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Levels of 3-monochloropropane-1,2-diol (3-MCPD) in selected processed foods from the Brazilian market

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ABSTRACT

In the present study, selected commercial processed foods available in the Brazilian market (232 samples) were analyzed for the first time in relation to 3-monochloropropane-1,2-diol (3-MCPD) content using a validated gas chromatography-mass spectrometry method. Most of the samples (66%) did not show quantifiable levels of the contaminant. However, high concentrations were verified in some samples of specific food groups. Maximum values reported were 2529 μg/kg for foods containing hydrolyzed vegetable protein (HVP), such as soups, seasonings and ready-to-eat meals, 4405 μg/kg for soy sauces, 156 μg/kg for foods and beverages containing malt-derived ingredients, 716 μg/kg for breads, and 49 μg/kg for smoked foods. This study highlights the importance of monitoring the 3-MCPD content in the HVP that is added to food as a savory ingredient.

1. Introduction

3-Monochloropropane-1,2-diol (3-MCPD) is a food processing contaminant that belongs to a group of chemicals called chloropropanols. It was originally discovered in acid hydrolyzed vegetable protein (HVP) (Velíšek et al., 1978) and subsequent studies demonstrated their presence also in soy sauces manufactured with acid-HVP (MAFF, 1999) as well as in several processed foods and food ingredients, such as cereal-based products, malt-derived ingredients and smoked foods (Hamlet, Jayaratne, & Matthews, 2002). More recent investigations have shown that 3-MCPD is present in food not only in its free form but also as mono- or di-esters with fatty acids, at much higher concentrations (Svejkovská et al., 2004; Zelinková, Svejkovská, Velíšek, & Doležal, 2006).

The presence of 3-MCPD in the diet is a concern due to its toxicological properties. The main target organ for 3-MCPD toxicity is the kidney, with chronic oral exposure resulting in nephropathy, tubular hyperplasia and adenomas (FAO/WHO, 2002, 2007). Male infertility and suppression of the immune function were also induced by 3-MCPD in rats (Kwack et al., 2004; Lee et al., 2004). In additional, clear evidence of carcinogenic activity in male rats, based on the increased incidences of kidney renal tubule carcinomas and Leydig cell tumors, was observed (Cho et al., 2008). The International Agency for Research on Cancer (IARC) classified 3-MCPD as a "possible human carcinogen (group 2B)" (IARC, 2012). However, the contaminant has not demonstrated a significant genotoxic potential *in vivo* (El Ramy, Ould Elhkim, Lezmi, & Poul, 2007; Onami et al., 2014) and a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight (bw) is currently established for this compound (SCF, 2001; FAO/WHO, 2002, 2007).

The occurrence of 3-MCPD in foods and food ingredients can mainly result from: the reaction between chloride ions and lipid components during thermal treatment, the use of acid-HVPs produced with hydrochloric acid, the migration from coating materials treated with epichlorohydrin, the reaction between chloride ions and 3-hydroxyacetone during the smoking processes, or from the enzyme-catalyzed hydrolysis of their esters (Hamlet & Sadd, 2009; Léon, Yusà, Pardo, & Pastor, 2008).

The levels of the contaminant may vary according to the product and the applied process. The highest concentrations of free 3-MCPD are usually reported in HVPs produced with hydrochloric acid as well as in soy sauces (Christova-Bagdassarian, Tishkova, & Vrabcheva, 2013; Crews et al., 2003; Fu et al., 2007; Macarthur et al., 2000; Nyman, Diachenko, & Perfetti, 2003; UK FSA, 2001; Wong, Cheong, & Seah, 2006). Concentrations of 3-MCPD in products other than HVP and soy sauces have also been reported in more recent surveys (Chung, Kwong, Yau, Wong, & Xiao, 2008; EFSA, 2013; Léon et al., 2008; UK FSA, 2010).

Considering the risks that the presence of 3-MCPD in foods could pose to human health, a number of risk management strategies were adopted,

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including the establishment of a maximum level of 0.02 mg/kg for liquid products containing 40% dry matter by the European Commission (EC, 2006) and the development of a Code of Practices by the Codex Alimentarius Commission (CAC, 2008). Furthermore, a monitoring plan concerning the presence of 3-MCPD in foods was recently recommended to European Union Member States (EC, 2014).

The methods described in the literature for the analytical determination of 3-MCPD usually employ gas chromatography-mass spectrometry (GC-MS) and require a previous derivatization step to enhance volatility and sensitivity. The most common derivatization reagents are boronic derivatives (e.g. phenylboronic acid), heptafluorobutyryl derivatives (e.g. heptafluorobutyrylimidazole) and dioxolane derivatives (Baer, de la Calle, & Taylor, 2010; Wenzl, Lachenmeier, & Gökmen, 2007). More recent procedures report the use of high performance liquid chromatography coupled to fluorescence detection (Hu, Cheng, Guo, Zhang, & Qi, 2013) and electrochemical sensor (Sun et al., 2014) as alternatives to GC-MS methods.

In view of the lack of data on the levels of 3-MCPD in its free form in commonly consumed food items in Brazil, the objective of this study was to use a validated GC-MS method to provide a comprehensive database on the occurrence of this contaminant in selected local foods.

2. Materials and methods

2.1. Reagents and solvents

Extrelut™ NT20 refill packs were acquired from Merck (Darmstadt, Germany). Heptafluorobutyrylimidazole (HFBI) was obtained from Regis Technologies (Morton Grove, IL, USA). Sodium chloride and sodium sulfate (analytical grade) were from Labsynth (Diadema, SP, Brazil). Hexane, 2,2,4-trimethylpentane, ethyl acetate and diethyl ether (chromatographic grade) were purchased from Tedia (Fairfield, OH, USA) and Vetec (Duque de Caxias, RJ, Brazil). Water was purified by reverse osmosis (Gehaka, São Paulo, SP, Brazil).

2.2. Standards and reference materials

3-MCPD and 3-MCPD-d_5 were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively, at a purity of 98%. Individual stock solutions of both standards at 1 mg/ml were prepared by dissolving the compounds in ethyl acetate. Intermediate (100 μg/ml) and work solutions (2 and 10 μg/ml for 3-MCPD and 3-MCPD-d_5, respectively) were also prepared in ethyl acetate.

Two reference materials of soy sauce were purchased from the Food Analysis Performance Assessment Scheme (FAPAS®, Central Science Laboratory, United Kingdom): T2625 with an assigned value of 47.9 μg/ml and T2626 with an assigned value of 22.2 μg/ml.

2.3. Samples

A total of 232 samples including foods containing HVP, soy sauces, breads, foods containing malt-derived ingredients (malt, malt extract and malt flour) and smoked foods, were purchased at supermarkets from the city of Campinas, SP, Brazil, between September 2010 and December 2011. The sampling was made considering previous data reported by other countries in relation to the occurrence of 3-MCPD. For most of the products, at least 3 different brands and 2 different lots of each brand were collected depending on the availability. The samples were analyzed as bought after proper homogenization and the analysis was carried out in duplicate.

2.4. Determination of 3-MCPD

The sample preparation was carried out according to Brereton et al. (2001) which corresponds to the AOAC Official Method 2000.01 — *Determination of 3-Chloro-1,2-Propanediol in Foods and Food Ingredients*. Briefly, the homogenized sample was weighed into a 250 mL beaker. The internal standard solution of 3-MCPD-d_5 was added to the test portion, followed by NaCl 5 M. The mixture was homogenized and, after sonication, the Extrelut™ NT20 refill pack was added and mixed thoroughly. The mixture was transferred to a glass chromatographic column and 3-MCPD was eluted with diethyl ether. The extract was concentrated to a small volume (±5 mL) in a rotary evaporator and derivatized with heptafluorobutyrylimidazole (HFBI) before injection into the chromatographic system.

2.5. GC-MS analysis

The analyses were performed into an HP 6890 gas chromatograph equipped with an MSD 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Helium (grade 5.0, purity 99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The Programmable Temperature Vaporizing (PTV) injector was operated in the splitless mode under the following temperature program: 100 °C, 500 °C/min to 280 °C (held until the end of the run). The separation was performed on a 60 m × 0.25 mm, d_f 0.25 μm HP-INNOWAX capillary column (Agilent Technologies) and the oven temperature program was: 50 °C (held for 1 min), 5 °C/min to 150 °C, 50 °C/min to 240 °C (held for 2 min). The mass spectrometer was operated in positive electron ionization mode (+EI) with 70 eV of electron energy. Selected ion monitoring (SIM) was used for the detection of the following ions: *m/z* 289*/275/291 for 3-MCPD and *m/z* 294*/278/456 for 3-MCPD-d_5 (*quantifier ions).

3. Results and discussion

3.1. Method performance

The performance of the method was initially evaluated considering the chromatography conditions adopted for this analysis, which employs a polar column (polyethylene glycol) instead of the non-polar (5% phenyl–95% dimethylpolysiloxane) stationary phase used in the procedure described by Brereton et al. (2001). As in this column an interfering compound was observed at the original quantifier ions (*m/z* 253 and 257 for 3-MCPD and 3-MCPD-d_5, respectively), quantification of 3-MCPD was carried out using *m/z* 289 and 294, respectively.

Linearity, limits of detection (LOD) and quantification (LOQ), accuracy and precision were evaluated by analyzing standard solutions, reference materials, and commercial blank samples of soy sauce, gravy, whole bread and smoked tuna, according to the guidelines laid down by the Brazilian Institute of Metrology, Standardization and Industrial Quality (INMETRO, 2007). Good linearity was observed in a concentration range from 0 to 312.5 μg/kg ($r^2 > 0.996$). Samples with content exceeding the validated range were reanalyzed by diluting the final extract before injection. LODs were set between 0.9 and 3.2 μg/kg and LOQs between 3.0 and 10.7 μg/kg, considering the four evaluated matrices. Bias varied from −4.0 to +22.6% for five replicates of each reference material (all results were within the satisfactory range). Recovery and precision were evaluated by fortifying blank samples at 12.5, 62.5 and 250 μg/kg of 3-MCPD. Mean recoveries varied from 96.5 to 114.3%, and coefficients of variation ranged from 1.9 to 6.9% for repeatability (same day, six replicates for each spike level) and from 4.0 to 10.3% for within-laboratory reproducibility (same laboratory, two different days, 12 replicates for each spike level), which are considered acceptable.

3.2. Levels of 3-MCPD in processed foods

Table 1 shows the levels of 3-MCPD in foods containing HVP. The concentrations varied from not detected to 2529 μg/kg and the contaminant was found in quantifiable amounts in 36 samples (61%). Seasonings showed the highest % of positive results (100%). The highest

Table 1
3-MCPD levels in foods containing hydrolyzed vegetable protein ($N = 59$).

Sample	$N/N+$	3-MCPD (μg/kg)	
		Mean	Min–Max
Sauces[a]	9/4	108	nd–512
Instant noodles[b]	5/4	126	nd–233
Stock cubes	8/6	16	nd–67
Seasonings	8/8	12	5.1–24
Snacks	7/1	3–6	nd–22
Soups	4/3	43	nd–87
Vegetarian meals	6/5	102	nd–192
Frozen ready-to-eat meals	5/4	980	nd–2529
Meats[c]	7/1	71 - 75	nd–500

N = number of samples; $N+$ = number of samples above the limit of quantification (LOQ = 3.7 μg/kg); nd = results below the limit of detection (LOD = 1.1 μg/kg). To calculate the mean, values below the LOD and LOQ were treated according to the World Health Organization (WHO, 2002). The range indicates different results for the lower and upper bounds for a group of data with less than 40% of quantifiable results.
[a] Excluding soy sauce.
[b] 3-MCPD was determined in the seasoning.
[c] Including frankfurter, chicken nuggets and hamburger.

concentrations of 3-MCPD were observed in frozen ready-to-eat meals produced by a specific company (results obtained from 2 different meals and 2 different lots of each product). The data demonstrated that the addition of HVP may be an important source of contamination by 3-MCPD. The occurrence of this contaminant in hamburger and chips was already associated to the addition of HVP in other studies (Chung et al., 2008; UK FSA, 2004).

The presence of 3-MCPD in HVP arises from their formation during the hydrochloric acid mediated hydrolysis step of the manufacturing process. During this hydrolytic stage, chloride reacts with residual lipids and phospholipids present in the raw material, resulting in the formation of 3-MCPD. To minimize the concentration of 3-MCPD in the final product, three main approaches may be adopted. The first of these involves careful control of the acid hydrolysis step; the second, subsequent neutralization to minimize 3-MCPD formation; and the third employs the use of sulfuric acid as a substitute for hydrochloric acid in the hydrolysis step (CAC, 2008). Therefore, the final content of 3-MCPD in HVP is significantly affected by its mode of production and may vary from producer to producer.

Although the levels of 3-MCPD in the HVP added to the products are not known, the results suggest that the ingredient may have concentrations even higher in some cases. As an example, if 0.5% of HVP had been added to a soup containing 87 μg/kg of 3-MCPD and considering that this ingredient is the only source of the contaminant, a concentration of 17.4 mg/kg could be estimated for the HVP used in formulation. It should be noted that maximum regulatory limits between 0.02 and 1 mg/kg are currently established in several countries (Velíšek, 2009). However, there is no regulation concerning the levels of this contaminant in HVP in Brazil so far.

Table 2
3-MCPD levels in soy sauces and similar products ($N = 61$).

Sample	$N/N+$	3-MCPD (μg/kg)	
		Mean	Min–Max
Soy sauce (natural fermentation)	35/3	32–33	nd–663
Soy sauce (containing HVP)	4/4	2200	144–4405
Soy sauce (process not informed)	6/0	0.0–0.9	nd
Sauces containing soy sauce[a]	16/0	0.0–0.9	nd

N = number of samples; $N+$ = number of samples above the limit of quantification (LOQ = 3.0 μg/kg); nd = results below the limit of detection (LOD = 0.9 μg/kg). HVP = hydrolyzed vegetable protein. To calculate the mean, values below the LOD and LOQ were treated according to the World Health Organization (WHO, 2002). The range indicates different results for the lower and upper bounds for a group of data with less than 40% of quantifiable results.
[a] Yakissoba, shimeji, oriental, tonkatsu and tare sauces.

The results obtained for the presence of 3-MCPD in soy sauce and similar products are presented in Table 2 (sample categories were defined according to information provided on the label). The concentrations varied from not detected to 4405 μg/kg and levels above LOQ were reported in 7 out of 61 samples. The large variation found in the concentrations of 3-MCPD in soy sauces might be related to the process used to obtain the product, which can be based on natural fermentation or chemical hydrolysis.

Sauces obtained by using natural fermentation, which required several months to be concluded, contain no or little 3-MCPD. Considering the analyzed samples of this category, only 3 out of 35 products showed quantifiable levels of 3-MCPD. One of them presented 8.6 μg/kg while concentrations of 456 and 663 μg/kg were obtained for the other two samples that corresponded to soy sauces from different lots produced by the same company. The presence of 3-MCPD in these samples could be an indicative of a partially natural fermentation process. The contaminant was not found in quantifiable levels in samples for which the process was not informed on the label and in sauces containing soy sauce.

On the other hand, the chemical hydrolysis of proteins, which reduces the time to produce soy sauces, can lead to the formation of high levels of 3-MCPD if hydrochloric acid is used in the process. In some cases, HVP is added directly to the products as an ingredient of formulation (Velíšek, 2009). In this study, all samples containing HVP as declared on the label ($N = 4$) presented 3-MCPD levels above the LOQ (range: 144–4405 μg/kg), which suggests the use of a highly contaminated ingredient, probably obtained by hydrolysis with hydrochloric acid. Data obtained from other countries showed maximum values of 3-MCPD in soy sauces (containing or not HVP) up to 0.2, 33, 148, 876 and 1779 mg/kg in Hong Kong, Japan, Australia, United States and European Union, respectively (FAO/WHO, 2007).

It should be mentioned that maximum regulatory limits of 0.02 (for a liquid product containing 40% of dry matter) and 1 mg/kg are currently established in Europe and in Canada, respectively, for the presence of 3-MCPD in soy sauces (Velíšek, 2009). For comparison purposes, when the positive results ($N = 7$) were adjusted for 40% of dry matter, levels between 20 and 7048 μg/kg were obtained, showing that 6 out of 7 samples would be above the maximum concentration set up by the European Commission (EC, 2006).

Table 3 presents the results obtained in relation to the occurrence of 3-MCPD in foods and beverages containing malt-derived ingredients. As can be observed, the levels ranged from not detected to 156 μg/kg. Concentrations above the LOQ were reported in 11 samples including granola, crackers and toast. Surveys carried out in the United Kingdom showed maximum levels of 134 μg/kg in crackers and 16 μg/kg in beers while the contaminant was not detected in malted milk beverages and breakfast cereals (UK FSA, 2001).

Table 3
3-MCPD levels in foods and beverages containing malt-derived ingredients ($N = 52$).

Sample	$N/N+$	3-MCPD (μg/kg)	
		Mean	Min–Max
Granola	6/4	52	nd–156
Cereal bar	4/0	0.0–3.2	nd
Corn flakes	6/0	0.0–4.5	nd–<10.7
Cracker	5/5	21	14–32
Toast	2/2	25	24–25
Chocolate drink powder	6/0	0.0–4.5	nd–<10.7
Beer[a]	23/0	0.0–3.2	nd

N = number of samples; $N+$ = number of samples above the limit of quantification (LOQ = 10.7 μg/kg); nd = results below the limit of detection (LOD = 3.2 μg/kg). To calculate the mean, values below the LOD and LOQ were treated according to the World Health Organization (WHO, 2002). The range indicates different results for the lower and upper bounds for a group of data with less than 40% of quantifiable results.
[a] Pilsen, dark, premium and non-alcoholic beer.

Although the formation of 3-MCPD in malt and derived ingredients seems to be unavoidable and levels up to 247 and 850 μg/kg have already been reported in dark malt and malt extract (Hamlet et al., 2002), the low proportion of positive results (21%) suggests that these ingredients may not contribute as a significant source of contamination by 3-MCPD to foods in which they are added. Therefore, these results indicate that the occurrence of 3-MCPD in granola, crackers and toast might be due to its formation during thermal processing rather than the addition of malt and derived ingredients to the formulation.

The results concerning the presence of 3-MCPD in samples of bread are shown in Table 4. The concentrations varied from not detected to 716 μg/kg (11 samples above LOQ) and the highest levels were observed in French bread and Italian bread. Data reported in the literature show 3-MCPD concentrations in bread ranging from not detected to 76 μg/kg. In the bread crust and toast, these values may reach up to 275 and 679 μg/kg, respectively (Hamlet & Sadd, 2009).

Studies conducted in model systems simulating fermented dough have suggested that glycerol is an important precursor of 3-MCPD in baked cereal products (Hamlet, Sadd, & Gray, 2004). Moreover, the formation of the contaminant in these products may be affected by lipids as well as sugar and organic acids. Fermentation time and pH may also have a significant impact on the final levels of 3-MCPD in breads (Breitling-Utzmann, Hrenn, Haase, & Unbehend, 2005; Hamlet & Sadd, 2005; Hamlet et al., 2004). Long fermentation times and low pH have been associated to higher concentrations of the contaminant, which is justified by the increase on the levels of glycerol due to the metabolism of the yeast and on the release of organic acids. These observations could explain the high concentrations found in Italian bread since its production usually involves the partial use of pre-fermented dough with a reduced pH.

Table 5 shows the levels of 3-MCPD found in smoked foods. The concentrations varied from not detected to 49 μg/kg and the contaminant was observed above the LOQ in 14 samples (47%). The highest % of quantifiable results was reported in sausage and the highest levels were found in cheese. Surveys carried out in Germany and United Kingdom concerning the presence of 3-MCPD in smoked meats showed concentrations ranging from <10 to 47 μg/kg in bacon and from <5 to 74 μg/kg in sausages and ham (Kuntzer & Weisshaar, 2006; UK FSA, 2001).

The data reported in the present study were used to estimate the intake of 3-MCPD by the Brazilian population considering information on food consumption of selected products (Arisseto, Vicente, Furlani, & Toledo, 2013). The authors concluded that the dietary intakes of 3-MCPD correspond to 3–5% and 16–25% of the PMTDI for average and high consumer, respectively, and the overall risk to human health due to the exposure to this contaminant is low. However, it was highlighted that a potential concern may arise for consumers of frozen ready-to-eat meals with high concentrations of 3-MCPD, which were not taken into account in this preliminary evaluation. For illustration purposes, the consumption of 250 g in a day of a meal containing 2529 μg/kg could result in a 3-MCPD intake of 10.5 μg/kg bw/day, considering an individual body weight of 60 kg. This value is approximately 5-fold higher than the PMTDI and may pose some risk for these target consumers. Besides that, the high concentrations of 3-MCPD fatty acids esters found in refined vegetable oils and fats may represent an additional concern (Arisseto, Marcolino, & Vicente, 2014; EFSA, 2013; Zelinková et al., 2006). The use of these ingredients in cereal-based products, breads, soups, stock cubes and snacks, for example, should be considered as a potential source of contamination, resulting in an exposure to free 3-MCPD even higher.

Table 4
3-MCPD levels in breads ($N = 30$).

Sample	$N/N+$	3-MCPD (μg/kg)	
		Mean	Min–Max
Whole bread	4/1	2.8–11	<10.7–11
White loaf bread	5/0	0–10.7	<10.7
French bread	6/4	43	nd–104
Corn bread	2/1	8.8	<10.7–12
Bread roll	5/2	7.3	nd–21
Pitta bread	3/0	0.0–8.2	nd–<10.7
Knead bread	2/0	0–10.7	<10.7
Italian bread	3/3	450	18–716

N = number of samples; $N+$ = number of samples above the limit of quantification (LOQ = 10.7 μg/kg); nd = results below the limit of detection (LOD = 3.2 μg/kg). To calculate the mean, values below the LOD and LOQ were treated according to the World Health Organization (WHO, 2002). The range indicates different results for the lower and upper bounds for a group of data with less than 40% of quantifiable results.

Table 5
3-MCPD levels in smoked foods ($N = 30$).

Sample	$N/N+$	3-MCPD (μg/kg)	
		Mean	Min–Max
Provolone cheese	6/6	22	6.0–49
Sausage	8/4	6.2	nd–15
Bacon	6/2	3.0–5.6	nd–12
Turkey breast	3/1	2.0–3.2	nd–6.0
Turkey breast *paté*	2/0	0.0–1.8	nd
Chicken breast	1/0	0.0–5.9	<5.9
Bologna	2/1	3.9	nd–6.8
Tuna	2/0	0.0–1.8	nd

N = number of samples; $N+$ = number of samples above the limit of quantification (LOQ = 5.9 μg/kg); nd = results below the limit of detection (LOD = 1.8 μg/kg). To calculate the mean, values below the LOD and LOQ were treated according to the World Health Organization (WHO, 2002). The range indicates different results for the lower and upper bounds for a group of data with less than 40% of quantifiable results.

4. Conclusions

The present study reported the results of a survey on 3-MCPD in 232 samples of processed foods and beverages commercialized in Brazil, which is, to our knowledge, the first information about the occurrence of this contaminant in the country. It was observed that the addition of HVP may be an important source of contamination by 3-MCPD in several foods, including sauces and ready-to-eat meals. Therefore, it is important to monitor and control the 3-MCPD content in the HVP that is added to food as a savory ingredient as well as to stimulate the reduction of 3-MCPD levels during the production of acid-HVP since no regulation is established in Brazil for this contaminant so far. Significant concentrations were also found in some types of bread and other cereal-based products, which may represent an additional concern due to the use of refined oils and fats containing high levels of 3-MCPD fatty acids esters.

Contributors

E. Vicente contributed to the conception of the work, designed data collection, analyzed and discussed the data, and drafted and revised the paper. A.P. Arisseto contributed to the conception of the work, designed data collection, analyzed and discussed the data, and drafted and revised the paper. R.P.Z. Furlani contributed to the conception of the work, designed data collection, analyzed and discussed the data, and drafted and revised the paper. V. Monteiro contributed to the acquisition of data. L.M. Gonçalves contributed to the acquisition of data. A.L.D. Pereira contributed to the acquisition of data. M.C.F. Toledo contributed to the conception of the work, designed data collection, analyzed and discussed the data, and drafted and revised the paper.

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