Glyphosate (Roundup): Fate in Aquatic Environment, Adverse Effect and Toxicity Assessment in Aquatic organisms

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Received: 17 June 2019; Revised: 28 August 2019; Accepted: 26 September 2019

Abstract

Glyphosate ($C_3H_8NO_5P$) is an organic substance in phosphonomethyl glycine group consisting of phosphorus in its structure. Its well-known trading name is Roundup, which is globally applied as herbicide. In Thailand, it has been widely applied, which results in it occupy in the top order of imported ago-chemical even though it was banned in many countries. Glyphosate application can cause contamination in groundwater and nearby surface water. Recently, there have been studies indicating that glyphosate contamination causes adverse effects on aquatic organism and is bio-accumulated and bio-magnified through food chain and finally reaches to human beings as top consumer. Glyphosate causes alterations in behavior, physiology, DNA, chromosome and bio-chemistry and moreover mortality in the case of exposure in high level. In this case, it may effect on aquatic population which is an important fishery stock in the future. In Thailand, there are lacks of fundamental data and knowledge thus it requires more studies or tests to fulfill this gap. As the importance mentioned above, the effect of glyphosate should be studied for developing the protection and management plan to reduce glyphosate contamination in aquatic organisms and environments.

Keywords: acetycholinesterase, aquatic organism, glyphosate, herbicide, roundup

Introduction

Glyphosate or N-(phosphonomethyl glycine) has many trading names such as Roundup, Roden and Accord in USA. In Thailand, there are more names such as Cowboy, Spark, Phosate, Brace, Fire and Clean Up. Glyphosate application has been continuously raised since it was firstly produced in 1950 by Swiss scientist, Dr. Henri Martin. At first, its herbicide capability was not known. Then, it was developed by scientists of Monsanto and applied as herbicide in 1970 (Dill et al., 2010). In 2012, 100,000 tonnes of glyphosate was imported into Thailand which was 5 times higher than insecticide. Most applications have been occurred in agricultural sector than forestry, garden and weed controlling sectors (Jitrapat, Watawanichakul, & Muangphra, 2015). In 1995, Pimentel (1995) reported that only 0.1% reached onto target organisms and the rest contaminated into the environment: aquatic organisms, soil and air. Glyphosate can adsorb onto clay particle surface while it less absorbs onto sand particle. Thus, it risks to be contaminated in the aquatic environment of both surface and ground waters (Borggard & Gimsing, 2008) because it is an ultimate sink of all wastes. Therefore, aquatic organisms are risk to expose and contaminate with glyphosate which will be further bioaccumulated and biomagnifed in food chain and eventually causes adverse effects to human as a top consumer. Even though, World Health Organization (WHO) 1994; Zouaoui, Dulaurent, Gaulier, Moesch, and Lachatre (2013) reported that pure glyphosate is non-toxic to human being; however, glyphosate being applied in agricultural sector is mixed with surfactants such as polyoxyethykeneamine (POEA) and alkylpolyphosphate amine. Giesy, Dobson, and Solomon (2000) reported that Roundup can penetrate into cell membrane, especially gill cell of fish and other aquatic organisms because it has POEA as the surfactants.

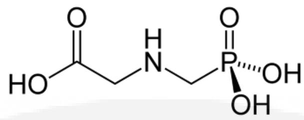
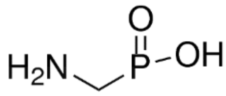
Recently, we found an increasing of glyphosate pesticide application, which resulted from generic glyphosate formulation development and production. Based on its worldwide application, glyphosate effects and toxicity in the aquatic environment and non-target species need to be reviewed and more importantly studied. Many researchers have tried to fill the knowledge gap of glyphosate effects in the aquatic environment such as determination of the effects of sub-lethal glyphosate concentrations in carp in Louisiana, US. The researchers studied the effect of sub-lethal concentrations of glyphosate; from five commercial pesticides including Roundup, on an aquatic snail species, *Pseudosuccinea columella* by examining the DNA damage in bullfrog tadpoles. In addition, they found that 23 different pesticides effect on aquatic plant and another study indicated that glyphosate can attribute to eutrophication in the case of primary producers such as diatoms using it as a source of phosphorus. Based on this evidence, glyphosate in ‘below detectable level’ can induce eutrophication and then causing adverse effects to aquatic organism habitats and aquatic resources. However, this degradation may be lower severe as compared to the effect of high levels of phosphorus generated (Buffin & Jewell, 2001).

In Thailand, study of contamination, effect and assessment of glyphosate is limited. Therefore, this review aimed to gather relevant information of glyphosate including structure, physical and chemical properties, occurrence and determination in the aquatic environment, toxicity assessment, current status and future perspectives of glyphosate in Thailand and finally to suggest solution to glyphosate contamination for usefulness in aquatic environment management.

1. Structure, Physical and Chemical Properties of Glyphosate

Glyphosate is a systemic and nonselective herbicide that was first identified as an herbicide in 1970 by Monsanto scientist and then was released in commercial formula in four years later (Annett, Habibi, & Hontela, 2014). The chemical name of glyphosate is N-(phosphonomethyl) glycine and its herbicidal activity is to eliminate broad leaved grass and sedge species (Cox, 1995). The properties of glyphosate are showed in table 1. After entering organism body, it blocks enzyme evolving in amino acids and protein manufacture and then affected plants die within a few days. However, glyphosate is never used in pure form, it is always mixed with other chemical ingredients, especially surfactants for improving penetration into plant cells.

Table 1 Physical and chemical properties of glyphosate and its primary metabolite AMPA

Common name	Glyphosate	AMPA
IUPAC Name	N-(Phosphonomethyl) glycine	(Aminomethy) phosphonic acid
Chemical formula (acid)	$C_3H_8NO_5P$	CH_6NO_3P
Chemical structure	 Glyphosate	 Aminomethy phosphonic acid
CAS number	1071-83-6 (acid)	1066-51-9
Molecular weight	169.09 g mol^{-1}	111.04 g mol^{-1}
Physical state and color	Crystalline powder, white	Crystalline powder, white
Melting point	200° - 230° C	120° C
Water solubility	10,000 - 15,700 mg/L at 25° C	-
Octanol/water partition coefficient (log K_{ow})	-4.59 to -1.70	-
Half life of glyphosate	7 - 142 days (in water)	76 - 240 days (in soil)

Source: Slightly adapted from Giesy et al. (2000)

However, glyphosate-containing herbicides are intently applied for controlling weed or clearing vegetation field but the rest may expose to plants, animals, invertebrates and microorganisms. Figure 1 shows many routes that glyphosate can expose to organisms via contacting (insect exposed to sprayed glyphosate), feeding (organism eating treated crops), exposing to contaminated water in both surface and ground waters or their contaminated habitats (Friends of the Earth Europe, 2013).

Figure 1 Scheme of glyphosate cycle and contamination

Many reports indicated that there are many impacts of glyphosate to ecology; for example, glyphosate accumulated in soil may be assimilated into roots and plant tissues. Moreover, it may be released into soil solution and then stimulate fungi growth and cause virulence of pathogens making plant susceptible to disease. In this situation, Roundup causes adverse effects to not only target organisms but also beneficial food microorganisms that functions as starter cultures in the food industry (Figure 2). However, the data is not sufficiently clear resulting in WHO (1994) suggested that biological activity of sediment- and soil-bound glyphosate in the environment should be more studied, and the further toxicity to organisms are needed as well (Buffin & Jewell, 2001).

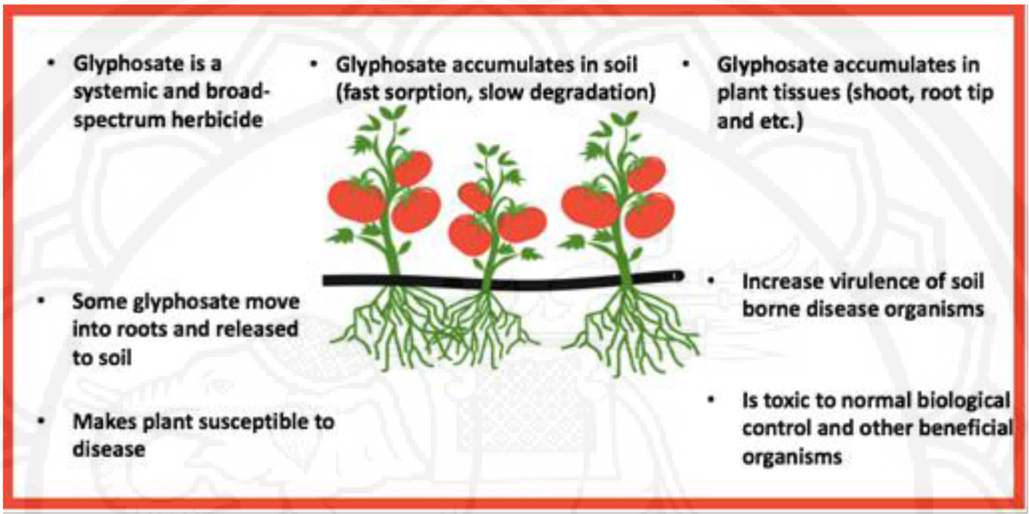


Figure 2 Glyphosate accumulation in soil and plant tissue

2. Occurrence of Glyphosate in Aquatic Environment

Because of glyphosate-based application, the level of overall herbicide has been lowered resulting in its contamination in surface water (Shipitalo, Malone, & Owens, 2008). Thus, the contamination of glyphosate-based herbicides or its metabolites in the aquatic environment has been increasingly found. They are found in many routes to surface water; for example, drifting during application or being carried by surface runoff after application (Borggaard & Gimsing, 2008). Many studies reported that glyphosate in the concentrations of 1.7 to 5.2 mg a.e./L could cause adverse effects; however, this contamination level may be reached in the case of accidental spills or direct overwater application (Giesy et al., 2000; Glozier et al., 2012; Annett et al., 2014). In the aquatic environment, it was found that glyphosate half-life is 7 - 14 days (Giesy et al., 2000). It can bond with many species of metal ion (Defargea, Spiroux de Vendômoisb, & Séralini, 2018) resulting in lowering bioavailability of nutrients. Moreover, glyphosate released into the aquatic environment may accumulate in organisms and bottom sediments. As generally known, all pesticides can be degraded in the environment by natural factors including light, temperature, and aquatic organism such as some bacteria as show in figure 3 (Mercurio, Flore, Muellera, Carter, & Negri, 2014). However in the case of excessive and long term application, biodegradation process cannot accomplish those pesticides causing water resource degrade.

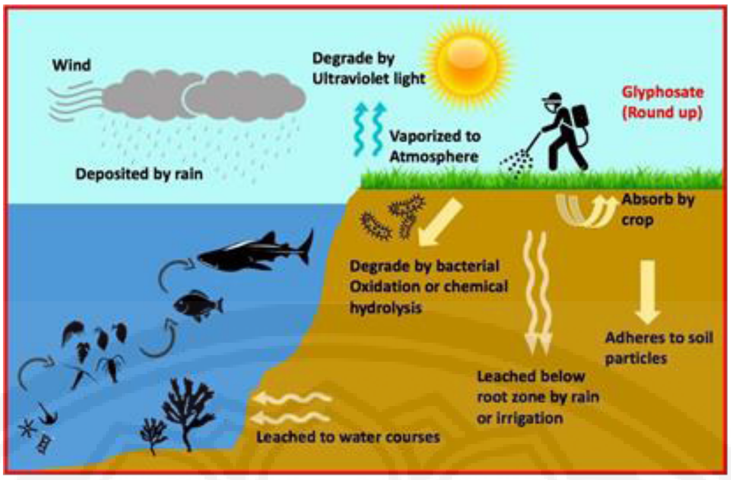


Figure 3 Distribution and transportation of glyphosate into aquatic organism and environments

Glyphosate and the mixed surfactants, polyoxyethylene amine (POEA) or MON 0818, can introduce adverse effects to exposed organisms and then the chemical can be magnified through aquatic food chain (Figure 4). They can be passed from protozoa, mussels, crustaceans, frog fish and finally human as the top consumer as same as terrestrial food chain. Generally, glyphosate having POEA as surfactant is highly toxic when compared to those without these surfactants. In addition, aquatic organisms express more sensitive to POEA than terrestrial one. For example, it expressed toxicity in micro crustacean (*Artemia salina*) and young zebrafish (*Danio rerio*) (Rodrigues et al., 2016; Van Bruggen et al., 2018).

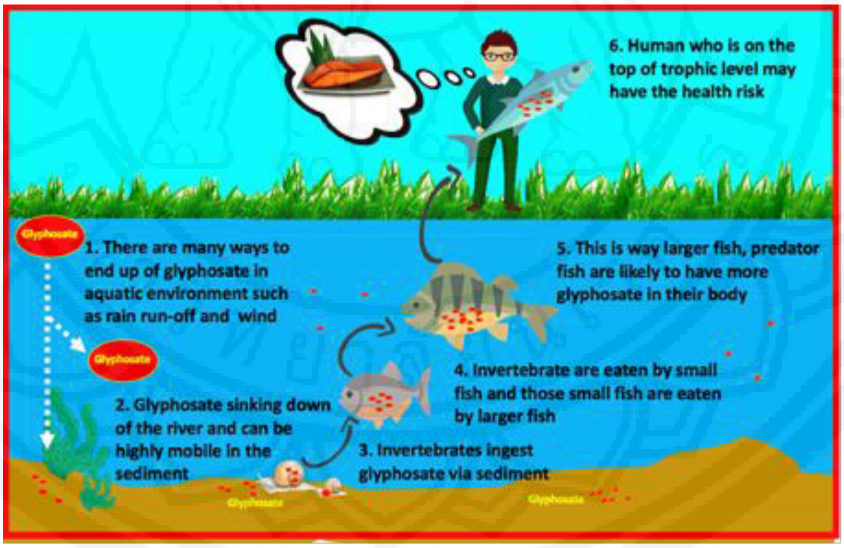


Figure 4 Biomagnification of glyphosate in aquatic food chain

3. Toxicity Assessment

The glyphosate N-phosphomethyl-glycine is an herbicide widely applied in many commercial formulations having glyphosate as the active ingredient because of its effective action and low toxicity to mammals (Corbera , Hidalgo, Salvado, & Wieczorek, 2005). Glyphosate (Roundup Transorb; RDT) is an example of herbicide having glyphosate as the active principle, which has been classified as a toxicant in the environment (Class III). In 1998, it was firstly released into the American market for controlling weed in the field of sugarcane, coffee and plants of the genus Citrus. Its main function related to other formulations, which is a rapid translocation of about 60 min. A component causing rapid translocation is surfactant used in the formulation. This technology is called Transorb, which enables the faster delivery of the product with larger quantities into the root of the weed. In addition, it offers low risk of loss by rain or run-off.

Surfactant is an important part in the herbicide structure because it plays a crucial role in many processes such as adhesion, wetting, spreading on the leaf, and being uptake into the leaf. Many types of surfactants have been used. Usually, they are highly toxic, which are responsible for the toxicity of the products. The surfactant used in RDT is polyoxyethylene amine (POEA) (15%) mixed with other unspecified surfactants (Howe et al., 2004).

The effects of glyphosate and its mixed surfactants on non-target aquatic organisms were inevitably indicated. Many studies have evaluated both of lethal and sub-lethal effects based on mortality, growth, biomass, weight, density, length, pigments, mobility, reproduction, and metabolism. The results were shown in LC (lethal concentration), EC (effective concentration) and IC (inhibition concentration). Additionally, values of NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) were suggested in some reports. In some case which dose-response curves could not be evaluated because of experimental design, its effect was reported in concentrations of treatments and obtained outcomes expressing in percentage of control values. The published effects of glyphosate in both acute and chronic are shown in Table 2. Expected environmental concentration (EEC) is placed as a reference value for relating their aquatic toxicity to realistic exposure levels (Perez, Sullivan, Michael, & Harris, 2012).

Table 2 Toxicity assessment of glyphosate in aquatic organisms

Species	Assessed chemical	Assessed parameter	Effect concentration	Exposure reference
Asian sea bass (*Lates calcarifer*)	Glyphosate	LC_{50}	LC_{50} - 24 h 5.57 mg/L LC_{50} - 48 h 3.55 mg/L LC_{50} - 72 h 2.5 mg/L LC_{50} - 96 h 0.76 mg/L	Thanomsit, Wattanakornsiiri, and Nanthanawat (2016)
Guppies (*Poecilia reticulata*)	Glyphosate	LC_{50}	LC_{50}-96 h 12.01± 0.1 ppm	Sadeghi and Hedayati (2014)
Nile tilapia (*Oreochromis niloticus*)	Glyphosate	LC_{50}	LC_{50} - 24 h 17.5 ppm LC_{50} - 48 h 17.1 ppm LC_{50} - 72 h 16.9 ppm LC_{50} - 96 h 16.8 ppm	Jiraungkoorskul et al. (2002)
Brine shrimp (*Artemia* spp.)	Glyphosate	LC_{50}	LC_{50}-24 h 0.028 ppm LC_{50}-24 h 0.019 ppm	Ali, Jamal Mohamed, Arun Kumar, and Akbar John. (2018)
H. huso *A. stellatus* *A. persicus*	Glyphosate, N-(phosphonomethyl) glycine	LC_{50}	LC_{50} - 96 h 26.4 mg/L LC_{50} - 96 h 23.2 mg/L LC_{50} - 96 h 27.5 mg/L	Filizadeh and Islami (2011)
Juvenile African catfish (*Clarias gariepinus*, Burchell 1822)	Glyphosate	LC_{50}	LC_{50}-24 h 34.72 (31.02-37.20) mg/L LC_{50} - 48 h 31.90 (28.12-33.89) mg/L	Ani et al. (2017)

Table 2 (Cont).

Species	Assessed chemical	Assessed parameter	Effect concentration	Exposure reference
			LC_{50} - 72 h 27.40 (24.98-29.30) mg/L LC_{50} - 96 h 24.60 (21.95-26.54) mg/L	
Daphnia magna	Glyphosate (Aqueous solution of 40 % b.w.)	EC_{50}	EC_{50} -48 h 3.7-10.6 mg a.i./L	Cuhra, Traavik, & BØhn. (2013)

In assessment of glyphosate-based herbicide toxicity, it is mainly based on relative insensitivity, mortality endpoint for calculation. Although, these endpoints generally expresses in population level, it is also found in concentrations that are responsible for behavioral, cellular and metabolic changes. However, it needs more investigations on the effects relevant to predicted exposure concentrations for determining the long-term effects of glyphosate-based herbicide exposure in the aquatic environment (Annett, Habibi, & Hontela 2014).

4. Effect of Glyphosate to Aquatic Organisms

Though WHO (1994) and Zouaoui et al. (2013) reported that pure glyphosate had no direct effect onto human; however, glyphosate has been applied in mixture. Lipok, Studnik, and Gruyaert (2010) and Moore et al. (2012) pointed-out that glyphosate-based herbicides are in a variety of formulations. It contains same active ingredient, being mixed with various surfactants, adjuvants and other chemicals. In the aquatic organisms risky to expose with to glyphosate, they showed sensitivity to glyphosate in commercial formulas is highly species-specific. Frequently, there is a greater difference in sensitivity between two related species than between species with taxonomical separation as found in the study of Giesy et al. (2000). They reported that Roundup product having glyphosate and surfactant in the structure could pass through cell membrane causing aquatic organisms risk to expose with and accumulate glyphosate. Moreover, Annette et al. (2014) reported that surfactant portion in the formulation of glyphosate-based herbicides was the primary source of its toxicity. This finding makes it quite difficult for assigning toxicity to a particular chemical. On the other hand, it has been found that POEA which is most tested surfactant is applied in various commercial formulations. In the case of wide applications in agriculture, it is concluded that aquatic organisms may risk exposing with these constituents.

Many reports showed the effect of glyphosate to aquatic organisms. Walker, Hopkin, and Peakall. (2006) indicated that the toxicity depended on exposure time and concentration such as acute effect of Roundup to movement of *Moina macrocopa* and chronic effect Round Up to growth and anti-oxidant enzyme activity (catalase) which can be used as bio-indicator of oxidative stress and reproductive in *M. macrocopa* (Jitrapat et al., 2015). In freshwater crayfish (*Cherax quadricarinatus*), which chronic exposed to glyphosate for 50 days, expressed a decrease in muscle protein as well as slower somatic growth. This might be related to an increasing in mobilization of energy serves in response to glyphosate stress (Frontera et al., 2011). Glyphosate can destroy central nervous system, which plays an important role in controlling body function consisting of 3 parts: brain controlling all activities of the body, spinal cord being the pathway of nerve signal for both incoming and outgoing nerves from the brain, and motor neuron controlling the functions of various organs. Therefore, glyphosate affects the behavior of aquatic organisms.

Teleost fish is proved to be an appropriate bio-indicator for evaluating the toxicity and effects of contaminants in animals because its biochemical responses are similar to those of mammals and other vertebrates. *Prochilodus lineatus* is an economic important neotropical fish, which is generally found in rivers of the south and southeast regions of Brazil and is classified as a potential bio-indicator (Martinez & Souza, 2002; Camargo & Martinez, 2006).In fish, adverse effects, which can be assessed, consist of abnormal swims gill tissue degradation, changes in liver structure, brain acetylcholine esterase level, oxidative stress, genotoxicity, reproductive organs, endocrine disruption and mortality (Modesto & Martinez, 2010; Mené ndez - Helman, Ferrey, Santos Afonso, & Salibian, 2012; Thanosit et al., 2016) (Figure 5). Moreover, a report indicated that glyphosate affects energy metabolism, free radical processes, acetylcholineesterase activity (Langiano & Martinez, 2008), and immune responses of histological in hepatocytes in some fish such as *Oreochromis niloticus* and *Cyprinus carpio* (Szarek, Siwicki, Andrzejewska, Terech-Majewska, & Banaszkiewicz, 2000; Jiraungkoorskul et al., 2003).

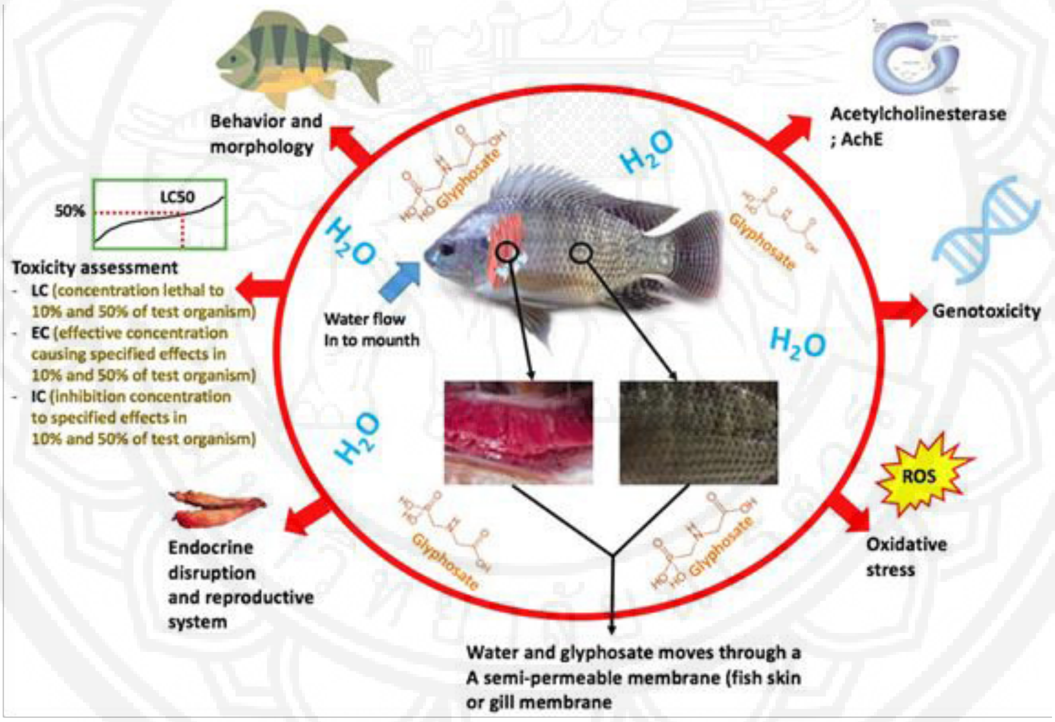


Figure 5 Adverse effects of glyphosate on fish

Recently, the effects of glyphosate have been widely studied and found that it can inhibit acetylcholinesterase activity in organisms. As generally known, acetylcholinesterase is the enzyme playing a crucial role in splitting acetylcholine (neurotransmitter) to choline and acetate. After acetylcholinesterase being inhibited, acetylcholine will accumulate and further affect to central and peripheral nervous systems causing muscle twitching. Thus, it can be assessed for herbicide exposure by monitoring functions of acetylcholinesterase as found in the study of Glusczak, Santos, Crestani, and Pimental Veieira (2006), which found that the effect of glyphosate to acetylcholinesterase activity in bonny fish (*Leporinus obtusidens*), which exposed to herbicide for 96 h at the concentrations of 0, 3, 6, 10 and 20 mg/ L and found that acetylcholinesterase activity in brain was higher than that of muscle ($p<0.05$). Brain acetylcholinesterase activity was 13.8 ± 0.76 μ mol/min/g protein compared to 6.1 ± 1.31 μ mol /min/g protein in muscle. They concluded that acetylcholinesterase activity might be a good early bio-indicator of herbicide exposure of *Leperinus obtusidens*. In 2012, Menendez-Heelman, Ferrey, Santos Afonso, and Salibian studied the toxicity and effect of glyphosate in *Cnesterodon decemmaculatus*, which exposed to glyphosate in the concentrations of 1, 17.5 and 35 mg/L. They found that the fish could survive in all concentrations and acetylcholinesterase activity was in the range of 23-36% . They concluded that acetylcholinesterase could be applied as bio-indicator of glyphosate, which affects to brain.

Moreover, the alteration in tissue of aquatic organism could be an alternative way in assessing glyphosate contamination in the aquatic environment. From assessing these changes, the results could give information about effects in cellular level and could be an appropriate indicator for chronic effect (expose to low concentration in longer period) (Walker et al., 2006). For example, Thanomsit et al. (2016) studied the effect of glyphosate to gill of sea bass and found the tissue alteration in exposed fish. They suggested that it could be applied as warning signal for glyphosate contamination in the aquatic environment. In fish, exposed to glyphosate in the concentration of 2.5 mg/L for 72 h, it was found edema of gill filament compared to fish in the control group, illustrating normal appearance of gill filament and secondary lamellae (Figure 6). That finding is in agreement with the result of Braz-Mota, Sadaus-Henrique, Duarte, Val, and Almeida-Val (2015) which reported that Amazon fish (*C. macroponum*) was exposed to Roundup expressing symptom in gill tissue.

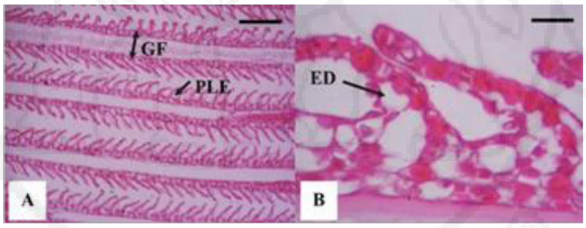


Figure 6 Transverse section of fish in non-exposed and exposed to glyphosate (A) Non-exposed fish showing normal appearance of gill filament and secondary lamellae; where GF: Gill filament, PLE: Primary lamellae epithelium and (B) Fish exposed to glyphosate in the concentration of 2.5 mg/L for 72 h, where ED: Edema (Thanomsit et al., 2016)

5. Measurement of Glyphosate in Aquatic Organisms and Aquatic Environment

In Thailand, contamination and accumulation of glyphosate (GLYP)-containing herbicide, Roundup, has been increasing, especially in sediment, water and aquatic organisms. In general, the assessment can be classified to 2 types: direct and indirect. For direct assessment, the selection of analytical methodologies is based on high polarity of glyphosate. It generally adds feature to form ionic species, and no chromophores groups determining its end product become more complex. Thus, the measurement of this pesticide needs additional processes, which can be quantified by chromatographic methods. However, these methods performance depend on the sensitivity and recovery (Melo, De Nucci, Trape, Jacobucci, & Rosa, 2018). For indirect measurement, acetylcholinesterase is measured by enzyme activity using Spectrophotometer (Yang, Ge, Song, Li, & Chen, 2015) or by immunological technique (You et al., 2016; Thanomsit et al., 2017).

The popular technique for direct measurement of glyphosate is High-performance liquid chromatography (HPLC). Generally, the development of analytical methods for HPLC in measuring is based on derivatization reactions, which end product can be measured by chromatographic technique (Melo et al., 2018). Mercurio et al. (2014) reported that HPLC technique can be applied to measure glyphosate concentration and its stability in sea water of Great Barrier Reef. They found that its half-life was 47 day at 25° C in light and 267 day in dark. Glyphosate concentration in natural sea water was 10 μl/L and then being decreased to 1.42 μl/ L by bacterial consumption. Another technique for direct measurement of glyphosate is Gas Chromatography (GC). It is widely applied technique requiring derivatization by mixing with two compounds: trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE) in excess. These compounds convert glyphosate to AMPA in only a single reaction step (Melo et al., 2018).

Gruber and Munn (1998) suggested that acetylcholinesterase level can be applied as bio-indicator for monitoring and evaluating glyphosate accumulation in organism. Acetylcholinesterase concentration can be measured in both fish brain and blood and the level depending on exposure time and concentration, which expose. For example, Glusczak et al. (2006) studied the effect of glyphosate to acetylcholinesterase activity in bonny fish (*Leporinus obtusidens*) being exposed to glyphosate and they found that acetylcholinesterase in brain of exposed fish was higher than that in muscle. Thus, it can be indicated that acetylcholinesterase may be an appropriate warning signal for herbicide exposure in *Leperinus obtusidens*. In 2010, Modesto and Martinez found that Roundup (glyphosate) at the concentration of 10 mg / L affected to acetylcholinesterase level in brain and muscle of *Prochilodus lineatus* after exposure for 6, 24 and 96 h. acetylcholinesterase activity in brain and muscle of exposed fish was higher than that of the controlled fish after 6 h of exposure. After 24 h, acetylcholinesterase in brain kept higher compared to significant lower ($p<0.05$) in muscle. After 96 h, acetylcholinesterase activity in both of brain and muscle in exposed fish was lower than that in the control.

6. Current Status in Thailand and Future Perspectives

For Thailand, glyphosate application trend is increasing, while agricultural area is still the same. In this phenomenon, many problems are increasing. Pesticide intoxication is a major public health problems, being resulted from intensive applications of pesticides. The trend of pesticide imported has been leveled up from about 110,000 tons in 2007 to approximately 172,000 tons in 2013. Herbicides were highest proportion (62-79%) followed by insecticides (12-23%) and fungicides (5-11%) (Tawatsin, Thavara, & Siriyasatien, 2015).

In this decade, Glyphosate isopropylammonium and paraquat dichloride were the major herbicides being applied at 27 and 13 million kilograms in 2013, respectively (Table 2). Other herbicides such as 2, 4-D sodium salt, 2, 4-D dimethyl ammonium, ametryn, atrazine, butachlor, diuron and acetochlor were also heavily applied in this country with over a million kilograms (of each one per year). They are generally applied for controlling weeds in agricultural fields. They are classified into broad and selective (with specific target) herbicides (Tawatsin et al., 2015).

Table 3 Top ten imported herbicides, insecticides and fungicides by active ingredients (a.i.) into Thailand in 2013

Rank	Herbicides		Insecticides		Fungicides	
	Name	**a.i. (Kg)**	**Name**	**a.i. (Kg)**	**Name**	**a.i. (Kg)**
1	glyphosate isopropylammonium	27,994,397	chlorpyrifos	1,193,302	mancozeb	1,513,307
2	paraquat dichloride	13,823,092	cartap hydrochloride	663,197	carbendazim	644,246
3	2,4-D sodium salt	6,361,633	carbaryl	592,587	propineb	548,961
4	2,4-D dimethyl ammonium	6,121,701	cypermethrin	504,931	captan	472,197
5	ametryn	4,621,614	carbosulfan	432,191	copper hydroxide	459,518
6	atrazine	4,284,683	isoprocarb	382,785	propiconazole	354,286
7	butachlor	2,368,861	dichlorvos	320,994	difenoconazole	347,803
8	diuron	1,776,238	chlorpyrifos+ cypermethrin	263,009	phosphonic acid	245,669
9	acetochlor	1,164,241	fenobucarb	215,289	fosetyl-aluminium	233,929
10	propanil	987,142	profenofos	189,467	metalaxyl	152,848

Source: Tawatsin et al. (2015)

Tawatsin et al. (2015) reported that farmers and farm workers usually work in the field for about 8-10 h a day during the growing season with pesticide applications for 3-8 days each month. Moreover, Thai farmers are not aware of pesticide hazards; in addition, about half of them apply pesticides in the concentrations, which are higher than that of recommendations. And, they applied pesticides without personal protective equipment during spraying and harvesting their products, which may be contaminated. In this situation, farmers and farm workers pose high potential risk of pesticide exposure. In fact, some pesticides; especially, glyphosate can be natural degraded but in the case of intensive and long application natural process cannot accomplish all pesticides causing contamination with water quality degradation. From all the problems mentioned above, many agencies are aware of the contamination and accumulation of glyphosate in the environment.

Thailand Pesticide Alert Network is another agency paying attention in this problem by gathering information and suggesting the limitation of glyphosate application because it is identified as potential carcinogen by WHO. Glyphosate can bind with heavy metals and cause chronic kidney disease. The US Endocrinology Society stated that glyphosate is a substance that interferes with the functioning of endocrine glands (EDCs). Glyphosate is associated with many diseases such as diabetes, obesity, Alzheimer's. In the case of mother receiving glyphosate in high concentration in any routes, her baby will also receive that substance. Although some organizations do not conclude exactly about carcinogenic property of glyphosate; however, in the case of high concentration it may effect on aquatic organisms and water quality (Thanomsit et al., 2017)

In Thailand, there is a lack of study about the effect of glyphosate contamination on aquatic organism and environment although there are many studies in other countries. For example, the effect of Roundup (glyphosate) on economic fish Nile Tilapia (Jiraungkoorskul et al., 2002), Climbing perch (Sawasdee, Phuthonghin, & Kunapratom., 2016), Asian sea bass (Thanomsit et al., 2016) and Water fleas (*Moina macrocopa*) (Jitrapat et al., 2015). Moreover, there was a study performed in egg of apple snail (Thanomsit et al., 2018).

Jiraungkoorskul et al. (2002) studied the acute toxicity (histopathological effects) of Roundup (glyphosate) in Nile tilapia (*Oreochromis niloticus*) and found that adult fish were more tolerant than the young fish. And, it was found histopathological changes in gills, liver and kidneys. For gills, filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm were found. In addition to adult Nile Tilapia, Thanomsit et al. (2019) reported that glyphosate could effect to egg and embryo of Common carp (*Cyprinus carpio*) by considering in morphological alteration, embryonic development, hatching rate, and abnormality in egg and embryo. They found that the percentage of alteration in egg being exposed to glyphosate in the concentration of 5 ppm was higher than that in the control; however, it was lower when compared to 10 ppm of exposure ($p<0.05$). The abnormal percentage in egg of the control, 5 ppm and 10 ppm of exposure were 6.67± 2.31%, 36.67± 7.02% and 66.00± 9.17%, respectively. The abnormal percentage in embryo of 5 ppm and 10 ppm of exposure were 4-5 times higher than the control. Moreover, the effect of highest exposure (10 ppm) to hatching rate was significant ($p<0.05$) higher than that of the control and 5 ppm of exposure. The abnormality in egg and embryo found were the alteration in outer membrane, gill, eyes, and parts that develop into the brain resulting in abnormalities, slow development, and low survival rate.

Sawasdee et al. (2016) studied glyphosate toxicity on Climbing Perch (*Anabas testudineus*) embryos exposed to glyphosate at the concentrations of 0.02, 0.05 and 0.2 mg/L and found that glyphosate at all concentrations affected the fish embryos by increasing mortality and decreasing hatching rate. And, Thanomsit et al. (2016) studied on the effect of glyphosate in Asian sea bass after exposure for 24, 48, 72 and 96 h at the concentrations of 0, 2.5, 5, 7.5 and 10 mg/L. The exposed fish expressed abnormal swim (spinning and losing balance) with faded color on their body. The gums were pale, swollen belly and hemorrhage in various parts of the body. The mortality rate was increased with an increasing in exposure time. The changes in gill tissue occurred in 3 phases. Phase 1 was swelling, agglomeration, and changes in the tissues of the gill area and the lifting of the tissue of the epithelium. The changes that occurred in the second phase were blood congestion. And stage 3, the most severe changes were swelling and necrosis of gill.

Moreover, Jitrapat et al. (2015) studied the effect of Roundup on Water fleas (*Moina macrocopa*) and found that the 48h-EC_{50} of Roundup in *M. macrocopa* was 3.97 mg/L. The size of water fleas depended on concentration and exposure time. The body length of neonates significantly increased except on day 7. Finally, Thanomsit et al. (2019) studied on glyphosate (commercial form) in hatchery of apple snail egg. Hatching rate in exposed egg was over 90% and not significantly different from the control ($p>0.05$). Average hatching rate in the control was 94± 1.97%, while it was 92.88± 3.16% in the exposure treatment. The alteration of apple snail eggs after exposed to glyphosate can be classified into 5 groups: abnormal egg shape, abnormal egg shell, abnormal embryo, disintegrating tissue, and connection of tissue cells. And, the latest study in Thailand was "Acute Toxicity of Glyphosate on embryonic development of Climbing perch (*Anabas testudineus*)". The embryo was exposed with glyphosate at the concentrations of 0.02, 0.05 and 0.2 mg/L. and examined the effect by monitoring the three endpoints: mortality, hatching rate and heart rate. The results indicated that glyphosate in all concentrations affected embryos with increased mortality and decreased hatching rate. There was not found significant differences in the head and tail bud stage, somite stage, and heart rate as comparing to the control. However, there was significant difference ($p<0.05$) in mortality and hatching rate (Sawasdee et al., 2016).

7. Guidelines and Management of Glyphosate in Thailand

For glyphosate application in Thailand, it was the most imported herbicide especially during the year 2012-2016. Most of them were imported from China. In 2016, the imported glyphosate was 61,801,858.54 kilograms, which was higher than 4 years ago. In the importation, there are various forms of glyphosate consisting of 95% glyphosate, 88.8% glyphosate-ammonium salt, 48-95% glyphosate - Isopropyl ammonium. The most imported form was glyphosate-isopropyl ammonium that is limited in application by determining it to be in Category 3 of hazardous substance. It must be registered and permitted before undertaking the business. In 2017, the committee to solve the problems of high-risk pesticides application consisting of the preventative from the Ministry of Agriculture and Cooperatives, the Ministry of Natural Resources and Environment, the Ministry of Industry and the Ministry of Public Health, announced the cancellation of the application of 2 substances: paraquat and chlorpyrifos, in December, 2019. And, glyphosate application is limited the watershed, public areas, rivers, water resources and communities, especially schools, child center and hospital. Based on all the information in this article, it shows the structure, composition, causes of contamination, the effects of glyphosate on aquatic animals, including effects on eggs, larvae or even adults, which are the important problems to aquatic organisms and fishery resources. In the case of no planning, it may affect to the numbers of aquatic organisms and the environment as well as humans may experience problems with food shortages or health risks if consuming aquatic foods with glyphosate contamination. Therefore, the problems should be managed seriously in both: (1) glyphosate application for weed control, using it in the appropriate amount and using it correctly to meet the objectives, and (2) avoiding and being careful of contamination of such substances into public water resources.

Conclusion and Suggestions

In conclusion, a large amount of pesticides consisting of herbicides, insecticides and fungicides have been annually imported into Thailand. In the case of not properly and excessive application, especially glyphosate, it certainly affects to human health and environments, although identifying the true extent of these is quite difficult. Several modes of action relevant to glyphosate-based herbicide toxicity; such as induction of oxidative stress damage, acetylcholinesterase inhibition, and genotoxicity, have been investigated in both target and non-target organisms. Currently, there is no consensus on a single mechanism of glyphosate toxicity; it is likely composed of multiple mechanisms depending on its formula and species. Thus, it still needs to identify the mode of action of glyphosate toxicity in a wide variety of organisms. It is suspected that the current glyphosate effect in Thailand is now underestimated and moreover its chronic toxic effects are likely inconsiderable. For limiting the intensive application of glyphosate, it is crucial to promote the organic farming practices which applies bio-pesticides or biological agents to control weeds instead of herbicides.

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