EVALUATION OF RISK POTENTIAL OF PAHS AND PESTICIDES IN SOILS

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ABSTRACT

Extracts of various environmental samples were examined for dioxin-like activity. Analytical data indicate that heating oil, road dust and seashore sediment were contaminated with polycyclic aromatic hydrocarbons (PAHs). The sum of 10 PAH concentrations ranged from 10^1 to 10^3 ng/g for solid samples and from 10^{-1} to10^0.g/L for liquid samples. The aims of the present study are to describe aryl hydrocarbon receptor (AhR)-mediated responses to extracts of various environmental samples, to estimate the contribution of PAH to the responses, and to propose methods for simple, inexpensive and practical primary screening and monitoring of dioxin-like compounds. The dioxin-like activity was tested in terms of 7-ethoxyresorufin *O*-deethylase (EROD) activity. Significant activities were detected in many samples, especially agricultural and forest soils and road dust. The 10 PAHs accounted for about 20 and over 30% EROD activity of the crude extracts of soils and road dust, respectively. On the other hand, the sum of 10 PAHs concentrations and EROD activity of municipal waste incinerator ash were in the same range as those in the river sediment. The contributions of 10 PAHs to EROD activity were estimated to exceed 60 %. Our results indicate that agricultural, forest and rice field soils and road dust should be analyzed in greater detail.

KEY WORDS PAHs, EROD, Hep G2, dioxin-like activity, primary screening

INTRODUCTION

Although large-scale contamination by PAHs and dioxins is of grave concern, the present methods for analyzing these compounds are complicated and expensive. Chemical analysis cannot determine the by-products of contaminants that act in a manner similar to dioxin in the human body. Biological tests are expected to represent the integrated effects of all chemicals and do not provide any specific information about the identity of the chemicals concerned. Thus, a simple and inexpensive primary screening method that combines both chemical analysis and biological test is desired.

The toxic effects of planar polychlorinated dibenzo-*p*-dioxins, dibenzofurans (PCDD/Fs), biphenyls (PCBs) and, at least in part, polycyclic aromatic hydrocarbons (PAHs) are linked to specific aryl hydrocarbon receptor (AhR)-mediated processes [(1)]. A very good correlation was found between the rank order of PCDF congeners relating their *in vivo* toxicity in rats to their CYP.A1-inducing potencial [(2)].

7-Ethoxyresorufin is specifically dealkylated by the cytochrome P450 isozyme, CYP.A1, yielding the strongly fluorescent resorufin [(3)]. 7-Ethoxyresorufin *O*-deethylase (EROD) activity is currently measured in S9 fractions or microsomes prepared from homogenized tissue or cell samples. However there exist some disadvantages inherent to these procedures when applied to cultured hepatocytes. Harvesting monolayers, disruption of cells, and preparation of subcellular fractions require precise handling of samples and involve a relatively long working time to produce reproducible results. Donato *et al.* (1993) developed a simple method for measuring EROD activity by using hepatocytes cultured on microplates.

Some human P450 species have notably different catalytic activities against various carcinogens from those predicted on the basis of studies using experimental animals, and the gene expression sometimes differs between human P450s and animal orthologues, although some structural and functional characteristics are shared [(5,6)]. Thus, we may use human hepatocytes although we cannot use them for routine experiments. Hep G2 [(7)] is a highly differentiated human hepatoma cell line that retains many cellular functions often lost by cells in culture. This cell line also has the enzymes involved in the phase . and phase. metabolism of xenobiotics, and it has been used as an *in vitro* system instead of human normal hepatocytes to study drug metabolism and toxicity [(8, 9, 10, 11)].

Therefore, we combined the simple *in vitro* EROD assay and Hep G2, and added a slight modification to make it useful for the primary screening of chemical risks and for practical monitoring. Kenmotsu (1998) developed a simple method for measuring PAH concentration. Several PAHs have been found to induce AhR-mediated activities, according to a study on individual compounds [(13)].

In the present investigation, we analyzed both PAH concentration and EROD activity of various environmental extracts using simple methods and estimated the contribution of PAHs to EROD activity.

MATERIALS AND METHODS

Materials

7-Ethoxyresorufin, .-glucuronidase (Type H-3) and Eagle's minimum essential medium (MEM) were purchased from Sigma Chemical Co. (St. Louis); fetal bovine serum was obtained from TRACE Biosciences PTY Ltd. (Aus); auto-POW MEM eagle, sodium pyruvate and nonessential amino acids (NEAAs) were from ICN Biochemicals Inc. (Ohio); PAHs were from Wako Pure Chemical Industries Ltd. (Osaka); resorufin was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo); the modified Lowry protein assay reagent kit was obtained from PIERCE Chemical Co. (Rockford). All other reagents were of guaranteed reagent grade.

Cells, cell culture and medium

The human hepatocellular carcinoma cell line, Hep G2 (ATCC HB 8065), at passage 74, was supplied by the America Type Culture Collection (U.S.A.). The cells were maintained in MEM containing 10% FBS, NEAAs and pyruvate, in a CO_2 incubator kept at 37. with humidified 5%CO_2-95% air atmosphere. For EROD assay, Hep G2 (at passage 83) frozen in liquid N_2 was seeded on 96-well microplates at a density of about 4.10^4 cells/well. When the cells reached confluence, the medium was replaced with a fresh one, and test samples dissolved in dimethylsulfoxide (DMSO) were added to the cells 12hrs before the experiments. The final concentration of DMSO was lower than 1%. This concentration showed little toxic effect on the cells. To eliminate artifactual effects due to DMSO, the same amount of DMSO was added to control cultures.

7-Ethoxyresorufin O-deethylase (EROD) activity

The determination of EROD activity was performed according to Donato *et al*. (1993) except that MEM without phenol red and 70 units of .-glucuronidase were used instead of MEM, together with 15 Fishman units of.-glucuronidase and 120 Roy units of arylsulfatase [14]. To define the optimal administration time for the measurement of EROD activity in Hep G2, different incubation times ranging from 3 to 36hrs were investigated using the same cell preparation. After exposure for 12-24hrs to benzo[k]fluoranthene (B[k]f) and benzo[a]anthracene (B[a]a) (0.63mg/L), EROD activities reached maxima and then began to decline sharply (data not shown). Previous investigations demonstrated that in the case of TCDD, CYP.A1-mediated luciferase activity was maintained with time, while in the case of PAHs, the activity declined after 24hr [15, 16]. Therefore, to evaluate the contribution of PAH to dioxin-like activity, the standard administration time of 12hrs was chosen for subsequent experiments. Fluorescence was measured using a high-performance liquid chromatograph (Shimadzu, LC-10AD) at 550nm excitation and 585nm emission [17]. For cellular protein measurements, monolayers were washed with phosphate-buffered saline (pH 7.2) and 120.L of 1M NaOH was added per well. After 2 hrs at room temperature, 80.L of sample was discarded and protein was assayed in the plate with a modified Lowry protein assay reagent kit and a microplate reader (Bio-Rad, model550).

Induction equivalent factor and 10PAHs' contribution to EROD activity

EROD activity is defined as the ability of inducing CYP.A1 and, measured as resorufin production by using dose-response curves. It was estimated according to the concentration of samples producing a response equivalent to 50% of the maximal response of B[k] f (ECB[k]f50%), because the samples produced different levels of maximal induction of resorufin. The ECB[k]f50% of B[k]f was divided by the ECB[k]f50% of each samples to determine relative potency to B[k]f (induction equivalent factor : IEF) [13]. In this study, since B[k]f exhibited the strongest activity among the 10 PAHs and could be obtained easily, it was used as an inducer in a reference experiment

The contribution of 10 PAHs to EROD-inducing potential of the samples was estimated by determining the inducing potential of each pure chemical. However Jones and Anderson (1999) reported that additive interaction of PCBs and PAHs for reporter gene assay of CYP.A1 decreased as the number of compounds increases, almost additive interaction was found for induction of EROD activity in Hep G2, rat hepatoma H4 E cells and primary cultures of rat hepatocytes using the 49 PCDDs mixtures [18, 19]. Because there were only B[k]f and benzo[a]pyrene (B[a]p) that could induce significant EROD activity at the concentrations detected in this study, the effects of individual PAH was assumed to be additive.

Samples

Most of the samples were collected in 2001-2002 at the Okayama Prefecture, Japan. As solid samples, surface river sediment (3 rivers, middle stream and estuarine samples were collected for each), seashore sediment (two sampling sites), agricultural soil, rice field soil, forest soil, fly ash and bottom ash of municipal waste incinerator (three sampling sites) and road dust (six sampling sites, each in July and November) were collected.

Mineral oil samples were purchased from the filling service station. The road dust samples were obtained in 1999 from the trucks that cleaned the national road.

There are about 195, 63 and 43 millions people in Okayama Prefecture, Okayama city and Kurashiki city respectively [20]. Mizushima, southern coastal part of Kurashiki city, is well known as the large scale industrial area in Japan. There are the petrochemical complexes, the steelworks, the machine factories and their related industries. Biochemical oxygen demand at three rivers was ranged from 0.9 to 1.9mg/L in fiscal 2001 [21].

Sample extraction and chemical analysis

Sample extraction and chemical analysis were carried out as previously described [12]. The solid samples (5-10g-dry, <2mm) were extracted with *n*-hexane after stirring with 1N KOH dissolved in ethanol at room temperature for 15hrs. An aliquot (50%) of the crude extract was semi-purified by passing through a glass column filled with silica gel/5% H_2O for solid samples. PAHs analysis was performed for the semi-purified extracts by gas chromatography / mass spectrometry (Shimadzu, QP5050) using a 30-m DB-5MS column (J . W Scientific, Folsom, California). The following PAHs were analyzed: benzophenone (Bp), phenanthrene (Phe), anthracene (Anth), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (B[a]a), chrysene (Chry), benzo[b]fluoranthene (B[b]f), benzo[k]fluoranthene (B[k]f), benzo[a]pyrene (B[a]p). Most of these PAHs have been well studied [16, 22, 23] and can be analyzed easily and precisely. Since this study aims at the simple analysis of PAHs and strongly adsorbed organic contaminants that cannot be extracted with *n*-hexane

probably does not affect human health, recoveries were not determined. After PAH analysis, crude extracts and semi-purified extracts were transferred to 50.L of DMSO for the determination of EROD activity.

RESULTS

The sum of 10 PAH concentrations is indicated by ' Total PAHs '. They were about 100- to 10,000-fold higher in solid samples (shown in .,10^{1}-10^{3}ng/g) than in liquid ones (shown in .,10^{-1}-10^{0}.g/L) except for mineral oil samples (Fig.1). The highest PAH concentrations were detected in heating oil, seashore sediments and road dust. Phe was ranged from 38 to 66% of total PAHs in the liquid samples except mineral oil samples. Furthermore adding Bp and Flu make it up to 62-83%. In heating oil and gasoline, Bp and Phe, which are relatively highly hydrophilic, were comprised more than 94% of their total PAHs. Road dust tended to contain much more total PAHs in November than in July. The road dust where traffic is heavy contained much more PAHs than that where traffic is light. In general, road dust samples exhibited the same PAHs composition and contained low amounts of hydrophilic PAHs such as Bp and Phe (4-17% of total PAHs). Relative contents of PAHs were: B[b]f and Pyr (19 and 17% of total PAHs on average respectively) > Flu, Chry and B[a]p (14, 13, 12) > Phe and B[a]a (8, 8) > Bp, B[k]f and Anth (3, 4, 4). Sum of major 5 PAH could account for 54-83% of total PAHs.

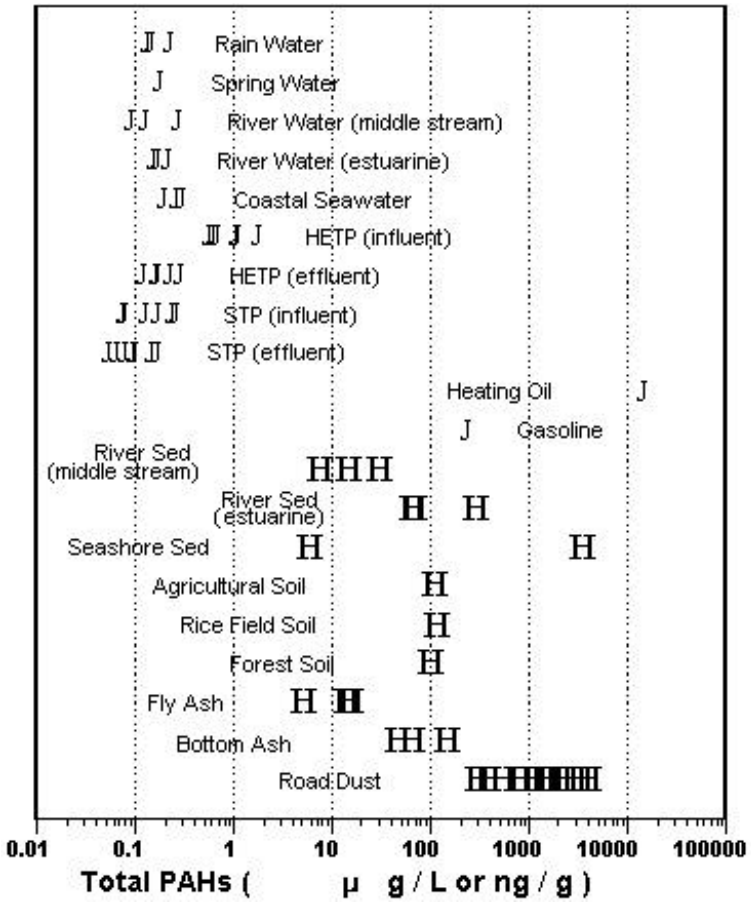

Fig.1 Levels of PAHs in various extracts, "Total PAHs2 shows the sum of 10 PAHs

The same results were obtained each other in the three municipal waste incinerators. Total PAH in the ash were in the same range as those in the river sediment. Total PAHs detected in the bottom ash were higher by a factor of 5-10 than those detected in the fly ash. In contrast with the road dust, ratio of hydrophilic PAHs (Bp and Phe) to the total PAHs was high in both of the bottom ash and fly ash samples (48-68% for the bottom ash, 63-90% for the fly ash). Moreover adding Flu and Pyr to Bp and Phe made the ratio up to 82-90% in bottom ash and 90-92% in fly ash.

Bp and Phe accounted for 50-89% of total PAHs in the middle stream sediments, but only 10-41% in the estuarine sediments. As shown in Fig.2, comparing the middle stream and the estuarine river sediments, each PAH concentration was increased from several to 40-fold. Correlation analysis of the data from the river sediments revealed that the magnifications were strongly correlated with the $\log P_{ow}$, showing a correlation coefficient of 0.78-0.88. When the same types of analysis were performed for the water samples obtained from the three rivers, good correlations were also found in the two rivers (r^2=0.69 and 0.88). However changes of ratio of Bp and Phe to the total PAHs between middle stream and estuarine river water samples were less than those of sediment samples (89% in the middle stream to 81% in the estuarine, 68% to 42%, 67% to 32%).

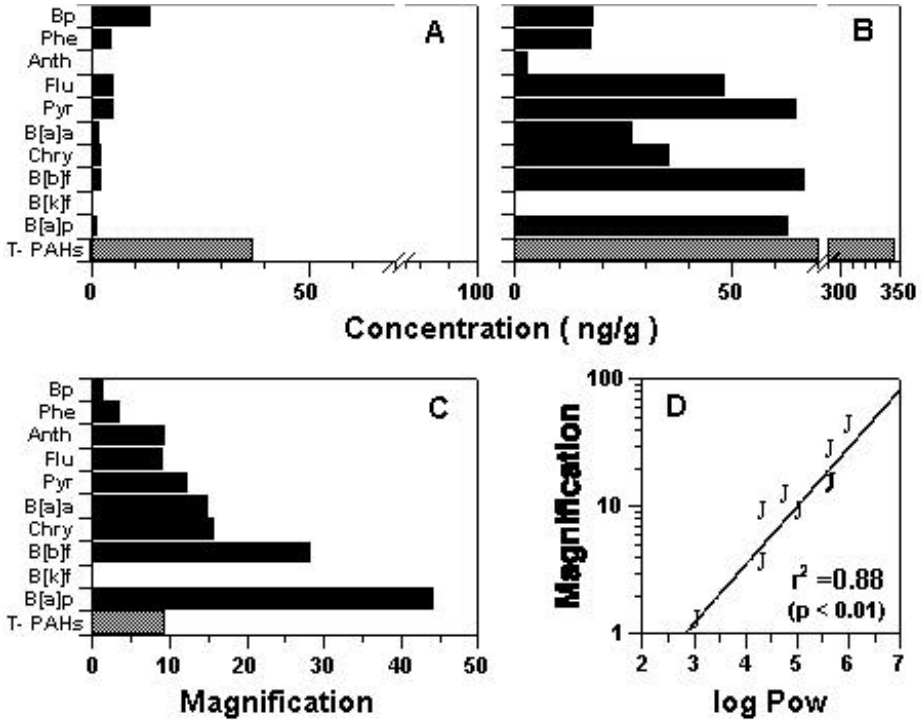

Figure 2

Although composition of 10 PAHs in the soil samples was similar to one another and to that in the ash samples, soils samples contained a relatively high percentage of B[b]f. Sum of Bp, Phe, Flu, Pyr and B[b]f were 66-81% to total PAHs.

Calculated IEFs for crude and semi-purified extracts are summarized in Fig.3. As the loss of 10 PAHs due to the semi-purification was less than about 10% (data not shown), the difference in EROD activity between crude and semi-purified extracts was considered to be caused by organic compounds other than the 10 PAHs. IEFs for crude and semi-purified extracts were about 100- to 1,000,000-fold higher in solid samples (10^{-10}-10^{-5}) than in liquid ones (10^{-11}-10^{-7}). Although most of the samples indicated the same result as in the case of total PAHs, soil samples and influent samples from the HETP showed different results. The highest EROD activities were detected for the crude extracts from agricultural soil, forest soil and road dust and for the semi-purified extracts from road dust, agricultural soil and rice field soil. Higher activities were obtained from the extracts of soil than that expected from the chemical analysis of PAHs. Significant activity was found in the extract of spring water that was thought not to contain any contaminants. On the other hand, although heating oil and gasoline contained relatively large amounts of PAHs, they did not have significant EROD activities.

As well as chemical analysis, it was found that IEFs of crude and semi-purified extracts of ashes were in the same range as those in the river sediments and IEFs of bottom ash were slightly higher than those of fly ash. With IEFs >60% and >75% for the crude and semi-purified extract respectively, the 10 PAHs accounted for a higher percentage of EROD activity than the other samples.

Strong EROD-inducing potential was observed for the extracts of road dust. Many crude extracts showed higher activity than their semi-purified counterparts. The contribution of 10 PAHs to EROD activity varied considerably between samples, ranging from 0 to 93 % for crude extracts and from 0 to 113 % for semi-purified extracts. In most instances, the dose-response curves obtained from the road dust revealed a decrease at the highest dose (Fig.4. A). However, whole cell protein content was not affected (data not shown).

IEF- values similar to those in strongly contaminated road dust were found in soil samples. Furthermore, the dose-response curves did not decrease at the highest dose and their maximum levels were higher than those in any other environment samples (Fig.4. C) and each of the 10 PAH. The contribution of 10 PAHs was lower than that in the case of road dust samples (15-36% for crude extracts, 21-36% for semi-purified extracts).

DISCUSSION

Although the combination of chemical analysis and CYP.A1-related bioassays have been used in previous studies on fly ash [22], river sediments [16, 24], waste disposal site sediments [25] and total suspended particles [14], it has not been used in comparison of the effects of on various environmental samples. This study was designed to determine the AhR-mediated responses of various environmental samples and to estimate the contribution of PAHs. As a result, it was found that the contribution of PAHs to EROD activity differed among various environmental samples, thereby indicating the existence of unidentified substances that have CYP.A1-inducing potential.

Because the extraction method used here can extract PCBs, dibenzofuran and dibenzo-*p*-dioxin [12] and there is only little loss of PAHs by the semi-purification, it is assumed that EROD activities can be induced by PAHs, dioxins and other dioxin-like organic compounds and the difference in EROD activity between crude and semi-purified extracts can be induced by organic compounds adsorbed to silica.

Ten PAHs in the many river sediment extracts could not account for their EROD activity. It is suggested that in such sediments, AhR agonists are organic compounds except for the 10 PAHs and may have similar chemical characteristics to the 10 PAHs. Moreover, the fact that PAH magnifications correlated well with $\log P_{ow}$ in river sediments and even river water samples may indicate that more hydrophobic PAHs and AhR agonists are more easily adsorbed and accumulated by the sediments and afterward stirred up to the river water on the suspended solids, because the PAHs in river water samples were thought to be not dissolved but mainly adsorbed on suspended solids for their hydrophobicity. This aspect is supported by the next two facts. One is that PAH concentrations in the sediment samples were higher than those in the river water samples. Another is that changes of composition of 10 PAHs between middle stream and estuarine were larger in the sediment samples than those in the river water samples.

In the HETP samples, EROD activities were not detected after the semi-purification of the crude extracts. Therefore, it can be assumed that organic compounds except PAHs and dioxins induced EROD activities and had the same origin as the 10 PAHs. Since the extracts from the effluent samples could not induce EROD activity, the AhR agonists might be removed or inactivated by the treatment process. On the other hand, effluent showed higher EROD activity than influent in the STP samples, suggesting the production of contamination of the inducers during the treatment process. The inducers may not be 10 PAHs and the AhR agonists which contained in the HETP samples, because 10 PAHs could not account for EROD activity fully and EROD-inducing potential was not lost after the semi-purification.

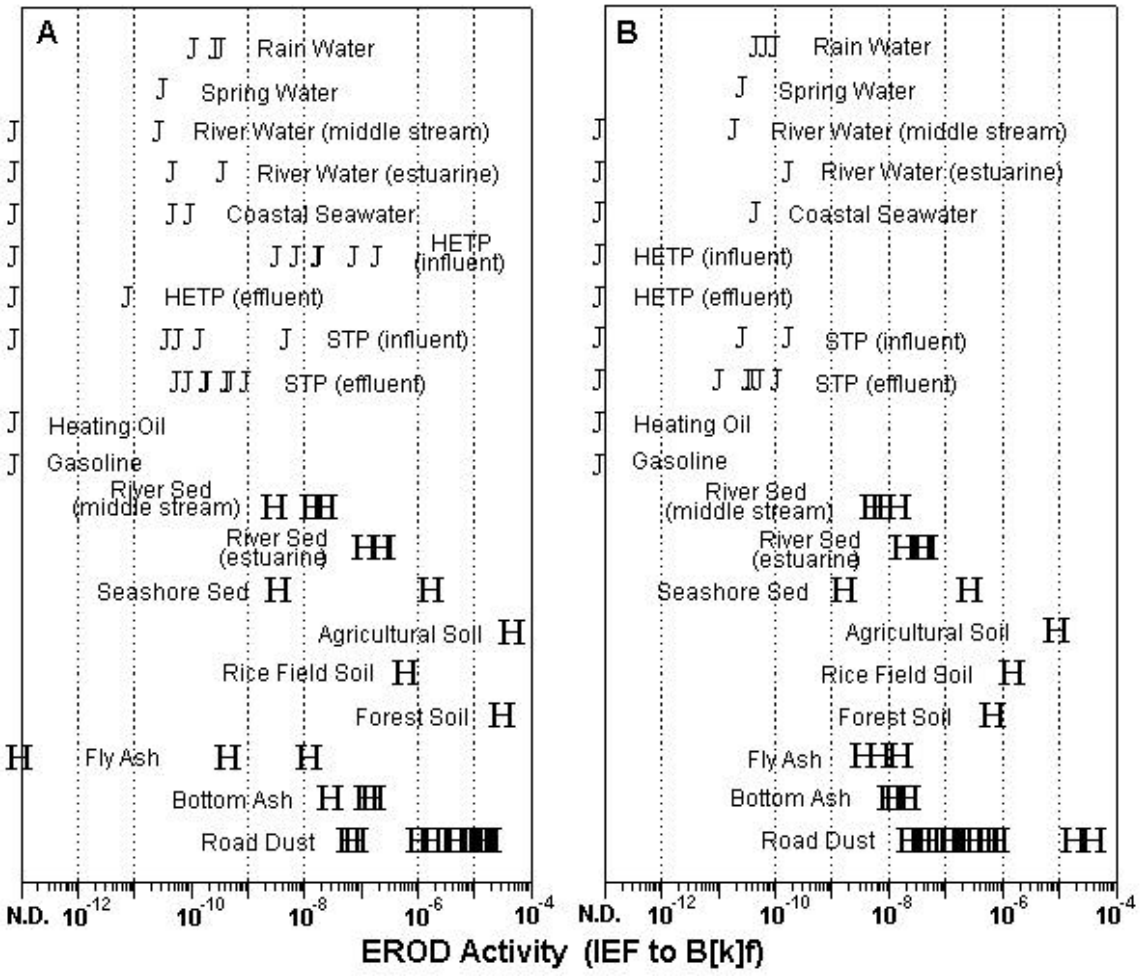

Figure 3

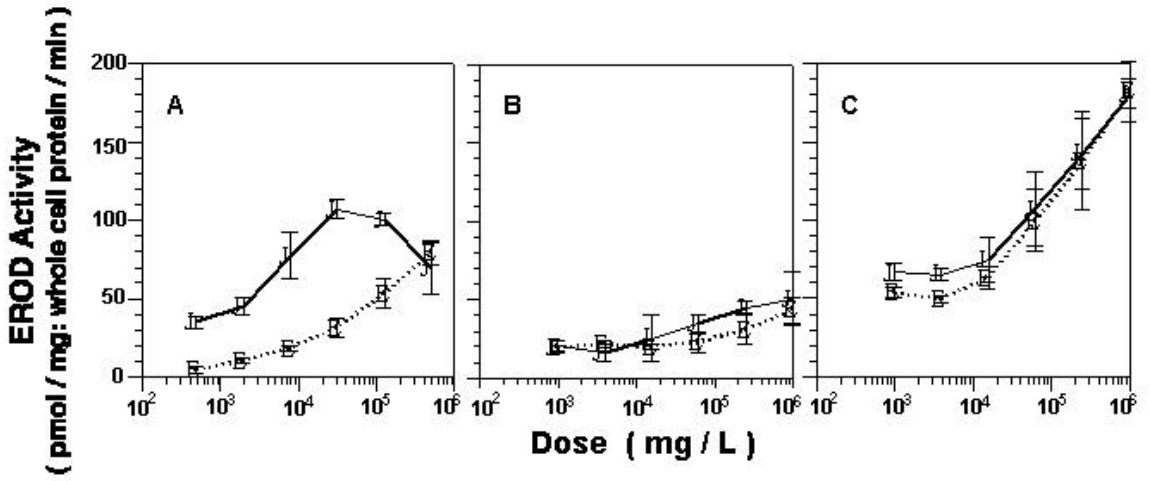

Figure 4 : Typical Dose-response curves of EROD activity for crude extracts (A Road dust , B Bottom ash, C agricultural soils)

Because composition of 10 PAHs was quite different between mineral oil and road dust, PAHs detected in road dust may not originate mainly from fuel oil but mobile exhaust gas, and may be affected subsequently by water elution and sunlight degradation. The contribution of the 10 PAHs to EROD activity varied considerably between samples and differences between crude and semi-purified extracts were also observed. These findings indicate that in the road dust, the synergistic interactions of PAHs with other dioxin-like compounds may have a major contribution to the induction of EROD activity and that we may not be able to classify these road dust samples as a group. The fact that whole cell protein content was not affected at the highest dose is suggesting that decrease of EROD activity in the dose-response curve was not caused by cytotoxicity. Similar results was reported previously for environmental samples [26] and chemical compounds [27].

Soil samples were found to exhibit potent EROD-inducing activity in spite of the fact that the total PAHs were not found at relatively high concentrations. Furthermore, the EROD activities increased in a dose-dependent manner up to the highest dose and maximal resorufin formation was 2- to 2.5-fold higher than B[k]f's, which is known to be a potent AhR agonist [13]. Although strong EROD activities were also detected in the road dust samples, their dose-response curves were clearly different at the highest dose with soil samples'. This fact indicates that AhR agonists are different between soil and road dust samples. In the mouse hepatoma cell line, Hepa-1, the fraction containing PAHs inhibited CYP.A 1 induction at higher concentrations, whereas the fraction containing dioxins did not [24]. In the present surface sediment of the Lake Shinji in Japan, the contribution of pentachlorophenol herbicides, chloronitrofen herbicides and incineration to the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents concentration was estimated to be about 60, 10 and 30%, respectively [28]. These findings indicate that EROD activities observed in the soil samples were induced not by PAHs but by pesticides, herbicides and their by-products. However, there may exist natural AhR agonists in humus soil, because significant activity was found in the extract of spring water.

Although ashes are considered to be harmful in general, their total PAHs and EROD activities were in the same range as those in the river sediments and most of their activities were due to the 10 PAHs. This may indicate that these ashes contain only little dioxin-like organic compounds other than B[k]f and B[a]p. The bottom ashes had more total PAHs and slightly greater EROD activity than the fly ash. This finding might reflect incineration parameters such as temperature and oxygen supply.

In conclusion, the concentrations of PAH and dioxin-like CYP.A1-mediated activity (EROD activity) were determined in extracts of various environmental samples in order to screen the levels and toxicities of dioxin-like contaminants. The combination of EROD assay using 96-well microplates and PAH analysis with alkali pretreatment and *n*-hexane extraction was shown to be useful for the primary screening of chemical risks and practical monitoring. In this study, we could rank various environmental samples in terms of PAH concentration and EROD activity. As a result, we found that municipal waste incinerator ash, which is generally considered to contain many dioxin-like compounds, actually has low dioxin-like activity. Furthermore, its activity is due to the 10 PAHs. On the other hand, road dust and, unexpectedly, soil induced strong EROD activity that could not be accounted for by the 10 PAHs. For these samples, further identification and toxicological characterization of dioxin-like compounds are needed.

REFERENCES

Safe, S. (1993): Development of bioassays and approaches for the risk assessment of 2,3,7,8-tetrachlorobenzo-*p*-dioxin and related compounds. *Environ. Health Perspect.* **101**: 317-325.

Safe, S.H. (1986): Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Ann. Rev. Pharmacol. Toxicol.* **26**: 371-399.

Burke, M.D., Thompson, S., Elcombe, C.R., Halpert, J., Haaparanta, T. and Mayer, R.T. (1985): Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol*. **34**: 3337-3345

Donato, M.T.,Gomez-Lechon, M.J. and Castell, J.V. (1993): A Microassay for measuring cytochrome P450 ,A1 and P450 ,B1 activities in intact human and rat hepatocytes cultured on 96-well plates. *Anal. Biochem.* **213**: 29-33

Gonzalez, F.J. (1989): The molecular biology of cytochrome P-450s. *Pharmacol. Rev*. **40**: 243-288

Guengerich, F.P. (1992): Characterization of human cytochrome-P450 enzymes. *FASEB J.* **6**: 745-748

Knowles, B.B., Howe, C.C. and Aden, D.P. (1980): Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science* **209**: 497-499

Doostdar, H., Burke, M.D., Melvin, W.T. and Grant, M.H. (1991): The effects of dimethylsulphoxide and 5-aminolaevulinic acid on the activities of cytochrome P450-dependent mixed function oxidase and UDP-glucuronosyltransferase activities in human HepG2 hepatoma cells. *Biochem. Pharmacol*. **42**: 1307-1313

Patel, D.K., Shockcor, J.P., Chang, S.Y., Sigel, C.W. and Huber, B.E. (1991): Metabolism of a novel antitumor agent, crisnatol, by a human hepatoma cell line, Hep G2, and hepatic microsomes. Characterization of metabolites. *Biochem. Pharmacol*. **42**: 337-346

Grant, M.H., Duthie, S.J., Gray, A.G. and Burke, M.D. (1988): Mixed function oxidase and UDP-glucuronyltransferase activities in the human Hep G2 hepatoma cell line. *Biochem. Pharmacol*. **37**: 4111-4116

Sassa, S., Sugita, O., Galbraith, R.A. and Kappas,A. (1987): Drug metabolism by the human hepatoma cell, Hep G2. *Biochem. Biophys. Res. Commun*. **143**: 52-57

Kenmotsu, K. (1998): Multiresidue determination of polycyclic aromatic hydrocarbons (PAHs) in environmental samples. *J. Res. Environ*. **34**: 1361-1369

Jones, J.M. and Anderson, J.W. (1999): Relative potencies of PAHs and PCBs based on the response of human cells. *Environ. Toxicol. Pharmacol*. **7**: 19-26

Nakama, A., Kuroda, K. and Yamada, A. (1995): Induction of cytochrome P450-dependent monooxygenase in serum-free cultured Hep G2 cells. *Biochem. Pharmacol*. **50**: 1407-1412

Postlind, H., Vu, T.P., Tukey, R.H. and Quattrochi,L.C. (1993): Response of human *CYP1*-luciferase plasmids to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polycyclic aromatic hydrocarbons. *Toxicol. Appl. Pharmacol*. **118**:255-262

Vondracek, J., Machala, M., Minksova, K., Blaha, L., Murk, A.J., Kozubik, A., Hofmanova, J., Hilscherova, K., Ulrich, R., Ciganek, M., Neca, J., Svrckova, D., and Holoubek,I. (2001): Monitoring river sediments contaminated predominantly with polyaromatic hydrocarbons by chemical and in vitro bioassay techniques. *Environ. Toxicol. Chem.* **20**: 1499-1506

Hanioka, N., Tatarazako, N., Jinno, H., Arizono, K. and Ando,M. (2000): Determination of cytochrome P450 1A activities in mammalian liver microsomes by high-performance liquid chromatography with fluorescence detection. *J. Chromatography B* **744**: 399-406

Lipp, H.P., Schrenk, D., Wiesmuller, T., Hagenmaier, H. and Bock, K.W. (1992): Assessment of biological activities of mixtures of polychlorinated dibenzo-p-dioxins (PCDDs) and their constituents in human HepG2 cells. *Arch. Toxicol*. **66**:220-223

Schrenk, D., Lipp, H.P., Wiesmuller, T., Hagenmaier, H. and Bock, K.W. (1991): Assessment of biological activities of mixtures of polychlorinated dibenzo-*p*-dioxins: comparison between defined mixtures and their constituents. *Arch. Toxicol*. **65**:114-118

Okayama Prefecture Annual Report 2000, http://www.pref.okayama.jp/kikaku/toukei/tounen/frame01.htm, Statistics management division, Okayama Prefecture.

The 2001 fiscal report of the analysis of public waters, http://www.pref.okayama.jp/seikatsu/kankanri/13koukyou.htm, Environmental management division, Okayama Prefecture.

Till, M., Behnisch, P., Hagenmaier, H., Bock, K.W. and Schrenk, D. (1997): Dioxinlike components in incinerator fly ash : a comparison between chemical analysis data and results from a cell culture bioassay. *Environ. Health Perspect*. **105**: 1326-1332

Mabey, W.R., Smith, J.H., Podoll, R.T., Johnson, H.L. and Mill, T. (1982): Aquatic fate process data for organic priority pollutants, EPA 440/4-81-014 . Washington: U.S. Environmental Protection Agency.

Matlova, L., Machala, M., Nezveda, K., Granatova, M. and Nevorankova,Z. (1995): Biochemical screening of highly toxic aromatic contaminants in river sediment and comparison of sensitivity of biological model systems. *Chemosphere* **30**: 1363-1371

Nakama, A., Yoshikura, T. and Fukunaga,I. (1997): Induction of cytochrome P450 in Hep G2 cells and mutagenicity of extracts of sediments from a waste disposal site near Osaka, Japan. *Bull. Environ. Contam. Toxicol*. **59**: 344-351

Nito, S., Todoroki, N., Misumi, M., Nakahama, T. and Inouye,Y. (2001): Biological evaluation of the pollution of rivers flowing into Tokyo bay with the 7-ethoxycoumarin *O*-deethylase (ECOD) activity induced by river sediment extracts in HepG2 cells. *J. Health Sci*. **47**: 118-122

Delescluse, C., Ledirac, N., De Sousa, G., Pralavorio, M., Botta-Fridlund, D., Letreut, Y. and Rahmani, R. (1997): Comparative study of CYP,A1 induction by 3-methylcholanthrene in various human hepatic and epidermal cell types. *Toxocol. in Vitro* **11**: 443-450

Masunaga, S. (2000): The origin of and change in, dioxin pollution in Japan - the contribution of dioxins in herbicides -. *J. Material Cycles Waste Management* **11**: 173-181