Available online at www.sciencedirect.com
ScienceDirect
Chemosphere 70 (2007) 20–28
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

In vitro toxicity assay using human bronchial epithelial cell, Beas-2B, for the screening of toxicological risk of dioxin-like compounds sampled from small sized Korean waste incineration plants

Mi-Hee Ha, Sang-Yee Ham, Dong-Hoon Lee, Jinhee Choi *

Faculty of Environmental Engineering, College of Urban Science, University of Seoul, 90 Jeonnong-dong, Dongdaemun-gu, Seoul 130-743, Republic of Korea

Received 28 February 2007; received in revised form 29 May 2007; accepted 15 July 2007
Available online 11 September 2007

Abstract

To test the suitability of cell bioassay as a tool for screening the toxicological risk of dioxin-like compounds, an *in vitro* toxicity assay was performed using samples obtained from small sized Korean waste incineration plants. Stress-related gene expression, cell viability, apoptosis, DNA damage and cell cycles were investigated as toxicological indicators of the polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/DFs) exposed human bronchial epithelial cell, Beas-2B. Of the stress-related genes, the expressions of the aryl hydrocarbon receptor (AhR), cytochrome P450 (CYP) and p53 genes were most significantly induced by exposure to PCDDs/DFs. Exposure of Beas-2B cells to PCDDs/DFs sampled from waste incinerators was sufficient for the expression of noticeable cytotoxic and genotoxic effects. Increased number of cells in the G1 phase in PCDDs/DFs treated samples suggests PCDDs/DFs might lead to alteration in the cell cycle. Statistical tests revealed significant correlations between the PCDDs/DFs concentration and the AhR and CYP gene expression/cell viability/DNA damage. Different from AhR-mediated bioanalytical assay using genetically modified cell line, the present study has been focused on the evaluation of toxicological effects of dioxin-like compounds using normal human cell line. The results of this study have demonstrated that PCDDs/DFs samples from waste incinerators can be applied to cell bioassays for the evaluation of the toxicity of dioxin-like compounds obtained from field samples, and the use of stress-related gene expression assay and cytotoxic/genotoxic test systems would appear to be relevant for preliminary screening of the risk associated with dioxin-like chemicals from waste incinerators.

Keywords: PCDDs/DFs; Cell bioassay; Stress-related gene expression; Cytotoxicity; Genotoxicity

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs) are highly persistent, bioaccumulative and toxic chemicals of global concern (Dickson and Buzik, 1993; Wu et al., 2001; Hays and Aylward, 2003). Together with co-planar PCBs, PCDDs/DFs are commonly known as dioxin-like chemicals. Their major sources are combustion related, usually resulting in their immediate release to the atmosphere (Alcock et al., 2001). The Stockholm Convention on persistent organic pollutants was negotiated internationally for the protection of human health and the environment (Godduhn and Duffy, 2003).

Studies on the toxicity of dioxin-like chemicals have been widely conducted on cells or animals using high doses of 2,3,7,8-TCDD (Van der Weiden et al., 1992; Parrott et al., 1995; Vogel and Abel, 1995; Puga et al., 2000; Matsumura, 2003). However, limited study has been conducted on environmentally relevant field sample. In real environments, human populations are exposed to dioxin-like

* Corresponding author. Tel.: +82 2 2210 5622; fax: +82 2 2244 2245.
E-mail address: jinhchoi@uos.ac.kr (J. Choi).

0045-6535/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2007.07.055

chemicals as complex mixtures of compounds and at lower levels than under laboratory experimental conditions. Therefore, although mechanistic studies with exposure to high doses of 2,3,7,8-TCDD have great values, the toxic effects of dioxin on human health need to be more realistically evaluated.

This study employed a combination of instrumental analyses and an *in vitro* toxicity assay, to evaluate the suitability of the cell bioassay as a tool for preliminary screening of the toxicological risk of dioxin-like compounds sampled from waste incinerators. The first part of this study comprises chemical analysis conducted on the sampled from stack of small sized Korean waste incinerators. In the second part, an *in vitro* toxicity assay was presented performed on human bronchial epithelial cells, Beas-2B, treated with dioxin-like compounds sampled from stack of small sized Korean waste incinerators, using stress-related gene expression, cell viability, apoptosis, DNA damage and cell cycles as the toxicological indicators. As potential stress-related genes, the genes encoding dioxin receptors (aryl hydrocarbon receptor; AhR-1, AhR-2), phase I xenobiotic metabolism enzymes (cytochrome P450; CYP1A2, CYP1B1), antioxidant enzymes thioredoxin reductase; TR, heme oxygenase-1; HO-1, cupper, zinc-superoxide dismutase; Cu, Zn-SOD, manganese-superoxide dismutase; Mn-SOD, the tumor suppressor protein (p53) and cell cycle regulator protein (cyclin-dependent kinase inhibitor; p21) were investigated.

2. Materials and methods

2.1. Sampling and chemical analysis

Thirteen small sized (capacity: bellow 200 kg day^{-1}) waste incinerator plants were selected for the investigation of the concentration of PCDDs/DFs in stacks (Table 1). The Korean Standard Testing Method for PCDDs/DFs (Ministry of Environment, Republic of Korea, 1996) was used for sampling, pre-treatment (extraction), and analysis. Collection of PCDDs/DFs from the incineration stack was achieved using a sampling train, consisting of a probe, a cylindrical filter assembly, which allowed insertion of a thermocouple (silica fiber thimble, 90 mm in length and 25 mm outer diameter, Whatman: at least 99% efficiency on 0.3 μm dioctyl phthalate smoke particles), three impingers (two filled with 150 ml distilled water, the other empty), a sorbent (XAD-2) module, which allowed insertion of a thermocouple and two impingers (one filled with 150 ml ethylene glycol, the other empty). After spiking 2 ng of 37 Cl-2,3,7,8-TCDD to the sorbent, two traverse-point sampling was isokinetically (isokinetic factor of 95–105%) performed over the respective cross-sections at the stack for about 2 h. The operating conditions of the incineration plant, (i.e., temperature, pressure, etc.,) were recorded, and the constituents of fuel gas, such as O_2, CO, SO_2 and NOx, measured using the electrochemical sensors of a gas analyzer at the sampling point every minute. All samples were separated into their liquid and solid phases using glass wool filter (GF/B; 1.0 μm). After separation, the liquid phase samples were extracted with dichloromethane, using liquid–liquid extraction, with the solid phase extracted using Soxhlet-Dean stock extraction with toluene as solvent. After the extractions, the PCDDs/DFs were subjected to a series of clean-up processes, such as, KOH/H_2SO_4/water, multi-silica gel and alumina columns, and activated carbon column cleanup, as conducted by Kim et al. (2006). The concentrations of PCDDs/DFs in the final extracts were determined using high resolution gas chromatography (HRGC; GC-8060, Fison Mainz-Kastel, Germany) and high resolution mass spectrometry (HRMS; Autospec Ultima, Micromass, Manchester, UK), with a SP-2331 column (60 m × 0.3 mm × 20 μm, supelco, Bellefonte, PA, USA).

2.2. Cell culture and treatment

The human bronchial epithelial cells, Beas-2B, were maintained in DMEM/F12 (GIBCO BRL Life Technologies,

Table 1
The characteristics of small size waste incinerators in Korea

Sample number	Location	Waste type	Capacity (kg/h)	Type	Operating time (h/day)	Feeding type	Incineration process
1	Inchon	IW	40	Floor	4	Batch	CC–CY–WS–Stack
2	Gyeongsangnam-Do	IW	95	Stoker	Under 8	Batch	CC–CY–Stack
3	Gyeongsangnam-Do	IW	95	Floor	4	Batch	CC–CY–Stack
4	Jeollabuk-Do	IW	195	Liquid injection	24	Continuous	CC–WS–Stack
5	Jeollabuk-Do	IW	180	Stoker	Under 8	Batch	CC–HE–CY–AP–SDA/BF–WS–Stack
6	Jeollanam-Do	MSW	150	Stoker	Under 8	Batch	CC–CY–Stack
7	Daegu	IW	195	Stoker	Under 8	Batch	CC–HE–CY–WS–Stack
8	Gyeongsangbuk-Do	MSW	195	Stoker	Under 8	Batch	CC–WHB–CY–SDA/BF–Stack
9	Gyeongsangbuk-Do	MSW	195	Stoker	Under 8	Batch	CC–WHB–CY–BF–Stack
10	Suwon	IW	188	Stoker	Under 8	Batch	CC–WHB–MC–SDA/BF–Stack
11	Seoul	MSW	195	Stoker	Under 8	Batch	CC–WHB–CY–SDA/BF–Stack
12	Gyeongsangnam-Do	MSW	190	Stoker	Under 8	Batch	CC–CY–Stack
13	Gangwon-Do	MSW	195	Stoker	8-16	Batch	CC–WHB–CY–AP–SDA/BF–Stack

MSW: municipal solid waste, IW: industrial waste, CC: combustion chamber, HE: heat exchanger, CY: cyclone, AP: air preheater, SDA: spray dryer absorber, BF: bag filter, WS: wet scrubber, VS: venturi scrubber and MC: multi cyclone.

Rockville, MD, USA), supplemented with 10% (v/v) fetal bovine serum and 1% antibiotics, at 37 °C in a CO_2 atmosphere. The solvents used for PCDDs/DFs analyses were evaporated using a rotary evaporator, and the PCDDs/DFs were re-dissolved in acetone for the cell treatment. The treated and control cells were incubated for 24 h, and then harvested and kept −80 °C, prior to the analysis.

2.3. Reverse transcription–polymerase chain reaction (RT–PCR)

Expression of AhR-1, AhR-2, CYP1A2, CYP1B1, TR, HO-1, Cu, Zn-SOD, Mn-SOD, p53 and p21 genes was examined in a semi-quantitative manner, using reverse transcription–polymerase chain reactions (RT–PCR). Gene expression analyses were performed on treated and control cells, using a two-step method, employing RT Premix and RCR Premix Kits (Bioneer Co., Seoul, Korea). The PCR products were separated through electrophoresis on a 1.5% agarose gel (Promega, Madison, WI, USA), and visualized with ethidium bromide (Bioneer Co.). All the tests were replicated at least three times. Primers for the detection of the stress-related genes were designed based on sequences retrieved from GenBank™. Following the RT–PCR analyses, the relative densities of the protein and DNA bands were determined using an image analyzer, the Gel Documentation system (Vilber Lourmat TFX-20.M, Marne la Vallee, France), coupled to a Kodak 1D 3.6 camera (Kodak EDAS 290, Rochester, NY, USA).

2.4. Cell viability assay

The cell viability was measured using 3-[4,5-dimethylthiazol-2-yl]-2,5-di phenyltera zolium bromide (MTT) reagent, as described by Mosmann (1983).

2.5. Flow cytometry

Flow cytometry was performed on the treated and control cells for analyses of the cell cycle and apoptosis. Propidium iodide (PI) stained cells were analyzed using a flow cytometer (BD Science, San Jose, CA, USA), as described by Nicoletti et al. (1991). The effect on apoptosis was determined by the increase in the proportion of sub G1 hypodiploid cells.

2.6. DNA gel electrophoresis

Apoptosis in the treated cells was also evaluated by electrophoretic demonstration of DNA fragmentation. After treatment, genomic DNA was extracted from the cells, using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany), subjected to electrophoresis on a 1.5% agarose gel, containing 0.1 g ml^{-1} of ethidium bromide and visualized under ultraviolet light.

2.7. Comet assay

The Comet assay was performed on treated and control cells using an image analysis system (Komet 5.5, Kinetic Imaging Limited, Nottingham, UK), as described by Tice et al. (2000).

2.8. Data analysis

The statistical differences between the untreated (control) and treated cells were determined with the aid of the parametric *t* test. The Pearson test was conducted for correlation studies. All the statistical analyses were conducted using SPSS 12.0.1 (SPSS Inc., Chicago, Illinois, USA). An alpha level of 0.05 was used to determine significance in all the statistical analyses.

3. Results and discussion

Thirteen small sized waste incineration plants were selected for the investigation of the concentrations of PCDDs/DFs in stacks. Six were municipal solid waste (MSW) incinerators and seven were industrial waste (IW) ones. Concentrations of PCDDs/DFs ranged from 0.165 to 837 ng-TEQ $(N\,m^3)^{-1}$ (Table 2). Most samples contained small amounts of dioxin-like compounds. The concentrations in six samples were below 10 ng-TEQ $(N\,m^3)^{-1}$, those of nine under 50 ng-TEQ $(N\,m^3)^{-1}$ and only three samples had concentrations greater than 100 ng-TEQ $(N\,m^3)^{-1}$. The percentages of PCDDs and PCDFs were 57 and 43%, respectively.

As dioxin-like chemicals are known to induce alterations in the expressions of many stress response genes, the stress-related gene expression profiles were investigated in the treated and control cells (Fig. 1). The most frequently used endpoint in the bioassays of dioxins has been the induction of CYP1A1, which is under the control of the AhR signal transduction pathway (Safe, 1986; Whitlock, 1990; Quattrochi et al., 1994; Xu et al., 2000; Mandal, 2005). As expected, the expressions of the AhR and CYP genes increased on exposure to samples containing high dioxin concentrations (Table 2; samples No. 11, 12 and 13). The AhR-1 and AhR-2 gene expressions increased on exposure to samples containing high concentrations of PCDDs/DFs (samples No. 11, 12 and 13); however, increases also occurred at relatively low levels (samples No. 2, 3 and 4), which might be due to mixture effects. The expression of the CYP1A2 gene increased on exposure to most samples, and the degree of increase was more pronounced at high dioxin concentrations (samples No. 11, 12 and 13) than at low concentrations. Among dioxin-induced cellular toxicity, oxidative stress is the most studied (Alsharif et al., 1994; Nebert et al., 2000; Slezak et al., 2000; Dalton et al., 2002). In this study, however, exposure to dioxin-like compounds did not lead serious alteration in the expressions of oxidative stress-related genes; increased HO-1 and CuZn-SOD gene expressions and decreased TR gene

Table 2
Concentrations of PCDDs/DFs from Korean small size waste incinerators analyzed using high resolution gas chromatography and high resolution mass spectrometry

Sample number	2378 TCDF	12378 PCDF	23478 PCDF	123478 HxCDF	123678 HxCDF	234678 HxCDF	123789 HxCDF	1234678 HpCDF	1234789 HpCDF	OCDF	PCDFs	2378 TCDD	12378 PCDD	123478 HxCDD	123678 HxCDD	123789 HxCDD	1234678 HpCDD	OCDD	PCDDs	PCDDs/DFs
1	0.003	0.003	0.061	0.009	0.017	0.026	0.000	0.008	0.001	0.000	0.128	0.010	0.011	0.002	0.006	0.005	0.003	0.000	0.037	0.165
2	0.000	0.000	0.132	0.016	0.032	0.034	0.002	0.010	0.001	0.000	0.227	0.021	0.000	0.003	0.004	0.003	0.001	0.000	0.032	0.259
3	0.040	0.020	0.285	0.025	0.046	0.067	0.006	0.018	0.003	0.001	0.511	0.064	0.058	0.009	0.017	0.012	0.009	0.002	0.171	0.682
4	0.013	0.011	0.375	0.064	0.128	0.531	0.025	0.132	0.037	0.060	1.378	0.061	0.102	0.017	0.042	0.038	0.073	0.036	0.369	1.747
5	0.360	0.120	1.840	0.110	0.210	0.260	0.040	0.040	0.01	0.000	2.990	0.180	0.280	0.050	0.060	0.040	0.020	0.000	0.630	3.620
6	0.074	0.104	1.617	0.262	0.483	0.339	0.051	0.112	0.019	0.004	3.066	0.272	0.675	0.111	0.123	0.147	0.100	0.015	1.443	4.509
7	0.674	0.302	5.014	0.447	0.937	0.962	0.087	0.199	0.026	0.007	8.656	1.169	1.474	0.227	0.257	0.190	0.088	0.010	3.415	12.071
8	1.230	0.390	8.100	0.730	1.520	1.500	0.160	0.260	0.040	0.010	13.930	1.900	2.590	0.260	0.300	0.230	0.100	0.010	5.400	19.330
9	2.280	0.689	12.200	0.976	1.721	2.358	0.259	0.401	0.053	0.039	20.976	2.020	2.066	0.283	0.489	0.356	0.196	0.033	5.444	26.420
10	4.500	1.850	28.960	2.040	3.660	3.190	0.300	0.320	0.060	0.010	44.890	4.530	7.600	0.570	0.740	0.600	0.160	0.010	14.220	59.110
11	2.790	3.310	41.770	5.870	9.300	11.710	0.900	2.800	0.590	0.180	79.220	3.700	19.710	3.660	10.020	11.950	5.190	0.490	54.720	133.940
12	11.770	3.810	88.630	6.990	13.610	10.760	1.420	1.720	0.180	0.030	138.930	17.890	16.980	1.480	1.960	1.570	0.480	0.030	40.410	179.330
13	19.896	18.147	376.536	43.493	88.355	82.066	12.029	16.978	2.663	0.623	660.780	48.570	82.405	12.275	15.598	12.706	5.007	0.335	176.895	837.680

*ng-TEQ $(Nm^3)^{-1}$.

expression were observed in limited PCDDs/DFs treated samples. The dioxin-induced oxidative stress response may be regulated at the post-transcriptional level. Expression of the p53 gene, a central factor in cellular stress responses, increased in most of PCDDs/DFs treated samples, while increases in the expression of the cell cycle regulator protein, the p21, occurred in limited dioxin-like compound treated samples (samples No. 11 and 12).

In order to verify the cytotoxic property of PCDDs/DFs released from waste incinerators, the effect of dioxin on cell viability was studied in Beas-2B cells (Fig. 2). Statistically significant cell death occurred in the cells treated with PCDDs/DFs concentrations greater than 50 ng-TEQ $(N\,m^3)^{-1}$. Decreases in the cell viability were about 10–20% that of the control group.

To verify if the dioxin-induced cell death observed in Fig. 2 was due to apoptosis, flow cytometry analysis was conducted, using PI staining, to quantify the number of cells with a sub-diploid DNA content. As shown in Fig. 3, 24 h after PCDDs/DFs treatment, the numbers of cells localized in the sub-diploid DNA peak were not significantly changed. Because DNA ladder formation is an important phenomenon in apoptosis, DNA fragmentation was also examined. However, the DNA ladder, a typical apoptosis marker, was not observed with any of the treatments.

The cytotoxic effects of PCDDs/DFs may indicate early cellular changes, with possible biological consequences, which should be considered in preliminary evaluation of the risk of populations exposed to these chemicals *in vivo*. Despite the constraints in the extrapolation of *in vitro* to *in vivo* data in humans, the cytotoxicity of the studied compounds requires special attention, in view of the major damage they cause to cell function, which could result in the inability of cells to proliferate. These disturbances frequently appear long before genotoxic effects are manifested, or even in the absence of the latter. Thus, the cytotoxic effect may be considered as an earlier indication of cellular damage, with possible biological consequences; therefore, this should be taken into account in preliminary evaluation of the risk to populations exposed *in vivo*, as already suggested by Ekwall (1983).

In order to verify the genotoxic property of PCDDs/DFs released from waste incinerators, DNA damage, especially DNA strand breaks, was measured in Beas-2B cells treated with dioxin-like compounds, using the Comet assay (Fig. 4). From the images of treated cells and the calculated tail, as well as the olive tail moments, the amount of DNA strand breaks increased on exposure to all dioxin-treated samples.

Genotoxic parameters are currently the most valuable biomarkers for environmental risk assessment, and many reports linking the DNA damage to subsequent molecular, cellular and tissue level alterations have been published (Ohe et al., 2004). DNA strand breaks are potential premutagenic lesions, which are sensitive markers of genotoxic damage. The overall results, shown in Fig. 4, suggest

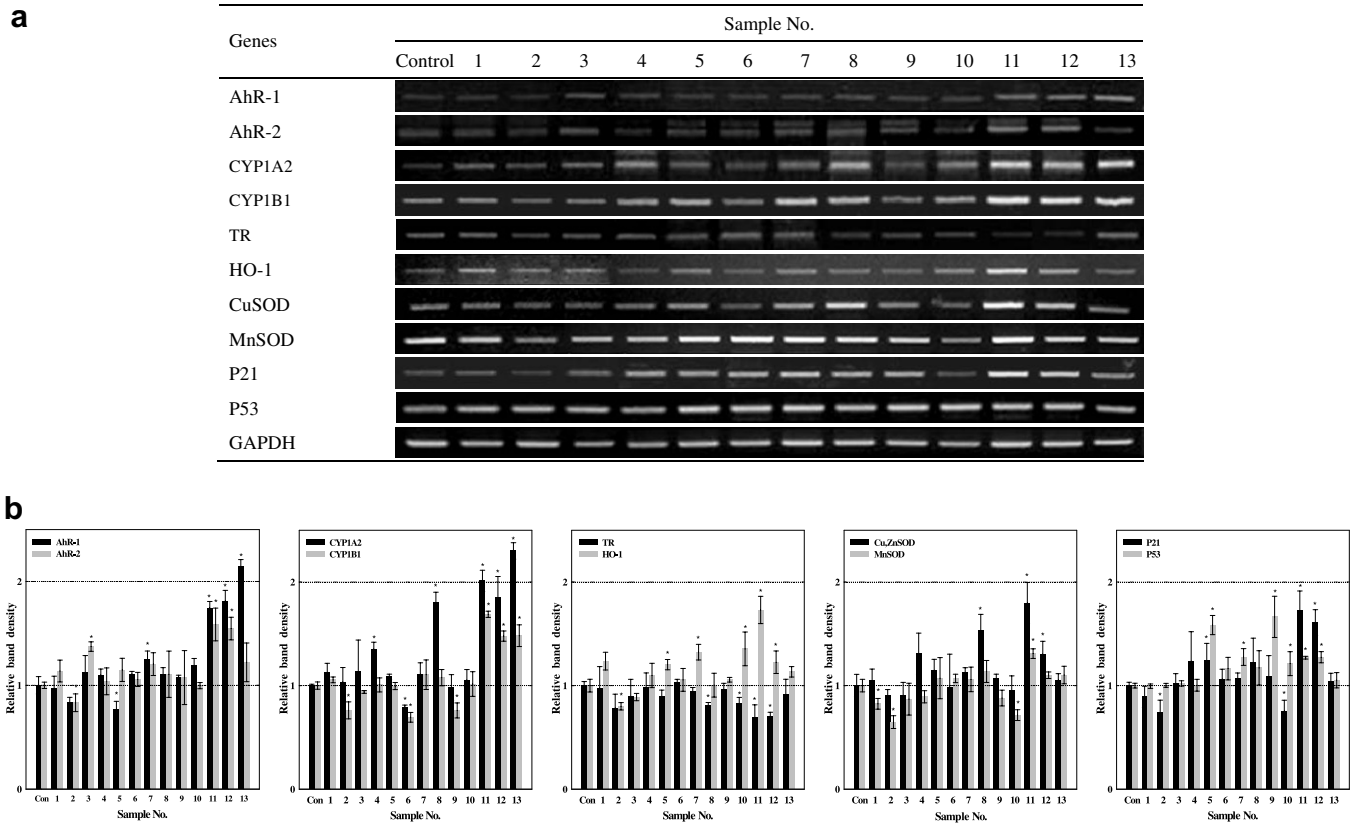

Fig. 1. Stress-related gene expression profiling in Beas-2B cells exposed to PCDDs/DFs. Prior to exposure, PCDDs/DFs were sampled from small sized Korean waste incineration plants, and their concentrations were analyzed. The cells were treated with PCDDs/DFs for 24 h prior to gene expression analyses. Electrophoresis gel images (a). Densitometric values of stress-related gene expressions, normalized using GAPDH mRNA (b: data are presented in arbitrary units compared to control; control = 1, $n = 3$, mean ± standard error of the mean, $*p < 0.05$).

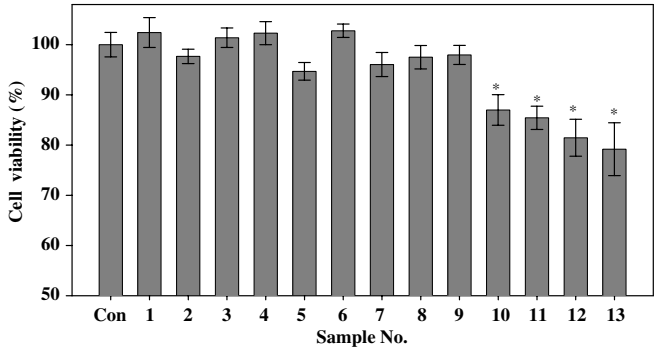

Fig. 2. Cell viability in PCDDs/DFs exposed Beas-2B cells. Prior to exposure, PCDDs/DFs were sampled from small sized Korean waste incineration plants, and their concentrations were analyzed. The cells were treated with PCDDs/DFs for 24 h prior to the MTT assay ($n = 3$, mean ± standard error of the mean, $*p < 0.05$).

exposure of Beas-2B cells to PCDDs/DFs sampled from the stacks of waste incinerator is sufficient for the expression of noticeable genotoxic effects. The relationships between genotoxic responses and physiological symptoms are complicated due to physiological compensatory mechanisms. Thus, a genotoxic indicator may not give a reliable prediction of the toxic effects under physiological condition and is, therefore, only ever likely to indicate exposure to chemicals possessing genotoxic properties. However, as the mere presence of potentially carcinogenic genotoxic compounds is a major concern to human health, with the sensitive and rapid detection of genotoxic properties in field samples can be considered important, although it does not necessarily include physiological alteration. Considering the importance of genotoxic indicators in environmental monitoring, the measurement of DNA damage could provide useful information for the monitoring and risk assessment of dioxin-like compounds from waste incinerators.

In order to identify the effects of PCDDs/DFs on the cell cycle, flow cytometry analysis was conducted on both control and treated samples (Table 3). The tumor suppressor protein, p53, elicits its normal functions by acting mainly as a transcription factor, which regulates genes contributing to the cell cycle, DNA repair and apoptosis. It is well known that upon activation, p53 is considered to determine the fate of cells, based on the severity of the damage. It can halt cell cycle progression and direct damage repair. In case of extensive, non-repairable damage, p53 induces apoptosis (Shonov and Haupt, 1999; Shen and White, 2001). The cell cycle analysis revealed the number of cells in the G1 phase increased on exposure to all PCDDs/DFs samples com-

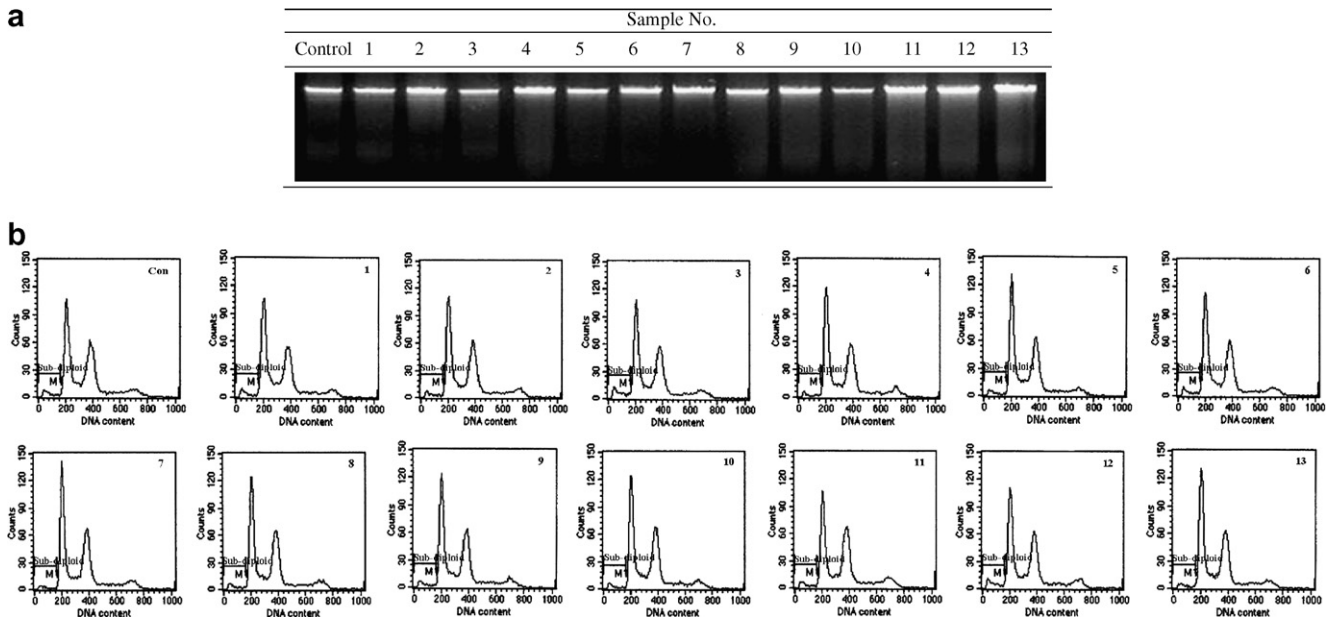

Fig. 3. Apoptosis analysis in PCDDs/PCDFs exposed Beas-2B cells. Prior to exposure, PCDDs/DFs were sampled from small sized Korean waste incineration plants, and their concentrations were analyzed. The cells were exposed to PCDDs/PCDFs for 24 h prior to a DNA fragmentation test (a) and flow cytometry analysis (b).

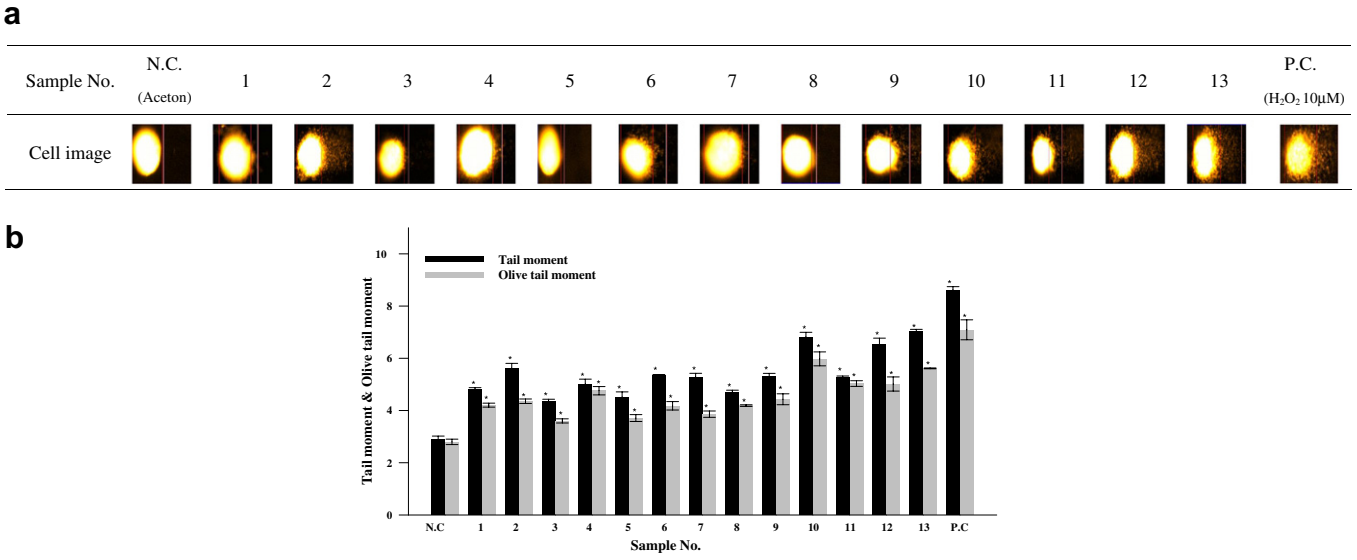

Fig. 4. DNA damage in PCDDs/DFs exposed Beas-2B cells. Prior to exposure, PCDDs/DFs were sampled from small sized Korean waste incineration plants, and their concentrations were analyzed. The cells were exposed to PCDDs/DFs for 24 h, and DNA strand breaks investigated using the Comet assay. Photographical images (a), and the tail and olive tail moments of both treated and control cells (b) (N.C.: negative control and P.C.: positive control).

pared to the control. This phenomenon, along with increases in the p53 and p21 gene expressions (Fig. 1) and no evidence of apoptosis (Fig. 3), suggests PCDDs/DFs might lead to alteration in the cell cycle, especially, arrest of cells in the G1 phase.

To identify any correlation between the toxicological indicators and the dioxin exposure level, Pearson correlation tests were conducted, using the log concentration of PCDDs/DFs (Table 4). Statistical tests revealed significant negative correlations between the log concentration of PCDDs/DFs and the results from MTT assay (cell viability). Positive significant correlation was observed between the log concentration of PCDDs/DFs and the AhR-1, CYP1A2, CYP1B1 and MnSOD gene expressions. Tail and olive tail moments, results from Comet assay (DNA damage) were also positively correlated with the log concentration of PCDDs/DFs. Overall correlation data suggest that the toxicity of dioxin-like compounds sampled

Table 3
Cell cycles in PCDDs/DFs exposed to Beas-2B cells

Sample numbers	G1	S	G2
Control (untreated cell)	37.6	25.7	36.7
1	40.9	25.6	31.6
2	41.2	23.0	35.8
3	41.4	21.7	37.0
4	42.6	23.9	33.6
5	38.0	29.3	32.8
6	40.6	23.8	35.7
7	41.6	23.1	35.3
8	41.3	22.8	35.9
9	40.8	24.3	34.9
10	38.0	26.3	35.6
11	40.6	23.8	35.7
12	42.0	21.6	36.5
13	42.3	22.9	34.8

Prior to exposure, PCDDs/DFs were sampled from small sized Korean waste incineration plants and their concentrations were analyzed. The cells were exposed to PCDDs/DFs for 24 h and the flow cytometry analysis was performed.

from waste incinerators may be mediated by the induction of CYP through AhR activation, in which, as a consequence leads to cyto- and geno-toxicities. However, a correlation study alone cannot prove any causal relationships between these parameters. Direct experimental demonstrations of the wider relationships between the chemical concentrations and biochemical/cellular toxic responses are needed to establish the causal relationships of the potential toxicity of field dioxin samples. Characterization of the causal relationships between *in vitro* toxic responses and the *in vivo* bioassay response, epidemiological evidence would help to define the hazards of field sampled dioxin-like compounds. The potential of the parameters tested in this study as toxicological indicators for the environmental monitoring and risk assessment of dioxin-like chemicals requires validation in the context of their specificities and sensitivities.

The relationship between the toxicological endpoints associated with aryl hydrocarbon receptor mediated mechanism of action was addressed by conducting the correlation tests among toxicological indicators (Table 5). Statistic analysis revealed that the response of AhR1 was significantly correlated with that of cell viability (MTT), phase I enzyme (CYP1A2) and DNA damage (tail moment). These results suggest among observed toxicity by dioxin-like compound, these phenomena may be more directly mediated by the activation of AhR.

Pollution induced by dioxin-like chemicals is caused by a complex mixture of compounds, making the exhaustive analyses of the contaminants present in polluted environments impossible, which limit the possibility of intensive toxicological studies. Therefore, rapid and sensitive tools are needed for screening the hazardous properties of such chemicals prior to intensive toxicological investigations and risk assessment (Park and Choi, 2007). The detection of dioxin-like compounds can be achieved using analytical

Table 4
Pearson coefficient of correlations between the log concentrations of PCDDs/DFs from small sized Korean waste incineration plants and toxicological indicators measured in PCDDs/DFs exposed Beas2-B cells

	MTT	AhR-1	AhR-2	CYP1A2	CYP1B1	TR	HO-1	CuSOD	MnSOD	P21	P53	G1	S	G2	M	Tail	Olive tail
2378TCDF	−.860(**)	.666(*)	.318	.648(*)	.518	−.606(*)	.301	.241	.401	.310	.412	−.076	−.170	.517	−.248	.700(*)	.572
12378PCDF	−.890(**)	.760(**)	.350	.666(*)	.585(*)	−.584(*)	.378	.272	.466	.333	.313	−.026	−.201	.502	−.259	.749(**)	.641(*)
23478PCDF	−.869(**)	.803(**)	.451	.718(**)	.644(*)	−.410	.464	.364	.557(*)	.444	.360	.000	−.153	.379	−.232	.690(**)	.647(*)
123478HxCDF	−.859(**)	.845(**)	.456	.738(**)	.670(*)	−.388	.482	.398	.583(*)	.464	.290	.070	−.197	.366	−.238	.702(**)	.678(*)
123678HxCDF	−.859(**)	.846(**)	.446	.737(**)	.668(*)	−.383	.468	.391	.581(*)	.456	.281	.082	−.204	.365	−.245	.707(**)	.674(*)
234678HxCDF	−.836(**)	.853(**)	.455	.783(**)	.695(**)	−.353	.488	.446	.581(*)	.499	.266	.140	−.199	.304	−.276	.670(*)	.693(**)
123789HxCDF	−.821(**)	.849(**)	.484	.767(**)	.728(**)	−.229	.564	.384	.575	.435	.218	.099	−.069	−.001	−.193	.647(*)	.660(*)
1234678HpCDF	−.813(**)	.890(**)	.480	.800(**)	.713(**)	−.325	.479	.463	.619(*)	.523	.204	.227	−.265	.306	−.248	.652(*)	.670(*)
1234789HpCDF	−.784(**)	.859(**)	.474	.772(**)	.708(**)	−.291	.511	.494	.640(*)	.544	.213	.178	−.194	.261	−.279	.602(*)	.665(*)
OCDF	−.645(*)	.727(*)	.193	.796(**)	.654(*)	−.239	.448	.440	.405	.399	.089	.287	.115	−.560	−.461	.514	.650(*)
2378TCDD	−.842(**)	.799(**)	.443	.707(**)	.615(*)	−.395	.412	.337	.537	.419	.334	.051	−.222	.430	−.198	.756 (**)	.674(*)
12378PCDD	−.869(**)	.786(**)	.334	.672(*)	.604(*)	−.579(*)	.393	.342	.503	.368	.235	.050	−.260	.516	−.297	.690(**)	.623 (*)
123478HxCDD	−.827(**)	.824(**)	.491	.709(**)	.674(*)	−.372	.535	.443	.653(*)	.504	.328	.033	−.167	.368	−.260	.627(*)	.615(*)
123678HxCDD	−.825(**)	.850(**)	.544	.750(**)	.727(**)	−.381	.595(*)	.498	.663(*)	.549	.304	.061	−.170	.324	−.238	.602(*)	.638(*)
123789HxCDD	−.809(**)	.851(**)	.547	.735(**)	.727(**)	−.375	.613(*)	.515	.675(*)	.561(*)	.281	.067	−.176	.322	−.234	.595(*)	.644(*)
1234678HpCDD	−.723(**)	.839(**)	.553	.736(**)	.708(**)	−.272	.604(*)	.550	.705(**)	.605(*)	.253	.142	−.188	.270	−.230	.515	.606(*)
OCDD	−.620	.744(*)	.334	.719(*)	.692(*)	−.294	.594	.530	.581	.523	.106	.207	.102	−.431	−.392	.427	.570
PCDFs	−.825(**)	.825(**)	.508	.717(**)	.680(*)	−.381	.537	.451	.631(*)	.513	.320	.042	−.182	.374	−.226	.633(*)	.637(*)
PCDDs	−.859(**)	.818(**)	.453	.735(**)	.658(*)	−.388	.475	.392	.569(*)	.467	.336	.038	−.162	.356	−.248	.686(**)	.665(*)
PCDDs/DFs	−.853(**)	.822(**)	.469	.732(**)	.667(*)	−.391	.494	.411	.587(*)	.482	.332	.039	−.167	.363	−.244	.674(*)	.660(*)

See text for definitions of abbreviations. Asterisks indicate significant correlations ($^{*}p < 0.05$; $^{**}p < 0.01$).

Table 5
Pearson coefficient of correlations among toxicological indicators measured in PCDDs/DFs exposed Beas2-B cells

	AhR-1	AhR-2	CYP1A2	CYP1B1	TR	HO-1	CuSOD	MnSOD	P21	P53	G1	S	G2	M	Tail	Olive tail
MTT	−.572(*)	.348	−.611(*)	−.483	.380	−.435	−.039	−.244	−.013	−.315	.052	.077	−.263	.136	−.738(**)	−.679(*)
AhR-1		−.191	.639(*)	.342	−.126	.228	.479	.419	.236	.506	.417	−.546	.448	.024	.562(*)	.500
AhR-2			−.156	.059	.087	.286	.030	.387	.210	.134	−.027	.010	−.047	.400	−.535	−.612(*)
CYP1A2				.726(**)	−.451	.075	−.025	.160	.052	.063	.481	−.499	.242	.394	.489	.292
CYP1B1					−.443	.593(*)	.112	.404	.413	.104	.454	−.207	−.200	.118	.116	−.067
TR						−.256	−.154	−.358	−.166	.050	−.354	.426	−.328	−.286	.050	.154
HO-1							.401	.684(**)	.525	.425	.057	.082	−.144	−.279	−.031	−.090
CuSOD								.761(**)	.669(*)	.717(**)	.116	−.128	.134	−.299	−.269	−.090
MnSOD									.805(**)	.763(**)	.123	−.057	.035	−.159	−.327	−.252
P21										.688(**)	.262	.033	−.208	−.322	−.435	−.388
P53											−.206	.146	.168	−.293	−.017	.078
G1												−.825(**)	.199	.217	.021	−.235
S													−.686(**)	−.446	−.231	.064
G2														.348	.386	.144
M															−.020	−.227
Tail																.864(**)

See text for definitions of abbreviations. Asterisks indicate significant correlations ($^{*}p < 0.05$; $^{**}p < 0.01$).

chemistry methods, but also using animal cell bioassays. The results of this study have demonstrated that PCDDs/DFs samples from waste incinerators can be applied to cell bioassays for the screening of the toxicity of dioxin-like compounds from field samples. Cell bioassays have the advantages of integrating the toxic potency of all compounds in a sample and are more rapid and inexpensive than analytical chemistry techniques (Schirmer et al., 2004). This is particularly important if many samples need to be assessed. Toxicity screening using cell bioassay would be ideal for large scale field monitoring, such as, in site-investigations of large contaminated areas or for the long-term monitoring of waste incinerators (Xiao et al., 2006).

Most of cell bioassays conducted on field dioxin samples are bioanalytical screening tests using genetically altered cell (e.g. H4IIE-luc, RLT2.0), such as, AhR-mediated enzyme immunoassay (EIA) or the reporter gene assay (e.g. chemical-activated luciferase gene expression; CALUX; Sanderson et al., 1996; Behnisch et al., 2001a,b; Koh et al., 2002, 2004; Yoo et al., 2006). Different from numerous other bioanalytical studies, our study is a biomarker assay, which has been focused on the evaluation of toxicological effects of dioxin-like compounds in human normal cell line. The application of a normal cell line is complementary to the use of chemical analysis or bioanalytical techniques, as the former can provide toxicity information; whereas, the latter focus on the detection of dioxin-like compounds.

4. Conclusions

Suitability of cell bioassay as a tool for screening the toxicological risk of dioxin-like compounds was evaluated using an *in vitro* toxicity assay on the samples obtained from small sized Korean waste incineration plants. From the results of the present study, the use of stress-related gene expression assay and cytotoxic/genotoxic test systems would appear to be relevant for preliminary screening of the human health effects of dioxin-like chemicals from waste incinerators. Thus, the application of the cell bioassay for testing waste incineration plant samples can be regarded as an early warning tool for the initiation of more detailed cause-analyses and to guide subsequent chemical identification.

Acknowledgement

This work was accomplished through the generous support of the Korean Ministry of Environment through the Ecotechnopia-21 Project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2007.07.055.

References

Alcock, R.E., Sweetman, A.J., Jones, K.C., 2001. A congener-specific PCDD/F emissions inventory for the UK: do current estimates account for the measured atmospheric burden. Chemosphere 43, 183–194.

Alsharif, N.Z., Lawson, T., Stohs, S.J., 1994. Oxidative stress induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin is mediated by the aryl hydrocarbon (Ah) receptor complex. Toxicology 92, 39–51.

Behnisch, P.A., Hosoe, K., Sakai, S.-I., 2001a. Bioanalytical screening methods for dioxins and dioxin-like compounds – a review of bioassay/biomarker technology. Environ. Int. 27, 413–439.

Behnisch, P.A., Hosoe, K., Sakai, S.-I., 2001b. Combinatorial bio/chemical analysis of dioxin and dioxin-like compounds in waste recycling, feed/food, humans/wildlife and the environment. Environ. Int. 27, 495–519.

Dalton, T.P., Puga, A., Shertzer, H.G., 2002. Induction of cellular oxidative stress by aryl hydrocarbon receptor activation. Chem. Biol. Interact. 141, 77–95.

Dickson, L.C., Buzik, S.C., 1993. Health risks of "dioxins": a review of environmental and toxicological considerations. Vet. Hum. Toxicol. 35, 68–77.

Ekwall, B., 1983. Screening of toxic compounds in mammalian cell cultures. Ann. N.Y. Acad. Sci. 407, 64–77.

Godduhn, A., Duffy, L.K., 2003. Multi-generation health risks of persistent organic pollution in the far north: use of the precautionary approach in the Stockholm Convention. Environ. Sci. Policy 6, 341–353.

Hays, S.M., Aylward, L.L., 2003. Dioxin risk in perspective: past, present, and future. Regul. Toxicol. Pharmacol. 37, 202–217.

Kim, S.C., Lee, K.C., Kim, K.H., Kwon, M.H., Song, G.J., 2006. Enrichment of PCDDs/PCDFs in the cooling system of municipal solid waste incineration plants. Waste Manag.. doi:10.1016/j.wasman.2006.07.007.

Koh, C.H., Khim, J.S., Villeneuve, D.L., Kannan, K., Giesy, J.P., 2002. Analysis of trace organic contaminants in sediment, porewater and water samples from Onsan Bay, Korea: instrumental analysis and in vitro gene expression assay. Environ. Toxicol. Chem. 21, 1796–1803.

Koh, C.-H., Khim, J.S., Kannan, K., Villeneuve, D.L., Senthilkumar, K., Giesy, J.P., 2004. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-TCDD equivalents (TEQs) in sediment from the Hyeongsan River, Korea. Environ. Poll. 132, 489–501.

Mandal, P.K., 2005. Dioxin: a review of its environmental effects and its aryl hydrocarbon receptor biology. J. Comp. Physiol. B, Biochem. Syst. Environ. Physiol. 175, 221–230.

Matsumura, F., 2003. On the significance of the role of cellular stress response reactions in the toxic actions of dioxin. Biochem. Pharmacol. 66, 527–540.

Ministry of Environment, Republic of Korea, 1996. The Korean Standard Testing Method for Dioxins and Furans.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Nebert, D.W., Roe, A.L., Dieter, M.Z., Solis, W.A., Yang, Y., Dalton, T.P., 2000. Role of the aromatic hydrocarbon receptor and (Ah) gene battery in the oxidative stress response, cell cycle control, and apoptosis. Biochem. Pharmacol. 59, 65–85.

Nicoletti, I., Migliorati, G., Pagliacci, M.C., Grignani, F., Riccardi, C., 1991. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J. Immunol. Methods 139, 271–279.

Ohe, T., Watanabe, T., Wakabayashi, K., 2004. Mutagens in surface waters: a review. Mutat. Res. 567, 109–149.

Park, S.-Y., Choi, J., 2007. Cytotoxicity, genotoxicity and ecotoxicity assay using human cell and environmental species for the screening of the risk from pollutant exposure. Environ. Int. 5, 5. doi:10.1016/j.envint.2007.03.014.

Parrott, J.L., Hodson, P.V., Dixon, D.G., 1995. Rainbow trout hepatic mixed-function oxygenase induction by polychlorinated dibenzo-*p*-dioxins (PCDDs) as a function of time and tissue concentration. J. Toxicol. Environ. Health. 46, 301–316.

Puga, A., Barnes, S.J., Chang, C., Zhu, H., Nephew, K.P., Khan, S.A., Shertzer, H.G., 2000. Activation of transcription factors activator protein-1 and nuclear factor-kB by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem. Pharmacol. 59, 997–1005.

Quattrochi, L.C., Vu, T., Tukey, R.H., 1994. The human CYP1A2 gene supports Ah-receptor binding and promoter-specific induction. FASEB J. 8, A1250.

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

Sanderson, J.T., Aarts, J.M., Brouwer, A., Froese, K.L., Denison, M.S., Giesy, J.P., 1996. Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-*O*-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. Toxicol. Appl. Pharmacol. 137, 316–325.

Schirmer, K., Dayeh, V.R., Bopp, S., Russold, S., Bols, N.C., 2004. Applying whole water samples to cell bioassays for detecting dioxin-like compounds at contaminated sites. Toxicology 205, 211–221.

Shen, Y., White, E., 2001. p53-Dependent apoptosis pathways. Adv. Cancer Res. 82, 55–84.

Shonov, R.Y., Haupt, Y., 1999. The cellular response to p53: the decision between life and death. Oncogene, 6145–6157.

Slezak, B.P., Hatch, G.E., DeVito, M.J., Diliberto, J.J., Slade, R., Crissman, K., Hassoun, E., Birnbaum, L.S., 2000. Oxidative stress in female B6C3F1 mice following acute and subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol. Sci. 54, 390–398.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F., 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen. 35, 206–221.

Van der Weiden, M.E.J., Van der Kolk, J., Bleumink, R., Seinen, W., van den Berg, M., 1992. Concurrence of P450 lAl induction and toxic effects after administration of a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rainbow trout (Oncorhynchus mykiss). Aquat. Toxicol. 24, 123–142.

Vogel, C., Abel, J., 1995. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on growth factor expression in the human breast cancer cell line MCF-7. Arch. Toxicol. 69, 259–265.

Whitlock, J.P.J., 1990. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. Annu. Rev. Pharmacol. Toxicol. 30, 251–277.

Wu, W.Z., Schramm, K.W., Xu, Y., Kettrup, A., 2001. Accumulation and partition of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) in the muscle and liver of fish. Chemosphere 43, 633–641.

Xiao, R., Wang, Z., Wang, C., Yu, G., 2006. Soil screening for identifying ecological risk stressors using a battery of in vitro cell bioassays. Chemosphere 64, 71–78.

Xu, L., Li, A.P., Kaminski, D.L., Ruh, M.F., 2000. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin induction of cytochrome P4501A in cultured rat and human hepatocytes. Chem. Biol. Interact. 124, 173–189.

Yoo, H., Khim, J.S., Giesy, J.P., 2006. Receptor-mediated in vitro bioassay for characterization of Ah-R-active compounds and activities in sediment from Korea. Chemosphere 62, 1261–1271.