

## Assessment of Dioxin-Like Soil Contamination in Mexico by Enzyme-Linked Immunosorbent Assay

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**Abstract** In this work, we describe the results of a preliminary soil assessment program for the detection of dioxins at different sites in Mexico performed by immunoassay. We studied five different sectors considered relevant sources of dioxins: Anaversa and Tekchem industrial areas where organochlorine pesticides were manufactured and released by accidental explosions, secondary smelters, brick kilns, and rural dwellings. In the context of the Agency for Toxic Substances and Disease Registry (ATSDR) guidelines, only the brick kilns sites can be considered as low-risk areas. The dioxin concentrations detected in the vicinity of the Anaversa and Tekchem chemical plants and secondary smelters exceed the screening level of 0.05 ppb set by the ATSDR, and therefore further site-specific studies are needed. The dioxin levels found in all soot samples from indigenous dwellings where wood is used for indoor cooking were above the evaluation level. Considering that the studied areas are representative examples of dioxin sources in less developed countries, our work demonstrates the useful

application of dioxin immunoassays as a tool for dioxin screening for environmental assessment programs in developing countries.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) have never been produced intentionally, but are unwanted toxic byproducts of many industrial and combustion chemical processes (ATSDR 2002). Almost all possible congeners are released from these sources. Due to their chemical, physical, and biological stability and long-range transport, PCDDs are ubiquitous environmental contaminants found in soil (Lorber et al. 1998; Nadal et al. 2002), sediment (Hilscherova et al. 2003), air (Quass et al. 2004), and food (fish, meat, and cow's milk) (Huwe 2002; Tsutsumi et al. 2008). In 1999, the United Nations Environmental Program published dioxin national inventories for 15 countries mostly from Europe and North America (UNEP 1999). In this report the PCDDs sources were categorized into nine major sectors: (1) waste incineration; (2) iron and steel industry; (3) nonferrous metals (primary and secondary plants for the generation of copper, aluminum, zinc or lead); (4) power plants fuelled with coal, gas, crude oil or wood; (5) industrial combustion plants; (6) small combustion units (i.e., domestic stoves); (7) road transport; (8) mineral products production (generation of cement, lime, glass, brick, etc.); and (9) others (wood chips, accidental fires, etc.). The percentage share of the above sectors varies among different countries, with waste incineration being the major source in many countries. However it should be noted that dioxin emissions from regulated sources are declining in developed countries, although surprisingly backyard trash burning is an increasingly important source (EPA 1998).

Although dioxin emission sources in Western Europe (Quass et al. 2004) and Northern America (EPA 2006) are

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well described, data on dioxin contamination in developing countries is still limited. A study recently performed in Thailand demonstrated that dioxin release sources are similar to those found in industrialized countries about 10–15 years ago (Fiedler 2001). On the other hand, unlike in most industrialized countries, municipal waste incineration is not the highest contributor to dioxins released into the atmosphere of some developing countries. Thus, secondary copper smelting, cement kilns, and electric arc furnaces for steel production are responsible for the most significant portion of dioxin into the environment of Taiwan (Chen 2004). In Uruguay, biomass incineration (sugar cane fields and rice skin) have been considered the major source of PCDDs (Fiedler et al. 2002). In Mexico, domestic trash burning and landfill fires are considered among the most important sources of dioxin emissions (Costner 2006).

Dioxin emission inventories reported by the majority of countries are based on a mathematical model, applying emission factors for dioxins and furans provided by the United Nations Environment Programme; further PCDDs sources that have not yet been identified or quantified may also exist (PNUMA 2005). Some of these, which have not been considered in the primary inventory, might have significant local impact (di Domenico et al. 1998; Senger 1991; Mocarelli 2001).

The presence of chlorinated organic compounds in accidental industrial fires has been reported as a source of dioxin release. The Seveso and Monsanto disasters are two examples of industrial explosions involving 2,4,5-trichlorophenol, which released dioxin into the environment (di Domenico et al. 1998; Senger 1991; Mocarelli 2001). Accidental industrial fires have not been evaluated in Mexico as potential sources of dioxin emissions. In 1991 in Córdoba, Veracruz, a fire broke out due to a short circuit in equipment used to mix pesticides in the Anaversa chemical plant, likely creating conditions that favored dioxin formation. The enterprise declared that, when the fire broke out, the plant contained 2,4-D and pentachlorophenol, both chemicals involved with dioxin released (EPA 2000).

Some pesticide production processes such as the manufacture of chlorophenols, chlorobenzenes, and chlorobiphenyls can form PCDDs and polychlorinated dibenzofurans (PCDFs) (EPA 2000). The Tekchem chemical plant located in Salamanca, Guanajuato, produced 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) from 1958. The highest DDT production, close to 80,000 tons per year, was reached around 1968, but production was later controlled to 25,000 tons per year. Use and production of DDT were discontinued in 1998 (Caballero 2001). This site is considered in this work for potential dioxin release, although it was not considered in the Mexican inventory. It is of interest for our study due to the potential local

implications, as communities surrounding this industry are of low socioeconomic level with soil-floor housing.

Assessment of environmental dioxin levels in the environment of developing countries is needed. However, analysis of these compounds by the traditional method using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC-HRMS) requires complicated sample cleanup, specialized equipment, and a highly trained analyst. This analytical technique is very expensive and time consuming. Worldwide there are few qualified laboratories that can perform these analyses. In Mexico, the cost and time required for analysis often severely limit the sampling effort. Alternative rapid and inexpensive bioanalytical methods have been evaluated either to replace the reference chromatographic method or at least to alleviate analysis costs by their use in preliminary screening (Harrison and Eduljee 1999; Behnisch et al. 2001; Denison et al. 2001; Roy et al. 2002; Focant et al. 2001). Several attempts have been made to develop sensitive and selective immunochemical techniques for dioxin analysis using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as an analyte, because of its recognized higher potential toxicity (Stanker et al. 1987; Vanderlaan et al. 1988; Langley et al. 1992; Harrison and Carlson 1997). In order to avoid using the toxic congener on a routine basis, highly sensitive polyclonal-antibody-based enzyme-linked immunosorbent assays (ELISAs) using the like less toxic to handle 2,3,7-trichloro-8-methyldibenzo-*p*-dioxin (TMDD) as an analytical surrogate standard have been developed (Sanborn et al. 1998; Sugawara et al. 1998). Furthermore, it has been demonstrated that immunochemical recognition of congeners can be used as an estimate of the toxic equivalents (TEQs) for a variety of real samples, such as biota (Shan et al. 2001), human milk (Sugawara et al. 2002), industrial waste, uncontrolled combustion (Hirobe et al. 2006; Trindade et al. 2008), human serum, and soil and sediments (Nichkova et al. 2004; Van Emon et al. 2008). A soil and sediment extraction and cleanup method using a multilayered silica gel column has been developed and validated with the aim of achieving simple, cost-effective, and efficient removal of the matrix interferences from the ELISA method (Nichkova et al. 2004).

The objective of this work is to perform a preliminary assessment of dioxin soil and soot contamination at several sites in Mexico using the enzyme-linked immunosorbent assay as a cost-effective method for dioxin screening. Soils from four different areas, representing examples of some of the major suspected sources of dioxins in Mexico, such as the Anaversa and Tekchem chemical plants, brick kilns, secondary smelters and soot from rural dwellings, were the subjects of this study. We discuss the results in terms of the utility of immunoassays for environmental assessment programs in developing nations.

## Materials and Methods

### Chemicals and Instrumentation

The preparation of the surrogate standard, 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (TMDD), the coating antigen (III-BSA), and the antibody (7598) used in the immunoassay were previously reported (Sanborn et al. 1998; Shan et al. 2001; Sugawara et al. 2002). Tween 20, 3,3',5,5'-tetramethylbenzidine, goat anti-rabbit immunoglobulin G (IgG) conjugated to horseradish peroxidase (HRP), and bovine serum albumin (BSA) were purchased from Sigma Chemical Company (St. Louis, MO). PBS (pH 7.5) is a phosphate-buffered saline solution ( $8 \text{ g l}^{-1} \text{ NaCl}$ ,  $1.15 \text{ g l}^{-1} \text{ Na}_2\text{HPO}_4$ ,  $0.16 \text{ g l}^{-1} \text{ KH}_2\text{PO}_4$ ,  $0.2 \text{ g l}^{-1} \text{ KCl}$ ). PBST is PBS with 0.05% Tween 20 (v/v). Coating buffer is 100 mM carbonate–bicarbonate buffer (pH 9.6). Citrate buffer is a 100 mM citrate–acetate buffer (pH 5.5). Solvents (hexane and toluene) used in the cleanup procedures were of HPLC grade. All other reagents were prepared from reagent-grade chemicals obtained from Fisher Scientific (Pittsburgh, PA). Microtiter plates used for preparing standard and sample dilutions were from Dynex Technologies (Chantilly, VA). ELISA experiments were performed in high-binding 96-well microtiter plates (Nunc, Roskilde, Denmark). Absorbances were read with a Biorad Benchmark microplate reader (BIORAD, USA) in dual-wavelength mode (450–650 nm).

### Soil Samples

Samples were collected in 2003. Thirty-seven soil samples from different relevant sites were included in this study: the Anaversa chemical plant site (Córdoba, Veracruz;  $n = 6$ ),

brick kiln sites (Guadalupe, Zacatecas;  $n = 14$ ), secondary smelter sites (Aguascaliente;  $n = 5$ ), and the Tekchem organochlorine pesticide manufacturing plant (Salamanca, Guanajuato;  $n = 12$ ; Fig. 1). Surface and subsurface soil samples were collected at 1–3 and 20–30 cm depth, respectively, with thoroughly cleaned, stainless-steel scoop. Rocks, vegetation, and debris were removed. Specifics about each sampling point are indicated in the corresponding tables presented in the “Results and Discussion” section. Three indoor soot samples were collected from the ceilings of indigenous dwellings (Lacanja, Chiapas) where wood was used as a fuel for cooking. All samples were transported to the laboratory in glass containers. In order to obtain efficient and reproducible extraction of the dioxins, the samples were dried in a drying oven at 70°C for 24 h and were then ground in a mortar using a pestle covered with aluminum foil and subsequently sieved through a (600- $\mu\text{m}$  mesh) sieve and kept under refrigeration (4°C) until analysis. The analysis was made within the first 2 months after collection. Control soil samples were polycyclic aromatic hydrocarbon (PAH)-contaminated standard reference (Material Natural Matrix Certified Reference Material, CRM 105-100, Resource Technology Corporation, Laramie, WY) and industrial sandy soil (PCDDs, PCDFs) certified reference material BCR 529 (EUR 18863 EN) (total WHO TEQ 6.65  $\mu\text{g/g}$ ) obtained from LGC Standards.

### Sample Preparation

Ten grams of soil (soot) sample were placed in a 125-ml glass flask with 1 g white quartz sand, then 30 ml hexane was added into each flask and shaken for 3 h. After separation of solvent, another 25 ml solvent was added and extracted for 1 h. The extracts were combined and cleaned

**Fig. 1** Distribution of soil samples taken at the Tekchem organochlorine pesticide chemical plant. Arabic numbers indicate the location of the sampling points in the proximity of the plant



by passing through Whatman filter paper (pore size 125 mm) and evaporated to 1 ml. Then the extracts were cleaned up using a three-layer H<sub>2</sub>SO<sub>4</sub>/silica-gel column (Sugawara et al. 2002) and 40 ml hexane as eluent. Silica gel (70–250 mesh, Sigma) was activated overnight at 170°C. Forty-four percent sulfuric acid–silica was prepared by mixing 2.4 ml concentrated sulfuric acid with 10 g activated silica. The multilayered silica gel column was packed in a 20-ml polypropylene filtration column (Supelco) loaded with the following material: 0.5 g activated silica, 2 g 44% H<sub>2</sub>SO<sub>4</sub>–silica, 1 g activated silica, and 1 g anhydrous sodium sulfate (Nichkova et al. 2004; Trindade et al. 2008). The eluate was evaporated under nitrogen stream, 10 ml before total evaporation 500 µl DMSO-Triton X (0.01% Triton X-100 in dimethyl sulfoxide) was added. Afterwards, the hexane was evaporated and another 500 µl DMSO-Triton X was added.

## ELISA

The ELISA procedure was performed as previously reported (Shan et al. 2001). Microplates were coated overnight at 4°C with 100 µl/well of the coating antigen at 1:14,000 dilution in 0.05 M carbonate–bicarbonate buffer. After the coated plates were washed five times with PBST, 200 µl blocking solution (0.05% BSA in PBS) was added and incubated for 30 min at room temperature. After another washing step, 50 µl/well antiserum 7598 at 1:3,000 dilution in PBS with 0.2% BSA and 50 µl/well of inhibitor (TMDD or sample) solution (prepared in DMSO containing 0.01% Triton X-100:PBS, 1:1, v/v) were added. The plate was incubated for 90 min at room temperature and then washed five times. Goat-anti-rabbit IgG-HRP (100 µl/well) diluted 1:3000 in PBST (PBS-Tween 20, 0.05% pH 7.5) was added and incubated for 60 min at room temperature. Following another washing step, tetramethylbenzidine (TMB) substrate solution (100 µl/well; 3.3 µl 30% H<sub>2</sub>O<sub>2</sub>, 400 µl 0.6% TMB in DMSO per 25 ml acetate buffer, pH 5.5) was added. Color development was stopped after 10 min with 2 M H<sub>2</sub>SO<sub>4</sub> (50 µl/well), and absorbance was measured at 450–650 nm to obtain the difference (450–650). All experiments were conducted in triplicate. Standard curves with serial dilutions of TMDD were obtained by plotting absorbance against the logarithm of analyte concentration (Sugawara et al. 1998). These were fitted to a four-parameter logistic equation:  $y = (A - D) / [1 - (x/C)^B] - D$ , where  $A$  is the maximum absorbance with no analyte,  $B$  is the curve slope at the inflection point,  $C$  is the concentration of analyte giving 50% inhibition, and  $D$  is the minimum absorbance at infinite concentration. For the analysis of dioxin in soil samples, DMSO extracts were diluted with 50% DMSO-Triton X-100: PBS (1:50, 1:100 and 1:200) and these dilutions were used as inhibitor

solution. Dilutions 1:10, 1:5 and 1:400, 1:800 were required for samples with low and high PCDDs concentrations respectively. The cross-reactivity of TMDD/TCDD is 130%, but TMDD is less toxic to handle (Shan et al. 2001).

## HRGC-HRMS

A soil sample pool (three subsamples) from the Tekchem chemical plant was analyzed by ELISA through the sulfuric acid–silica gel cleanup methodology previously mentioned and by HRGC-HRMS. The aim of these analyses was to assess the accuracy of the overall ELISA-based method, including the sample cleanup. The overall HRGC-HRMS analytical method (including cleanup) has been validated through many International Intercalibration Studies (UNEP 2005). The HRGC-HRMS analysis using the Swedish Standard SS-EN 1948:1-3 (ECS 1997) is briefly summarized below.

<sup>13</sup>C-labeled standards were added to the sample extraction. The sample was Soxhlet-extracted with toluene for 16 h. PCDDs, PCDFs and polychlorinated biphenyls (PCBs) were purified first in a multistep silica column followed by a basic alumina column. The final step was made on a carbon column. The carbon column was used to separate the PCDDs/PCDFs and planar PCBs from the rest of the PCBs. The final extract was fortified with 30 µl tetradecan and 40 µl <sup>13</sup>C-labeled recovery standard before the solvent was evaporated to the final volume. HRGC-HRMS analyses was performed on the sample with a 60-m DB-5 (J&W Scientific, Folsom, CA) directly attached to the VG instrument (70/70S). The HRMS instrument was operated in electron ionization (EI) mode with single-ion registering. Quantification was done by relating peak areas from the sample to peak areas of a standard that contained specific amounts of all toxic isomers and all <sup>13</sup>C-labeled standards.

## Results and Discussion

### ELISA Performance

The statistical analysis of 50 ELISA calibration curves, run in triplicate on different days, showed that the immunoassay was sensitive and reproducible. The average 50% inhibition concentration (IC<sub>50</sub>) was 181 ± 61 pg TMDD/ml. The limit of detection (LOD) (defined as 90% of the maximum response) was 8.0 ± 6 pg TMDD/ml, and the quantification limit (LOQ) (defined as 80% of the maximum response) was 34 ± 32 pg TMDD/ml. The average coefficient of variation (CV) for three TMDD in DMSO samples spiked at 200, 100, and 50 pg/ml concentrations

evaluated by triplicate on three consecutive days, with a total of nine results averaged for each concentration, was 14, 17, and 36%, respectively.

### Method Validation

In this study, we used a sample preparation procedure similar to the methodology developed for dioxin analysis in soil and sediment samples differing in degree of contamination, soil type, and total organic matter (Nichkova et al. 2004). The method is based on hexane extraction and cleanup by multilayered silica gel column, followed by organic solvent exchange with DMSO-Triton X-100 and ELISA measurement (Nichkova et al. 2004). It has been demonstrated that multilayered (44% H<sub>2</sub>SO<sub>4</sub>) silica gel column cleanup provides sufficient removal of the ELISA interferences for most sediment samples with total organic content between 0.6 and 23%. These HRGC-HRMS validation studies ( $n = 13$ ) revealed that the immunoanalytical method is suitable for TEQ screening of dioxin in sediments with accuracy of 60–113% and precision of 13–33% relative standard deviation (Nichkova et al. 2004).

Based on our previous experience, we used a sulfuric acid silica-gel column in order to eliminate immunoassay interferences present in the hexane extracts from soil and soot samples collected from Mexican sites. The strong acidic environment of the sulfuric acid treatment would likely remove polyaromatic hydrocarbons (PAHs), acidic polar interferences, and other contaminants (Till et al. 1997). In order to test the efficiency of the PAH removal we performed the cleanup procedure, passing dioxin certified soil standard (BCR529) and PAH standard reference material (CRM 105-100) through different numbers of sulfuric acid columns. The obtained hexane extracts were exchanged to DMSO-Triton X and analyzed by TMDD at dilutions of 1:50, 1:100, and 1:200 for BCR529 and up to 1:800 dilution for CRM 105-100 without sulfuric acid silica column, and at 1:10, 1:20, and 1:40 dilutions for CRM 105-100, with three sulfuric acid silica columns. Table 1 presents the results of this procedure. It can be observed that, without any cleanup, the PAH standard soil sample gives an ELISA response of 1,004 ng TMDD equivalents/g due to cross-reactivity with PAHs and/or the presence of other specific and nonspecific interferences. The first acid silica column significantly reduces the matrix effect (64.8 ng TMDD equiv./g) and the use of three columns results in a consistent response that is equivalent to 1.6% of the response before any cleanup. Furthermore, the control experiment with the dioxin soil standard demonstrates that the detected 4.3 ng TMDD equiv/g correlates very well with the theoretical concentration of 4.94 ng TMDD equiv./g estimated based on the HRGC-HRMS-determined concentrations of the congeners recognized by

**Table 1** Matrix effect evaluation: dioxin concentration (ng TMDD equiv./g) determined by ELISA for dioxin and PAH soil standards using different numbers of H<sub>2</sub>SO<sub>4</sub> silica gel columns in the cleanup procedure

Clean-up column number	Dioxin concentration (ng/g)			
	0	1	2	3
Dioxin standard <sup>a</sup>	4.3 (21)	3.6 (18)	4.7 (13)	4.2 (22)
PAH standard <sup>b</sup>	1004.0 (15)	64.8 (12)	25.7 (1)	16.0 (27)
PAH + dioxin <sup>c</sup>	1078.0 (19)	78.8 (2)	33.0 (12)	27.0 (25)

<sup>a</sup> Certified soil reference material BCR 529 (EUR 18863 EN)

<sup>b</sup> PAH-contaminated standard reference material (CRM 105-10)

<sup>c</sup> This sample is a combined sample of the dioxin and PAH soil standards

All samples were analyzed in three-well replicates with three dilutions, the value represents the arithmetic mean and the CVs are shown in parenthesis

the antibody. This sample was a sandy soil and, according to our experience, little or no matrix effects are usually observed for sandy type of soils that are free of humic substances. In addition, no loss of dioxins is observed during the application of multiple consecutive columns.

We applied this approach to evaluate the efficient removal of the matrix effect for different soil and soot samples (Table 2). In general, a constant value ( $\pm 20\%$ ) of dioxin concentration can be obtained after using two columns, with the obvious exception of soot sample 343. Therefore, we decided to apply this procedure to the analysis of the remaining soil and soot samples from different Mexican sites. We would like to note that, alternatively, the cleanup can be performed by simple washing (shaking) of the hexane phase with concentrated sulfuric

**Table 2** Matrix effect evaluation: dioxin concentration (pg TMDD equiv./g) determined by ELISA in soil and soot samples using different numbers of H<sub>2</sub>SO<sub>4</sub> silica gel columns in the cleanup procedure

Number of columns	Dioxin concentration (pg/g)			
	0	1	2	3
SAL 1 <sup>a</sup>	1 540 (20)	691 (35)	685 (45)	–
SAL 9 <sup>a</sup>	1 456 (19)	60 (18)	53 (51)	–
Copper smelter <sup>a</sup>	943 (35)	587 (17)	636 (12)	–
Soot 5 <sup>b</sup>	33 007 (16)	344 (59)	389 (69)	387 (39)
Soot 279 <sup>b</sup>	55 094 (14)	110 (51)	165 (52)	134 (20)
Soot 343 <sup>b</sup>	59 731 (5)	605 (14)	357 (30)	319 (57)

<sup>a</sup> Soil samples were collected from areas around the Tekchem chemical plant and a copper smelter

<sup>b</sup> Soot samples were collected from rural dwellings where wood is being used for indoor cooking

All samples were analyzed in three-well replicates with three dilutions, on three different days; the value represents the arithmetic mean and the CVs are shown in parenthesis

acid followed by centrifugation and collection of the hexane layer (Nichkova et al. 2004). However, the column procedure allows higher throughput, it is more convenient to perform, and it avoids the use of large volumes of concentrated sulfuric acid.

Finally our aim was to test the accuracy of the method. However, due to the high cost of HRGC-HRMS analysis we were able to perform instrumental analysis of only one representative soil sample for the validation of the immunochemical method. According to HRGC-HRMS results the dioxin level in a soil sample pool collected around the Tekchem chemical plant for organochlorine pesticides was 220 TEQ pg/g. Considering the HRGC-HRMS concentration of each congener (data not shown) and the cross-reactivity of the antibody, a theoretical level of 188 TMDD equiv./g was estimated. The dioxin concentration found by the immunoassay was 214 pg TMDD equiv./g with a CV of 21%, corresponding to an accuracy above 90%.

## Analysis of Soil and Soot Samples from Mexican Sites

### Chemical Plants

PCDDs are formed as unwanted impurities during the manufacturing of chlorophenols, chlorobenzenes, and chlorobiphenyls. Consequently, disposal of industrial wastes from manufacturing facilities producing these compounds may result in release of PCDDs into the environment (EPA 1998). We evaluated the soil contamination in the vicinity of two chemical plants for pesticides: the Anaversa pesticide formulation plant located in the urban area of Córdoba, Veracruz, and the Tekchem chemical plant that manufactured organochlorine pesticides between 1957 and 1998, located in Salamanca, Guanajuato. The Anaversa explosion in 1991 was one of Mexico's worst pesticide disasters. Thirty-eight thousand liters of pesticides were burnt in the fire, including 18,000 l of methyl parathion, 8,000 l of paraquat, and 3,000 l of 2,4-D. According to a company inventory, over 1,500 l of pentachlorophenol, malathion, benzene hexachloride, and lindane also went up in smoke. Unofficial research indicates that, 11 years after the accident, there had been more than 170 deaths related to this accident. Many of the original 1,500 people who were poisoned and who did not immediately die have since died. Twelve years after the accident there are still new cases of people affected by cancer as well as genetic, respiratory, and other problems. For both sites, surface (1–3 cm) and subsurface (20–30 cm) soil samples were collected within 500 m of the chemical plants from areas currently available for children's recreation. The dioxin levels determined by the immunochemical method are presented in Table 3. For the Anaversa plant sites, no significant

**Table 3** Levels of dioxin-like compounds (pg TMDD equiv./g) in soil samples collected around chemical plants

Surface sample	Concentration (pg/g)	Subsurface sample	Concentration (pg/g)
Anaversa chemical plant			
ANA 3	55 (55)	ANA 1	36 (62)
ANA 4	45 (58)	ANA 2	48 (57)
Background	ND	ANA 5	74
Tekchem chemical plant for organochlorine pesticides (1957–1998)			
SAL 1	813 (41)	SAL 3	101 (39)
SAL 2	37 (35)	SAL 12	151 (41)
SAL 4	ND	SAL 13	202 (39)
SAL 5	37 (28)	Background	ND
SAL 8	37 (55)		
SAL 9	75 (52)		
SAL 10	528 (7)		
SAL 11	52 (41)		

All samples were collected within 500 m of the chemical plant in areas available for children's recreation. *ND* not detected. Each sample was studied in three-well replicates with three dilutions. The value represents the arithmetic mean and CVs are shown in parenthesis. The Tekchem chemical plant sampling points are shown in Fig. 1

difference between surface and subsurface soil samples was observed. However, subsurface dioxin concentrations appear higher than the surface ones at the Tekchem chemical plant. In general, levels were higher in closer proximity to the plant (Fig. 1).

### Brick Kilns

These are small-scale low-technology combustion kilns that are fired with inexpensive and highly polluting fuels, such as used tires, domestic garbage, used motor oil, and wood scraps (Costner 2006). The kilns are a leading source of air pollution in some cities and a serious health hazard to those who live near them (Joshi and Dudani 2008). These traditional kilns can produce between 4,000–80,000 bricks per bake, with 1–4 bakes per month (Costner 2006). In Mexico, more than 13,000 brick kilns are distributed all over the country (Costner 2006). In general, they are located in brick production areas named “ladrilleras,” each including 20–100 kilns. We analyzed 14 surface soil samples collected around kilns from Zacatecas in two different brick production areas. None of the samples showed detectable levels (estimated LOQ = 3.4 pg TMDD/g dry soil).

### Secondary Smelters

These are industries primarily engaged in recovery of nonferrous metals and alloys from new and used scrap and

**Table 4** Dioxin levels (pg TMDD equiv./g) in surface soil samples collected in secondary smelter areas

Sample	Concentration (pg/g)	Sample	Concentration (pg/g)
Lead smelter	ND	Copper smelter	872 (46)
Iron smelter	149 (38)	Copper smelter	442 (25)
Background	ND		

ND not detected. Each sample was studied in three-well replicates with three dilutions. The value represents the arithmetic mean and the CVs are shown in parenthesis

cross. Scrap metal and metal wastes may contain organic impurities such as plastics, paints, and solvents. Furthermore, the processing of some metals (e.g., aluminum, copper, and magnesium) utilizes chemicals such as NaCl, KCl, and other salts (EPA 1998). Table 4 shows the levels found in surface soil samples collected at three different secondary smelters. Higher levels were found at the copper smelter sites. In this regard, it is interesting to note that copper is a good catalytic agent for dioxin production (EPA 1998).

#### Rural Dwellings

PCDDs can be formed during thermal processes, including in stack gases and solid residues from any combustion process, e.g., bottom ashes, slags, and fly ash. PCDDs levels in ranking from 4–42,048 pg/g were found in soot from home-heating systems using wood and coal as fuel (UNEP 1999). Soot is a carbon-rich material surrounded by hydrocarbons coming from incompletely burnt wood, besides the lipid content probably emanated from wood resins and food preparation (13% w/w lipid content, data not shown); it is therefore a suitable concentrating material for lipophilic compounds such as PCDDs/PCDFs. In this work, we showed that the dioxin levels found in three soot samples collected from rural dwellings were the higher than all sites evaluated (Table 5).

The US Agency for Toxic Substances and Disease Registry (ATSDR) published an interim policy guideline based on current understanding of the toxicology and epidemiology associated with PCDDs (De Rosa et al. 1997). These guidelines and procedures apply to human exposure for direct ingestion of soils contaminated with dioxin and dioxin-like compounds in residential areas. Three different levels have been considered: (1) screening ( $\leq 0.05$  ppb TEQs), (2) evaluation ( $>0.05$  to  $<1.0$  ppb TEQs), and (3) action level ( $\geq 1.0$  ppb TEQs). If one or more soil sampling value exceeds the screening level, further site-specific evaluations are needed. If a complete or potentially complete exposure pathway is identified, then the extent of exposure and public health implications are further evaluated. If the

**Table 5** Levels of putative dioxin-like compounds (pg TMDD equiv./g) in soot samples collected in rural dwellings

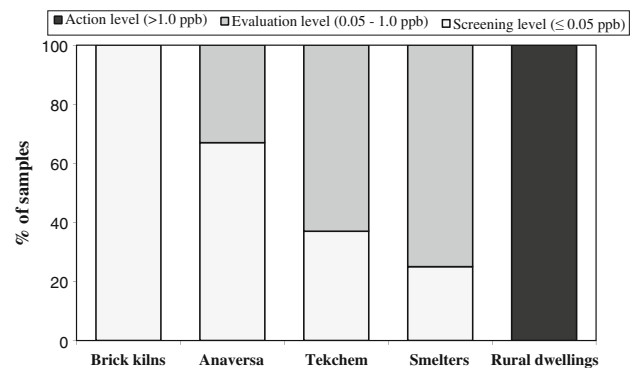
Sample	Concentration (pg/g)
Soot 11	2,419 (11)
Soot 4	5,550 (12)
Soot 6	5,967 (8)

Each sample was studied in three-well replicates with three dilutions. The value represents the arithmetic mean and CVs are shown in parenthesis

concentrations in residential soils exceed 1 ppb TEQs, ATSDR health assessors should consider judging the site a public health hazard and consider site-specific public health recommendations or actions to prevent or prohibit exposure.

Figure 2 summarizes the data on the dioxin soil and soot contamination obtained in our work in the context of the ATSDR's guidelines. It can be observed that a limited number of samples can be considered a public health hazard. None of the studied samples had a dioxin concentration above the action level of 1.0 ppb. However, an important percentage of soil samples in almost all the areas had dioxin concentrations within the evaluation range (0.05–1 ppb). Only the brick kilns fired with wood scraps can be considered as low-risk sites in terms of PCDDs concentrations in soil. However, in other areas of Mexico, kilns are fired with more polluting fuels, such as used tires, domestic garbage, and used motor oil (Costner 2006). The emission factor for wood combustion is 0.82 ng TEQ/kg (EPA 1998), while the factor for used oil combustion is 2.0 ng TEQ/kg (Bremmer et al. 1994). Thus, a higher concentration of PCDDs can be expected in those brick kiln areas where these other fuels are used.

Considering the percentage of samples with concentrations above 0.05 ppb, and taking into account the size of the potentially exposed population, four sectors deserve further studies: the areas in the vicinity of the Anaversa plant, the Tekchem chemical plant for organochlorine



**Fig. 2** Distribution of soil (kilns, plants, and smelters) and soot (rural dwellings) samples according to ATSDR guidance levels. The figure illustrates the percentages of samples that exceed the evaluation level or screening level recommended by ATSDR for each analyzed site

pesticides, secondary smelters, and rural dwellings. The putative levels of dioxin-like compounds found in all three of the soot samples collected from indigenous dwellings where wood is used for indoor cooking were above the evaluation level. It has been estimated that approximately 50% of the world's population and up to 90% of rural households in developing countries still rely on coal or unprocessed biomass material in the form of wood, dung, and crop residues for fuel (Bruce et al. 2000). In Mexico, 27 million people use wood as an energy source, while 19 million use it as a fuel for indoor cooking; a daily consumption of 2 kg of wood per person has been calculated (Torres-Dosal et al. 2008). Therefore, numerous individuals are exposed to dioxins and further more detailed assessment is urgently needed.

## Conclusions

Five different sectors considered relevant sources of PCDDs in Mexico were evaluated in this study, all of them with an important presence in less developed countries. The putative levels of dioxin-like compounds found in most of the areas suggested the need for further evaluation of the extent of exposure and public health effects. Our work is a demonstration of the utility of the immunoassays as an analytical screening tool for assessment of dioxin-like contamination in developing countries, where instrumental analysis cannot be easily performed due to its high cost. In this scenario, HRGC-HRMS can be used only for confirmation purposes.

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