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The Influence of Bisphenol A on adults Humour and Anxiety

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The Influence of Bisphenol A on adults Humour and Anxiety

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TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

The Influence of Bisphenol A on adults Human and Anxiety

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To my parents
and to my lovely sister.

Title: The Influence of Bisphenol A on Adults Humour and Anxiety

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The Influence of Bisphenol A on Adults Humour and Anxiety.

Abstract:

Background/Objective: Bisphenol A (BPA) is one of the most common environmental endocrine disrupter, present ubiquitously in our daily life. Several animal and human studies conclude that there is an association between BPA exposure on early neurodevelopment ages and behavioral alterations, but to date, only few animal studies had study the influence of BPA on a mature neurodevelopment brain. Also, it has been proposed that gender interferes with this association. With our study, we aim to investigate the relation between BPA levels on adulthood and several psychoparameters.

Material and Methods: Urinary BPA concentration and psychoparameters were evaluated in a cohort of 30 young adults. A gender-dependent association was also analyzed. The outcomes measured were based on the Positive and Negative Affect Schedule (PANAS), Hospital Anxiety and Depression Scale (HADS) and Perceived Stress Scale (PSS) scores, translated and validated for the portuguese population.

Results: Urinary BPA was detected in 24 individuals, with a mean of 0.34ug/L. BPA concentration was significantly associated with lower levels of negative affects (B= -9.54; p-value = 0.038). Also, a negative association was found with anxiety (B= -3.65; p-value=0.108) and depressive (B= -2.41; p-value=0.193) indicators. A positive association was shown with higher levels on PSS (B= 4.12; p-value= 0.360). No gender influence was established in the main analyses.

Conclusions: These results suggest that BPA can influence behavioral parameters in a young adult population.

Body Text:

Introduction:

Bisphenol A (BPA) is one of the most common environmental endocrine disrupter, with a production of > 8 billion pounds each year and>100 tons released into the atmosphere yearly (1).

BPA is a monomer used in the manufacture of polycarbonate plastics and epoxy resins, found extensively in our daily life including baby bottles, reusable water bottles, reusable food containers, papers, toys, water pipes, drinking containers, eyeglass lenses, sports safety equipment, dental monomers, medical equipment and tubing, and consumer electronics. BPA has been shown to leach from food and beverage containers, and some dental sealants and composites under normal conditions of use (2, 3). In according to several studies, in humans, BPA can be detected in serum, urine, amniotic fluid, follicular fluid, placental tissue, and umbilical cord blood (2). Ingestion is considered the major route of BPA exposure in humans, although transdermal exposure and inhalation are also possible routes. Indeed, there are works pointing out that BPA was detected in 99 % and 92.6 % of urine samples from German (4) and from US general population (5) , respectively.

Several studies have been conducted to evaluate the impact of BPA on human health. Lang et al. found a positive association between urinary BPA levels and diabetes, heart disease and liver toxicity (6); other associations that have been detected are with: obesity, endometrial hyperplasia, endometriosis and PCOD, chromosomal abnormalities and recurrent miscarriage (2).

In addition to the issues referred, other major concern about BPA levels and human health is its impact on neurobehavioral. Several animal studies conclude that there is an association between BPA exposure and behavioral alterations, namely anxiety and depression-like behavior. However these results are contradictory in relation to gender dependence/ independence association, increase/ decrease in anxiety and depression and the time of exposure (7-14). Most studies have been realized in rodents with prenatal or perinatal exposure and only few have been taken in account the exposure in young adulthood, a time when also important neuronal alteration can take place (15-

17).
To date, only few human studies have been realized, all of them in children with less than 11 years of age and also with contradictory conclusions (18-22).
There is a lack of data about this compound exposure in adulthood and neurobehavioral dysfunctions. To address more information to this issue, in the present work, we aim to study the putative association between the levels of urinary BPA and anxious and depressive symptoms in a population of 30 young adults. Since BPA is an endocrine disruptor, the study was realized in a male and in a female sample.

Methods:

Selection of the individuals: The Ethics Committee of Centro Hospitalar São João**,** EPE - Hospital de São João/Faculty of Medicine of the Porto University and informed consent was obtained from all study participants. The individuals of this study were volunteers that respond to an advertisement published in the student community of the Faculty of Medicine of Porto University. Inclusion criteria were: age between 20 and 23 years old and attending clinical practice. From the volunteers 15 male students and 15 female students were randomly selected.

Data Source: demographical and psychometric data were collected from self-report inventories that individuals responded in a controlled environment. The scales used were the Perceived Stress Scale, the Hospital Anxiety and Depression Scale and the Positive and Negative Affect Schedule, all of them translated and validated for the portuguese population (23-25).

Perceived Stress Scale measures the degree to which respondents perceive their lives to be stressful, uncontrollable, unpredictable, and overloaded during the previous month. The PSS consists of 10 items using five-point scales, each ranging from 0 (never) to 4 (very often). Half the items are reverse coded, and all the scores are then summed to obtain a total score (26).

Hospital Anxiety and Depression Scale records levels of depression and anxiety symptoms. HADS consists in 14 statements, 7 related to anxiety symptoms and 7 related to depression levels, score on a four-point scale. The sum score of each subscale (levels anxiety and depression symptoms) ranges from 0 to 21 (27).

Positive and negative affect: This scale instructs participants to rate to what extent they generally have experienced 20 different feelings or emotions (10 positive affects and 10 negative affects) during the previous weeks, from 1 (very slightly) to 5 (extremely) (28).

Urinary BPA Concentrations:

The volunteers were asked to collect the first urine of the morning, to a proper recipient, being advised not to practice vigorous exercise the day before. The urines were collected in the same day and stored at -20ºC until analysis. Later, total urinary BPA concentrations were measured according to the procedure described by Mansilha et al. (29), using a solid phase extraction combined with liquid chromatography tandem mass spectrometry. The urine samples were incubated with E. coli beta-glucuronidase to obtain the free and the conjugated forms. The chromatographic analyses were carried out in a Thermo Trace-Ultra Gas Chromatograph coupled to an Ion trap mass detector thermo polaris. The limit of detection (LOD) was 0.0019 µg/L and the Limit of Quantification was 0.0062 µg/L. The method accuracy was 68%. The BPA levels were normalized with urinary creatinine concentrations.

Covariates:

Covariates were selected based on whether they were known or suspected risk factors according to the literature. We included the following potential confounding variables: social-economic status, chronic disease, medications, smoking and alcohol consumption. Since all the individuals were medical students and none was doing any pharmacological treatment, we did not consider education and medication as confounders.

Statistical Analysis:

Descriptive statistics was used to analyze social-demographic factors. To control urine dilution BPA concentration was divided by urinary creatinine to obtain normalized values. The scales outcomes and the BPA-creatinine-standardized-concentrations followed a normal distribution and were examined as continuous variables. Individual differences in respect to the psychometric scores and BPA concentrations were estimated using Student's t-tests. We performed multiple linear regression analyses using urinary BPA as the independent variable, and adjusting for various confounders: gender, socioeconomic status, smoking and alcohol habits. Next, we did the same analyzes but comparing results by gender. All statistical analyses were performed using IBM® SPSS Statistics, version 21.0. All results were considered statically significant when p-value was less than 0.05.

Results:

A total of 30 individuals participated in the study. Six were excluded: two didn't have detectable levels of urinary BPA (LOD < 0.0019 µg/L); three had detectable levels, but they were too high for the calibration curve used. The last individual was excluded because he had outliers scores in the PSS and in the negative PANAS scales. No other parameters invalidated the participation of the volunteers. The mean age was 23 years old (range 22-24); 12 were female and 12 were male (50.00%). All were single and higher educated; no one suffered from psychiatric disorders or was taking medication (See Table 1).

Urinary BPA concentration was standardized with creatinine level, to diminish the error associated with urine dilution. The levels ranged from 0.04 µg/L to 1.35 µg/L, with a mean of 0.34 µg/L (SD 0.33). The difference between female and male urinary BPA-creatinine-standardized levels was not statically significant (table 2). The scales were normally distributed; Table 3 shows the mean and the standard deviation for each scale: the Positive PANAS scale had a mean score of 33.38, with a minimum value of 25 and maximum of 45. The mean negative PANAS was 22.04, ranging from 11 to 34; The HADS anxiety subscale had values between 3 and 15, with a mean of 7.71. Depression subscale had lower levels: mean of 3.50, extending from 0 to 9. The PSS showed the values between 10 to 34, being the mean 22.58. None of the scales presented significant differences among females and males. The Pearson correlation between the scales is presented in table 4: although they evaluate different parameters, they are related to each other. The associations between urinary BPA and the psychoparameters evaluated, are showed in table 5. After correction for the co-varieties, the analyses revealed: a negative association statically significant was found between urinary BPA and negative PANAS scores (Beta unstandardized= -9.54; R2= 0.29; p-value = 0.038). BPA is also negatively associated with anxiety (Beta unstandardized= -3.65; R2= 0.24; p-value=0.108) and depression (Beta unstandardized= -2.41; R2=0.11; p-value=0.193) on HADS. A positive association was found with higher levels on PSS (Beta unstandardized= 4.12; R2=0.14; p-value= 0.360). Although the beta unstandardized calculated for the gender is very low, taking into account the literature and the fact that our sample is small and can therefore dissipate some interactions, we did a separate analyses for females and males. Table 6 presents the results by sex: Positive PANAS score have different values on females and males: in the first, BPA is positively associated with positive affects (Beta unstandardized= 8.91; R2=0.25; p-value=0.283) while the opposite happens in males (Beta unstandardized= 6.73; R2=0.34; p-value=0.181). The others parameters show equal interactions although females have stronger associations in the negative affects (R square=0.44 in females versus R square= 0.22 in males) and depression levels (R square=0.39 versus R square= 0.23). The anxiety parameter and the perceived stress have the same level of association in both genders (R square= 0.33 for females, and R square=0.35; R square= 0.31 for females, and R square=0.38, respectively).

Discussion:

Bisphenol A is increasing its importance because of its adverse effects in various systems, namely in Central Nervous System with consequent neurobehavioral disruptions. Nevertheless, the majority of the studies in this area are: 1) with animal models and 2) the human studies, focus their attention in early development stages, namely on pre-natal, postnatal and childhood exposure. Our study is pioneer since it is the first that establish a relation between neurobehavioral alterations and BPA exposure in adulthood on humans. Currently, increasing evidence points to alterations in various psychometric parameters, although the data are controversial: some studies point to an anxiogenic effect, others to a null or even anxiolytic action; the same incongruence also persists in relation to depression levels and other parameters (not discuss in this article). In our study, we concluded that BPA is associated with an anxiogenic effect (lower scores in the subscale anxiety of HADS) and with inferior levels of depressive symptoms and negative affects (measured with the subscale depression of HADS and with the PANAS). However, BPA was positively associated with higher levels of perceived stress. Our findings are in agreement with the data presented by Farabollini et al. (13) and Perera et al. (21), but in disagreement with others, such as Hong et al. (22) that reported a positive association not only with anxiety but also with depression scores. Some attention must be taken when interpreting this evidence: the concentrations of BPA depends on the exposure, for example through food and beverage containers, dental products, coating of CDs, DVDs, electronic equipment, automobiles and sports equipment, among others materials. Thus, high levels of BPA could be associated with life styles that in the short term can result in wellness (and therefore inferior levels of anxiety and depressive symptoms and negative affects) but, with prolonged exposure, may lead to harmful effects, evidenced later in life. Other issue in debate is the possibility of different responses to BPA between female and male individuals. Although our results didn't achieve a p-value < 0.05, a stronger association was observed in females in relation to negative and depressive scores. Once again, regarding this point, data is inconclusive both in animal and in human studies. To refer some: Braun et al. (18) as Hong et al. (22) reported null gender interaction whereas Perera et al. (21) and Harley et al. (20) conclude that boys and girls have different responses to BPA. This response may depend on several factors, namely dose, routes and age of exposure, as is evidenced by Braun et al. (18) and Perera et al. (21), that reported alterations in child behavior with prenatal but not with childhood exposure. Several studies present possible mechanisms underlying the disruptions induce by BPA on early neurodevelopment: alterations in neocortical and spatiotemporal gene expressions (30); disruptions in both dendritic and synaptic development (31); interference with estrogenic, androgen and thyroid signaling (32); changes in the dopaminergic system (33). However, there is lack of information about the influence of this endocrine disruptor on a mature central nervous system, such as the individuals of our study. Luo et al. (15) attempted to elucidate this point: they reported that mice exposed during puberty to BPA had significant lower levels of AChE activity in the hippocampus in the adulthood; which can explain altered anxiety patterns. Future studies are needed to establish which periods are BPA-susceptible, levels needed, the effects induced and the mechanisms underlying it.

There were some limitations to this study. A single first urine sample may not predict the real BPA exposure, due to its short biological half-life and to temporal variability of BPA urinary concentration (1). In fact, the median concentration of our sample (0.36 ng/mL unadjusted and 0.36 ng/mL creatinine adjusted) is lower than other reports (2.2 ng/ml unadjusted and 1.63 ng/mL creatinine adjusted). Another point that needs to be further evaluated is the possible routes of BPA exposure, for example diet and dental products, which can explain the extreme BPA concentrations of some samples (namely 3 values very high and two inferior to the LOD). Our sample size was very modest, which reduced the statistical power and also impended to conclude about a gender-dependent association. Other point that took our attention was that our sample

presented higher levels of perceived stress and negative affects, compared to the general population with the same age (26, 28). Extend the study to other demographic groups may overcome this issue.

In summary, our findings suggest possible effects of BPA on psychometric parameters on young adults. Additional studies are necessary to evaluate the action on behavior of this endocrine disruptor, present ubiquitous in our daily life.

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Table 1 - Social-demographical characteristics of the individuals included in the study

Demographic Variables	Total (N=24)	Female (N=12)	Male (N=12)
Age (years): M (SD)	23.00 (0.30)	22.92 (0.29)	23.08 (0.30)
Civil state (single)	100.00	100.00	100.00
Education (Higher education,%)	100.00	100.00	100.00
Socioeconomic status			
Lower middle class (%)	41.70	41.70	41.70
Upper middle class (%)	58.30	58.30	58.30
Chronic Diseases (%)	0.00	0.00	0.00
Psychiatric disorders	0.00	0.00	0.00
Chronic Medication (%)	0.00	0.00	0.00
Smoking (%)			
Never	45.80	66.70	25.00
Ocasional	37.50	25.00	50.00
Less than 10/day	4.20	0.00	8.30
11-20/day	12.50	8.31	16.70
Alcohol Habits (%)			
Never	0.00	0.00	0.00
1 /month	16.70	25.00	8.30
2-4/month	66.70	75.00	58.30
2-3/week	16.70	0.00	33.30

M- Mean; SD – Standard Deviation;

Table 2- First morning urine BPA unadjusted and creatinine adjusted concentrations

Urinary Values	All (N:24)	Female (N:12)	Male (N:12)	p-value
Bisphenol Unadjasted (ug/L) (M, SD)	0.37 (0.23)	0.35 (0.21)	0.39 (0.26)	0.688
Bisphenol Creatinine Adjusted (ug/L) (M,SD)	0.34 (0.33)	0.39 (0.30)	0.28 (0.36)	0.462

[a] Creatinine-adjusted urinary BPA concentrations were calculated by dividing urinary BPA concentrations by urinary creatinine concentrations, to control for urine dilution.
M – Mean; SD- Standard Deviation; p-value (<0,05).

Table 3 – Distribution of the PANAS, HADS and PSS score outcomes in the cohort of 24 individuals

Scales Mean (minimum and maximum)	All (N:24)	Female (N:12)	Male (N:12)	p-value
PANAS				
Positive	33.38 (25, 45)	32.75 (25, 45)	34.00 (29, 40)	0.567
Negative	22.04 (11, 34)	22.17 (11,34)	21.92 (12, 32)	0.932
HADS				
Anxiety	7.71 (3, 15)	7.50 (3,15)	7.92 (3, 13)	0.771
Depression	3.50 (0, 9)	3.50 (1,9)	3.50 (0, 8)	1.00
PSS	22.58 (10, 34)	22.08 (10,27)	23.08 (15, 34)	0.714

PANAS - Positive and negative affect; HADS - Hospital Anxiety and Depression Scale; PSS - Perceived Stress Scale. p- value (< 0,05)

Table 4 – Pearson Correlation between the PANAS, HADS and PSS

		PANAS positive	PANAS negative	HADS anxiety	HADS depression	PSS
PANAS positive	Pearson correlation p-value	1	-0.36 0.077	-0.39 0.62	-0.45 0.28	0.24 0.257
PANAS negative	Pearson correlation p-value	-0.39 0.077	1	0.73 0.000	0.71 0.000	-0.53 0.008
HADS anxiety	Pearson correlation p-value	-0.39 0.062	0.73 0.000	1	0.61 0.002	-0.66 0.000
HADS Depression	Pearson correlation p-value	-0.45 0.028	0.713 0.000	0.61 0.002	1	-0.51 0.011
PSS	Pearson correlation p-value	0.24 0.257	-0.53 0.008	-0.66 0.000	-0.51 0.011	1

N=24

p-value < 0,05

HADS - Hospital Anxiety and Depression Scale; PANAS - Positive and negative affect Scale; PSS - Perceived Stress Scale

Table 5 – Adjusted association between urinary bisphenol A concentration and the scores from the PANAS, HADS and PSS.

Scales (N=24)	Beta unstandardized	95 % CI	p - value	R square
PANAS				
positive	0.16	-7.41, 7.74	0.964	0.09
negative	-9.54	-18.50, -0.58	0.038	0.29
HADS				
anxiety	-3.65	-8.02, 0.89	0.108	0.24
depression	-2.41	-6.12, 1.33	0.193	0.11
PSS	4.12	-5.10, 13.33	0.360	0.14

The analyses were adjusted for socioeconomic status, smoking, alcohol habits and gender (relatively to gender, beta-undstandardized for each scale between -0.15 and 0.96)

Creatinine-standardized values were used for urinary bisphenol A concentration.

p-value < 0,05

CI - confidence inter; HADS - Hospital Anxiety and Depression Scale; PANAS - Positive and negative affect Scale; PSS - Perceived Stress Scale.

Table 6 – Comparison of the adjusted association between urinary bisphenol A concentration and the scores from the PANAS, HADS and PSS in females and in males.

	Female (N=12)				Male (N=12)			
Scales	Beta unstardardized	95 % CI	p - value	R square	Beta unstardardized	95 % CI	p - value	R square
PANAS								
positive	8.91	-9.23, 27.06	0.283	0.25	-6.73	-17.43, 3.97	0.181	0.34
negative	-12.06	-28.11, 3.98	0.119	0.44	-8.69	-29.78, 12.41	0.362	0.22
HADS								
anxiety	-7.44	-.18.29, 3.40	0.148	0.33	-4.05	-11.60, 9.43	0.245	0.35
depression	-4.25	-10.85, 2.36	0.172	0.39	-0.94	-8.52, 6.63	0.777	0.23
PSS	7.11	-12.30, 26.53	0.415	0.31	2.94	-13.81, 19.70	0.690	0.38

The analyses were adjusted for socioeconomic status, smoking and alcohol habits. Creatinine-standardized values were used for urinary bisphenol A concentration.

p-value < 0,05

CI - confidence inter; HADS - Hospital Anxiety and Depression Scale; PANAS - Positive and negative affect Scale; PSS - Perceived Stress Scale; SE, standard error.

Acknowledgments

I would like to express my gratitude towards Professor Conceição Calhau for the guidance, encouragement and opportunities provided, without her this work wouldn't be possible. Furthermore I would like to acknowledge with much appreciation Professor Rui Coelho for introducing me to Mental Health and the possibility of developing this project. In addition, a thank you to Professor Margarida Figueiredo for the amiability and total disposal offered. I also would like to acknowledge Dr. Luísa Sá and the others investigators of the group for the patience, availability and sympathy.
I would like to thank Francisco, for the tolerance, assistance and interest with this work. Last but not least, I would like to thank my colleagues for the availability to participate in this project.

Annexes:

1. Instructions for authors of The International Journal of Clinical Neurosciences and Mental Health

2. Positive and Negative Affect Schedule (PANAS)

3. Hospital Anxiety and Depression Scale (HADS)

4. Perceveid Stress Scale (PSS)

5. Ethical Committee approval of the The Ethics Committee of Centro Hospitalar de São João, EPE - Hospital de São João/Faculty of Medicine of the University of Porto

INSTRUCTIONS FOR AUTHORS

Contents

1. Aims and Scope

The International Journal of Clinical Neurosciences and Mental Health is an open-access peer-reviewed journal published trimonthly by ARC Publishing.

Our goal is to provide high-quality publications in the areas of Psychiatry and Mental Health, Neurology, Neurosurgery and Medical Psychology. Expert leaders in these medical areas constitute the international editorial board.

The journal publishes original research articles, review articles, drug reviews, case reports, case snippets, viewpoints, letters to the editor, editorials and guest editorials.

The International Journal of Clinical Neurosciences and Mental Health follows the highest scientific standards, such as the CONSORT / STROBE guidelines and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (ICJME).

The journal offers:

- Trusted peer review process
- Fast submission-to-publication time
- Open-access publication without author fees
- Multidisciplinary audience and global exposure

2. Types of papers

The International Journal of Clinical Neuroscience and Mental Health publishes scientific articles in the following categories:

- Original research articles.
- Reviews.
- Drug reviews.
- Case reports.
- Case snippets.
- Viewpoints.
- Letters to the editor.
- Editorials and guest editorials.

2.1. Original research articles

The International Journal of Clinical Neurosciences and Mental Health welcomes original clinical research related with psychiatry, mental health, medical psychology, neurosurgery and neurology.

Reports of randomized clinical trials should follow the CONSORT Guidelines and reports of observational studies should comply with STROBE Guidelines.

Body text of an Original Research Article should have no more than 4000 words (word count excludes title page, abstract, acknowledgments, references and tables). A maximum of 6 illustrations (figures or tables) are allowed. Supplementary online material may be submitted at the editor discretion.

2.2. Review articles and Drug Reviews

Review articles on CNS-related drugs, psychiatry, mental health, medical psychology, neurosurgery and neurology topics are welcome. Both invited and unsolicited submissions are accepted.

Manuscripts should be limited to a maximum of 4,500 words, excluding title page, abstract, acknowledgments, references and tables.

2.3. Case reports and case snippets

Case Reports and Case Snippets should have no more than 750 and 500 words, respectively (word count excludes references); one figure or table can be included.

Only highly meaningful Case Reports are accepted, including major educational content or major clinical findings. Case Snippets should describe a diagnosis or therapeutic challenge.

2.4. Viewpoints

Viewpoints should provide an expert opinion on important topics for medical research or practice, with possibility for covering social and policy aspects. This section encourages dialogue and debate on relevant issues with expert views based on evidence.

Viewpoints are limited to 1500 words (word count excludes references) and can include one figure or table.

2.5. Letters to the Editor

Letters to the Editor should share views on published articles, any findings insufficient for a research article or present ideas of any subject in the scope of the journal.

Letters to the Editor have a maximum of 600 words (including references) and can include one figure or table.

2.6. Editorials and Guest Editorials

Authors are invited by the Editor-in-Chief to comment on specific topics and express their opinions. Editorials and Guest Editorials have a maximum of 1,000 words and can include one figure or table.

3. Manuscript Submission

These instructions advise on how the manuscript should be prepared and submitted. Manuscripts that do not comply with the guidelines will not be considered for review.

All manuscripts should be prepared in A4-size or US-letter size, in UK or US English.

Manuscripts should be submitted in *.doc and *.pdf formats, in the appropriate section of the journal website: IJCNMH online submission.

3.1. Cover Letter

A cover letter should be submitted together with the manuscript, in *.doc or *.pdf format, addressed to the Editor-in-Chief.

A template for the cover letter is available for download.

The cover letter should contain statements about originality of your publication, Ethics Committee approval and informed consent (if applicable), conflicts of interest and why in your opinion your manuscript should be published.

3.2. Manuscript Preparation

The manuscript must be divided in 2 files: the Title page (submitted in *.doc format and *.pdf formats) and the Manuscript body (submitted in *.doc and *.pdf formats).

Title page

This should be submitted as a separate file from your manuscript (to assure anonymity in the peer review process) and should include:

- Article title.
- Authors' names, titles (e.g. MD, PhD, MSc, etc.) and institutional affiliations.
- Corresponding author: name, mailing address, telephone and fax numbers.
- Keywords (maximum of 10).
- A running head (up to 50 characters).
- Abstract word count (up to 250 words).
- Body text word count.
- The number of figures and tables.

Manuscript body:

The Manuscript body must be anonymous, not containing the names or affiliations of the authors. Manuscript body must be structured in the following order: title, abstract, body text, acknowledgements, references, tables, and figures captions/legends.

- The text must be formatted as follow:
- Arial fonts, size: 11 points.
- Single line spacing (see paragraph menu).
- Aligned to the left (not justified).

Showing continuous line numbers on the left border of the page. For MS Word you can add line numbers by going to: Page Layout -> Line Numbers -> select "Continuous"; for OpenOffice: Tools -> Line Numbering -> tick "Show numbering".

Title

A descriptive and scientifically accurate article title should be provided.

Abstract (250 words maximum)

An abstract should be prepared for Original Research Articles, Review Articles and Drug Reviews.

Should be structured and include: background/objective, material and methods, results, and conclusions. These sections should be separated by the respective headings.

If the publication is associated with a registered clinical trial, the trial registration number should be referred at the end of the abstract.

Body text

Original research articles

Original research articles should be structured as follows:

Introduction: Should present the background for the investigation and justify its relevancy. Claims should be supported by appropriate references. Introduction should end by stating the objectives of the study.

Methods: Should allow the reproduction of results and therefore must provide enough detail. Appropriate subheadings can be included, if needed.

Results: Should include detailed descriptions of generated data. This section can be separated into subsections with concise self-explanatory subheadings.

Discussion and Conclusions: Should be brief but comprehensive and well argued, summarise and discuss the main findings, their clinical relevance, the strengths and limitations of the study, future perspectives with suggestion of experiments to be addressed in the future.

Review articles and Drug Reviews

These types of articles should be organized in sections and subsections.

Acknowledgements

This section should name everyone who has contributed to the work but does not qualify as an author. People mentioned in this section must be informed and only upon consent should their names be included along with their contributions. Financial support (with grant number, if applicable) should also be stated here.

Any conflict of interests should be declared. If authors have no declaration it should be written: "The authors declare no conflict of interests".

References

References citation in the text should be numbered sequentially along the text, within brackets.

The use of a reference management tool (such as Endnote or Reference Manager) is recommended. References must be formatted in Vancouver style.

Only published or accepted for publication material can be referenced. Personal communications can be included in the text but not in the references list.

Tables

Tables should be smaller than a page, without picture elements or text boxes. Tables should have a concise but descriptive title and should be numbered in Arabic numerals. Table footnotes should explain any abbreviations or symbols that should be indicated by superscript lower-case letters on the body table.

Figures

Figures should have a concise but descriptive title and should be numbered in Arabic numerals. If the article is accepted for publication, the authors may be asked to submit higher resolution figures. Copyright pictures shall not be published unless you submit a written consent from the copyright holder to allow publishing.

Each figure file shall not be larger than 30MB.

Figures should be tested and printed on a personal printer prior submission. The printed image, resized to the intended dimensions, is almost a replication of how the picture will look online. It shall be clearly perceived, non-pixelated nor grainy. Only flattened versions of layered images are allowed. Each figure can only have a 2-point white space border, thus cropping is strongly advised. For text within figures, Arial fonts between 8 to 11 points should be used and must be readable. When symbols are used, the font information should be embedded.

Photographs should be submitted as *.tif or *.eps at high-resolution (300 dpi or more). Graphics should be submitted in *.eps format. MS Office graphics are also acceptable.

All figures, tables and graphics should have white background and not transparent.

Lines, rules and strokes should be between 0.5-1.5 points for reproducibility purposes.

3.3. Supporting Information

Code of Experimental Practice and Ethics

The minimal ethics requirements are those recommended by the Code of Ethics of the World Medical Association (Declaration of Helsinki). Authors should provide information regarding ethics on research participants, patient informed consent, data privacy as well as competing interests. If the authors have submitted a related manuscript elsewhere should disclose this information prior submission.

Nomenclature

All units should be in International System (SI). Drugs should be designated by their International Non-Proprietary Name (INN).

3.4. Submission Checklist

Please ensure you have addressed the following issues prior submission:

- Details for competing interests.
- Details for financial disclosure.
- Details for authors contribution.
- Participants informed consent statement.
- Contributor copyright authorization of figures included in the manuscript, not produced by the authors and subjected to copyright.
- Authorship, affiliations and email addresses are correct.

- Cover letter addressed to the Editor-in-Chief.
- Identification of potential reviewers and their email addresses (to be introduced at the online submission platform).
- Manuscript, figure and tables comply with the author guidelines, including the correct format, SI units and standard nomenclature.
- Separated files for Title page (*.doc and *.pdf) and Manuscript body (*.doc and *.pdf)—4 in total.
- Manuscript body does not contain the names or affiliations of the authors.

If you have any questions, please contact ijcnmh@arc-publishing.org

4. Overview of the Editorial Process

The International Journal of Clinical Neurosciences and Mental Health aims to provide an efficient and constructive view of the manuscripts submitted to achieve a high quality level of publications. The editorial board is constituted by expert leaders in several areas of medicine particularly in Clinical Neuroscience and Mental Health.

Once submitted, the manuscript is assigned to an editor which evaluates and decides whether the manuscript is accepted for peer-review. At this initial phase, the editor evaluates if the manuscript fulfils the scope of the journal according to the content and minimum quality standards. For peer-review, one or two additional expert field editors will comment on the manuscript and decide on whether it is accepted for publishing with minor corrections or not accepted for publishing. The editor may ask authors to resubmit after major revision. Decision is based on technical and scientific merits of the work. Reviewers can be asked to be disclosed or stay anonymous. Authors can exclude specific editors or reviewers from the process, upon submission, a rational should be provided.

Upon evaluation, an email is sent to the corresponding author with the decision. If accepted, the manuscript enters the production process. It takes approximately 6-7 weeks for the manuscript to be published.

4.1. Appeal Process

The editors will respond to appeals from authors which manuscripts were rejected. Their interests should be sent to the Editor.

Two directions can be followed:

- If the Editor does not accept the appeal, further right to appeal is denied.
- If the Editor accepts the appeal, a further review will be asked. After the new review, the editor can reject or accept the appeal. If rejected, nothing else can be done, if accepted the author is able to resubmit the manuscript.

The reasons for not accepting a manuscript for consideration can be:

- The manuscript does not follow the scope of the journal.
- The manuscript has potential interest but there are methodological concerns after peer-review or editorial examination.

Positive and Negative Affect Schedule - PANAS

Esta escala consiste num conjunto de palavras que descrevem diferentes sentimentos e emoções. Leia cada palavra e marque a resposta adequada no espaço anterior à palavra. Indique em que medida sentiu cada uma das emoções durante a última semana:

1- Nada ou muito Ligeiramente.
2 - Um Pouco
3 - Moderadamente
4 - Bastante
5 - Extremamente

___ Interessado
___ Orgulhoso
___ Perturbado
___ Irritado
___ Excitado
___ Encantado
___ Atormentado
___ Remorsos
___ Agradavelmente surpeendido
___ Inspirado
___ Culpado
___ Nervoso
___ Assustado
___ Determinado
___ Caloroso
___ Trémulo
___ Repulsa
___ Activo
___ Entusiasmado
___ Amedrontado

ESCALA DE ANSIEDADE E DEPRESSÃO HOSPITALAR – HADS

Este questionário foi construído para ajudar a saber como se sente. Pedimos-lhe que leia cada uma das perguntas e faça uma cruz (X) no espaço anterior à resposta que melhor descreve a forma como se tem sentido na última semana. Não demore muito tempo a pensar nas respostas. A sua reacção imediata a cada questão será provavelmente mais correcta do que uma resposta muito ponderada.
Por favor, faça apenas uma cruz em cada pergunta.

1. Sinto-me tenso/a ou nervoso/a:
( ) Quase sempre
( ) Muitas vezes
( ) Por vezes
( ) Nunca

2. Ainda sinto prazer nas coisas de que costumava gostar:
( ) Tanto como antes
( )Não tanto agora
( )Só um pouco
( ) Quase nada

3. Tenho uma sensação de medo, como se algo terrível estivesse para acontecer:
( ) Sim e muito forte
( ) Sim, mas não muito forte
( ) Um pouco, mas não me aflige
( ) De modo algum

4. Sou capaz de rir e ver o lado divertido das coisas:
( ) Tanto como antes
( )Não tanto como antes
( ) Muito menos agora
( ) Nunca

5. Tenho a cabec¸a cheia de preocupaçõess:
( ) A maior parte do tempo
( ) Muitas vezes
( ) Por vezes
( ) Quase nunca

6. Sinto-me animado/a:
( ) Nunca
( ) Poucas vezes
( ) De vez em quando
( ) Quase sempre

7. Sou capaz de estar descontraidamente sentado/a e sentir-me relaxado/a:
( ) Quase sempre
( ) Muitas vezes
( ) Por vezes

( ) Nunca

8. Sinto-me mais lento/a, como se fizesse as coisas mais devagar:
( ) Quase sempre
( ) Muitas vezes
( ) Por vezes
( ) Nunca

9. Fico de tal forma apreensivo/a (com medo), que até sinto um aperto no estômago:
( ) Nunca
( ) Por vezes
( ) Muitas vezes
( ) Quase sempre

10. Perdi o interesse em cuidar do meu aspecto físico:
( ) Completamente
( )Não dou a atenção que devia
( ) Talvez cuide menos que antes
( ) Tenho o mesmo interesse de sempre

11. Sinto-me de tal forma inquieto/a que não consigo estar parado/a:
( ) Muito
( ) Bastante
( )Não muito
( ) Nada

12. Penso com prazer nas coisas que podem acontecer no futuro:
( ) Tanto como antes
( )Não tanto como antes
( ) Bastante menos agora
( ) Quase nunca

13. De repente, tenho sensações de pânico:
( ) Muitas vezes
( ) Bastantes vezes
( ) Por vezes
( ) Nunca

14. Sou capaz de apreciar um bom livro ou um programa de rádio ou televisão:
( ) Muitas vezes
( ) De vez em quando
( ) Poucas vezes
( ) Quase nunca

MUITO OBRIGADO PELA SUA COLABORAÇÃO.

Perceived Stress Scale

Para cada questão, pedimos que indique com que frequência se sentiu ou pensou de determinada maneira, **durante o ultimo mes**. Apesar de algumas perguntas serem parecidas, existem diferenças entre elas e deve responder a cada uma como perguntas separadas. Responda de forma rápida e espontânea. Para cada questão, escolha a alternativa que melhor se ajusta à sua situação.

0 - Nunca. 1 - Quase nunca. 2 - Algumas vezes. 3 - Frequentemente. 4 - Muito frequentemente.

1. No último mês, com que frequência esteve preocupado(a) por causa de alguma coisa que aconteceu inesperadamente?
2. No último mês, com que frequência se sentiu incapaz de controlar as coisas importantes da sua vida?
3. No último mês, com que frequência se sentiu nervoso(a) e em stresse?
4. No último mês, com que frequência sentiu confiança na sua capacidade para enfrentar os seus problemas pessoais?
5. No último mês, com que frequência sentiu que as coisas estavam a correr à sua maneira?
6. No último mês, com que frequência sentiu que não aguentava com as coisas todas que tinha para fazer?
7. No último mês, com que frequência foi capaz de controlar as suas irritações?
8. No último mês, com que frequência sentiu ter tudo sob controlo?
9. No último mês, com que frequência se sentiu furioso(a) por coisas que ultrapassaram o seu controlo?
10. No último mês, com que frequência sentiu que as dificuldades se estavam a acumular tanto que não as conseguia ultrapassar?

Parecer

Título do Projecto: Níveis de poluentes ambientais e parâmetros psicométricos numa população de jovens adultos

Nome do Investigador Principal: Rui Coelho e Conceição Calhau

Entidade Promotora: Faculdade de Medicina da Universidade do Porto

Serviço onde decorrerá o Estudo: NA

Objectivo e concepção do Estudo:

São objectivos deste projecto de investigação:

1. Caracterização de alguns parâmetros de saúde mental como sintomas depressivos, nível de ansiedade, nível de stresse, sintomas positivos e sintomas negativos, e, em paralelo, quantificar níveis plasmáticos de poluentes ambientais tais como organoclorados e ftalatos.
2. Correlacionar o perfil psicológico com os níveis de contaminação.

Os poluentes avaliados são alteradores endócrinos, existindo evidências na literatura para uma relação como alteradores neuroendócrinos e metabólicos, pelo que é pelos autores considerada relevante a investigação apresentada. O recrutamento de voluntários (15 do sexo M e 15 do sexo F) será de jaez de conveniência, entre os estudantes dos 4º, 5º e 6ºs anos do MIMED, com divulgação através de edital no Sigarra e por e-mail dinâmico da FMUP (autorização da realização do estudo pela Sr.ª Directora do Curso do MIMED).

A metodologia do projecto, e para que se alcancem os objectivos próprios da investigação, prevê a realização de colheitas sanguíneas para doseamento de poluentes ambientais, como organoclorados e ftalatos. Todas as despesas inerentes serão suportadas pela equipa de investigação.

Serão ainda realizados questionários para avaliação da saúde mental.

O projecto é financiado pela FCT (as despesas serão indirectamente alocadas a projectos já em curso!)

Benefício/risco: Benefícios: informação sobre os próprios resultados obtidos. Riscos: apenas o inerente colheita sanguínea e o incómodo de se deslocarem ao Departamento de Bioquímica da FMUP para essa mesma colheita.

Respeito pela liberdade e autonomia do sujeito de ensaio: Será solicitado consentimento aos participantes no estudo, mediante informação esclarecedora que possa validar a liberdade da sua decisão.

Confidencialidade dos dados: Serão codificados os dados a obter.

Elo de ligação: NA

Indemnização por danos: NA

Continuação do tratamento: NA

Propriedade dos dados: "*Os dados obtidos serão propriedade exclusiva do promotor/Investigador, é assim referido, tal como a indicação da existência de critérios de publicação dos resultados da investigação.*" Trata-se de um projecto que visa ainda a realização do projecto de opção de uma aluna do 5º ano, como é transparente na informação a disponibilizar aos participantes. A publicação dos dados é, assim, previsível.

Currículum do investigador: Adequado à investigação

Data previsível da conclusão do estudo: Janeiro 2014

Conclusão:

Considerados os objectivos e a metodologia que lhe será dedicada, o parecer da CES é favorável à realização deste projecto de investigação, na sua actual definição.

Porto e H.S.João, 2013-07-18

O Presidente da CES

Doutor Filipe Almeida

7. SEGURO

a. *Este estudo/projecto de investigação prevê intervenção clínica que implique a existência de um seguro para os participantes?*

SIM ☐ (Se sim, junte, por favor, cópia da Apólice de Seguro respectiva)
NÃO ☐
NÃO APLICÁVEL ☒

8. TERMO DE RESPONSABILIDADE

Eu, Rui Manuel Coelho e Conceição Calhau, abaixo-assinado, na qualidade de Investigadores responsáveis, declaramos por nossa honra que as informações prestadas neste questionário são verdadeiras. Mais se declara que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos. Aceitamos, também, a recomendação da CES de que o recrutamento para este estudo se fará junto de voluntários que não tenham participado em outro estudo.

Porto, 13 / _Maio / 2013

Rui Coelho

Os Investigadores Responsáveis

A Comissão de Ética para a Saúde tendo aprovado o parecer do Relator, aguarda que o Investigador/Promotor esclareça as questões nele enunciadas para que possa emitir parecer definitivo.

2013-06-27

Prof. Doutor Filipe Almeida
Presidente da Comissão de Ética

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO CENTRO HOSPITALAR DE S. JOÃO

emitido na reunião plenária da CES de ___/___/___

Considerado que foram cabalmente os esclarecimentos prestados pelo investigador

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

2013-07-18

Prof. Doutor Filipe Almeida
Presidente da Comissão de Ética