes greatly to pesticide distribution.[39] Also, elimination of the applied pesticide may be affected by climate conditions, such as wind or moisture.[22]

Acknowledgments

The authors thank ARES-CCD (Académie de Recherche et d'Enseignement Supérieur – Commission pour la Coopération au Développement) for its financial support within the framework of the DELTAQUASAFE project.

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