STUDY ON THE PHYSIOLOGY OF ENVIRONMENT POLLUTION AND THE EFFECT OF HEATING ON THE MALATHION RESIDUE ANALYZED USING RADIOTRACER METHOD

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Abstract

The aim of this study is to determine, the comparison of absorption by aquatic plants *Hydrilla verticillata* and Water hyacinth (*Eichhomia crassipes*) of malathion insecticide residues in water and comparison of malathion concentration in tissues of fish fed with of contaminated water plants (Water hyacinth) with tissues of fish, which was not fed contaminated water plant. The effect of heating the contaminated fish tissue, on its level in tissues of rats that consume it. For the first experiment (aquarium filled with 3 litre of water + *H. verticillata* 100 gr + Water hyacinth 100 gr + 20 uci ^{14}C-labeled malathion); for the second experiment (the first aquarium filled with 3 litre of water + 30 tails of goldfish + 20 uci ^{14}C-labeled malathion; second aquarium filled with 3 litre of water + Water hyacinth 100 gr + 30 tail of goldfist + 20 uci ^{14}C –labeled malathion. For the third experiment (most of contaminated fish tissue in the second experiment was dried at room temperature and then given to 30 mice and partly heated and then given to another 30 mice). Malathion levels were then analyzed by using a liquid scintillation counter LSC-753 (Aloka). The results of all treatments were compared using the Student t-test. It can be concluded, *H. verticillata* was more efficient compared to the enceng gondok in absorbing the insecticide malathion residues in water; malathion concentration in the tissues of fish fed Water hyacinth was higher than those of fish not fed Water hyacinth; contaminated fish tissue residues of malathion, although be heated, can not be lowered significantly, levels in the tissue.

Keywords: ^{14}C-labeled malathion, Hydrilla verticillata, water hyacinth

1. Introduction

Malathion is one of organophosphor insecticide group which is widely used to eradicate insects in the field of health, agriculture, livestock and household, and have a high toxicity to insects, while relatively low toxicity to mammals, so that it is broad-used Matsumura [1]. Malathion kills insects by way of stomach poison, direct contact and through inhalation/breathing. Malathion has very specific properties, can inhibit cholinesterase of acetylcholine (asetilcholinesterase inhibitor) in the body. Insecticides undergo biotransformation in the blood, liver. Some of malathion can be metabolized in mammal's liver and the decrease of the number in the body occurs via esterase hydrolysis pathway. Insecticide level in various tissues increases with the length of time of administration, and then (after ± 2 months) in general showed a decline, even though still in continuing administration [1]. Rai *et al.* [2] reported that organophosphor carbaryl residues in meat (0.0541 mg/mL), eggs (0.0506 mg/mL), and milk (0.0453 mg/mL), were above threshold value based on the FAO/WHO [3]. The report of the Institute of Ecology, University of Padjadjaran, year 1978/1979 [4]. Indicated that the water spinach, velvet-leaf, cassava from Cianjur also contained a high insecticide residues. Even the vegetables sold in the wet market still contained residues of insecticides of 2-4 mg/kg [5]. This residue exceeds the level of ADI (acceptable daily intake) as recommded by the FAO/WHO 1986 [3], which is 0.02 mg/kg for malathion. On the other hand, Isaac *et al.* [6] reported that the susceptibility test of *Aedes aegypti* to malathion indicates the effective dose of malathion has increased from 0.04% to 5%. Daniel [7] also reported that there was an indication that the larval and adult mosquito of *A. aegypti* in Indonesia was resistant to insecticides, including malathion, and this would increase the level of residue around the environment. Insecticide dissolved in the water in the low levels, if it is used in long term, will not cause death, but can cause problems in animals or human physiology that utilize that water. In addition, Mehta *et al.* [8] reported that the organophosphate (endosulfan and chlorpyrifos) could cause changes in shape, size and rupture of lymphocytes cell. Sameeh *et al.* [9] accounted for the malathion could cause degenerative and renal tubular epithelial cell necrosis in rats. Based on the reports mentioned above, the environmental pollution in Indonesia needs to be examined to address and minimize the residue in the aquatic environment and also need to know the pathway of movement from aquatic plants and aquatic animals, and then to in-land mammals (rats).

The purpose of this study was compare the ability of the residue absorption of malathion between *Hydrilla verticillata* and Water hyacinth; and comparison of malathion concentration in tissues of fish fed with of contaminated water plants (Water hyacinth) with tissues of fish, which was not fed contaminated water plant. The effect of heating the contaminated fish tissue, on its level in tissues of rats that consume it.

2. Methods

Materials and tools: goldfish, *H. verticillata*, Water hyacinth, Rats, HNO_3 35%, Diethil ether, ^{14}C labeled malathion, rat food, aquariums, water heater, the appliance section, rat cages, gloves, liquid scintillation Spectrometer, LSC- 753 (Aloka), a solution sintilator (toluens), a small tube.

The first step of the study was to compare the absorption ability of water plants *H. verticillata* and small water hyacinth (*Eichhomia crassipes*) to the Malathion residues in water. An aquarium was filled with water of 3 litres, *H. verticillata* 100 gr, Water hyacinth 100 gr and 20 μ*Ci of* ^{14}*C-labeled* malathion in liquid form (malathion = o, o-dimethyl S-1,2-di(ethoxycarbonyl phosphrodithionate) ordered from the Radiochemical Centre Ltd., Amersham, England. Allowed for 24 hours. After 24 hours *H. verticillata* and Water hyacinth were taken out, and pseudo wet ashing with HNO_3 (material samples, *H. verticillata* and Water hyacinth, respective inserted into a small test tube, add 0.35 mL 35% HNO3, then heated above the water heater at 90 °C for 4 hours). Fluid obtained, mixed with 4 mL solution sintilator (toluene), then left for 24 hours), then counted using liquid scintillation counter, LSC-753, Aloka. The second study was to compare the levels of malathion in fish tissue fed with Water hyacinth with tissues of fish, which was not fed contaminated water plant (Water hyacinth). One aquarium filled with 3 litre of water, 30 gold fish, 20 μ*Ci* ^{14}*C-labeled* malathion. One aquarium again filled with 3 litre of water, 30 gold fish, Water hyacinth 100 gr and 20 μ*Ci* ^{14}*C-labeled* malathion. Allowed for 24 hours. After 24 hours fish tissues (intestine, liver, kidney, meat, scales and brain) were taken and pseudo wet ashing with HNO_3 for 4 hours, then counted using liquid scintillation counter (liquid scintillation spectrometer, LSC-753 (Aloka). If the activities of a radioactived-material is known, the weight can be easily calculated. If you need to know the weight of ^{14}C, this statement will help you, but you have to know also the half-life of ^{14}C, the Avogadro number, and the atomic weight of ^{14}C.

Subsequent treatment was to determine the effect of heating the contaminated fish tissue, on its level in tissues of rats that consume it. Most of contaminated fish tissue in the first experiment was dried at room temperature and then given to 30 mice and partly heated (heated over a water heater at 90 °C for 4 hours), and then given to another 30 mice), that had been fasted for 24 hours. Both treatments were allowed 17 hours. After 17 hours, the food eaten, and mice from the two treatments were killed and organs were collected (liver, kidney, intestines, meat, testes, brain). All the tissues were then undergone pseudo wet ashing with HNO_3 for 4 hours. Concentration of malathion of all treatments were counted using liquid scintillation counter LSC-753 (Aloka).

Data analysis was performed using Student's t-test "Steel and Torrie 1995 [10]". Research was conducted at the Department of Physiology, Pharmacology and the Department of Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, IPB, Bogor.

3. Results and Discussion

Table 1 shows that the leaves and stems of *H. verticillata* can absorb malathion in large amounts with the concentration factor = 0.9870 with 0.0494% of absorption size and was significantly ($P < 0.05$) higher than the Water hyacinth (concentration factor = 0, 3580, with the size of absorption = 0.0179%). This happened probably because most of the Water hyacinth stems and leaves are above water, although according to Matsumura [1], malathion absorbed by the roots will also flow into

Tabel 1. The Average of Concentration Factor and Absorption Size of ^{14}***C-labelled*** **Malathion in** ***H. verticillata*** **and** ***Enceng Gondok*** **(Water hyacinth) After 12 Hours**

Material	Concentration Factor	Size of the Absorption (%)
H. verticillata	0,9870 ± 0.08	$0{,}0494 \pm 0.005^{a}$
Water hyacinth	0,3580 ± 0.04	$0{,}0179 \pm 0.002^{b}$

Different superscript in the same column indicates significantly different

1) Concentration factor = FK = Concentration (aktiviti, uci), per-gram tissue organisme in an aquatic, divided Concentration (aktiviti, uci) per gram in the medium of origin, or concentration in food, are eaten by animals)
2) Measurement of absorption = sample (cpm) of tissues, reduced back ground, divided by FT x 100%;
3) FT = (standard, reduced back ground) multiplied by a radioactive substance given (uci) divided by the standard (uci), in food

the stems and leaves. Hasim [11] reported that Water hyacinth can clean up heavy metal pollutants (Cr = chromium, Cd = cadmium, Hg = mercury, Ni = nickel). Based on this research data *H. verticillata* would be more efficient to use as a cleaner for residual insecticide malathion in aquatic environment, moreover, malathion is very fast to be hydrolised and excreted by the plants [1].

In Table 2 fish tissue from the aquarium that did not contain Water hyacinth, the largest concentration factor occurs in intestinal = 3.0581 with absorption size = 0.1529%. Furthermore, successive absorption size in kidney = 0.0957, liver = 0.0525, scales = 0.0253, meat = 0.0245, in the brain = 0.0179. Goldfish intestine from the aquarium containing Water hyacinth, size of absorption = 0.2977 significantly different ($P < 0.05$) higher when compared with intestinal from the aquarium that did not contain Water hyacinth = 0, 1529. In the liver of the fish that were in the aquarium containing Water hyacinth the absorption size = 0.1993 significantly different ($P < 0.05$) higher compared to the liver of fish from the aquarium that did not contain Water hyacinth = 0.0525. In kidney, brain, scales and meat of the fish from the aquarium containing Water hyacinth showed no significant difference when compared with kidney, brain, scales and meat of the fish from the aquarium that did not contain Water hyacinth. From the data obtained showed that malathion residue could enter the aquatic animals through food, also without food, probably enter the body through the gills, skin or scales. This means animals or humans who are taking a bath or associated with insecticide residues in water have to be careful, although not drink it. Especially if we take a look at the researchers report that the eggs, milk, meat, vegetables had been contaminated by insecticide residues [2-5].

From the data in Table 3 showed that rats fed with malathion residues contaminated-aquatic animal tissue (fish) in the form of raw tissue or heated tissues, will also be concentrated in organs with different concentrations. Sequence based on the largest size of the absorption is: intestinal, kidney, liver, meat, testes, brain. In Table 3 seen that the lowest levels was in the brain (0.0115); This occurs because of the existence of Blood Brain Barrier that loering the rate of passage of the insecticide [1].

From the data in Table 3 showed that malathion could be concentrated in almost all kinds of mammal body tissues with lower levels than those of in aquatic animals. This ocurs because in the mammal's body, malathion molecule is quickly hydrolyzed and excreted [1]". The average size of absorption of malathion in the

Tabel 2. The Average of Concentration Factor and Absorption Size of ^{14}*C-labeled* Malathion in Goldfish in two Aquariums *with and without* Water hyacinth (WH) After 12 Hours

Rats Organ	Concentration Factor without WH	Concentration Factor with WH	Absorption Size without WH	Absorption Size with WH
Intestine	3.0581 ± 0.32	5.9541 ± 0.49	0.1529 ± 0.013[a]	0.2977 ± 0.03[b]
Kidney	1.9154 ± 0.15	2.0581 ± 0.21	0.0957 ± 0.012	0.1029 ± 0.015
Liver	1.0500 ± 0.013	3.9850 ± 0.35	0.0525 ± 0.009[a]	0.1993 ± 0.02[b.]
Brain	0.3580 ± 0.040	0.3850 ± 0.04	0.0179 ± 0.002	0.0193 ± 0.002
Scales	0.5560 ± 0.061	0.3820 ± 0.037	0.0253 ± 0.0028	0.0191 ± 0.003
Meat	0.4890 ± 0.051	0.4780 ± 0.045	0.0245 ± 0.003	0.0239 ± 0.003
Total	7.4265 ± 1.059	13.242 ± 2.318	0.369 ± 0.0053	0.662 ± 0.116

WH = Water hyacinth

Tabel 3. The Average of the Comparison of the Absorption Size in % and in mg of Malathion Insecticides in Rats Tissues After 24 Hours Fed Auatic Animals Tissues-Goldfish (Raw and After Heating) after Contaminating with *Malathion Isotop*^{14}C

Rats Organs	Absorption Size			
	Raw Tissues		Processed Tissues	
	(%)	(mg)	(%)	(mg)
Intestine	0,1050 ± 0.015	4.80×10^{-18}	0,0980 ± 0.01	4.48×10^{-18}
Kidney	0,0905 ± 0.01	4.14×10^{-18}	0,0835 ± 0.005	3.82×10^{-18}
Liver	0,0590 ± 0.008	2.7×10^{-18}	0,0480 ± 0.004	2.20×10^{-18}
Brain	0,0115 ± 0.007	5.26×10^{-19}	0,0130 ± 0.009	5.96×10^{-19}
Testes	0,0158 ± 0.008	7.27×10^{-19}	0,0175 ± 0.008	8.01×10^{-19}
Meat	0,0203 ± 0.006	9.29×10^{-19}	0,0185 ± 0.005	8.46×10^{-19}
Total	0.3021 ± 0.041	13.82×10^{-18}	0.2785 ± 0.037	12.74×10^{-18}

Changing from uci, become mgr: Size absorption divided by one hundred, then multiplied with the total radioactivity in an aquarium, and then multiplied by dpm (number of atoms that disintegrate per minute), and then multiplied again by the atomic weight substance, and divided by avogardo numbers.

organs of mice fed with fresh aquatic animal tissue (given raw meat) in the intestine (0.1050%), kidneys (0.0905%), liver (0.0590%), meat (0.0203%) higher than in the organs of rats fed with heated aquatic animal tissue, in the intestine (0.0980), kidney (0.0835), liver (0.0480), meat (0.0185), but not significantly different.

The total size of absorption of all combined organs of rats (intestine, kidney, liver, meat, testes and brain) that fed raw aquatic animal tissue (0.3021%) higher than the combined rat tissues (intestine, kidney, liver, meat, testes, brain), which fed heated fish tissue = 0.2785%, but not significantly different. So the process of heating the malathion residues contaminated animal organs not significantly reduce the contained residues in the tissues of animals that consume contaminated tissue. Foods derived from aquatic animals that have been contaminated by malathion if comsumed by mammals (rodents) or probably humans will also absorped into their organs.

In fact, malathion enters mammal'sbody through food, respiration, skin, while its excretion can be through urine, feces and other metabolic fluid [1]. From these results, the highest levels found in the digestive tract, so be careful in consuming contaminated animal intestinal organs, because the research from Sammeh *et al.* [9], stating that malathion can cause vacuole formation, degenerative, liver cell necrosis, cytoplasm granulation and increasing of uric acid level. Renata *et al.* [12], also reported that malathion could affect the increase of acid phosphates and alkaline phosphates which would indicate the occurrence of degeneration and lysis of liver cells. "Babu *et al.* [13], reported that malathion could inhibit the function of the liver for metabolism (changing) the drug substance in the liver. Renata *et al*. [12] reported that malathion in a longer period of time, could damage the central nervous system by inhibiting asetilcholinesterase of the brain.

4. Conclusion

H. verticillata was more efficient than water hyacinth in absorbing malathion residues in water. Malathion concentration in tissues of fish fed Water hyacinth was higher than in the tissue of fish which were not fed Water hyacinth; Malathion residues in water could be absorbed by aquatic plants and enter aquatic animal through food, gills, scales and skin, also could enter mammal's body through contaminated aquatic foods consumed. Heating process of malathion residue contaminated animal tissue, could not significantly reduce levels in organs of animals that consume it.

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