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DEVELOPMENT OF BIO-MONITORING TOOL IN WASTE WOOD RECYCLING USING THE CALUX ASSAY

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Introduction

A large amount of waste wood has been and will continue to be released each year. Instead of waste incineration and landfill, recycling has been accelerated in waste wood disposal these years. In addition to paper and paperboard, fiberboard products, particleboard, another items made of recovered waste wood could be included in mulch and animal bedding. However, the use of waste wood as animal bedding poses the potential concern about hazardous contaminants not sufficiently removed from waste flows such as demolition wastes. Since animal bedding is used in direct contact with livestock and then thrown into pasture or farm soil possibly after composed, potential contaminants pose a risk of dermal, inhalant and oral exposure. Some contaminants potentially including wood preservatives such as Chromated Copper Arsenate (CCA), polycyclic aromatic hydrocarbons (PAHs), chlorophenols (CPs) and their impurities, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs) have a high possibility to be accumulated in the body of cattle, resulting in hazardous effects even on humans through meat and dairy products. To control their contamination levels in food chain, an integrated and systematic approach coupled with limit-setting and monitoring for the presence of dioxin in food and feed which entered into force in EU (IP/01/1698, IP/01/1670) is being designed in Europe. In addition to food and feed, waste wood materials such as animal bedding may also have to be monitored to avoid contamination of food chain.

The objectives of this study were to investigate the chemical characteristics of waste wood samples and to develop the CALUX (Chemical Activated LUciferase eXpression) bioassay as a rapid and cost-effective screening/monitoring method and a contributive tool to risk management in the waste wood recycling process. Waste wood samples including litters were selected and firstly analyzed in terms of PCDD/DFs and Co-PCBs. Then it was investigated whether the CALUX bioassay was successfully applied to the waste wood samples in combination with clean-up procedure for the detection of dioxin-like compounds.

Materials and Methods

Samples

To design clean-up procedure, waste wooden sleeper which appeared to contain high concentration levels of PCP, PCDD/DFs and PAHs[1] was used. Sleeper sample was a cross-section of used railroad sleeper processed by pressure treatment with PCP in combination with creosote. Litter samples made of waste wood were sampled from seven different manufacturers (A - G) in Hokkaido, Japan. Waste wood chips aimed at energy recovery, dust of litter and litter made of virgin sawdust were also sampled. Wood subsamples were collected by sawing several grams of materials.

Chemical Analysis

Wood samples were Soxhlet-extracted with toluene for 16 h. As the further chemical clean-up for the determination of PCDD/DFs and Co-PCBs, the Japanese Industrial Standard (JIS) method K 0312 was adapted. PCDD/DFs and Co-PCBs were identified and quantitated by high-resolution capillary column gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS).

CALUX assay

Crude extracts from wood samples were prepared by DMSO (dimethylsulfoxide)/ n-hexane extraction[2]. Then, the extracts were processed to the silica gel-H_2SO_4 (44 %) reflux treatment (50 g of silica gel-H_2SO_4 was mixed with the crude extracts) at 70°C for 60 min.

For the sleeper sample, silica gel-H_2SO_4 reflux treatment was repeated four times and each fraction was separately collected in addition to the crude extracts. Based on the comparison of CALUX results (*i.e.*, CALUX-TEQ) of each fraction with chemical analysis results (*i.e*,, WHO-TEQ), optimal reflux number of times was determined. Then, for the litter samples, assay fractions were obtained after DMSO/n-hexane extraction and after the silica gel-H_2SO_4 reflux treatment with the optimized number of times.

All the fractions were evaporated and replaced with DMSO for the CALUX bioassay.

Recombinant rat hepatoma H4IIE cell line stably transfected with the AhR-controlled luciferase–cDNA construct[3] (DR-CALUX® cell line) was obtained from Bio Detection Systems B.V. (Amsterdam, The Netherlands) and the CALUX assay was carried out as described by Behnisch *et al*[4].

The TCDD standard dose-response curve was fitted using a cumulative fit function using Slide Write Plus Ver. 6.00 (Advanced Graphics Software). CALUX assay-determined TEQs (CALUX-TEQs) for the tested fractions were obtained from their dilutions so that their luciferase activities were in the reproducible lower part of the linear range corresponding to 1- 4 pM in TCDD.

Results and Discussion

Concentrations of PCDD/DFs and Co-PCBs in Samples

Chemical concentrations of PCDD/DFs and Co-PCBs in the sleeper and litter samples were shown in Table 1.

Table 1. PCDD/DFs and Co-PCBs concentration levels in the sleeper and litter samples (pg/g)

	Sleeper	Litter dust B	Natural sawdust litter	Waste wood Litter and fuel chips		
				Mean	Min	Max
PCDDs	11,000,000	420	N.D.	340	7.2	1,400
TEQ	17,000	0.32	0	0.17	0	0.85
PCDFs	980,000	270	N.D.	44	N.D.	200
TEQ	4,000	0.43	0	0.0010	0	0.0095
PCDD/Fs	12,000,000	690	N.D.	390	10	1,600
TEQ	21,000	0.73	0	0.17	0	0.86
Co-PCBs	6,700	1,200,000	1,300	7,400	1,300	37,000
TEQ	0.93	240	0.19	2.0	0.19	8.6
Total TEQ	21,000	240	0.19	2.1	0.19	8.6

The concentrations of PCDD/DFs showed significantly high values in the sleeper sample. The possibility of pressure treatment with creosote in combination with PCP [1] was suggested.

Concentrations of PCDD/DFs in the litter samples were one order of magnitude greater than those in natural sawdust, but their levels didn't clearly reflect the presence of PCP-treated waste wood in the litter samples. Litter dust contained relatively high concentration of Co-PCBs (1,200 ng/g, 0.24 ng-TEQ/g). The source of this contamination is unknown. However, the relationship between PCB levels in litter and its risk should be discussed, and its risk control also need to be taken into consideration.

Optimization of Clean-up Procedure for Waste Wood using CALUX assay

Fig.1 shows the ratio between CALUX-TEQ values and WHO toxicity equivalent (WHO-TEQ) values obtained by congener-specific chemical analysis (CALUX-TEQ/WHO-TEQ ratio) at each level of clean-up procedures for the sleeper sample. The CALUX-TEQ value was remarkably reduced through a single silica gel-H_2SO_4 reflux treatment and after this the values became stable in spite of the repetition of reflux treatment. For the sample after DMSO/n-hexane extraction, CALUX-TEQ/WHO-TEQ ratio was 7,100,000, which became, however, 1.1 just after a single silica gel-H_2SO_4 reflux treatment. The CALUX-TEQ values for the four refluxed samples showed good agreement with WHO-TEQ values. These results showed that labile AhR ligands such as PAHs in samples were effectively removed by the single proposed reflux method, and this clean-up was available for the 'devoted' detection of PCDD/DFs and Co-PCBs in wood samples. The results were consistent with our CALUX results previously reported for fly ash[4] and PCB-containing mineral oil[5] samples.

Bio-TEQ and Chemical TEQ for Litter Samples

CALUX -TEQ values, WHO-TEQ values and their ratio for the litter samples were summarized in Table 2. CALUX-TEQ/WHO-TEQ ratios ranged from 0.058 to 22. For litter B, litter dust B and litter C, CALUX-TEQ values underestimated WHO-TEQ values, and the CALUX-TEQ/ WHO-TEQ ratios were 0.058, 0.24 and 0.94, respectively. In these samples, WHO-TEQ values of Co-PCBs had great contributions to total WHO-TEQ values, which suggests the underestimation is due to antagonistic effect of non dioxin-like PCBs considered to exist to a remarkable extent together with Co-PCBs.

Target setting for the POPs monitoring and further application of the CALUX method to various types of wood samples are required in the future research. However, practical use of the CALUX assay found a way out as a rapid and cost-effective screening tool for dioxin-like POPs in the recycling process of biomass.

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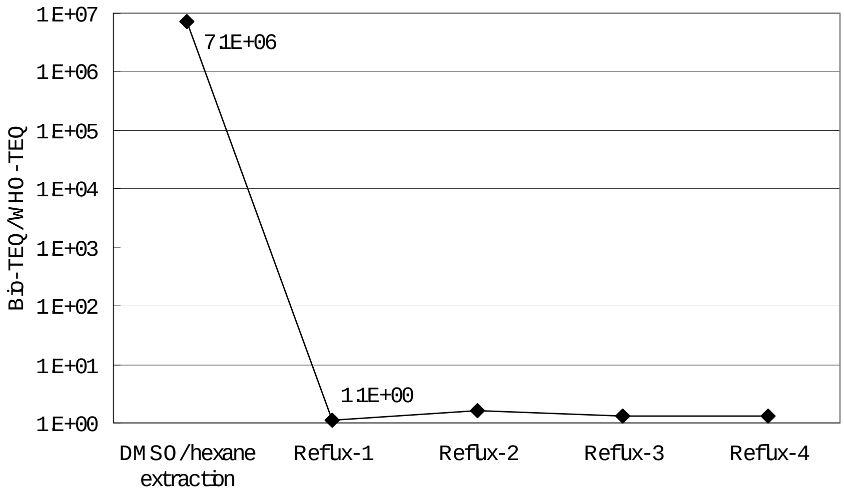

Figure 1. The ratio between CALUX-TEQ and WHO-TEQ at each level of clean-up procedure for the sleeper sample

Table 2. CALUX-TEQ values, WHO-TEQ values and their ratio

	CALUX-TEQ (pg/g)	WHO-TEQ (pg/g)			CALUX-TEQ /Total WHO-TEQ
		PCDD/DFs	Co-PCBs	Total	
Fuel chip A	2.2	0.39	0.36	0.76	2.9
Litter A	3.3	0.32	0.42	0.74	4.4
Litter B	5.2	0.0052	5.6	5.6	0.94
Litter dust B	14	0.73	240	240	0.058
Fuel chip C	6.5	0.015	3.6	3.6	1.8
Litter C	2.1	0.010	8.6	8.6	0.24
Litter D	11	0.86	0.22	1.1	10
Litter E	6.0	0.032	0.24	0.27	22
Litter F	2.2	0.031	0.20	0.23	9.7
Litter G	1.2	0.0066	0.19	0.19	6.1
Plywood litter G	N.D.	0	0.26	0.26	-
Sawdust litter	N.D.	0	0.19	0.19	-

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