Pathological Changes of Suspected Tetrachloro dibenzo-ρ-dioxins/Tetrachloro dibenzofurans Toxication in Beef Cattle

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ABSTRAK

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Kontaminasi tetrachloro dibenzo-ρ-dioxins (TCDDs) dan tetra chlorinated dibenzofurans (TCDFs) dapat mempengaruhi kesehatan masyarakat dan hewan seperti kanker, gangguan reproduksi, keracunan kulit dan gangguan neuorologi. Tujuan penelitian ini adalah mempelajari perubahan patologis kontaminasi TCDD/Fs dalam pakan pada berbagai jaringan tubuh sapi potong yang mana TCDD/Fs dideteksi dengan GC MS/MS. Hasil penelitian menunjukkan bahwa POPs (seperti DDT, heptakhlor, aldrin, dieldrin dan endrin) sebagai pemicu pembentukan dioksin terdeteksi pada semua sampel kecuali air minum ternak. Konsentrasi total OC dalam tanah antara tt – 42,73 μg/kg, rumput (3,30 – 27,66 μg/kg), air sumur (0,82 – 1,00 μg/kg), konsentrat (3,90 μg/kg), serum (tt – 13,08 μg/kg) dan daging (tt – 100,72 μg/kg). Lebih lanjut nilai TEQ residu TCDDs/Fs pada daging sapi di Yogyakarta adalah 4.496,66 - 20.642,40 pg/g dan 717.13 pg/g (daging sapi) dan 0.037 pg/g (jaringan otak) di Solo (Jawa Tengah). Konsentrasi residu TCDD/TCDFs dalam daging sapi berada diatas batas maksimum residu (BMR) yakni sebesar 2 pg/g. Pakan ternak diketahui sebagai sumber utama kontaminasi dioksin pada daging. Perubahan makroskopik meliputi anemia, kaheksia, fibrosis hati, atropi jantung, penyumbatan rumen, konstipasi usus, perdarahan ginjal dan petechiae pada otak. Secara mikroskopis terlihat deplesia limpa, vakuolisasi interseptum, haemoragi dan akumulasi hemosiderin. Jantung mengalami degenerasi, fragmentasi dan pucat pada serabut otot dan pembengkakan inti sel. Hati terlihat pucat, degenerasi sel epitel dan kongesti. Paru – paru mengalami pneumonia, oedema pulmonum dan haemoragi ringan. Usus halus terlihat haemoragi dan infiltrasi sel mononuklear, neutrofil dan eosinofil. Otak mengalami perdarahan, perivascular cuffing dan terdapat intranuclear inclusion bodies. Hewan mengalami enteritis haemoragika, encephalitis dan degenerasi hati.

Kata Kunci: TCDDs, TCDFs, POPs, Produksi Ternak, Matriks, GC MS/MS

ABSTRACT

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The contamination of tetrachlorodibenzo-ρ-dioxins (TCDDs) and tetra chlorinated dibenzofurans (TCDFs) may affect human or animal health such as cancer, reproductive failure, dermaltoxicities and neurologic effects. The present study describes the effects of TCDD/TCDFs contamination in feed to various tissues of beef cattle to which TCDD/TCDFs were detected byGC MS/MS. The results revealed that POPs (DDT, heptachlor, aldrin, dieldrin and endrin) as a precursor for dioxins were detected in all samples except drinking water. The total concentration of OC in soils was Nd – 42.73 μg/kg, grasses (3.30 – 27.66 μg/kg), well water (0.82 – 1.00 μg/kg), feed mill (3.90 μg/kg), sera (Nd – 13.08 μg/kg) and meats (Nd – 100.72 μg/kg). Futhermore, the TEQ residues of TCDDs/TCDFs in beef were 4496.66 - 20642.40 pg/g from Yogyakarta, and 717.13pg/g (beef) and 0.037 pg/g (brain tissues) from Solo (Central Java). The concentration of TCDD/TCDFs residues in beef was above the maximum residue limit (MRL) at 2 pg/g. Animal feeds is regarded as the main source of dioxins contamination in meats. Macroscopic changes were general anaemia, cachexia, fibrotic liver, athropic heart, ruminal impaction, constipated intestinal, haemorrhagic kidney, and ptechiae in the brain. Microscopically were depleted spleen vacuolation of interseptum, haemorrhages and accumulation of hemosiderin. Heart shows degeneration, fragmentation and pale cardiac muscle and swollen nuclei. Liver was pale, degeneration of epithelial cells and congestion. Lungs were pneumonia, oedema pulmonum and mild haemorrhage. Intestines showed haemorrhage and infiltration of mononuclear cells, neutrophyls and eosinophyls. Brain was haemorrhage, perivascular cuffs and intranuclear inclusion bodies. The animal was suffering from haemorrhagic enteritis, encephalitis, and hepatic degeneration.

Key Words: TCDDs, TCDFs, POPs, Animal Products, Matrices, GC MS/MS

INTRODUCTION

Tetrachloro dioxins and related compounds such as TCDDs/TCDFs and PCBs are toxic compounds of aromatic tetracyclic hydrocarbon derived from wastes of chemical processes or natural activities such as forrest fires, combustions, incinerators, household heating, and pulp and paper industry (McKay 2002; EC 2006a; EC 2006b). The contamination of dioxins becomes a public concern due to their undegradable properties in the environment and toxic effects in human health. As these compounds are highly non-polar lipophilic with longer biological half-lives, the dioxins may accumulate in food chains (Froescheis et al. 2000).The contaminated-food intake appears as the main source for human and animal exposures (Startin & Rose 2003). Chronic exposures in humans cause reproductive and developmental disorders, neurological effects, dermal toxicity, immunological changes and carcinogenic effects (Bencko 2003; Schwarz & Appel 2005; Wang et al. 2009; WHO 2010).

The exposures of dioxins in human as well as livestock are mostly resulted from intake of contaminated food (SCAN 2000; Bocioa et al. 2007), where they are regarded as the major source of exposure. Several studies have reported that the dioxins-contaminated feed were mainly due to (1) the use of contaminated clay; (2) the drying process of contaminated grass; and (3) the contaminated feed from treated timber using preservatives; and (4) the contaminated feed with wastes originating from industrial sources (Malisch 2000; Hoogenboom et al. 2009; Tlustos 2009a; Tlustos 2009b).

Animal products such as milk, eggs and meats are also significant sources for the contamination of dioxins (van Larebeke et al. 2001; Schmid et al. 2002). As milk is a nutritious source and the mammary gland may secrete several xenobiotic substances, it is known as risk factor for dioxins toxicities (Licata et al. 2004). The concentration of dioxins in milk depends on their concentration in pasture or commercial feed being consumed by cattle (Lorber & Winters 2007; Kleter et al. 2009).

The development of analytical techniques has facilitated the determination of dioxins in different matrices such as liquid samples of blood, milk and drinking water and solid samples of feed, tissues and soils. The techniques are therefore possible to monitor human exposure to dioxins and food safety of animal origin (Link et al. 2005). Monitoring of dioxins in foods of animal origin is important, since human exposures particulalry for younger age may cause adverse effect in brain development and the immune system (Weisglas-Kuperus et al. 2000). The present study is to asses the effects of TCDD/Fs contamination in feed to various tissues of beef cattle raised in a suspected dioxins contaminated areas in particular around an organic waste landfill area and a volcanic area, and to identify the potential sources of dioxins contamination in beef cattle.

MATERIALS AND METHODS

Sample collection

The present study was carried out to determine the concentration of dioxins in animal products and environmental matrices around the farms and to investigate their pathological changes in animal tissues. The locations of study was divided into two types following the previous study (Indraningsih & Sani 2014) including: (1) a volcanic area that had been errupted recently in Yogyakarta. The beef cattle farms were established surrounding the mountain and (2) an organic waste landfill is the place where beef cattle are being raised in this location in Central Java.The environmental matrices and blood samples were collected directly from the farms based on a tracing back of the previous study reported by Indraningsih & Sani (2014). Sample collection was carriedout in suspected dioxins and/or POPs-contaminated areas of Solo (Central Java) and Yogyakarta. Samples consisted of soils, water, public-waste ashes, pastures, blood of beef cattle and meat. The organic wastes were collected from the waste landfill in Solo consisting vegetables wastes, market wastes etc.

A number of 113 samples in this study was collected from Provinces of Yogyakarta (72 samples) and Central Java (41 samples), consisting 40 sera, 21 sera, 24 meats, 9 ovals, 6 soils, 6 water, 3 organic waste, 3 grasses and 1 feedmill of rice bran. Approximately 25-50 gram of matrices were collected from both areas and was 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. Blood were collected individually from beef cattle at the volume of 10 ml using plain–blood sample tubes. All field samples were processed for measurement of organochlorine pesticides or POPs using GC-ECD (Thermo Finigan). The dioxins were analysed from meats, animal tissues and feed using GC MS/MS (Thermo Scientific).

Solvents and standards

All solvents used were suprasolve grade and purchased commercially from Merck-Millipore (Germany) including hexane, toluene, dichloromethane and ethyl acetate. All standard solutions were the isomers of isotope $^{13}C_{12}$ (EDF-5999 of 99% purity) for internal standard solutions; a clean-up standard (EDF-6999 isotope $37Cl_4$) of 96% purity; a labelled 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 precission and recovery standard solution (EDF-7999-10X) were purchased from Cambridge Isotope Laboratories (USA). The coloumn chromatography consisting alumina, carbon and silica were purchased from Thermo Scientific (Germany).

Sample-preparation procedure

The procedure of sample preparation is according to the method released by the EPA Method 1613 using FMS/Fluid Management System (EPA 1994). The method is for determination of tetra– through octa–chlorinated dibenzo-*p*-dioxins (TCDDs) and dibenzofurans (TCDFs) in water, soil, sediment, sludge, tissue, and other sample matrices by gas chromatography mass spectrometry (GC MS/MS). Samples were homogenised using dissection and/or mortar followed by an addition of hydromatrix powder, Na_2SO_4 anhydrate to dehydrate the samples and 20 µl labeled compounds following a method of EPA 1613. The FMS was then performed on dried products powder using an internal standard solution with *n*-Nonane as a solvent. This was followed by subsequent clean-up steps using a clean-up standard solution of 96% purity (EDF-6999 Isotope $37Cl_4$).

Instrumental analysis

The quantification of POPs or organochlorine pesticides residue was performed by GC-ECD (Thermo Finnigan) according to the methods described by Schenck & Wagner (1995) and Lehotay et al. (2005). The TCDD/Fs were quantified by GC MS/MS (Thermo Scientific) in MID mode and a Trace GC coupled to a MAT-95 XP mass spectrometer (Thermo Scientific). The GC MS/MS was supported with a CTC A 200S autosampler at 10000 resolving power (10% valley definition). Instrumental conditions and purity control criteria were according to the EPA 1613 method (EPA 1994). The limit of detection (LOD) for each congener was determined as the concentration in the extract which produced in two different ions and was monitored with a signal to noise ratio of 3:1 (CD 2004). The WHO-98 toxicity equivalent factors (TEF) were used to calculate the TEQ (Van den Berg et al.1994). Residues of TCDD/F were calculated using XCalibur program installed in the GC MS/MS.

Pathological examination

Clinical and pathological examination was also performed for beef cattle suspected to expose with POPs and/or dioxins and dioxin-like compounds. An exposed cattle was selected from a waste land fill area where the animal was grown on this area. Clinical examination was performed to the selected animal. Necropsy was carried out to examine pathological changes following a routine procedure for necropsy. Microscopic examination was carriedout on tissues showing lesions including liver, kidney, lungs, intestinals and brain. The tissues were stained with hematoxylin eosin (HE) and examined under light microscope.

RESULTS AND DISCUSSION

Analysis of persistent organic pollutants contamination in beef cattle

Organochlorines were detected in organic wastes, water, whole blood cells and tissues of beef cattle in Central Java. The POPs including DDT, heptachlor, aldrin, dieldrin and endrin were detected in organic wastes; faeces; meats, liver, spleen, heart, kidneys, lung, rumen and brains, but not in the intestines, lymphonodes and water (Table 1). There were four compounds of POPs detected in organic waste, such as DDT (Nd – 22.3 μg/kg); heptachlor (Nd – 1.9 μg/kg); Aldrin (1.5-7.3 μg/kg); and Endrin (Nd – 6.7 μg/kg). The POPs were also detected in faecal sample although the concentration was less than in organic waste, including DDT (1.2 μg/kg); heptachlor (0.7 μg/kg); aldrin (2.8 μg/kg); dieldrin (0.4 μg/kg); and endrin (2.1 μg/kg). The lesser amounts of POPs in faecal samples appear to be due to the metabolic process for contaminated feed intake. The compounds seem to be deposited in various tissues of the animal, and lesser amounts were excreted through urine and/or faeces as shown in Table 1. The dieldrin was not detected in organic waste but was found in faecal samples.

The residues of organochlorines were also found in meats, spleen and heart at the concentration range of 1.1-2.8 μg/kg (heptachlor); 0.8-7.3 μg/kg (aldrin); 0.2-0.6 μg/kg (dieldrin); and 0.2-3.1 μg/kg (endrin). These POPs were also detected in spleen and heart. POPs were not detected in intestines, but in ruminal content consisting DDT (2.2 μg/kg); heptachlor (5.4 μg/kg); dieldrin (0.4 μg/kg); and endrin (0.4 μg/kg).

Table 1. The concentration of organochlorines residue in different matrices collected from organic waste landfill area in Central Java

Samples (*n*)	Concentration of organochlorines (μg/kg)									Total OC (μg/kg)
	Concentration of POPs					Concentration of other OCs				
	DDT	Hepta.	Aldrin	Dieldrin	Endrin	Endosulfan	Lind.	Meth.	Chlor.	
OW (3)	Nd-22.3	Nd-1.9	1.5-7.3	Nd	Nd-6.7	0.4-39.1	1.0-2.5	Nd	Nd	3.9-77.9
DW (2)	Nd	Nd	Nd	Nd	Nd	Nd-9.6	Nd	Nd	Nd	Nd-9.6
Well water (1)	Nd	Nd	Nd	Nd	Nd	Nd	2.9	Nd	0.2	3.2
Faeces (1)	1.2	0.71	2.8	0.4	2.1	9.0	1.4	0.58	Nd	18.1
Meats (4)	Nd	1.1-2.8	0.8-7.3	Nd-0.4	Nd-3.1	Nd-3.7	0.3-0.8	Tt - 06	Nd	5.2-18.1
Livers (1)	Nd	1.5	Nd	Nd	Nd	Nd	1.9	Nd	Nd	3.4
Spleen (1)	Nd	1.1	2.2	0.2	0.2	Nd	Nd	Nd	Nd	3.7
Heart (1)	Nd	1.2	1.8	0.6	0.2	Nd	Nd	Nd	Nd	4.2
Kidney (1)	0.9	Nd	Nd	0.2	0.3	Nd	Nd	Nd	Nd	1.4
Lungs (1)	Nd	1.0	Nd	0.1	0.6	Nd	Nd	Nd	Nd	1.7
Lymphnodes (1)	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Intestines (1)	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Rumen (1)	2.2	5.4	Nd	0.4	0.4	Nd	Nd	Nd	Nd	8.5
Brain (1)	Nd	0.7	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.7

OW= Organic waste; DW= Drainage water; POPs= Persistent organochlorine pollutants; Hepta.= Heptachlor; (n)= Number of samples; Nd= Not detected; Meth.= Methoxychlor; ppb= Part per billion; Chlor.= Chlorpyrifos; Lind.= Lindane

Only heptachlor was detected in the liver as much as 1.5 $\mu g.kg^{-1}$.This may be due to the impaction of ruminal pylorus where undigested waste present in the stomach as shown in the patological examination. The presence of POPs residue such heptachlor (0.7 μg/kg) in brain tissues of the cattle should be taken into consideration. The dichloro diphenyl trichloroethane (DDT) was detected in organic wastes (Nd – 22.3 μg/kg), faeces (1.15 μg/kg), kidneys (0.9 μg/kg) and ruminal content (2.2 μg/kg). The organic wastes seem to be as the source of POPs contamination in beef cattle raised on this area.

Samples of Yogyakarta including soils, fodder and feedmill were collected from area around Mount Merapi that had been recently erupted between 2010 and 2011. Meats and blood samples were collected from a slaughtering house at Giwangan. Table 2 shows the concentration of organochlorine residues in environmental matrices and animal products collected in an erupted volcanic mount in Yogyakarta.

Although most POPs were detected in soils, grasses, feedmills, drinking water, sera and even meats, clinical cases of organochlorines toxicities were not found during the field visits. This was due to the concentration of POPs residues was below the maximum residue limits (≤200 μg/kg) which may not exert clinical signs of POPs toxicities in beef cattle.The animals that were suspected being toxicated by POPs were only showed cachectic, less activity, reduce in appetite and reduce in body weight.

The POPs (DDT, heptachlor, aldrin, dieldrin and endrin) were detected in all samples except drainage water. The total OC detected in soils was Nd – 42.7 μg/kg, grasses (3.3-27.7μg/kg), well water (0.8-1.0 μg/kg), feedmill (3.9 μg/kg), sera (Nd – 13.1μg/kg) and meats (Nd – 100.7 μg/kg). The dichloro diphenyl trichloroethane (DDT) was detected only in meats at the concentration of Nd – 19.8 μg/kg. While heptachlor was detected in soils, grasses, well water, sera and meats at the range of concentration residue was 0.1-35.8 μg/kg. Aldrin was between 2.6-6.6 μg/kg, dieldrin between 0.3-14.9 μg/kg and endrin between 0.8-2.7 μg/kg. This study indicates that the animal products of beef cattle in Yogyakarta were contaminated by POPs as detected in sera at the concentration of POPs

Table 2. The concentration of organochlorines residue in different matrices collected around an errupted volcanic mount in Yogyakarta

Types of OC	The concentration of organochlorine pesticide residues (μg/kg)						
	Soils (6)	Grasses (2)	Drainage water (1)	Well water (2)	Feedmill	Sera (20)	Meats (20)
POPs			Nd				
DDT	Nd	Nd	Nd	Nd	Nd	Nd	Nd-19.8
Heptachlor	Nd-35.8	0.8-3.3	Nd	0.1-0.7	Nd	Nd-3.8	Nd-20.5
Aldrin	Nd-2.6	Nd	Nd	Nd	Nd	Nd-6.6	Nd
Dieldrin	Nd	Nd	Nd	Nd-0.3	Nd	Nd-8.0	Nd-14.9
Endrin	Nd-2.7	Nd	Nd	Nd	2.0	Nd-0.8	Nd-2.4
Other OC			Nd				
Endosulfan	Nd-1.7	Nd	Nd	Nd	Nd	Nd-5.7	Nd-45.1
Lindane	Nd	Nd-0.9	Nd	Nd-0.7	1.9	Nd-0.3	Nd-15.4
Methoxychlor	Nd-0.3	Nd	Nd	Nd	Nd	Nd-5.0	Nd
Chlorpyrifos	Nd	1.6-24.4	Nd	Nd	Nd	Nd	Nd
Total OC	Nd-42.7	3.3-27.7	Nd	0.8-1.0	3.9	Nd-13.1	Nd-100.7

POPs= Persistent organochlorine pollutants; Ppb= Part per billion; Nd= Not detected; (n)= Number of samples

between 0.3-8.0 μg/kg and meats between 2.4-20.5 μg/kg. Soils appear to be the source of contamination of POPs in animal products with the concentration of POPs between 2.6-35.8 μg/kg.

Analysis of TCDDs/TCDFs in beef cattle farms using GC MS/MS

The analysis of TCDDs/TCDFs was carried out for animal tissues in particular meats collected from Central Java and Yogyakarta. Samples of animal tissues were collected from the necropsied cattle for a pathological study as above included kidneys, brain, meats, ruminal content and faeces. Meats were collected from Giwangan Animal Slaughtering House in Yogyakarta, where the origin of beef cattle was selected only from the areas suffered from the eruption of Mount Merapi recently. The determination of TCDDs/TCDFs was undertaken by GC MS/MS supported by automated sample preparation using 17 congeners as shown in Table 3 below. The results show that dioxins can be detected in all samples from both locations with a total TEQ, including Central Java: kidneys (99649.85 ρg/g), meat (1024.47ρg/g), ruminal contents (2016.51 ρg/g) and faeces (281313.86 ρg/g) and Yogyakarta: meats with a range between 6423.73 to 29489.14 ρg/g. The concentration of dioxins and dioxin – like in these samples seem to be above the maximum residue limit (MRL) stated by EC (2006) such as meat (4.5 ρg/g) and oval (12.0 ρg/g).

There were 4 of 17 congeners detected from meat of Central Java including 12378 – PeCDF, 23478 – PeCDF, 123478 – HxCDF, and 1234789 – HpCDF with a range of TEQ between 6.81 and 134.69 pg/g. Both 2378 – TCDF and 2378 – TCDD were not detected in this sample. Furthermore 7 congeners were detected in meats of Kulonprogo and Bantul (Yogyakarta) consisting 2378 – TCDD, 12378 – PeCDF, 23478 – PeCDF, 123478 – HxCDF, 123678 – HxCDF, 234678 – HxCDF and OCDF; 5 congeners in Ambarketawang (A): 2378 – TCDD, 23478 – PeCDF, 123478 – HxCDF, 1234678 – HpCDF, and OCDF; 4 congeners in Ambarketawang (B): 23478 – PeCDF, 123678 – HxCDD, 1234678 – HpCDF, and OCDF. The 2378 – TCDD was not detected in meats collected from 4 locations.

Food safety is becoming a vital issue at present day due to the incidence of dioxins contamination in food of animal origin and animal feed (Lorber & Winters 2007; Kleter et al. 2009). The concentration of dioxins and dioxin-like in meats and milk is depending 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 (van Larebeke et al. 2001; Schmid et al. 2002). The property of PCDDs/Fs is water soluble, but its solubility is increasing in organic solvents and fats with increasing chlorine content (Geyer et al. 2002; McKay 2002). Since their lipophilic nature and long biological half-

Tabel 3. The concentration of dioxins and dioxin-like in animal tissues from Central Java and Yogyakarta detected by GC MS/MS

Congeners	Concentration of dioxins in animal tissues (ρg/g)								
	Central Java					Yogyakarta			
	Solo					Kulonprogo	Bantul	Amb (A)	Amb (B)
	Kidney	Meat	Rumen content	Brain	Faeces	Meat (5)	Meat (3)	Meat (7)	Meat (5)
2378 – TCDF	6.08	0	0.96	0	21.19	0	0	0	0
2378 – TCDD	37.90	0	0	0	0	80.38	10.13	77.57	0
12378 – PeCDF	17.42	35.17	0	0.04	0	15.24	0.01	0	0
23478 – PeCDF	864.35	6.81	0	0	0	759.75	166.91	31.46	52.07
12378 – PeCDD	27.19	0	0	0	0	0	0	0	0
123478 – HxCDF	402.16	28.23	52.06	0	0	307.58	214.41	450.46	0
123678 – HxCDF	0	0	0	0	0	38.50	230.75	0	0
123789 – HxCDD	0	0	0	0	0	0	0	0	0
234678 – HxCDF	18471.65	0	0	0	0	8330	2621.70	0	0
123478 – HxCDD	0.61	0	0	0	0	0	0	0	0
123678 – HxCDD	0.61	0	0	0	0	0	0	0	4727.96
123789 – HxCDF	0	0	0	0	0	0	0	0	0
1234678 – HpCDF	102.0	0	350.28	0	0	0	0	4585.69	1117.78
1234678 – HpCDD	0	0	0	0	56241.58	0	0	0	0
1234789 – HpCDF	0	134.69	0	0	0	0	0	0	0
OCDD	0	0	0	0	0	0	0	0	0
OCDF	0.01	0	0.01	0	0	0.01	0.02	0.01	0.02
TOTAL TEQ ($pg.\mu l^{-1}$)	19929.97	204.89	403.30	0.04	56262.77	1284.75	3242.93	5145.20	5897.83
TEQ ($pg.g^{-1}$)	69754.50	717.13	1411.55	0.13	281313.86	4496.66	11350.26	18008.21	20642.40

Amb. = Ambarketawang
(n) = Number of samples

lives, the PCDDs/Fs and dioxin-like PCBs will acumulate in the food chain (Froescheis et al. 2000; Startin & Rose 2003). Most dioxins exposure in animals and humans are mainly through food intake (Liem et al. 2000). 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 (Bencko 2003).

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 between 1,284.75 and 5,897.83 pg/g and 204.89 pg/g respectively. The concentration of dioxins residues in these meat samples seem to be exceeded the maximum residue limit (MRL) stated by EC (2006a) as much as 4.5 pg/g. 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.

Although the analytical equipment available in this institute is GC MS/MS triple quadropole (Thermo Scientific) that is usually used for screening analysis, the present results of dioxins residues in animal products and environmental matrices is the first report in Indonesia. The development of analytical techniques has facilitated the analysis of dioxins and dioxin-like in different samples including environmental matrices, blood, animal products and animal feeds. The techniques are therefore possible to conduct human exposure to dioxins and food safety monitoring by measuring dioxins and dioxin-like in blood plasma or serum (Covaci et al. 2002; Koppen et al. 2002; Link et al. 2005). This present study was conducted to asses potential sources of dioxins contamination in beef cattle raise around suspected contaminated areas such as natural disaster (erruption of volcanic mountains and floods) and public waste landfills.

Clinical and pathological effects of suspected dioxins contamination in beef cattle

Clinical and pathological examination was carriedout to a suspected dioxin-exposed beef cattle. The beef cattle was selected from a herd of cattle raised on an organic waste landfill in Central Java. Necropsy was undertaken to a selected beef cattle showing poor condition for pathological examination. The beef cattle was exsanguinated at both blood vessels: jugular vein and carotid artery. Clinical changes included general anemia, cachectic, anoretic, enlargement of abdominal flank as tympany-like, fatigue, diarhoea with smelt and dark faeces and dermatitis. Macroscopically showed the animal was pale and anemia, cachexia, shiny and watery skeletal muscle. Liver was mottled on the capsular surface, hardened and fibrotic (Figure 1). Heart was pale and athropy. Kidneys were ptechiae and hemorrhages. Gastrointestinal revealed as ruminal impaction due to undigested materials such as plastics and other harder materials, constipation and ballooning intestines containing watery materials and gas. Brain was congested and hemorrhages (Figure 2). Microscopic changes were seen in some organs of beef cattle such as liver, gastrointestinal tracts, kidney, spleen, lungs and brain. Liver was seen as degeneration of hepatic cells, congestion, accumulation of mononuclear cells around the portal tracts, and fibrosis (Figure 3). Reticulum showed necrosis of villi mucosa and tunika muscularis (Figure 4). Intestines were haemorrhagesand infiltration of lymphocytes, macrophages, neutrophyls and eosinophyls. Spleen was seen as deplesia of red pulp followed by vacuolisation of interseptum, haemorrhages and accumulation of hemosiderin. Heart was showing degeneration and fragmentation of cardiac muscle, swollen nuclei and pale cardiac muscle. Lungs were pneumonia, oedema pulmonum and mild haemorrhage. Brain was haemorrhage, congestion, perivascular cuffs and intranuclear inclusion bodies (Figures 5 and 6). The animal was then diagnosed as haemorrhagic enteritis, encephalitis, pneumonia, hepatic degeneration, cardiomyopathy and splenonecrosis.

Figure 1. Liver of a beef cattle raised on a waste landfill showing ptechiae on the capsular surface, haemorrhagic and fragile (arrow)

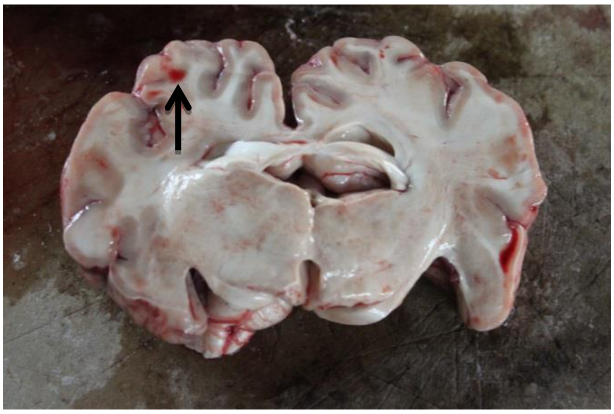

Figure 2. Brain of a beef cattle raised on a waste landfill showing petechiae on medula oblangata (arrow)

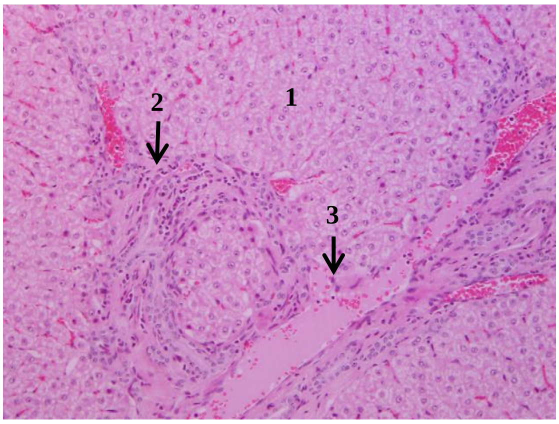

Figure 3. Liver of a beef cattle shows hepatic degeneration (1), infiltration of mononuclear cells (2), congestion and necrosis of blood vessels. HE.x200

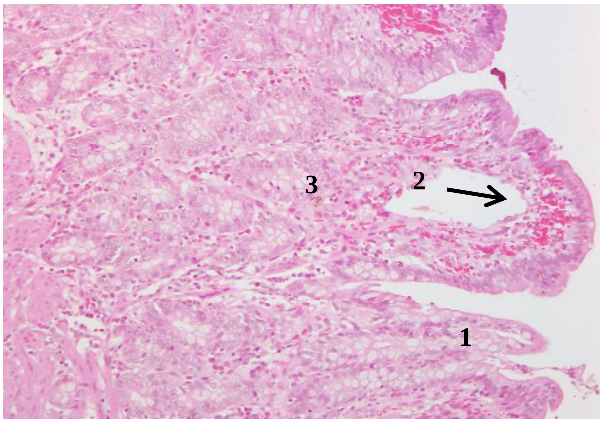

Figure 4. Reticulum of a beef cattle raised shows necrosis of villi mucosa (1) and hemorrhages (2), and accumulation of hemosiderin (3). HE x100

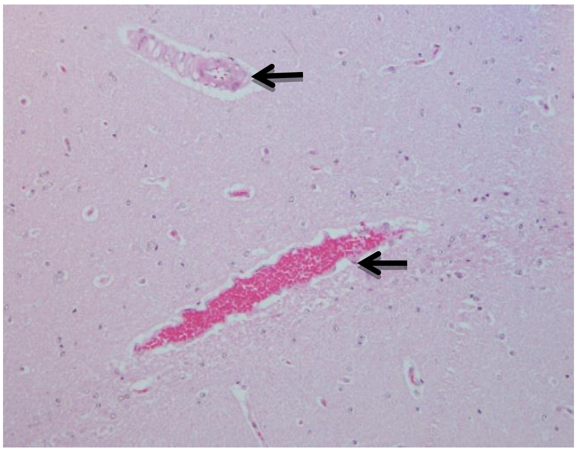

Figure 5. A brain tissue collected from beef cattle shows encephalitis indicated by perivascular cuffing and congestion (arrows). HE. 200X

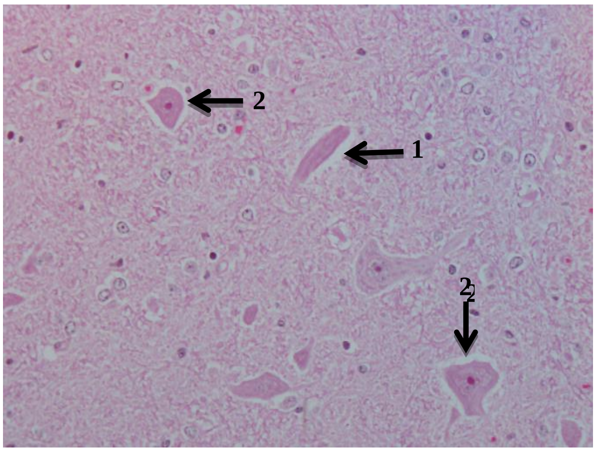

Figure 6. A brain tissue collected from beef cattle shows encephalomyopathy indicated by neuron necrosis (1), nucleolysis of neuron (1) and intranuclear inclusion bodies in neuron (2). HE. 200X

CONCLUSION

It is concluded that the TCDDs/Fs were detected in meats, ovals and environmental matrices, with a total TEQ between 6423.73 and pg/μg in Solo. The concentration of TCDDs/Fs residues in meats was exceeding the maximum residue limit (MRL) at 4.5 pg/g. High level of dioxins 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. Gross pathology of TCDD/Fs toxication was shown as general anaemia, cachexia, shiny and moist skeletal muscle, fibrotic liver, heart atrophy, renal haemorrhagy, and petechiae in brain. The results show that dioxins residues were detected in animal products and environmental matrices is the first report in Indonesia. Microscopically included deplesia of red pulp of spleen followed by vacuolisation of interseptum, haemorrhages and accumulation of hemosiderin. Heart shows degeneration and fragmentation of cardiac muscle, swollen nuclei and pale muscle. Liver was pale, degeneration of epithelial cells and congestion. Lungs were pneumonia, oedema pulmonum and mild haemorrhage. Intestines showed haemorrhage and infiltration of lymphocytes, macrophages, neutrophyls and eosinophyls. Brain was haemorrhage, perivascular cuffs and intranuclear inclusion bodies.

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