

Identification of fatty foods with contamination possibilities by plasticizers when stored in PVC film packaging

Identificação dos alimentos gordurosos com possibilidades de contaminação por plastificantes quando acondicionados em filme de PVC

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Abstract

Poly-(vinyl chloride) (PVC) requires the addition of plasticizers - additives that give flexibility and malleability for its processing into flexible film. The most used ones are: di-(2-ethylhexyl) adipate (DEHA) and di-(2-ethylhexyl) phthalate (DEHP). Toxic effects of DEHP have been observed by several authors. Phthalates are being replaced by alternative substances in PVC flexible products, because of their possible toxicological effects. DEHA is a substitute for phthalates widely used as a plasticizer in PVC materials for involving food. Some authors have shown that the exposure to DEHA also induces toxicity. A cross-sectional study was performed to identify which fatty foods carry the possibility of contamination by DEHP and DEHA. Eighteen different foods with at least 3% (m/m) fat and the possibility of being wrapped in plastic film were determined. This study suggested that all foods were subject to contamination by DEHP and DEHA in those conditions - in decreasing consumption order of 96 to 22% in the convenience sample. New guidelines on the limits of DEHA and DEHP established by the Brazilian legislation, as additives in PVC film for packaging fatty food, are still relevant to ensure human health.

Keywords: PVC; DEHA; DEHP; fatty foods; packaging; toxicity.

Resumo

O poli-(cloro de vinila) (PVC) necessita da adição de plastificantes, que são aditivos que conferem flexibilidade e maleabilidade para a sua transformação em filme flexível. Os mais utilizados são o adipato de di-(2-etil-hexila) (DEHA) e o ftalato de di-(2-etil-hexila) (DEHP). Efeitos tóxicos do DEHP foram observados por vários autores. Em virtude dos possíveis efeitos toxicológicos dos ftalatos, esses estão sendo substituídos por substâncias alternativas em produtos flexíveis de PVC. O DEHA é um substituto dos ftalatos amplamente utilizado como plastificante em filmes de PVC para materiais que envolvam alimentos. Alguns autores demonstraram que a exposição ao DEHA também causa toxicidade. Realizou-se um estudo seccional para identificar os alimentos gordurosos com possibilidades de contaminação por DEHA e DEHP. Foram determinados 18 alimentos diferentes, com no mínimo 3% (m/m) de gordura e com a possibilidade de acondicionamento em filme de PVC. Esse estudo sugeriu que todos os alimentos estavam passíveis de contaminações por DEHA e DEHP naquelas condições, na ordem de consumo decrescente de 96% a 22% na amostra de conveniência. Novas orientações com relação aos limites estabelecidos pela legislação brasileira de DEHA e DEHP, como aditivos em filmes de PVC para acondicionar alimentos gordurosos, continuam pertinentes para assegurar a saúde humana.

Palavras-chave: PVC; DEHA; DEHP; alimentos gordurosos; embalagem; toxicidade.

1 Introduction

Food quality is directly related to food safety and depends on a range of variables, tangible as well as intangible, which require traceability. The most studied factors that influence the quality of food are the physical, chemical and biological ones, acting on food during the time period from production to consumption (PASCUET, 1996; BARROS, 2005).

The choice of packaging is always related to the product to be packaged (PASCUET, 1996). Packaging materials traditionally used in the market are: glass, paper, metal and plastic. However, in recent decades, the large growth of the plastics industry has

encouraged the production of polymeric packaging because they are extremely versatile (AUTIAN, 1980; BRISTON; KATAN, 1974; GILBERT, 1980; ABRANTES, 1998). Poly-(vinyl chloride) (PVC) is one of the used polymers because it can be easily processed and the materials involved in its formulation have a low cost and offer a wide range of properties. The main features that make PVC widely used for packaging food are: transparency, good impact resistance, good barrier properties to gases and water vapor, chemical resistance, and sanitary factors, which allow for consumers external observation of the quality

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aspects of products (FERNANDES; GARCIA; PADULA et al., 1987; NERIN; GANCEDO; CACHO et al., 1992).

The incorporation of additives such as plasticizers, thermal stabilizers, lubricants, impact modifiers and others is essential for the processing of PVC resin in the finished product, which gives the product appropriate specifications for each application. The typically used plasticizers are adipates, phthalates and epoxidized soybean oil. The range of plasticizers concentrations used in plastic is 20 to 40% (m/m) of the polymer mass. The proportion of these plasticizers is carefully chosen to provide adequate permeability to oxygen, carbon dioxide and water vapor (FERNANDES; GARCIA; PADULA et al., 1987; BARROS et al., 2006).

Flexible PVC films are widely used in food packaging as well as in domestic use and retail marketing. PVC requires the addition of plasticizers, which are additives that bring in flexibility and malleability. The most used ones are: di-(2-ethylhexyl) adipate, DEHA, di-(2-ethylhexyl) phthalate, and DEHP, which are not chemically bound to the PVC polymer and, subject certain conditions, may migrate from the plastic to product that is in contact with the PVC film. Fatty foods are more likely to absorb these plasticizers, because of their fat solubility. Toxicity of DEHP has been reported by several authors (DIRVEN et al., 1993; DOULL et al., 1999; KLIMISH et al., 1992; DAVID et al., 2000; POON et al., 1997).

The most relevant toxicological effect is due to the hepatocarcinogenic property of this substance in rats and mice associated with the induction of hepatic peroxisome proliferation (DIRVEN et al., 1993). The identification of risk exposure to DEHP in humans is difficult, because humans and primates are less sensitive to peroxisome proliferation than rats and mice (STOTT, 1988). However, in workshop on health risks caused by the DEHP, participants concluded that this substance could possibly induce peroxisome proliferation in humans and it is possibly considered carcinogenic to humans (SCHULZ, 1989).

Recent studies have suggested that DEHP is an endocrine disrupter (LATINI; VERROTI; DE FELICE et al., 2004). The endocrine system is a system of hormones that control organism development, growth, reproduction and behavior. This system is found in all animals, including humans, and it has three essential parts: (1) the glands that secrete hormones; (2) the hormones themselves; and (3) the hormone receptors in each cell. There are eight types of glands that make up the human endocrine system: the pituitary; the hypothalamus; the parathyroid; the thyroid; the thymus; the pancreas; the adrenal; and the gonads (ovaries in females and tests in males) (TRUSSEL, 2001).

According to Latini (2005), the analysis of biochemical effects resulting from exposure to DEHP in the levels of steroid and gonadotroph hormones should be part of a full risk assessment in human populations.

Although the results are based on a relatively small sample, which does not have statistically significant evidence, Swan et al. (2009) suggested that exposure to DEHP and di-(n-butyl) phthalate, DBP, during pregnancy, could interfere in the development of the brain, blocking the action of the testosterone male hormone. The researchers have analyzed urine samples from pregnant women at the middle of the pregnancy term,

and when their children were 4 and 7 years old these women were asked to track how their children play. The study found that boys exposed to high doses of DEHP and DBP during pregnancy had a lower tendency to play with cars, trains, toy guns and participate in typically male games, such as fights. These authors suggested a full investigation under this view.

Because of the possible toxicological effects of phthalates, they are being replaced by substitutes in flexible PVC products. The DEHA is a substitute for phthalates widely used as a plasticizer in PVC for materials involving food. When these films are used in contact with fatty foods like cheese or some kind of fat meat, there may be a significant migration of DEHA to food in quantities that exceed the limit of specific migration of DEHA, 18 mg.kg⁻¹ of food, proposed by the Scientific Committee for Food of the European Union (DALGAARD et al., 2003).

Silva and Delgado (2007) showed that exposure to DEHA *in utero* and during lactation induced mortality in prior and postnatal offspring, delayed development and caused changes in specific parameters of the male reproductive system in *Webster* mice. They reported a significant reduction of absolute and relative weight; seminal vesicle; and suggested that the offspring had been more vulnerable to adverse effects of DEHA during lactation than during pregnancy.

Grob et al. (2007) showed that for small packs with a high ratio of contact surface area/volume, present European legislation tolerates extremely high migration in terms of concentration in the food, since limits are applied as migration per surface area. Overall migration limit (OML) may exceed 1,000 mg.kg⁻¹, which is an order of magnitude above the tolerance for any other type of food contaminant. With the present rules for specific migration (SM), the tolerable daily intake (TDI) may be exceeded with less than 100 g of food, particularly when a fat consumption reduction factor (FRF) is applied. The OML and the SM of DEHA from PVC cling films into cheese demonstrated that such high migration is not just theoretical extrapolation, but it is encountered in reality. They suggested that the legal limit in terms of concentration in food should be converted to migration per surface area of contact - in case of small packages, and the stipulated limit of the relationship between contact area and volume of food should suppose 20 dm².L⁻¹. Then, the proposed OML of 60 mg.kg⁻¹ would correspond to 3 mg.dm⁻². Below that ratio of area and volume, the value of migration limit should be calculated proportionally to the surface area of contact, especially for smaller packages.

The National Sanitary Surveillance Agency (ANVISA), Resolution n. 105 (BRASIL, 1999) establishes the maximum of 3% (m/m) of DEHP, in the plastic mass, if the material to get into contact with foods contained more than 5% fat, and at that time it did not indicate a restriction to DEHA limit; however, it indicated a concern with these plasticizers and health. Currently in force, Resolution - RDC n. 17 (BRASIL, 2008) approves the "Technical Regulation on the positive list of additives for plastics intended for production and packaging equipment in contact with food", where the specific migration limit (SML), expressed in mg.kg⁻¹ of simulants for DEHA, is 18 mg.kg⁻¹. In the case of DEHP, the limit is lower (1.5 mg.kg⁻¹) and only for use as a plasticizer in materials and reusable objects that are in

contact with non-greasy foods or as an agent for the process at concentrations up to 0.1% in the final product.

The purpose of this paper was to perform a cross-sectional study, through a field survey, for the identification of fatty foods with contamination possibilities by DEHP and DEHA in PVC film packaging.

2 Materials and methods

2.1 Materials

Eighteen different foods were chosen with at least 3% (m/m) of fat in their nutritional composition to determine the main foods for the purpose of standardization in the food questionnaire form (PINHEIRO et al., 1998; FISBERG et al., 2005; PHILIPPI, 2002; INSTITUTO..., 1997) and the packaging possibilities in PVC film, both for domestic use as well as for retail sale.

2.2 Methods

Food survey

The food survey was used as a tool for cross-sectional study in order to obtain the food frequency, the convenience sample and to know how much and which fatty foods with packaging possibilities in PVC films were the most consumed. The sample population was chosen at the Seventh Regional Administration of the State of Rio de Janeiro, which includes the following neighborhoods: 'Praça da Bandeira', 'Tijuca' and 'Alto da Boa Vista'.

Before starting the food survey, participants were told that the objective of the study was to investigate how much and which foods showed on the questionnaire form they consumed. They were told to read an explanation and a consent form advising that all the information collected would be kept strictly confidential and that their names would not appear in any analyses or published reports. They signed the referred consent form. They were also told they could give up the study at any time without any explanations. All participants volunteered for the study and did not receive any financial help for their participation.

Feeding frequency studies were carried out through interviews according to the food history of each participant and were put in standard questionnaire form, as recommended by (FOOD...; WORLD..., 1995). The studies covered the main foods with packaged possibilities in PVC film, with at least 3% (m/m) fat in their nutritional composition and evaluated the consumption according to Pinheiro et al. (1998), Fisberg et al. (2005), Philippi (2002) and IBGE (INSTITUTO..., 1997), following an experimental design similar to that carried by Abrantes et al. (2005). The standard questionnaire form used for the dietary recall can be seen in Appendix I.

Statistical analysis

Two thousand consumers were interviewed at random, where 41.25 % (825) were men and 58.75% (1,175) were women, respecting the age group, without being obliged to

declare anything in writing. To obtain a convenience sample representing a population of 85,100 people aged between 18 and 49 years old, a total of 180,992 inhabitants were the object of the present study (INSTITUTO..., 2008). The sampling plan was used in accordance with NBR 5426 (ASSOCIAÇÃO... 1985) and Abrantes et al. (2005).

The cross-sectional study began in March 2007 with the studies for the preparation of the standard questionnaire form. The interviews took place between May and October 2007 and were held in 4 different large supermarkets in the studied region in different days and times. The questionnaire forms were marked with the possibility of 18 different colors, one for each food, with 126 alphanumeric subgroups, to identify and quantify the frequency of consumption of each food for each subject with scores from zero to six, which represented the absence or the frequency of consumption of each food in the day, week and month as the standardization in the food form (PINHEIRO et al. 1998; FISBERG et al. 2005; PHILIPPI, 2002, INSTITUTO..., 1997).

For this study, the eating score was developed based on the dietary recall, where the food intake was treated as an ordinal variable and received seven values as follows: zero points, when the individual reported that he/she had never eaten that food; one point, when the item was consumed less than once a month; two points, for consumption from one to three times per month; three points, for once a week consumption; four points, for two to four times a week; five points, for once a day; and six points, for twice or more times a day. The total score was obtained by adding up the frequency of each food showed in the food form. The sum of the 18 items constituted the numerator of the preliminary score, in order to consider the items altogether and answer the entire food questionnaire. Thus, estimated the total number of points that each food could have been referred to as the largest food consumption (two or more times a day), among all the items that every person had responded. The preliminary score was then calculated by dividing the points that the food obtained by the total possible points for each case. This concern was relevant, since not all individuals answered to all items. The eating score was calculated by adding the total points scored, divided by the maximum points for each food, according to the number of items completed, multiplied by 100 (FONSECA, 1996; PINHEIRO et al. 1998; FISBERG et al. 2005; PHILIPPI 2002; INSTITUTO..., 1997; LAPPONI, 2001; ABRANTES et al., 2005).

The data were mapped, organized, typed and recorded in *Excel Program-Windows* and assessed for frequency and amount of consumption in percentage, total and average. Means were compared using the t-Student test and analysis of variance (ANOVA). Estimates of central tendency were also tested using the t-Student test, and differences between proportions by χ^2 test, considering the significance level of 95%. The assurance and the validity of the scores were evaluated using two methods: observation of the correlation coefficients between the items that composed the food score and observation of the correlation coefficients between each item and eating score (GOMES, 1976; COSTA NETO, 1977; SALVINI et al., 1989; RODRIGUES, 1993; VIEIRA, 1988; LAPPONI, 2001; SOARES; SIQUEIRA, 2002;

Table 1. The percentage of food consumption, total consumption and average consumption.

Foods with at least 3% (m/m) fat	*Fat (g/100 g)	Percentage of consumption by the convenience sample	Quantification of total consumption (kg/day)	Average consumption per person (g/day)
Chicken Breast (raw)	9,26	96	299,09	156
Lean Beef (raw) Standard	5,72	95	177,00	96
Mozzarella Pizza	9,85	91	965,76	555
Chicken Thighs Whole (raw)	15,30	82	85,12	76
Mozzarella Cheese	21,60	76	46,50	30
Ground Meat (raw)	19,53	73	137,40	94
Fudge Cream	5,90	71	86,76	60
Chicken Wing	12,20	65	98,60	76
Pork Sausage (raw)	27,50	63	139,44	109
Minas cheese	19,00	62	103,86	83
Cheese plate	27,80	59	76,48	65
Pork Ham	23,20	57	56,44	49
Simple White Cake Ready	8,36	54	76,32	71
Chicken Ham	3,40	51	48,48	47
Mortadella	29,43	47	22,03	23
Chicken Sausages	13,76	42	103,92	111
Lean Pork	9,10	38	80,80	100
Butter Bulk	81,10	22	19,80	10

*Source: Pinheiro et al. (1998); Fisberg et al. (2005); Philippi (2002); IBGE (INSTITUTO..., 1997).

CALLEGARI-JACQUES, 2004; FISBERG et al. 2005). The field research data analysis was completed in July 2008.

3 Results and discussion

Table 1 was completed according to the answers of each consumer, where the results of the percentage rate of consumption, the total consumption and the average consumption of foods in the study were evaluated. It was observed that, although the mozzarella pizza is in third place on the food frequency, it was the only food with the highest numbers of total consumption, and when it was measured by average consumption, it moved to the first place in this regard.

Because of their fat content, the results showed that there are contamination possibilities by DEHP and DEHA in all foods evaluated in this study, when packed in flexible PVC film, considering that the relevant legislation is not sufficiently clear - not stating the determination of use of DEHP and DEHA, specifically, for fatty foods.

4 Conclusions

The information results compiled and evaluated in the questionnaire form suggested that all foods in the survey which were stocked in PVC film could be contaminated by DEHP and DEHA. We should reflected on the use of these films for food packaging, with technical and scientific criticality, especially regarding the limits set for DEHA and DEHP in our legislation and the government oversight to the matter. Specific laws should state its use in fatty foods and not greasy ones, as well as the respective DEHA and DEHP limits. This study shows the importance of a research that can verify the migration of plasticizers into food, when PVC films are used for packaging. New guidelines on the limits of DEHA and DEHP,

as additives in PVC film for packaging foods are still relevant in the multidisciplinary area of the Sanitary Surveillance, so that it can ensure food safety in this regard, therefore ensuring human health. This study is directly linked to the scientific experiment of author in her PhD in Sanitary Surveillance at INCQS / FIOCRUZ.

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References

- ABRANTES, S. M. P. **Uso da eletroforese capilar para a determinação da migração química em alimentos em contato com embalagens.** 1998. 182 f. Tese (Doutorado em Química Orgânica)-Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 1998.
- ABRANTES, S. M. P. et al. Exposição do ser humano ao ftalato e adipato de di-(2-etil-hexila) provenientes de filmes flexíveis de PVC. **Revista Higiene Alimentar**, v. 19, n. 130, 2005.
- ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS - ABNT. Planos de amostragem e procedimentos na inspeção por atributos. In: ABNT. **NBR 5427**: Guia para a utilização da norma NBR 5426. Rio de Janeiro: ABNT, 1985.
- AUTIAN, J. *Plastics*. In: CASSARETT & Doull's Toxicology. The Basic Science of Poisons. 2. ed. New York: Macmillan, 1980. p. 531-536.
- BARROS, H. D. Metrologia, alimentos e saúde. In: SEMINÁRIO RIO-METROLOGIA E II FÓRUM DE METROLOGIA NA SAÚDE, 3., 2005, Rio de Janeiro. **Anais...** Rio de Janeiro: Rede de Tecnologia, 2005. p.137-151
- BARROS, H. D. et al. Filme de PVC pode Contaminar Alimentos? **Revista Brasileira de Vigilância Sanitária (REVISIA) = Brazilian Journal of Health Surveillance**, v. 2, 2006.

- BRASIL. Resolução nº 105, de 19 de maio de 1999. Regulamentos técnicos: disposições gerais para embalagens e equipamentos plásticos em contato com alimentos. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 20 maio 1999. Seção 1, n. 95, p. 21.
- BRASIL. Resolução - RDC nº 17, de 17 de março de 2008. Regulamento Técnico sobre lista positiva de aditivos para materiais plásticos destinados à elaboração de embalagens e equipamentos em contato com alimentos. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 18 mar. 2008. Seção 1, n. 53, p. 42.
- BRISTON, J. H.; KATAN, L. **Plastics in contact with food**. London: Trade Press, 1974. 466 p.
- CALLEGARI-JACQUES, S. M. **Bioestatística: princípios e aplicações**. Porto Alegre: Artmed, 2004. 255 p.
- COSTA NETO, P. L. O. **Estatística**. 8. ed. São Paulo: Edgard Blücher, 1988. 264 p.
- DALGAARD, M. et al. Di(2-ethyl-hexyl) adipato (DEHA) induced development toxicity but not antiandrogenic effects in pre- and postnatally exposed Wistar rats. **Reproductive Toxicology**, v. 17, p. 163-170, 2003. [http://dx.doi.org/10.1016/S0890-6238\(02\)00149-1](http://dx.doi.org/10.1016/S0890-6238(02)00149-1)
- DAVID, R. M. et al. Chronic toxicity of di-(2-ethyl-hexyl) phthalate in rats. **Toxicological Sciences**, v. 55, p. 433-443, 2000. PMID:10828276. <http://dx.doi.org/10.1093/toxsci/55.2.433>
- DIRVEN, H. A. A. M. et al. Metabolites of the plasticizer di-(2-ethyl-hexyl)-phthalate in urine samples of workers in polyvinylchloride processing industries. **International Archives of Occupational and Environmental Health**, v. 64, p. 549-554, 1993. PMID:8314612. <http://dx.doi.org/10.1007/BF00517699>
- DOULL, J. et al. A cancer risk assessment of di-(2-ethyl-hexyl)-phthalate: application of the new U.S. EPA risk assessment guidelines. **Regulatory Toxicology and Pharmacology**, v. 29, p. 327-357, 1999. PMID:10388618. <http://dx.doi.org/10.1006/rtp.1999.1296>
- FOOD AND AGRICULTURE ORGANIZATION - FAO; WORLD HEALTH ORGANIZATION - WHO. **Recommended International Standard for Health in Food and Nutrition Section**. New York, 1995.
- FERNANDES, M. H. C.; GARCIA, E. E. C.; PADULA, M. **Migração de Componentes de Embalagens Plásticas para Alimentos**. Campinas: ITAL, 1987. 153 p.
- FISBERG, R. M. et al. **Inquéritos Alimentares: métodos e bases científicas**. Barueri: Manole, 2005. 334 p.
- FONSECA, M. J. M. **Hábitos Alimentares entre Funcionários de Banco Estatal do Rio de Janeiro**. 1996. 173 f. Dissertação (Mestrado em Saúde Pública)-Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, 1996.
- GILBERT, S. G. Low molecular weight components of polymers used in packaging. **Environmental Health Perspectives**, v. 11, p. 47-52, 1980.
- GOMES, F. P. **Curso de Estatística Experimental**. 6. ed. Piracicaba: USP, 1976. 430 p.
- GROB, K. et al. European legal limits for migration from food packaging materials: 1. Food should prevail over simulants; 2. More realistic conversion from concentrations to limits per surface area. PVC cling films in contact with cheese as an example. **Food Control**, v. 18, p. 201-210, 2007. <http://dx.doi.org/10.1016/j.foodcont.2005.09.015>
- INSTITUTO BRASILEIRO DE GEOGRAFIA ESTATÍSTICA - IBGE. Estudo Nacional da Despesa Familiar - ENDEF. **Tabelas de Composição de Alimentos**. 2. ed. Rio de Janeiro: IBGE, 1997.
- KLIMISCH, H. J. et al. Di-(2-ethyl-hexyl) polymers used in packaging phthalate: a short-term repeat inhalation toxicity including fertility assessment. **Food and Chemical Toxicology**, v. 30, p. 915-919, 1992. [http://dx.doi.org/10.1016/0278-6915\(92\)90175-K](http://dx.doi.org/10.1016/0278-6915(92)90175-K)
- LAPPONI, J. C. **Estatística usando excel**. 2. ed. São Paulo: Lapponi treinamento, 2001. 450 p.
- LATINI, G.; VERRI, A.; DE FELICE, C. Di-(2-ethylhexyl) phthalate and endocrine disruption: a review. **Current Drug Targets - Immune, Endocrine & Metabolic Disorders**, v. 4, p. 37-40, 2004. PMID:12476795. <http://dx.doi.org/10.2174/1568008043340017>
- LATINI, G. Monitoring phthalate exposure in humans. **Clinica Chimica Acta**, v. 361, p. 20-29, 2005. PMID:12476795. PMID:16004980. <http://dx.doi.org/10.1016/j.cccn.2005.05.003>
- NERIN, C.; GANCEDO, P.; CACHO, J. Determination of bis(2-ethyl-hexyl) adipate in food products. **Journal of Agricultural and Food Chemistry**, v. 40, p.1833-1835, 1992. <http://dx.doi.org/10.1021/jf00022a020>
- PASCUET, N. **Legislação da União Européia e IDA com respeito a contaminantes provenientes de embalagens e sua implantação nos países-membros: Fortalecimento do Comitê Codex Alimentarius**. Campinas: 1996. 27 p.
- PHILIPPI, S. T. **Tabela de Composição de Alimentos: Suporte para Decisão Nutricional**. 2. ed. São Paulo: Coronário, 2002. 135 p.
- PINHEIRO, A. B. V. et al. **Tabela para Avaliação de Consumo Alimentar em Medidas Caseiras**. 4. ed. Rio de Janeiro: Produção independente, 1998. 75 p.
- POON, R. et al. Subchronic oral toxicity of di-n-octyl phthalate and di-(2-ethyl-hexyl) phthalate in the rat. **Food and Chemical Toxicology**, v. 35, p. 225-239, 1997. [http://dx.doi.org/10.1016/S0278-6915\(96\)00064-6](http://dx.doi.org/10.1016/S0278-6915(96)00064-6)
- RIO DE JANEIRO. Prefeitura Municipal. Instituto Pereira Passos - IPP. **Dados populacionais**. Rio de Janeiro: IPP. Disponível em: <<http://www.armazemdedados.rio.rj.gov.br>>. Acesso em: 03 jul. 2008.
- RODRIGUES, P. C. **Bioestatística**. 2. ed. Niterói: Eduff, 1993. 268 p.
- SALVINI, S. et al. Food based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. **International Journal of Epidemiology**, v. 18, p. 858-867, 1989. PMID:2621022. <http://dx.doi.org/10.1093/ije/18.4.858>
- SCHULZ, C. O. Assessing human health risks from exposure to di-(2-Ethylhexyl). Phthalate (DEHP) and Related Phthalates: Scientific Issues. **Drug Metabolism Reviews**, v. 21, n. 1, p. 111-120, 1989. PMID:2696632. <http://dx.doi.org/10.3109/03602538909029958>
- SILVA, A. V. P.; DELGADO, I. F. **Avaliação da toxicidade do plastificante adipato de di-(2 etil-hexila) - DEHA sobre o sistema reprodutivo de camundongos machos expostos in utero e durante a lactação**. 2007. 126 f. Dissertação (Mestrado em Vigilância Sanitária)-Instituto Nacional de Controle em Qualidade em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, 2007.
- SOARES, J. F.; SIQUEIRA, A. L. **Introdução à estatística médica**. 2. ed. Belo Horizonte: Coopmed, 2002. 300 p.
- STOTT, W. T. Chemically induced proliferation of peroxisomes: implications for risk assessment. **Regulatory toxicology and pharmacology**, v. 8, 125-159, 1988. [http://dx.doi.org/10.1016/0273-2300\(88\)90026-8](http://dx.doi.org/10.1016/0273-2300(88)90026-8)
- SWAN, S.H. et al. Prenatal phthalate exposure and reduced masculine play in boys. **International Journal of Andrology**, v. 32, p. 1-9, 2009.
- TRUSSEL, R. R. Endocrine disruptors and the water industry. **Journal American Water Works Association**, v. 93, n. 2, p. 58-65, 2001.
- VIEIRA, S. **Introdução à bioestatística**. 5. ed. Rio Comprido: Campus, 1988. 294 p.

Appendix I

Ministry of Health

Oswaldo Cruz Foundation - National Institute of Quality Control in Health, Brasil Av., 4365, CEP 21040-900, Manguinhos, Rio de Janeiro, RJ, Brazil

Food Survey

Food Frequency Questionnaire

Required to be resident of the regional VII Tijuca/RJ

Name (optional):

Sex: () Woman () Man

Income (optional):

Foods	Quant.(g)	Never	- 1 × month	1 to 3 times months	1 × per week	2 to 4 × per week	1 × daily	2 × or + daily (determine)
Minas Cheese								
Mozzarella Cheese								
Cheese plate								
Mortadella								
Pork Ham								
Chicken Ham								
Chicken Sausages								
Pork Sausage (raw)								
Lean Beef (raw) - Standard								
Ground Meat (raw)								
Chicken Breast (raw)								
Chicken Wing								
Chicken Thighs Whole (raw)								
Lean Pork								
Fudge Cream								
Mozzarella Pizza								
Simple White Cake Ready								
Bulk Butter								