Pesticide exposure, oxidative stress, and metabolic syndrome among male farmers exposure to pesticide

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Pesticide exposure, oxidative stress, and metabolic syndrome among male farmers exposure to pesticide

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This certifies that the Doctoral Dissertation of Kang Myeong Lee is approved.

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The Graduate School
Yonsei University
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그리고 논문 진행 동안 많은 도움을 주신 예방의학교실 및 평생건강관리센터 모든 선생님들께 감사의 마음을 전합니다.

예방의학교실에서 생활하면서 힘들고 어려운 상황이 생길 때마다 옆에서 용기와 힘이 되어준 현숙정선생님, 김동숙선생님, 그리고 수현이 너무 많은 힘이 되었고, 즐거운 시간이었습니다. 고맙습니다. 그리고 논문 실험을 하면서 물심양면 힘이 되어주신 최홍순선배님과 박준호선배님, 호영오빠 진심으로 머리 숙여 감사드립니다. 또한 논문을 쓰는 동안 많은 도움을 준 윤진하선생님 감사합니다.

그리고 일이 힘들거나 어려울 때 항상 옆에서 끊임없는 애정으로 용기와 지혜를 주는 나의 영원한 친구인 현양이, 혜은이, 수연이, 정말 많이 고맙고 당신들이 너무 너무 좋습니다.

마지막은 무한한 사랑으로 어려울 때 항상 옆에서 걱정해주고, 힘이 되고 버팀목이 되어주신 나의 부모님께 고마움과 감사함을 전합니다. 두 분의 헤아릴 수 없이 가득한 사랑과 관심, 인내가 없었다면 지금의 저는 결코 없었을 것입니다. 그 무엇으로도 다 표현할 수 없을 만큼의 헌신적인 두 분의 사랑에 항상 감사하며 모든 일에 최선을 다하는 사람이 될 것을 약속하겠습니다. 그리고 저의 든든한 동생인 강부와 이번에 결혼한 강오와 올케에게도 지면을 통해서 고마움을 전합니다. 옆에서 큰 힘이 되어주시고 사랑으로 대해 주신 작은아빠, 작은 엄마, 삼촌, 강능이, 강인이에게도 감사의 마음을 전하고 싶습니다.

이 외에도 고마운 분들이 많이 계셨지만 이름 하나하나를 되새기지 못함을 죄송하게 생각하며 아낌없는 사랑을 주신 아버지, 어머니께 저의 작은 결실인 이 논문을 바칩니다.

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ABSTRACT

Pesticide exposure, oxidative stress, and metabolic syndrome among male farmers exposure to pesticide

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Background and Objectives

Pesticide exposure and accumulation in the human body significantly affect human health. Pesticide exposure results in the generation of reactive oxygen species in vitro and vivo, which in turn causes biomolecule oxidation, resulting in cell necrosis or tissue damage.

Several studies have examined the relationship between oxidative stress and diseases, such as cardiovascular disease, hypertension, diabetes, and cancer. Therefore, the objective of this study was to measure malondialdehyde and isoprostane which has been used as an index of lipid injury, 8-OHdG, which has been used as an index of DNA damage, and dialkyl-phosphate, which has been used to quantify pesticide exposure, and to investigate the relationship among pesticide exposure, oxidative stress, and metabolic syndrome.

Subjects and Methods

This study was a cross-sectional study that evaluated 84 male farmers exposure to pesticide. In this study, 8-OHdG, isoprostane, and MDA were measured as oxidative stress indices, and dialkyl-phosphate(DMP, DEP, DMTP, and DETP) excreted in the urine was also measured to evaluate pesticide exposure. A logistic regression analysis was performed to investigate the relationship among metabolic syndrome, oxidative stress biomarkers, and pesticide metabolites. In addition, a linear regression analysis was applied to determine the relationship between the oxidative stress and pesticide metabolites.

Results

The group with metabolic syndrome (19 subjects) had a higher concentration of oxidative biomarkers than the group without metabolic syndrome (65 subjects) ($p < 0.05$). The logistic regression analysis revealed a higher concentration of 8-OHdG (odds ratio 3.8, 95% CI 1.23 - 11.71), isoprostane (odds ratio 4.4, 95% CI 1.34 - 14.52), and MDA (odds ratio 6.0, 95% CI 1.28 - 27.69) in the group with metabolic syndrome than in the group without metabolic syndrome.

A Correlation analysis was performed for PEI, CEI, and DAP as well as the concentration of the oxidative stress biomarkers. The PEM significantly and positively correlated to the levels of 8-OHdG, isoprostane, CEI, and DMP. CEI showed a correlation to 8-OHdG. DMP, DEP, and DETP showed a positive correlation to 8-OHdG, isoprostane, and MDA.

A correlation analysis was adjusted some demographic characteristics, such as age, smoking, drinking, and exercise to determine the relationship between pesticide exposure and oxidative stress.

The 8-OHdG, isoprostane, and MDA levels were significantly related to the

DMP (ß = 0.320), DEP (ß = 0.390), and DETP (ß = 0.082); DMP (ß = 0.396), DEP (ß = 0.508), and DETP (ß = 0.504); and DMP (ß = 0.432), DEP (ß = 0.508), and DETP (ß = 0.329) levels, respectively.

When the logistic regression results for the exposure indices (i.e., the dialkyl-phosphate levels and the PEM and CEI) were calibrated to account for age, smoking, drinking, and exercise, significant differences were observed between the groups with and without metabolic syndrome with respect to DMP (odds ratio 2.5, 95% CI 1.09−5.86) and DEP (odds ratio 5.0, 95% CI 1.62−15.52) levels.

In addition, although the group with metabolic syndrome showed higher odds ratios for DMTP and DETP than the group without metabolic syndrome, there were no significant statistical differences in the odds ratios.

Conclusion

The concentration of oxidative stress biomarkers was higher in the group with metabolic syndrome than in the group without metabolic syndrome, and there was a positive correlation between the pesticide metabolites and oxidative stress biomarkers. Indicators of oxidative stress was associated with a pesticide metabolite DMP, DEP, and DETP. Therefore, Pesticide exposure and oxidative stress, metabolic syndrome were relevant.

Key words: farmers, pesticide exposure, oxidative stress, pesticide metabolite, metabolic syndrome

ABBREVIATION

- 8-OHdG: 8-hydroxy-2'-deoxyguanosine
- Isoprostane: 8-iso-prostaglandin F2α
- MDA: malondialdehyde
- GPx: glutathione peroxidase
- Oxidized-LDL: oxidized low density lipoprotein
- BMI: Body mass index
- SBP: systolic blood pressure, DBP: diastolic blood pressure
- LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol
- FBS: fasting blood sugar
- TG: triglycerides
- PEI: pesticide exposure index
- CEI: cumulative exposure index
- Mets: metabolic syndrome
- DMP: dimethylphosphate
- DEP: diethylphosphate
- DMTP: dimethylthiophosphate
- DETP: diethylthiophosphate
- DAP: dialkyl-phosphate

I. INTRODUCTION

1. PREFACE

Pesticides are agricultural chemicals that protect crops and stored products, partially by exterminating harmful insects. Pesticides can be classified into several different types according to their purpose, such as insecticides, fungicides, herbicides, acaricides, rodenticides, nematicides, and plant growth regulators. Pesticides can also be classified according to chemical composition (organophosphates, organochlorines, carbamates, pyrethroids, sulfur, and urea)[1)] or powder type (emulsifier, wettable powder, soluble powder, powder, particle powder, and smoke powder).

Pesticides are extensively used throughout the world and influence the human body through long-term exposure[2)]. For instance, the accumulation of dichlorodiphenyltrichloroethane, benzene hexachloride, and endosulfan causes cardiovascular diseases and hypertension[3)]as well as other health problems in humans. Cardiovascular disease and several risk factors for type 2 diabetes can be considered as a single disease group called metabolic syndrome which is characterized by insulin resistance and various metabolic abnormalities. Previous studies have suggested that the use of organochlorine pesticides (OCPs) is highly related to the risk of metabolic syndrome and to type 2 diabetes[4-5)].

Pesticides produce reactive oxygen species (ROS) in vitro and vivo, which in turn causes biomolecular oxidation, resulting in cell necrosis and tissue damage. In addition, organophosphorus compounds inhibit acetylcholinesterase, which causes oxidative stress. Thus, pesticide compounds increase the levels of free radicals and decrease the effect of antioxidants or free-radical-scavenging enzymes[6-7)].

Malondialdehyde and 8-iso-prostaglandin (isoprostane) are used to evaluate lipid injury, 8-hydroxy-2´-deoxyguanosine (8-OHdG) is used to evaluate protein and lipid injury, and glutathione peroxidase (GPx) is used to evaluate antioxidant activity. These biomarkers are also used to evaluate oxidized low-density lipoprotein (oxidized-LDL). Oxidative stress can be caused by several factors including environmental pollutants, ultraviolet rays, pesticides, cancer, stress, heart disease, cataract, diabetes, and hypertension.

Previous studies performed in humans and animals indicated that organophosphorus pesticides particularly caused such oxidative stress[8-11]. Organochlorine, carbamate, and pyrethroid pesticides have also show toxicity[12-13]and oxidative stress may cause acute and chronic inflammatory diseases[14].

Plasma cholinesterase (butyrylcholinesterase and pseudocholinesterase) has been studied as a possible indicator of pesticide exposure (organophosphorus and organochlorine pesticides). It shows a process similar to the dissolution of acetylcholine by the muscle relaxant succinylcholine. In this process, acetylcholine is immediately degraded by plasma cholinesterase into succinylmonocholine and choline, and the succinylmonocholine is dissolved into succinic acid and choline. Succinylcholine has been widely studied as a biomarker because of its low activity and delayed metabolism[15-18]. Pesticide exposure can thus be usually measured by evaluating the cholinesterase activity in the blood by sampling the blood before and after such exposure. However, this method is qualitative and not quantitative.

A different method for evaluating pesticide exposure is to quantify the levels of urinary dialkyl-phosphate (DAP), which is an organophosphorus metabolite. This method shows excellent sensitivity for biological monitoring and can be used to

quantitatively evaluate the pesticide exposure to the human body. The DAP detected in urine is usually metabolized as dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), and diethylthiophosphate (DETP). The amount of pesticide exposure can thus be quantitatively evaluated by analyzing the amount of these metabolites.

Thus, in this study, subjects who spray pesticides were divided into 2 groups, i.e., with or without metabolic syndrome. Information on pesticide exposure, e.g., the use of pesticides and the duration of use, was collected. In addition, the levels of MDA and 8-isoprostane, which represent an index of lipid injuries, and 8-OHdG, which is an index of DNA injuries, were measured. DAP was measured to quantitatively evaluate the relationship among pesticide exposure, oxidative stress, and metabolic syndrome.

2. BACKGROUND

2.1. Pesticide

Pesticide exposure in humans is classified according to inhalation, skin exposure, and oral exposure. Farmers are usually exposed through inhalation and skin exposure. Such exposure badly affects their health.

Organophosphorus and carbamate pesticides have been widely used in recent years. Organophosphorus pesticides cause acute and subacute toxicity to varying degrees by suppressing cholinesterase activity, which causes symptoms such as dyspnea and convulsions. In particular, acetylcholinesterase (AChE) can be affected by such organophosphorus and carbamate pesticides[19].

A previous study on the chronic toxicity of pesticides showed that the cognitive ability and exercise performance of children in areas sprayed with pesticides were significantly decreased compared with those of children from non-sprayed areas[20].

Pesticide exposure was also found to be significantly related to lung cancer, pancreatic cancer, colon cancer, rectal cancer, leukemia, hematologic malignancy, lymphoma, multiple myeloma, bladder cancer, prostate cancer, brain tumor, and skin cancer[21]. In particular, it has been reported that organophosphorus pesticides such as crotoxyphos, dichlorvos, famphur, diazinon, fonofos, malathion, and phorate, increased the risk of leukemia, non-Hodgkin's lymphoma, and prostate cancer. In addition, chlorpyrifos resulted in increased lung cancer rates upon direct exposure[22].

Several studies have been performed to assess the influence of pesticide exposure on human health and the environment. Chronic pesticide exposure has also been shown to increase the risk of depression[23], in sprayers and their partners[24].

Pesticide compounds have been shown to affect free radicals, antioxidants, and free-radical-scavenging enzymes[25], and organophosphorus pesticides have been particularly shown to inhibit AChE and increase lipid peroxidation[26]. Oxidative stress caused by pesticide exposure also affects erythrocytes and lymphocytes, which leads to health impairment[25]. Thus, the pesticide exposure can be considered to generally increase oxidative stress.

Cholinesterase enzyme activity has been measured in the blood to monitor the exposure to organophosphorus pesticides. However, this method presents a disadvantage in which it is not sufficiently sensitive for occupational and non-occupational settings, and it is not possible to evaluate the exposure quantitatively. A different method for evaluating the exposure is to measure organophosphorus pesticide biomarkers such as DAP in urine[27].

2.2 Metabolic syndrome

Cancer, cerebrovascular disease, heart disease, and diabetes are the 4 most important causes of death[28], and hypertension, diabetes, cerebrovascular disease, and heart disease are the 4 major diseases in Korea[29].

People with metabolic syndrome have a two-to-threefold increased risk of cardiovascular diseases and a fivefold increased risk of type 2 diabetes[30]. Metabolic syndrome can be defined as a combination of some risk factors for cardiovascular disease (coronary artery and cerebrovascular disease) and type 2 diabetes, such as fat content (abdominal fat content), dyslipidemia (increases in triglyceride levels and decreases in HDL-cholesterol levels), hypertension, and insulin resistance.

Fat content and insulin resistance are the most important factors that determine

the metabolic syndrome. Adipocytokine levels, inflammation, oxidative stress, cortisol vascular abnormalities, hypertension, fat metabolism abnormalities, and genetic factors also play a role in metabolic syndrome[31-32].

2.3 Oxidative stress

Reactive oxygen species (ROS) are generated as a result of physiological cellular activity. Although the oxidative stress is normally balanced by the activity of antioxidants, imbalances can result from the excessive generation of oxidants such as reactive oxygen or a lack of antioxidants[33].

In healthy individuals, the concentrations of reactive oxygen and antioxidants are balanced. However, oxidative stress occurs when this balance is disturbed by high concentrations of ROS. Increased ROS caused by oxidative stress can be caused by various lifestyle habits including smoking, drinking, overweight, and stress as well as environmental factors including air pollutants, heavy metals, organic solvents, ozone, and pesticides (Figure 1). Damage from oxidative stress underlies the pathology of aging, atherosclerosis, autoimmune diseases, Parkinson's disease, Alzheimer's disease, and cancer[34-35]. ROS can be measured directly through electron spin resonance or indirectly through the concentration of the oxidants of lipids, protein, and DNA.

In lipid peroxidation, free radicals are generated in cell membranes, which have abundant phospholipids. In particular, a polyunsaturated fatty acid group can easily be reacted with free radicals, and the peroxidation of lipids in fatty acids causes a chain reaction. As a result, many lipid peroxidants are generated because of a single free radical substrate. Levels of malondialdehyde (MDA), oxidized LDL, and isoprostane are used as indices of such lipid peroxidation.

MDA can be analyzed by reaction with thiobarbituric acid (TBA),[36] and which is quantitatively analyzed in the urine using 2,4-dinitrophenylhydrazine (DNPH). The concentration of MDA in the urine is significantly increased in rats exposed to endrin, alachlor, paraquat, smokeless tobacco, adriamycin, and cadmium[37]. In addition, It has been used as a biomarker for lipid peroxidation in most studies on diabetes. Patients with hyperlipidemia or diabetes have shown a higher serum concentration of the thiobarbituric acid reactive substance (TBARS) than that shown by normal controls[38-39].

Isoprostane levels can be used as a different index for measuring lipid peroxidation. Isoprostanes are generated from the oxidation of arachidonic acids, and their concentration is increased by hypercholesterolemia, cigarette smoking, hyperhomocysteinemia, diabetes mellitus, overweight, obesity, and hypertension[40-42].

DNA is a major target of ROS. Although more than 20 adducts involved in DNA injury have been recognized, 8-OHdG is the most highly investigated because of its mutagenic capacity[43]. The 8-OHdG concentration in urine was found to be significantly increased in the patients with type 2 diabetes who also showed increased oxidative DNA injuries[44].

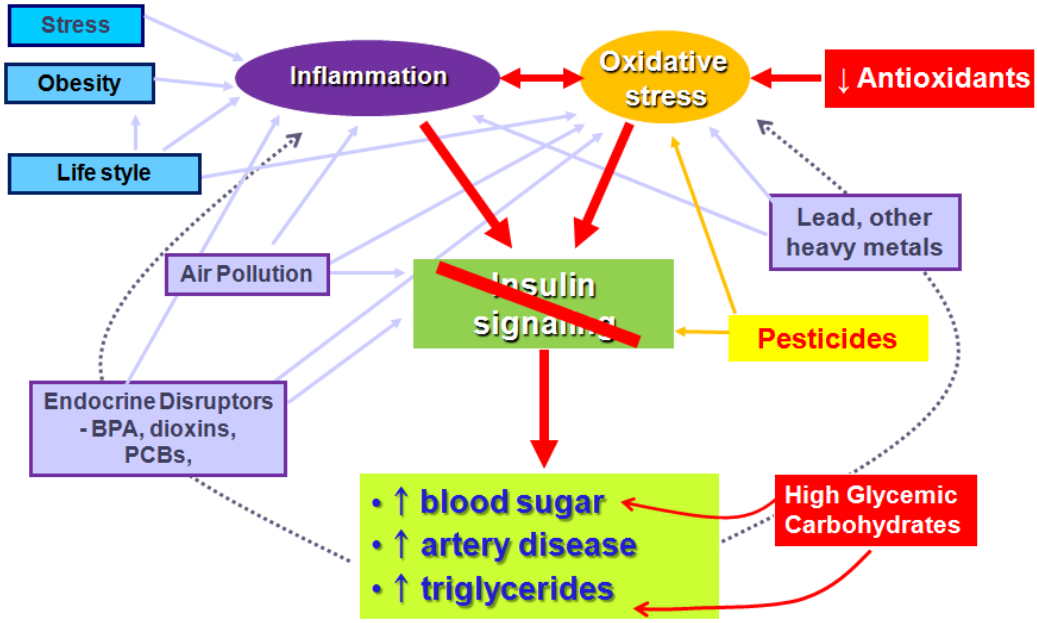


Figure 1. Environmental factors of disrupt insulin signaling and inflammatory metabolism

In addition to MDA and 8-OHdG as discussed above, glutathione, which is an antioxidant that exists in all cells, and other different ROS-scavenging enzymes can be used as indices of oxidative stress and antioxidant activity[45].

II. SUBJECTS AND METHODS

1. Study subjects

This study was a cross-sectional study of 104 male farmers exposure to pesticide who were selected from 126 farmers among the total 197 subjects between May and August 2011. In total, 11 subjects who had stomach cancer, liver cancer, cancer of the large intestine, lung cancer, bladder cancer, type B and C hepatitis, angina pectoris, stroke, and myocardial infarction were excluded. 9 subjects who did not answer the questionnaire were also excluded.

Thus, 84 subjects were selected for the blood tests. The survey focused on general items (e.g., age, smoking, and drinking), pesticide-related items (e.g., spraying of pesticides, duration of spraying pesticides, days of spraying, types of pesticides, amount of pesticides, and method for spraying pesticides), items for observing compliance with regulations related to the spraying of pesticides, health-related items (e.g., diseases in the circulatory system, musculoskeletal system, respiratory system, digestive system, endocrinology and metabolism, cancer, depression, renal failure, and atopic dermatitis), and experiences of pesticide poisoning.

2. Definition of the metabolic syndrome

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), the World Health Organization (WHO), the European Group for the Study of Insulin Resistance (EGIR), and the American Association of Clinical

Endocrinologists (AACE) have defined diagnostic criteria for metabolic syndrome[46-48].

We adopted the NCEP ATP III guidelines, in which the components of the metabolic syndrome are: Metabolic syndrome was diagnosed on the concomitant presence of at least three of the following five features; 1) waist circumference ≥ 90 cm (men), ≥80 cm (women); 2) blood pressure ≥130/85 mmHg or under medication; 3) triglyceride ≥150 mg/dL; 4) fasting glucose ≥110 mg/dL; 5) HDL cholesterol <40mg/dL (men), <50mg/dL (women).

3. Metabolic data collection

Fasting glucose, total cholesterol(TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein(LDL), and cholesterol were measured using enzymatic methods (ADVIA 1650, Simens, Tarrytown, NY, USA)[49].

4. Oxidative stress biomarker assays

4.1. 8-OHdG

8-Hydroxy-2´-deoxyguanosine (8-OHdG) is a promutagenic lesion in DNA that is generated in response to a number of chemicals that induce oxidative stress,[50] and it is used as a biomarker of oxidative stress in DNA.[51] The measurement tool was a high-performance liquid chromatography-triple tandem mass detector (HPLC-MS/MS; Agilent 6410, Agilent). 8-OHdG was purchased from Calbiochem

(CA, USA), and 2´-deoxyguanosine was purchased from Sigma (St Louis, MO, USA).

15N5-2´-deoxyguanosine and 5´-triphosphate (15N5-dGTP) were purchased from Martek (Columbia, MD, USA), and alkaline phosphate was purchased from Roche (Mannheim, Germany). Ammonium acetate and methyl alcohol (MeOH) were purchased from Sigma. Pre-treated urine samples were subjected to solid-phase extraction. Figure 2 shows the preprocessing.

The HPLC-MS/MS mass spectrometer was equipped with an Agilent (2.1 × 100 mm × 3.5 μm) column. Ammonium acetate and methyl alcohol were used as the mobile phase with a column flow of 0.16 mL/min. The applied injection volume was 6 μL for the quantification mode (MS SIM mode + MRM mode).

4.2. Isoprostane

Isoprostane was used to detect lipid peroxidation in urine. The measurement tool was a high-performance liquid chromatography-triple tandem mass detector (HPLC-MS/MS; Agilent 6410, Agilent). Figure 3 presents the preprocessing and analysis methods. The HPLC-MS/MS mass spectrometer was equipped with a PGC (Hypercarb 5 μm × 150 mm × 1.0) column. Water and acetonitrile with MeOH were used as the mobile phase with a column flow of 60 mL/min. The applied injection volume was 10 μL for the quantification mode (MS SIM mode + MRM mode).

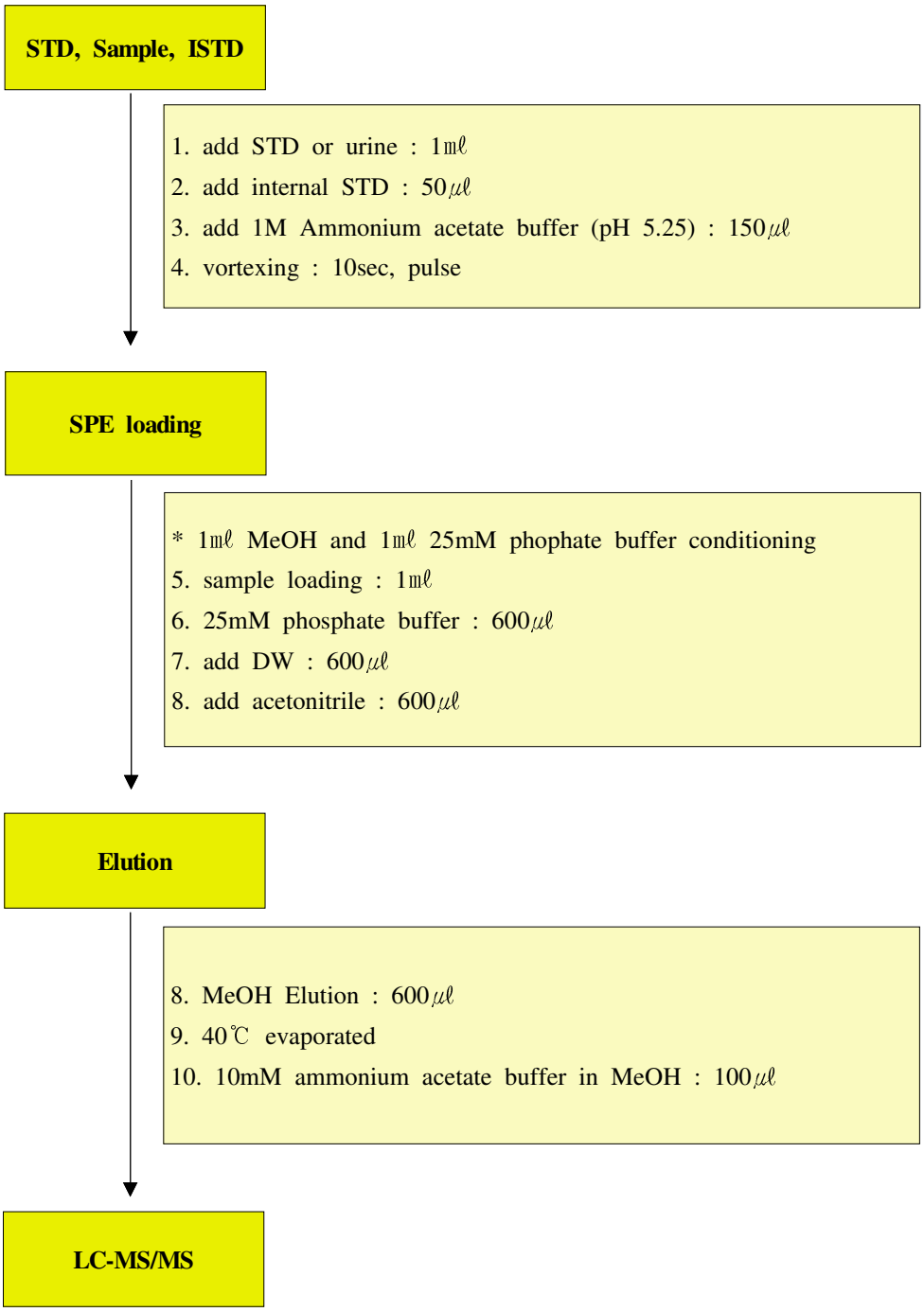


Figure 2. 8-OHdG analysis procedures

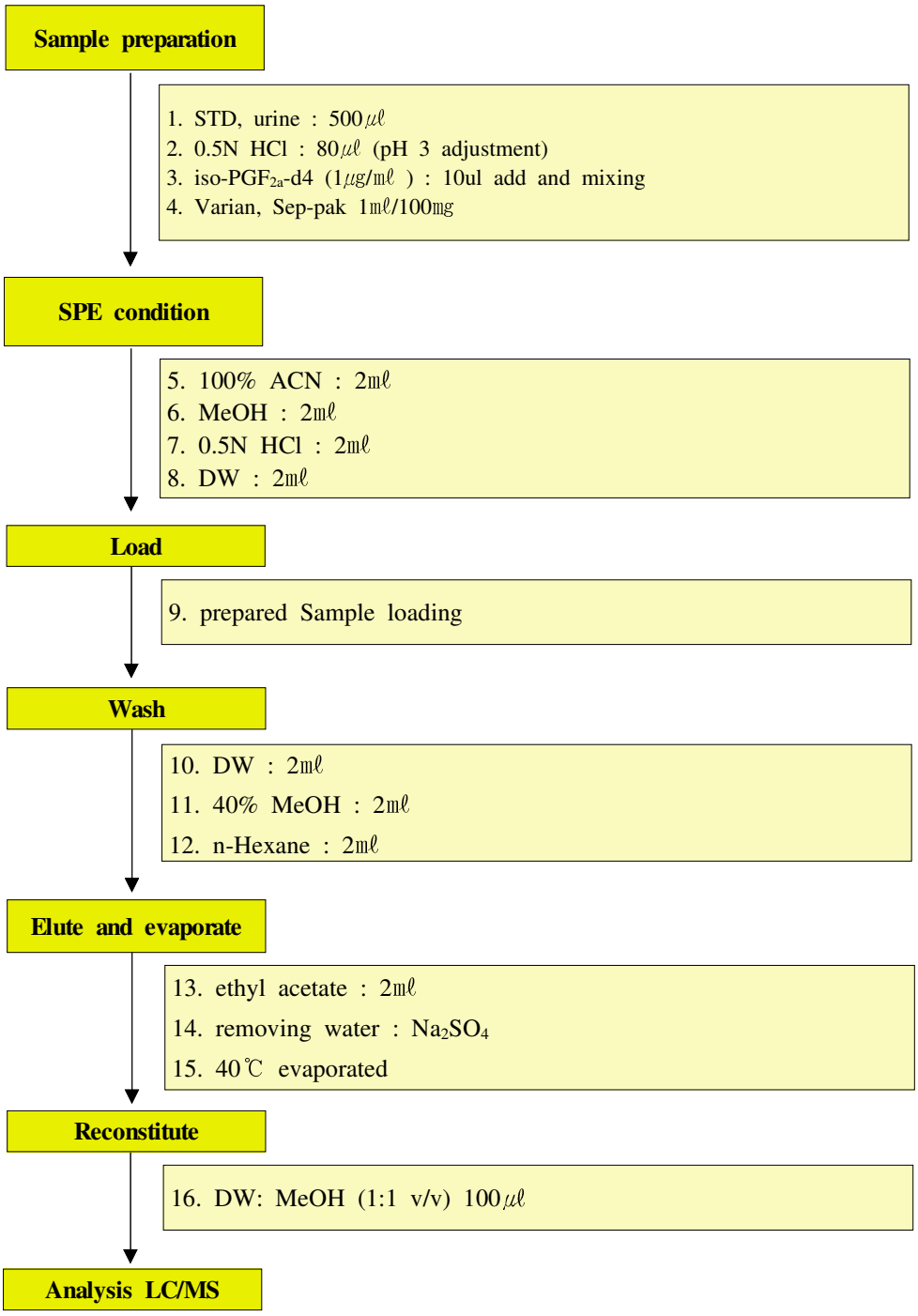


Figure 3. Isoprostane analysis procedures

4.3. MDA

Hydrolyzing 1,1,3,3-tetramethoxypropane (TMP. 99%, Sigma) and 1.0N HCl generated a MDA standard. Analysis was conducted with HPLC after derivatization with 2,4-DNPH. TMP (165㎕, 1mmol) and 835㎕ 1.0N HCl were mixed and were diluted ten-fold with 1.0 N HCl.

Samples were incubated at 40℃ for 30min and 100mM TMP hydrolysate (malondialdehyde, MDA) was added. A final level of MDA produced through hydrolysis was calculated by measuring the optical density at 245nm and using maximum molar extinction coefficient (ε=13,700). After centrifuging the urine samples at 4000rpm for 10min, the supernatant was isolated for analysis (Figure 4).

Urine samples of workers were collected in polyethylene bottles from the first urination in the morning. They were kept at -85℃ until further analysis. The urinary MDA levels of each individual will be corrected according to urine creatinine values, which were measured using an automated method based on the Jaffe reaction. Analysis method was used HPLC equipment.

Analysis was performed on a HPLC, Waters separation Module Alliance 2695 and Water 2487 Dual λ Absorbance detector equipped with a Higgins (4.6 × 250 ㎜ × 5㎛) column. Mobile phase with a column flow of 1㎖/min was used as a acetonitrile (40%) and 20mM K_2PO_4 (60%) in phosphate acid 100㎕. Injection volume was 20㎕ of solution into the system and analysed under the following conditions.

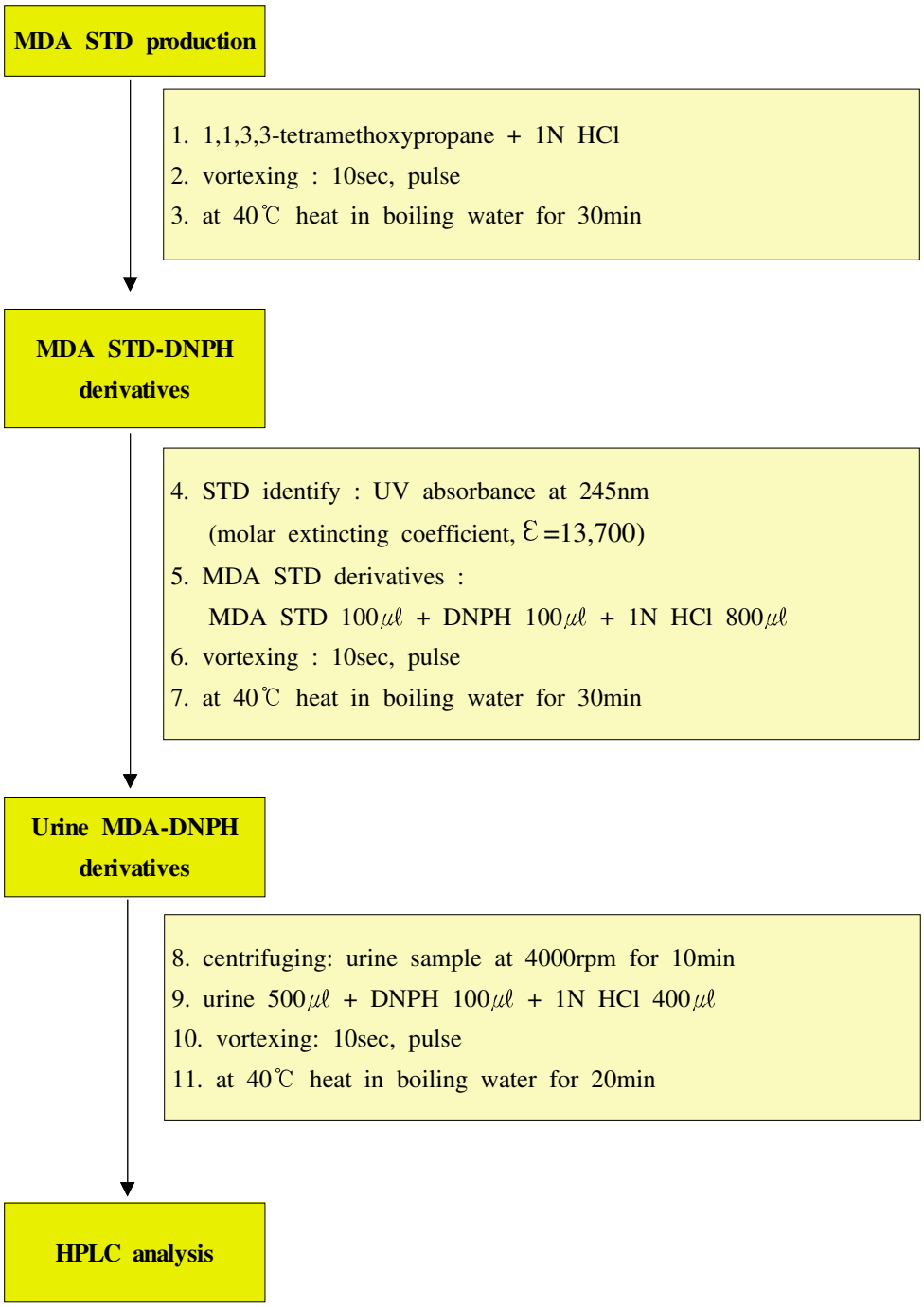


Figure 4. MDA analysis procedures

5. Pesticide metabolites

Organophosphate pesticides were analyzed as metabolites of pesticides. When organophosphate pesticide exposure occurs, it is metabolized via dealkylation, hydrolysis, and isomerization.

Dialkyl-phosphate (DAP) in the urine can be used as a metabolite to indirectly measure organophosphate pesticide exposure. The metabolites of DAP are measured as dimethylphosphate (DMP), diethylphosphate (DEP), dimethyl phosphorothioate (DMTP), diethyl phosphorothioate (DETP), dimethyl dithiophosphate (DMDTP), and diethyl dithiophosphate (DEDTP). Most organophosphate pesticides are metabolized from DAP, but ethyl p-nitrophenyl phenylphosphorothioate (EPN) is metabolized from p-nitrophenol. The amount of metabolites discharged in the urine is closely related to the amount of organophosphate pesticide exposure (Figure 5).

In the survey of the state of the use of pesticides, the recently applied pesticides were investigated and duplicated answers in the name of the pesticides were allowed. Appendix 1 shows the state of the use of pesticides in subjects.

In this study, 4 metabolites (DMP, DEP, DMTP, and DETP) among the 6 target metabolites were analyzed. Table 1 presents a summary of the substances metabolized from the organophosphate pesticides.

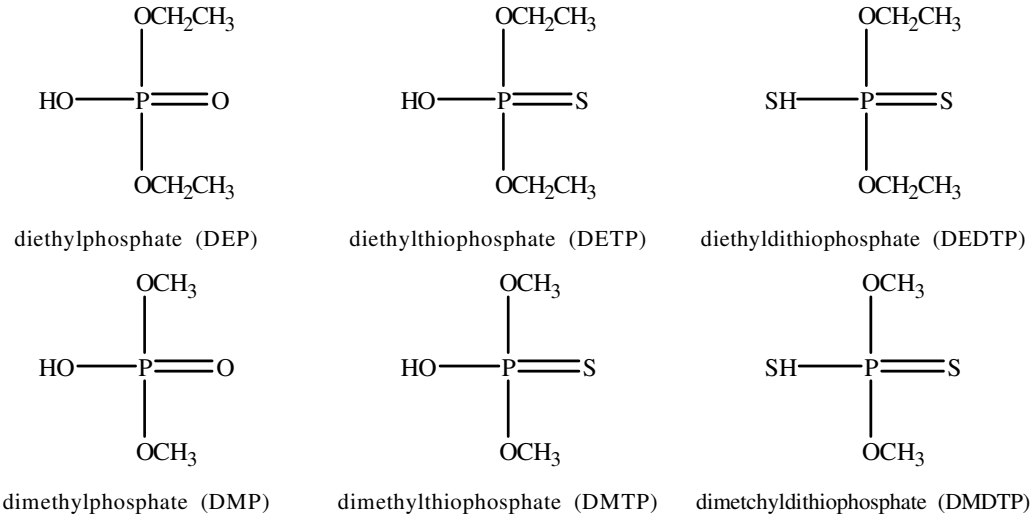


Figure 5. Structures of dialkyl-phosphate metabolites of organophosphophate pesticides

Table 1. Dialkyl phosphate metabolites and organophosphate pesticides[52-54)]

OP	DMP	DEP	DMTP	DETP
Acephate	◎		◎	
Azinphos methyl	◎		◎	
Chlorethoxyphos		◎		◎
Chlorpyrifos		◎		◎
Chlorpyrifos methyl	◎		◎	
Coumaphos		◎		◎
Dichlorvos(DDVP)	◎			
Diazinon		◎		◎
Dicrotophos	◎			
Dimethoate	◎		◎	
Disulfoton		◎		◎
Ethion		◎		◎
Ethyl parathion		◎		◎
Fenitrothion	◎		◎	
Fenthion	◎		◎	
Isazophos-methyl	◎		◎	
Malathion	◎		◎	
Methamidophos	◎		◎	
Methidathion	◎		◎	
Methyl parathion	◎		◎	
Mevinphos	◎			
Naled	◎			
Oxydemeton-methyl	◎		◎	
Parathion		◎		◎
Phorate		◎		◎
Phosalone		◎		◎
Phosmet	◎		◎	
Primiphos-methyl	◎		◎	
sulfotepp		◎		◎
Temephos	◎		◎	
Terbufos		◎		◎
Tetrachlorvinphos	◎			
Tribufos		◎		
Trichlorfon	◎			

5.1. Standard and materials

DMP (98%), DMTP (98%) and dibutylphosphate (DBP, 99%), used for an internal standard (I.S), was purchased from Acros Chimica.

DEP (98%) was purchased from Supelco and DETP (98%), 2,3,4,5,6-pentafluorobenzyl bromide (derivatization reagent, 99.9%, PFBBr), toluene (99.5%) and n-hexane (99.9%) from Sigma Aldrich. Diethyl ether, acetonitrile, which are HPLC grade, hydrochloric acid (37%, HCl) and sodium sulfate anhydrous (99%, Na_2SO_4), sodium disulfite ($Na_2S_2O_5$), potassium carbonate (K_2CO_3) were obtained from Burdick & Jackson. Gases used by the instrumentation had a minimum purity of 99.99%. Water used through out the experiments was distilled and deionized to 18MΩ with a Millipore Milli-Q system (Millipore Co., Bedford, MA, USA).

5.2. Gas chromatography and mass spectrometer conditions

Electron ionisation mass spectrometric analysis was performed on a GC-MS Hewlett-Packard 6890 (gas chromatography) and HP 5973 mass spectrometer system equipped with a DB-5MS (30m × 0.25mm × 0.25um) capillary column. Gas (helium) with a column flow of 1 mL/min was used as a carrier gas.

Injection volume was 2μL of solution into the system in the splitless mode (split 20:1 at 1 min) and the column temperature was initially held at 80℃ for 1 min, raised to 250℃ at 20℃/min, held for 10 min. The injector temperature was 250℃. The ion source (detector) and interface temperatures were set at 280℃ and 300℃, respectively.

An auto-tune of the mass spectrometer using pentafluorotributylamin (PFTBA, tuning standard) was performed before the analysis.

5.3. Standard preparation and analytical procedure

DMP, DEP, DMTP and DETP were prepared at a concentration of 1000 mg/L in MeOH, and diluted with MeOH to each working standard solution at concentrations ranging from 3 to 100 mg/L. The standard solutions were stored in the dark at 4℃. A flow picture of the urinary DAP determination procure is shown in Figure 6. Five milliliters of urine was pipetted into a 15 mL screw top glass test tube, and 50 μL of internal standard solution (50 μg/L DBP), 5g of NaCl, 1 mL of 6M HCl, 50 mg of $Na_2S_2O_5$, and 5 mL of diethylether-acetonitrile (1:1, v/v) were added.

After shaking for 5min and vortex 1min, the test tube was centrifuged (3000rpm for 5min). The organic phase (upper layer) containing DAP was transferred into a new screw-top glass test tube containing 15 mg K_2CO_3. The residuals were re-extracted with 5 mL of diethylether-acetonitrile (1:1, v/v) and then shaked for 5 min, vortexed, and centrifuged (3000 rpm for 5 min). The supernatant obtained from the second extraction was combined with the first extract.

The resulting extract was evaporated at 45℃ to dryness with a gentle nitrogen stream. To the dried extracts, 15 mg K_2CO_3, 1 mL of ACN, and 50 μL of PFBBr were added and incubated in a water bath at 70℃ for 60min with occasional swirling. Afterwards, 5 mL of water and 5 mL of n-hexane were added, and shaked the mixture for 5 min, vortexed, and centrifuged.

The upper layer containing PFB-DAP was transferred to new test-glass tubes. The extraction was then repeated with 5 mL of n-hexane and the supernatant obtained from the second extraction was combined with the first extract. And extraction materials was evaporated at 45℃ to dryness with a gentle nitrogen stream. The residue was dissolved in 100 μL of toluene and injected into GC/MSD.

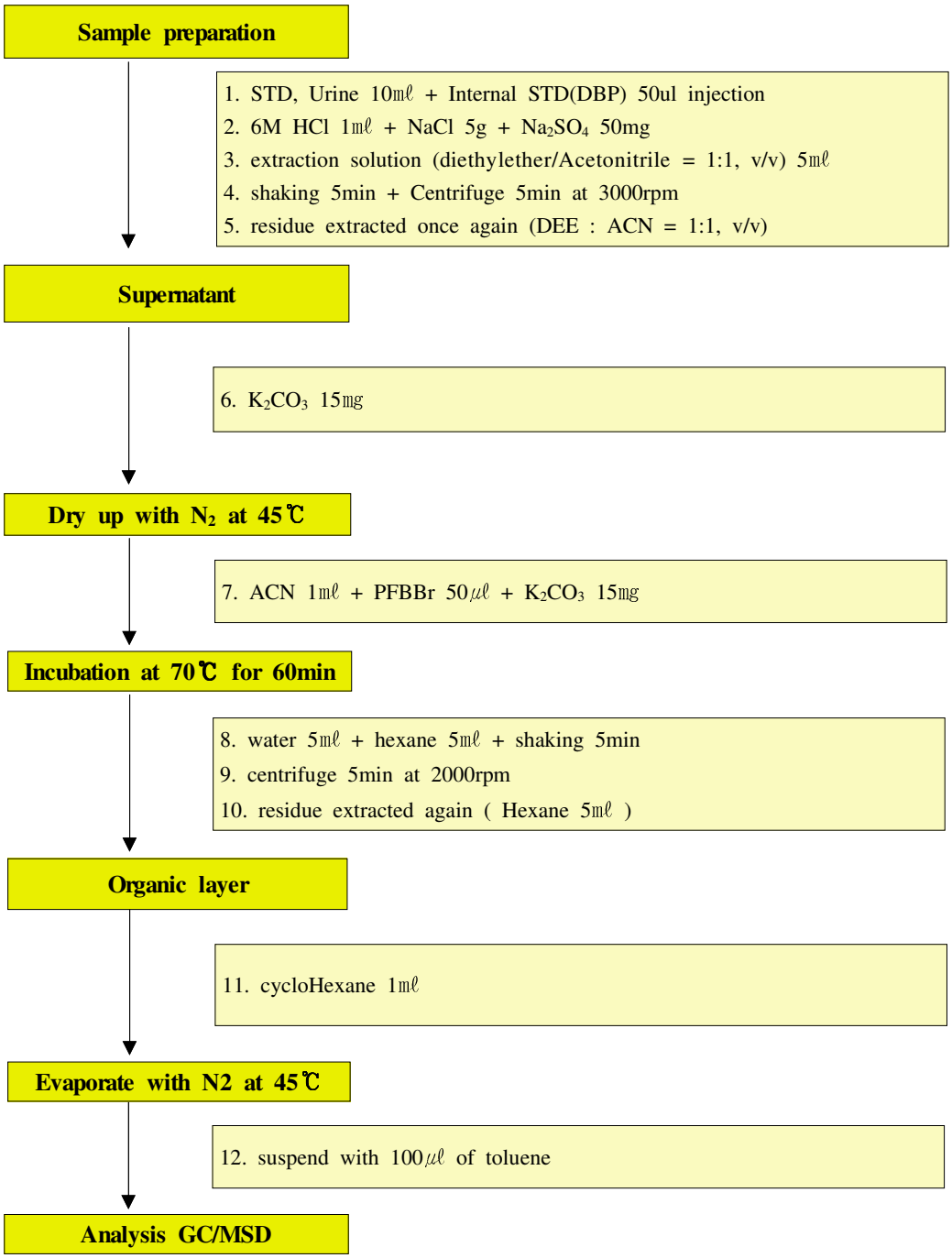


Figure 6. Analytical procedure for urinary dialkyl-phosphate

6. Pesticide exposure index

A chronic disease and cancer risks associated with pesticides, agricultural exposures and lifestyle factors[55]. Exposure to pesticides may occur while transporting, mixing, applying chemicals, through cleaning or repairing equipment. Factors affecting the level of exposure include type of activity (e.g. application, mixing), method of application (e.g. backpack, hand spray, speed sprayer), use of personal protective equipment (PPE) (e.g. gloves, respirators, face-shields, boots or overalls), and personal work habits and hygiene (e.g. changing into clean clothes or taking bath after the use of pesticide). The challenge was to incorporate these exposure modifiers into an estimation of intensity of pesticide exposure.

Pesticide exposure index were used to estimate the intensity of exposure to individual pesticides using the intensity level. In the literature Dosemeci[56], there was applied to modify the intensity levels.

*** Intensity level =**

(mixing status + application method + equipment repair status) × PPE[56]

Mixing status was a two-level variable, based on never mixing and mixed (values of 0 and 9, respectively). Application method was a six-level variable, based on does not apply, use of aerial-aircraft, application in furrow, use of boom tractor, use of backpack, use of hand spray, and speed sprayer (values of 0, 1, 2, 3, 8, 9, and 9, respectively). Status of repairing equipment was two-level variable, based on not repairing and repairing equipment (0 and 2, respectively). PPE use was categorized as an eight-level variable based on the percentage of protection during pesticide spray.

The pesticide exposure and cumulative exposure index were calculated as follows:

* PEM = pesticide exposure month

Spraying year × spraying day per year / 30day

CEI = cumulative exposure index

Intensity level × spraying year × spraying day per year

7. Oxidized LDL and glutathion peroxidase(GPx)

The serum concentrations of oxidized LDL and GPx were measured using the enzyme-linked immunosorbent assay (ELISA, Kit-Cayman Chemical Company, USA).

8. Evaluation of detection limits

In general, the detection limits can be evaluated using instrumental detection limits (IDL) and method detection limits (MDL) methods. In this study, we used MDL method which is a method recommended by the 40 CFR, US EPA (Environmental Protection Agency).

The results for this method were obtained by multiplying the standard deviation of the results obtained from the repetitive measurement of n samples by the t-distribution value at a 99% of confidence, where the values of t (n-1, α = 0.01) were obtained from the following table.

$$\mathbf{MDL} = t_{(n-1,\ \alpha=0.01)} \times S$$

No. of samples	3	4	5	6	7	8	9	10
t-statistic	6.96	4.54	3.75	3.36	3.14	3.00	2.90	2.82

9. Statistical analysis

SPSS 18.0 was used to evaluate the relationship among pesticide exposure level, metabolic syndrome, and oxidative stress. The data were expressed as frequencies, means, and standard deviations. We used the *t*-test, Mann-Whitney *U* test, or chi-square test to compare the subjects with and without metabolic syndrome.

Demographic and lifestyle characteristics (i.e., age, smoking, drinking, and exercise), and pesticide characteristics (i.g., number of spraying years, number of spraying days per year, and spraying time), and biomarker levels (i.e., 8-OHdG, MDA, GPx, oxidized LDL, isoprostane, HDL-cholesterol, LDL-cholesterol, triglycerides, pseudo-cholinesterase, SBP, DBP, total cholesterol) were evaluated.

Metabolic syndrome was diagnosed based at least 3 of the following 5 featrues; waist circumference, arterial pressure, triglyceride level, fasting glucose level, and HDL-cholesterol.

A logistic regression analysis was used to calculate the risk for metabolic syndrome associated with oxidative stress and pesticide exposure. To better evaluate the relationship among 8-OHdG, MDA, isoprostane, and other variables, we used Spearman's Rank correlation coefficients.

Linear regression analyses were used to assess the relationship between the levels of pesticide metabolites and oxidative stress biomarkers. Logistic regression and linear regression analyses adjusted for age, smoking, drinking, exercise. From the logistic regression models, 95% confidence intervals (CI) were computed. A P value < 0.05 was taken as being statistically significant.

Oxidative stress biomarkers and pesticide metabolites concentration were natural log-transformed to account for skewed distribution for linear regression models and logistic regression analysis.

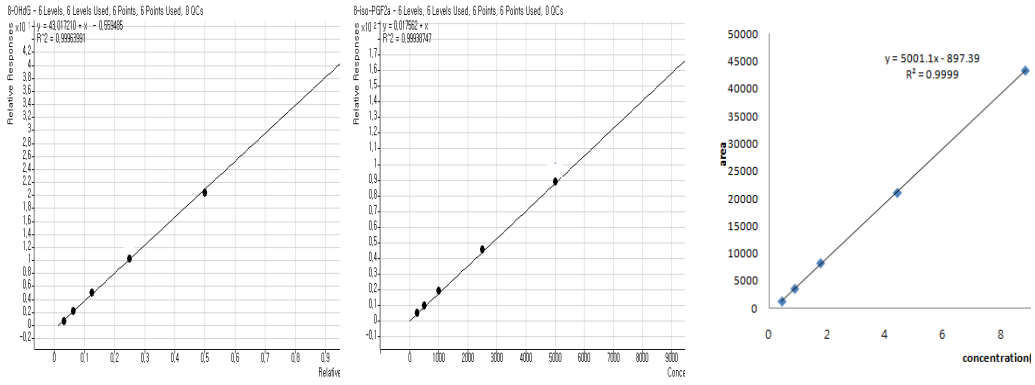
III. RESULTS

1. Quality control

1.1 Oxidative stress biomarkers

The calibration curve of 8-OHdG, isoprostane, and MDA were produced at a range of 0.5 μg/L～20 μg/L, 0.005 ng/mL～1 ng/mL, 0.443 umol/L～8.850 umol/L, respectively and the linear regression equation for the curve is presented in Figure 7.

The detection limit of 8-OHdG (0.053 μg/L), isoprostane (0.162 pg/mL) and MDA (0.0437 umol/L) was calculated by using the method proposed by the US EPA (Environmental Protection Agency) (Appendix 9-11).



8-OHdG isoprostane MDA

Figure 7. The calibration curve of oxidative stress biomarkers

1.2 Pesticide metabolites

A. Orgaonophosphate chromatogram and mass spectra

The organophosphate metabolites were obtained using PFBBr and analyzed by GC/MSD. The results are presented as a retention time and chromatograms (Appendix 2).

The retention times of DMP, DEP, DMTP, and DETP were 6.72, 7.40, 7.81, and 8.38 respectively. In addition, the retention time of the internal standard substance of DBP was 9.26. Appendix 3-6 represent the mass spectra in each metabolite. Appendix 7 shows the mass spectra of the internal standard substance.

DMP-PFBBr was obtained as a form of R-$OC_6F_5CH_2Br$ in which the derivative ions were formed as m/z 306, 110, and 194.

DEP-PFBBr was characterized by m/z 334, 258, and 197. DMTP-PFBBr was characterized by m/z 322, 211, and 110. DETP-PFBBr was characterized by m/z 350, 213, and 274 (Table 2).

Table 2. Retention time and fragment ions of the dialkyl-phosphates

DAP-PFBBr derivatives	Retention time (min)	Detected masses (m/z)
DMP	6.72	306, 110, 194
DEP	7.40	334, 258, 197
DMTP	7.81	322, 211, 110
DETP	8.38	350, 213, 274
DBP	9.26	335, 279, 258

B. Calibration curves and recovery ratio

The derivative reaction can be implemented by adding their own standard and internal standard substances and a qualitative analysis was applied using GC/MSD. Then, the results can be obtained as a linear relationship of the concentration with respect to the areal ratio, which can be obtained by dividing the area in each substance by the area of the internal standard substance (Table 3).

Table 3. Calibration curve and linear equation

DAP	Linear range(μg/L)					Slope	Intercept	Correl
DMP	5	10	20	40	100	150.178	-0.7674	0.99237
DEP	5	10	20	40	100	100.585	-1.5345	0.99775
DMTP	5	10	20	40	100	298.404	0.4249	0.99051
DETP	5	10	20	40	100	225.758	-3.8363	0.99198

C. Recovery ratio and method detection limits (MDL)

By using the method proposed by the US EPA (Environmental Protection Agency), DMP, DEP, DMTP, and DETP were calculated as 0.84 μg/L, 1.35 μg/L, 0.59 μg/L, and 2.06 μg/L, respectively.

The mean absolute recoveries ratio were 77.25−129.00% for DMP, 65.02−88.12% for DEP, 85.32−143.64% for DMTP, and 76.82−148.44% for DETP with pooled urine spiked with DAP (Table 4).

Table 4. Recovery, MDL data of analytical procedure

	Pooled urine spiked concentration (μg/L of urine)	N	DMP	DEP	DMTP	DETP
Mean recovery [a](%)	10	3	90.44	77.49	143.64	148.44
	20	3	129.00	88.12	85.32	97.44
	80	3	77.25	65.02	86.07	76.82
SD recovery (%)	10	3	7.47	5.57	14.76	18.72
	20	3	8.14	3.78	7.87	7.46
	80	3	8.98	8.77	5.43	2.84
RSD recovery (%)	10	3	8.27	7.19	10.28	12.61
	20	3	6.31	4.29	9.23	7.66
	80	3	11.62	13.49	6.32	3.69
MDL (μg/L)		5	0.84	1.35	0.59	2.06

Abbreviations: N, number of observations; DMP, dimethylphosphate; DEP, diethylphosphate; DMTP, dimethylthiophosphate; DETP, diethylthiophosphate; MDL, method detection limits; SD, standard deviation; RSD, relative standard deviation

[a] Recovery given by adding the standards on derivatization step.

2. General characteristics of the study subjects

The results of the comparison of general characteristics between groups are presented in Table 5. The groups with and without metabolic syndrome were investigated as 19 and 65 subjects respectively.

The non-metabolic and metabolic syndrome groups showed a large distribution of ages between 61 and 70 years. In the non-metabolic syndrome group, there were 28 and 23 nonsmoking and smoking subjects, respectively; there were 17 and 42 nondrinking and drinking subjects, respectively. In addition, 16 subjects exercised.

In the metabolic syndrome group, there were 9, 6, and 4, nonsmokers, past smokers, and recent smokers, respectively. There were 13 recent drinkers, and 17 subjects exercised. As mentioned above, there were no significant differences in these general items between the subjects with and without metabolic syndrome.

Table 5. Demographic characteristics of metabolic syndrome and non-metabolic syndrome

	non-Mets (n=65)	Mets (n=19)	P value
Demographic characteristics			
Age, n(%)			0.238
~59	23 (35.4)	3 (15.8)	
60~69	29 (44.6)	12 (63.2)	
70~	13 (20.0)	4 (21.1)	
Smoking, n(%)			0.396
Non-smoking	28 (43.8)	9 (47.4)	
Past-smoking	13 (20.3)	6 (31.6)	
Smoking	23 (35.9)	4 (21.1)	
Drinking, n(%)			0.928
Non-drinking	17 (26.6)	5 (26.3)	
Past-drinking	5 (7.8)	1 (5.3)	
Drinking	42 (65.6)	13 (68.4)	
Exercise, n(%)			0.318
Non-exercise	49 (75.4)	17 (89.5)	
Exercise	16 (24.6)	2 (10.5)	

Data expressed chi-square test

3. Oxidative stress and metabolic characteristics according to metabolic syndrome

In the group without metabolic syndrome, the average concentration of 8-OHdG was 0.808 μg/g creatinine. The isoprostane level was determined as 0.259 ng/mg creatinine. The MDA level was determined as 0.120 μmol/g creatinine. In the group with metabolic syndrome, the average concentration of 8-OHdG was 1.303 μg/g creatinine. The isoprostane level was 0.418 ng/mg creatinine. The MDA level was 0.188 μmol/g creatinine. The group with metabolic syndrome showed a significantly higher concentration of biomarkers of oxidative stress than the group without metabolic syndrome.

The BMI, waist circumference, SBP, TG level, and fasting blood glucose level were all significantly higher in the group with metabolic syndrome than in the group without metabolic syndrome, whereas the HDL levels was significantly lower ($p<0.05$). The DBP, LDL level, and total cholesterol level were not significantly different (Table 6).

Table 6. Metabolic characteristics of metabolic syndrome and non-metabolic syndrome

	non-Mets (n=65)	Mets (n=19)	P value
Metabolic characteristics	mean±SD	mean±SD	
8-OHdG, μg/g creatinine	0.808 ± 0.38	1.303 ± 0.92	0.034
Isoprostane, ng/mg creatinine	0.259 ± 0.14	0.418 ± 0.25	0.023
GPx, nmol/min/ml	125.02 ± 14.97	127.58 ± 20.49	0.550
Oxidized LDL, U/L	36.42 ± 9.40	39.25 ± 10.48	0.265
MDA, μmol/g creatinine	0.120 ± 0.05	0.188 ± 0.10	0.038
(pseudo)cholinesterase×10^3, U/L	7.956 ± 1.54	8.352 ± 1.96	0.360
BMI, kg/m^2	22.97 ± 2.45	25.14 ± 2.46	0.001
Waist circumference, cm	82.47 ± 6.02	89.34 ± 4.95	0.000
SBP, mmHg	126.94 ± 15.53	136.47 ± 10.79	0.014
DBP, mmHg	79.00 ± 10.97	78.37 ± 10.63	0.632
LDL, mg/dL	109.88 ± 27.89	113.58 ± 28.03	0.613
HDL, mg/dL	53.97 ± 13.83	46.00 ± 8.56	0.020
TG, mg/dL	113.28 ± 46.13	192.84 ± 86.22	0.001
FBS, mg/dL	97.81 ± 16.50	110.95 ± 26.25	0.010
Total cholesterol, mg/dL	173.63 ± 28.81	182.84 ± 29.38	0.226

Data expressed mean ± standard deviation with student T-test and Mann-whitney U test

4. Relationship of oxidative stress and metabolic characteristics

Table 7 shows the relationship between the use of pesticides and the levels of pesticide metabolites according to the presence of metabolic syndrome. In subjects with and without metabolic syndrome, the average agricultural duration was approximately 36 and 40 years, respectively, which was not significantly different. The duration of spraying pesticides was also similar (approximately 24 years).

The subjects without and with metabolic syndrome sprayed pesticides for 9 and 15 days per year, respectively, which represented a significant difference ($p < 0.002$).

In the calculation of the pesticide exposure month as an index of pesticide exposure, the indices of the groups without and with metabolic syndrome were 4.98 and 9.54, respectively, which represented a significant difference ($p < 0.05$).

The cumulative exposure index proposed by Dosemeci[56] can also be used to evaluate the wearing of protective equipment and observance of spraying regulations. The indices of the groups without and with metabolic syndrome were 159.24 and 314.84, respectively; however, this difference was not significant. In addition, the values of DMP and DEP in the group without metabolic syndrome were 0.798 μg/g creatinine and 1.324 μg/g creatinine, respectively.

In the group with metabolic syndrome, the values of DMP and DEP were 0.945 μg/g creatinine and 2.063 μg/g creatinine, respectively. The difference between groups was significant ($p < 0.05$). Although the other metabolites such as DMTP, DETP, and total DAP showed higher levels in the group with metabolic syndrome, the differences did not reach significance.

Table 7. Pesticide characteristics and pesticide metabolite of metabolic syndrome and non-metabolic syndrome

	non-Mets (n=65)	Mets (n=19)	P value
Pesticide characteristics			
Farming duration	36.68 ± 14.28	40.63 ± 13.32	0.326
Spraying year, year	24.08 ± 10.09	24.43 ± 8.27	0.907
Spraying day per year, day	8.85 ± 6.09	15.07 ± 6.33	0.002
PEM	4.98 ± 5.37	9.54 ± 6.53	0.004
CEI	159.24 ± 185.82	314.84 ± 347.67	0.075
Pesticide metabolites (μg/g creatinine)			
DMP	0.798 ± 0.96	0.945 ± 0.54	0.020
DEP	1.324 ± 1.15	2.063 ± 1.55	0.034
DMTP	2.897 ± 6.24	4.406 ± 10.29	0.826
DETP	4.760 ± 9.12	5.133 ± 4.78	0.101
Total DAP	9.629 ± 14.89	12.419 ± 11.01	0.074

Data expressed mean ± standard deviation with student T-test and Mann-whitney U test.

Abbreviations:
PEM: pesticide exposure month(spraying year×spraying day per year /30day)
CEI: cumulative exposure index (intensity level×spraying year×spraying day per year),
Total DAP : total dialkylphosphate

5. Correlation analysis for oxidative stress biomarkers, pesticide exposure indices, and pesticide metabolites

In a simple correlation analysis, 8-OHdG showed a significant positive correlation with MDA ($r = 0.471$), CEI ($r = 0.240$), DMP ($r = 0.285$), DEP ($r = 0.396$), and DETP ($r = 0.361$) ($p < 0.05$). Isoprostane showed a significant positive correlation to MDA ($r = 0.461$), DMP ($r = 0.484$), DEP ($r = 0.578$), and DETP ($r = 0.603$) ($p < 0.05$). MDA showed a significant positive correlation to DMP ($r = 0.532$) and DEP ($r = 0.506$), and DETP ($r =0.367$) ($p < 0.05$). In addition, PEM, which is used as an index of pesticide exposure, showed a significant positive correlation to 8-OHdG ($r = 0.326$), isoprostane ($r = 0.408$), CEI ($r = 0.771$), and DMP ($r = 0.280$) (Table 8).

Table 8. Spearman's Rank correlation coefficient between pesticide metabolite and biomarkers of oxidative stress

	8-OHdG	Isoprostane	GPx	Oxidised-LDL	MDA	total DAP	CEI	DMP	DEP	DMTP	DETP	PEM
8-OHdG	1											
Isoprostane	0.187	1										
GPx	0.138	0.007	1									
Oxidised-LDL	0.225	0.154	0.054	1								
MDA	0.471**	0.461**	0.146	0.029	1							
TotalDAP	0.243	0.316*	-0.013	-0.033	0.207	1						
CEI	0.240*	0.226	-0.142	0.069	0.133	-0.001	1					
DMP	0.285*	0.484**	0.058	0.017	0.532**	0.701**	0.096	1				
DEP	0.396**	0.578**	0.128	0.027	0.506**	0.758**	0.102	0.723**	1			
DMTP	0.119	0.178	-0.056	-0.070	0.196	0.570**	-0.035	0.231	0.206	1		
DETP	0.361**	0.603**	0.089	0.043	0.367**	0.813**	0.057	0.766**	0.895**	0.246*	1	
PEM	0.326**	0.408**	-0.110	0.137	0.254	0.075	0.771**	0.280*	0.186	-0.045	0.142	1

Abbreviations: total DAP; total dialkyl-phosphate, CEI; cumulative exposure index (intensity level × spraying year × spraying day per year), PEM; pesticide exposure month (spraying year × spraying day per year / 30 day) *p<0.05, **p<0.01

6. Logistic regression analysis of oxidative stress and metabolic syndrome

To verify the relationship between the oxidative stress biomarkers and the metabolic syndrome, a logistic regression analysis was implemented before applying a calibration according to the presence of metabolic syndrome and after applying a calibration for the terms of age, smoking, drinking, and exercise. Then, the odds ratios of 8-OHdG, isoprostane, and MDA as well as a 95% confidence interval were obtained.

In the case of the 8-OHdG before applying the calibration, the concentration of 8-OHdG in the group with metabolic syndrome was 3.3 times (95% CI 1.205–9.167) higher than that in the group without metabolic syndrome. In addition, after the calibration for the terms of age, smoking, drinking, and exercise was applied, the 8-OHdG in the group with metabolic syndrome was 3.8 times (95% CI 1.232–11.707) higher than that in the group without metabolic syndrome.

The concentration of isoprostane in the group with metabolic syndrome before the calibration was applied was 4.0 times higher (95% 1.378–11.551) than in the group without metabolic syndrome. Moreover, the concentration of isoprostane in the group with metabolic syndrome was 4.4 times (95% 1.347–14.518) higher than that in the group without metabolic syndrome after the calibration for the terms of age, smoking, drinking, and exercise was applied.

The concentration of MDA in the group with metabolic syndrome before the calibration was applied was 5.4 times higher (95% 1.293–22.664) than that in the group without metabolic syndrome. Moreover, the concentration of MDA in the group with metabolic syndrome was 6.0 times (95% 1.276–27.693) higher than that in the group without metabolic syndrome after the calibration for age, smoking, drinking, and exercise was applied (Table 9).

Table 9. Logistic regression analysis of oxidative stress and metabolic syndrome

Independent variables	Crude		Adjusted‡	
	OR	(95% CI)	OR	95% CI
8-OHdG* (μg/g creatinine)	3.324	1.205 - 9.167	3.798	1.232 - 11.707
Isoprostane* (ng/mg creatinine)	3.990	1.378 - 11.551	4.423	1.347 - 14.518
MDA* (μmol/g creatinine)	5.414	1.293 - 22.664	5.945	1.276 - 27.693

Adjusted‡: age, smoking history, alcohol drinking, exercise

*Oxidative stress biomarker concentration : log transformation

7. Logistic regression analysis of pesticide metabolites and metabolic syndrome

To investigate the relationship between pesticide metabolites and the metabolic syndrome, a logistic regression analysis was implemented before applying a calibration according to the presence of the metabolic syndrome and after applying a calibration for age, smoking, drinking, and exercise. The ratios of DMP, DEP, DMTP, DETP, PEM, CEI, and total DAP as well as a 95% confidence interval were then obtained.

Before the calibration was applied, the concentration of DMP in the group with metabolic syndrome was 2.0 times (95% CI 0.961−4.190) higher than that in the group without metabolic syndrome, but the difference was not significant. Moreover, after the calibration for age, smoking, drinking, and exercise was applied, the concentration of DMP in the group with metabolic syndrome was 2.5 times (95% CI 1.096−5.858) higher than that in the group without metabolic syndrome.

The concentration of DEP in the group with metabolic syndrome before the calibration was applied was 2.7 times (95% CI 1.132−6.517) higher than that in the group without metabolic syndrome. In addition, the concentration of DEP in the group with metabolic syndrome was 5.0 times (95% CI 1.618−15.522) higher than that in the group without metabolic syndrome after the calibration for age, smoking, drinking, and exercise was applied.

The concentrations of DMTP, DETP, and total DAP in the group with metabolic syndrome before the calibration was applied were 1.074 times (95% CI 0.731−1.577), 1.6 times (95% CI 0.899−2.927), and 1.012 times (95% CI 0.977−1.048) higher, relatively, than those in the group without metabolic syndrome.

Moreover, although the group with metabolic syndrome showed concentrations of DMTP, DETP, and total DAP that were 1.031 times (95% CI 0.672−1.582), 1.8 times (95% CI 0.952−3.494), and 1.012 times (95% CI 0.974−1.052) higher, relatively than those in the group without metabolic syndrome after the calibration for age, smoking, drinking, and exercise was applied, the difference was not significant.

The concentration of PEM in the group with metabolic syndrome before the calibration represented was applied was 1.1 times (95% CI 1.031−1.236) higher than that in the group without metabolic syndrome. The concentration of PEM in the group with metabolic syndrome was 1.2 times (95% CI 1.048−1.290) higher than that in the group without metabolic syndrome after the calibration for age, smoking, drinking, and exercise was applied.

The concentration of CEI in the group with metabolic syndrome before the calibration was applied was 1.002 times (95% CI 1.000−1.004) higher than that in the group without metabolic syndrome. The concentration of CEI in the group with metabolic syndrome was 1.002 times (95% CI 1.000−1.005) higher than that in the group without metabolic syndrome after the calibration for age, smoking, drinking, and exercise was applied (Table 10).

Table 10. Logistic regression analysis of pesticide metabolite and metabolic syndrome

Independent variables	Crude		Adjusted‡	
	OR	(95% CI)	OR	95% CI
DMP, (μg/g creatinine)*	2.007	0.961 - 4.190	2.534	1.096 - 5.858
DEP, (μg/g creatinine)*	2.717	1.132 - 6.517	5.012	1.618 - 15.522
DMTP, (μg/g creatinine)*	1.074	0.731 - 1.577	1.031	0.672 - 1.582
DETP, (μg/g creatinine)*	1.622	0.899 - 2.927	1.824	0.952 - 3.494
Pesticide exposure month	1.129	1.031 - 1.236	1.163	1.048 - 1.290
Cumulative exposure index	1.002	1.000 - 1.004	1.002	1.000 - 1.005
Total dialkyphosphate* (μg/g creatinine)	1.012	0.977 - 1.048	1.012	0.974 - 1.052

Adjusted‡ : age, smoking history, alcohol drinking, exercise

* Pesticide metabolite concentration : log transformation

8. Linear regression analysis of oxidative stress biomarkers and pesticide exposure

To investigate the influence of pesticide exposure on oxidative stress among farmers exposure to pesticide, a linear regression analysis was performed using 8-OHdG, isoprostane, and MDA as dependent variables with the application of a calibration for the demographic characteristics of age, smoking, drinking, and exercise.

The 8-OHdG level showed a significant relation to DMP (ß = 0.320, p = 0.020), DEP (ß = 0.390, p = 0.004), DETP (ß = 0.082, p = 0.015), and PEM (ß = 0.302, p = 0.020), and CEI (ß = 0.297, p = 0.020). In addition, isoprostane was related to DMP (ß = 0.396, p = 0.003), DEP (ß = 0.508, p = 0.000), DETP (ß = 0.504, p = 0.000), total DAP (ß = 0.302, p = 0.036), and PEM (ß = 0.434, p = 0.000).

MDA was related to DMP (ß = 0.432, p = 0.001), DEP (ß = 0.508, p = 0.000), and DETP (ß = 0.329, p = 0.014). Thus, the levels of the pesticide metabolites were significantly associated with the levels of oxidative stress biomarkers among farmers exposure to pesticide who were exposed to organophosphate pesticides (Table 11) (Figure 8, 8-1).

Table 11. Linear regression analysis of pesticide metabolites and oxidative stress biomarkers

Independent variables	8-OHdG (μg/g creatinine)*			Isoprostane (ng/mg creatinine)*			MDA (μmol/g creatinine)*		
	B	ß	P-value	B	ß	P-value	B	ß	P-value
DMP, (μg/g creatinine)*	0.238	0.320	0.020	0.329	0.396	0.003	0.320	0.432	0.001
DEP, (μg/g creatinine)*	0.306	0.390	0.004	0.471	0.508	0.000	0.426	0.508	0.000
DMTP, (μg/g creatinine)*	0.009	0.019	0.897	-0.020	-0.051	0.732	-0.001	-0.002	0.987
DETP, (μg/g creatinine)*	0.207	0.082	0.015	0.342	0.504	0.000	0.180	0.329	0.014
Total dialkyl-phosphate (μg/g creatinine)*	0.155	0.220	0.116	0.209	0.302	0.036	0.140	0.224	0.105
Pesticide exposure month	0.029	0.302	0.020	0.040	0.434	0.000	0.015	0.195	0.155
Cumulative exposure index	0.001	0.297	0.020	0.001	0.213	0.106	0.000	0.182	0.171

Adjusted : age, smoking history, alcohol drinking, exercise

*Oxidative stress biomarker and pesticide metabolites : log transformation

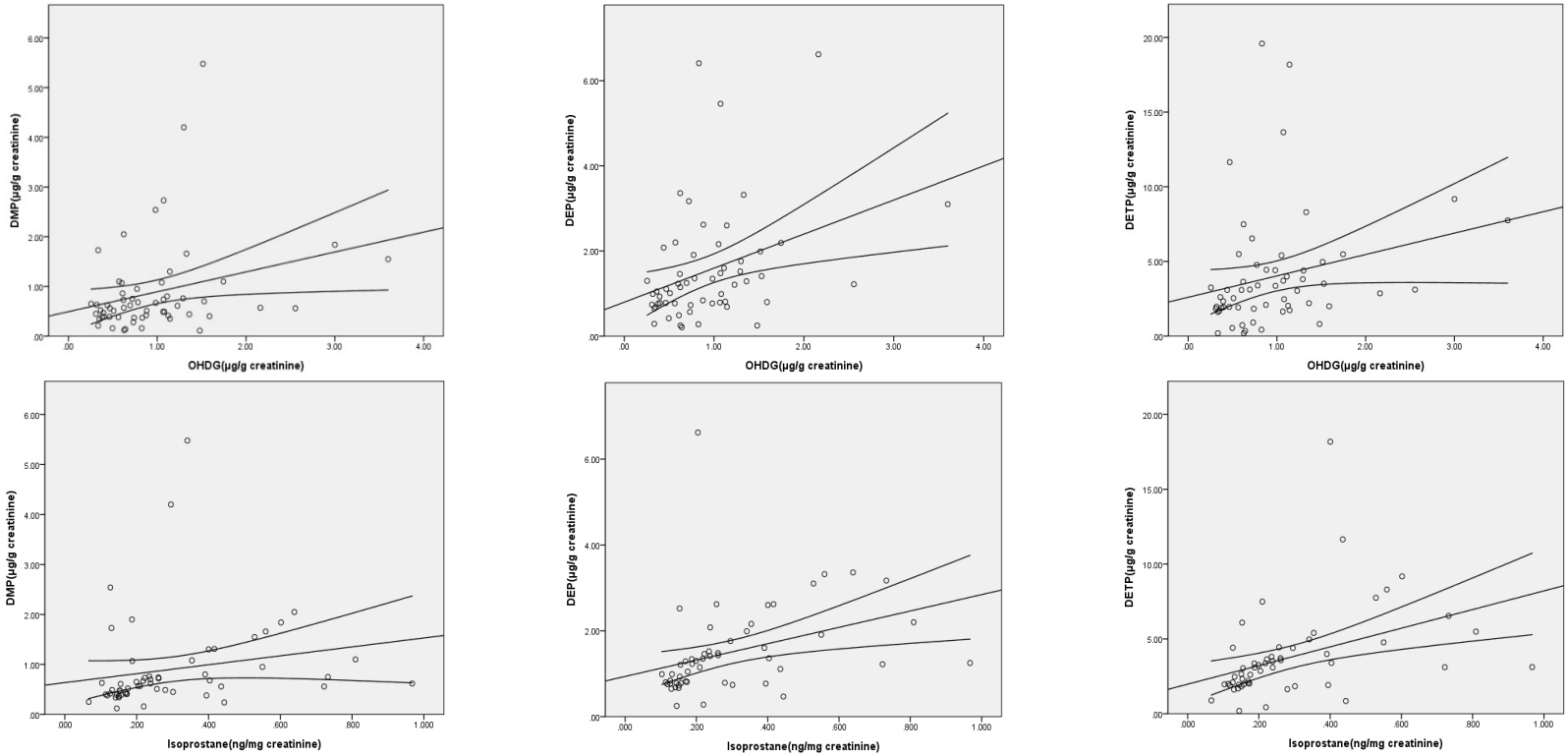


Figure 8. Scatter diagram between oxidative stress biomarkers and pesticide metabolites among farmers exposure to pesticide

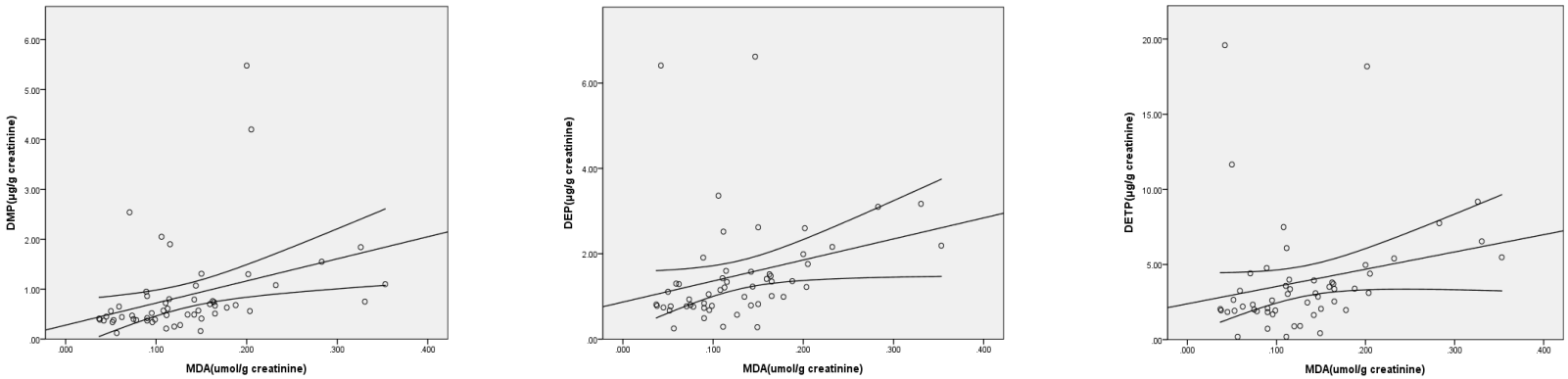


Figure 8-1. Scatter diagram between oxidative stress biomarkers and pesticide metabolites among farmers exposure to pesticide (continue)

IV. DISCUSSION

In this study, MDA and isoprostane were used as lipid injury indices for farmers exposure to pesticide. Moreover, 8-OHdG and DAP were measured as a DNA injury index and a quantitative indicator of pesticide exposure, respectively, to collect the information on the use of pesticides, DNA injury, and oxidative stress in farmers who spray such pesticides.

The influence of the oxidative stress caused by pesticide exposure on metabolic syndrome was also investigated. Oxidative stress damages lipids, proteins, and DNA and thereby causes diseases[57]. Factors that increase oxidative stress are determined as habitual (e.g., smoking, drinking, obesity, and stress) and environmental (e.g., pesticides, heat, pollution, heavy metals, plasticizers, ozone, and organic solvents)[58].

In particular, pesticides increase oxidative stress, leading to an imbalance between oxidants and antioxidants[59]. It has been reported that organophosphorus pesticides disturb the cytochrome p 450 system in the liver and affect the transfer system related to mitochondrial membranes[60] In addition, it has been known that organophosphorus pesticides cause oxidative stress by inhibiting enzymatic and nonenzymatic antioxidant defenses[61-62]. Other studies have shown that acute and chronic pesticide exposure affect internal secretions and the pancreas[63-64]. The use of organochlorine pesticides is highly related to the risk of metabolic syndrome and particularly type 2 diabetes[4-5]. Therefore, it is important to investigate how oxidative stress caused by pesticide exposure affects the metabolic syndrome.

In this study, oxidative stress biomarkers, such as 8-OHdG, isoprostane, and MDA, were measured in subjects with and without metabolic syndrome. The

group with metabolic syndrome presented a higher concentration of oxidative biomarkers than the group without metabolic syndrome ($p < 0.05$) (Table 6).

The logistic regression analysis revealed that the group with metabolic syndrome showed a higher concentration of 8-OHdG (odds ratio 3.8, 95% CI 1.23−11.71), isoprostane (odds ratio 4.4, 95% CI 1.347−14.52), and MDA (odds ratio 6.0, 95% CI 1.28−27.69) than the group without metabolic syndrome (Table 9).

Oxidative stress and pesticide metabolite showed dose response relationship to increased number of metabolic syndrome component (Appendix 8). In the precedent studies, it has been recognized that the relationship between the pesticide and the oxidative stress is highly related.

The PEM (spraying years × spraying days per year/30 days), CEI (intensity × spraying years × spraying days per year), and DAP levels (DMP, DEP, DMTP, and DETP) as well as PEM were positively correlated to the levels of 8-OHdG, isoprostane, CEI, and DMP. CEI, DMP, DEP, and DETP also represented a positive correlation to 8-OHdG (Table 8).

The MDA and isoprostane levels directly represent lipid peroxidation resulting from exposure to organophosphorus pesticides. Thus, our findings are consistent with those of other studies in which the oxidative stress was increased by the toxicity of synthetic .pyrethroid, organochlorine, and carbamate pesticides, including xenobiotics[65-66].

In the linear regression analysis (Table 11), a correlation analysis was performed after demographic characteristics, such as age, smoking, drinking, and exercise, were adjusted to determine the relationship between pesticide exposure and oxidative stress. As a result, 8-OHdG, isoprostane, and MDA were significantly related to DMP (ß = 0.320, p = 0.020), DEP (ß = 0.390, p =

0.004), and DETP (ß = 0.082, p = 0.015); DMP (ß = 0.396, p = 0.003), DEP (ß = 0.508, p = 0.000), and DETP (ß = 0.504, 0.000); and DMP (ß = 0.432, p = 0.001), DEP (ß = 0.508, p = 0.000), and DETP (ß = 0.329, p = 0.014), respectively. Organophosphorus pesticide mediated formation of ROS that activate protein kinase. Organophosphorus pesticide generated ROS formation and oxidative stress have been shown to be associated with apoptosis in different tissues[67-68].

A logistic regression analysis in a previous study showed that exposure to organophosphorus pesticides such as coumaphos (odds ratio 1.26, 95% CI 1.03−1.55), phorate (odds ratio 1.22, 95% CI 1.06−1.42), terbufos (odds ratio 1.17, 95% CI 1.02−1.35), and trichlorfon (odds ratio 1.85, 95% CI 1.03−3.33) were highly associated with diabetes. Longitudinal studies in Australia and USA have also shown an important role of pesticides in diabetes mortality. It is therefore necessary to manage the pesticide exposure in farmers because type II diabetes plays an important role in coronary artery disease and heart disease[69].

In this study, the relationship between DAP levels, PEM, and CEI and metabolic syndrome suggests that DAP, PEM, and CEI are important factors in metabolic syndrome. In addition, the logistic regression analysis after adjustment for age, smoking, drinking, and exercise showed significant differences between subjects with and without metabolic syndrome with respect to DMP (odds ratio 2.5, 95% CI 1.09−5.86) and DEP (odds ratio 5.0, 95% CI 1.62−15.52). In addition, although the group with metabolic syndrome showed higher odds ratios for DMTP and DETP than the group without metabolic syndrome, the difference was not significant (Table 10). There are dose response relationship between pesticide metabolite and number of metabolic syndrome component (Appendix 8).

Our results suggest that pesticide exposure increases oxidative stress, and the increased oxidative stress plays a role in metabolic syndrome[70-72]. Moreover, the

present results support the findings of previous studies showing direct relationships between pesticide exposure and metabolic syndrome. Thus, pesticide exposure should be controlled for farmers and the population at large. It is necessary to strictly control the access to pesticides, to minimize their use, and to replace highly toxic pesticides with those of low toxicity.

Prospective studies are required to verify the causal relationships between pesticide exposure and chronic diseases. In addition, it is necessary to consider the application and exposure characteristics of the pesticides and to exactly reproduce previous pesticide exposures[73]. Moreover, further studies are required in a larger farmer population to ensure statistical power, and a proper measurement of the biomarkers is required to reduce misclassification biases in the measurements of pesticide exposure and its influences on health.

The major limitation of this study is the cross-sectional design. The association between lipid soluble toxins and metabolic syndrome or insulin resistance may be explained by reverse causality. Some of the results were not statistically significant because of the small size.

V. REFERENCES

1. Lee WJ, The roles of mercury and pesticides on Alzheimer's Disease. Graduate School of Complementary Alternative Medicine, Pochon CHA University 2008.
2. Carmen Gomez, Manuel J. Bandez and Ana Navarro, Pesticides and impairment of mitochondrial function in relation with the parkinsonian syndrome. Frontiers in Bioscience 2007;12: 1079-1093.
3. Chandra, Pangtey BS, Modak DP, et al., Biological monitoring of chlorinated pesticides among exposed workers of mango orchards: a case control study in tropical climate. Bull environ Contam Toxicol 1992;48: 295-301.
4. Lee DH, Lee IK, Jin SH, Steffes M, Jacobs DR Jr, Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults: Results from the National Health and Nutrition Examination Survey 1999-2002. Diabetes Care 2007;30(3): 622-628.
5. Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR Jr, Relationship between serum concentrations of persistent organic pollutants and the prevalence of met abolic syndrome among non-diabetic adults: Results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia 2007;50(9): 1841-1845.
6. Serdal Ogut et al., Oxidative stress in the blood of farm workers following intensive pesticide exposure, Toxicology and Industrial Health 2011;27(9): 820-825.
7. Agrawal D, Sultana P. and Gupta GSD, Oxidative damage and changes in the glutathione redox system in erythrocytes from rats treated with hexachlorocyclohexane. Food and chemical Toxicology 1991;29: 459-462.

8. Almedia MG, et al., Pro and antioxidant parameters in rat liver after short-term exposure to hexacholorobenzene. Hum Exp Toxicol 1997;16: 257-261.

9. Banerjee BD et al., Biochemical effects of pesticides on lipid peroxidation and free radicals scavengers. Toxicol Lett 1999;107: 33-37.

10. Malkovics B, Effects of organophosphates on the antioxidant systems of fish tissue. Acta Biol Hung. 1995;46(1): 9-11.

11. Abdollahi M, et al., Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion comound. Comp bio Biochem Physiol C. Toxicol Pharmacol. 2004a;133: 29-39.

12. Ranjbar A, Pasalar P, Sedighi A, Abdollahi M. Induction of oxidative stress in paraquat formulating workers. Toxicol Lett 2002;131: 191-194.

13. Lee CH, Kamijima M, Kim H, et al., 8-Hydroxydeoxyguanosine levels in human leukocyte and urine according to exposures to organophosphorus pesticides and paraoxonase 1 genotype. Int Arch Occup Environ Health 2006;80: 217-227.

14. Samar Basu, Fatty acid oxidation and isoprostanes: Oxidative strain and oxidative stress, Prostaglandins, Leukotrienes and Essential Fatty Acids 2010;82: 219-225.

15. Anne Valle, Daniel T. O'connor, et. al., butyrylcholinesterase: Association with the metabolic Syndrome and identification of 2 Gene Loci Affecting Activity. Clinical Chemistry 2006;52(6): 1014-1020.

16. Evans FT, Gray PW, Lehmann H, Silk E, Sensitivity to succinylcholine in relation to serum cholinesterase. Lancet 1952;1: 1229-1230.

17. Harris H. Enzymes and drug sensitivity, The genetics of serum cholinesterase

'deficiency' in relation to suxamethonium apnoea Proc R Soc Med, 1964;57: 503-506.

18. Yen T, Nightingale BN, Burns JC, Sullivan DR, Stewart PM, Butyrylcholinesterase (BCHE) genotyping for post-succinylcholine apnea in an Australian population. Clin Chem 2003;49: 1297-1308.

19. Kim JH, and Kim YH, Ingibition of Acetylcholinesterase Activity on the Organophosphorus and Carbamate Pesticides, J. of the Korean Environmental Sciences Society 1998;7(1): 52-56.

20. Elizabeth A. Guillette, Maria Mercedes Meza, Maria Guadalupe Aquilar, Alma Delia Soto, and Idalia Enedina Garcia, An Anthropological Approach to the Evaluation of Preschool Children Exposed to pesticides in Mexico. Environmental Health Perspectives 1998; 106(6): 347-353.

21. Weichenthal S, Moase C, Chan p, A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort. Environ Health Perspect 2010;118(8): 1117-1125.

22. Lee WJ, Blair A, Hoppin JA, Lubin JH, Rusiecki JA, et al., Cancer incidence among pesticide applicators exposed to chlorpyrifos in the Agricultrual Health Study. J Natl Cancer Inst 2004;96(23): 1781-1789.

23. Beseler CL, Stallones L, A cohort study of pesticide poisoning and depression in colorado farm resident. Ann Epidemiol 2008;18(10): 768-774.

24. Beseler C, Stallones L et al., Depression and pesticide exposures in female spouses of licensed pesticide applicators in the Agricultural Health Study cohort. J Occup Environ Med 2006;48(10): 1005-1013.

25. Banerjee, B.D, et al., Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicology Letters 1999;107: 33-77.

26. Shahin Shadnia, et al., Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. Human & Experimental Toxicology 2005;24: 439-445.

27. Barr DB, Needham LL, Analytical methods for biological monitoring of exposure to pesticides: a review. J Chromatogr B 2002;778: 5-29.

28. Changes in leading causes of death (2006), Korean National Statistical Office. http://nso.go.kr/

29. Chronic Disease Information (2003-2007), Health Insurance Review & Assessment Service. http://hira.or.kr

30. K George MM Alberti, Paul Zimmet, Jonathan Shaw, The metabolic syndrome-a new worldwide definition from the International Diabetes Federation Consensus. Lancet 2005;366 (9491): 1059-1062.

31. Miranda PH, De Fronzo RA, Robert M. Califf RM, Guyton JR, Tex SA, et al., Metabolic syndrome: Definition, pathophysiology, and mechanisms. Am Heart J 2005;149: 33-45.

32. Steinberger J, Daniels SR, Eckel RH, Hayman L, Robert H, Lustig RH, et al., Progress and challenges in metabolic syndrome in children and adolescents: A scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in the Young Committee of the Council on Cardiovascular Disease in the Young; Council on Cardiovascular Nursing; and Council on Nutrition, Physical Activity, and Metabolism. Circulation 2009;119; 628-47.

33. Kohen R, Nyska R, Oxidation of bilogical systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicologic pathology 2002;30: 620-650.

34. Gulam Waris and Haseeb Ahsan, Reactive oxygen species: role in the development of cancer and various chronic conditions, journal of carcinogenesis. 2006;5(1): 14.

35. Davies KJ, Oxidative stress: the paradox of aerobic life. Biochem Soc Symp 1995;61: 1-31.

36. Draper HH, Hadley M, Malondialdehyde determination as index of lipid peroxidation. Meth Enzymol 1990;186: 421-431.

37. Loeckie LZ, John HM, Jan NC, Noco PV, Biomarkers of free radical damage applications in experimental animals and in humans. Free Radical Biology & Medicine 1999;26: 202-226.

38. Wolff SP, Jiang ZY, Hunt JV, Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med. 1991;10: 339-352.

39. Nacíitarhan S, Ozben T, Tuncer N, Serum and urine malondialdehyde levels in NIDDM patients with and without hyperlipidemia. Free Radic Biol Med 1995;19: 893-896.

40. Chehne G, Oguogho A, Lupattelli G, Budinsky AC, Palumbo B, Sinzinger H, Increase of isoprostane 8-epi-PGF_2 after restarting smoking Prostaglandins Leukotrienes, Essential Fatty Acids 2001;64: 307-310.

41. Frei B, Forte TM, Ames BN, Cross CE. Gas phase oxidants of cigarette smoke induce lipid peroxidation changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. Biochem J 1991;277: 133-138.

42. Pilz H, Oguogho A, Chehne F, Lupattelli G, Palumbo, Sinzinger H, Quitting cigarette smoking results in a fast improvement of in vivo oxidative injury determined via plasma, serum, and urinary isoprostane. Thromb Res 2000;99:

209-221.

43. Germadnik D, Pilger A, Ewertz M, Tjonnenl and A, Overvad K, Poulsen HE, Oxidative DNA damage estimated by 8-hydroxyde-oxyguanosine excretion in humans: influence of smoking, gender and body mass index Carcinogenesis 1992;13: 2241.

44. Leinone J, Lehtimaki T, Toyokuni S, Okada K, Tanaka T, et al., New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabet esmellitus. FEBSLett 1997;417: 150-152.

45. Cutler RG, Antioxidants and aging. Am J Clin Nutr 1991;53: 373-379.

46. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) JAMA. 2001;285: 2486-2497.

47. Alberti KG, Zimmet PZ, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15: 539-553.

48. Beck Nielsen H, General characteristics of the insulin resistance syndrome: prevalence and her it ability. European Group for the study of Insulin Resistance (EGIR) Drugs 1999;58(Suppl 1): 7-10.

49. Koh JH, et al., Optimal Waist Circumference Cutoff Values for Metabolic Syndrome Diagnostic Criteria in a Korean Rural Population. J Korean Med Sci 2010;25(5): 734-737.

50. Kasai H, et al., Life style and urinary 8-hydroxydeoxyguanosine, a marker of

oxidative DNA damage: effects of exercise, working conditions, meat intake, body mass index, and smoking. Jpn. J. Cancer Res 2001;92(1): 9-15.

51. Toraason, Mark., 8-Hydroxyguanosine as a biomarker of workplace exposure. Biomarkers 1999;4(1): 3-26.

52. satsakis AM, Barbounis MG, et al., Determination of dialkyl-phosphates in human hair for the biomonitoring of exposure to organophosphate pesticides, Journal of Chromatography B 2010;878: 1246-1252.

53. Angelina Duggan, Gail Charnley et al., Dialkyl phosphate biomonitoring data: assessing cumulative exposure to organophosphate pesticides, Regulatory Toxicology and Pharmacology 2003;37: 382-395.

54. Roberto Bravo, Lisa M Caltabian, et. al., Measurement of dialkyl-phosphate metabolites of organophosphorus pesticides in human urine using lyophilization with gas chromatography-tandem mass spectrometry and isotope dilution quantification. Journal of Exposure Analysis and Environmental Epidemiology 2004;14: 249-259.

55. Alavanja MC, Sandler DP, McMaster SB, Zahm Sh, McDonnell CJ, Lynch CF, Pennybacker M, Rothman N, Dosemeci M, Bond AE, Blair A. The agriculture health study. Environ Health Perspect 1996;104: 362-369.

56. Dosemeci M, Michael CR, et al., A quantitative approach for estimating exposure to pesticide in the agricultural health study. Ann Occup Htg 2002;46(2): 245-260.

57. Kohen R, Nyska A, Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol 2002;30: 620-650.

58. Kelton Tremellen, Oxidative stress and male infertility-a clinical perspective,

human Reproduction Update, 2008;14(3): 243-258.

59. Ahmed RS, Vandana Seth, Pasha ST & Banerjee BD, Influence of dietary ginger(Zingiber officinalis Rosc) on oxidative stress induced by malathion in rats. Food and Chemical Toxicology 2000;38: 443-450.

60. Singh VK, Jyoti, Krishna Reddy MM, Kesavachandran C, Rastogi, SK, Siddiqui, MK. Biomonitoring of organochlorines, glutathione, lipid peroxidation and cholinesterase activity among pesticide sprayers in mango orchards. Clin Chim Acta 2000;377: 268-272.

61. Soltaninejad K, Abdollahi M Current opinion of the science of organophosphate pesticides and toxic stress: A systematic review. Med Sci Monit 2009;15: 75-90.

62. Lukaszewicz Hussain A, Role of oxidative stress in organophosphate insecticide toxicity－Short review. Pest Biochem Physiol 2010;98: 145-150.

63. Kamath V, Rajini PS, Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. Toxicology 2007;231: 137-146.

64. Rahimi R, Abdollahi M, A review on the mechanisms involved in hyperglycemia induced by organophosphorus pesticides. Pesti Biochem Physiol. 2007;88: 115-121.

65. Stevenson DE, Kehrer JP, Kolaja KL, Walborg EF, and Klaunig JE, Effect of dietary antioxidants on dieldrin induced hepato toxicity in mice. Toxicology Letters 1995;75: 177-183.

66. Prakasam AS, Sethupathy S, Lalitha, Plasma and RBCs antioxidant status in occupational male pesticide sprayers. Clinica Chimica Acta 2001;310: 107-112.

67. Oral B, Guney M, Demirin H, Ozguner M, Giray SG, Take G, Mungan T,

Altuntas I, Endomertrial damage and apoptosis in rats induced by dichlorvos and almeliorating effect of antioxidant vitamins E and C. Reprod Toxicol 2006;22(4): 783-790.

68. Yu F, Wang Z, Ju B, Wang Y, Wang J, Bai D, Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamins C and E. Exp Toxicol Pathol 2008;59(6): 15-23.

69. Everett CJ, Matheson EM, Biomarkers of pesticide exposure and diabetes in the 1999-2004 National Health and Nutrition Examination Survey, Environment International 2010;36: 398-401.

70. Stevenson DE, Kehrer JP, Kolaja KL, Walborg EF, and Klaunig JE, Effect of dietary antioxidants on dieldrin-induced hepato-toxicity in mice. Toxicology Letters 1995;75: 177-183.

71. Soltaninejad K, Abdollahi M, Current opinion of the science of organophosphate pesticides and toxic stress: A systematic review. Med Sci Monit 2009;15: 75-90.

72. Lukaszewicz Hussain A, Role of oxidative stress in organophosphate insecticide toxicity－Short review. Pest Biochem Physiol 2010;98: 145-150.

73. Loewenherz C, Fenske RA, Simcox NJ, Beltarny G, Kalman D, Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central washington state. Environ Health Perspect 1997;105: 1344-1353.

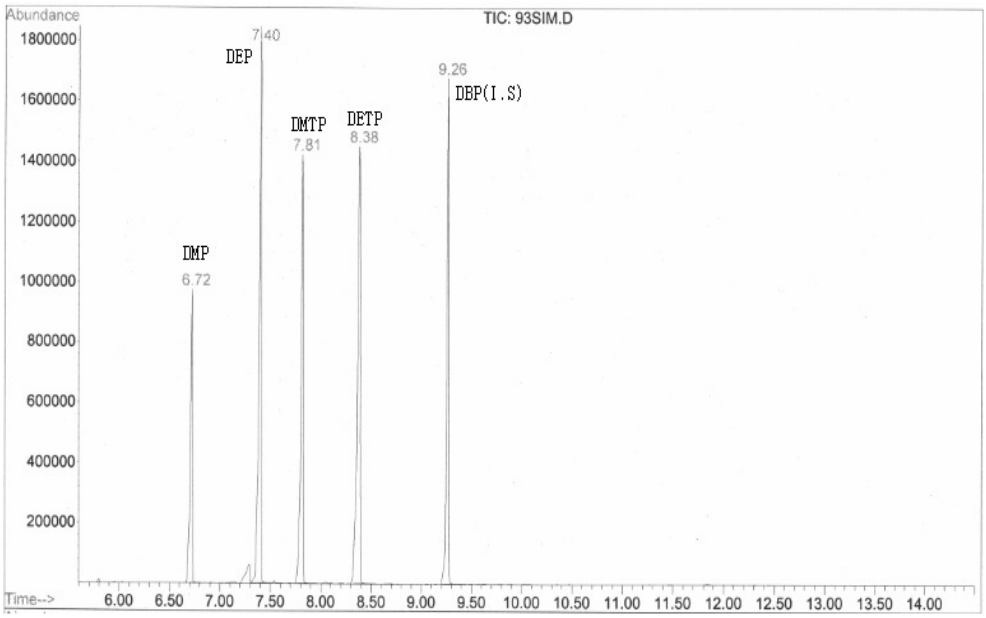
APPENDIX

Appendix 1. Status of pesticide use

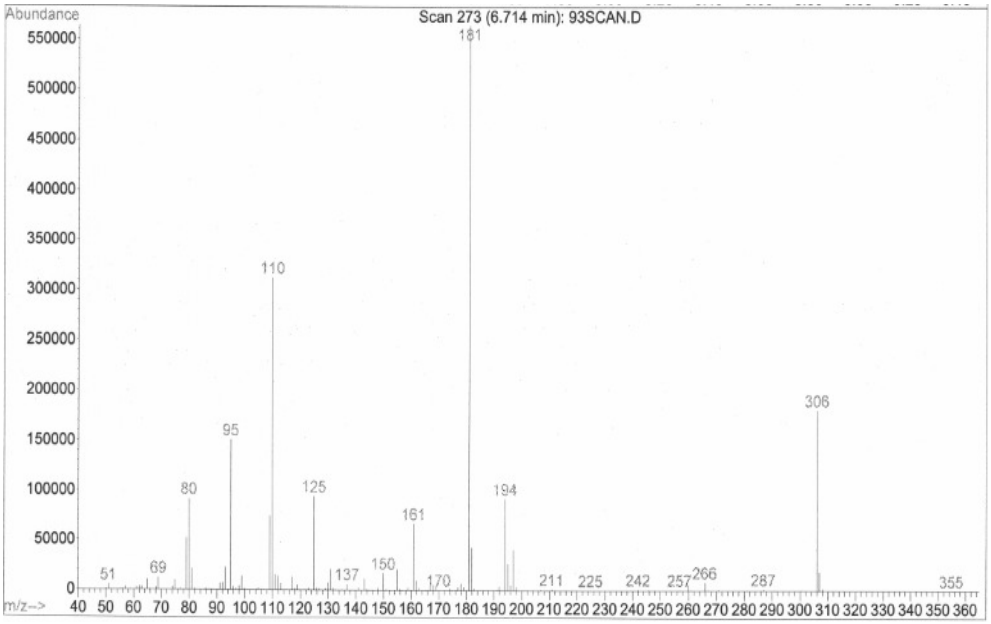
pesticide name	available component	N
butachlor	chloroacetanilide	43
paraquat dichloride	Bipyridilium	38
carbofuran	carbamate	25
mancozeb	organic sulfur	22
chlorothalonil	organochlorine	19
fenitrothion	organophosphate	14
mefenacet+pyrazosulfuron-ethyl	mixture (acetanilide+sulfonylurea)	12
alachlor	chloroacetanilide	11
tricyclazole	Triazole	9
dithianon	Quinone	9
IBP	organophosphate	8
machine oil	Emulsifiers	6
deltamethrin	synthetic pyrethroid	6
glyphosate	organophosphate	5
s-metolachlor	acetanilide	5
dichlorvos	organophosphate	5
cartap hydrochloride	cartap	5
chlorpyrifos	organophosphate	5
propineb	dithiocarbamate	5
benomyl	benzimidazoles	4
mancozeb+metalaxyl	mixture	4
acetamiprid+etofenprox	mixture (chloronicotinil+synthetic pyrethroid)	4
pendimethalin	dinitroaniline	3
molinate+pyrazosulfuron-ethyl	mixture (thiocarbamate+sulfonylurea)	3
bentazone	pH regulator	3

phorate	organophosphate	3
acephate	organophosphate	3
sulfur	inorganic sulfur	3
isoprothiolane	organic sulfur	3
fluazinam	dinitroaniline	3
carbendazim, kasugamycin	carbamate, Antibiotics	3
phosphamidon	organophosphate	2
fenobucarb	carbamate	2
methidathion	organophosphate	2
cypermethrin	synthetic pyrethroid	2
probenazole+thiacloprid	mixture(probenazole+chloronicotinil)	2
glyphosate-ammonium	Surfactants	1
glyphosate ammonium+oxyfluorfen	Surfactants	1
bensulfuron-methyl+molinate	mixture(sulfonylurea+carbamate)	1
endosulfan	organochlorine	1
ethoprophos	organophosphate	1
methomyl	carbamate	1
trichlorfon	organophosphate	1
hexaconazolr	Triazole	1
daimuron+imazosulfuron+oxaziclomefone	mixture (phenylurea+sulfonylurea+oxazinone)	1
bensulfuron-methyl+indanofan	mixture	1
butachlor+pyrazolate	chloroacetanilide+pyrazol	1
imidacloprid	chloronicotinil	1
azoxystrobin	strobilurin	1
glufosinate ammonium	Surfactants	1
chlorpyrifos, imidacloprid	organophosphate, imidazolin	1

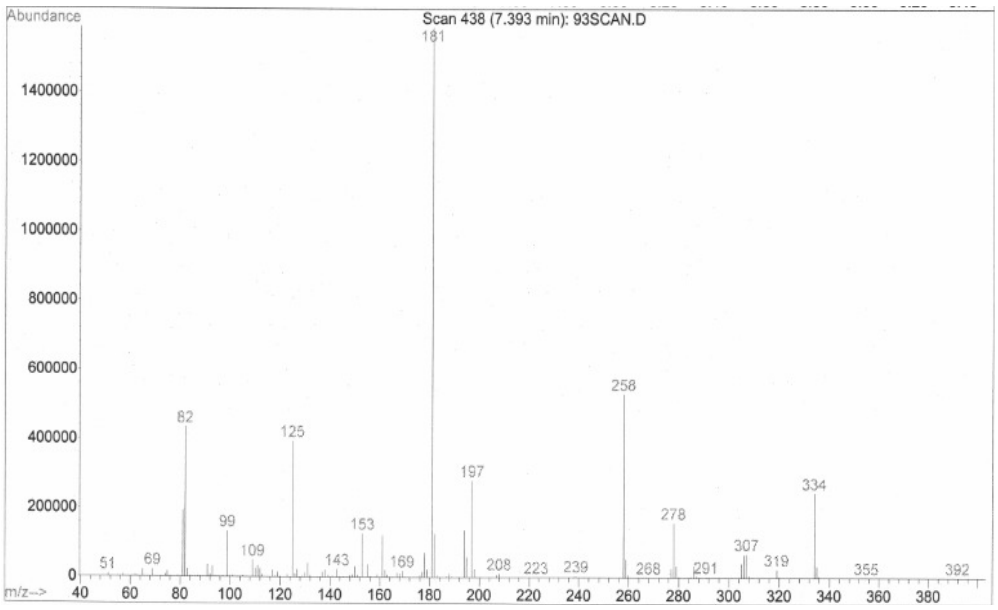
Appendix 2. Chromatogram of dialkyl-phosphates-PFBBr



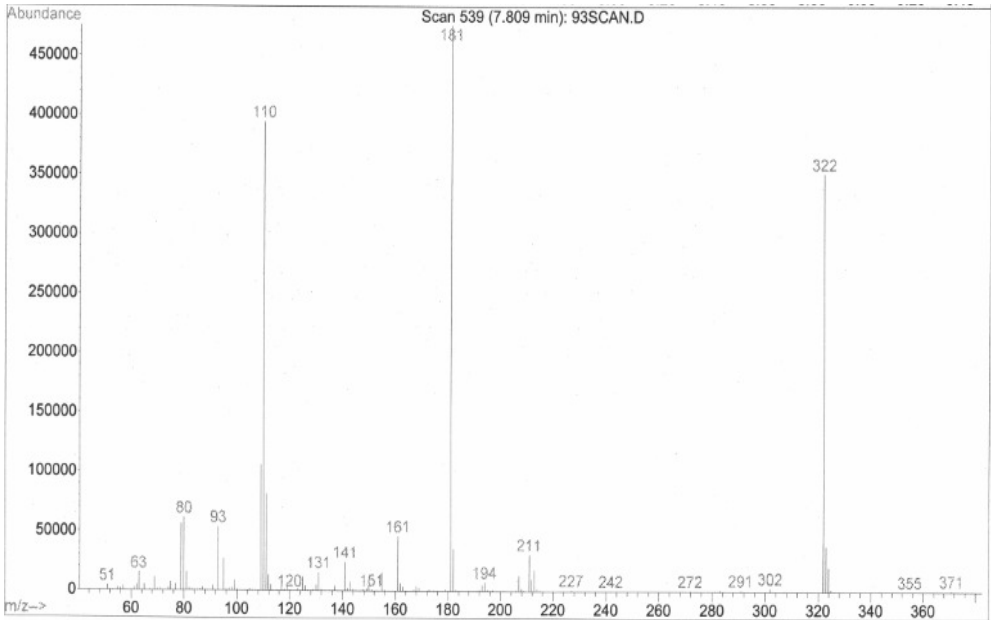
Appendix 3. Mass spectra of DMP



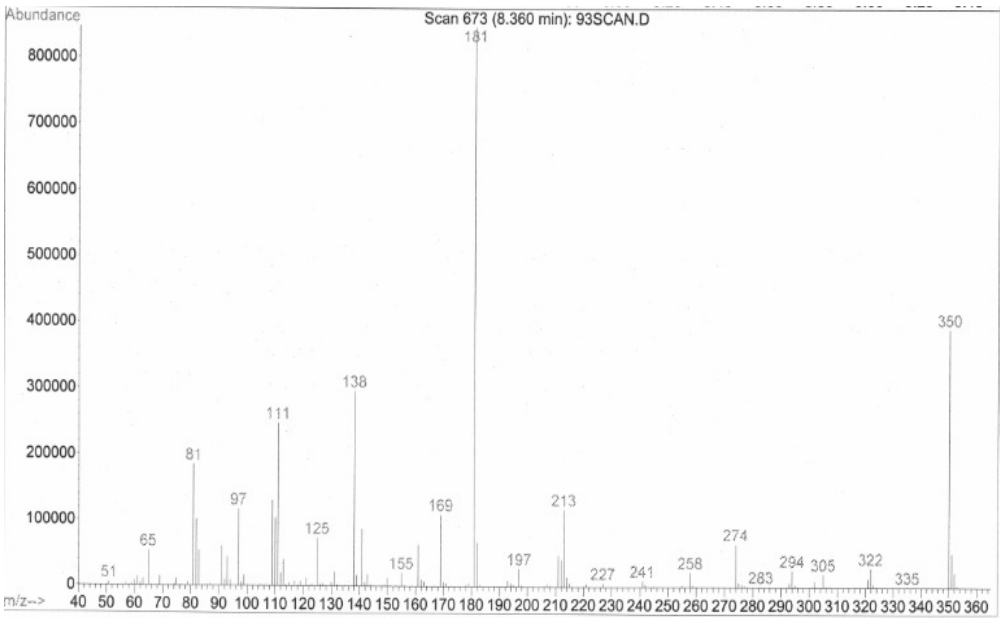
Appendix 4. Mass spectra of DEP



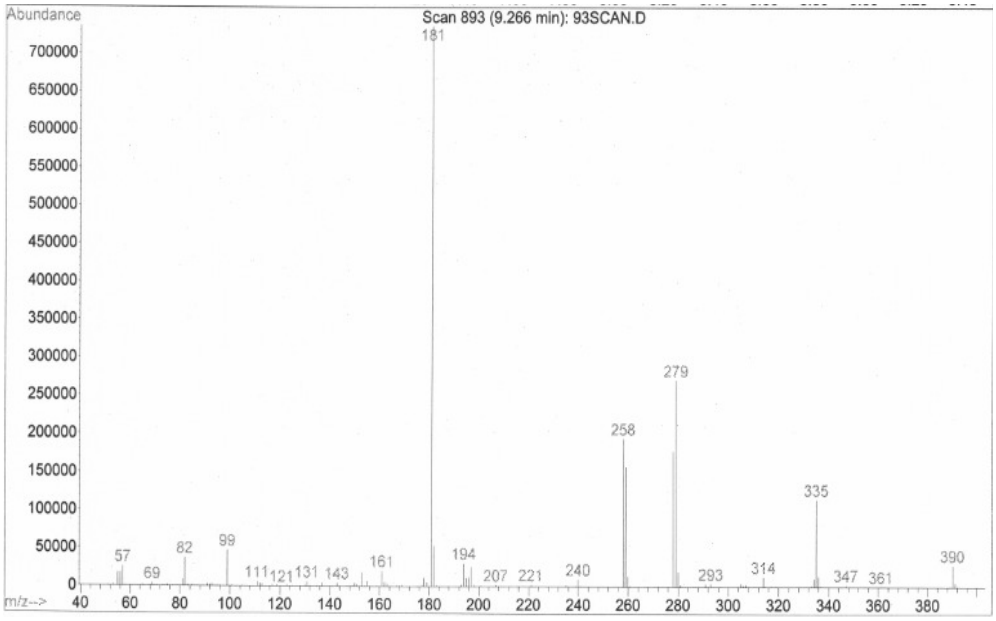
Appendix 5. Mass spectra of DMTP



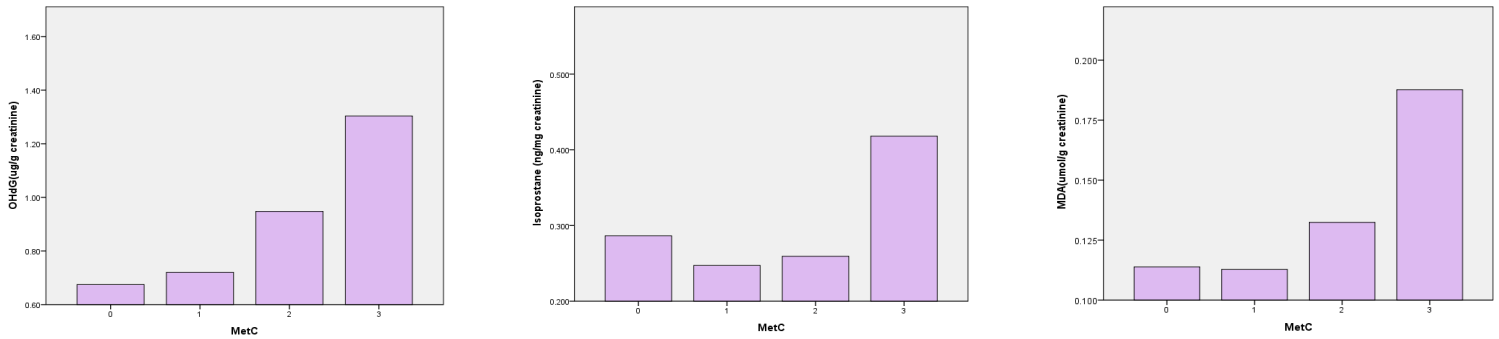
Appendix 6. Mass spectra of DETP



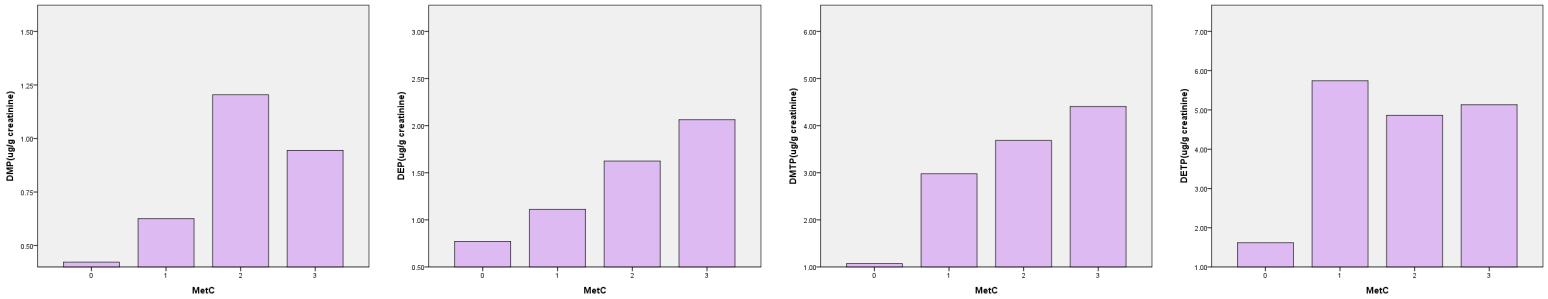
Appendix 7. Mass spectra of DBP



Appendix 8. Effects of oxidative stress biomarkers and pesticide metabolites on metabolic syndrome component number



a) Effects of oxidative stress biomarkers on metabolic syndrome component number



b) Effects of pesticide metabolites on metabolic syndrome component number

Appendix 9. Recovery, repeatability, and MDL of 8-OHdG

	concentration (μg/L)	N	8-OHdG (Mean)	SD	RSD(%)
Recovery(%)	2.5	2	101.8	1.30	1.29
	10	2	100.6		
	20	2	99.2		
Repeatability	2.5	2	2.597	0.023	0.9
MDL(μg/L)		3	0.053		

Abbreviations: N, number of observations; MDL, method detection limits; SD, standard deviation; RSD, relative standard deviation

Appendix 10. Recovery, repeatability, and MDL of isoprostane

	concentration (ng/mL)	N	Isoprostane (Mean)	SD	RSD(%)
Recovery(%)	2.5	2	101.8	1.30	1.29
	10	2	100.6		
	20	2	99.2		
Repeatability	2.5	2	2.619	0.055	2.1
MDL(pg/mL)		3	0.162		

Abbreviations: N, number of observations; MDL, method detection limits; SD, standard deviation; RSD, relative standard deviation

Appendix 11. Recovery, repeatability, and MDL of MDA

	concentration (umol/L)	N	MDA (Mean)	SD	RSD(%)
Recovery(%)	1.77	3	91.87	4.92	5.36
Repeatability	2.5	2	2.619	0.055	2.1
MDL(umol/L)		3	0.043		

Abbreviations: N, number of observations; MDL, method detection limits; SD, standard deviation; RSD, relative standard deviation

국 문 요 약

농작업자에서의 농약노출, 산화스트레스, 대사증후군에 대한 연구

배경 및 목적:

농약에 노출이 되면 건강에 많은 영향을 주는데 특히, vitro와 vivo에서 활성산소종이 생성되고 생성된 래디칼은 생체분자를 산화시킴으로서 세포의 괴사 또는 조직손상이 일으킨다. 이처럼 증가된 산화스트레스는 심혈관 질환, 고혈압, 당뇨, 암과 같은 질병과의 관련성이 강조되고 있어, 이에 본 연구는 농약에 노출된 농작업자를 대상으로 지질손상 지표인 MDA, isoprostane와 DNA 손상지표로 8-OHdG, 농약 대사물질 (diakyl-phosphate)을 측정하여 농약노출, 산화스트레스, 대사증후군과의 관련성을 알아보고자 한다.

대상 및 방법:

본 연구는 단면연구로 2011년 5월부터 2011년 8월까지 농작업자 중 농약에 노출된 작업자 84명을 대상으로 하였다. 산화스트레스 지표로는 8-OHdG, isoprostane, MDA를 측정하고, 농약 노출에 대해서는 요 중으로 배출되는 dialkyl-phosphate 측정하였다. 대사증후군과 산화스트레스 지표, 농약 대사물질과의 관련성을 파악하기 위해 로지스틱회귀분석 및 상관분석을 실시하였다.

결과:

대사증후군 있는 그룹 (19명)과 없는 그룹 (65명)으로 구분하여 산화스트레스 지표인 8-OHdG, isoprostane, MDA를 측정한 결과 대사증후군 있는 그룹이 없는 그룹에 비해서 산화스트레스 지표의 농도가 높은 것을 확인하였다 ($p<0.05$). 로지스틱회귀분석 결과에서도 대사증후군이 없는 그룹에 비해 있는 그룹에서 8-OHdG (odds ratio 3.8, 95% CI 1.23-11.71), isoprostane (odds ratio 4.4, 95% CI 1.347-14.52), MDA (odds ratio 5.4, 95% CI

1.29-22.66)의 농도가 높은 것을 확인하였다.

농약 노출 평가와 산화스트레스 지표와의 상관분석 결과 농약노출지수 (PEM)는 8-OHdG, isoprostane, 누적노출지수 (CEI), DMP와 유의한 양의 상관성을 보였으며, CEI는 8-OHdG와 상관성을 보였다. 농약 대사물질인 DMP, DEP, DETP는 8-OHdG, isoprostane, MDA와 양의 상관성을 보였다.

농약 노출이 산화스트레스에 미치는 관련성을 파악하기위해 인구학적 특성을 보정하였다. 8-OHdG는 DMP (ß=0.320), DEP (ß=0.390), DETP (ß=0.082)와 유의한 관련성을 보였고, isoprostane은 DMP (ß=0.396), DEP (ß=0.508), DETP (ß=0.504)와 유의한 관련성이 있었으며, MDA는 DMP (ß=0.432, p=0.001), DEP (ß=0.508, p=0.000), DETP (ß=0.329, p=0.014)와 관련성이 있었다.

Dialkyl-phosphate와 노출지표인 PEM, CEI를 대사증후군과의 관련성을 살펴본 결과 인구학적 특성을 보정하여 로지스틱회귀분석 결과 DMP는 대사증후군이 없는 그룹에 비해 있는 그룹에서 DMP (odds ratio 2.5, 95% CI 1.09-5.86), DEP (odds ratio 5.0, 95% CI 1.62-15.52)는 통계적으로 유의한 차이가 있었다. DMTP와 DETP는 대사증후군이 없는 그룹에 비해 대사증후군이 있는 그룹에서 odds ratio가 높았지만 통계적으로 차이는 없었다.

결론:

대사증후군 증상이 있는 농약에 노출된 작업자에서 산화스트레스 지표인 8-OHdG, isoprostane, MDA의 농도가 증가하였고, 대사증후군이 있는 그룹에서 농약 대사물질인 DMP, DEP, DETP의 농도가 높았다. 산화스트레스 지표는 농약 대사물질과 관련성이 있었다. 따라서 농약 노출은 산화스트레스, 대사증후군과의 관련성이 있음을 확인할 수 있었다.

핵심이 되는 말 : 농작업자, 농약 노출, 산화스트레스, 농약대사물질, 대사증후군