Characterization of population exposure to organochlorines: a cluster analysis application

Caracterização da exposição de população a organoclorados: uma aplicação da análise de cluster

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Abstract

This study aimed to show the results from a cluster analysis application in the characterization of population exposure to organochlorines through variables related to time and exposure dose. Characteristics of 354 subjects in a population exposed to organochlorine pesticides residues related to time and exposure dose were subjected to cluster analysis to separate them into subgroups. We performed hierarchical cluster analysis. To evaluate the classification accuracy, compared to intra-group and inter-group variability by ANOVA for each dimension. The aggregation strategy was accomplished by the method of Ward. It was, for the creation of clusters, variables associated with exposure and routes of contamination. The information on the estimated intake doses of compound were used to weight the values of exposure time at each of the routes, so as to obtain values proxy exposure intensity. The results showed three clusters: cluster 1 (n = 45), characteristics of greatest exposure, the cluster 2 (n = 103), intermediate exposure, and cluster 3 (n = 206), less exposure. The bivariate analyzes performed with groups that are groups showed a statistically significant difference. This study demonstrated the applicability of cluster analysis to categorize populations exposed to organochlorines and also points to the relevance of typological studies that may contribute to a better classification of subjects exposed to chemical agents, which is typical of environmental epidemiology studies to a wider understanding of etiological, preventive and therapeutic contamination.

Keywords: Multivariate statistical methods. Cluster analysis. Organochlorines. Exposure. Environmental health. Environmental epidemiology.

Resumo

O presente trabalho objetivou apresentar os resultados da aplicação da análise de agrupamento (cluster) para a caracterização da exposição a organoclorados, através de variáveis relacionadas ao tempo e à dose de exposição. Características de 354 sujeitos de uma população exposta a resíduos de pesticidas organoclorados relacionadas ao tempo e à dose de exposição foram submetidas à análise de cluster para separá-las em subgrupos. Foi realizada a análise de cluster hierárquico. Para avaliar a precisão da classificação, foram comparadas a variabilidade intragrupo e a variabilidade inter-grupos através do teste de ANOVA para cada dimensão. A estratégia de agregação foi realizada pelo método de Ward. Para criação dos clusters, foram comparadas as variáveis associadas à exposição e às rotas de contaminação. As informações referentes às doses estimadas de ingesta do composto foram usadas para ponderar os valores de tempo de exposição a cada uma das rotas, de forma a obter valores *proxy* de intensidade de exposição. Os resultados indicaram 3 clusters: o *cluster* 1 (n = 45), com características de maior exposição; o *cluster* 2 (n = 103), de exposição intermediária, e o cluster 3 (n = 206), de menor exposição. As análises bivariadas realizadas com os grupos evidenciaram que são grupamentos com diferença estatisticamente significativa. Este estudo evidenciou a aplicabilidade da análise de cluster para categorizar populações expostas a organoclorados, e aponta para a relevância de estudos tipológicos que possam contribuir para uma melhor classificação de sujeitos expostos a agentes químicos, condição típica dos estudos de epidemiologia ambiental, para uma mais ampla compreensão dos aspectos etiológicos, preventivos e terapêuticos da contaminação.

Palavras-chave: Métodos estatísticos multivariados. Análise de agrupamento. Organoclorados. Exposição. Saúde ambiental. Epidemiologia ambiental.

Introduction

The concept of exposure in the area of environmental epidemiology is defined as a contact limited in time and space between an individual and one or more biological, chemical and physical agents[1]. In contrast, the assessment of exposure is identified from the exposures that occur or that can be predicted in human populations[2]. This can be a complex event, requiring the analysis of different aspects of contact between individuals and substances.

Exposure assessment methods can be used to identify and define groups with higher or lower exposure. Depending on the exposure assessment proposal, the numerical value can be an estimate of intensity, rate, speed, duration and frequency of contact or exposure. According to the International Program of Chemical Safety (IPCS), one of the ways to estimate exposure can be the separate assessment of concentration of exposure and time of contact, as well as their combination a posteriori. Based on this, evaluators focus on the determination of the concentration of substances in the environment or location and compare this information with the time and ways in which an individual or population groups have come into contact with such substances[2].

Among the substances most frequently studied in recent years are the organochlorines, which are compounds of carbon, hydrogen and oxygen. They are extremely persistent in the environment and accumulate in several environmental compartments. Their persistence in the environment is defined by the time that it takes a chemical product to lose at least 95% of its activity, i.e. to decompose into simpler structures, basically CO_2 and H_2O, under usual environmental conditions and uses. It takes between one and three weeks for products that are not persistent to be degraded; those with moderate persistence, between one and 18 months; and those that are persistent, two or more years[3-7]. These compounds cannot be easily measured in human beings, due to their lipophilic nature and low availability in the blood tissue. Additionally, estimates of organochlorines require high-precision technology and, at times, do not reflect the actual contamination of individuals[8]. Bearing this in mind, investigative programs aimed at this area have sought alternative forms of classification of individuals' exposure to perform studies that quantify the magnitude of the association between the contamination of compounds and different health outcomes[9-12].

Cluster analysis aims to divide the elements of a sample into groups so that elements belonging to the same group are similar to one another, with regard to the variables (characteristics) measured in them, and so that elements in distinct groups are heterogeneous for these characteristics. The term "Cluster Analysis", first used by Tyron[13], in fact includes a variety of algorithms with distinct classifications, all aimed at an important question found in several areas of research: how to organize data observed in structures that make sense or, in other words, how to develop taxonomies capable of classifying the data present in different classes. It should be emphasized, among other things, that these classes must be classes that occur "naturally" in the set of data[14].

There were no studies in the literature that had used cluster analysis to verify the joint relations among variables associated with exposure to chemical substances, particularly organochlorines. The majority of international studies use data on the serum concentration of compounds, a solution that creates two problems: a) blood is not the main biological matrix of concentration of compounds; b) investigative programs with less funding or relying on more precarious logistics do not have the resources to collect or analyze any biological matrices.

Consequently, the present study aimed to present the results of the application of cluster analysis to characterize exposure to organochlorines, using variables associated with the exposure time and dose.

Methods

Study data

The data used in this study originated from a research project which is one of the main products of the Cooperation Term 74/2010 (process 25000.153491/2010-00) established between the *Instituto de Estudos em Saúde Coletiva* (IESC – Institute of Collective Health Studies) of the Federal University of Rio de Janeiro (UFRJ) and the *Coordenação de Vigilância em Saúde Ambiental do Ministério da Saúde* (CGVAM/MS – Brazilian Ministry of Health Coordination for Environmental Health Surveillance) in 2011.

This Cooperation Term, entitled "Analysis of the Health Effects Resulting from Exposure to Organochlorine Compounds in the district of Cidade dos Meninos, Duque de Caxias, RJ, Brazil", aims to analyze the database developed by the *Instituto Nacional do Câncer* (INCA – National Cancer Institute) in 2007, based on a partnership between the INCA and CGVAM.

This partnership had the purpose of conducting a health survey of the population living in the area known as Cidade dos Meninos, including clinical data collection, physical examination and laboratory tests. This survey was completed in 2007 and the database developed was sent to the Ministry of Health.

In 2011, the Ministry of Health requested that the IESC/UFRJ analyze this database, in accordance with the aforementioned Cooperation Term.

The data maintained in the database do not have any type of identification, so that the analyses do not include any approaches of procedures that may violate the human rights established by the Ethics Code. This is a secondary database whose archives have been granted to the Ministry of Health under number 47/2012.

Statistical analysis

Hierarchical cluster analysis was performed in the present study. Intra-group variability (which is small if the classification is good) and inter-group variability (which is great if the classification is good) were compared to assess the accuracy of the classification, using the ANOVA test for each dimension (case of variable)[15,16]. The cluster analysis aimed to classify the objects of analysis (for example, individuals of a population) that are known for their characteristics, in groups that are intra-group homogeneous and inter-group heterogeneous. The objective techniques of clusters reduce subjectivity, as they quantify the similarity or dissimilarity among individuals. Among the several methods or algorithms of classification of groups are those that use hierarchical techniques. In this case, the partition occurs from an undefined group number, in which the major groups are divided into minor sub-groups, gathering individuals that have similar characteristics. The classification of individuals in distinct groups is performed from a cluster function (distance or similarity) and a mathematical cluster criterion. The Euclidean distance is the dissimilarity measure most frequently used in clusters, although there are several distance measures[16-18].

When *p* x *n* data are considered, given an *X* matrix in a dimensional space *p*; the Euclidean distances between entities *Xi,* and *Xj* are expressed by:

$$d_{ij} = [X_i - X_j] = \left[\sum_{k=1}^{P} (X_{i,k} - X_{j,k})^2\right]^{1/2}$$

Both the Euclidean distance, a dissimiliarity measure, and the correlation coefficient, a similarity measure, can be used to determine groups. To achieve this, there are two cluster methods, hierarchical and non-hierarchical. Several cluster criteria are possible in the hierarchical methods; of these, the Ward method (1963) was selected for the present study. This method searches for partitions that minimize loss associated with each cluster (Everitt, 1974; Bussab et al., 1990; Mingoti, 2005). This loss is quantified by the difference between the sum of squared errors of each pattern and the mean of the partition where it is included. The sum of squared errors is defined by:

$$SQD = \sum_{i=1}^{n} x_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} x_i\right)^2$$

where *n* is the total number of cluster elements and xi is the i-th cluster element.

The method, rather than putting together the two classes with the lowest distance (similarity), gathers instead two classes in a way that the resulting class has minimum dispersion, compared to all classes that could be formed in a certain stage of its algorithm[17]. The algorithm calculates the dispersion of each new class comprised of two original classes. The distance between objects (study subjects) to be classified must be a squared d^2 (Euclidean, reduced Euclidean or distance from c^2). If the {k} and {k'} classes are grouped together, the increase in intra-group variance, also known as cluster level $\Delta_{(k \cup k')}$, is expressed by:

$$\Delta_{(k \cup k')} = \frac{n_k . n_{k'}}{n_k + n_{k'}} d^2_{(G_k, G_{k'})}$$

where G_k and $G_{k'}$ correspond to the gravity centers of classes k and k', whereas d^2 represents the Euclidean distance[18].

Microsoft Excel spreadsheets, version 2010, were used to organize data, while the SPSS 20.0 software was used to analyze clusters.

Results

The cluster technique used was the ascending hierarchical classification. The ascending or hierarchical cluster method starts with individual objects, i.e. the subjects. In the beginning, there are as many groups as there are subjects (n = 354). Those who were most similar were grouped together and the initial groups were joined according to their similarities. As the similarities decrease, all sub-groups are gathered into a single group. Aiming to select a good partition, a cluster level whose value was not very high was selected, i.e. with low change of the initial distances between objects[15,18].

Selection of cluster variables

Variables whose ancillary theoretical model identified itself with the behavior associated with exposure and routes of contamination were considered to create the clusters.

Organochlorines are part of a group of compounds classified as persistent organic pollutants (POPs). This attribution is due to three basic characteristics: environmental persistence, bioaccumulation (with resulting biomagnification in the trophic chain) and high toxicity. Innumerable studies have been performed and the harmful effect of these substances on the environment and public health has been confirmed, which has led to the restriction and even prohibition of its use in several countries. Properties such as low volatility, chemical stability, lipid solubility, low biotransformation and degradation rates, which caused these chemicals to become highly efficient pesticides, also banned them due to their high persistence in the environment, bioconcentration and biomagnifications in various food chains[19].

Because of their persistence in the environment, organochlorines have a greater chance of entering several food chains and remaining in the ecosystem for an undetermined period of time. What causes these compounds to be harmful, apart from their persistence, is the fact that they are liposoluble and easily eliminated. They remain stored in the fatty tissue of the animal chain, so that animals become reservoirs of such products. There are reports that nearly 96% of human exposure to organochlorines and dioxines occurs through food intake, especially that of animal origin such as fish, meat, eggs, milk and dairy products[20]. The following variables were used to define the clusters: time of residence in the area, time of contact with the soil (in occupational activities, agriculture etc.), time of animal husbandry, time of consumption of meat produced in the area, time of consumption of milk, eggs and derivatives, all of which are continuous variables measured in years.

Toxicology considers the dose (intensity) x time relation to assess how toxic any chemical compound is. All these variables were adjusted for age, using a person time calculation in which each year of exposure[8] was taken into consideration to calculate the accumulated exposure.

During the Study on Risk Assessment, due to the limited amount of data on environmental dispersion of contaminants out of the target areas and to the lack of data on all target contaminants, soil and food (eggs) samples were collected to analyze the levels of contaminants[21-23]. The sample was systematic, using a grid based on two imaginary straight lines situated, respectively, 50 m and 100 m from the shoulders of the Camboaba Road, the main access to this area. The sampling points were defined at 500 m from these straight lines, using the main gatehouse to Cidade dos Meninos as the starting point. Beyond the proposed sampling points on the grid, other points were established in the immediate area surrounding the houses located along a stretch of the Camboaba Road. Data on the concentration of each contaminant detected in the soil samples in the main areas of emission in the Cidade dos Meninos were compared to reference values of the Dutch legislation for residential soils (MRT – Maximum Risk Tolerance – value and intervention)[24].

Tests were performed by a private laboratory in the city of Rio de Janeiro (limit of detection of the method: 0.01µg/kg for all compounds analyzed; the p'DDE, p'DDD, and p'DDT were quantified by the semi-quantitative method). With regard to food, eggs were selected because they store great amounts of organochlorine pesticides and, together with cow milk, represent a basic source of animal protein for locals. Pesticide levels in milk samples obtained from cows raised in the Cidade dos Meninos were used to calculate the exposure dose in foods. Sample collections were performed with milk producers located during the study. Each sample was based on the daily production of each farmer[22.23].

The analyses of pesticides of food samples were performed by a private laboratory in the city of Rio de Janeiro (limit of detection of the method: 0.01µg/kg for all compounds analyzed; the p'DDE, p'DDD, and p'DDT were quantified by the semi-quantitative method). The levels of residues of pesticides in foods were compared to the maximum limits of residues established by the European Food Safety Authority[24], which is considered the most complete and strictest international standard.

The doses of environmental exposure estimated by the Studies on the Assessment of Risk to Human Health performed in this area[21,25] used the following formulas:

Estimated dose per soil intake (Edsi)

$$Edsi = \frac{CxIRxEFx10^{-6}}{BW}$$

where C = concentration of contaminant on the surface soil (mg/kg); IR = soil intake rate; EF = exposure factor; and BW = body weight.

Estimated dose per food intake (Edf)

$$Edf = \sum_{i=1}^{n} \frac{CixRixEF}{BW}$$

where C = concentration of contaminant in the food group (MG/g); Ri = rate of intake of food group (g/day); EF = exposure factor; and BW = body weight.

The information about the estimated doses were used to weight the exposure time values for each of the routes, so that the proxy values of intensity of exposure could be obtained.

Definition of clusters

The number of clusters to be used does not follow a fixed pattern, as it depends on the researchers' objective. There is not a standard statistical criterion for inference, such as tests or similar procedures. A frequently used procedure is the stopping rule, which analyzes a certain measure of similarity or distance among clusters at each successive step, with the cluster solution being defined when the measure of similarity exceeds a specific value, i.e. when the distance between two points is greater than a value predefined by researchers.

Clusters were subsequently formed by the individual combination of subjects, one by one, in different groups, until the formation of the estimated number through modeling (data driven), which was three in this particular case.

The results indicated that cluster 1 (n = 45), here identified, has characteristics of exposure that can be considered as more intense than those identified for cluster 2 (n = 103), of intermediate exposure, which has a higher intensity than cluster 3 (n = 206).

Table 1 identifies the mean values of each of the variables used for the creation of clusters, with the respective ANOVA test. In contrast, Table 2 shows data on certain potential confounders of any analysis of organochlorines for health outcomes indicated by the literature[26], revealing that they are clusters with statistically significant differences, i.e. heterogeneous groups among themselves, although homogeneous internally.

The predictive validity of clusters can be assessed in a prospective way, observing the behavior of each sub-type with regard to the different effect modification variables, such as sex, age or stage of life when exposure began. Although the study design does not enable the predictive validity of typology to be assessed, it is possible nonetheless to discuss the monitoring priorities. Clusters maintained different exposure patterns; therefore, their information could be used to categorize the exposed population and prioritize the group with the greatest exposure to screen for outcomes associated with organochlorines in the health care network, especially endocrine, reproductive, neurological, hepatic and cancer outcomes.

Conclusion

One of the aims of Environmental Health is to prevent harm to health caused by chemical contaminants present in the

Table 1 - Average values of environmental exposure times according to exposure groups.
Tabela 1 - *Valores Médios dos tempos de exposição ambiental segundo grupos de exposição.*

Time variable	Group 1 (n=45)		Group 2 (n=103)		Group 3 (n=206)		Total (n=354)		p valor*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Residence in the area	43.09	11.71	24.02	11.20	10.94	9.48	18.83	14.99	< 0.001
Contact with soil	42.71	11.83	21.81	12.40	10.50	9.90	17.89	15.29	< 0.001
Animal husbandry	10.33	16.75	8.33	12.09	3.53	7.04	5.79	10.65	< 0.001
Meat consumption	44.29	9.87	23.86	11.07	10.85	9.48	18.89	15.07	< 0.001
Egg consumption	44.33	9.88	24.21	11.10	10.84	9.49	18.99	15.12	< 0.001
Milk and dairy product consumption	43.91	10.72	23.89	11.16	10.74	9.56	18.78	15.14	< 0.001

* Obtained with the ANOVA test. / ** obtido através do teste de ANOVA.*

Table 2 - Bivariate analysis of potential confounding according to exposure group.
Tabela 2 - *Análise Bivariada de potenciais confundidores segundo grupo de exposição.*

Variable	Group 1 (n=45)		Group 2 (n=103)		Group 3 (n=206)		Total (n=354)		p-value*
	n	%	n	%	n	%	n	%	
Was born in the area									
No	30	11.6	89	34.4	140	54.1	259	100.0	0.001
Yes	15	15.8	14	14.7	66	69.5	95	100.0	
Beginning of exposure after the age of 5 years									
No	22	12.60	68	38.90	85	48.60	175	100.0	< 0.001
Yes	16	16.20	16	16.20	67	67.70	99	100.0	
Sex									
Male	16	8.90	64	35.60	100	55.60	180	100.0	0.001
Female	32	18.40	36	20.70	106	60.90	174	100.0	
Age group									
0 – 9 years	0	0.00	0	0.00	3	100.0	3	100.0	< 0.001
10 – 19 years	0	0.00	0	0.00	30	100.0	30	100.0	
20 – 29 years	0	0.00	0	0.00	38	100.0	38	100.0	
30 – 39 years	0	0.00	3	4.20	69	95.80	72	100.0	
40 – 49 years	5	4.80	52	50.00	47	45.20	104	100.0	
50 – 59 years	27	34.60	36	46.20	15	19.20	78	100.0	
60 years and more	13	44.80	12	41.40	4	13.80	29	100.0	
Micro-area of the ESF**									
1	9	10.6	18	21.2	58	68.2	85	100.0	
2	10	10.9	30	32.6	52	56.5	92	100.0	<0.001
3	13	18.8	35	50.7	21	30.4	69	100.0	
4	12	12.1	30	30.3	57	57.6	99	100.0	

* Obtained with the Pearson chi-square test. ** ESF – Estratégia Saúde da Família (Family Health Strategy)
** Obtido através do teste de qui-quadrado de Pearson. ** ESF – Estratégia Saúde da Família*

environment, maintaining the levels of exposure at values that do not represent an unacceptable risk. To achieve this, this risk must be identified and quantified through the biological assessment of human exposure[27].

The study of common characteristics among the populations exposed to chemical substances is important to develop a program to reduce such exposure[28]. In Brazil, there are few programs aimed at exposure prevention and risk reduction, unlike the reality of countries such as Canada, Australia, France and the United States.

One limitation of this study is that the technique used does not enable inferences of findings to be made (due to the lack of a gold standard). Nonetheless, this technique was useful for the study of these relationships and for the design of the profile of sub-groups of this population, some of which must be prioritized by actions aimed at controlling this health problem.

It is expected that the common characteristics found in this study will point towards the planning of strategies of organochlorine exposure prevention programs and promotion of future studies based on the hypotheses raised. Future studies analyzing population groups who have been exposed to accidents with organochlorines or who are using these substances at home or work, or even those living in places where exposure has not been eliminated, will be useful to increase knowledge about the associated factors and to assess the joint relations of these factors among groups.

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