

EVALUATION OF ORGANIC ENDOCRINE DISRUPTORS IN WATER AT BRAZILIAN MULTIPURPOSE REACTOR – RMB INSTALATION AREA

Larissa L. Silva¹, Elaine A. J. Martins², Marycel E. B. Cotrim³ and Maria A. F. Pires⁴

¹ Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
¹ larissa.limeira07@gmail.com

² elaine@ipen.br

³ mecotrim@ipen.br

⁴ mapires@ipen.br

ABSTRACT

The study of pollutants with organic and inorganic characteristics in groundwater and surface waters of a given region is an important tool in the assessment of pollution. Endocrine interferers are synthetic or natural chemicals that have the ability to act on the endocrine system of humans and animals by mimetizing natural hormones and may produce adverse effects on organisms, even in low concentrations (μg or $\text{ng}\cdot\text{L}^{-1}$). Anthropic activities are the major source of input of endocrine disruptors into the environment. The Brazilian government has a project to construct a multipurpose reactor, Brazilian Multipurpose Reactor (RMB), at the Iperó city, to improve the nuclear research Brazilian capacity. The object of this research in to analyze 14 organic compounds that may be present in the groundwater and surface waters of the RMB installation area. This is an unprecedented and extremely important study for the evaluated region; since it will provide guidance on the degree of contamination of the local waters before the construction begins. The study will also make it possible to verify if the construction of the RMB will offer environmental issues to the place. For the determination of the compounds of interest, a developed and validated analytical method was used. This methodology consists of the concentration of the samples by solid phase extraction (SPE) followed by quantification by gas chromatography coupled to the mass spectrometry detector (GC/MS). The water column particulate was also evaluated by ultrasonic extraction, followed by quantification by GC/MS. The results reveal that some of the compounds were found and it was due to anthropic activities in the vicinity of the regions. By initial analysis it was possible verify river that cross the RMB area present values below $0.05 \mu\text{g L}^{-1}$.

1. INTRODUCTION

The lack of water is already pointed out as the main reason for conflicts between nations in the not too distant future. Industrial, agricultural and domestic activities, besides causing a greater consumption of water, also cause a higher degree of contamination of this water ^[1].

Human's civilizations have always settled their communities close to water bodies and nowadays, throughout the world, the highest population densities are still located in coastal

regions or near water bodies ^[2]. It is not only water availability but also its quality to meet basic human needs that is worrying the world. In large urban centers, it is necessary to seek quality water in areas that are increasingly far from the places of consumption. This fact greatly increases the costs of funding, distribution and investment in the sector ^[1;3].

One of the main contributors to the alteration of surface water quality and availability is the anthropic activity, mainly due to the composition of the domestic and industrial sewage that, even after treatment, present concentrations of organic pollutants that are considered responsible for the degradation of water bodies. Water resources in Brazil, both superficial and groundwater, are important instruments for economic and social development, including for the sustainability of the public and private sector ^[3-4].

Contemporary society has been demanding rapid and efficient mechanisms to control environmental contamination processes, as soon as environmental protection becomes more important with each passing day ^[5]. Several agents, such as nutrients (phosphorous, nitrogen), inorganic (toxic metals) and organic compounds (agrochemicals) can alter the quality of water bodies, and/or increase the eutrophication effect, offering health risks for the population. In the aquatic environment, pollution can occur due to urbanization, with industrialization and agricultural expansion also generating its effects. Polluting sources can be classified as point loads, or diffuse loads. In the punctual case, it is by the discharge of effluents, and in the diffuse load case, by the defluence of urban and rural areas ^[6-8].

The aim of this work was to evaluate the characteristics of 14 organic compounds considered endocrine disruptors: diethyl phthalate (DEP), dibutylphthalate (DBP), nonylphenol (NNP), bisphenol A (BPA), pentachlorophenol (PCP), caffeine (CAF), androstane (AND), estrone (E1), estradiol (E2), ethinylestradiol (EE2), progesterone (PROG), coprostanol (COP), cholesterol (CHOE) and cholestanol (CHOA) in surface and groundwater in the area of a new construction project, the Brazilian Multipurpose Nuclear Reactor (RMB), to be installed in the adjacent area of the Aramar Experimental Center of the Technological Center of the Navy in São Paulo (CTM-SP) located in the municipality of Iperó, São Paulo. At Table 1 it is presented a classification of studied compounds.

Table 1. Classification of study compounds

HORMONES	ANTHROPOGENIC	SYNTHETIC
Androstane	Caffeine	Diethyl phthalate
Estrone	Coprostanol	Nonylphenol
Estradiol	Cholesterol	Pentachlorophenol
Ethinylestradiol	Cholestanol	Dibutylphthalate
Progesterone	-	Bisphenol A

This study may contribute to a future assessment if the work of building the RMB, will interfere in the environment.

2. MATERIALS AND METHODS

2.1. Sampling points

Initial information was established for the RMB-PMRA, Pre-Operational Environmental Radiological Monitoring Program, where it was proposed the chemical and radiological monitoring of several environmental matrices, both in the project area and on other significant locations as control samples. In order to monitor the impact area, sampling sites of different environmental matrices that make up the program were preselected: air (particulate material, gases and atmospheric water), water (rainwater, surface water, and groundwater), soil, sediment, agricultural products, milk and pasture. For water, there are ten sites: 4 to surface water and 6 for groundwater. The descriptions of collection points are presented in table 2.

Table 2. Descriptions of collection points

Collection point identification	Collection point description	Geographic coordinates
PC2	Ribeirão do Ferro, upstream of the RMB - Surface water	23°17'25" S 74°09'219"W
P6	Ribeirão do Ferro, downstream of the RMB - Surface water	23°25'23" S 74°11'043"W
P7	Rio Sorocaba, downstream of the Ribeirão do Ferro discharge point - Surface water	23°26'66" S 74°13'599" W
P8	Rio Sorocaba, upstream of the Ribeirão do Ferro discharge point, after the Ipanema River discharge point in the Sorocaba River - Surface water	23°41'69" S 74°14'130" W
P9	São Benedito Farm, downstream of the RMB groundwater water	23°18'84" S 74°13'191" W
LAKE	São Benedito Farm (Lake) - Surface water	23°18'84" S 74°13'191" W
IBAMA Stream	Source of Ibama little river - Surface water	23°16'84" S 74°10'834" W
PC3-120m	Ipanema Farm - Lake Well (\pm 120m deep) - groundwater water	23° 43'58" S 74°06'833 W
PC3-130m	Ipanema Farm - water well (\pm 130m deep) - groundwater water	23° 43'58" S 74°06'833 W
SABESP	Entrance of public supply water-Sarapuí - groundwater water	21°15'03" S 73°82'411" W

2.2. Sample collection

The surface water samples were collected directly from the rivers using a polypropylene recipient and without the addition of preservatives. For groundwater samples, the collection was realized using pumps. Both collection and sampling procedures followed the ANA/CETESB National Guide on Collection and Preservation of Samples ^[9]. Previously decontaminated

amber glass bottles were used for sample conditioning. Until the analysis of organic compounds the samples were kept refrigerated. For the accomplishment of the work, conservation, storage and analysis of the samples, the structure of the Chemistry and Environment Center laboratories of IPEN / CNEN-SP was used.

2.3. Determination of endocrine disruptors

The following solvents and reagents are used for this procedure: dichloromethane (DCM), J.T. Baker, methanol (MeOH), J.T. Baker, acetone, hexane, acetonitrile, ultrapure water (Barnstead™ Purification Systems), hydrochloric acid (HCl), Merck and BSTFA/TMCS (N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (Aldrich). Standards of the studied compounds: diethyl phthalate (DEP), dibutylphthalate (DBP), nonylphenol (NNP), bisphenol A (BPA), pentachlorophenol (PCP), caffeine (CAF), androstane (AND), estrone (E1), estradiol (E2), ethinylestradiol (EE2), progesterone (PROG), coprostanol (COP), cholesterol (CHOE) and cholestanol (CHOA) from Sigma-Aldrich.

The procedure for preparing the water samples from the studied area for chromatographic analysis was adapted from an existing methodology developed in Center of Chemistry and Environment laboratory^[3].

Solid Phase Extraction and CG-MS determination

In the laboratory, samples were filtrated through PTFE membrane with 0.45 µm porosity, acidified to pH 3 with hydrochloric acid solution (v/v 1:1) 3 before solid phase extraction.

The cartridges used in the extraction (C18, SUPELCO, filled with octadecyl) were conditioned with 5 mL of methanol (MeOH), followed by 5 mL of MeOH/H₂O ultrapure solution at pH 3 (1: 9 v/v). A volume of 1 L of the sample was percolated into the cartridge at a flow of approximately 6 mL min⁻¹ and the cleanup was made with methanol/H₂O (v/v 1:9). For complete removal of water, the cartridge was placed under vacuum and then centrifuged for 20 min. at 2500 rpm (rotation per minute). The elution of the analytes was made twice with 5 mL DCM/MeOH (6:4 v/v) each. The extract was completely dried in a N₂ smooth flow. Finally, 100 µL of the derivatizing reagent (BSTFA/TMCS), oven-heated at 100 °C for 30 min.) was added to the dry residue in the vial. After cooling, DCM was used to dilute the sample to 1 mL.

The chromatographic analyzes were carried out on the same day as the derivatization of the analytes. The matrices interfere in the determinations of all compounds, then the degree of interference of each of the matrices was considered.

The particulate material retained on the filter membrane was extracted in an ultrasonic bath with 15 mL of acetonitrile and then 15 mL of 1:1 acetone-hexane solution. Then the supernatant was filtered with the aid of a Millex microfilter with 0.45 µm PTFE membrane into a round bottom flask. The solvent was evaporated to approximately 0.5 mL in a rotary evaporator under a temperature of 50°C. Finally, 250 mL of ultrapure water was added for the SPE cleanup step, followed by the same procedure for the water samples, followed by GC/MS analysis.

3. RESULTS AND DISCUSSION

Surface and groundwater samples collected during days 3, 4 and 5 in October 2016 were filtered, concentrated and analyzed according to the methodology described above. This collection campaign was a very dry season. The results of the concentrations obtained for each analyzed organic compounds were compiled and can be observed in Tables 3 and 4. These results are referent to the collect points described in Table 2.

Table 3: Results of the water samples analysis collected in October 2016 at points PC2, P6, P7, P8 and P9 in the RMB area.

Compounds	LOQ $\mu\text{g L}^{-1}$	PC2	P6	P7	P8	P9
Diethyl phthalate	0.0017	0.046±0.007	0.037±0.005	0.084±0.007	0.056±0.004	0.23±0.03
Nonylphenol	0.0024	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Caffeine	0.0030	0.013±0.002	0.80±0.03	0.92±0.05	0.027±0.002	< LOQ
Pentachlorophenol	0.0019	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Dibutylphthalate	0.0017	0.015±0.001	0.0050±0.0004	0.032±0.006	0.0074±0.0004	0.017±0.001
Androstane	0.0047	< LOQ	< LOQ	0.12±0.01	< LOQ	< LOQ
Bisphenol A	0.0014	0.029±0.001	0.008±0.001	0.070±0.004	< LOQ	< LOQ
Estrone	0.0015	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Estradiol	0.0014	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ethinylestradiol	0.0012	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Progesterone	0.0112	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Coprostanol	0.0039	< LOQ	< LOQ	0.27±0.02	< LOQ	< LOQ
Cholesterol	0.0022	< LOQ	< LOQ	0.32±0.02	< LOQ	< LOQ
Cholestanol	0.0022	< LOQ	< LOQ	0.031±0.004	< LOQ	< LOQ

LOQ = Limit of quantification

Table 4: Results of the of water samples analysis collected in October 2016 at points PC3 120m, PC3 130m, Sabesp, Lake and Ibama stream in the RMB area.

Compounds	LOQ $\mu\text{g L}^{-1}$	PC3 120m	PC3 130m	SABESP	LAKE	IBAMA stream
Diethylphthalate	0.0017	0.29±0.02	0.24±0.02	0.18±0.01	0.08±0.01	0.053±0.004
Nonylphenol	0.0024	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Caffeine	0.0030	< LOQ	< LOQ	< LOQ	< LOQ	0.010±0.001
Pentachlorophenol	0.0019	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Dibutylphthalate	0.0017	0.021±0.002	< LOQ	< LOQ	0.0023±0.0004	0.022±0.001
Androstane	0.0047	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Bisphenol A	0.0014	0.016±0.001	0.007±0.001	0.011±0.001	< LOQ	< LOQ
Estrone	0.0015	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Estradiol	0.0014	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ethinylestradiol	0.0012	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Progesterone	0.0112	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Coprostanol	0.0039	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cholesterol	0.0022	0.022±0.003	< LOQ	< LOQ	< LOQ	< LOQ
Cholestanol	0.0022	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = Limit of quantification

According to the results on table 3 and 4, low concentrations of diethylftalate, caffeine, dibutylphthalate and bisphenol-A compounds were observed in practically all of collection points. At points PC3-120m and PC3-130m, these higher results to diethylphthalate can be principally justified by the material utilized on the confection of the reservatory of water which can be plastic, or may be because these sites have greatest anthropogenic activity. Besides that, the visual observation and the data obtained in the field, at the moment of the collection, help in the understanding of the impact that the urban occupation offers to the quality of the waters of the region. Although the low concentrations of the compounds above mentioned were found, these are indicators of anthropogenic activities and should be monitored as they may affect surface water quality.

As the results for waters, in the Tables 5 and 6 can be observed results for the particulate material from the same water samples collected in October 2016. The analyzed compounds were obtained by PTFE membranes extraction. These results also are referent to the collect points described in Table 2.

Table 5: Results of the particulate material of samples analysis collected in 2016, at points PC2, P6, P7, P8 and P9 in the RMB area.

Compounds	LOQ µg L ⁻¹	PC2	P6	P7	P8	P9
Diethyl phthalate	<i>0.0017</i>	0.018±0.001	0.018±0.001	0.023±0.001	< LOQ	0.022±0.001
Nonylphenol	<i>0.0024</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Caffeine	<i>0.0030</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Pentachlorophenol	<i>0.0019</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Dibutylphthalate	<i>0.0017</i>	0.033±0.006	0.33±0.01	0.015±0.003	0.010±0.001	0.014±0.002
Androstane	<i>0.0047</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Bisphenol A	<i>0.0014</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Estrone	<i>0.0015</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Estradiol	<i>0.0014</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ethinylestradiol	<i>0.0012</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Progesterone	<i>0.0112</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Coprostanol	<i>0.0039</i>	< LOQ	< LOQ	0.006±0.001	< LOQ	< LOQ
Cholesterol	<i>0.0022</i>	< LOQ	< LOQ	0.008±0.002	< LOQ	< LOQ
Cholestanol	<i>0.0022</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = Limit of quantification

Table 6: Results of the particulate material of samples analysis collected in 2016, at points PC3 120m, PC3 130m, Sabesp, Lake and Ibama stream in the RMB area.

Compounds	LOQ µg L ⁻¹	Ibama stream	LAKE	PC3-120	PC3-130	Sabesp
Diethyl phthalate	<i>0.0017</i>	0.019±0.001	0.016±0.002	0.010±0.002	0.019±0.001	0.026±0.001
Nonylphenol	<i>0.0024</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Caffeine	<i>0.0030</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Pentachlorophenol	<i>0.0019</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Dibutylphthalate	<i>0.0017</i>	0.007±0.001	0.025±0.005	< LOQ	0.014±0.002	< LOQ
Androstane	<i>0.0047</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Bisphenol A	<i>0.0014</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Estrone	<i>0.0015</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Estradiol	<i>0.0014</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ethinylestradiol	<i>0.0012</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Progesterone	<i>0.0112</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Coprostanol	<i>0.0039</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cholesterol	<i>0.0022</i>	< LOQ	< LOQ	0.010±0.002	< LOQ	< LOQ
Cholestanol	<i>0.0022</i>	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

LOQ = Limit of quantification

It could be observed that most of the compounds have higher affinity with the water compartment because the results were bigger in water than in particulate material considering the same points for both samples (water and particulate). With the exception of the sterols (coprostanol, cholesterol and cholestanol) which probably had more affinity with the particulate material.

4. CONCLUSIONS

The results obtained in this initial research showed that the place of the RMB project is preserved, but some water bodies around the area, like Sorocaba River, already present small concentrations of organic compounds considered endocrine disruptors, probably due to anthropogenic activities. These results indicate this area needs for a greater attention. Although low concentrations of few compounds, (diethylphthalate, dibutylphthalate, caffeine and bisphenol-A in water and coprostanol, cholesterol, dibutylphthalate and diethyl phthalate in particulate material), have been observed, constant monitoring is necessary to minimize the impact in the studied region.

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