Fate and Toxicity of Abamectin in Exposed Fish

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Abstract

Abamectin is one of the worldwide applied insecticides generally known in many trade names such as A.G.BA, Dimatin, Agrotin, Abama, and Jacket. It occurs in fermentation process of soil bacterium; *Streptomyces avermitilis*, which produce avermectin B1a and avermectin B1b. These substances have the same physical and toxic properties but avermectin B1a shows higher insecticidal property. Thus, most insecticide in this family composes of at least 80% avermectin B1a. Because of its high toxicity, it is applied in quite few amounts. It is classified as contact and semi-systemic insecticide thus it can control most insects such as thrips, lime butterfly, and rice leaffolder. However, it could be released and contaminated into the waters by both intently and non-intently. Many researches showed its harmful effects on aquatic environment especially freshwater fish; for example, histology changes in many organs and degenerative changes in brain, gill and kidney. Based on above information, the study on structure, mode of action, and toxicity of abamectin is required.

Keywords: Abamectin; Fish; Toxicity; For Examples; Histology was Changed in Many Organ and Degeneratives were Changed in Brain; Kidney and Gill

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Introduction

Generally, water pollution is more serious now than in the past. Many varieties of contaminants; natural and man-made, have been found in all part of the waters. For anthropogenic source, it is the chemical which is produced for use in industrial and agricultural purposes. Insecticide pollution is a great problem, especially in developing countries. In these countries, the application of insecticide is for protecting agricultural yield. The excessive insecticides find their way into aquatic environments. From urban area, it is swept by rainfall runoff, atmospheric deposition into the waters. Moreover, another important source is the insecticide being discharged from municipal and industrial area [1] - [5]. These pollutants are highly toxic to non-target organisms which inhabit in contaminated environments nearby agricultural area. Insecticides have harmful effect on the growth, survival and reproduction of aquatic organisms. Many recent researches indicated that the mortality of fish in various streams, lakes, and ponds worldwide has been increased which relate to an increasing in insecticide contamination from agriculture.

Abamectin is an insecticide widely used for controlling animal parasites and insects. In the case of excessive application, it can be released and cause aquatic pollution. Based on its chemical structure, abamectins can be rapidly photodegraded in water and transformed to be a less bioactive compounds (t1/2 = 4-21 h) [6]. It is readily adsorbed onto the solid surface; organic matter, soil and sediment particles. In some case, it can reach and contaminate groundwater. However, the most serious problem caused by abamectin is posing a risk to the aquatic environment [1].

Structural of Abamectin

Abamectin (ABM) is a product of *Streptomyces avermitilis* naturally occurrs in fermentation process. Abamectin is a mixture of homologues B1a (at least 80%) and B1b. The chemical structure of Abamectin is shown in Figure 1 and Table 1 [7].

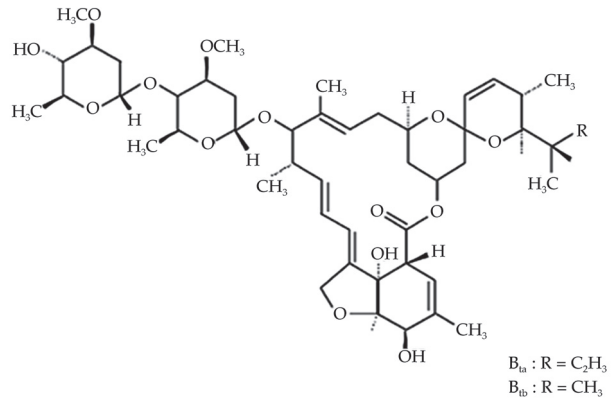

Figure 1 Chemical structure of Abamectin [7]

Table 1 Main structural characteristic of Abamectin [8]

Common name :	Abamectin (BSI, draft E-ISO, ANSI); abamectine (f) draft F-ISO
Chemical name :	Avermectin B1
Appearance :	Abamectim is a colorless to yellowish Crystalline powder
Molecular weight :	873.11
Water solubility :	Insoluble
Solubility in other solvent :	Vs in acetone, methanol, toluene, Chloroform and ethanol.
Vapor pressure :	Negligible
Composition	A mixture containing’80% avemectin B1a (i) and £20% avermectin B1b (ii).
Chemical class :	Insecticide/miticide
Acute toxicity :	Abamectin is highly toxic to insects and May be highly toxic to mammals as well
Effects on birds :	Abamectin is practically nontoxic to birds
Effects on aquatic organisms :	Abamectin is highly toxic to fish and Extremely toxic to aquatic invertebrates

Fate of abamectin in aquatic environment

Firstly, there were many reports that abamectin is classified in avermectin subfamily of macrocyclic lactones. It is produced from fermentation process performed by the soil microorganism, Streptomyces avermitilis. It consists of a mixture of homologues B1a and B1b and containing a minimum of 80% B1a and a maximum of 20% B1b. Table 1 shows that avermectin B1 can be used as both pesticides and anti-helminthic drug in animal. However, the studies on the fate of abamectin have been mostly performed in avermectin form especially in the aquatic. In some studies on the mobility of avermectin which were conducted under simulated conditions, the results indicated that the highest level of avermectin could be found atter 24 hr (0.052 ppb) in the water and 48 hr (0.091 ppb) in sediment after releasing. Half-life of avermectin in water was 4 days, and 2 - 4 weeks in sediment. Avermectin can be absorbed strongly on the surface of sediment or soils (K = 4940). Under simulated runoff conditions, avermectin with a concentration up to 16 ppb introducing into an aquatic environment was in undetectable levels in water or sediment (Minimum Detection Limit, MDL, = 0.1 ppb). Based on this study, avermectin application in the field conditions would cause minimal contamination of aquatic ecosystems through drift or runoff [9]. Fate of avermectin in the environment is controlled by 2 processes; hydrolysis and photodegradation. A study indicated that hydrolysis was not an important process to breakdown avermectin B1a. Avermectin was incubated in the aqueous solutions (25^{o}C) in various pH; 5, 7 and 9. After 28 days, about 95% of the avermectin was recovered. However, some researchers suggested that photodegradation is a dominant process to transform avermectin. Delta 8, 9-isomer of avermectin B 1a, which derived from photodegradation has similar toxicological properties to the parent compound [9].

Mode of actions and Metabolism of Abamectin

In abamectin action mechanism, c-aminobutyric acid (GABA) system and chloride channels play an important role. GABA receptors act like a regulating agent to control the neural basal tone of the brain [10] and in all neurons of the central nervous system (CNS). For the symptoms of abamectin, it was found in many tested animals such as rainbow trout [7] and tilapia [8], [11]. Additionally, some studies showed genotoxic effects of abamectin [12].

An increasing of GABA-binding raises flow rate of chloride ions into the cell which ultimately cause hyperpolarization and elimination of signal transmission [5]. In detail, enzyme acetylcholinesterase action is blocked by insecticide resulting in signs and symptoms of intensive cholinergic stimulation. Abamectin is classified as neurotoxins which has an effect on sodium and potassium channels in neurons transmission. It can be observed by a decreasing in potassium permeability, the inhibition of cadmodulin, Na/K ratio, and Ca^{2+}ATPase activity. In 2007, El-Said, M.M. [11] indicted that fish exposed to abamectin for 7 days showed a significant increase in the level of sodium (Na^+) ions (hypernatraemia) and chloride (Cl^-) ions (hyperchloraemia). This could be described by the stimulation of Na^+-K^+ ATPase activity which led to raise of influx of Na^+ and Cl^- ions and/or may cause the reabsorption function in the kidney. In contrast, hyponatraemia and hypochloraemia were observed in fish after exposed to abamectin tested for 14 days. This may be described that abamectin disturbs Na^+-K^+ ATPase or carbonic anhydrase (CA) or both of these enzymes.

After getting into fish's body, abamectins are quickly transformed and concentrated in various tissues and may be bio-magnified along the food chain and ultimately to the top consumer. Abamectin which concentrated in the body can be released and cause death or long-term damage. In 2011, Al-Kahtani, M.A. [8] showed that abamectin disturbed oxygen consumption and some biochemical substances (total protein, carbohydrate and cholesterol in liver, muscle, kidney and gills) of exposed tilapia fish (*Oreochromis niloticus*). They indicated that oxygen consumption rate clearly declined after exposure. In addition, other bio-chemical indicators were also decreased in all tissues compared with control.

Its bioaccumulation rate in exposed fish depends on many factors in both insecticide and fish. In the part of fish, the rate is influenced by the species, development stages, and the amount of fat in each tissue and the diet of fish. For insecticide, it depends on chemical and physical properties. To enhance elimination and detoxification of insecticide exposed fish respond by developing complex detoxification mechanisms such as releasing of many enzymes to metabolize that xenobiotic substance. However, metabolized or transformed insecticide may potentially alter its activity and toxicity. The enzymes playing the important role in biotransformation can be classified into 2 phases. In Phase I, cytochrome P450 enzymes; including CYP1A and CYP3A, transforms the xenobiotic compounds to be a more polar compound. There are many families of cytochrome P450 enzymes in fish [13]. In the liver of some freshwater fish, CYP1A, CYP2B, CYP2E1, CYP2K1 and CYP3A have been recently identified [14] and found that it act an important

role to detoxify organophosphate and carbamate insecticides [15]. Riga, M. et al. [16] studied the relationship between cytochrome p450 (CYP392A16) and intermediate metabolitezed of abamectin and found that a cytochrome P450 could metabolize abamectin to be a less toxic hydroxyl-form as shown in Figure 2.

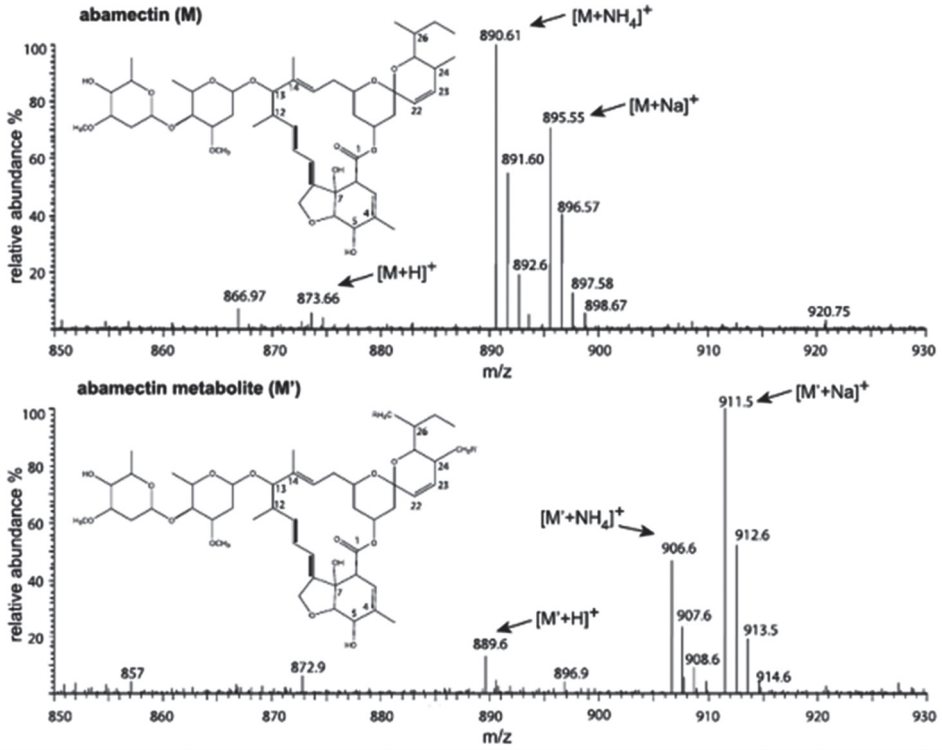

Figure 2 Mass spectrum of the substrate abamectin and its metabolite hydroxyl-abamectin. Upper panel: Mass spectra of the substrate abamectin incubated in 20 pmol CYP392A16 and 62.5 mM abamectin for 1 h without NADPH. Lower panel: Mass spectra of the metabolite hydroxyl-abamectin (HO-groups correspond to either R or R0 on the shown structure (indicated in red)) incubated in 20 pmole CYP392A16 and 62.5 mM abamectin for 1 h with NADPH [16].

Evaluation of abamectin toxicity in fish

After the fish, both of caged and wild are exposed to insecticides, they absorb the insecticide in gills, skin or gastrointestinal tract [1], [11]. Because of its high lipophilic property, insecticides easily filtrate biological membranes causing sensitivity of fish to the insecticide. In sub-lethal toxicity testing (one tenth or more of LC_{50} dose in moderate

periods), fish organs or other biological process in its body are affected such as; respiratory, hepatic, haematopoietic, nervous, cardiovascular, and reproductive and immune systems. For biomarkers perspective, insecticides may lead to changes in the blood biochemical parameters and haematological profile of fish thus this alteration can be used as biomarker to monitor water pollution [17] - [19].

Based on U.S. Food and Drug Administration (FDA) regulations, the LD_{50} of a substance was set to control and manage environmental pollution. The LD_{50} indicates the estimated dose causing 50% mortality in exposed animal under the defined conditions. Each LD_{50} test must be performed at least two routes of exposure; oral and parenteral depending on the nature of the substance and targeted animal. The other routes are inhalation, dermal, and other possible routes. For aquatic animals, toxicity is showed in mediun lethal concentration (LC_{50}) which is the estimates causing 50% mortality in experimental animals [20]. For acute effect, toxicity of the substance; abamectin for this case, depends on the exposed species, for example most sensitive fish, 96 h LC_{50} of rainbow trout is 3.2 μgL^{-1}. Meanwhile, the least sensitive fish species such as carp has 96 h LC_{50} at 42 μgL^{-1} [7]. The major component of abamectin; avermectin B1a, having water insolubility at (7.8 μgL^{-1}) and high KO_w at 9900 [21], [6]. However, Van den-Heuvel, W.J.A. et al. [22] found that abamectin could not concentrate in bluegill sunfish because it's rapidly eliminated during depuration period.

Van den Heuvel, W.J.A. et al. [22] studied the bioconcentration and depuration of abamectin in the bluegill sunfish (*Lepomis macrochirus*). They were exposed to 0.099 lg/l abamectin for 28-day exposure period. After examining, abamectin residue in tissue of whole fish were 6.8 lg/kg. After 14 days of depuration period (after 28 day of exposure), the residue clearly decreased to 0.32, 0.27 and 0.53 lg/kg, respectively [22]. Additionally, Jenč ič, V. et al. [7] studied the toxic effect of abamectin in rainbow trout (*Oncorhynchus mykiss*) and found histology changes in organs indicating a direct toxicity such as degenerative changes in brain and kidney and a minor extent in fish liver. This finding was in agreement with the study of El-Said, M.M. [11] who found that Tilapia (*Oreochromis niloticus*) exposed to abamectin at both concentrations comprising 50.48 μ/L and 103.68 μ/L for 14 days showed necrosis of lamella in gills and kidneys, infiltration of acidophils leukocyte in gills, and degenerative changes in kidney tubules. Figure 3 and 4 show the histological changes in exposed Tilapia (*O. niloticus*) [11].

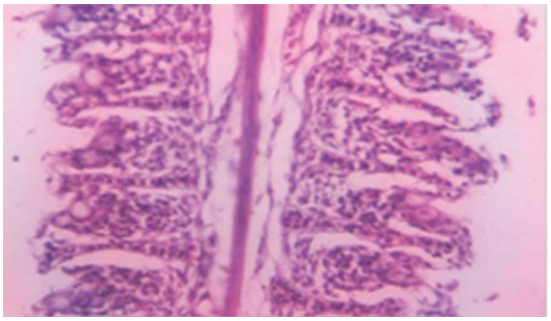

Figure 3 Gills of Tilapia (*Oreochromis niloticus*) after exposed to 50.48 μL-1 abamectin for 14 days showing necrosis of lamella (HXE X 160) [11]

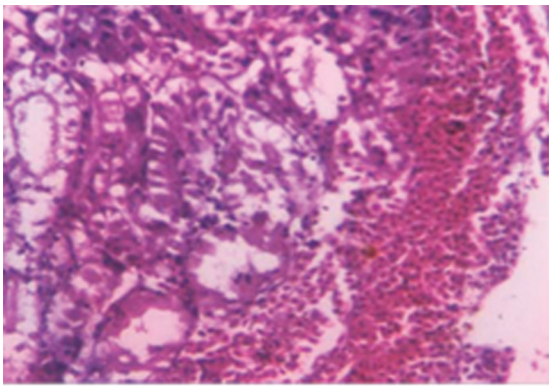

Figure 4 Kidney of Tilapia (*O. niloticus*) exposed to 50.48 μL^{-1} abamectin for 14 days showing focal haemorrhages between the vacuolar degenerated renal tubules (HXE X 40) [11]

In addition, Jenčič, V. et al. [7] found that abamectin caused a negative effect on rainbow trout observed by histological technique. In the liver, single cell necrosis was found, (Figure 5) and mononuclear foci were also observed in some exposed fish. In the brain, vacuolar degeneration of neurons and enlarged number of glial cells (Figure 6) were identified.

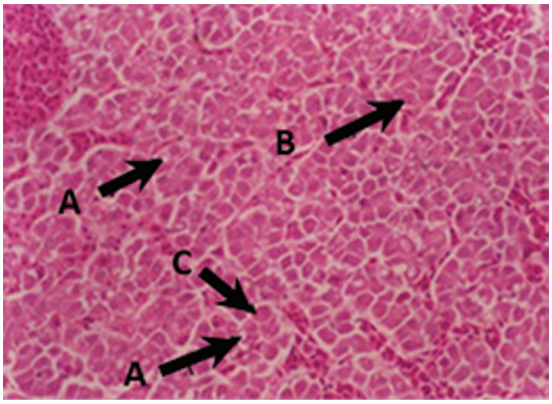

Figure 5 Histological alterations observed in liver; vacuolar degeneration (A), single cell necrosis (B) and normal hepatocyte (C) (H&E, 250x) [7]

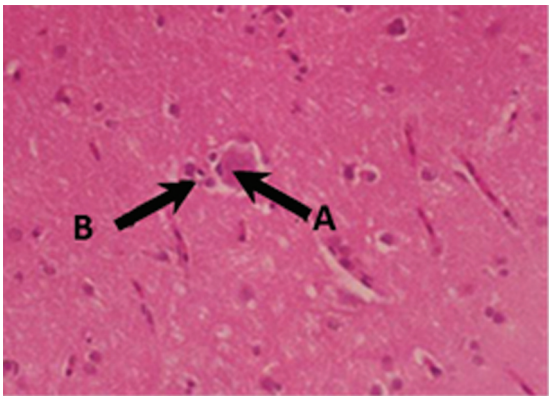

Figure 6 Histological alterations observed in brain; degenerated neuron cell (A), surrounded with empty space and glial cells (B) (H&E, 400x) [7]

Current status of abamectin in Thailand and future perspective

For Thailand, the volume of imported agricultural pesticide is very high, which is about 16,000 million Thai Baht (522.4 million US$). Abamectin insecticide is about 1.8% which was worth 623 million Thai Bath (in 2009) because it was not expensive. In the local market, a liter of abamectin costs only 300-580 Thai Bath. Although it is very effective insecticide, it should be properly and legally applied. In a case study of abamectin using to control plant hopper, the Department of rice found the outbreaks of plant hopper since early February 2012. One of the dominant causes of the outbreak is the abuse of abamectin which ultimately results in insecticide-resistance in the hopper. To avoid that resistance in the hopper, the Department of rice recommended the local farmers to use abamectin

insecticide properly. However, it is applied to control other insect such as cotton leafhopper or cotton jassid. It causes 100% mortality in 3 staged larva of cotton leafhopper while bio-insecticide derived from fungus *Metarhizium anisopliae* can cause only 83.33% mortality [23]. However, abamectin could result in water pollution when it is swept into the river by water run-off. To prevent this problem, abamectin insecticide should be used as much as necessary or applied in other forms. In Thailand, the lack of supportive evidence for abamectin insecticide application is still the problem, thus it require more study in this issue.

Conclusion

Abamectin is one of the worldwide applied insecticides generally known in many trade names such as A.G.BA, Dimatin, Agrotin, Abama, and Jacket. It is occurred in fermentation process of soil bacterium. It is a high potential insecticide. However, it may cause water pollution via water run-off. It can cause harmful effect in edible fish. Thus, it should be applied as much as necessary and legally to avoid water contamination.

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