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Pesticide Metabolites, Anti-Thyroid Peroxidase and Thyroid Stimulating Hormone Status in School Children: A Preliminary Study in Agriculture Areas in Indonesia

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Abstract

Pesticides, which commonly use in agriculture areas, are classified as endocrine disrupting chemicals (EDCs). The study aimed to analyze status of pesticide metabolites, anti-thyroid peroxidase (anti-TPO) antibodies and thyroid stimulating hormone (TSH) status in school children of agriculture areas. Thiswasa cross sectional study. Study subject consisted of 48 elementary school students in Brebes District. Examination of pesticide metabolites used urine sample to detect diethylthiophosphate (DETP) and dimethyl dithiophosphate (DMDTP),using LC MS-MS. Both TSH and anti-TPO used serum sample and were examined by ELISA. Data was analyzed using chi square test (α 0.05).

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Mean level of urine DETP was 0.00423 ppm (ranged 0.000-0.043 ppm, SD 0.0099), while urine DMDTP was 0.00953 ppm (ranged 0.00-0.064 ppm, SD 0.019). Anti-TPO ranged 59.29-491.47 IU/ml, with mean level of 167.48 IU/ml (SD 14.19 IU/ml). TSH ranged 1.23-31.56 ml U/L and mean level of 6 ml U/L (SD 5.35 ml U/L). Pesticide metabolites detected in 31.25% of subjects, while positive anti-TPO ($>$200 IU/ml) found in 25% of subjects. Subclinical hypothyroidism (TSH $\geq$4.5 mI U/L) was detected in 47.9% of subjects, while 52.1% was euthyroid. Mean levels of urinary iodine excretion (UIE) was 607.27 µg/L, which above requirements UIE level ($\geq$200 µg/L). Proportion of school children who have subclinical hypothyroidism and pesticide metabolites were 66.7%; subclinical hypothyroidism and anti-TPO were 41.7%; while pesticide metabolite and anti-TPO were 20%. There was no significant association between pesticide metabolites and subclinical hypothyroid incident (p-value 0.08, PR 1.69, 95% CI: 0.97-2.9). There was also no significant association between pesticide metabolites and anti-TPO (p-value 0.61, PR 0.83, 95% CI 0.39-1.75). Proportion of school children with positive pesticide metabolites who suffer from subclinical hypothyroidism were higher (66.7%) than negative subclinical hypothyroidism (33.3%). Iodine above requirements may serve as a risk factor of hypothyroidism and autoimmune thyroid disease.

Keywords: pesticide metabolites; thyroid peroxidase antibody; thyroid stimulating hormone; subclinical hypothyroidism; school children.

1. Introduction

Subclinical hypothyroidism in children, which is characterized by low tetraiodothyronine levels (FT4), is an abnormal and elevated level of sensitive Thyroid Stimulating Hormones (TSH) [1,2]. Studies from the National Health and Nutrition Examination Survey (NHANES III) to the US population over 12 years of age between 1988 and 1994 showed prevalence of subclinical hypothyroidism was 4.3% [3]. Hypothyroidism in infants and children significantly affect the growth and development if not diagnosed and left untreated properly [4]. Hypothyroidism can affect the appearance of goiter, which may lead to low learning achievement in elementary school children. A study showed incidence of goiter in school children was 97%, and school children with goiter has lower average grade point compared to non-goiter students [5] .

Pesticides, chemicals that widely used in agricultural areas, are classified as Endocrine Disrupting Chemicals (EDCs). One of the disrupted hormones by exposure to pesticides is a thyroid hormone. Organophosphate affects the immune response, one of which is the production of antibodies either in animals and humans [6, 7]. TPO activity can be inhibited by anti-TPO [8]. TPO is a key enzyme thyroid to catalyzed for iodination and coupling reactions for the synthesis of thyroid hormones [9].Working in agricultural fields are risk factors of TPO activity. Elevated titers of anti-TPO were found in 20.37% (22 out of 108) greenhouse workers who used chlorpyrifospesticide [10].

Based on our previous study, prevalence of hypothyroidism in school children in an agricultural area was 32%. In addition, we found more than 50% pesticide metabolites in the urine of school children. Iodine intake in school children was in normal range. Therefore, we assume the hypothyroidism did not relate to iodine intake. School children in agricultural areas in Brebes Districts usually help their parents or play around agriculture field. This condition increases their risk to pesticides exposure.

2. Material and Method

This was an observational, cross sectional study. Subjects were 48 out of 50 school children in 4th grade of State Elementary School of Dukuhlo 01, Brebes District. Informed consent and medical examinations were carried out before the blood and urine specimen collection and interviews. Urine specimens as much as 100 ml and 10 ml of blood specimens were taken for each child. Spot samples of urine were collected from children at the beginning of morning. Half of urine sample (50 ml) was put into a urine specimen cup, labeled, and transferred on cool pack (-4 ^{o} C) to the BiochemLab Angler Surabaya for pesticide metabolites analysis. Another half was transported to GAKY Laboratory Faculty of Medical, Diponegoro University for urinary iodine excretion (UIE) analysis. The blood serum was labeled and transferred on ice in to the Cito Laboratory-Tegal for thyroid hormone analysis. Another half was transported to GAKY Laboratory Faculty of Medical, Diponegoro University for anti-TPO analysis. Examination of the types and levels of pesticide metabolites in the urine (ppm) was conducted by liquid chromatography tandem mass spectrophotometry method (LC MS-MS), examination of thyroid hormone status (TSHs, FT4, T3) and anti-TPO by ELISA, while urinary iodine (UI) by colorimetric method with acid digestion. History of exposure in children was measured by interview and observation using questionnaire. Cut of point for hypothyroidism was ≥4.5 mI U / L TSHs [3], anti-TPO ≥ 200 IU / ml [11], and UIE≥200 ug / L [12]. Data was analyzed using descriptive statistics and chi-square test at α 0.05 error level. Ethical clearance was issued by Health Research Ethics Committee of the Faculty of Public Health, University of Diponegoro Number 117 / EC / FKM / 2014.

3. Results

Dukuhlo village is one of the agricultural areas. Like other agricultural areas in Brebes District, onion is the main product. The use of pesticides by onion farmers is very high. Subject was consisted of 27 males and 21 females of 4th grade students. Most parents only completed elementary school (79.2%). Father's occupation is mostly as traders (62.5%), while most of mothers work as farm workers (37.5%). A total of 47 school children (97.9%) lived in the village Dukuhlo since birth.

Mean of anti-TPO in school children was 167.48±14.19 IU / ml. A total of 12 school children (25%) has anti-TPO (≥ 200 IU / ml). Examination of pesticide metabolites consisted of DETP and DMDTP. Mean DETP was 0.00423±0.0099 ppm. Mean DMDTP was 0.00953±0.019 ppm. A total of 31.3% school children was detected to have positive pesticides metabolites. Mean level of TSHs was 6±5.35 ml U/L. prevalence of subclinical hypothyroidism was 47.9% (23 out of 48 subjects). The average level of FT4 was 16.89±1.00 mI U/L. Mean levels of T3 on school children was 1.84±0.29 mI U/L.

Mean of iodine excretion in the urine of school children was 607.27±30.46 µg / L, ranged 412 µg/L-616 µg/L(SD 30.46 µg / L). Based on the table 1, all subjects (100%) had urinary iodine above requirement (≥200 µg / L). As for the risk to pesticides exposure, we found most subjects are involved in agricultural activities such as separating or cleaning onion (81.3%), and look for the onions (62.5%). In addition, almost all subjects played outside the house (95.8%), mostly in farm areas (81.3%)

Table 1: Anti-TPO, pesticide metabolites in urine, thyroid hormones and activities of school children

Variable	Min.	Max.	Mean	SD	n=48	%
Anti-TPO(IU/ml)	59.29	491.47	167.48	14.19		
DETPin urine (ppm)	0.00	0.043	0.00423	0.0099		
DMDTPin urine (ppm)	0.00	0.064	0.00953	0.019		
TSHs (mlU/L)	1.23	31.56	6	5.35		
FT4 (mlU/L)	13.54	25	16.89	1.99		
T3 (mlU/L)	1.27	2.93	1.84	0.29		
UIE (µg/L)	412	616	607.27	30.46		
anti-TPO (>200 IU/L)					12	25
Presence/positive pesticide metabolites in urine					15	31.25
Hypothyroidism (TSHs≥4,5 mlU/L)					23	47.9
Above requirements UIE (>200 µg/L)					48	100
Children involved in agriculture activities					39	81.3
Children seeking onion					30	62.5
Children playing in outdoor					46	95.8
Children playing in agriculture area					39	81.3

Anti-TPO= anti-thyroperoxidase antibodies; DETP=diethylthiophosphate; DMDTP=dimethyldithiophosphate; TSHs=sensitive thyroid stimulating hormone; UI=urinary iodine

The relationship between independent variables and dependent variable (not showed in table)

There was no relationship between presence/positive pesticide metabolites and anti-TPO on 4thgrade school children (p-value 0.59). Proportion of school children with anti-TPO who suffer hypothyroidism was 41.7% (5 students) compare to anti-TPO who not suffer hypothyroidism 58.3% (7 students). There was no correlation between the anti-TPO and the incidence of hypothyroidism (p-value 0.62). The school children with presence/positive pesticide metabolites in urine who suffer from subclinical hypothyroidism was 10 subjects (66.7%). There was also no relationship between presence/positive pesticide metabolites and incidence of hypothyroidism (p-value 0.08). We found 35.9% school children with farming activities have presence/positive pesticide metabolites in urine. However, there was no relationship between their involvement in farming activities and the presence/positive of pesticide metabolites (p-value 0.38). Proportion of school children who collected onion and have presence/positive pesticide metabolites was 30%. There was also no relationship between collecting onion and presence/positive pesticide metabolites (p-value 1).

4. Discussion

4.1 Pesticide exposure

Pesticide metabolites urine is a good method to determine the exposure of pesticides in the human body [13]. In general, exposure to pesticides that occurs in agricultural areas can also occur in the home. Children of farm workers are particularly vulnerable to exposure to pesticides because they may be exposed to pesticides from various ways such as found near agricultural areas, exposure brought into the home of his parents, and the milk of the mother [14].

In our study, a total of 15 students (31.3%) were detected positive DETP and DMTP in urine. This proved that one third of school children have been exposed to pesticides. The DETP and DMTP metabolite is the types of organophosphate pesticide groups [15]. Children who live in the area of agriculture or family as a farm worker can exposed to organophosphate pesticides [16]. Our study was in accordance with Eskenazi et.al that children living in agricultural areas were exposed to higher levels of pesticides than children in other places [17], because pesticides from farming may get into homes through family members, pesticides flowing body of water, breast milk of women work in agriculture fields, as well as playing near agricultural land.

Based on our observations, all subjects reside in the area of agriculture of onion. Most of subjects helped their parents to clean soil attached to the onion, release dry seed of onion, and collect good onion in the dump onion garbage. In addition, they play around the onion farming. These conditions allow school children getting pesticides exposure. Theoretically, the exposure of pesticide may be through skin, mouth and respiratory tract [18]. During their activities at onion farming, these children are likely to be exposed on the dust/soil containing pesticides through inhalation or ingestion. According to Budi Gunawan, onion farmland in Brebes District contained chlorpyriphos pesticide residues in alluvial soil, which ranged 0.39 to 0.72 mg / kg soil [19]. Another pesticides residues, prophenophos, also found in onion of Brebes District with levels of 0.481 ppm [20]. The active ingredients of pesticides used in agriculture in Brebes District include: chlorpyripos, propineb, alpha sipermetrin, fipronil, prophenophos, chlorantanilliprol, abamectin, and deltamethrin [21].

4.2 Anti-TPO

Anti-TPO is a strong marker for autoimmunity of thyroid disease on human. The anti-TPO is more sensitive than thyroglobuline antibody (TGAb) for predicting hypothyroidism. There were a significant different for sensitivity between the two markers, i.e. 98.1% and 61.8% for anti-TPO and anti-TGAb, p-value< 0,005. Hypothyroidism itself is the most common dysfunction of thyroid in patients with anti-TPO and TGAb [22]. An experimental study showed that TPO activities are inhibited by anti-TPO [8]. Prevalence of anti-TPO in patient group with normal high of TSH (2.5–5.49 IU/ml) is higher (18.6%) than in patients group with low normal of TSH (0.36–2.49 IU/ml) 3%. [23]. Our study was contrast to those other studies as we revealed proportion school children with anti-TPO and suffer hypothyroidism are lower (41,7%) than proportion of school children with anti-TPO without hypothyroidism (58.3%).

4.3 Thyroid hormone and subclinical hypothyroidism

Thyroid hormones in this study include free tyroxine (fT4), triiodothyronine (T3) and TSHs. Standard for TSH 0.2± 4.5mU/l, fT4 8±20 pmol/l and T3 1,2±2,7 nmol/l [11]. To determine whether school children have hypothyroidism, the most sensitive indicator is TSHs. TSHs level of 4.5 mI U/L is considered as cut of point to determine hypothiroidism [3,24,25].

Our study obtained 47.9% subclinical hypothyroidism in 4th grade elementary Dukuhlo 01 school children. The proportion was greater compared to hypothyroidism on State Elementary School of Dukuhlo 02, another state elementary school at the same area. Only 26% from 27 students of Dukuhlo 02 elementary school experienced of hypothyroidism [26]. The proportion of sub clinical hypothyroidism in our study also higher compared to studies from several areas. In Pakistan, subclinical hypothyroidism prevalence was 8.43% from 83 samples of children aged 6-11 years [27]. A study in Purbalingga showed 40% students of elementary school aged 9-12 years had subclinical hypothyroidism with levels of TSH> 4 mU / L [28]. In Ponorogo, proportion of clinical hypothyroidism in 135 elementary school children aged 9-11 years was 21.5%, while subclinical hypothyroidism was 12.6% [29].

Thyroid hormones T4 and T3 are produced through a series of peroxidation reactions require iodide iodine?, hydrogen peroxide, enzymes thyroid peroxidase, and iodine acceptor protein TG [30]. Hydrogen peroxide produced by the enzyme activity dual oxidase (DUOX / ThOX oxidase) located in the apical pole of thyroid follicular cells [31,32]. Thyroid peroxidase (TPO) facilitate covalent binding of iodide to iodine oxidation reduces H_2O_2 and where they bind to different thyrosine residues in proteins thyroglobulin and form tyrosinesmonoiodinated (DIT) or thyrosinesdiiodinated (MIT) [31,32]. Two diiodotyrosyl, or one monoiodotyrosyl and one diiodotyrosyl, joined by oxidation to form thyroxine [3,5,3 ', 5'-tetraiodothyronine (T4)] and 3,5,3'-tri-iodothyronine (T3) [33].

Although we found organophosphate pesticides (DETP and DMDTP), this organophosphate pesticide group actually does not have a mechanism to inhibits activity of TPO enzyme. Unlike the carbamate pesticide groups such as Mancozeb, which has a mechanism of inhibiting TPO enzyme [34]. The mechanism of organophosphate pesticide groups is increasing reactivity Caspase 3 [35].

Mancozeb is a type of carbamate pesticides that can inhibit the formation of thyroid hormones by inhibiting the enzyme thyroid peroxidase (TPO) so as to reduce the production of T3 and T4 [34]. This is because the function of TPO is to oxidize iodine to be T3 or T4 [36,31,32]. With decreasing levels of T3 / T4 in the blood it will stimulates hypofisis to produce TSH, so that high TSH levels. [34]. However, organophosphate pesticides group does not have a binding mechanism TPO enzyme, in contrast with carbamate pesticide groups (mancozeb) which has a mechanism for inhibiting the enzyme TPO [34]. There was no measurement for metabolites of carbamate pesticide in the urine.

4.4 Iodine intake

Iodine enter with food and drinks in the form of iodine (I^o), which will be converted to iodide (I^-) in the stomach. Iodide is absorbed in the gastrointestinal tract and is distributed in the extracellular fluid, saliva, gastric secretions and breast milk. [37]. Adequacy of iodine is essential for the normal functioning of the thyroid gland [38]. Examination of IUE in the urine is very important because almost all of iodine (90%) is excreted via the urine, thus UIE can describe a person's intake of iodine. At UIE content measurement, the method recommended by WHO and used throughout the world is a method acid digestion with a solution of ammonium persulfate. [39.40]. According to WHO the recommended daily iodine intake requirement is 150 µg/day for adults, 200 µg/day for pregnant and lactating women, and 50-120 µg/day for children [12]. However, in our study the level of UIE was all (100%) above (≥200 µg/L) daily requirement. Above requirement of iodine intake may serve as a risk factor of hypothyroidism [41,12] and thyroid autoimmunity disease [12].

It is interesting that the level iodine intake in the students of State Elementary School of Dukuhlo 01 is high enough. UIE levels ranged 201-299 µg/L were a risk factor of iodine-induced hyperthyroidism between 5-10 years after getting iodized salt. While the levels of ≥ 300 µg/L will have risk on iodine induces autoimmune hyperthyroidism and thyroid disease in vulnerable groups [12]. From our observation and confirmation to health officers and teachers, students never receive iodine supplementation. Therefore, most likely they get iodine intake through food or drink. However, the food that they eat everyday does not reflect an adequate intake of iodine. The possibility is there are other things that lead to the accumulation of iodine in the body.

4.5 Relationships between independents and dependent variables

No relationship between the positive/presence of pesticide metabolites and anti-TPO activity was found in our study. But this study clearly revealed the majority of students have pesticide metabolites DETP and DMDTP in urine. DETP and DMDTP are belongs to organophosphate pesticides [15]. Our study was against previous studies that showed organophosphate affects immune response, especially in antibody production [6,7]. Thrasher et al. reported that chlorpyrifos exposures lead to higher allergic reaction and sensitivity to antibiotics, followed by decreasing of CD5 cells, increasing of CD26 cells and autoantibody [42]. According to Simescu et al, there was elevated anti-TPO titer in greenhouse workers who using pesticide type of chlorpyrifos, trichloropyridinol (TCP), carbofurane, cypermethrin, and dimethoate [10].

In this study, there was no relationship between anti-TPO and hypothyroidism. Based on Kaczur et al, the TPO activity is inhibited by anti-TPO [11]. Disruption of TPO activities will lead to dysfunction of thyroid hormone synthesis. More than 85.6% male and 86.0% female who have TSH >4 U/ml also have positive anti-TPO. This indicates autoimmunity is an important factor in the occurrence of hypothyroidism [8]. Although our study did not show correlation of anti-TPO with hypothyroidism in school children of Brebes District, they still needs to be warn about the harmful of pesticides, because organophosphate pesticide can trigger autoimmunity.

There was also no relationship between the positive pesticide metabolites and the incidence of hypothyroidism on school children 4th grade State Elementary School of Dukuhlo 01, Bulakamba, District Brebes. However, the proportions of school children with positive pesticide metabolites who suffer from subclinical hypothyroidism (66.7%) were higher than positive pesticide metabolites without subclinical hypothyroidism (33.3%). A previous study showed chlorpyrifos, one of organophosphate pesticides, caused a significant slowdown in weight, thyroid weight loss, decreased T3 and T4 and TSH hormone levels on mice. Histologically, there was a decrease in the size of the follicles and the amount of colloids, major degeneration of follicular cells, thickening of the collagen fibers, and narrowing of blood vessels. Histochemically, there was a decrease in PAS reaction. By immune histochemistry, an increase of enzyme caspase-3 and weak expression of thyroglobulin protein were revealed. In ultra structure, the tested mice had smaller heterochromatic nucleus, mitochondrial degeneration and little secretion granules. In morphometric, there was significant decrease in size of the thyroid follicles, percentage of colloidal area and percentage area of collagen fibers [35].

5. Conclusion

Proportion of school children positive pesticide metabolites and suffer subclinical hypothyroidism were higher (66.7%) than negative subclinical hypothyroidism (33.3%). Urinary iodine excretion above requirement may as a risk factor of hypothyroidism and thyroid autoimmune disease.

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References

[1] Paul B. Kaplowitz. Subclinical Hypothyroidism in Children: Normal Variation or Sign of a Failing Thyroid Gland?.Int J Pediatr Endocrinol . 2010:281453. Published online 2010 Jun 13. doi:10.1155/2010/281453

[2] Alice Monzani, Flavia Prodam, Anna Rapa, Stefania Moia, Valentina Agarla, Simonetta Bellone, Gianni Bona. Natural history of subclinical hypothyroidism in children and adolescents and potential effects of replacement therapy: a review. European Journal of Endocrinology. 2013:168:R1–R11.

[3] Joseph G. Hollowell, Norman W. Staehling, W. Dana Flanders, W. Harry Hannon, Elaine W. Gunter, Carole A. Spencer, and Lewin E. Braverman. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab.2002;87:489-499

[4] Mark D Kilby, 2011. The Role of Thyroid Hormones in Placental and Fetal Nervous System Development, e-theses repository, University of Birmingham.

[5] Apoina K. Associatin between struma and grade point average in school children of Elementary School in agriculture area in Brebes District (research report in Bahasa Indonesia), Faculty of Public Health Diponegoro University, Semarang. 2011.

[6] Casale GP, Cohen SD, Di Capua RA. The effects of organophosphate-induced cholinergic stimulation on the antibody response to sheep erythrocytes in inbred mice. Toxicol Appl Pharmacol. 1983;68:198-205.

[7] Johnson VJ, Rosenberg AM, Lee K, Blakley BR. Increased T-lymphocyte dependent antibody production in female SJL/J mice following exposure to commercial grade malathion. Toxicology. 2002;170:119-129

[8] V. Kaczur, Gy. Vereb, I. MolnaÂr, G. KrajczaÂr, E. Kiss, N.R. Farid, and Cs. BalaÂzs Effect of anti-thyroid peroxidase (TPO) antibodies on TPO activity measured by chemilumine scence assay. Clinical Chemistry 43, No. 8, 1997

[9] McLachian,S.M., Rapport B. The molecular biology of thyroid peroxidase: Cloning, expression and role as autoantigen in autoimmune thyroid disease. Endo. Rev. 1992: 13,192-206.

[10] M. Simescu, C. Podia Igna, E. Nicolaes Cu, I. Ion, A.C. Ion, A. Caragheorgheopol M. Negru, M. Pribu, A.Kochanska-Dziurowicz& Anita Stanjek-Cichoracka. Multiple pesticide exposure of greenhouse workers and thyroid parameters., Int. J. Sus. Dev. Plann2014. Vol. 9, No. 1: 15–28.

[11] T Bjøro, J Holmen, Ø Kruger, K Midthjell, K Hunstad, T Schreiner, L Sandnes and H Brochmann (2000).Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trøndelag (HUNT), European Journal of Endocrinology 143:639–647.

[12] WHO, UNICEF, ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination : a guide for programme managers. – 3rd ed, 2007.

[13] Denise Wessels, Dana B. Barr, and Pauline Mendola. Use of Biomarkers to Indicate Exposure of Children to Organophosphate Pesticides: Implications for a Longitudinal Study of Children's Environmental Health. Environmental Health Perspectives • 2003. VOLUME 111 | NUMBER 16 | December

[14] Lambert W.E., Lasarev M., Muniz J., Scherer J., Rothlein J., Santana J., and McCauley L. Variation in organophosphate pesticide metabolites in urine of children living in agricultural communities. Environ Health Perspect. 2005: 113(4): 504–508

[15] Dana B. Barr, Roberto Bravo, Gayanga Weerasekera, Lisa M. Caltabiano, Ralph D. Whitehead, Jr., Anders O. Olsson, Samuel P. Caudill, Susan E. Schober, James L. Pirkle, Eric J. Sampson, Richard J. Jackson, and Larry L. Needham, Concentrations of Dialkyl Phosphate Metabolites of Organophosphorus Pesticides in the U.S. Population, Environmental Health Perspectives, February 2004 VOLUME112 | NUMBER2

[16] Quandt S.A., Arcury T.A., Rao P., Snively B.M., Camann D.E., Doran A.M., Yau A.Y., Hoppin J.A., and Jackson D.S. Agricultural and residential pesticides in wipe samples from farm worker family residences in North Carolina and Virginia. Environ Health Perspect2004: 112(3): 382–387

[17] Eskenazi B., Bradman A., and Castorina R. Exposures of children to organophosphate pesticides and their potential adverse health effects. Environ Health Perspect.1999: 107(3): 409–419

[18] Johnson MP, Easter EP and Horstman SW. Personal Protective Equipment for Pesticide Aplicators, Kentucky University,1992.

[19] Budigunawan AN, chlorpyrifos Residue Analysis in Soil Alluvial After Planting Onion in Brebes, Unpublished Research, Bogor Agricultural University, 2004

[20] Soeriatmadja, R. E., A.L.H. Dibyantoro and I. Sulastrini. Residual insecticides in vegetable crops in vegetable production centers lowland province of Central Java and Yogyakarta. Bul. Penel. Hort. 1993.25 (3): 72-78

[21] Moekasan and RS Basuki, Spodoptera resistance Status oxigua the onion crop origin Cirebon district, Brebes and Tegal to insecticides commonly used by farmers in the area. J. Hort2007 17 (4): 343-354

[22] Ajit S. Shinto, MBBS, DRM,DNB,PGDHA, LeenaPachen, BSc, NMT,DNMT, T.K. Sreekanth, BSc, MRT, DMRIT, Deepu George, MD. Prevalence of Antithyroid Antibodies in Histologically Proven Autoimmune Thyroid Diseases and Correlation with Thyroid Dysfunction in South India. Thyroid Science 5(9):1-5, 2010

[23] Ana Sofia Zelaya, MD; Angela Stotts, PhD; Shahla Nader, MD; Carlos A. Moreno, MD, MSPH. Antithyroid Peroxidase Antibodies in Patients With High Normal Range Thyroid Stimulating Hormone. Fam Med 2010;42(2):111-5

[24] Canaris GJ, Manowitz NR, Mayor G, Ridgway EC.The Colorado thyroid disease prevalence study. Arch Intern Med.2000;160:526-534

[25] Surks, M. I, Ortiz, E, Daniels, G. H, Sawin, C. T, Col, N. F, Cobin, R. H, Franklyn, J. A, Hershman, J. M, Burman, K. D, Denke, M. A, Gorman, C, Cooper, R. S, & Weissman, N. J. (2004). Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. Journal of the American Medical Association, January 2004), 0098-7484, 291(2), 228-238

[26] Apoina K. Factors related to goiter in school children of elementary school in agriculture area. Proceeding of National Seminar Iodine Disorder" Role of National Institute of Health Research and Development (NIHRD) for bridging IDD/GAKI elimination", (in Bahasa Indonesia),Yogyakarta, 2012

[27] Muhammad Ramzan, Irshad Ali, Faiqah Ramzan, Faiza Ramzan, Muhammad Haris Ramzan. Prevalence of Sub Clinical Hypothyroidism in School Children (6-11 years) of Dera Ismail Khan. J Postgrad Med Inst 2012; 26(1): 22-8

[28] Mohamad Samsudin. The prevalence of hypothyroidism in the Regional School Age Children Replete Iodine (in Bahasa Indonesia), BP2 GAKI, National Institute of Health Research and Development (NIHRD),

Ministry of Health (Indonesia)2012.

[29] Sri Supadmi. Serum Ferritin and Hypothyroidism in Primary School Age Children (in Bahasa Indonesia), BP2 GAKI National Institute of Health Research and Development (NIHRD), Ministry of Health (Indonesia), 2011.

[30] Kambe, F., Y. Nomura, T. Okamoto, and H. Seo. Redox regulation of thyroid-transcription factors, Pax-8 and TTF-1, is involved in their increased DNA-binding activities by thyrotropin in rat thyroid FRTL-5 cells. MolEndocrinol1996.10(7):801-12

[31] Pachucki, J., D. Wang, D. Christophe, and F. Miot. Structural and functional characterization of the two human ThOX/Duox genes and their 5'-flanking regions. Mol Cell Endocrinol2004.214(1-2):53-62

[32] Wang, D., X. De Deken, M. Milenkovic, Y. Song, I. Pirson, J.E. Dumont, and F. Miot. 2005. Identification of a novel partner of duox: EFP1, a thioredoxin-related protein. J BiolChem 280(4):3096-103

[33] Stevens and J. Lowe, 1997. Human Histology, pp. 258–261, Times Mirror, Barcelona, Spain

[34] Mark D. Miller, Kevin M. Crofton, Deborah C. Rice,and R. Thomas Zoeller,2009. Thyroid-Disrupting Chemicals: Interpreting Upstream Biomarkers of Adverse Outcomes Environmental Health Perspectives • volume 117 | number7 |July 2009

[35] Abeer M. Shady, and Fayroz I. Noor El-Deen, Effect of Chlorpyrifos on Thyroid Gland of Adult Male Albino Rats, Egypt. J. Histol. Vol. 33, No. 3, Sep., 2010: 441 – 450

[36] WHO. Possible developmental early effects of endocrine disrupters on child health, Geneva, Switzerland, 2012.

[37] David G, Dolores S. Basic and clinical endocrinology. Greenspan’s eight edition. States of America: McGraw Hills companies; 2007

[38] Delange F. The disorders induced by iodine deficiency. Thyroid 1994;4(1):107–28.

[39] Arita E, Evinaria. The pattern of food consumption, nutritional status and its relationship with learning achievement in elementary school in the village of Kuta endemic areas GAKI Dame Empire District of Dairi North Sumatra province. Faculty of Public Health, University of North Sumatra; 2004.

[40] Rachmawati B. Effect of storage time at room temperature (26-34 degrees Celsius) of the iodine content in the urine(in Bahasa Indonesia) Clinical Pathology, Faculty of Medicine, University of Diponegoro Semarang:; 1997

[41] YuqianLuo, Akira Kawashima, Yuko Ishido, Aya Yoshihara, Kenzaburo Oda, Naoki Hiroi, Tetsuhide Ito, Norihisa Ishii and Koichi Suzuki. Review:Iodine Excess as an Environmental Risk Factor for Autoimmune

Thyroid Disease. Int. J. Mol. Sci. 2014, 15, 12895-12912; doi:10.3390/ijms15071289

[42] Jack D. Thrasher Ph.D. , Roberta Madison & Alan Broughton. Immunologic Abnormalities in Humans Exposed to Chlorpyrifos: Preliminary Observations, Archives of Environmental Health: An International Journal, 1993. 48:2, 89-93, DOI: 10.1080/00039896.1993.9938400.