Rapid Bioassay for the Determination of Dioxins and Dioxin-like PCDFs and PCBs in Meat and Animal Feeds

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Abstract

Over the past several years, the numerous contamination incidents have raised concerns over the presence of halogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and related chemicals in foods and feeds. Here we applied a sensitive recombinant mouse hepatoma cell (H1L1.1c2) bioassay for the determination of dioxins and dioxin-like polychlorinated dibenzofurans (PCDFs) and biphenyls (PCBs) in meat and animal feeds. These cells responded to TCDD-like chemicals with dose-dependent induction of firefly luciferase activity, and the minimal detection limit of TCDD in the cell was 16 fg. Induction equivalency factors determined for pure TCDD-like polychlorinated dibenzo-*p*-dioxins (PCDDs), PCDFs, and PCBs in the bioassay were well-correlated with the World Health Organization's toxic equivalency factors. To determine the applicability of the bioassay system to detect those compounds presence in meat and feed samples, cell bioassays for 17 TCDD-like PCDDs and PCDFs congeners-spiked lipid extracted from beef or animal feed were performed. Mean recoveries of TCDD-like chlorinated PCDDs and PCDFs congeners from spiked beef or feed fat ranged from 61.2 to 122.3%. Within-laboratory coefficients of variation for analysis as index of precision were lower than 5.2%, and the calculated limits of detection and quantitation were 0.33 and 1 pg toxicity equivalency quantity (TEQ)/0.5 g fat, respectively. Correlation between bioassay- and high-resolution gas chromatography–mass spectrometry (HR-GC–MS)-determined TEQs for 10 meat samples was 0.85, with 1.2 times higher in bioassay than HR-GC–MS. The correlation between bioassay- and HR-GC–MS-determined TEQs in 10 animal feed products was 0.81, with 2.1 times higher in bioassay than HR-GC–MS. Overall, these results demonstrated that the recombinant cell bioassay can be used for the rapid detection and quantitation of PCDDs and dioxin-like PCDFs and PCBs in meats and animal feeds

Introduction

Polyhalogenated aromatic hydrocarbons (PHAHs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), induce a wide variety of toxic effects including tumor promotion, immunotoxicity, lethality, birth defects, and alterations in hormone metabolism and responsiveness (1,2). The trials for decreasing human exposure to dioxins are important for human health because 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) has been classified as a human carcinogen by International Agency for Research on Cancer, and dioxins including dioxin-like chemicals are persistent and body-accumulative compounds with high hazardous impact on progeny (3,4). Animal products that contain a large portion of fat are major exposure sources of dioxin to humans; likewise, animal feeds are major dioxin sources to livestock animals (5–7). A representative example is the Belgium dioxin scare caused by dioxin-tainted animal feeds (8).

Most countries and international expert groups on risk assessment have recommended or established tolerable daily intake for humans or regulatory control levels of dioxins for animal products and feeds as low as picogram or part-per-trillion levels (9,10). Monitoring the dioxin levels in foods and animal feeds provides baseline values for identification of harmful contamination and helps develop strategies for decreasing or preventing contamination in the human diet (11). However, dioxin analysis through either conventional or modern analytical technique as high-resolution gas chromatography–mass spectrometry (HR-GC–MS) analysis involves time-consuming, exhaustive sample preparation with a number of hazardous organic chemicals that add to the cost (12). Several bioassay systems, including immunoassay and enzyme assay, have been introduced as alternative ways to screen a large number of samples rapidly and inexpensively; however, their sensitivity and specificity have not been satisfied fully.

Recently, genetically engineered cell lines specifically responding to dioxins have been developed (13–15). The recombinant cell line expressing aryl hydrocarbon receptors (AhR) was made intrinsically by transfection of the host cells with plasmid containing dioxin responsive elements (DREs) inserted into potent promoter and linked to sensitive reporter genes such as luciferase reporter genes (15).

This study was performed to evaluate the applicability of a recombinant mouse hepatoma cell line for rapid and cost-

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effective determination of contaminating levels of dioxins and dioxin-like PCDFs and PCBs in meat and animal feeds.

Experimental

Materials

All chemicals used were of pesticide analysis or high-performance liquid chromatography (HPLC) grade. 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PnCDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,4,7,8-HxCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1,2,3,4,6,7,8-HpCDD), octachlorodibenzo-*p*-dioxin (OCDD), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF), octachlorodibenzofuran (OCDF), and PCBs (PCB 105, 118, 126, 156, and 169) were purchased from ChemService Co. (Westchester, NY). The Cambridge Isotopes Laboratory Method 1613 Precision and Recovery Standards Solution (#EDF-7999, containing 100 ng toxicity equivalency quantity (TEQ)/100 µL nonane, CIL Co., Andover, MA), which contains 17 different 2,3,7,8-substituted PCDD/F congeners, was evaporated under nitrogen and dissolved in 1 mL of dimethyl sulfoxide (DMSO) to generate the diluted working PCDD/F stock solution (100 ng TEQ/mL DMSO).

Recombinant cell line

H1L1.1c2 cells, recombinant mouse hepatoma cells, were used for dioxins bioassay. Mouse hepatoma cells (Hepa1c1c7) were transfected with plasmids (pGudLuc1.1) containing luciferase reporter gene under control of four dioxin responsive elements, and the stably transfected cells induced luciferase by response to TCDD and related AhR agonists (15).

Preparation of "clean" and spiked fat samples for calibration curve of bioassay

To prepare a series of concentrations of standard meat and feed samples for determination of the dioxin level, "clean" fat samples were used. Fat was extracted from 100 g of ground beef that contained a large portion of fat or from 400 g of fattening pig feeds (National Livestock Cooperative Federation, Korea) by mixing and thorough shaking with 5 volumes of *n*-pentane for 30 min in separatory funnels. The *n*-pentane fraction was collected by filtering through glass-wool covered with anhydrous sodium sulfate (anhydrated in a dry oven at 150°C for 16 h prior to use), and the resulting samples were evaporated using a Savant automatic speed vacuum evaporator. The extracted fat was dissolved in an equal volume (w/v) of *n*-hexane, mixed with activated carbon (1:20, w/w of fat) and swirled at 180 rpm for 30 min in water bath at 45°C. The activated carbon was removed by filtering the mixture twice through ashless filters (No. 41, Whatman). The filtrate was then passed twice through a glass-wool plugged funnel containing anhydrous sodium sulfate. The filtered *n*-hexane fat sample was evaporated using a Savant automatic speed vacuum evaporator followed by a slow stream of nitrogen, and the final dried material was collected as "clean fat." Several different concentrations of standards spiked to clean fat were prepared. Firstly, a mixture of 17 different 2,3,7,8-substituted PCDD/F congeners was diluted into an aliquot of clean fat that had been dissolved in 4 volumes of *n*-hexane/diethylether (97:3, v/v) to a final concentration of 100 pg TEQs/g fat (dioxin stock fat). A series of standard fat samples containing different dioxin TEQ levels (0, 0.2, 0.4, 2, 5, and 10 pg TEQ/0.5 g fat) were prepared in 4-fold volume (w/v) of *n*-hexane/diethylether (97:3, v/v) by diluting aliquots of the dioxin stock fat with clean fat.

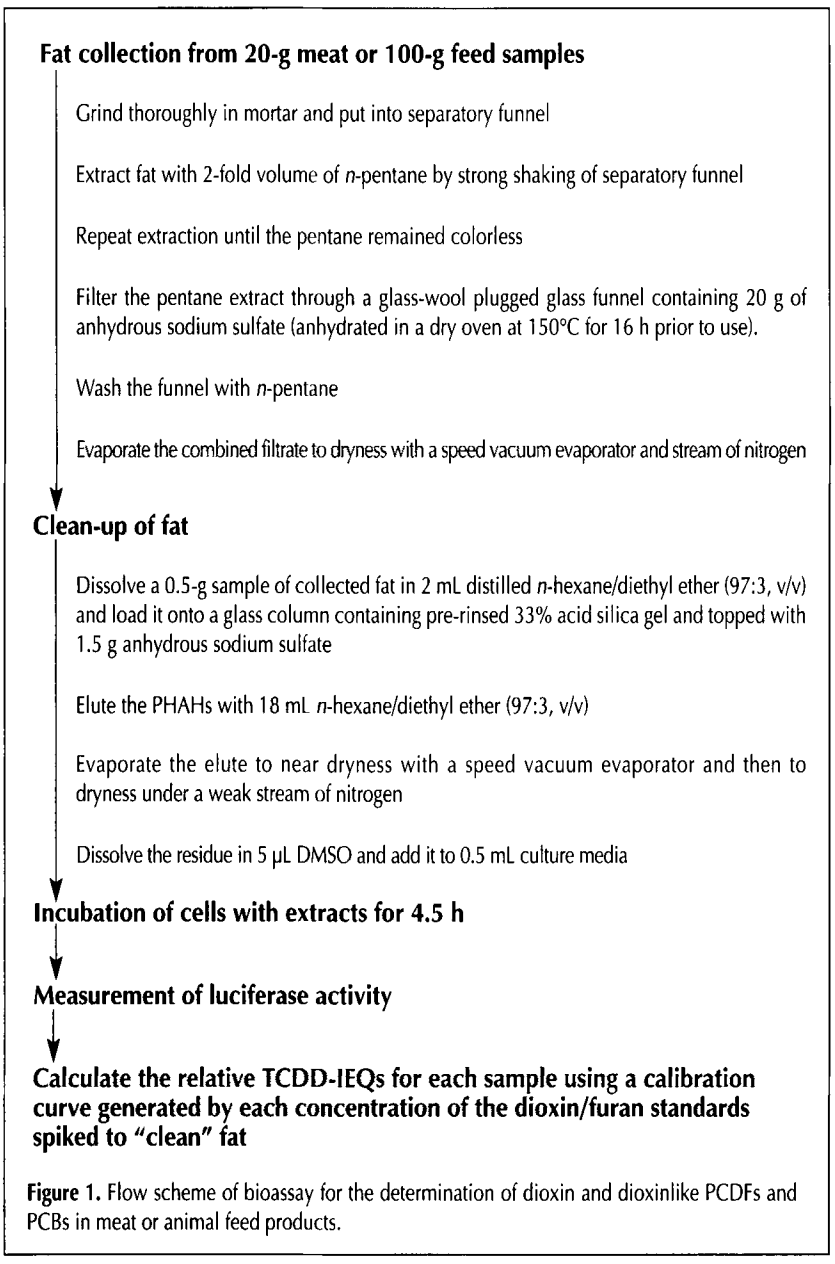

Figure 1. Flow scheme of bioassay for the determination of dioxin and dioxinlike PCDFs and PCBs in meat or animal feed products.

Sample preparation for bioassay of dioxins in beef

The generalized scheme for preparation of meat samples for luciferase cell bioassay is outlined in Figure 1. Each fat sample was collected from 10 beef (brisket constituted with lean and fat) obtained from several meat retail stores located in Anyang City, Korea. Among them, 5 samples were beef imported from the U.S., and another 5 samples were beef produced in Korea. Each beef fat sample was trimmed out from 20 g of briske, ground thoroughly with a mortar and pestle, and then divided into two parts for bioassay and HR-GC–MS analysis. The ground beef fat was put into a separatory funnel and then extracted into a 2-fold volume of *n*-pentane by thorough and strong shaking of the funnels. The extraction step was repeated until the pentane remained colorless. The fat extracted in *n*-pentane was passed through a glass-wool plugged glass funnel containing anhydrous sodium sulfate. The glass funnel was washed with *n*-pentane, and the combined filtrates were evaporated to dryness with a Savant speed vacuum evaporator and subsequently under a mild stream of nitrogen. The weight of fat extract per sample was measured. The fat contents were 16.8 ± 3.1% for beef samples after *n*-pentane extraction. A 0.5-g aliquot of fat sample was dissolved in 2 mL of distilled *n*-hexane/diethylether (97:3, v/v) and loaded onto a 33% acid silica gel column prepared as described. Silica gel (0.063–0.2 mm for column chromatography, Merck, Inc., Rahway, NJ) was activated at 140°C for 16 h in a dry oven and then cooled in desiccators prior to use. Preparation of the 33% acid silica gel involved combining with ratio of 6.7 of activated silica gel and 3.3 of H_2SO_4 in a glass bowl and fully mixing the materials with a glazed porcelain spatula until the H_2SO_4 blebs were fully mashed. To prepare 33% acid silica gel columns, glass syringes (15 mm × 90-mm length) containing a glass filter (AP1504700, Millipore Co., Bedford, MA) at the bottom were packed with 8 g of 33% acid silica gel and layered with 1.5 g of anhydrous sodium sulfate. The resulting columns were rinsed with 12 mL of *n*-hexane followed by 12 mL of *n*-hexane/diethyl ether (97:3, v/v) and used for sample clean-up. After rinsing, a 0.5-g aliquot of fat sample dissolved in 2 mL of distilled *n*-hexane/diethylether (97:3, v/v) was loaded on column, and PCDDs and dioxin-like PCDFs and PCBs were eluted with 18 mL of *n*-hexane/diethylether (97:3, v/v). The eluate was collected in a 50-mL glass centrifuge tube from the moment of sample loading. Fat trapped by acid silicagel turned the silicagel from white to orange in color, and PCDDs and dioxin-like PCDFs and PCBs were eluted with *n*-hexane/diethylether (97:3, v/v). The collected solution was evaporated to dryness using a Savant speed vacuum evaporator and then under a weak stream of nitrogen. The dried residue was resuspended in 5 µL DMSO, mixed with 0.5 mL tissue culture medium, and then added to H1L1.1c2 cells followed by incubation for 4.5 h at 37°C.

Sample preparation for bioassay of dioxins in animal feeds

The whole flow scheme for preparation of feed samples for the cell bioassay was almost the same as that described for beef samples and is outlined in Figure 1. A feed fat sample was collected from each of the 10 100-g animal feed samples (5 feeds for fattening pigs and 5 feeds for laying hens) obtained from the feed plants of the National Livestock Cooperative Federation located in Kunpo City, Korea. Fat from each feed product after *n*-pentane extraction was divided into two parts for bioassay and HR-GC–MS analysis. The fat contents were 7.4 ± 1.1% for pig-fattening feeds and 3.7 ± 1.5% for laying-hen feeds after *n*-pentane extraction.

Recombinant cell culture condition and bioassay

Recombinant H1L1.1c2 cells were grown in alpha minimal essential medium (Gibco™/InVitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum, 50 IU/mL penicillin, and 50 µg/mL streptomycin (16). Cells (2×10^5) in the described culture media were plated into 24-well cell culture plates and grown for 24 h in a humidified atmosphere (5% CO_2/95% air) at 37°C. Cells were washed twice with warm phosphate-buffered saline (PBS) [0.01M sodium phosphate, pH 7.2, with 0.85% (w/v) NaCl], followed by the addition of 0.5 mL of growth media containing 5 µL of DMSO or the test compounds or sample extracts in 5 µL DMSO and further incubation for 4.5 h at 37°C. After incubation, culture plates were loaded on ice to stop the reaction, and the cells were washed twice with cold PBS and lysed with 100 µL of lysis reagent in luciferase kit (Promega, Madison, WI) by rotating the culture plate on ice for 15 min. Cell lysates were transferred into 0.5 mL microcentrifuge tubes and centrifuged for 3 min at 13,000 × *g* in a refrigerated (4°C) centrifuge. An aliquot (25 µL) of each lysate sample was added into a 96-well plate, and luciferase activity was measured in an EG&G LB96V microplate luminometer (Berthold GmbH & Co., Germany) after an automatic injection of 50 µL of Promega stabilized luciferase reagent. Protein content in each well after luciferase measurement was also analyzed by adding 100 µL fluorescamine solutions (500 µg/mL acetone) and reading fluorescence intensity at excitation and emission wavelengths of 380 and 460 nm, respectively, with the fluorometer. In cases where the protein content was significantly different from vehicle control (5 µL DMSO), the luciferase activity data were rejected for cytotoxicity of the sample or rough handling of the cells.

HR-GC–MS analysis

Dioxins in meat and animal feeds were determined by HR-GC–MS according to U.S. EPA Method 1613 (17). Each fat sample trimmed out from 10 beef samples and ground with a mortar and pestle was subjected to soxhlet extraction for 18–24 h, and the fat concentration was determined gravimetrically. The fat contents were 17.2 ± 3.3% for beef samples after soxhlet extraction. Fat from animal feed product was extracted by *n*-pentane and then used for analysis. The resulting fat samples were spiked with $^{13}C_{12}$-TCDD and $^{37}Cl_4$-2,3,7,8-TCDD standards (internal standards to allow determination of sample recovery) and then mixed thoroughly. The fat was removed on a gel-permeation column, and the fraction containing dioxins was further purified through Al_2O_3 and then a graphitized carbon column as described in Method 1613. The content of the 2,3,7,8-substituted PCDD/Fs was determined using an HR-GC–MS (AutoSpec-Ultima NT with HP6890, Micromass Co., Manchester, U.K.) with two selected ions in recording mode (17).

Induction equivalency factors (IEF) or TEQ calculations

For the determination of TCDD IEFs for pure PCDDs, PCDFs, and PCBs in the bioassay, dose-response curves for each chemical were generated. EC_{50} values for each chemical (i.e., the concentration of each chemical producing half of the maximal luciferase activity induced by 2,3,7,8-TCDD) were obtained by fitting the dose-response curve using a sigmoidal fitting program (Origin, version 6.0). The bioassay-IEF for individual compounds was calculated by dividing the EC_{50} value of 2,3,7,8-TCDD with the EC_{50} value obtained for the compound of interest. TEQs for sample extracts measured by bioassay were calculated with linear regression equation of the calibration curve obtained using each concentration of the mixture of 2,3,7,8-substituted PCDD/Fs spiked to clean fat. TEQs for sample extracts measured by HR-GC–MS analyses were calculated using World Health Organization (WHO) TEF values (9).

Results

TCDD-inducible luciferase activity in recombinant cells

The dose dependence of luciferase induction in H1L1.1c2 cells was determined by incubation of the cells with increasing concentrations of TCDD for 4.5 h. Induction of luciferase was dose-dependent, with a maximal induction by 500pM TCDD (11-fold of control), EC_{50} as 10.29pM, and a minimal detection limit as 0.1pM, which is equal to 16 fg (Figure 2).

PCDD-, PCDF-, and PCB-inducible luciferase activity in recombinant cells

The dose-responses for 2,3,7,8-congeners of PCDD/F and some coplanar PCBs were also observed (Figure 3). The shape of the curves for the compounds was comparable to that of TCDD when shifted to a higher dose range. The maximum responses induced by the chemicals were also similar except for PCB 126 (22% higher response than TCDD) and PCBs 105 and 118 (31–33% lower response than TCDD). The EC_{50} values

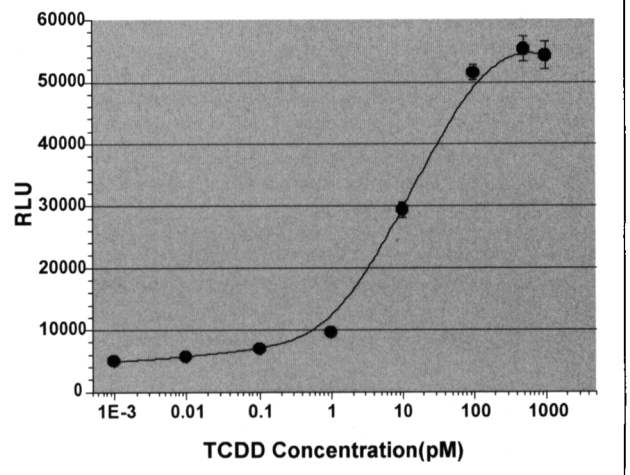

Figure 2. Dose-response curve for the induction of luciferase activity by 2,3,7,8-TCDD in recombinant mouse hepatoma cell line (H1L1.1c2). Values are mean ± SE (n = 6).

Table I. Luciferase Induction Equivalency Factors (Bioassay-IEFs) in H1L1.1c2 Cells for Selected PHAHs

Compound	EC50 (pM)*	Bioassay-IEF	WHO-TEF('98)
2,3,7,8-TCDD	10.29	1	1
1,2,3,7,8-PnCDD	14.80	0.70	1
1,2,3,4,7,8-HxCDD	139.2	0.074	0.1
1,2,3,4,6,7,8-HpCDD	1185	0.0087	0.01
2,3,7,8-TCDF	98,601	0.0001	0.001
OCDD	123.8	0.083	0.1
OCDF	113,921	0.00009	0.0001
PCB105	110,972	0.000093	0.0001
PCB118	64,245	0.00016	0.0001
PCB126	120.8	0.085	0.1
PCB156	18,423	0.00056	0.0005
PCB169	1267	0.0081	0.01

* EC_{50} values obtained by sigmoidal fitting dose-responsive curves of bioassay for a number of different dioxins, furans and coplanar PCBs using the Origin curve-fitting program.

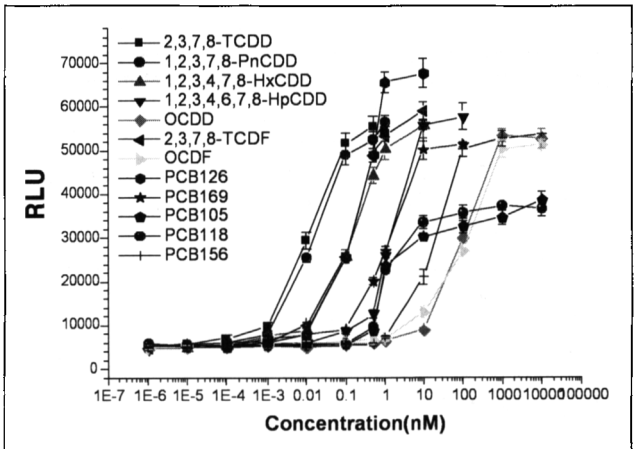

Figure 3. Dose-response curves obtained in the bioassay with a number of different dioxins, furans, and coplanar PCBs. Data points are mean ± SE of nine replicates by three independent measurements.

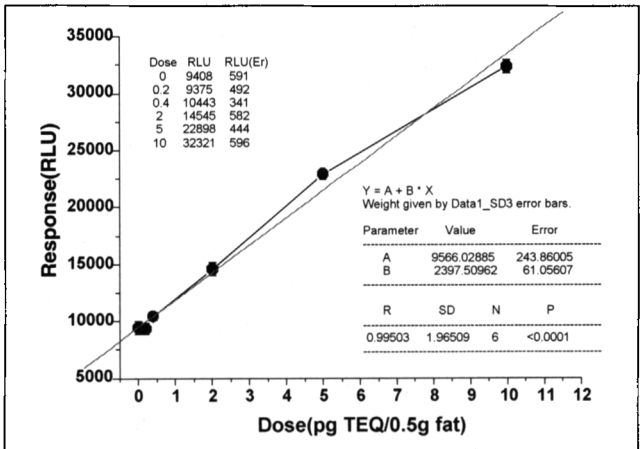

Figure 4. Calibration curve for each concentration of 17 mixed-congeners of dioxins/furans spiked to clean fat. Data are mean ± SE from eight independent experiments with triplicates of each dosed sample (n = 24).

of dioxins, PCDFs, and PCBs were determined by the sigmoidal fitting method (Origin program 6.0) and fixing the value of maximal response as that induced by 500pM TCDD. There was a good correlation between the 1998 WHO-TEF and the IEFs calculated from cell bioassay system (Table I).

Recoveries and limits of detection (LOD) and quantitation (LOQ) of dioxins in bioassay

Figure 4 shows the calibration curves for dioxins obtained from eight independent within-laboratory experiments over a period of two months. The luciferase activities induced by 0, 0.2, 0.4, 2, 5, and 10 pg TEQ of 17 different 2,3,7,8-substituted PCDDs and PCDFs standards spiked to 0.5 g of clean fat extracted from beef or porcine feed products and then processed with the same treatment for samples showed the same luciferase activities as those of standards spiked to elution solution without clean fat (data not shown). The within-laboratory coefficients of variation (CVs) ranged from 1.8 to 5.2%, and mean recoveries of the dioxins spiked to clean fat ranged from 61.2 to 122.3% (Table II). The LOD and LOQ calculated from eight different experiments with triplicates for each sample were 0.33 pg TEQ/0.5 g fat and 1.00 pg TEQ/0.5 g fat, respectively.

Correlation between bioassay- and GC–MS-determined TEQs in beef and animal feeds

Ten imported or domestic beef samples were analyzed using bioassay and HR-GC–MS. Contents of the 17 mixed 2,3,7,8-substituted PCDD and PCDF congeners were determined by HR-GC–MS analysis, and then TEQ concentrations were calculated using the 1998 WHO-TEFs. Correlation between bioassay- and GC–MS-determined dioxin levels was 0.85. The HR-GC–MS analysis revealed no samples that exceeded the tolerance level of 5 pg TEQ/g fat. Bioassay-determined dioxin levels were approximately 1.2 times higher than GC–MS-determined level with y-intercept value 0.64 (Figure 5). As for the animal feeds, the correlation between bioassay- and GC–MS-determined dioxin levels was 0.81. Neither the GC–MS analysis nor the bioassay revealed any samples that exceeded the Belgian tolerance level 0.75 ng TEQ/kg feed product. Bioassay-determined dioxin levels of animal feed products were approximately 2.1 times higher than the GC–MS-determined levels with y-intercept value 1.85 (Figure 6).

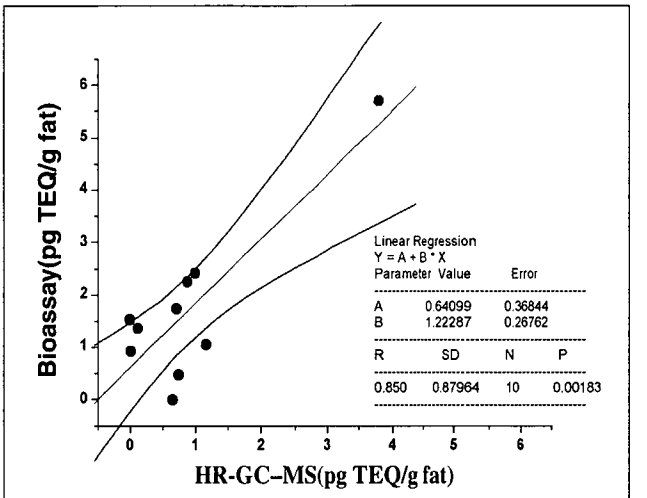

Figure 5. A comparison of the dioxin-TEQs determined by bioassay with those by HR-GC–MS for 10 meat samples. Linear regression curve and 95% confidence ranges are expressed with 10 data points.

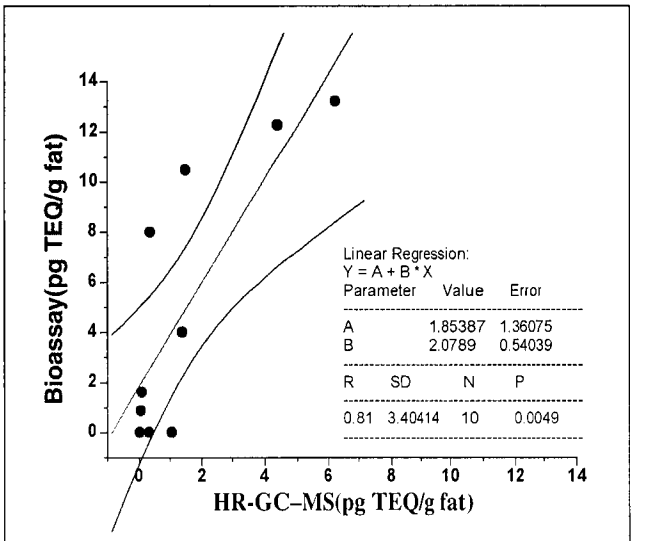

Figure 6. A comparison of the dioxin TEQs determined by bioassay with those by determined by HR-GC–MS for 10 animal feed samples. Linear regression curve and 95% confidence ranges are expressed with 10 data points.

Table II. Within-Laboratory Variation, Recovery, and Limits of Detection and Quantitation

TCDD Content* (pg TEQ/0.5 g fat)	CV (%)	Recovery (%)	LOD† (pg TEQ/0.5 g fat)	LOQ‡ (pg TEQ/0.5 g fat)
0.2	5.2	122.3 ± 6.1	0.33	1.00
0.4	3.3	72.1 ± 2.4		
2	4.0	61.2 ± 1.9		
5	1.9	67.6 ± 1.4		
10	1.8	66.4 ± 1.2		

* Results from eight separate analyses of triplicates for each sample.
† Limit of detection (LOD). The LOD = 3.3σ/S, where σ is the standard deviation of the y-intercept and S is the slope of the regression line of the calibration curve.
‡ Limit of quantitation (LOQ). The LOQ = 3 × LOD.

Discussion

In this study, we can see that the recombinant mouse hepatoma cell line (H1L1.1c2) is useful for bioassay systems with high sensitivity and throughput. These results are very comparable to those reported by Garrison et al. (16), and the sensitivity was greater than that observed in another recombinant rat hepatoma cell line, H4IIE cells, that contains the similar DRE-luciferase reporter plasmid (13). The differences in responsiveness of the cell

lines are mainly caused by species differences in the reactivity of AhRs, its chaperone proteins (heat shock proteins), and/or transacting factors in each cell types (16). Human AhR is less reactive than that of the rat, and guinea pigs and mice have well-operated AhR, which may be the reason for the high vulnerability of these species to dioxin compared with other species (18).

The concept of TEFs was introduced for the assessment of health risks of closely related chemicals that have identical action mechanisms but different toxic potencies. PCDDs, PCDFs, and coplanar PCBs are ubiquitous environmental toxicants and contaminated with various patterns and toxicological potencies (19). IEFs of PCDDs, PCDFs, and coplanar PCBs obtained from their IE_{50}s compared to that of TCDD in our study showed good correlation with TEFs established by the WHO in 1998 (9). It means that toxicity induced by dioxins and dioxin-like PCDFs and PCBs is mediated by AhR and DREs. That is, the induction of luciferase activity in recombinant mouse hepatoma cells exposed to dioxins and dioxin-like chemicals is mediated by the binding of the ligands with AhR that intrinsically exists in cytoplasm and then the translocating of the ligand-receptor complex to the specific binding sites on DNA, DREs, which stimulates transcription of adjacent genes (luciferase reporter gene) (15). The quantities of dioxins and dioxin-like contaminants in the tested samples can be estimated directly from the luciferase activities induced in the recombinant cell lines.

HR-GC–MS analysis is needed to understand the precise concentration of known isomers and congeners contaminating food or feed and to trace back the sources with the patterns, even though it gives little information about biological impact of samples and is time-consuming and requires expensive solvents. In this study, it took approximately 2 days for bioassay from fat collection to luciferase measurement for about 20 samples. It is a high-throughput screening system compared to HR-GC–MS analysis, which usually took more than 7 days for 20 samples. In addition, we have an idea of the actual toxicological potencies of the sample, so the bioassay may be used for screening toxic samples rapidly.

CVs and recovery values of the bioassay satisfied the requirements for the validation of analytical methods in the criteria of precision and accuracy, which should be lower than 35% in CV and within –50 to +20% in recoveries when the concentration is lower than 1 ppb according to the recommendations of CODEX, EU EMEA, and U.S. FDA (20–22). The LOD and LOQ calculated from eight different within-laboratory experiments performed in triplicate (n = 24) according to the VICH guidelines on validation of analytical procedures (20) covered the tolerances or guidelines established in several countries for dioxins in meats and animal feeds, which ranged from 1 to 5 pg TEQ/g food fat and from 0.75 to 2 ng TEQ/kg feed product (9,10,22).

In this study, the TEQ values of dioxins obtained by bioassay for beefs and animal feed products were 1.2 and 2.1 times higher than those by HR-GC–MS with correlations of 0.85 and 0.81, respectively. Coplanar PCBs, of which PCB126 or PCB169 are 1/10 part or 1/100 part of 2,3,7,8-TCDD in TEFs, were not included in our HR-GC–MS analysis. In addition, this bioassay cell line may theoretically respond to any compounds capable of binding and activating AhR and DREs. Furthermore, compounds involved in any dioxin signal transduction pathways may interfere the responsiveness of the assay. Glucocorticoid hormones, benzo(a)pyrene, benzimidazole drugs, α-naphthoflavones, and brominated compounds may be referred to as interfering compounds for their properties of inducing cytochrome P450 1A enzyme groups (13,14). Considering those points, it can be explained why the bioassay showed higher values than instrument analysis. Further studies on whether those compounds are removed or destroyed fully during the sample preparation need to be performed. It is clear that the luciferase activities induced in the recombinant cells by the exposure to the sample extracts reflects the toxicological potencies of samples. As for efficient strategies for reduction and prevention of dioxin contamination in food and feeds to secure human health, it is recommended that sensitive and rapid bioassay screening be preliminarily performed on large numbers of samples, followed by GC–MS analysis for confirmation of the positive samples and for elucidation of dioxin patterns.

In conclusion, the bioassay system using recombinant mouse hepatoma cells that contain luciferase gene under TCDD-inducible control of dioxin-responsive elements is a valuable tool, allowing the screening of relatively large number of meat or feed samples with low cost and determining sensitively the toxic level of dioxins and dioxin-like PCDFs and PCBs contaminated in food and animal feeds.

Acknowledgments

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