POLYCHLORINATED DIBENZO-*p*-DIOXINS AND DIBENZO-FURANS IN LIVER OF PIGLET ACCORDING TO DISEASES

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Introduction

Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food web and pose a risk of causing adverse effects to human health and the environment[1]. Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are found in a variety of foods. Ninety per cent of human exposure to POPs comes from food[2]. The major food sources are fish and seafood and dairy products[1]. A number of studies also reported high levels of PCDD/Fs in animals and public concern about PCDD and PCDF levels in animals has been raised[3-4].

Globally, there has been increase in the breeding of pig more than cattle. Koreans are consuming pork more than beef and chicken[5]. Pigs contaminated by POPs are a serious public health issue[6].

Porcine reproductive and respiratory syndrome (PRRS) has become endemic and now is an important cause of pneumonia in 3.24-week-old pigs in swine producing countries around the world. Several research groups have demonstrated increased severity of clinical disease in animals coinfected with PRRS virus (PRRSV) and different bacteria, including Streptococcus suis, Haemophilus parasuis, Salmonella choleraesuis and Mycoplasma hyopneumoniae[7]. Exposure of PRRSV-infected pigs to different S. suis strains resulted in different clinical manifestations and severity of disease, indicating that the strain of bacteria can influence the clinical disease induced in a coinfection model[8]. However, the mechanisms of the interaction between PRRSV and bacterial infections remains unclear[7].

The purpose of this study was to evaluate interrelation between the levels of PCDD/Fs in liver of piglets and diseases of piglet.

Materials and methods

1) Standars and Reagents

The following ^{13}C-labeled and ^{37}Cl-labeled PCDD/Fs solutions for EPA 1613 were purchased from Wellington laboratories Inc.(Ontario, Canada). For the quantification of low concentrations of PCDD/Fs, calibration standards CS1/2 through CS4 were used. All organic solvents were of ultra-residue grade analysis (J.T Baker, Philipsburg, NJ, USA). Anhydrous sodium sulfate and sulfuric acid from E. Merck (Darmstadt, Germany) were used.

2) Samples

It was consisted two experiments that analysis of PCDD/Fs in pig liver according to diseases samples and analysis of PCDD/Fs in liver. In first, Twenty 3-4 week-old piglets were assigned to 4 groups of 3 piglets each. The diseases of piglet were Bacillus nature diseases(Salmonella spp and Streptococcus suis) and Virus diseases (PRRS:Porcine Reproductive and Respiratory Syndrome**,** Lung), PRRS(Lymph, Intestine).

In second, Ten 7 day-old piglets were assigned to 2 groups of 5 piglets each. The diseases of piglet were Virus diseases(North America type, European type).

3) Analytical condition

The preparation of samples(Fig. 1) was based on the EPA method 1613 with automated sample cleanup for trace analysis(PowerPrep^{TM}, FMS).

The instrumental analysis was performed on a JEOL(Akishima, Tokyo, Japan) MStation JMS-700D high resolution mass spectrometer (B/E configuration) equipped with a Agillent (Palo Alto, CA, USA) 6890 Plus gas chromatograph. The capillary GC column 30 m•0.32 mm, coated with a DB-5MS stationary phase (film thickness 0.25um) was used. Samples were injected in splitless mode at an injector temperature of 280 ℃ and at an initial column temperature of 160℃. After 1 min., the temperature was ramped at 20℃/min to 200℃, at

5℃/min up to 235℃, and at 3℃/min up to 310℃. The latter temperature was held for 3 min. The ion source was operated at 260 C, the electron energy was 38 eV. The mass spectrometer was tuned to a mass resolution of 10,000. When signal to noise ratio (S=N) for a given peak was lower than 3, congeners were recorded as nondetected (n.d.) for quantification. Quantification was carried out Tetra- through Octa-Chlorinated Dioxins and Furans by the isotopic dilution method.

Results and Discussion

1) The comparison of bacteria diseas and virus disease

All 17 of the 2,3,7,8-chloro-substituted dibenzo-p-dioxins and dibenzofurans included in the WHO methodology were analyzed. No detectable concentrations of PCDD/Fs were observed in piglet liver according to the diseases of Salmonella spp. and Streptococcus. In piglet liver according to the diseases of PRRS(lung), all 17 of the 2,3,7,8-chloro-substituted OCDD and dibenzofurans were not detected on two samples. Only 2,3,7,8-chloro-substituted OCDD were detected on one sample. In the diseases of PRRS(lymph and intestine), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PCDD), the most potent dioxins, was observed in one sample. 2,3,7,8-TCDF, 1,2,3,4,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF were detected(Table 1). 1,2,3,4,6,7,8-HpCDF was detected in 3 samples. Salmonella spp. and Streptococcus is Bacillus nature diseases. PRRS is Virus diseases. The concentrations of PCDD/Fs in piglet liver from Virus diseases was higher than the concentrations from Bacillus nature diseases.

The recoveries of sampling and clean-up standards for dioxin congener which indicated the loss of the procedure. The recoveries of sampling and clean-up standards ranged 73-110% which were under the required range. Method blanks have been also run.

2) The comparison of PRRS in north American type and European type

The PCDD/Fs in piglet liver from PRRS of North America type were detected more than from PRRS of Europe(Table 2). The concentrations of PCDD/Fs in piglet liver from PRRS of North American type were observed higher than from PRRS of European type.

As a result of congener specific analysis, the concentrations determined in this study are in agreement with results published by reports[9]. The concentrations of PCDD/Fs from the piglet of Virus disease were more than from Bacillus nature disease.

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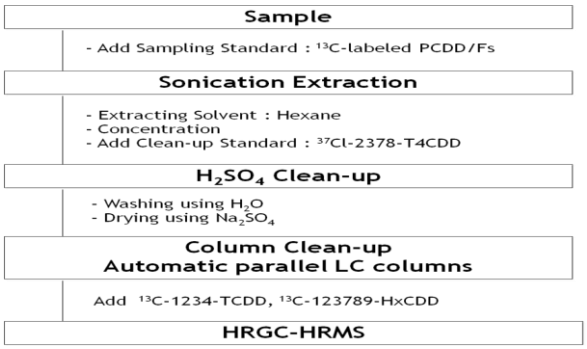

Fig. 1 Schematic diagram for analytical procedure

Table 1. The concentrations of PCDD/Fs in piglet liver according to diseases samples

(pgWHO-TEQ/g)

Compounds	Salmonella spp. and Streptococcus suis			Streptococcus suis			PRRS (Lymph, Intestine)			PRRS(Lung)		
	1	2	3	4	5	6	7	8	9	10	11	12
2,3,7,8-TCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8-PeCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.4300	N.D	N.D	N.D	N.D
1,2,3,4,7,8-HxCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.0055	N.D	N.D	N.D	N.D
1,2,3,6,7,8-HxCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8,9-HxCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,4,6,7,8-HpCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
OCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2,3,7,8-TCDF	N.D	N.D	N.D	N.D	N.D	N.D	0.0550	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8-PeCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2,3,4,7,8-PeCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,4,7,8-HxCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,6,7,8-HxCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8,9-HxCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2,3,4,6,7,8-HxCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,4,6,7,8-HpCDF	N.D	N.D	N.D	N.D	N.D	N.D	0.0067	0.0045	0.0042	N.D	N.D	N.D
1,2,3,4,7,8,9-HpCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.0049	N.D	N.D	N.D	N.D
OCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.0002
ΣPCDD/F	N.D	N.D	N.D	N.D	N.D	N.D	0.0617	0.4449	0.0042	N.D	N.D	0.0002

N.D: not detected

Table 1. The concentration of PCDD/Fs in pig liver according to Bacillus nature diseases

(pgWHO-TEQ/g)

Compounds	PRRs of North American type					PRRs of European type				
	NA-1	NA-3	NA-5	NA-7	NA-15	EU-1	EU-3	EU-5	EU-7	EU-10
2,3,7,8-TCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8-PeCDD	N.D	N.D	N.D	N.D	0.2095	N.D	N.D	N.D	N.D	N.D
1,2,3,4,7,8-HxCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,6,7,8-HxCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8,9-HxCDD	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,4,6,7,8-HpCDD	N.D	0.0011	N.D	N.D	0.0028	N.D	N.D	N.D	N.D	N.D
OCDD	N.D	N.D	0.0001	N.D	0.0001	0.0003	0.0002	0.0001	N.D	N.D
2,3,7,8-TCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8-PeCDF	0.0065	0.0031	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
2,3,4,7,8-PeCDF	0.0650	0.0437	N.D	N.D	0.1396	N.D	N.D	N.D	N.D	N.D
1,2,3,4,7,8-HxCDF	0.0208	N.D	N.D	N.D	0.0349	N.D	N.D	N.D	N.D	N.D
1,2,3,6,7,8-HxCDF	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
1,2,3,7,8,9-HxCDF	N.D	N.D	N.D	0.0183	N.D	N.D	N.D	N.D	N.D	N.D
2,3,4,6,7,8-HxCDF	N.D	0.005	N.D	N.D	0.0349	N.D	N.D	N.D	N.D	N.D
1,2,3,4,6,7,8-HpCDF	0.0036	N.D	0.0028	N.D	N.D	N.D	N.D	0.004	N.D	N.D
1,2,3,4,7,8,9-HpCDF	N.D	0.001	N.D	N.D	0.0063	N.D	N.D	N.D	N.D	N.D
OCDF	N.D	N.D	N.D	N.D	0.0001	N.D	N.D	N.D	N.D	N.D
ΣPCDD/F	0.0959	0.0539	0.0029	0.0183	0.4282	0.0003	0.0002	0.0041	0	0

N.D: not detected