Characterization of Paint Samples Used in Drinking Water Reservoirs: Identification of Endocrine Disruptor Compounds

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Abstract

Several

migration tests are performed from various epoxy paint samples that, according to the regulation, can be used in food reservoirs such as drinking water reservoirs. The level of the organic compounds capable of producing migrations to water with special attention to endocrine disruptor compounds (EDCs) are identified and estimated by closed loop-stripping analysis (CLSA) and liquid-liquid extraction (LLE) methods coupled with gas chromatography (GC)-mass spectrometry (MS). Bisphenol A, a strong endocrine disruptor, is found in all migration experiments. Its concentration level reaches between 0.02 and 0.03 mg/cm². The higher concentration corresponds with benzylic alcohol, which is used as a solvent and curing agent in epoxy paint. Other EDCs identified in the migration tests are phthalates, 4-nonylphenol, and t-butylphenol. The main non-EDCs identified are solvents, antioxidants, and rubber-like compounds. No great differences are found in the use of metallic plates or concrete slabs for migration experiments; only additional compounds related with the pretreatment of the concrete wall have been identified, too. In the study of a drinking water sample the same organic compounds identified in the migration test is not seen. This is probably because of the dynamic situation in a drinking water reservoir. Finally, a GC profile of a direct epoxy paint analysis is shown. The main peak identified is bisphenol A diglycidyl ether, monomer, and an active principle of the polymerization of epoxy resins based on bisphenol A. In addition, we report the recoveries of a selected group of EDCs using CLSA and LLE methods coupled with GC-MS.

Introduction

An extensive group of organic compounds with an assorted chemistry nature are considered as endocrine disruptor compounds (EDCs). Epidemiological and in vivo studies (ecotoxicological tests with fish) related these compounds with a reduction in the quality of sperm, gonadal and mammalian cancer, and other damages in the genital tract caused by hormonal modulation mechanisms (1). Today, there is a growing awareness about levels of and the exposure to these compounds in the environment because of their high risk to human health and the growing number of substances catalogued as EDCs by in vitro detection tests such as the Vitellogenin or E-screen test (2,3).

The hormonal activity of some chemical compounds was wellknown some decades ago. For instance, in 1938 an unidentified hormonal activity for bisphenol A was published. After World War II, scientists located high levels of xenoestrogens (or EDCs related with human activity) in the environment because of their increased use in industrial and domestic areas. In 1990 only some pesticides, catalogued as dangerous for other negative effects, formed the EDCs list. The endocrine disruptor concept, the rules for specific studies of endocrine activity, and the awareness of human health and environmental exposure were established at the European Workshop on the Impact of Endocrine Disruptors on Human Health and Wildlife (Weybridge, U.K.) in 1996. Alkylphenols, bisphenols, phthalates, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, dioxins-furans (PCDD/F), some metals (Pb, Cd, and Hg), and organotin compounds have been finally included in the EDCs list.

EDCs can be found in different products of daily domestic use such as fertilizers, detergents, paintings, preservatives, and plasticizers. The monomer bisphenol A diglycidyl ether (BADGE), the synthesis product resulting from the reaction between bisphenol A (4,4'-(1-methylethyldiene)bisphenol) and epichlorhydrin, is the main compound used in the manufacture of epoxy-resins-based paints. Despite their higher price, epoxy resins are widely used as a coating for food packaging materials as well as for drinking water reservoirs because of their high quality (4). Therefore, migrations of specific components from epoxy resins to the foods were located as a result of the contact between these products and the food (5–8); the migration of bisphenol A in inner painted cans is a typical example cited in the literature (4,7). The EDC fixes at 10 mg/dm² (measured as the weight of the global dry residue), which is the maximum leached level in global migration tests for

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products that may hold contact with food.

In Japan, the production of bisphenol A was 250,000 tons per year (9) between 1994 and 1996, while in the USA 850,000 tons were produced during 1999. Ninety percent of this annual production was used in the manufacture of epoxy resins (26%) and polycarbonate plastics (61%) industries, whereas 10% was employed to make flame-retardant compounds, thermal paper, brake fluid, coating, and other nonepoxy resins. Although bisphenol A has a weak toxicity to aquatic organisms, its estrogenic activity was well-known many years ago, and today it is probably one of the most studied endocrine disruptors (10).

There is less information available about other compounds identified in the formulations of epoxy-resins-based paints (or epoxy paints) that could also have endocrine disruptor properties. Paint additives, which are commonly denominated curing agents, give epoxy paints specific characteristics of polymerization, drying, antifouling, plasticity, antiburning, electrical properties, heat resistance and antioxidize capacities. Alkylphenols, phthalates, alcohols, amines, amides, and anhydrides are the most important curing agents added to epoxy paint formulations.

4-Nonylphenol (4-NP) is an alkylphenol commonly identified in the aquatic environment (i.e., wastewater effluents, rivers, sediments, and natural waters) (11–13). 4-NP is widely used in the industry as the raw material. It is also the main biodegradation byproduct for nonionic surfactants such as polyethoxylated nonylphenols (14), which is one of the most widely used surfactants in the world. Another use for 4-NP is as a curing agent in epoxy paints and resins to help in the polymerization and drying processes. 4-NP is one of the most important EDCs from the alkylphenols group, because it is estrogenic in biotests and in vivo studies at different levels (6, 15–18). Several European countries have adopted actions beginning in 1986 to decrease the use of polyethoxylated nonylphenols, because their breakdown products (including 4-NP) were known to be toxic to aquatic organisms (19).

The purpose of this study is to give an overview about the EDCs and other compounds leached from epoxy paints used in drinking water reservoirs by coupling the migration test with chromatographic techniques. We have performed several tests with various paint samples that, according to the regulation EC 90/128 (20), can be used in food reservoirs (such as drinking water reservoirs) to identify and estimate the level of the organic compounds that are capable of producing migrations to water. We have also calculated the recoveries of the different EDCs found in the previously mentioned experiments when the closed loop-stripping analysis (CLSA) and liquid–liquid exraction (LLE) methods coupled with gas chromatography (GC)–mass spectrometry (MS) are employed.

Experimental

Samples

Four types of samples related to epoxy paints were analyzed. The first set consisted of three different epoxy paints over stainless metallic plates (approximately 1 dm²) (labeled as paint 1, 2, and 3, respectively) that were sent to our lab for bisphenol A leaching analysis. The second set consisted of two concrete slabs painted with an epoxy-paint in order to simulate the surface of drinking

water reservoirs; slab A represented the first coat with the pretreatment nonepoxy paint of the stone and slab B represented the final process, which included a second coat with the epoxy paint. A sample of drinking water from a reservoir that was recently painted with the same slab paints and then a raw epoxy paint was also analyzed directly.

Reagents

Bisphenol A, alkylphenols, phthalates, and anthracene D10 were from Aldrich (Munich, Germany) and used for recovery experiments. Mono- and diethoxylated isomers of 4-NP were synthesized in our laboratory. Carbon disulfide from Merck (Parmstadt, Germany) for spectroscopy was used as the elution solvent for CLSA analysis. The 1-chloroalkanes (C₅, C₆, C₁₂, C₁₆) were from Fluka (Buchs, Switzerland) and the C₈, C₁₀, C₁₄, and C₁₈ were from Aldrich. Acetone r.a. (Carlo Erba, Milan, Italy) and dichloromethane (Promochem, Wesel, Germany) were bidistilled over glass in our laboratory and were used for stock and standard preparations and liquid extractions, respectively. Milli Q (Millipore S.A., Bedford, MA) ultrapure water was used for all recovery and migration experiments.

Migration tests

The migration tests of the metallic plates and slabs were carried out according to established methods in the EC (20) and Spanish regulations (21). Briefly, the painted metallic plate or slab was doused in a vessel containing 1 L of Milli-Q ultrapure water with a contact time of five days at 40–45°C and preserving evaporation. Finally, the plate or slab was removed and the migration water was split in two aliquots (0.5 L) to carry out organic analysis by CLSA and LLE techniques and further GC–MS identification of the organic compounds leached from the painted plate/slabs.

CLSA

Analyses were carried out in a commercial CLSA apparatus (Brechbüler, Schieren, Switzerland) according to the method developed by Grob et al. (22). One liter of water samples (or dilutions) was spiked with 1-chloroalkanes (C_5 , C_6 , C_{10} , C_{12} , C_{16} , and C_{18}) to give a final concentration of 400 ng/L for each compound. We used 1.5 mg of activated carbon filters to trap the organic compound that was stripped from the water during 1 h. Temperatures of 45°C and 55°C were used for the waterbath and carbon filter, respectively. After stripping, the filters were spiked with C_8 and C_{14} 1-chloroalkanes at the same concentrations of the spiked water samples. The filters were then extracted twofold with 10 µL of CS₂ to obtain a final volume of 20 µL.

LLE

Water samples of 0.5 L in volume (pH 7) were twofold extracted with 70 mL of bidistilled dichloromethane in 5 min of manual shaking. The organic extract was dried over sodium sulfate and concentrated to 500 μ L with a rotavapor and finally under a gentle stream of nitrogen. A semiquantitation was performed using anthracene-d10 as the internal (sandwich injection) or external standard.

Analysis of paint

A few milligrams of paint was introduced in a vial and 2 mL of methylene chloride were added. The covered vial was put in an

ultrasonic bath for 20 min to dissolve and homogenize the solution. The extract was stored at -20° C for 24 h and then filtered through a column of anhydrous sodium sulfate. This step was repeated until the extract was completely transparent and without precipitates. Finally, the volume extract was adjusted for GC–MS purposes.

GC-MS analysis (instrumental conditions)

GC-MS analyses were carried out on a VG Fisons TRIO-1000 MS equipped with a Fisons GC 8000 Series GC. For CLSA extracts the injection mode was 1 μ L "cold on column" (5 s) in a 50-m × 320-µm-i.d. CP-Sil 19CB (0.25-µm film thickness) fused-silica column (Chrompack, The Netherlands) with 2 m of deactivated precolumn. The GC temperature program was 30°C (5 min) to 280°C (10 min) at a rate of 3°C/min. Helium was the carrier gas, and the linear velocity was set to 42 cm/s at 30°C. For LLE extracts the following conditions were used: 1-µL splitless mode (200°C and 1 min) in a DB-5 30-m × 250-µm-i.d. × 0.25-µm film, fusedsilica column (J&W, Folsom, CA). The GC temperature program was 60°C (5 min) to 285°C (10 min) at a rate of 6°C/min. Helium was the carrier gas (42 cm/s at 60°C). The MS was operated in electron impact mode (70 eV). The transfer line and ion-source temperatures were 200°C and 250°C, respectively. Mass spectra were acquired by scanning from 35-450 daltons with a 1-s decade. The identification was carried out by a library search using Wiley 6.0 and NISH 98.

Results and Discussion

Table I shows the recovery obtained for a group of selected EDCs in water applying CLSA and LLE extraction techniques. For LLE, efficiencies close to 100% were obtained for all the EDCs studied, and only a significant efficiency decrease for 4-NP deriva-

Table I. Recovery of Selected EDCs in Different Water

	LLE	(at 10 µg	g/L)	CLSA (at 1 µg/L)			
	Milli-Q water	River water	Tap water*	Milli-Q water	River water ⁺	Tap water*	
Bisphenol A	98	97	101	0	0	0	
4-p-Nonylphenol	91	87	87	78	70	81	
Nonylphenol	93	91	86	15	10	12	
monoethoxylated							
Nonylphenol	87	81	79	0	0	0	
diethoxylated							
4-t-Butyl-phenol	98	n.t.‡	n.t.	3	n.t.	n.t.	
4-t-Octyl-phenol	100	n.t.	99	43	n.t.	n.t.	
2-n-Octyl-phenol	105	n.t.	99	40	n.t.	n.t.	
Di-ethyl phthalate	95	n.t.	n.t.	0	0	0	
Di-butyl phthalate	97	n.t.	n.t.	45	42	51	
2-Ethylhexyl phthalate	93	n.t.	n.t.	2	0	0	

* Before use, free chlorine was removed by adding 1 mL of 0.1M ascorbic acid.

⁺ CLSA recovery affected by turbidity of water samples. River water was filtered before analysis.

* n.t., not tested.

tives was observed when the number of ethylene oxide units was increased (80% for diethoxylated 4-NP). No differences were observed for EDC recoveries when river, tap, or ultrapure water was used, which suggests that no matrix effects occurred.

The CLSA technique showed different efficiency responses for the EDCs studied. CLSA did not seem to be a suitable tool for EDC analysis, but the CLSA technique gave additional information about other volatile compounds (non-EDCs) that can produce odor characteristics in water. A large group of EDCs, (including bisphenol A) were not extracted; some alkylphenols have low to medium recoveries (75% for 4-NP or 40% for octylphenol). For the phthalates group, only di-*i*-butyl phthalate was recovered with a 45% efficiency. The low-efficiency results and great variability obtained for the EDCs studied using the CLSA technique was not caused by matrix effects because similar recoveries have been obtained with the three different water matrices used. The different results obtained with LLE and CLSA could be explained by the specific characteristics of the CLSA technique. CLSA was originally developed for the trace analysis of organic volatile compounds in water causing odor episodes, thus the efficiency decreased when the molecular weight and boiling point of the compound studied increased. Also, because the CLSA technique was based on the principle of gas-liquid extraction, compounds with high solubility such as alkylphenols were recovered less efficiently.

Figure 1 shows an example of an LLE–GC–MS profile corresponding with a metallic-plate migration test. Bisphenol A was identified in all experiments. The maximum concentration level was reached between 0.02 and 0.03 μ g/cm² of the painted metallic plate (Table II). Benzylic alcohol was also identified in all experiments, but at higher concentration levels. This compound is used as a solvent and curing agent for polymerization in epoxy paint bicomponents.

Other authors have found similar bisphenol A migration levels from bisphenol A epoxy-resins-based products. Brotons et al. (7) studied bisphenol A migration from lacquer coatings in food cans. They reported 0.05 μ g/cm² (10 μ g/can) as the average for several vegetable cans that were analyzed. Krishnan et al. (23) reported 3 μ g/L of bisphenol A in water autoclaved in polycarbonate flasks. Simal et al. (4) discussed EU food regulations (21,24) that establish a limit of 3 mg/kg bisphenol A in food or liquid used in a migration test (equivalent to 5 μ g/cm² for bisphenol A, considering that 1 L is in contact with 6 dm²).

The bisphenol A migration level found in the three samples analyzed agrees with the regulations (European Community and Spanish regulation) for food and water contact purposes in their leach level for global and bisphenol A migration. However, the estrogenic activity of bisphenol A has been measured by the E-Screen test to be 10^{-5} times the Estradiol-17 β activity (natural human estrogenic hormone) (16). This value and its wide use make bisphenol A an important xenoestrogen for human exposure similar to other EDCs as organochlorine pesticides. Recently, studies performed in different countries were trying to correlate the organochlorine pesticide bioaccumulation as a xenoestrogen burden in breast adipose tissues and female breast cancer (25,26). Bisphenol A could be included in this hypothesis in the near future.

In addition to bisphenol A, we identified a large group of addi-

tives leached from the plates, depending on the paint used (Table II). Alcohols are normally used as curing agents in epoxy paints. Benzylic alcohol is the main alcohol used, but we could also find other alcohols to be curing agents, such as 4-NP in plate 3 (Figure 1) or linear alcohols in plate 2 with C12 > C14 > C16 > C10 concentration levels by carbon number. Phthalates were also identi-



Table II. Levels of EDCs and Other Significant Compounds Found in the Studied Samples

				ppb	Quantitative*		
	Plate 1	Plate 2	Plate 3	Slab A	Slab B	Reservoir ⁺	Paint component B
Bisphenol A	0.03	0.02	0.02	n.d.‡	n.q. ^{§,**}	'n.d.	yes ⁺⁺
4-p-Nonylphenol	n.d.	n.d.	1.7	n.d.	n.d.	n.d.	no
Nonylphenol monoethoxylated	n.d.	n.d.	t.r. ^{‡‡}	n.d.	n.d.	n.d.	no
4-t-Butyl-phenol	n.d.	n.d.	4	n.d.	n.d.	n.d.	no
4-t-Octyl-phenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	no
Sum linear alcohols ^{§§}	n.d.	0.4	0.13	n.d.	n.d.	n.d.	yes
Sum phthalates	0.3	0.04	0.12	0.04	0.05	2	no
Benzaldehyde ^{§§}	n.d.	0.02	0.02	0.04	0.04	n.d.	no
Benzyl alcohol	5	n.q.***	3	63	180	> 1***	yes ^{‡‡‡}
Sum aromatic solvents ^{§§}	n.d.	1.02	0.02	0.01	n.d.	6	yes
Octanal ^{§§}	n.d.	n.d.	n.d.	n.d.	n.d.	3	no
2-Et-4ClMe-1,3-DXL§§§	n.d.	n.d.	n.d.	0.03	0.03	n.d.	no
Ketones ^{§§}	n.d.	n.d.	n.d.	0.02	n.d.	<1	no
Polypropylenglycols	n.d.	n.d.	n.d.	n.q.	n.q.	n.d.	no
Sum antioxidants§§	n.d.	n.d.	n.d.	0.07	0.07	n.d.	no

* Presence or absence.

[‡] Not detected.

§§§ 2-Ethyl-4-chloromethyl-1,3-dioxane.

fied in all the samples but at higher levels in plate 1, reaching concentrations of $0.3 \ \mu\text{g/cm}^2$ for the leached product. 4-NP has been reported as an EDC in in vivo and in vitro tests (6), which reduces testicular development in fish and has a positive response in E-screen and Vitellogenin assays, showing close estrogenic activity to bisphenol A (3). It is a world chronic pollutant in the environ-

> ment because of its relationship with anionic surfactants. Janssen et al. (27) calculated that the human daily exposure to 4-NP is equivalent to 0.001–4 ng, which is an extra input of Estradiol-17 β . With respect to the phthalate group, a recent study of eight phthalate esters proved that selected phthalate esters elicited weak estrogenic activity "in vitro" but produced no estrogenic responses "in vivo" when examining their effects on uterine wet weight and vaginal cell cornification (28). Basically, only butylhexyl phthalate could be considered as an EDC with an E-Screen activity ten times lower than bisphenol A or 4-NP.

> When CLSA was employed (Figure 2) several volatile compounds were identified at trace levels as aromatic solvents (mainly xylenes) and linear aldehydes. In addition to benzyl alcohol, linear alcohols and phthalates were the main compounds present. Finally, we also found benzaldehyde, which is formed by the transformation of benzyl alcohol and is responsible for the characteristic sweet odor that is present in migration water after experiments.

> Some authors specify that the migration range could be modified by specific water characteristics used in migration experiments (i.e., pH) or liquid simulated (4,7,23). This possibility has not been studied in this case, but it is feasible that aggressiveness and other physical–chemical properties of drinking water could modify leached values from the metallic plates studied.

> However, it is clear that leached products are related to the process of painting and drying. We believe that the epoxy-paint support in a migration experiment could play an important role in leaching processes. We have explored this possibility by trying to emulate normal conditions in drinking reservoirs and repeating the experiment of migration with a concrete slab simulating real conditions of painted wall reservoirs. In this study, two independent experiments were performed because the wall was not painted directly. First, a wall pretreatment was necessary in order to prepare the surface to be painted: this pretreatment is usually made with a coat of nonepoxy paint (slab A). Finally, the wall was covered with the epoxy paint (slab B). The leached compounds observed are summarized in Table II. Figure 3 shows the chromatographic profile of LLE-GC-MS for slab B. The same compounds identified in steel plate migration experiments were found in the slab migration experiment. Benzylic alcohol was the

⁺ Results correspond with real drinking water sample in contact with recently painted reservoir.

[§] Not quantitated.

^{**} An unidentified compound masked bisphenol A.

⁺⁺ The active principle of BADGE was identified with other bisphenol-related compounds.

^{‡‡} Traces.

^{§§} The results were mainly observed and quantitated by the CLSA technique.

^{***} The level was higher than 1 mg/cm².

⁺⁺⁺ No more precision was possible because the chromatographic profile was saturated.

^{###} In component A.





Figure 3. LLE–GC–MS profile from the migration test using slab B.



main identified compound. It was also possible to detect bisphenol A (coeluting with another compound), and several other unidentified compounds were found. We believe they are probably related to the paint used in the pretreatment wall because they were identified in both slabs, probably coming from polypropyleneglycol rubbers. We can conclude from the slab migration experiment that there are no substantial differences between the supports used; therefore, it is possible to correlate the leached compounds on the steel plates with those leached from drinking water reservoir walls.

In order to know the significance of this migration study in a real case, we analyzed a drinking water sample from a recently painted reservoir (according to paint specifications) with one of the epoxy paints studied. The same analytical procedure as described before was performed for CLSA and LLE. We did not identify bisphenol A or any other bisphenol derivative. Only some phthalates were found at low levels (below 2 ppb) as representatives of the EDCs group. Ozone and chlorine disinfection byproducts and aromatic solvents were identified in the drinking water sample (see Table II). These results indicated that leaching from reservoir walls can be minimized by a correct paint process, drving time, and watercleaning afterwards of the wall before its use. In addition, the leached products were continuously diluted from refilling by a "dynamic" process in drinking water reservoirs, making it easy to get the concentrations found in "statical" migration experiments; this was a great difference in relation to cans or other packings. In addition to bisphenol A. Zafra et al. (29) have recently reported the possibility of the formation of chlorinated bisphenol A derivatives as disinfection byproducts and its destruction by free chlorine. In real conditions (5 ppb of bisphenol A and between 1 and 1.5 ppm free chloride in finished drinking water) we have verified the total destruction of the bisphenol A with little contact time, but no chlorinated bisphenol A derivatives were found.

The last step of this work was to study the direct analysis of a bicomponent raw epoxy paint, which was used in plate 2. Component A, the curing agent, was characterized by GC–MS as benzylic alcohol. BADGE, the base product for polymerization in the epoxy resins formed using a molecule of bisphenol A with two of epichlorhydrin, was the main compound observed in the GC–MS profile obtained for component B (Figure 4). In addition to BADGE, we have also found other related compounds characterized by Simal et al. (30) as hydrolysis and synthesis byproducts as well as linear alcohols and aromatic solvents (xylenes) as additives. It is likely that the carriers and solvents were also present at parts-per-billion levels. No bisphenol A (as unreacted starting material) was found, probably because of its trace concentration levels with respect to BADGE.

Conclusion

LLE is a suitable tool to study EDCs in water. The efficiencies obtained were close to 100% with no matrix effect observed. Regardless of whether the compounds are EDCs, the CLSA technique has a low efficiency for these compounds, but it shows additional information over the organoleptic water characteristics and other minor organic compounds.

The main compound identified in all of the samples was benzyl alcohol. This compound is related to commercial painting formulations and is commonly used as a solvent or a curing agent. During the migration experiment, a strong odor (similar to bitter almonds) was perceived because of the benzaldehyde production from benzyl alcohol.

Bisphenol A was identified in all of the migration on the steel plates. The concentration levels reached $0.02-0.03 \mu g/cm^2$. These values were comparatively low according to the regulations, but we must take into account that the human and environmental health problem with EDCs is related to bioaccumulation and low-level chronic exposures, according to scientists.

Other EDCs frequently identified in the migration tests were phthalates and, depending on the paint used, 4-NP or *t*-butyl-phenol. The main non-EDCs identified were solvents, antioxidants, and rubber-like compounds.

The slab analysis showed the same leached compounds as those found on steel plates, but an additional compound related to the pretreatment of the wall (polypropylenglycols) before painting was identified.

Neither bisphenol A nor other EDCs found in the migration experiment were identified in a drinking water sample that came in contact with a recently painted reservoir. Drinking water reservoirs are dynamic systems, which makes a huge difference when extrapolating results obtained from the static migration experiments and also enables free chlorine in the drinking water to destroy bisphenol A completely.

A direct paint analysis showed the same group of compounds found in the migration tests. The main peak identified was BADGE, which is a monomer and active principle of the polymerization of epoxy resins based on bisphenol A.

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