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Residues of Persistent Organic Pollutants in Beef Cattle

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Abstract – **Tetrachloro dibenzo-*p*-dioxins (TCDDs) and tetrachloro dibenzofurans (TCDFs) are toxic compounds persistently present in the environment. The contamination of dioxins may affect human health shown as carcinogenic effects, reproductive failure, dermal toxicities and neurologic effects. The present study describes the determination of TCDDs/TCDFs in tissues of beef cattle in relation to the contamination of persistent organic pollutants(POPs) in solid matrices. The TCDDs/TCDFs were detected by GC MS/MS and POPs by GC-ECD. The analysis of POPs revealed that POPs (aldrin, dieldrin, DDT, endrin and heptachlor) were detected in rice straws, concentrate, and drinking water in Kulonprogo, Bantul and Klaten. The average concentration of POPs was 0.45μg.kg^{-1} (rice straws), 46.10 μg.kg^{-1} (concentrate) and 0.04 μg.kg^{-1} (drinking water) in Kulonprogo; 3.12 μg.kg^{-1} (rice straws), 0.84 μg.kg^{-1} (concentrate) and 3.22 μg.kg^{-1} (water) in Bantul and 1.92 μg.kg^{-1} (rice straws), 1.08 μg.kg^{-1} (concentrate) and 4.34 μg.kg^{-1} (drinking water) in Klaten. These matrices are regarded as the sources of POPs contamination in cattle. The POPs residues that were also detected in sera were 2.33 μg.kg^{-1} (Kulonprogo), 0.83 μg.kg^{-1} (Bantul), and 0.65 μg.kg^{-1} (Klaten) and in beef at 0.33 μg.kg^{-1} (Kulonprogo), 0.41 μg.kg^{-1} (Bantul), and 0.77 μg.kg^{-1} (Klaten). The TCDD/Fs were also detected in beef samples with a total TEQ from 1284.746 to 5897. 827 ρg.g^{-1}, and 204.893ρg.g^{-1} respectively. The concentrations of dioxins residues in these meat samples seem to be exceeding the maximum residue limit (MRL) at 4.5 ρg.g^{-1}. High concentration of dioxins residues in meat should be taken into consideration since it may have a direct effect on animals and also on the health of humans consuming it.**

Keywords -- **Dioxins, POPs, Meat, Feed Matrices, GC MS/MS.**

I. Introduction

Dioxins comprising tetrachlorinated dibenzo-ρ-dioxins (TCDDs), tetrachlorinated dibenzo-furans (TCDFs) and polychlorinated biphenyls (PCBs) are highly toxic compounds that may affect human health (European Commission, 2006ab). These toxicants may induce toxicity effects such as cancer, skin lesions, reproductive failures and endocrine dysfunctions (Schwarz and Appel, 2005; Wang *et al*., 2009;The World Health Organization, 2010). The dioxins are normally derived from wastes of chemical processes or natural disasterssuch as forest fires and volcanic eruption, combustions, waste incinerators, household heating, and pulp and paper industry (McKay, 2002; European Commission, 2006ab).

The compounds are hydrophobic and highly non-polar lipophilic that may cause accumulation in fatty content of food chains in a longer period of time (Ferrario and Byrne, 2000). The consumption of contaminated fatty food is then known as the primary pathway for human dioxins exposure (Startin and Rose, 2003). Chronic exposures in humans causes reproductive and developmental disorders, neurological effects, dermal toxicity, immunological changes and carcinogenic effects (Schwarz and Appel, 2005; Wang *et al*., 2009;The World Health Organization, 2010). Dioxins contamination was then regarded as significant food safety issues throughout the world in the 20th century such as crises of dioxins exposures in American continent and Europe. The Belgian crises in 1999 showed that leaking of mineral oil containing high PCDDs/Fscaused dioxinscontamination in poultry feed (Bernard et al., 2002; Pirard and DePauw, 2007), while a contamination of PCBs in oranges occurred in Brazil last decade (Malisch, 2000). There is however insufficient information available in Indonesia relating to dioxins contaminations in food products. On the other hand, the contamination of persistent organic pollutants and other organochlorinesare commonly reported in food chains in Indonesia.

The persistent organic pollutants (POPs) such as DDT, aldrin, dieldrin, endrin and heptachlor are non-degradable organochlorine pesticides and persistently occur in the environment. These compounds are known as a precursor for dioxins and dioxin-likes formation (Schmid et al., 2007; Shaw et al., 2013). The PCDDs/Fs are generally formed through high temperature combustion of organic materials containing chlorines such as POPs (Bumb et al., 1980; Weber and Kuch, 2003). Schmid et al (2007) reported that the dioxins were regarded as a metabolite of POPs particularly DDT. Although all organochlorine pesticides including POPs had been prohibited to use and distributed for agricultural purposes since 1990s in Indonesia, the residues of these compounds were still detected in animal products and environment matrices (Indraningsih et al., 2004; Indraningsih and Sani, 2006).

The purpose of this study is to determine the level of POPs residues in sera, beef and farm matrices such as animal feed and drinking water detected with GC-ECD in relation to the dioxins contamination in meat using GC MS/MS.

II. Materials and Method

A. Sample collection

Samples consisting of animal feed, drinking water, blood, and meat were collected from Kulonprogo, Bantul and Klaten. Meats were collected at the local Animal Slaughtering Houses, while others were collected directly from the farms. Approximately 25 – 50 gram of solid matrices were collected from both areas and kept in clean sampling bags or containers. About 200 – 500 ml of water samples were collected from irrigated water, wells and public water and kept in a sampling bottle individually. Bloods were collected individually from beef cattle at the volume of 10 ml using plain–blood sample tubes. These samples were brought for the analysis of POPs using GC-ECD (Thermo Finigan). Meats were also analyzed for tetrachloro dibenzo-*p*-dioxins (TCDDs) and tetrachloro dibenzofurans (TCDFs) using GC MS/MS (Thermo Scientific).

B. Materials

All solvents used were pro analysis or suprasolve grade and purchased commercially from Merck-Millipore (Germany) including hexane, toluene, dichloromethane and ethyl acetate. All standard solutions were mixed standard solutions containing aldrin, dieldrin, dichloro diphenyl trichlorethane (DDT), endrin and heptachlor; and the isomers ofisotope$^{13}C_{12}$(EDF-5999 of 99% purity) for internal standard solutions; a clean-up standard (EDF-6999 isotope 37Cl_4) of 96% purity; a labeled compound of EDF-8999 (Isotope stock solution); a calibration standard (EDF-9999-A isotope solutions CS_1 – CS_5) of 1/10 concentration; *n*-Nonane of 99% purity; and a precision and recovery standard solution (EDF-7999-10X) were purchased from Cambridge Isotope Laboratories (USA). Sodium sulphate of pro analysis grade was obtained from Merck-Millipore (Germany). The column chromatography consisting alumina, carbon and silica were purchased from Thermo Scientific (Germany).

C. Analysis of POPs using Gas Chromatography – Electron Capture Detector(GC-ECD)

Sample preparation procedure

Samples were put into a clean plastic bag or container and kept in a freezer at -20°C until further analysis. Preparation of samples was carried out following the method described by Schenck et al (1996) where 5 g sample was diluted and homogenized in 15 ml acetonitrile for 30 second at 11,000 rpm and centrifuged at 3,000 rpm. The filtrate was then diluted with distilled water and eluted into a cartridge C_{18} that has been conditioned with 6 ml petroleum ether, 6 ml acetone, 2X 6 ml methanol and 2X 6 ml distilled water with a flow rate at 3 drops per second.

The cartridge was then washed with 2X 5 ml distilled water. Samples were cleaned-up using flourisil in a syringe containing glass wool. The top layer was covered with Na_2SO_4 anhydrate and poured with 5 ml petroleum ether. The samples were re-eluted with 2% diethyl ether in petroleum ether. The eluent was evaporated until almost dried and diluted with hexane up to 1 ml volume for the detection of POPs using GC-ECD (Thermo Finnigan).

The detection of POPs using GC-ECD

The sample in hexane at 1 µl was injected into GC-ECD using a micro syringe. The operational conditions of equipment were 230°C of an injector, 250°C of a detector, 175 - 280°C column, 1 ml/minute for helium gas carrier, and 30 ml/minute for nitrogen carrier. The concentration of POPs was calculated as a ratio between peak area of a sample to peak area of standard multiplied by a dilution factor and divided by sample weight.

D. Analysis of TCDDs/Fs using Gas Chromatography Tandem Mass Spectrometry (GC-MS/MS)

Sample-preparation procedure

The procedure of sample preparation is according to the method released by the EPA Method 1613 using FMS/Fluid Management System (Environmental Protection Agency, 1994). The method is for determination of tetra– through octa–chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) in water, soil, sediment, sludge, tissue, and other sample matrices by gas chromatography mass spectrometry (GC MS/MS).Samples were homogenized using dissection and/or mortar followed by an addition of hydro matrix powder and Na_2SO_4 anhydrate to dehydrate the samples. The FMS was then performed on dried products powder using an internal standard solution with *n*-Nonane as a solvent. This was the followed by subsequent clean-up steps using a clean-up standard solution of 96% purity (EDF-6999 Isotope 37Cl_4).

Extraction of Lipids

Samples of solid matrices (soils, meat and animal feed) were subjected to solid material extraction procedure consisting of mixing withhexane, toluene, dichloromethane and ethyl acetate. The organic layer of this extraction was evaporated and the lipid residue was dried and weighed in order to calculate the levels of lipids in the matrices. The labeled quantification standards were added to each sample before the extraction. A toluene solution contains a mixture of the $^{13}C_{12}$ isomers of all the 17PCDDs/Fs congeners and 1 OCDF without $^{13}C_{12}$ non-ortho PCBs and $^{13}C_{12}$ mono-ortho PCBs.

Clean up

For the determination of PCDDs/Fs and OCDF, clean-up was performed according to theEPA Method 1613 (Environmental Protection Agency, 1994):

a. Silica Cleanup

The first step of clean up was carried-out for these samples by using a commercial silica column. Glass-wool was placed in a 15 mm ID chromatography column and filled with 1 g silica gel, 4 g basic silica gel, 1 g silica gel, 8 g acid silica gel, 2 g silica gel and 4 g granular anhydrous sodium sulphate. Pre-elute was performed to the column with 50 – 100mL of hexane and close the stopcock when the hexane is within 1 mm of the sodium sulphate. The eluate was discarded. The concentrated extract was added to the column and rinse twice with 1 mL portions of hexane. The CDDs/CDFs was eluted with 100 mL hexane, and the eluate was ready for further cleanup.

b. Alumina Cleanup

The second step of clean up was using an alumina column. A glass wool was placed in a 15 mm ID chromatography column and added with 6 g acid alumina or 6 g basic alumina. Pre-elute was performed with 50 – 100 mL of hexane and close the stopcock when the hexane is within 1 mm of the alumina. The concentrated extract was added to the column and rinse twice with 1 mL portions of hexane. The CDDs/CDFs was eluted with 20 mL methylene chloride: hexane (20:80 v/v) if using acid alumina or with 20 mL methylene chloride: hexane (50:50 v/v) if using basic alumina. The concentrated eluate was ready for further cleanup.

c. Carbon Cleanup

Finally, clean up was performed by using a carbon column. A glass column of 10 cm length and 10 mm ID was cut both ends to produce a 10 cm column and filled with glass wool and 0.55 g of Carbopak/Celite to form an adsorbent bed approximately 2 cm long. Pre-elute was carried out with 5 mL of toluene, 2 mL of methylene chloride: methanol: toluene (15:4:1 v/v), 1 mL of methylene chloride: cyclohexane (1:1 v/v), and 5 mL of hexane. The sample extract was transferred into the column and rinsed the sample container with 1 ml portions of hexane twice then transferred to the column. The interfering compounds were eluted with 3 ml portions of hexane, 2 mL of methylene chloride: cyclohexane (1:1 v/v), and 2 mL of methylene chloride: methanol: toluene (15:4:1 v/v). The CDDs/CDFs was eluted with 20 mL of toluene. The concentratedeluate was ready for injection into the GC MS/MS with an addition of 50 µl of *n*-nonane containing 2 ng/ml of standard solution ($^{13}C_{12}$ 1,2,3,4 TCDD).

Instrumental analysis

The residues quantification of POPs was performed by GC-ECD (Thermo Finnigan) following the methods described by Lehotay*et al*. (2005) and Schenck and Wagner (1995). The PCDDs/Fs were quantified by GC MS/MS (Thermo Scientific) in MID modeand a TraceGCcoupled to a MAT-95 XP mass spectrometer (Thermo Scientific). The GC MS/MS was supported with a CTC A 200S auto sampler at 10000 resolving power (10% valley definition).Instrumental conditions and purity control criteria were according to the EPA 1613 method (Environmental Protection Agency, 1994). The isotopic dilution method was applied for quantifying concentration and recovery levels. The limit of detection(LOD) for each congener was determined as the concentration in the extract which produced in two different ions to be monitored with a signal to noise ratio of 3:1 for the less sensitive signal (Commission Directive,2004). The WHO-98 toxicity equivalent factors (TEF) were used in calculating TEQ (Van den Berg *et al*., 1994).

III. Results and Discussion

A. Residues of persistent organic pollutants in meats and sera of beef cattle

Table 1 shows that the highest concentration of POPs residue consisting aldrin, dieldrin, DDT, endrin and heptachlor in beef samples was found in Klaten at the average concentration of 0.774 µg/kg followed by Bantul (0.412 µg/kg) and Kulonprogo (0.330 µg/kg). Heptachlor was the most common compound detected from beef samples (27 of 30 samples) in Kulonprogo, followed by DDT (12 of 15 samples) in Klaten and dieldrin (9 of 15 samples) in Bantul. The DDT was found to be the highest concentration of residue detected in beef in Klaten (2.049 µg/kg) and Bantul (0.768 µg/kg), but heptachlor was found as the highest concentration of residue detected in Kulonprogo (0.549 µg/kg).The concentration of DDT was detected in beef samples at 2.069µg/kg in Klaten followed by 0.768µg/kg in Bantul and 0.301µg/kg, while heptachlor was found as the highest concentration of residue detected in Kulonprogo (0.549 µg/kg).

The presence of POPs in beef samples in particular DDT should be taken into consideration since this compound had been banned for agricultural purposes in 1970s and all POPs were banned since 1990s. The DDT was detected in two districts (Bantul and Klaten) at high concentration of 0.768 µg/kgand 2.049 µg/kg subsequently. The results are suggested to be an important information since this compound was the first organochlorinebeing banned in Indonesia. The presence of DDT in beef samples might be caused by an earthmoving of the contaminated deep soils since excessive use of DDT being applied in the past or there was an illegal distribution of DDT. However, heptachlor was detected frequently in Kulonprogo than other POPs compound and districts where 27 out of 30 samples being positive to DDT at the average concentration of 0.549 µg/kg.

Furthermore, DDT was also detected in sera of beef cattle as much as 5 of 15 samples (Bantul), 8 of 15 samples (Klaten) and 10 of 30 samples (Kulonprogo) at an average concentration of 1.106 µg/kg, 0.871 µg/kg and 2.135 µg/kgsubsequently. Heptachlor was detected in sera at higher concentration (4.081 µg/kg) inKulonprogothan other POPs compounds such as aldrin (2.637 µg/kg) and DDT (2.135 µg/kg). While in Bantul, aldrin (1.706 µg/kg) had the highest concentration followed by DDT (1.106 µg/kg), while aldrin (1.005 µg/kg) was the highest contamination followed by DDT (0.871 µg/kg).

The average concentration of total POPs in sera appears higher than meat except for Klaten district. The average total residue of POPs is 2.329 µg/kg (sera) compare to 0.330 µg/kg (meat) in Kulonprogo and 0.826 µg/kg (sera) compare 0.412 µg/kg (meat) in Bantul, but the concentration of POPs in sera (0.645 µg/kg) was lower than meat (0.774 µg/kg) in Klaten. The concentration of heptachlor (4.081 µg/kg) appears as the highest concentration detected in Kulonprogo followed by aldrin at 2.637 µg/kg, but was frequently found in sera. However, aldrin was the highest concentration detected in sera ofBantul and Klaten at 1.706 µg/kg and 1.005 µg/kg respectively followed by DDT at 1.106 µg/kg and 0,871 µg/kg. DDT was frequently found in sera of cattle in Bantul and Klaten although their concentrations were lower than aldrin. On the other aldrin was frequently detected in sera of cattle in Kulonprogo to which nearly all samples (27 of 30 samples) were positive followed by heptachlor (22 of 30 samples).

The presence of DDT in sera of cattle indicates that the animals had been exposed by this compound and other organochlorine such as heptachlor and aldrin in Kulonprogo. The source of organochlorines contamination may be come from animal drinking water and animal feeds such as rice straws that commonly collected from and/or around the paddy fields. The pesticides are commonly practiced in these areas for rice production.

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Table 1: Residue levels of POPs in meat and sera of beef cattle from Kulonprogo, Bantul and Klaten.

No.	POPs	Average concentration of positive samples					
		Kulonprogo		Bantul		Klaten	
		Concentration (µg/kg)	Ratio	Concentration (µg/kg)	Ratio	Concentration (µg/kg)	Ratio
Beef							
1.	Aldrin	0.393 (0.073 – 0.984)	3/30	Nd	0/15	0.115 (0.121 – 0.834)	5/15
2.	Dieldrin	0.078 (0.024 – 0.308)	11/30	0.147 (0.065 – 0.255)	9/15	0.158 (0.061 – 0.255)	5/15
3.	DDT	0.301 (0.059 – 0.812)	4/30	0.768 (0.369 – 1.075)	4/15	2.049 (0.190 – 8.143)	12/15
4.	Endrin	Nd	0/30	0.334 (Tt – 0.334)	1/15	Nd	0/15
5.	Heptachlor	0.549 (0.09 – 1.220)	27/30	0.40 (0.052 – 1.227)	2/15	Nd	0/15
Average concentration		**0.330 (0.024 – 0.984)**		**0.412 (0.052 – 1.227)**		**0.774 (0.061 – 0.834)**	
Sera							
1.	Aldrin	2.637 (0.510 – 8.851)	27/30	1.706 (0.710 – 4.084)	4/15	1.005 (0.710 – 1.299)	2/15
2.	Dieldrin	1.897 (0.357 – 4.425)	11/30	Nd	0/15	Nd	0/15
3.	DDT	2.135 (0.158 – 6.053)	10/30	1.106 (0.539 – 1.690)	5/15	0.871 (0.351 – 1.690)	8/15
4.	Endrin	0.893 (0.174 – 1.611)	2/30	0.370 (Tt – 0.370)	1/15	0.370 (tt – 0.370)	1/15
5.	Heptachlor	4.081 (0.271 – 17.534)	22/30	0.121 (0.067 – 0.262)	4/15	0.335 (0,067 – 0.823)	4/15
Average concentration		**2.329 (0.158 – 17.534)**		**0.826 (0.067 – 4.084)**		**0.645 (0.067 – 1.690)**	

Limit of detection (LOD) GC-ECD:
Aldrin = 0.02 µg/kg
Dieldrin = 0.02 µg/kg
pp DDE = 0.02 µg/kg
Endrin = 0.69 µg/kg
Heptachlor = 0.02 µg/kg

Maximum residue limit (MRL):
Aldrin = 200 µg/kg
Dieldrin = 200 µg/kg
DDT = 500 µg/kg
Endrin = 200 µg/kg
Heptachlor = 200 µg/kg

Nd = not detected

B. Residues of persistent organic pollutants in feeds

The farmers in these areas are commonly fed their cattle with rice straws and commercial concentrate. Rice straws were collected after rice harvest from around the location of beef cattle production. The concentration of POPs in animal feeds is shown in Table 2. DDT, endrin and heptachlor were persistently detected from rice straws and concentrate in these locations. On the other hand, aldrin and dieldrin were not detected from rice straws in Klaten, but both compounds were detected in Kulonprogo and only aldrin was detected in Bantul. Aldrin and dieldrin were also detected concentrate from Bantul, but only aldrin was detected in Kulonprogo and dieldrin in Klaten. The concentration of POPs residue in rice straws of Bantul was found to be the highest contamination at 3.116 (0.389 – 12.365) µg/kg followed by Klaten at 1.922 (0.531 – 3.506) µg/kg and Kulonprogo at 0.446 (0.046 – 1.531) µg/kg. The concentration of DDT in rice straws was the highest contamination found in Bantul at 6.620 (0.876 – 12.365) µg/kg followed by heptachlor at 3.281 (1.643 – 4.919) µg/kg. Heptachlor (3.506 µg/kg) was the highest residue found in Klaten followed endrin (1.730 µg/kg), while in Kulonprogo was heptachlor (0.682 µg/kg) followed by DDT (0.533 µg/kg).

Concentrate shows different figure of concentrationsin which aldrin was having high concentration at 180.367 µg/kg in Kulonprogo, endrin (1.616 µg/kg) in Bantul and DDT (1.923 µg/kg) in Klaten. The total residue of POPs was higher in Kulonprogo (46.096 µg/kg) compared to Klaten (1,077 µg/kg) and Bantul (0.841 µg/kg).

The present study indicates that contaminated agricultural soils by POPs could affect animal feeds where the raw materials were grown on these soils. Eventually this situation affects animal health and products such as residual formation and poisoning of pesticides in beef cattle. These compounds were also known to have properties to be non-degradable in the environments. All of the POPs has been withdrawn from the agricultural activities since last two decades. DDT was the highest contamination in rice straws of Bantul at 6.620 µg/kg, followed by heptachlor (3,281 µg/kg) and aldrin (1.910 µg/kg). Heptachlor was the highest residue detected in rice straws of Klaten at 3.506 µg/kg followed by endrin (1.730 µg/kg) and DDT (0,531 µg/kg). Similar results were also shown in Kulonprogo where heptachlor was detected at 0.682 µg/kg, DDT (0.533 µg/kg) and aldrin (0.445 µg/kg).

The POPs were also detected in concentrate from these locations at the concentrations of 46,096 (0,115 – 325,473)

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µg/kg (Kulonprogo); 1.077 (0.285 – 2.834) µg/kg (Klaten); and 0.841 (0.050 – 7.412) µg/kg (Bantul). DDT was persistently appeared in concentrate of these three locations with concentrations of 1.923 µg/kg (Klaten), 0.730 µg/kg (Kulonprogo), and 0,586 µg/kg (Bantul). Aldrin (180.367 µg/kg) was the highest concentration of POPs residues detected in Kulonprogo, DDT (1.923 µg/kg) in Klaten and endrin (1,616 µg/kg) in Bantul.

Table 2: Residue levels of POPs in feeds of beef from Kulonprogo, Bantul and Klaten.

No.	POPs	Average concentration of positive samples					
		Kulonprogo		Bantul		Klaten	
		Concentration (µg/kg)	Ratio	Concentration (µg/kg)	Ratio	Concentration (µg/kg)	Ratio
I. Rice straws							
1.	Aldrin	0.445 (0.349 – 0.536)	2/8	1.910 (0.389 – 3.431)	2/2	Nd	0/1
2.	Dieldrin	0.204 (Nd – 0.204)	1/8	Nd	0/2	Nd	0/1
3.	DDT	0.533 (0.110 – 1.053)	4/8	6.620 (0.876 – 12.365)	2/2	0.531	1/1
4.	Endrin	0.366 (0.201 – 0.531)	2/8	0.654 (Nd – 0.654)	1/2	1.730	1/1
5.	Heptachlor	0.682 (0.046 – 1.531)	5/8	3.281 (1.643 – 4.919)	2/2	3.506	1/1
Average concentration		**0.446 (0.046 – 1.531)**		**3.116 (0.389 – 12.365)**		**1.922 (0.531 – 3.506)**	
II. Feed Concentrate							
1.	Aldrin	180.367 (0.210 – 325.473)	3/8	0.673 (Nd – 0.673)	1/6	Nd	0/3
2.	Dieldrin	Nd	0/8	0.281 (0.050 – 0.513)	2/6	0.285 (Nd – 0.285)	1/3
3.	DDT	0.730 (0.300 – 1.160)	2/8	0.586 (0.343 – 7.412)	4/6	1.923 (1.011 – 2.834)	2/3
4.	Endrin	3.094 (0.251 – 5.936)	2/8	1.616 (0.654 – 2.139)	3/6	0.725 (Nd – 0.725)	1/3
5.	Heptachlor	0.192 (0.115 – 0.269)	2/8	1.049 (Nd – 1.049)	1/6	1.376 (0.413 – 2.091)	3/3
Average concentration		**46.096 (0,115 – 325,473)**		**0.841 (0.050 – 7.412)**		**1.077 (0.285 – 2.834)**	
III. Drinking water							
1.	Aldrin	0.001 (Nd – 0.001)	1/14	Nd	0/2	Nd	1/1
2.	Dieldrin	0.103 (Nd – 0.103)	1/14	Nd	1/2	Nd	0/1
3.	DDT	Nd	1/14	3.218	1/2	4.340	0/1
4.	Endrin	Nd	0/14	Nd	1/2	Nd	0/1
5.	Heptachlor	0.011 (Nd – 0.011)	1/14	Nd	1/2	Nd	0/1
Average concentration		**0,038 (0,001 – 0,103)**		**3.218**		**4.340**	

Limit of detection (LOD) GC-ECD:
Aldrin = 0.02 µg/kg
Dieldrin = 0.02 µg/kg
pp DDE = 0.02 µg/kg
Endrin = 0.69 µg/kg
Heptachlor = 0.02 µg/kg

Maximum residue limit (MRL):
Aldrin = 200 µg/kg
Dieldrin= 200 µg/kg
DDT = 500 µg/kg
Endrin = 200 µg/kg
Heptachlor = 200 µg/kg

Nd= not detected

DDT was the only POPs detected in drinking water of cattle in Bantul and Klaten at the concentration of 3.218 µg/kg and 4.340 µg/kg respectively. The DDT was not detected in drinking water of cattle in Kulonprogo, but aldrin, dieldrin and heptachlor were found in drinking water at the concentration of 0.001 µg/kg, 0.103 µg/kg and 0.011 $\mu g.kg^{-1}$ subsequently. The highest total concentration of POPs was found in Klaten (4.340 µg/kg) followed by Bantul (3.128 µg/kg) and Kulonprogo (0.038 µg/kg). The present study indicates that DDT is a major contaminant in drinking water originally from natural wells. This is one of contamination source of POPs other than animal feeds.

C. Residues of tetrachlorodibenzo-ρ-dioxins andtetrachlorodibenzofurans (TCDDs/Fs)

Table 3 shows a concentration of TCDDs/Fs in beef samples collected from Kulonprogo, Bantul and Klaten. The residue of TCDDs/Fs in beef samples ofBantul (total TEQ = 3,242.93ρg/µl) was higher than Kulonprogo at total TEQ = 1,284.75ρg/µl, while the TCDDs/Fs was not detected in Klaten. High concentration of TCDDs/Fs residues in beef samples from Bantul and Kulonprogo may be due to animal feeds and drinking water as well as natural disasters such volcanic eruption and floods occurred in these areas. The beef cattle were fed on rice straws which commonly exposed to pesticides application for rice production. The results show that beef samples from Kulonprogo and Bantul were containing most toxic compounds of TCDDs/Fs such as 12378 – TCDD and 12378 – PeCDF. It should be taken into consideration since these two compounds may affect animal and/or human health as commonly noted as cancer, tumor and reproductive failure. The concentration of dioxins and dioxin – like in these samples seem to be above the maximum residue limit (MRL) stated by European Commission (2006a) such as meat (4.5 ρg/g) and oval (12.0 ρg/g).

Rice straws were found to be contaminated by most POPs such as aldrin, dieldrin, DDT, endrin and heptachlor (Table 2). DDT, endrin and heptachlor in particular were persistently detected in rice straws and concentrate of Kulonprogo, Bantul and Klaten. However, only DDT was detected in drinking water of cattle in Klaten (4.340 µg/kg) and Bantul (3.218 µg/kg) then three compounds of POPs such as aldrin, dieldrin and heptachlor were detected in Kulonprogo.

The source of dioxins exposure in human and animal is mainly through food intake particularly milk, eggs and meats (Schmid*et al*., 2002). The present development of analytical techniques has brought many laboratories to enable determining dioxins in different samples including animal feed and animal products. These techniques are therefore possible to monitor human exposure to dioxins and food safety (Koppen*et al*., 2002; Link *et al*., 2005). This study was carried out to determine the concentration of dioxins present in animal products and environmental matrices. The locations of study were divided into two different groups including: (1) a volcanic area where a volcanic mount has recently erupted in Yogyakarta and (2) an organic waste landfill where beef cattle are being raised on the landfill in Central Java.

Food safety becomes a vital issue due to the incidence of dioxins contamination in food of animal origin and animal feed (Lorber and Winters, 2007; and Kleter*et al*., 2009). The concentrations of dioxins and dioxin-like in meat depend on their concentration in pasture or other feed consumed by the animals. Animal products such as milk, eggs and meats are significant sources of dioxins and PCBs contamination for human and animals (Schmid*et al*., 2002). The property of TCDDs/Fs is water soluble, but its solubility is increasing in organic solvents and fats with increasing chlorine content (Geyer *et al*., 2002). Since their lipophilic nature and long biological half- lives, the PCDDs/Fs and dioxin-like PCBs will accumulate in the food chain (Startin and Rose, 2003).Most dioxins exposure in animals and humans are mainly through food intake. The chronic dioxins exposure in animals and humans causes a wide variety of toxic actions including reproductive and developmental effects, neurological and behavioral effects, dermal toxicity, immunomodulatory and carcinogenic effects (The World Health Organization, 2010; Wang *et al*., 2009).

The present study shows that dioxins and dioxin-like residues were detected in meats of beef cattle collected in Central Java and Yogyakarta, with a total TEQ from 1284.746 to 5897.827 $ρg/g^{-1}$ and 204.893 $ρg/g^{-1}$, respectively. The concentration of dioxins residues in these meat samples seem to be exceeded the maximum residue limit (MRL) stated by European Commission (2006a) as much as 4.5 $ρg/g^{-1}$. High concentration of dioxins residues in meat should be taken into consideration by the government and public since its toxicity effects in animal and human health. Animal feeds are presumed to be the source of dioxins contamination in meats as indicated from this experimental results that the samples were taken from an erupted of volcanic mount area in Yogyakarta and an organic waste landfill location in Central Java. This study also indicates that there was a correlation between POPs contamination detected in environmental matrices consisting soils, grasses etc and dioxins residue formation in meats.

Table 3: Residue levels of TCDDs/Fs beef samples from Kulonprogo, Bantul and Klaten.

No.	Congeners	Concentration of TCDDs/Fs in beef		
		Yogyakarta		Central Java
		Kulonprogo	Bantul	Klaten
1.	2378 – TCDF	0	0	0
2.	2378 – TCDD	80.39	10.13	0
3.	12378 – PeCDF	15.24	0.01	0
4.	23478 – PeCDF	759.75	166.91	0
5.	12378 – PeCDD	0	0	0
6.	123478 – HxCDF	307.58	214.41	0
7.	123678 – HxCDF	38.50	230.75	0
8.	123789 – HxCDD	0	0	0
9.	234678 – HxCDF	83.30	2621.70	0
10.	123478 – HxCDD	0	0	0
11.	123678 – HxCDD	0	0	0
12.	123789 – HxCDF	0	0	0
13.	1234678 – HpCDF	0	0	0
14.	1234678 – HpCDD	0	0	0
15.	1234789 – HpCDF	0	0	0
16.	OCDD	0	0	0
17.	OCDF	0.01	0.02	0
Total TEQ(ρg/µl)		**1284.75**	**3242.93**	**0**
Total residue (ρg/g)		**4496.66**	**11 350.26**	**0**

Notes: Kulonprogo = 1 pool of 2 samples Bantul = 1 sample Klaten = 1 pool of 15 samples

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Table 4: The total residue levels of TCDDs/Fs in relation to number of POPs detected in beef samples from Kulonprogo, Bantul and Klaten.

No.	Locations	Concentration of TCDDs/Fs		Residues of POPs	
		Total TEQ ($\rho g.\mu l^{-1}$)	Total residue ($\rho g.g^{-1}$)	Number of POPs detected/total samples	Range of total POPs residue ($\mu g.kg^{-1}$)
1.	Kulonprogo	1284.75	4496.66	27/30	0.024 – 0.984
2.	Bantul	3242.93	11 350.26	12/15	0.052 – 1.227
3.	Klaten	0	0	12/15	0.061 – 0.834

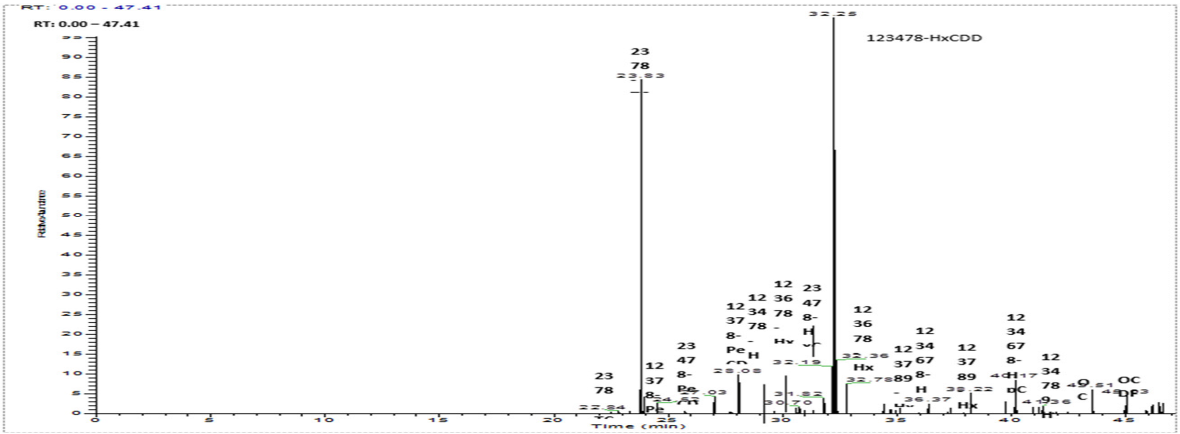

Fig.1. A chromatogram of TCDDs/ TCDFs residues in beef sample collected from Kulonprogo (Yogyakarta) using GC MSMS.

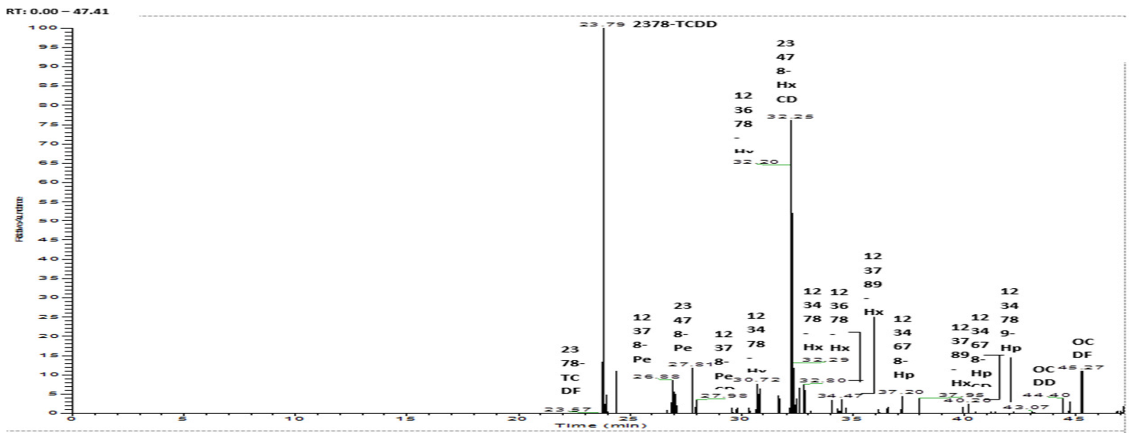

Fig.2. A chromatogram of TCDDs/ TCDFs residues in beef sample collected from Bantul (Yogyakarta) using GC MSMS.

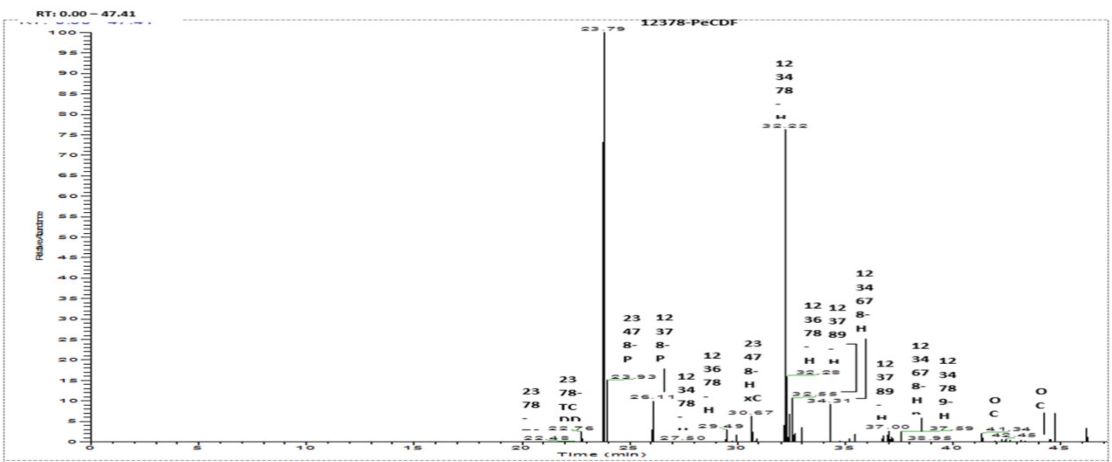

Fig.3. A chromatogram of TCDDs/ TCDFs residues in beef samples collected from Klaten (Central Java) using GC MSMS.

IV. Conclusion

It is concluded that dioxins and dioxin – like residues were detected in meats and environmental matrices, with a total residue between 4496.66 ρg/g (Kulonprogo) to 11,350.26 ρg/g (Bantul), but was not detected in meat samples of Klaten (Central Java). The concentration of dioxins and dioxin – like residues in meats were exceeding the maximum residue limit (MRL) at 4.5 ρg/g. High level of dioxins and dioxin – like concentration in meat should be taken into consideration by the government and public since their toxicity effects in animal and human health. Animal feeds are regarded as the source of dioxins contamination in meats. The results of this study showing that dioxins residues were detected in animal products and environmental matrices are the first report in Indonesia.

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