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Immunochemical Analysis of 2,4,6-Tribromophenol for Assessment of Wood Contamination

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2,4,6-Tribromophenol (2,4,6-TBP) has been used as a wood preservative and flame retardant and is a synthetic intermediate of the most important brominated flame retardants (BFR) produced. The use of TBP-contaminated wood materials in the food industry poses a risk of significant economical losses due to food contamination. In this work an efficient and reliable immunochemical method for analysis of TBP in wood samples has been established consisting of alkaline wood extraction followed by analysis on a microplate ELISA (enzyme-linked immunosorbent assay). TBP is efficiently extracted from wood samples in 10 min and directly measured after 10-fold buffer dilution to avoid matrix interferences. The analytical procedure has a limit of detection of 45 ng g^{-1} of TBP in wood (1.5 μg L^{-1} in extracts). The method has been applied to the analysis of contaminated real wood samples, showing that the levels of contamination can reach high TBP concentrations (up to 2000 μg L^{-1}). An excellent correlation was observed between TBP levels in wood extracts determined by ELISA and gas chromatography–mass spectrometry (GC-MS) analysis ($R^2 = 0.990$, $N = 19$). The precision found is below 22% CV. The immunoanalytical method developed is fast, reliable, and cost-effective, shows good high-throughput screening capabilities, and can be an excellent tool for assessment of wood contamination at lumber mills or related industries. The TBP ELISA has the potential to be used for environmental, food, and biological monitoring of brominated phenolic compounds considered nowadays as emerging pollutants.

KEYWORDS: 2,4,6-Tribromophenol; brominated flame retardants; ELISA; wood; pallets; lumber mill; GC-MS

INTRODUCTION

Brominated flame retardants (BFRs) represent major industrial chemicals whose use has increased dramatically over the past few decades (*1*). They are produced to prevent fires and thus can have a direct and obvious benefit. However, concerns are being raised because of their persistence, bioaccumulation, and potential for toxicity, both in animals and in humans (*2–4*). There are more than 75 different BFRs recognized commercially. Among them, 2,4,6-tribromophenol (TBP) can be used as a flame retardant in epoxy, polyurethane, plastics, paper, textiles, and others, although its main use is as an intermediate for the production of other commercial flame retardants and fire extinguishing media. Thus, TBP is used to produce brominated epoxy resins made from tetrabromobisphenol A (probably the largest application), tribromophenyl allyl ether, and 1,2-bis(2,4,6-tribromophenoxyethane), with the latter being the second most prevalent flame retardant used in acrylonitrile−butadiene−styrene resins (*5*). TBP is by far the most widely produced brominated phenol. The production volume of TBP was estimated at approximately 2500 tons/year in Japan and 9500 tons/year worldwide in 2001 (*6*). TBP has also been used as a pesticide and wood preservative. The solution of sodium tribromophenate is very effective in controlling insects, fungi, and bacteria in construction lumber, plywood timbers, railroad ties, fence posts, utility poles, landscape materials, and foundation materials. Although nowadays this use is not allowed in the European community and the United States, it remains still registered as a wood preservative in South America. Synthetic derivatives of TBP are also used as general fungicides in leather, textiles, paint, plastics, paper, and pulp industries. Moreover, it has been reported that brominated phenols can be formed from the biodegradation of other pollutants, such as brominated benzenes and some brominated diphenyl ethers (*7*).

Brominated phenols are generally not readily biodegradable and will persist in the environment. TBP has been detected in drinking waters, waste treatment facility effluents, sludges, and soils (*8, 9*). Although knowledge on TBP toxicity and its effects

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on humans and environment is still limited, risks for developmental neurotoxicity, embryotoxicity and fetotoxicity (*10*), endocrine disrupting potency (*11, 12*), estrogen-like activity (*13*), and induction of neuroblastoma cell differentiation (*14*) have been reported. Human exposure may occur through the food chain, inhalation, and dermal contact (*9, 15*). TBP has been found in human urine (*9, 16*) and in relatively high concentration in human serum samples (*3, 4*).

The increasing use of recycled wood and polymeric-based materials, which may previously have been impregnated with TBP and its derivatives, may represent a source of latent pollution likely to contaminate sensitive food products and potable water as a result of irresponsible storage. If wood treated with halophenols (TBP, chlorophenols) is used to manufacture food packaging, the food may become contaminated. The halophenols, which impart a "chemical/medicinal" taint, can be converted to their corresponding anisoles by microbial action, and these compounds may have much lower, "musty/moldy" taint thresholds (nanogram per liter to picogram per liter concentration in water) (*17, 18*). Thus, 2,4,6-tribromoanisole (TBA) has been found to cause powerful musty and earthy flavors in dried fruit packed in polyethylene film that was in contact with fiberboard cases contaminated with TBP (*17*). The study shows that TBP has been broken down into TBA by the ubiquitous fungus *Paecilomyces variotii*, isolated both from fiberboard cartons and timber floors of freight containers. Indirect pollution of pharmaceuticals by polyethylene stoppers contaminated through contact with wooden pallets impregnated with TBP has also been reported by Hoffman and Sponholz (*19, 20*). Furthermore, deterioration of drinking water organoleptic quality was attributed to the presence of TBA originated by TBP leaching from the storage tank coating (*21*). Recently, TBA was identified in wines with a significant "musty or corked" character (*22*). Analysis of the materials in contact with the atmosphere in this winery showed that the wooden roof timbers had been massively impregnated with TBP, which had gradually broken down into TBA due to the action of microflora in the atmosphere. Some of the paints assayed in the same winery also contained TBP as a flame retardant and/or fungicide. Atmospheric quality in storage areas is vital to ensure the chemical and organoleptic inertia of materials (plastics, corks) that come into contact with wine. Therefore, the continued use of TBP, either as a fungicide or as a synthetic intermediate of BFRs, requires its careful monitoring in wood materials in order to avoid costly food contamination.

Currently, laboratory methods for TBP and/or TBA are based on chromatographic techniques, usually gas chromatography coupled to electron capture (EDC) or mass (MS) spectrometry. Thus, these methodologies allowed the sensitive detection of TBPs in water (*23, 24*), urine, soil and sawdust (*9*), food (*17*), wine and wood samples (*22*). However, these methods require special equipment and trained personnel and have high cost. Rapid, inexpensive, and reliable techniques are needed for routine TBP screening of wood materials. Thus, the lumber industry distributes materials that are further in close contact with the everyday life of humans and food stuffs. Immunochemical methods may fulfill the efficiency requirements due to their simplicity, specificity, high detectability, and high-throughput sample processing capabilities.

In this work we demonstrate that ELISA can be an excellent tool to efficiently control contamination of wood samples by TBP and avoid further problems related to the undesirable musty/moldy off-flavors or to the potential adverse effects due to the presence of other more toxic BFRs.

MATERIALS AND METHODS

Reagents and Instruments. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions containing each of the halophenols at a concentration of 1 g L^{-1} were prepared in ethanol. The preparation of the immunoreagents used in this study has been described (*25, 26*). The pH and the conductivity of all buffers and solutions were measured with a pH meter, pH 540 GLP, and a conductimeter, LF 340, respectively (WTW, Weilheim, Germany). Polystyrene microtiter plates used for the ELISA analysis were purchased from Nunc Maxisorb (Roskilde, Denmark). Washing steps were carried out using a SLY96 PW microplate washer (SLT Labinstruments, Salzburg, Austria). Absorbances were read on a SpectramaxPlus (Molecular Devices, Sunnyvale, CA) at a single wavelength mode of 450 nm. The competitive curves were analyzed with a four-parameter logistic equation using the software SoftMaxPro v2.6 (Molecular Devices) and GraphPad Prism (GraphPad Software Inc., San Diego, CA). Unless otherwise indicated, data presented correspond to the average of at least two well replicates.

Wood Samples. Five blank (BW) and 14 contaminated (W) wood samples (1.5 cm × 0.7 cm × 0.7 cm, weight of 0.5 g) were provided by Micromaterials Research, Inc., and obtained from a lumber mill where TBP contamination was found.

Sample Extraction. Wood samples were extracted three consecutive times with 0.1 N NaOH (15 mL) at 60 °C (sand bath) for 2 min or just once for 10 min. For recovery studies samples were spiked by dipping them (2 min) into a 1 N NaOH solution (9 mL) containing different concentrations of TBP (20 mg L^{-1}, 2 mg L^{-1}, and 0.2 mg L^{-1}). After that, the samples were left to dry overnight at room temperature.

ELISA Analyses. The basic wood extracts were buffered with 100 mM PBS (100 mM phosphate buffer, 0.8% saline solution, pH 7.5) and neutralized with few drops of concentrated HCl to place the pH and conductivity values close to the buffer parameters (pH 7.5 and 15 mS cm^{-1}). Following, the extracts were diluted with 10 mM PBS (10 mM phosphate buffer, 0.8% saline solution, pH 7.5) to place the concentration of the samples within the linear range of the method. The samples were analyzed by ELISA following the protocol already described (*25, 26*) and using TBP as standard.

ELISA Matrix Effect Studies. TBP standard curves were prepared in extracts, prior to and after dilution with 10 mM PBS, and run in the competitive ELISA to compare parallelism with the standard curve prepared in just 10 mM PBS.

GC-MS Analyses. The aqueous solutions of the standards (10 mL) and the alkaline wood extracts (10 mL) were acidified (pH <3) with concentrated HCl and extracted with toluene (1 mL) by agitating the mixture on an automatic shaker at 300 rpm for 50 min. Recoveries for this process are higher than 70% as already reported (*27*). After separation of the phases by centrifugation, the toluene extract (100 μL) was silylated by adding BSTFA [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide, 2 μL] and incubated for 2 h at room temperature. 2,3,5,6-Tetrachloronitrobenzene was used as internal standard (IS). The TMS derivatives were then analyzed by GC-MS. For the GC-MS analysis, injections (1 μL) were splitless (48 s) with solvent delay (5 min). A HP-5MS [cross-linked 5% phenylmethylsiloxane, 30 m × 0.25 mm i.d. × 0.25 μm (film thickness)] column was used. The ion source temperature was set at 200 °C, and He was the carrier gas employed at 1 mL/min. The ionization energy was 70 eV. GC conditions were as follows: temperature program, 100−300 °C (7 °C/min); injector temperature, 250 °C. The ions 253/255/268 (2,4,6-TCP), 137/139/263/280 (2-B-4-CP), 307/309/324 (2,4-DBP), 387/389/402 (TBP), 287/289/304 (2,3,4,6-TtCP), and 203/259/261 (IS) were monitored in the SIM mode. For the SCAN mode, the mass range explored was 45–550. All data are reported as *m*/*z* (relative intensity). Calibration curves for TBP, 2,4-DBP, 2-B-4-CP, 2,4,6-TCP, and 2,3,4,6-TtCP were set up in the range 0.5−50 μg L^{-1}. The standard curves in all GC-MS analyses were built using toluene extracts of phenol standards prepared in water. In this way the losses during the extraction step were included. Calibration standards were analyzed with each analytical series. A reagent blank was also analyzed in each series. If the measured values of the sample extracts were above the linear range of the calibration graphs, the toluene extracts were diluted with toluene and injected again. The limits

Table 1. Features of the ELISA for TBP

parameter	value (mean ± SD)[a]
A_{max}	1.01 ± 0.11
A_{min}	0.02 ± 0.01
IC_{50}, $\mu g\ L^{-1}$	0.65 ± 0.13
dynamic range, $\mu g\ L^{-1}$ (80–20% inhibition)	0.26 ± 0.07 to 1.38 ± 0.39
slope	1.90 ± 0.32
LOD (90% inhibition), $\mu g\ L^{-1}$	0.15 ± 0.04
r^2	0.992 ± 0.01

[a] The values are extracted from the four-parameter equation used to fit the standard curve. The data reported correspond to the average of eight standard curves run in eight different days using two-well replicates for each concentration.

of detection (defined as a signal-to-noise ratio S/N of 3:1) corresponding to the halogenated phenol concentration in wood aqueous extracts (toluene:NaOH extraction ratio 10:1) were 0.25 $\mu g\ L^{-1}$ for TBP, 2,3,4,6-TtCP and 2,4-DBP, 2 $\mu g\ L^{-1}$ for 2-B-4-CP, and 0.13 $\mu g\ L^{-1}$ for 2,4,6-TCP.

Validation Studies. For the validation studies the alkaline wood extracts were divided in two fractions for ELISA (5 mL) and GC-MS (10 mL) analyses. The results obtained with both methods were used to perform correlation studies using a linear regression analysis.

RESULTS AND DISCUSSION

TBP ELISA. The ELISA used in this study uses immunoreagents previously developed for the analysis of chlorophenols in environmental and biological samples (*25, 26*). Because of the strong recognition of the brominated analogues, this method has been adapted in this work to the analysis of TBP residues in wood samples. The immunoassay features obtained are shown in **Table 1**. As can be observed, the detectability achieved [limit of detection (LOD) of 0.15 $\mu g\ L^{-1}$ TBP] is good enough to analyze TBP residues below the parts per billion level. This sensitivity could allow further application of the TBP immunoassay to the analysis of different environmental, biological, and food samples, such as water, soil, human serum and urine, wine, etc.

According to our previously reported data on IC_{50} values of 16 structurally related chloro- and bromophenols (*26*), recognition of the bromophenols was always much stronger than that of the corresponding chlorinated analogues. The recognition pattern has already been analyzed and discussed in detail previously (*26*). Thus, the most important cross-reactants in the TBP immunoassay are 2,4-dibromophenol (2,4-DBP, 22.4%), 2,4,6-trichlorophenol (2,4,6-TCP, 17.5%), 2-bromo-4-chlorophenol (2-B-4-CP, 6%), and 2,3,4,6-tetrachlorophenol (2,3,4,6-TtCP, 4.6%). Very poor recognition was observed for 4-bromophenol, 2,4- and 2,6-dichlorophenols, 2,6- dibromophenol, 2,3,5- and 2,4,5-trichlorophenols, 2,3,5,6-tetrachlorophenol, 2,4,6-trichloroanisole, 2,4,6-tribromoanisole, pentabromophenol, and pentachlorophenol. Thus, these data pointed out that the ELISA has the necessary features to be evaluated as a screening method for TBP.

Extraction of Halophenols from Wood. GC-MS analysis of halophenols is usually performed after their extraction from cork, wood, and/or fiberboard with organic solvents such as dichloromethane/acetic acid (*22*) and pentane/ether (*17*). The choice of extraction procedure in our study was driven by the aqueous compatibility with the ELISA method of detection. Thus, we decided to assess the extraction efficiency of a basic aqueous solution such as 0.1 N NaOH. The basic media would favor the solubility of the phenols by forming the corresponding salt. Moreover, the absence of organic solvent would limit the extraction of other components of the matrix rendering a cleaner extract. With this purpose, blank wood samples (BW-1) were spiked by dipping them into a solution containing different TBP concentrations (20 mg L^{-1}, 2 mg L^{-1}, and 0.2 mg L^{-1} TBP) and letting them dry overnight at room temperature. Extractions were performed with 15 mL of 0.1 N NaOH at 60 °C (sand bath) for 2 min. The process was repeated three consecutive times (extractions I, II, and III), and the concentration of each extract was analyzed by GC-MS, after acidification, extraction with toluene, and derivatization of the halophenols as TMS esters (*16*). The concentration of TBP in each wood extract (I, II, and III) is presented in **Table 2**. As can be observed, complete extraction was achieved after three consecutive extractions for all spiked levels. Moreover, between 60% and 70% of the TBP was already extracted in the first cycle, which pointed to the possibility to extract quantitatively TBP from wood samples in just a single step by slightly increasing the time. The extraction procedure was reproducible with a CV in the range of 4−15%.

Table 2. Evaluation of TBP Extraction from Wood Samples by GC-MS

TBP concn in spiking solution, mg L^{-1}	extraction	TBP concn in extract,[a] $\mu g\ L^{-1}$ ± SD	% recovery[b]
20	I	49.67 ± 1.98	61
	II	21.05 ± 1.30	26
	III	10.60 ± 1.50	13
	I + II + III		100
2	I	7.35 ± 0.36	73
	II	1.73 ± 0.21	17
	III	0.94 ± 0.08	10
	I + II + III		100
0.2	I	2.80 ± 0.17	66
	II	0.86 ± 0.13	20
	III	0.56 ± 0.06	13
	I + II + III		100

[a] Concentration in the aqueous extract is measured by GC-MS and is the average of at least three replicates. [b] Recovery is calculated on each extract from the total amount extracted.

Table 3. Bromophenol Levels in Contaminated Wood Extracts Determined by GC-MS and ELISA

sample	GC-MS TBP,[a] $\mu g\ L^{-1}$	ELISA TBP IR equiv,[b] $\mu g\ L^{-1}$
W-1	2029.85	2331.00 ± 88.00
W-2	2156.56	2328.00 ± 68.20
W-3	311.75	320.00 ± 14.00
W-4	311.90	354.80 ± 20.05
W-5	2031.91	1908.00 ± 80.00
W-6	247.70	313.10 ± 21.20
W-7	399.44	514.10 ± 72.31
W-8	484.39	548.80 ± 59.23
W-9	56.00	63.60 ± 1.84
W-10	68.12	61.0 ± 12.20
W-12	54.82	65.9 ± 8.08
W-13	54.70	49.6 ± 16.32
W-14	2200.61	2150 ± 183.30

[a] For GC-MS analysis, phenols were extracted with toluene and derivatized with BSTFA. Each sample was injected twice. [b] The concentration values obtained by ELISA correspond to the average plus the standard deviation of analysis made using two-well replicates.

Matrix Effect of Wood Extracts on the ELISA for TBP. As a blank sample with no detectable levels of halogenated phenols is desirable to evaluate matrix effects in the ELISA, blank wood sample BW-1 (see **Table 4**) was selected. Alkaline extract was prepared as described above and used to test the TBP standard curves. Prior to ELISA, the pH and conductivity of the wood extract was adjusted to the values of the assay buffer (PBS; pH = 7.5 and 15 mS cm^{-1}) by adding some drops of concentrated HCl and buffering the aqueous extract with 100 mM PBS (10% of the extract volume to accomplish a final

Table 4. Bromophenol Levels in "Blank" Wood Extracts Determined by GC-MS and ELISA

	GC-MS,[c] μg L^{-1}			
sample	TBP	2,4-DBP	TBP IR equiv[a] (TBP + 2,4-DBP), μg L^{-1}	ELISA TBP IR equiv,[b] μg L^{-1}
BW-1	ND	ND		ND
BW-2	0.44	0.35	0.52	0.39 ± 0.02
BW-3	0.55	1.28	0.92	1.03 ± 0.02
BW-4	0.90	1.25	1.18	1.29 ± 0.09
BW-5	0.77	1.35	1.07	1.19 ± 0.13

[a] IR equivalents were calculated from the concentration values found by GC-MS considering the cross-reactivity value of 2,4-DBP. [b] The concentration values obtained by ELISA correspond to the average plus the standard deviation of analysis made using two-well replicates. [c] For GC-MS analysis, phenols were extracted with toluene and derivatized with BSTFA. Each sample was injected twice. ND = not detected.

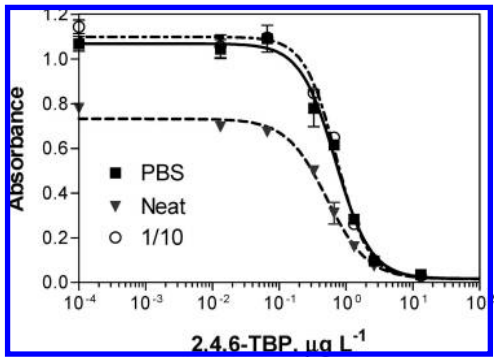

Figure 1. Matrix effect of aqueous wood extracts on the ELISA for TBP. A blank wood sample (0.5 g) was extracted with 15 mL of 0.1 N NaOH for 10 min. The basic wood extract was buffered with 10× PBS, and its pH was adjusted to 7.5. Standard curves were run in PBS, neat buffered wood extract, and wood extract diluted 10 times with PBS. A dilution factor of 1/10 is sufficient to avoid the undesirable matrix effects caused by the wood matrix.

concentration of 10 mM PBS). In this way possible effects of pH and salinity on the immunoassay were eliminated. Further dilutions of the neat wood extract were performed in 10 mM PBS buffer. As can be observed in **Figure 1**, the neat extract caused significant interference in the assay. However, this effect could be eliminated after a 10-fold dilution of the extract with PBS. Therefore, analyses can be made without the need to introduce a cleanup step. Considering this dilution factor, we can estimate a LOD of 1.5 μg L^{-1} TBP in wood extracts corresponding to 45 ng g^{-1} TBP in wood. This LOD is much lower than the TBP levels found in wood barrels (240–1300 ng g^{-1}) that were a potential source of contamination of wine with TBA (*22*). It is in the range of the total TBA levels (300 ng) in fiberboard that has been demonstrated to produce a musty taint in polyethylene-packaged sultanas (*17*). Therefore, the detectability achieved is sufficient to analyze wood extracts without the need to preconcentrate. Extraction is fast and easy, and just a simple buffer dilution is needed to perform the analysis. Combined with the high-throughput screening character of the microplate ELISA methods, the immunoanalytical procedure established can be an excellent tool to screen wood pallets or other materials at lumber mills, wineries, etc. to prevent food contamination with TBP.

Validation Studies. Alkaline extracts were prepared from blank and contaminated wood samples (N = 19) and split in two fractions to be analyzed by ELISA and by GC-MS. The ELISA measurements were performed after adjusting the pH and buffering the extracts. Following, the samples were serially diluted (starting 1/10) with PBS to ensure measurements within the dynamic range of the immunoassay (0.26–1.38 μg L^{-1}). Values obtained were expressed as ELISA TBP immunoreactivity equivalents (IR equiv) because of the potential concomitant presence of other halophenols cross-reacting in the immunoassay. The ELISA analyses revealed that contaminated wood samples were found to contain high levels of TBP (see **Table 3**). Thus, samples W-3, W-4, and W-6 were diluted 200 and 400 times, while samples W-1, W-2, W-5, and W-14 had to be diluted up to 2000 and 4000. These dilution factors are much higher than the necessary dilution to eliminate nonspecific matrix interferences. As can be observed in **Table 3**, wood extracts W1 and W2 contained the highest levels of 2331 and 2328 μg L^{-1} TBP IR equiv, respectively, while the less contaminated samples (W-13 and W-10) had concentration values of 49.6 and 61 μg L^{-1}, respectively. Surprisingly, wood samples labeled as "blank" by the lumber mill also contained detectable TBP IR equiv levels (see **Table 4**). The origin of this contamination could have been the same lumber mill, where other TBP- (or BFR-) treated wood samples were stored or during transport, since all wood samples were submitted in the same box or in the laboratory. It must be noticed that the estimated vapor pressure (0.76 × 10^{-2} Pa) indicates that TBP will exist in both the vapor and particulate phases in the ambient atmosphere (*8*).

Validation of the ELISA was then performed, analyzing the same extracts by GC-MS. For GC-MS analysis the extracts were acidified, extracted with toluene, and derivatized to form the corresponding TMS derivatives. In this case, analyses were performed in a SIM mode monitoring for TBP, 2,4-DBP, 2-B-4-CP, 2,4,6-TCP, and 2,3,4,6-TtCP, the most important cross-reactants of the assay. As can be observed in **Tables 3** (contaminated wood samples) and **4** (blank samples), the results obtained by ELISA for TBP matched very well those obtained by GC-MS. The levels of the cross-reactants were not very significant. Thus, 2,4-DBP was the only bromophenol detected in most of the samples although at very low levels (in the range 0.52−2.88 μg L^{-1}), while chlorophenols were not found in these wood extracts. Regarding blank samples, according to the GC-MS analysis the levels of 2,4-DBP were higher than those of TBP. As mentioned before, the contribution of this analyte to the immunoreactivity measured by ELISA is around 22%. Thus, in order to analyze the correlation, the concentration of 2,4-DBP was converted in GC-MS TBP IR equiv, applying this factor. The total GC-MS concentration expressed as TBP IR equiv matched then almost perfectly the TBP IR equiv concentration measured by ELISA (see **Table 4**).

A linear regression analysis was performed with all of the wood samples analyzed by both methods. As can be observed in **Figure 2**, an excellent correlation between both techniques was observed ($y = 1.05x + 12.4$, $R^2 = 0.990$). It should be noted that the GC-MS levels of 2,4-DBP found in the contaminated wood samples were so low in comparison with the TBP concentrations that they have negligible effect on the present correlation. The precision of the method including extraction and ELISA quantification was in the range from 4.8% to 22.4% RSD.

Summarizing, here we presented an immunochemical analytical method to analyze TBP in wood samples. The whole analytical procedure shows high-throughput screening capabilities since the extraction procedure is fast and simple and completely compatible with the ELISA method. The immunoassay takes about 90 min and can process many samples simultaneously. Thus, in the actual microplate setup used in this study about 20 samples can be

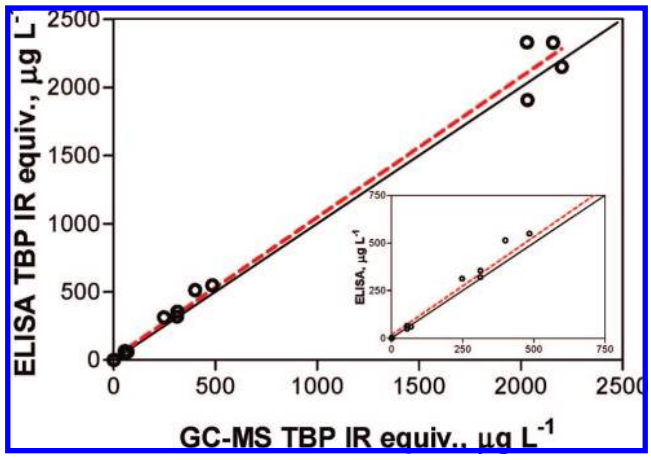

Figure 2. Correlation between TBP IR equiv determined by ELISA and GC-MS for the wood samples analyzed ($N = 19$). The GC-MS response graphed is defined as [TBP] + 0.22[2,4-DBP]. The black line indicates an ideal correlation ($X = Y$) between both methods.

analyzed in one plate using two-well replicates and two different dilution factors for each extract (total of four measurements for one sample). The whole analytical procedure is able to detect this analyte down to 45 ng g^{-1} wood (1.5 μg L^{-1} for extracts), considering that a minimum of 10-fold dilution factor is needed to ensure the absence of the nonspecific matrix response. The dynamic range of the immunoassay allows the analysis of TBP in wood extracts from 2.6 to 13.8 μg L^{-1}. Higher concentrations can be determined just by further PBS sample dilution. The excellent correlation between ELISA and GC-MS demonstrated that the present immunoassay is a reliable and accurate tool for TBP quantification in wood extracts. The data obtained show that TBP contamination may reach high concentration values and that contamination of other closely placed solid material may occur, as can be drawn from the contamination found in blank samples.

The analysis can be easily performed in lumber or other industry sectors. Although this work demonstrates the application of the ELISA for determination of TBP in wood samples, the immunoassay could be used for quality control of water, wine, cork stoppers, or other materials. Furthermore, the immunochemical method could be applied to large TBP occupational and environmental exposure studies where high-throughput screening is needed. This would provide new information on TBP distribution and exposure effects.

ABBREVIATIONS USED

BSTFA, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; BSA, bovine serum albumin; 2,4-DBP, 2,4-dibromophenol; 2-B-4-CP, 2-bromo-4-chlorophenol; CR, cross-reactivity; GC-MS, gas chromatography coupled to mass spectrometry; ELISA, enzyme-linked immunosorbent assay; anti-rabbit IgG-HRP, anit-rabbit immunoglobulin conjugated to horseradish peroxidase; IC_{50}, concentration of analyte giving 50% inhibition; KLH, keyhole limpet hemocyanin; LOD, limit of detection; Na-TBP, sodium tribromophenate; PBS, phosphate-buffered saline; PCP, pentachlorophenol; RSD, relative standard deviation; TBA, 2,4,6-tribromoanisole; TBP, 2,4,6-tribromophenol; 2,3,4,6-TtCP, 2,3,4,6-tetrachlorophenol; TMB, tetramethylbenzidine.

LITERATURE CITED

(1) Alaee, M.; Arias, P.; Sjodin, A.; Bergman, A. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **2003**, *29*, 683–689.

(2) Hale, R. C.; Alaee, M.; Manchester-Neesvig, J. B.; Stapleton, H. M.; Ikonomou, M. G. Polybrominated diphenyl ether flame retardants in the North American environment. *Environ. Int.* **2003**, *29*, 771–779.

(3) Thomsen, C.; Lundanes, E.; Becher, G. Brominated flame retardants in archived serum samples from Norway: A study on temporal trends and the role of age. *Environ. Sci. Technol.* **2002**, *36*, 1414–1418.

(4) Thomsen, C.; Lundanes, E.; Becher, G. Brominated flame retardants in plasma samples from three different occupational groups in Norway. *J. Environ. Monit.* **2001**, *3*, 366–370.

(5) Weil, E. D.; Levchik, S. A review of current flame retardant systems for epoxy resins. *J. Fire Sci.* **2004**, *22*, 25–40.

(6) IUCID. Data Set for 2,4,6-Tribromophenol. Ispra, European Chemical Bureau. International Uniform Chemical Information Database, **2003**.

(7) Bergman, A. Brominated flame retardants in a global environmental perspective. Workshop on brominated aromatic flame retardants, Skokloster, Solna, National Chemicals Inspectorate (KEMI), **1990**.

(8) Howe, P. D.; Dobson, S.; Malcolm, H. M. 2,4,6-Tribromophenol and other simple brominated phenols. *Concise Int. Chem. Assess. Doc.* **2005**, *66*, 1–47.

(9) Gutierrez, M.; Becerra, J.; Godoy, J.; Barra, R. Occupational and environmental exposure to tribromophenol used for wood surface protection in sawmills. *Int. J. Environ. Health Res.* **2005**, *15*, 171–179.

(10) Lyubimov, A. V.; Babin, V. V.; Kartashov, A. I. Developmental neurotoxicity and immunotoxicity of 2,4,6-tribromophenol in Wistar rats. *Neurotoxicology* **1998**, *19*, 303–312.

(11) Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Murk, A. J.; Kester, M. H. A.; Andersson, P. L.; Legler, J.; Brouwer, A. In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. *Toxicol. Sci.* **2006**, *92*, 157–173.

(12) Hassenklover, T.; Predehl, S.; Pilli, J.; Ledwolorz, J.; Assmann, M.; Bickmeyer, U. Bromophenols, both present in marine organisms and in industrial flame retardants, disturb cellular Ca^{2+} signaling in neuroendocrine cells (PC12). *Aquat. Toxicol.* **2006**, *76*, 37–45.

(13) Olsen, C. M.; Meussen-Elholm, E. T. M.; Holme, J. A.; Hongslo, J. K. Brominated phenols: characterization of estrogen-like activity in the human breast cancer cell-line MCF-7. *Toxicol. Lett.* **2002**, *129*, 55–63.

(14) Rios, J. C.; Repetto, G.; Jos, A.; del Peso, A.; Salguero, M.; Camean, A.; Repetto, M. Tribromophenol induces the differentiation of SH-SY5Y human neuroblastoma cells in vitro. *Toxicol. in Vitro* **2003**, *17*, 635–641.

(15) Hazardous Substances Data Bank. Tribromophenol. Available at http://toxnet.nlm.nih.gov, 1999.

(16) Nichkova, M.; Marco, M. P. Biomonitoring Human Exposure to Organohalogenated Substances by Measuring Urinary Chlorophenols Using a High-Throughput Screening (HTS) Immunochemical Method. *Environ. Sci. Technol.* **2006**, *40*, 2469–2477.

(17) Whitfield, F. B.; Hill, J. L.; Shaw, K. J. 2,4,6-Tribromoanisole: A potential cause of mustiness in packaged food. *J. Agric. Food Chem.* **1997**, *45*, 889–893.

(18) Adams, J. B.; Lock, S. J.; Toward, M. R.; Williams, B. M. Bromophenol formation as a potential cause of "disinfectant" taint in foods. *Food Chem.* **1999**, *64*, 377–381.

(19) Sponholz, W. R.; Grossmann, M. K.; Muno, H.; Hoffmann, A. The distribution of chlorophenols and chloroanisoles in cork and microbiological method to prevent their formation. *Ind. Bevande* **1997**, *26*, 602–607.

(20) Hoffmann, A.; Sponholz, W. R. Direct thermal analysis of solids—A fast method for the determination of halogenated phenols and anisoles in work. *Am. Lab.* **1997**, 22–23.

(21) Malleret, L.; Bruchet, A. A taste and odor episode caused by 2,4,6-tribromoanisole. *J.—Am. Water Works Assoc.* **2002**, *94*, 84–95.

(22) Chatonnet, P.; Bonnet, S.; Boutou, S.; Labadie, M. D. Identification and responsibility of 2,4,6-tribromoanisole in

musty, corked odors in wine. J. Agric. Food Chem. **2004**, *52*, 1255–1262.

(23) Blythe, J. W.; Heitz, A.; Joll, C. A.; Kagi, R. I. Determination of trace concentrations of bromophenols in water using purge-and-trap after in situ acetylation. J. Chromatogr. A **2006**, *1102*, 73–83.

(24) Polo, M.; Llompart, M.; Garcia-Jares, C.; Gomez-Noya, G.; Bollain, M. H.; Cela, R. Development of a solid-phase microextraction method for the analysis of phenolic flame retardants in water samples. J. Chromatogr. A **2006**, *1124*, 11–21.

(25) Galve, R.; Camps, F.; Sanchez-Baeza, F.; Marco, M.-P. Development of an Immunochemical Technique for the Analysis of Trichlorophenols Using Theoretical Models. Anal. Chem. **2000**, *72*, 2237–2246.

(26) Galve, R.; Nichkova, M.; Camps, F.; Sanchez-Baeza, F.; Marco, M.-P. Development and evaluation of an immunoassay for biological monitoring of chlorophenols in urine as potential indicators of occupational exposure. Anal. Chem. **2002**, *74*, 468–478.

(27) Nichkova, M.; Marco, M. P. Development and evaluation of C18 and immunosorbent solid-phase extraction methods prior immunochemical analysis of chlorophenols in human urine. Anal. Chim. Acta **2005**, *533*, 67–82.

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