Pesticide Exposure and the Level of Reverse Triiodothyronine on School Children in Brebes District—Indonesia

by Suhartono Suhartono

Advanced Science Letters
Vol. 23, 3525–3530, 2017

Pesticide Exposure and the Level of Reverse Triiodothyronine on School Children in Brebes District—Indonesia

Budiyono[1,*], Suhartono[1], Apoina Kartini[1], Soeharyo Hadisaputro[2], and Tjokorda G. D. Pambayun[2]

[1]*Faculty of Public Health Diponegoro University, Semarang, 50275, Indonesia*
[2]*Doctoral Program of Medicine and Health, Medical Faculty, Diponegoro University, Semarang, 50275, Indonesia*

Background: School children in agriculture area in Brebes District are involved agriculture activities. Pesticide metabolites were detected in 31.25% out of 48 school children. Pesticide activated de-iodinase type 3 enzymes and produce reverse Triiodothyronine (rT3). Reverse T3 influences uptake of cellular thyroid hormone. The study aimed to describe the pesticide exposure and level of rT3 in school children. *Method*: Subjects were 84 school children who lived in agriculture area in Brebes District, Indonesia. The study used cross-sectional design. Pesticide metabolites in urine were measured by HPLC with Triple Quadrupole Tandem Mass Spectrometry detector. Serum samples were examined by ELISA method for rT3 detection. Data were analyzed using Mann-Whitney tests ($\alpha = 0.05$). *Results*: In 51.2% out of 84 subjects, three of six types of dialkyl phosphate metabolite were detected in urine. The type of pesticide metabolites were diethylthiophospate (35.7%), dimethylthiophospate (28.6%), and dimethyldithiophosphate (8.3%). The mean levels of diethylthiophospate were 0.01 ± 0.019 ppm (0.001–0.1 ppm), of dimethylthiophospate 0.015 ± 0.034 ppm (0.001–0.14 ppm), and of dimethyldithiophosphate 0.042 ± 0.013 ppm (0.026–0.064 ppm). The mean level of rT3 was 323.21 ± 193.78 pg/ml (97.22–864.56 pg/ml). All subjects had rT3 above the normal level (25–75 pg/ml). There was a significance different between the mean level of rT3 among school children who were exposed and non exposed to pesticide ($p < 0.001$). *Conclusion*: Pesticides exposures are thought to increase the activity of D3 and have an impact on increasing the levels of rT3 level in school children in the agriculture area. All subjects may indicate cellular hypothyroidism and needs to be confirmed by assessed free T3/reverse T3 ratio.

Keywords: Pesticide Exposure, Reverse T3, School Children, Agriculture Area, De-Iodinase Type 3.

1. INTRODUCTION

Endocrine disrupting chemicals (EDC) are the compounds that alter the normal functioning of the endocrine system of both wildlife and humans.[1] Pesticide is one of the endocrine disruptors.[1–3] Thyroid hormone endocrine disruptors interfere with the synthesis of thyroid hormone (T4 and T3) by inhibiting thyroid peroxidase (TPO) activity, iodine uptake, and de-iodinase activity, thyroid hormone binding to transport proteins, and thyroid hormone metabolism and excretion which all result in the alteration of thyroid hormone levels.[4]

An experimental study found out the organophosphate pesticide (chlorpyrifos) reduces thyroid hormones (T3 and T4) level in mice blood serum, significantly and results hypothyroidism.[5,6] Chlorpyrifos causes reduction in triiodothyronine (T3) and thyroxine (T4).[7] Pesticide exposure reduces the level of thyroid hormone (fT4) 10–16% in greenhouse workers.[8] Increases dimethyl phosphate (DMP) followed by reduced the level of the total tri-iodothyronine (T3) serum.[9] Overt hypothyroidism exhibit reduces free T4 estimates and increased TSH levels correlates to growth and development.[10,11] Free T4 associates with birth weight.[13] Furthermore, hypothyroidism also correlates to the goiter[14,15] and development (cognitive)[16,17] and will affect growth and present a delay in bone age of children.[11]

Enzyme de-iodinase type 3 (D3) catalyzes the degradation of T4 converted to inactive T3 (reverse T3/rT3).[18,19] The reverse T3 blocks the receptor of T3 and reduces the conversion T4 to active T3.[19] Increase type III de-iodinase resulted in higher demand for T4 and T3.[20] D3 distributes in all tissues[21] and inactivates T3 at tissue and plasma level,[22] both de-iodinase type D1 and D2 in pituitary.[21] The conversion of T4 to active T3 catalyzed by enzyme de-iodinase type 1 (D1). D3 enzyme in tissues competes with D1 enzyme and D3 enzyme will convert T4 to rT3.[23]

Organophosphate pesticide exposure activates enzyme deiodinase type 3 (D3). A study in the Goldfish revealed level of D3 enzyme in mRNA of the liver were increased 2.66 and 4.50

*Author to whom correspondence should be addressed.

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fold in a group that was exposed to monochrotophos with the dosages of 0.01 and 0.1 mg/L.[24]

Children have a potential to be exposed the pesticides. One of the sources of pesticide exposure to children is the family member that has activity in the agriculture.[25] The children playground near an agriculture area can be contaminated pesticides and cause exposure.[26] Organophosphate pesticides (chlorpyrifos) are applied to the shallot plantation in Brebes District of, intensively (three times a week, respectively). Most of school children who live near the shallot planting and rice field are involved in agriculture activities and play in the area. Residents store the onion in the house and some of them spray onion for seed with pesticide for preservation.

A preliminary study in Brebes district showed that more than 50% out of 28 school children pesticide metabolites (dialkyl phosphate) were detected in urine. Pesticide metabolites were also detected in 31.25% out of 48 school children in the agriculture area in the district of Brebes.[27] De-iodinase enzymes are essential control points of cellular thyroid activity that determine intracellular activation and deactivation of thyroid hormones. Approximately 80–85% of T3 is generated outside the thyroid.[28] Local control of cellular thyroid levels is mediated through three different de-iodinase enzymes (type I or D1, type II or D2, type III or D3). Type III de-iodinase (D3) reduces cellular thyroid activity by converting T4 to the anti-thyroid reverse T3 (reverse T3) and it will influence uptake of thyroid hormones in cellular.[29] There is no study of reverse T3 in school children in Indonesia relating to pesticide exposure in agriculture area.

The aims of the study were to describe the potential pathways and sources of pesticide exposure in school children, the type, and level of pesticide metabolites in urine and the level of reverse T3. The level of reverse T3 in school children is compared in subjects who present and absent of metabolites pesticide in urine.

2. METHOD

This was a cross-sectional study including observational data and laboratory results. Subjects were 84 healthy school children in the 4th–5th grade of State Elementary School of Dukuhlo 01, district of Brebes. The subjects were taken by purposive sampling method. Subjects were 4th–5th grade because of technical consideration and it will easier to follow up. Students at the 1st–3th grade rejected and 6th grade prepared study for facing the final examination. Informed consent signed by parents. The parents and the students explain the purposes of the study. Then they explain about the students will be taken the blood specimen with the sterile disposable syringe and get a little pain. The blood specimen will be taken by professional staff from medical laboratory. Next, beside the blood, the students also collected urine specimen and will be interviewed according involvement in agriculture activities. Medical examinations were carried out before the blood and urine specimen collection and interviews. If the subjects have health problem according the research the researcher will responsible by serving medical doctor and medical center. The parents and the subjects will give the reward. The participation in the research is voluntary and they can reject or accept. Finally, the parents who understand the explanation and accepted they will sign the informed consent.

Urine specimens as much as 100 ml and 10 ml of blood specimens were taken from each subject, after the explanation about the study and method of urine collection. Samples of urine were obtained in May and September. Labeled and sterile filed cup (pot urine) delivered to the school children one day before the collection. Subjects collected the first urine sample into a cup in the morning[30] and then tightened and the cup was put into a plastic bag. Urine sample brought to the school by the school children and checked for the volume. If the urine sample did not meet the volume (100 ml), the student were given a labeled new cup and a spot sample was collected. If the student could not provide the sample during the collection, a spot sample was collected.[31] Every urine sample (100 ml) was put into the specimen cup, tightened by the sealer (parafilm) and put into a plastic bag. Then, urine specimen cups were put into a cool box with the cool pack (4 °C and no preservative) and transported (<24 hours)[32] to the Angler BiochemLab Surabaya for pesticide metabolites analysis. Examination of the types and level of pesticide metabolites in the urine (ppm) was conducted by high-pressure liquid chromatography tandem mass spectrophotometry method (HPLC-MS/MS) to detects 6 types of DAP metabolites: Diethyldithiophosphate (DEDTP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Dimethyldithiophosphate (DMDTP), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP).[33]

Analysis of pesticide metabolites was performed by LC-MS-MS[34] (HPLC with Triple Quadrupole Tandem Mass Spectrometry detector HPLC-MS/MS AB SCIEX API 4500™). Qualitative measurements were conducted by monitoring Ion Ratio of 2 multiple reaction monitoring (MRM) pairs for each compound. Quantitative determinations were calculated by single point matrix based calibration at RL. RL (Reporting Limit) is the practical Limit of Quantitation (LoQ). RL is measured in every analysis batch. The precision of the analysis batch had been checked and fulfilled the declared Laboratory Quality Control Criteria. A value below RL for reference only and not granted quantitatively. It means the sample detected below RL the value is out of range the calibration. Limit of Quantitation (LoQ) is the lowest level for which it has been demonstrated that criteria for accuracy and precision have been met, measured once during method validation. The results of analysis is in ppm (mg/L), it was classified into two categories, named ND (not detected) and the quantitative (ppm).

The blood pressure of the students were examined before the blood specimen collection by the medical doctor. The subjects who have blood pressure lower than ≤90/60 mmHg or higher ≥130/90 mmHg were excluded. When the student rejected the blood specimen collection, they were replaced by the healthy one. Ten ml of blood specimens was taken from school children using the disposable syringe and conducted by laboratory analyst. The blood specimen put into the labeled tube and tightened. Blood specimen then were centrifuged and the blood serum in the tube was labeled and put into a cool box with the cool pack and ice was transported to GAKY Laboratory Faculty of Medical, Diponegoro University for reverse T3 analysis. Examination of reverse T3 level (pg/ml) was used by ELISA method. History of exposure in children was measured by interview structure using a questionnaire. This was supplemented by eight semi-structured key informant interviews with village officers, health-care providers, teachers, student's parents. Data was analyzed using descriptive statistics and Mann-Whitney test at α 0.05 error level. The study design was approved by the Commission on Health Research Ethics of the faculty of Public Health at Diponegoro University. All participants gave written informed consent and signed by parents. Ethical clearance was issued by the Commission on Health Research Ethics of the faculty of Public Health at Diponegoro University Semarang Number 91/EC/FKM/2016.

3. RESULTS

Forty percent of 1.8 million peoples of Brebes District are farmers and farmworkers. A total area of shallot planting was 250 km^2 and the production of onion was 297609.9 ton in 2010. Location of the study was in agriculture area in Dukuhlo village Subdistrict Bulakamba Brebes District, an area with mostly shallot planting. Farmers or farmworkers sprayed pesticide more than 3 times a week for every planting (growing) season over 4 months in average. Chlorpyrifos, fipronil, profenophos was the most pesticide found in the agriculture and at home. Production of onion in Subdistrict Bulakamba was 507.706 quintal. Area of Dukuhlo village is 3.05 km^2 and the population is 8925 people. There are 3 elementary schools in the village, named State Elementary School of Dukuhlo 01, 02 and Islamic Elementary School. The study was the state elementary school of Dukuhlo 01 and the prevalence of goiter (increase TSH level) 47.9% of 48 students. The total number of students in Dukuhlo 01 is 243 students, consisting of 110 male and 133 female.

Of 84 subjects, 48.8% were male and 51.2% were female. The age average was 10.3 years old, with the minimal of 8 years and the maximal of 12.01 years old. Among the subjects, 32.1% of the fathers and 39.3% of mothers were farmers and farmworkers. Almost a half (45.2%) of all parents were farmers and farmworkers.

Most of the subjects (97.6%) lived in the surrounding agriculture area since they were born and play area near (≤60 meters) agriculture area was 76.2%. Numbered of 65.5% of school children involved in agriculture activities varied, there were help to bought pesticide, formulated pesticide, sprayed pesticide, washed clothes and sprayer equipment, removed grass and insect from shallot, brought onion to the home, seek and collected onion in the shallot field, cut the leaf of onion and clean attached soil on onion. The subjects involved in the agriculture activities every planting season was 60.7%.

The potential ways to be exposed of the pesticides for school children were: using the container of pesticide for toys (16.7%), using anti-mosquito (mosquito coils, mosquito repellent) that may contain pesticide (86.9%), storage onion at home (16.7%), presence of pesticide and sprayer equipment at home (29.8%), the onion in the house (36.9%), neighbors of subjects who storage onion (44.1%). As many as 51.2% subjects exposed to pesticide (positive) based on pesticide metabolite in urine.

The school children usually played near (≤60 meters) to agriculture areas. The frequent were 3 times per week. Length of involvement school children were involved in agriculture activity was 2.5 years. A frequency of involvement in agricultural activities per growing season with the average 2 times. The average duration to storing onion at home was 9.6 days. The average distance of homes from agricultural areas was 615.9 meters.

Farmer usually put onion on the floor in the living room, warehouse, in the ceiling above the kitchen, terrace, in the kitchen, and beside the house. The farmers also stored onions for seed in the ceiling above the kitchen and poured/sprayed it with pesticide to prevent decay for more than 4 months.

The data included 84 valid measurement for three types of DAP in urine samples. Three types of DAP were not detected, there were DEDTP, DEP, and DMP. Distribution of the other DAP metabolites in the school children samples analyzed are

Table I. Potential pathway to expose the pesticide in school children.

Potential pathways	$n=84$	%Yes
Lived in the near agricultural areas (<1 km)	82	97.6
Play area (near agriculture area/≤60 m)	64	76.2
Involved in agriculture activities	55	65.5
(a) Help buy pesticides	8	14.5
(b) Help formulated pesticides	4	7.3
(c) Help spray pesticide	3	5.5
(d) Help wash clothes or pesticides sprayer equipment	6	10.9
(e) Help remove insect from shallot plant	9	16.4
(f) Help remove grass	17	30.9
(g) Bring the yield of onion to the home	28	50.9
(h) Seek and collect onion in field	36	42.9
(i) Help cut the onion leaf	51	65.5
(j) Help clean attached soil from onion	10	18.2
Involved every planting season	51	60.7
Using pesticide containers for toys	14	16.7
Using anti-mosquito	73	86.9
Storage onion at home	14	16.7
Presence of pesticide and sprayer equipment in home	25	29.8
Presence of onion in the home	31	36.9
Neighbor's storage onion	37	44.1
Pesticide exposure status (positive)	43	51.2

Table II. Potential source and duration of exposure to pesticide in school children.

Variables	N	Mean	SD	Min.	Max.
Frequency of play in agriculture area (times/week)	64	3.02	2.07	1	7
Length of involvement in agricultural activities (year)	55	2.55	1.44	1	7
Frequency of involvement in agricultural activities per planting season (times/season)	49	2.08	1.29	1	7
The duration of storage yield of onion at home (day)	13	9.62	9.54	2	30
Distance between home and agricultural areas (meter)	74	651.91	838.48	3	7000

Table III. Type and level of DAP metabolites, pesticide exposure status, and the level reverse T3 in school children.

	N	Mean	Median	SD	Min.	Max.	p-value
DETP	30	0.01	0.005	0.019	0.001	0.1	
M	15	0.016	0.007	0.02	0.002	0.1	0.02
F	15	0.005	0.002	0.006	0.001	0.02	
DMTP	24	0.015	0.004	0.034	0.001	0.14	
M	10	0.03	0.005	0.051	0.001	0.14	0.5
F	14	0.005	0.004	0.004	0.002	0.018	
DMDTP	7	0.042	0.043	0.013	0.026	0.064	
M	3	0.033	0.027	0.012	0.026	0.047	0.1
F	4	0.049	0.0455	0.01	0.041	0.064	
rT3	84	323.21	233.8	193.8	97.22	864.6	
M	41	319.18	229.48	196.61	97.22	864.6	0.9
F	43	327.04	266.53	193.28	113.04	758.3	
rT3	84	323.21	233.8	193.78	97.22	864.6	
+	43	392.19	373.39	203.07	126.24	8646	<0.001
−	41	250.86	177.11	155.35	97.22	726.8	

Notes: M = male, F = female, + = presence pesticide, − = absence pesticide.

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presented in Table III. DAP metabolite detected in more than 50% of the subjects in total, with DETP being detected most frequently (35.7%). DMTP detected in 28.6% subjects and DMDTP detected in 8.3% subjects. The concentration of DETP was a significantly greater ($p = 0.02$) in male than female school children. There were no significant different concentration of DMTP (p-value 0.5) and DMDTP (p-value 0.1) in male and female. There was also no significant different the level of reverse T3 in male and female (p-value 0.9).

The average level of reverse T3 was 323.21 pg/ml. It was higher in the group with the present pesticide (positive) (392.19 pg/ml) than in group was absent of pesticide (250.86 pg/ml). There was a significance different the mean level of reverse T3 among school children who positive pesticide metabolite and negative pesticide metabolite ($p < 0.001$).

4. DISCUSSION

Some pesticide will undergo to air by evaporation and to soil directly after sprayed and washed by rain. More than 90% of pesticides used in California are prone to drift.[35] Over 98% sprayed insecticide reach non-target[36] and it undergoes to soil, air and water. Degradation of chlorpyrifos in the water is up to 75 days and the dissipative half-life (DT_{50}) in the soil depends on the soil characteristic. Degradation of pesticides in the soil is affected by hydrolysis mechanism that contained organic matter. Hydrolysis the pesticide is slower in clay minerals, humic, dissolved organic matter, and suspended sediment.[37] It will take longer time the presence of pesticides in the soil. The addition of organic matter to the soil, in the form of biochars, increase the persistence of chlorpyrifos from a DT_{50} of 21.3 days to 55.5 days, and to $158 + 10.1$ days in sterilized soil.[38] Based on previous study the residues of chlorpyrifos in the alluvial soil in field of shallot plantation in district of Brebes detected 0.39–0.72 mg/kg.[39] After harvest, the soil may contained pesticides from shallot field still attaching on onion and it will contaminated and exposed to school children. The pathway can be explained that the soil contacted to skin or dust from the soil inhaled when the school children cleaned soil from onion and then exposed to pesticides.

The pesticides also rested in onion crop for several days. The concentration of chlorpyrifos residues in onion crop in Brebes ranged 0.001–0.039 ppm and profenofos ranged 0.009–0.9902 ppm.[40] Other data showed chlorpyrifos detected in onion crop in Brebes ranged 0.0011–0.0052 ppm and profenofos concentration was 0.0063 ppm.[41] Mean concentrations of some organophosphate pesticide residues in different parts of onion varied. Confirmed by Akan et al., the levels of these pesticide residues in root ranged 11.2–59.08 μg/g; 13.54–131.04 μg/g in stem and 17.86–156 μg/g in leaf.[42] The presence of pesticides in the onion crop will contaminate and expose to the school children through skin and inhalation due to their activities to cut the stem and root from onion.

House is one of the potential sources of pesticide exposure because the yield of onion may contain pesticide is usually stored at home. Residues of many pesticides are found in and around the houses.[35] The pesticide that may contain in the onion will evaporate and contaminate to the school children.

Soil which attached on onion can drift on the floor of the living rooms. The duration to stored onion at home were varied (3–30 days). The longer to storage onion the more dust/drift leaved on the floor. The dust on the floor will contaminate to the residents, including school children. Dust also came from neighbors which stored onion at home.

Farmers or farmworkers stored onion for seed on the ceiling of the kitchen and sprayed/poured by the pesticide powder. Dust from stored the onions for seed on the ceiling that contain pesticide can fall out on the kitchen floor and tableware. The dust on the floor will stick to the skin or inhaled by the occupants of the house, including school children. Azinphos-methyl was the pesticide residue found most often in farmworkers home.[43] Confirmed by Lu, the mechanism of exposed children to pesticides through dust in the floor of the house contaminated to the hands and then absorbed to body. The pesticide detected in urine of children.[44]

School children can contaminate pesticide due to involvement in agriculture activities. They can direct contact with soil, onion crops (through root, leaf, and stem). Clothes and sprayer equipment used by parents (almost 50% parents as a farmer or farm worker) may contaminate pesticide residues. School children helped parents to cut the leaf, brought onion to the home, washed clothes and sprayer equipment and cleaned attach soil from the onion. The subjects did these activities with bared hands and didn't use personal protection devices such as rubber gloves or masker. It may absorb pesticide residues from onion leaf, stem, root, and soiled onion through contacted skin. Dust from soiled onion can inhale by school children that involved in agriculture activities. Pesticides can expose to the body through ingestion, inhalation, or dermal contact.[25,45]

Children lived in agricultural area can be exposed to pesticides used in agricultural production.[46] Pesticide at agriculture area can blow by the wind to the residential and playground because of the transformation. In gaseous phase pesticide can be transported over long distances through wind and rain.[47] Our finding showed amount 4 out of 74 respondents' home had the distance ≤60 meters and the average distance was 651.91 meters from agriculture area. The distance within 500 meters to a farm significantly ($p = 0.002$) associated with urinary dimethylalkylphosphates (DMAP).[48] Distance is closer to the agriculture area the more risky to get exposure to pesticide. Median pesticide concentrations in house dust ($p = 0.01$) and metabolite concentrations in urine ($p = 0.01$) were significantly higher in the children living near treated orchards (within 60 m) than those living more distance.[44] Several factors influenced magnitude of exposure by pesticide from microenvironment (home), there are concentration, duration, and frequency of exposure.[49] It may the major route of exposure to the pesticide in the school children was skin contact and inhalation. The other source of pesticide in the home is anti-mosquito (mosquito coils, mosquito repellent) and it is potential source of exposure to pesticide.

Our finding showed the mean level of reverse T3 were higher in subjects who positive pesticide metabolite compared to subjects who negative pesticide metabolite ($p < 0.001$). The mechanisms of EDCs involve divergent pathways including (but not limited to) estrogenic, anti-androgenic, thyroid, peroxisome proliferator-activated receptor, retinoid, and actions through other nuclear receptors; steroidogenic enzymes; neurotransmitter receptors and systems.[3] Estimated more than 70% of T4 is produced in the thyroid[50] is eventually de-iodination in peripheral tissues (liver, kidney),[28] either at the outer phenolic ring to form T3 or at the inner tyrosyl ring to form rT3.[51] T3 is the most metabolically active thyroid hormone[52] and 80–85% of T3 is generated outside the thyroid gland.[28]

Organophosphate pesticide activated enzyme de-iodinase type 3 (D3). The level of D3 enzyme in mRNA of the liver was increased in a group that exposed to organophosphate pesticide (monocrotophos).[23] De-iodinase type 3 (D3) catalyzed the degradation of T4 converted to reverse T3. Reverse T3 is a competitive inhibitor of T3, blocking T3 from binding to its receptor and blocking T3 effect, and reduces metabolism.[19] Endocrine disruptors interfere with the synthesis of thyroid hormone (T4 and T3) by de-iodinase activity result in the alteration of thyroid hormone levels.[4] Reverse T3 is a normal component of human serum and thyroglobulin.[28] Peripheral metabolism of T4 is the major source of rT3 (about 97%) and it is derived from T4 mono deiodination.[19] About 45–50% of the daily production of T4 is transformed into rT3.[28]

Higher reverse T3 level correlated to thyroid hormones (FT4 and FT3).[53] According to Holtorf, people have high reverse T3 and normal thyroid levels (TSH, T4), actually suffer from hypothyroidism. A reverse T3 level above 150 or a Free T3/Reverse T3 ratio that exceeds 0.2 (when the Free T3 is measured in pg/ml may indicate hypothyroidism.[19] A high reverse T3 demonstrates that there is either an inhibition of reverse T3 uptake into the cell and/or there is increased T4 to reverse T3 formation.[29] Reverse T3 is an excellent marker for reduced cellular T4 and T3 levels not detected by TSH or serum T4 and T3 levels.[19] Reverse T3 was associated with shorter survival in elderly.[52] Reverse T3 testing will be useful for clinical purpose.[10]

Based on reverse T3 parameter, all of the school children had above range levels of the laboratory reference (25–75 pg/ml) of reverse T3. The high or high-normal levels (150 ng/dl) of reverse T3 may indicate tissue/cellular hypothyroidism.[29] Elevated or high-normal rT3 is the reliable marker for reduced transport of thyroid hormones and an indication that a person has low cellular thyroid levels. The impacts of cellular hypothyroidism are weight gain, fatigue, stress, metabolism, aging, and development. It needs to assess free T3 and reverse T3 ratio to ensure proper diagnostic of cellular hypothyroidism by following-up the study. The local government needs to evaluate the organophosphate pesticide exposure to the school children, especially in agriculture area.

5. CONCLUSION

The concentration of DETP was significantly higher in male than female, however there was no difference in rT3 concentration among the genders. The mean level of reverse T3 were significantly higher in subjects who had positive pesticide metabolite compared to the subjects who had no pesticide metabolite. Pesticides exposures are thought to increase the activity of D3 and have an impact on increasing the levels of rT3 level in school children in the agriculture area. All of the subjects had above normal levels of reverse T3 and may indicate cellular hypothyroidism. These data will be important in evaluating the organophosphate pesticide exposure in the school children in agriculture area. These data can be used to determine the effectiveness of the regulation to reduce exposures to school children. The study can be followed up by assessed free T3 and reverse T3 ratio for the proper/confirmed diagnostic of cellular hypothyroidism.

Acknowledgments: The authors would like to thank the school children, parents, and head and staff of Government Elementary School of Dukuhlo 01 for their participation in the study and the students and laboratory staffs who collected the data. This project was funded by Diponegoro University (#1051-28/UN7.5.1/PG/2016).

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Received: 8 October 2016. Revised/Accepted: 31 December 2016.

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