7-ETHOXYRESORUFIN O-DEETHYLASE INDUCING SUBSTANCES IN SOIL SAMPLES FROM INCINERATION SITES FOR METAL RECLAMATION IN WAN-LI, TAIWAN, REPUBLIC OF CHINA

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1. INTRODUCTION

We previously reported that four surface soil samples from six incineration sites of waste electric wire and/or magnetic card for metal reclamation in Wan-Li, southern Taiwan, Republic of China were heavily polluted with PCBs, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)[1]. Numerous organic compounds were detected in fly ash from a municipal incinerator in Canada[2]. This indicated that other toxic substances as well sa PCBs, PCDFs and PCDDs might be generated in thermal process of waste combustion for metal reclamation at Wan-Li area. Therefore, we tried to detect toxic compounds in soil samples from waste incineration sites in Wan-Li using chick embryo microsomal 7-ethoxyresorufin O-deetylase (EROD) as a biological toxic indicator, because there was confirmed a close correlation between the EROD inducible potency and toxic effects (thymic atrophy and reduction of body weight gain, etc.) by toxic planar compounds such as PCDDs, PCDFs and non-ortho chlorine substituted coplanar PCBs (Co-PCBs), etc[3].

2. EXPERIMENTAL

Sampling

Surface soil samples were collected from ten incineration sites for metal reclamation from waste electric wires and magnetic cards in Wan-Li, southern Taiwan in February, 1991. In addition, two control surface soils were also sampled in Wan-Li in the same period.

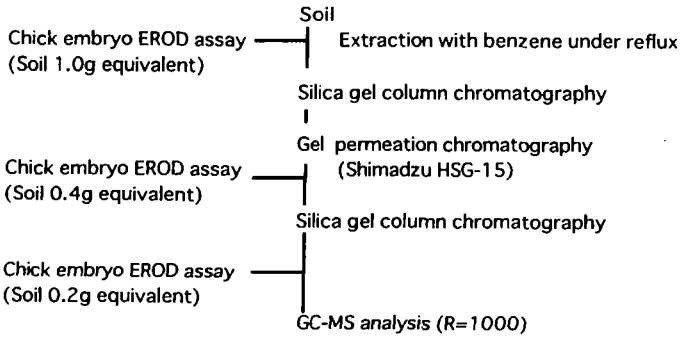

Figure 1. Outline of analytical method for detecting EROD producible compounds in soil samples

Extraction and fractionation

The sediment specimens were thinned in a thickness of 1 cm, left for 2 days at outdoor for complete dryness, and then pulverized into small pieces.

The procedure for analysis was outlined in Figure 1. Each poured samples (ca. 200 g) was extracted with 600 ml of benzene for 5 hours under reflux. The benzene extract was filtered through a 1 μm glass fiber filter in order to remove sediment particles. For EROD assay, a portion of each extract was concentrated to dryness by evaporation and dissolved with 1,4 -dioxane. After concentration, the remainders of two sample extracts with strong EROD inducible potency were respectively precleaned on a silica gel column (Merch, Kieselgel 60) with an eluent of acetone in order to remove polar compounds with high molecular weight. After complete replacing solvent with methylene chloride, each eluate was chromatographed into five fractions on a gel permeation column (two Shimadzu HSG-15 columns, 50 cm x 4.6 mm, solvent: methylene chloride, flow rate: 0.6 ml/min) using UV detector (254 nm) and fluorescence detector (Exciting: 365 nm, Emission: 430 nm) as a separation indicator. An aliquot of each fraction from two samples was tested for EROD inducible ability.

After complete changing of solvent with n-hexane, the remainder of each extract having strong enzymatic induction was separated into four fractions on a silica gel column (50 g, Merck, Kieselgel 60) with successive eluates of 250 ml of n-hexane, 300 ml of 20% methylene chloride in n-hexane, 200 ml of methylene chloride and 200 ml of acetone. A portion of each fraction was also assayed for EROD.

An aliquot of each strong EROD inducible fraction was analyzed for constituents by a Hewlett Packard 5890J gas chromatograph-JEOL mass spectrometer SX102 with a J&W DB-5 capillary column (30 m x 0.32 mm, 0.25 μm) in EI mode at a resolution of 1000.

Enzyme assay in chick embryo

After removal of the solvent under an N_2 stream, each extract or chromatographed fraction was dissolved in 1,4-dioxane. The 25 μl was injected into the air sac of White Leghon egg incubated for 16.5 days at 37.5℃ (two embryo per group, triplicate). On 2 days following the administration, livers from each group were isolated, washed with cold 1.15% KCl solution, and then mixed. The microsome was prepared by using a method of Guengerich[4].

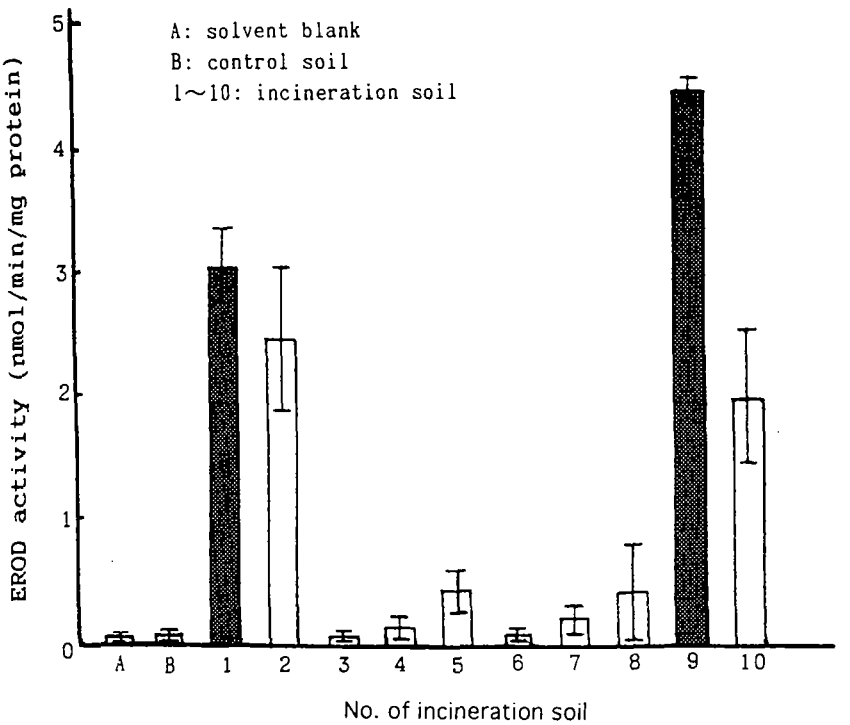

Figure 2. EROD activities of benzene extracts from control soils and incineration site soils A and B show solvent blank and control soil No. 1, respectively.

The activity of EROD was assayed according to the procedures of Nebert and Gelboin[5] and Pohl and Fouts[6], respectively.

3. RESULTS AND DISCUSSION

Firure 2 shows the results of EROD activity determinations of benzene extract from soils colected at control areas and combustion sites in Wan-Li. The activities of control sample Nos. 1 and 2 were 0.095 and 0.084 nmol/min/mg protein/1 g soil equivalent with a slightly higher level than that (0.063 nmol/min/mg protein/1 g soil equivalent) of solvent control group, indicating the control soils to contain a trace level of enzymatic inducing substances. As shown in Figure 1, there was a great difference in the EROD induction among soil samples from incineration sites. Soil sample Nos. 1, 2, 9 and 10 showed significantly high activities with 32, 26, 47 and 21 times greater, respectively than did the controls.

The strongest three samples of Nos. 1, 2 and 9 were collected from incineration sites of waste electric wires as a main combustion material.

From above the results, we tried to detect EROD positive compounds in benzene extracts from sample Nos. 1 and 9.

After silica gel chromatographic precleaning, benzene extracts of soil Nos. 1 and 9 were separated into five fractions (Fractions A to E) on a gel permiation column.

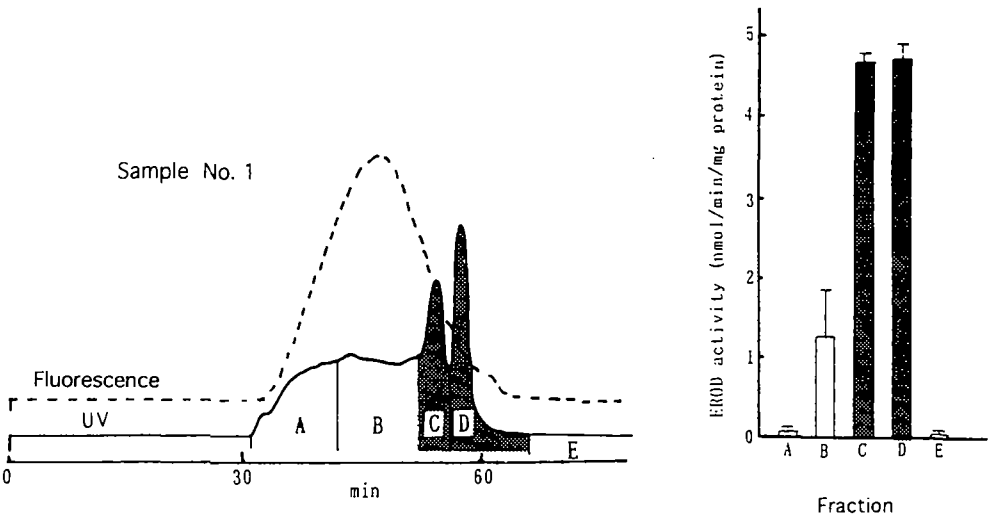

Figure 3 Gel permiation column chromatogram and EROD activities of benzene extract from soil sample No. 1

Figure 3 illustrates gel permiation chromatogram and EROD activities of each fraction of sample No. 1. Fractions C and D showed respectively high EROD activities with 4.70 and 4.75 nmol/min/mg protein/0.4 g soil equivalent, being equal to 470 and 475 times greater than that (0.01 nmol/min/mg protein/0.4 g soil equivalent) of Fraction E with the weakest producing activity.

There was also observed high activities with 5.57 in Fraction C and 1.39 nmol/min/mg protein/0.4 g soil equivalent in Fraction D. Other fractions all showed only a slight elevation.

From above the results, further separation was conducted about Fractions C and D of sample Nos. 1 and fraction C of 9 on a silica gel column.

In sample No. 1, n-hexane eluates (Fractions 1-C-a and 1-D-a, respectively) from silica gel column of both Fractions C and D showed significant elevated EROD levels with 2.64 and 1.98 nmol/min/mg protein/0.2 g soil equivalent. While Fraction C of sample No. 9 gave high activities in n-hexane (Fraction 9-C-a) and 20% methylene chloride in n-hexane eluates (Fraction 9-C-b), showing to be 3.33 and 2.58 nmol/min/mg protein/0.2 g soil equivalent, respectively.

Table 1 shows the results of each fraction from sample Nos. 1 and 9 by GC-MS analysis.

Fraction 1-C-a was found to contain di- through nonachlorinated biphenyls (DCBs through NCBs) as a major and pentachlorinated dibenzofurans (PeCDFs) as a minor. In Fraction 1-D-a, TCBs through HxCBs, penta- and hexachlorinated benzenes (PeCBzs and HxCBzs), and tri- and tetrachlorinated naphthalenes (TrCNs and TCNs) were found as majors and, PeCDFs were found as minors.

In sample No. 9, both of Fractions 9-C-a and 9-D-a contained only PCBs including di- through nonachlorinated biphenyls and di- through heptachlorinated biphenyls, respectively.

It is well known the EROD is strongly induced by a molecular size of 3 x 10 Å with planar compounds[7]. In this viewpoint, PCBzs are unlikely to give the enzymatic induction.

Table 1. Major and minor components detected in GC-MS total ion chromatograms of Fractions 1-C-a, 1-D-a, 9-C-a and 9-C-b

Fraction	Component	
	Major	Minor
1-C-a	PCBs (2Cl~9Cl)	PCDFs
1-D-a	PCBs (4Cl~6Cl), PCBzs (5Cl, 6Cl), PCNs (3Cl, 4Cl)	PCDFs
9-C-a	PCBs (4Cl~7Cl)	-
9-C-b	PCBs (4Cl~7Cl)	-

Table 2. Contributions of PCDDs, PCDFs, PCNs, Non-Co-PCBs, Mono-Co-PCBs and unknown substances for the total EROD activities of four active fractions from soil Nos. 1 and 9

Fraction	Contribution (%)					
	PCDDs	PCDFs	PCNs	Non-Co-PCBs	Mono-Co-PCBs	Unknown
1-C-a	2.1	9.3	-	4.6	1.8	82.2
1-D-a	1.5	20.5	6.0	1.9	0.2	69.9
9-C-a	0.1	6.0	-	94.0	-	-
9-C-b	-	-	-	38.0	-	62.0

On the other hand, the EROD inducing potency of a mixture of PCNs have not yet been reported until now. Therefore, using chick embryo assay, we determined the inducible potency of a commercial preparation of PCN isomer mixture (Halowax 1013), because this product has a similar constituent detected in Fraction 1-D-a of sample No. 1. Consequently, TCDD toxicity equivalency quantity (TEQ) of PCNs in Fraction 1-D-a was determined using log dose-response curve for EROD induction.

As shown in Table 1, numerous PCB isomers were found in all fractions. However, Co-PCBs having strong EROD induction abilities were not confirmed in GC-MS total ion chromatograms of all fractions. In addition, the detection of PCDFs in Fractions 1-C-a and 1-D-a suggests the co-presence of PCDDs, because both chemicals have similar chemical and physical properties.

Taking above things into consideration, we tried to analyze for Co-PCBs, PCDFs and PCDDs according our method[8]. TEQ levels of PCDDs and PCDFs, and mono-ortho chlorinated coplanar PCBs (Mono-Co-PCBs) were calculated using I-TEF method[9] and a report by Safe[10], respectively. While the calculation of non-ortho chlorinated coplanar PCBs (Non-Co-PCBs) was conducted using our previous data[11].

Table 2 illustrates the contributions (%) of PCDDs, PCDFs, Non-Co-PCBs, Mono-Co-PCBs and PCNs in the total EROD induction activities of four active fractions from soil sample Nos. 1 and 9.

In Fractions 1-C-a and 1-D-a of sample No. 1, the contributions of PCDFs gave higher values

with 9.3 and 20.5%, respectively than did PCDDs, PCNs, Non-Co-PCBs and Mono-Co-PCBs. On the other hand, Non-Co-PCBs were important substances in Fractions 9-C-a and 9-D-b of sample No.9, and especially, in the former fraction, the 94.0% of total TEQ was attributable to Non-Co-PCBs. Mono-Co-PCBs showed small figures with 0.0 to 1.8%. The effect of PCNs was recognized in only Fraction 1-D-a with a level of 6.0%.

As shown in Table 2, other fractions except Fraction 9-C-a were revealed to contain unknown EROD inducible compounds at levels of 62.0 to 82.2% of the total TEQ. This indicates that unknown highly toxic compounds might be present at a trace level in these fractions. Therefor, it is important to detect them.

4. REFERENCES

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