Synthesis, quantification and biological evaluation of halogeno Bisphenol derivatives

M. Doumas[1,2], A. Dupuis[1,2,3], M. Albouy-Llaty[1,2,4], N. Venisse[2,5], P.P. Eugene[1,2], M. Munier[6], P. Sibilia[6], P. Rodien[6], Y. Deceuninck[7], E. Bichon[7], B. Veyrand[7], P. Marchand[7], B. Le Bizec[7], V. Migeot[1,2,4], P. Carato[1,2*]

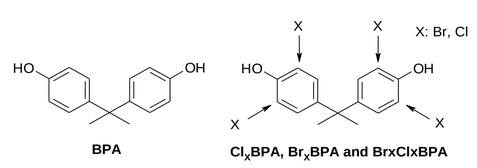
(1) Univ Poitiers, F-86000 Poitiers (2) CIC INSERM 1402, UFR Médecine Pharmacie, Poitiers (3) Service de Pharmacie, CHU, Poitiers (4) Pole Biospharm Services de Santé Publique, CHU, Poitiers
(5) Service de Toxicologie et Pharmacocinétique, CHU, Poitiers (6) INSERM 1083-CNRS 6016, Service d'Endocrinologie-Diabétologie-Nutrition (7) LABERCA, Oniris, INRA, Université Bretagne Loire, Nantes France

Introduction

Endocrine-disrupting chemicals (EDCs) alters functions of the endocrine system and causes adverse health effects in an intact organism. EDC include a wide variety of chemicals, such as pesticides, environmental pollutants and components used in the plastics industry.[1] Some EDCs are rapidly degraded in the environment or the human body or so widespread in their use like bisphenol A (BPA), that they can also have serious deleterious effects if exposure occurs during critical development periods. Indeed, extensive literature has shown adverse effects on animals following exposure to even a low dose of BPA, including developmental and reproductive toxicity, altered body weight, cancers and abnormally early puberty. BPA toxicity has been intensively investigated over the past decade, and it is now widely considered to have an estrogenic effect.[2]

Given the fact that in most drinking water treatment plants, routine operations are concluded by a chlorination step, the formation of chlorinated derivatives of BPA (ClxBPA) in drinking water is to be expected.[3-4] Last but not the least, estrogenic activity in ClxBPA may be higher than in parent compounds. The formation of brominated derivatives of BPA (BrxBPA) was also expected in presence of bromine, which was formed by oxidation of bromide during the chlorination step.

Recently, in our team, a cohort of a hundred of pregnant women was built in Deux-Sèvres (EDDS - France) and human biological environments were collected like urine, human breast milk[5-6] (colostrum) but also drinking water. The deal of this study, in a first time, was to synthesize halogeno BPA (Figure 1). In a second time, theses compounds were used as witness in an analytical study with LC/MS/MS to quantify them in drinking water and then evaluated *in vitro* on the follitropin receptor (FSHR).

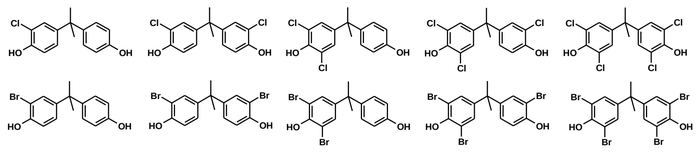


Figue 1 : BPA and BrxClxBPA derivatives

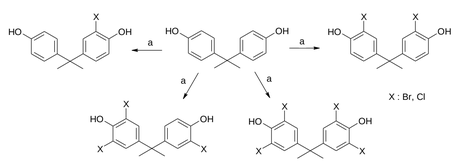
Synthesis of chloroBPA compounds and their conjugated analogues

For the synthesis of ClxBPA, BrxBPA and mixte BrxClxBPA (Figures 2, 3), we studied the nature of the reagent (NaClO, SO_2Cl_2, NCS, Br_2, NBS) and solvent ($CHCl_3$, THF, CH_3COOH or a mixture of CH_2Cl_2/THF). The best condition reactions were obtained with sulfuryl chloride and bromine as reagents in a mixture of CH_2Cl_2/THF (8/2). Then all halogeno BPA derivatives were synthesized according to these conditions, recently described in a publication (Scheme 1).[7]

The two compounds (Cl_2BPA and Br_2BPA) substituted by two halogeno atoms at the 2 and 6 positions on the same phenyl ring were prepared according scheme 2 by using protecting group as acetyl. The chloration or bromation of the acetylBPA afforded the corresponding 2,6-dichloro or dibromo acetylBPA which was deprotected in methanol with hydrochloric acid to give the corresponding 2,6-dihalogenoBPA.

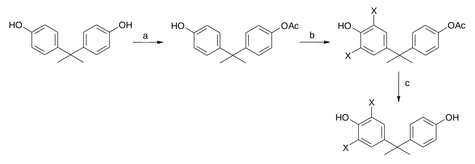


Figue 2 : ClxBPA and BrxBPA derivatives



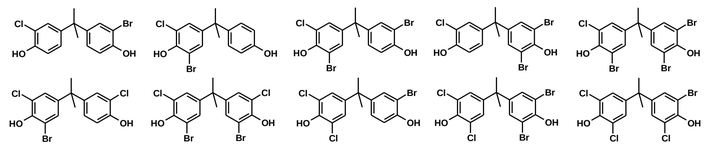
(a): SO_2Cl_2 or Br_2 (1-5 eq), CH_2Cl_2/THF, 0°C, 30 min, 40 – 69%

Scheme 1 : Synthesis of ClxBPA and BrxBPA



(a): Acetic anhydride, CH_2Cl_2/THF, pyridine, 0°C, 1 h, 33% (b): SO_2Cl_2 or Br_2 (3 eq), CH_2Cl_2/THF, 0°C, 30 min, 76-85% (c): HCl 3N, MeOH, rt, 4 h, 94%,

Scheme 2 : Synthesis of Cl_2BPA and Br_2BPA



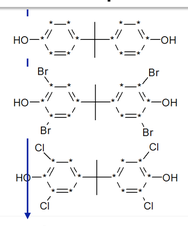
Figue 3 : BrxClxBPA derivatives

Quantification of Bisphenol and their halogeno derivatives

Analytical Protocol

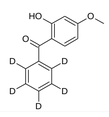
150 mL water sample

Internal standard addition



Chromabond HR-X Macherey-Nagel (0.5g/6mL)

External standard addition



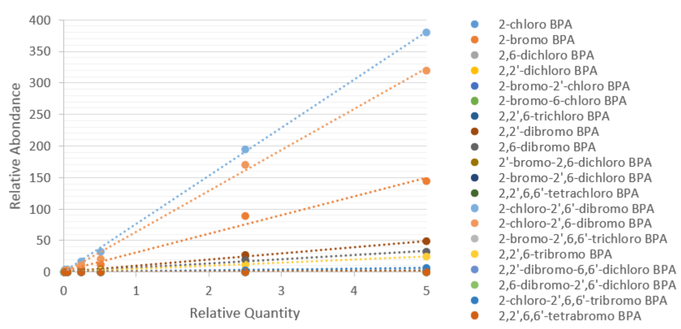
MSTFA derivatization

GC-MS/MS Agilent 7010 Optima-17MS 30 m x 0.25 mm, 0.25 µm

SRM and expected retention times

Analyte	Transition 1	Coll energy 1 (eV)	Transition 2	Coll energy 2 (eV)	retention time (min)
Bisphenol A	357.2>191.2	20	372.2>357.2	12	12.71
^{13}C-Bisphenol A	369.2>197.2	20	384.2>369.2	12	12.7
2-chloro bisphenol A	391.2>225.1	20	391.2>191.1	20	13.57
2,2'-dichloro bisphenol A	440.3>425.3	15	425.3>225.1	20	14.39
2,6-dichloro bisphenol A	425.3>73.0	30	425.3>191.1	10	14.26
2,2',6-trichloro bisphenol A	476.3>461.3	15	476.3>73.0	40	15.14
2,2',6,6'-tetrachloro bisphenol A	495.3>93.0	40	495.3>73.0	30	15.67
^{13}C-2,2',6,6'-tetrachloro bisphenol A	507.3>93	40	507.3>73	30	15.65
2-bromo bisphenol A	452.3>437.3	15	437.3>341.5	20	14.11
2,2'-dibromo bisphenol A	530.3>515.3	15	515.3>207.1	15	15.39
2,6-dibromo bisphenol A	515.3>355.2	25	515.3>287.3	15	15.44
2,2',6-tribromo bisphenol A	610.3>595.3	20	595.3>73.0	20	16.74
2,2',6,6'-tetrabromo bisphenol A	688.3>673.3	20	673.3>73.0	20	18.52
^{13}C-2,2',6,6'-tetrabromo bisphenol A	700.3>685.3	20	687.3>373.2	20	18.5
2-chloro-2',6,6'-tribromo bisphenol A	629.3>365.2	20	629.3>73.0	20	17.61
2-bromo-2',6,6'-trichloro bisphenol A	554.3>539.3	20	541.3>73.0	20	16.23
2,2'-dibromo-6,6'-dichloro bisphenol A	600.3>585.3	20	585.3>73.0	20	16.87
2-bromo-2'-chloro bisphenol A	468.7>73.0	25	470.7>73.0	25	14.91
2-bromo-6-chloro bisphenol A	468.7>73.0	25	470.7>73.0	25	14.83
2'-bromo-2,6-dichloro bisphenol A	502.7>73.0	40	504.7>73.0	40	15.56
2,6-dibromo-2',6'-dichloro bisphenol A	582.6>73.0	40	584.6>73.0	40	16.87
2-chloro-2',6-dibromo bisphenol A	547.0>73.0	40	548.7>73.0	40	16.12
2-chloro-2',6'-dibromo bisphenol A	546.7>73.0	40	548.7>73.0	40	16.16
2-bromo-2',6-dichloro bisphenol A	502.7>73.0	30	504.7>73.0	30	15.6
2-bromo-2',6,6'-trichloro bisphenol A	536.7>73.0	40	538.7>73.0	40	16.23
2-OH-4-MeO-BP d5	291.1>247.1	25			11.15

Calibration curves

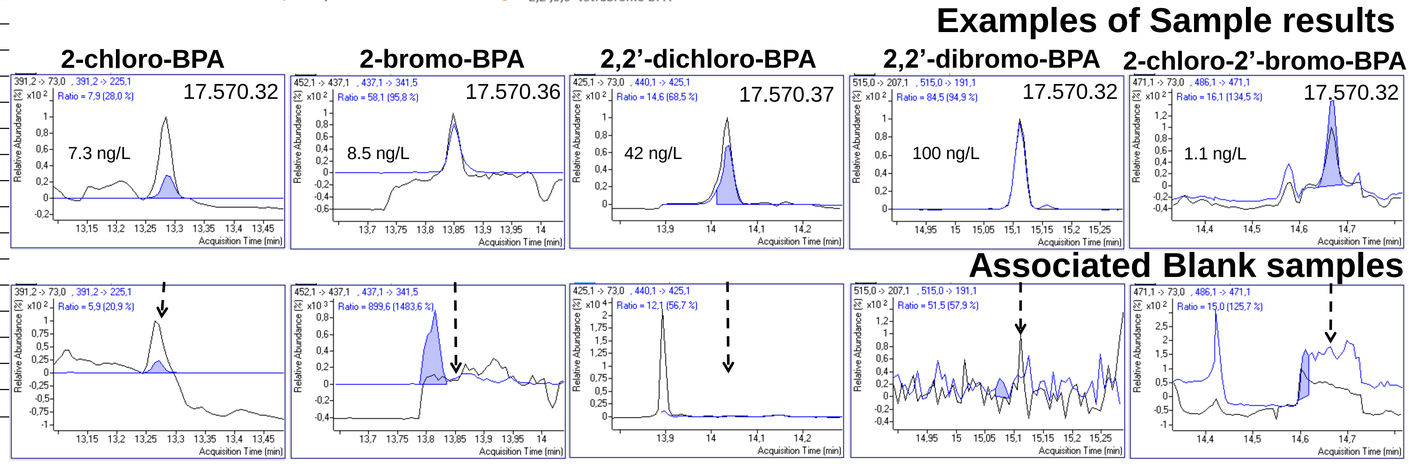


Limits of quantification

Analyte	LOQ (ng/L)
bisphenol A	23.2
2-chloro BPA	0.8
2-bromo BPA	5.9
2,6-dichloro BPA	3.0
2,2'-dichloro BPA	0.8
2-bromo-2'-chloro BPA	0.5
2-bromo-6-chloro BPA	0.4
2,2',6-trichloro BPA	1.2
2,2'-dibromo BPA	4.5
2,6-dibromo BPA	3.5

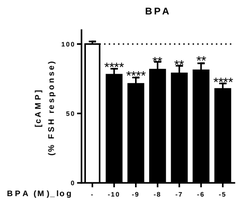
Analyte	LOQ (ng/L)
2'-bromo-2,6-dichloro BPA	0.3
2-bromo-2',6-dichloro BPA	0.5
2,2',6,6'-tetrachloro BPA	0.7
2-chloro-2',6'-dibromo BPA	4.4
2-chloro-2',6-dibromo BPA	5.5
2-bromo-2',6,6'-trichloro BPA	0.4
2,2',6-tribromo BPA	2.1
Sum of 2,2'-dibromo-6,6'-dichloro BPA and 2,6-dibromo-2',6'-dichloro BPA	0.7
2-chloro-2',6,6'-tribromo BPA	2.7
2,2',6,6'-tetrabromo BPA	1.0

Examples of Sample results



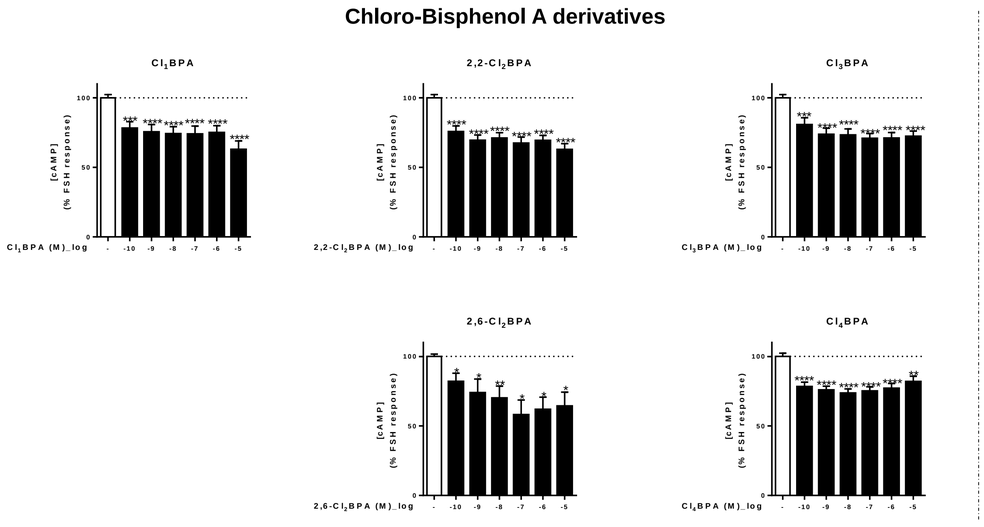
Evaluation *in vitro* on the follitropin receptor (FSHR)

Bisphenol A

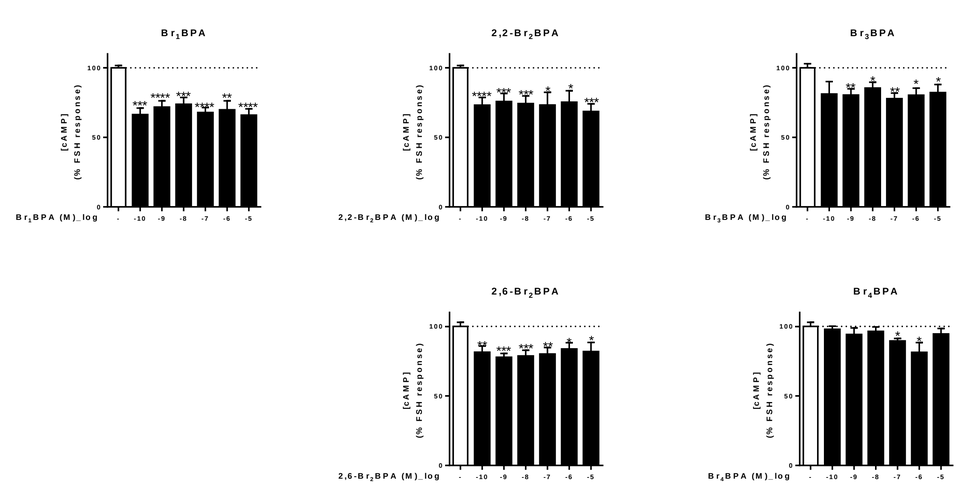


CHO-FSHR cells were incubated with FSH 10^{-10}M and increasing concentrations of halogeno bisphenol A were investigated. The cAMPc concentration measured in the presence of FSH alone was arbirtrarly set at 100% and the differences were evaluated using the Mann Whitney U test.

Chloro-Bisphenol A derivatives



Bromo-Bisphenol A derivatives



Conclusion

A series of twenty halogeno BPA compounds were synthesized and could be used as analytical standards and in biological evaluations for their potential endocrine disruptors. The study of the quantification of halogeno derivatives of BPA in water samples demonstrated their presence. The *in vitro* evaluation of ClxBPA showed their ability to interact with receptors as follitropin receptor (FSHR).

Ref: [1] K, Larsson, K, Ljung Björklund, B, Palm, M, Wennberg, L. Kaj, C.H. Lindh, A.G. Jönsson, M, Berglund *Environment Inter.* 73 (2014) 323-333 [2] L.N. Vandenberg, T. Colborn, T.B. Hayes, J.J. Heindel, D.R. Jacobs, Jr., Duk-Hee Lee, T. Shioda, A.M. Soto, F.S. Vom Saal, W.V. Welshons, R.T. Zoeller, J.P. Myers *Endocrine Reviews* 14 (2012) 378-455. [3] C. Li, Z. Wang, Y.J. Yang, J. Liu, X. Mao, Y. Zhang *Chemosphere* 125 (2015) 86-93 [4] M. Bourgin, E. Bichon, J.P. Antignac, F. Monteau, G. Leroy, L. Barritaud, M. Chachignon, V. Ingrand, P. Roche, B. Le Bizec *Chemosphere* 93 (2013) 2814-2822 [5] A. Cariot, A. Dupuis, M. Albouy-Llaty, B. Legube, S. Rabouan, V. Migeot Talanta 100 (2012) 175-182 [6] V. Migeot, A. Dupuis, A. Cariot, M. Albouy-Llaty, F. Pierre, S. Rabouan Environ. Sci. Technol. 47 (2013) 13791-13797. [7] M. Doumas, S. Rouillon, N. Venisse, C. Nadeau, P. Pierre Eugene, A. Farce, P. Chavatte, A. Dupuis, V. Migeot, **P. Carato**. Chlorinated and brominated bisphenol A derivatives: Synthesis, characterization and determination in water samples. Chemosphere (2018), 213, 434-442.