Persistent organic pollutants and stable isotopes in pinnipeds from King George Island, Antarctica

Caio V.Z. Cipro\textsuperscript{a,b}, Paco Bustamante\textsuperscript{b}, Satie Taniguchi\textsuperscript{a}, Rosalinda Carmela Montone\textsuperscript{a}

\textsuperscript{a}Universidade de São Paulo, Instituto Oceanográfico, Praça do Oceanográfico, 191, 05508-120 São Paulo-SP, Brazil
\textsuperscript{b}Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, 17042 La Rochelle Cedex 01, France

\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

In the present work, fat, skin, liver and muscle samples from \textit{Leptonychotes weddellii} (Weddell seal, \(n = 2\)) individuals, \textit{Lobodon carcinophagus} (crabeater seal, \(n = 2\)), \textit{Arctocephalus gazella} (Antarctic fur seal, \(n = 3\)) and \textit{Mirounga leonina} (southern elephant seal, \(n = 1\)) were collected from King George Island, Antarctica, and analysed for POPs (PCBs, organochlorine pesticides and PBDEs) and stable isotopes (\(\delta^{13}C\) and \(\delta^{15}N\) in all tissues but fat). PBDEs could be found in only one sample (\textit{L. weddellii} fat). Generally, PCBs (from 74 to 523 ng g\textsuperscript{-1} lw), DDTs (from 14 to 168 ng g\textsuperscript{-1} lw) and chlordanes (from 9 to 78 ng g\textsuperscript{-1} lw) were the prevailing compounds. Results showed a clear stratification in accordance with ecological data. Nonetheless, stable isotope analyses provide a deeper insight into fluctuations due to migrations and nutritional stress. Correlation between \(\delta^{15}N\) and pollutants suggests, to some degree, a considerable ability to metabolize and/or excrete the majority of them.

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\textbf{1. Introduction}

Antarctica, in spite of being the most isolated continent on Earth, has not escaped the deleterious effects of human activity. Its unique marine ecosystems and their endemic faunas are affected on local and regional scales by overharvesting, pollution and the introduction of alien species (Aronson et al., 2011). Persistent organic pollutants (POPs) are deposited in Antarctica following the process of global distillation, and the cold conditions of the Antarctic environment favour their persistence compared to temperate and tropical environments (de Wit et al., 2006). The storage of lipids as an energy source makes Antarctic food webs vulnerable to bioaccumulative chemicals, and top predators are the species exposed to the greatest risk (Loganathan et al., 1990; Loganathan and Kannan, 1991).

Pinnipeds and other marine mammals differ from terrestrial ones because their high rates of lactational energy transfer to the young, primarily because of the elevated milk lipid content (Carlini et al., 2000). This characteristic also contributes to the transfer of lipophilic contaminants to the young. In cetaceans and pinnipeds, more than 90% of organochlorine contaminants present in neonates are transferred through milk, greatly exceeding gestational transfer before birth (Addison and Stobo, 1993; Borrell et al., 1995). Because of their elevated trophic position in the marine environment, their relatively long life spans and their elevated energy requirements, pinnipeds can also be regarded as sentinel species for studying contaminant bioaccumulation and its deleterious effects (Ross, 2000). Limited information is available for both POP levels and isotopic ratios for Antarctic pinnipeds. In this context, the aim of the present work is to evaluate the occurrence and transfer of organic contaminants (organochlorine compounds and polybrominated diphenyl ethers) in Antarctic pinnipeds using stable isotope analysis (SIA) as an ecological tool to provide a deeper understanding of the results, since carbon and nitrogen supply data, respectively, on carbon sources exploited by consumers and trophic position (Lesage et al., 2002). \(\delta^{13}C\) values are generally used as a tracer of the habitat or the feeding zone of organisms (France, 1995; Hobson, 1999). \(\delta^{15}N\) values are particularly used as an indicator of the trophic position (TP) of organisms, and have been widely employed to calculate the absolute or relative trophic level of organisms in various ecosystems by measuring their concentrations in tissues of a suite of consumers, since they are enriched in \(\delta^{15}N\) relative to their food (Hobson and Welch, 1992; Lesage et al., 2001). Conversely, \(\delta^{13}C\) values vary little (1% per trophic level vs 3–5% from \(\delta^{15}N\)) along the food chain and are mainly used to determine sources of primary production in a trophic network (DeNiro and Epstein, 1978). In the marine environment, \(\delta^{13}C\) values can also indicate inshore vs offshore, or pelagic vs benthic, contribution to food intake (France, 1995). Furthermore, the knowledge of the food chain length is one key aspect for understanding the transfer of organic...
contaminants in marine food webs. Overall, SIA and derived TP and/or feeding zones of organisms may thus help to investigate the transfer of POPs in food webs of interest (Dietz et al., 2004).

2. Materials and methods

2.1. Sampling

Samples were collected from King George Island (62°05’S 58°23’W) in the austral summers of 2004/2005 and 2005/2006. Sampling was fully opportunistic, i.e. only from animals found already dead, with no signs of degradation. All samples were taken using previously n-hexane-rinsed instruments, stored in previously n-hexane-rinsed instruments, stored in previously n-hexane-rinsed instruments, and/or feeding zones of organisms may thus help to investigate the transfer of POPs in food webs of interest (Dietz et al., 2004).

2.2. Chemical analyses

Organochlorine (OC) and PBDE analyses were performed at the University of São Paulo (Brazil). Laboratory protocol was based on MacLeod et al. (1986) and quality assurance/quality control (QA/QC) followed guidelines described by Wade and Cantillo (1994). Extraction and clean-up were common for all contaminant analyses. Briefly, wet samples (0.25 g for fat, 2.5 g for liver) were ground with anhydrous Na2SO4 and surrogate (PCB 103) was added before extraction in a Soxhlet apparatus for 8 h with 80 mL of n-hexane and methylene chloride (1:1, v/v). The extract was concentrated (by rotoevaporation) to 1 mL and cleaned up in a column filled from top to bottom with 16 g alumina and 8 g silica gel (both 5% deactivated with water). The extract was eluted (100 mL of methylene chloride) and subsequently concentrated (also by rotoevaporation) to 500 μL. A further clean-up step was performed in an HPLC-size exclusion column system: two Phenogel 100 A (22.5 × 250 mm) and a 7.8 × 50 mm precolumns. Methylene chloride was used as mobile phase. A new roteoveaporation (up to 900 μL) followed, and finally, internal standard (100 μL TCMX, used to estimate surrogate recovery) was added to the purified extract prior to injection in the gas chromatograph.

OC analyses were run in a gas chromatograph equipped with an electron capture detector (GC-ECD, Agilent Technologies, model 6890 N). Hydrogen was used as carrier gas at constant pressure (13.2 psi, i.e. 91.01 kPa). The injector was operated in splitless mode and kept at 300°C (13.2 psi, i.e. 91.01 kPa). The capillary column used was a DB-5 (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness). The detector operated at 320°C using N2 as makeup gas at a flow rate of 58 mL min⁻¹. The oven was programmed as follows: 70°C for 1 min, 5°C min⁻¹ to 140°C (1 min), 1.5°C min⁻¹ to 250°C (1 min) and 10°C min⁻¹ to 300°C (5 min). The investigated compounds were PCBs (IUPAC Nos. 8, 18, 28, 31, 33, 44, 49, 52, 56, 60, 66, 70, 74, 77, 81, 87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126, 128, 132, 138, 141, 149, 151, 153, 156, 157, 158, 167, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 201, 203, 206 and 209), DDTs (p,p’-DDD, p,p’-DDE, p,p’-DDD, p,p’-DDE, p,p’-DDE, and p,p’-DDE), HCB, HCHs (α, β, γ, and δ isomers), chlordane (α- and γ-chlordane, heptachlor and heptachlor epoxide), mirex and drins (aldrin, dieldrin and endrin). Surrogate recovery ranged from 98% to 111%. Detection limits were set as three times the standard deviation (σ) of seven method blank replicates. Spiked matrices were recovered within the acceptance ranges (i.e. 40–130% for at least 80% of the spiked analytes) suggested by Wade and Cantillo (1994). Method validation was performed using NIST SRM 1945 (Organics in Whale Blubber). Blanks were included in every analytical batch (usually 10–12 samples) and all data were blank-subtracted.

PBDE analyses were performed in a gas chromatograph 6890 Plus attached to the MS 5973 N mass detector, with an HP-5MS column (30 m long × 250 μm internal diameter and internal film 0.25 μm thick). The congeners analysed were the IUPAC # 28, 47, 99, 100, 153, 154 and 183. The injector operated at 270°C. The oven was programmed as follows: 130°C for 1 min, 12°C min⁻¹ until 154°C (0 min), 2°C min⁻¹ until 210°C (0 min), 3°C min⁻¹ until 300°C (5 min). PCB 103 was used as surrogate and TCMX as internal standard.

Stable isotope analyses (SIA) were performed at the University of La Rochelle (France). Prior to SIA, samples were lyophilized and ground to obtain a fine powder. One aliquot of 100 mg of sample was placed in a test tube with 4 mL of cyclohexane to remove lipids. The mixture was shaken for an hour, then centrifuged for separation (as many times as needed, until the liquid phase, which is discarded, comes out clear) and dried at 50°C for 48 h. Purified samples were analysed using a Thermo Scientific Delta V Advantage, ConFlo IV interface (NoBlank and SmartEA) and Thermo Scientific Flash EA1112 Elemental Analyzer. Each injection corresponded to 0.4 ± 0.1 mg of sample encapsulated in tin cups, and there were no replicates. Pee Dee Belemnite and atmospheric nitrogen were used as standards for calculation of δ¹³C and δ¹⁵N, respectively. Based on replicate measurements of internal laboratory standards, experimental precision is of ±0.13‰ and ±0.20‰ for δ¹³C and δ¹⁵N, respectively.

Statistical tests were performed in Microsoft Excel (2007 version) and StatSoft Statistica (10.0) at P = 0.05 and non-parametric distribution.

3. Results and discussion

3.1. Organic pollutants

With regard to PBDE analyses, only one sample (fat from L. weddellii) presented concentration superior to the MDLs, which was 2.04 ng g⁻¹ lw (lipid weight), only for BDE #99. This congener is the second most present in the technical formula available in the Americas, with a profile reasonably similar to the Antarctic environmental results as in Yogui and Sericano (2008). However, another study (Corsolini et al., 2007) points out some interspecific differences that could be interpreted as being the result of the fractionation of the technical product. In fat samples from A. gazella pups, Schiavone et al. (2009b) reported an equally small concentration of PBDEs of 2.35 ng g⁻¹ ww. An important fact that might explain these apparently low values is the small distance from the base of the food web that some of these organisms occupy, due to the representativeness of euphausiids in their diets. This is specially the case for L. carcinophagus, which feed on krill, Euphausia superba, practically all year round (Berta et al., 2006). Such a specialized diet also appears for A. gazella when it forages in Antarctic waters (Berta et al., op. cit.). For comparison purposes, the closest trophic equivalent to L. weddellii in the northern hemisphere would be the grey seal, Halichoerus grypus, with the closest (yet slightly higher) δ¹³N values (data from Aubail et al., 2011). Since no significant difference in δ¹³N values is found for primary producers in either hemisphere (Horton et al., 2009; Mincks et al., 2008), the difference between H. grypus and L. weddellii (around 0.8‰) must be taken into account. Ilkonomou and Addison (2008) report averages for PBDEs in grey seals ranging from 27.8 up to 319 ng g⁻¹ lw depending on the remoteness of the collection site. Nevertheless.
this represents from one up to two orders of magnitude above the levels found in the single sample from the present work which overcame the MDLs. Higher values in the northern hemisphere can be explained by greater contamination as a result of the presence of industrialized countries in a greater extent. These outputs have been highlighted for POPs in general but also for other contaminants such as petroleum hydrocarbons (Poland et al., 2003). Another reason might be that food chains in the Arctic are somewhat longer than in the Antarctic (Aubail et al., 2011).

The results obtained for organochlorine compounds are shown in Table 1. As previously stated, L. carcinophagus allegedly has the lowest trophic level among the analysed species (Table 1). This fact can directly explain why this species displays the lowest OC and PBDE concentrations. There is a clear stratification between the species, in spite of different ecological niches, which will be further discussed in the stable isotope analysis section. As a basis for comparison, Yogui (2002) found DDTs, PCBs, mirex, chlordanes and HCB concentrations in L. weddellii from the same area of the present study in the same order of magnitude (i.e. 460 ng g⁻¹, 150 ng g⁻¹, 18 ng g⁻¹, 4 ng g⁻¹ and 2 ng g⁻¹ in lipid weight, respectively). However, Yogui (op. cit.) shows an inversion of the PCB ratio, indicating a decrease in the inputs from agricultural activity in relation to industrial ones during the last decade.

Quantitative data for A. gazella are in the same order of magnitude as previously reported in fat (Schiavone et al., 2009a,b), as well as for M. leonina in liver samples (juveniles reported in Miranda-Filho et al., 2007). Kajiwara et al. (2001) presented data in the same order of magnitude, in a general way, for fat and liver in pinnipeds (the California sea lion, Zalophus californianus, the northern elephant seal, Mirounga angustirostris and the harbour seal, Phoca vitulina) from California. When compared to M. leonina from the present study, OCs show slightly lower concentrations in M. angustirostris (Kajiwara et al., 2001). One must take into account that, even though collections by Kajiwara et al. (op. cit.) were made in the boreal summer, such an environment is not as seasonal as the Antarctic and therefore fluctuations due to nutritional stress should be lower.

L. weddellii show higher values (from one to two orders of magnitude) when compared to the ones found for one population in the same area of study by Vetter et al. (2003). However, when comparing our values to those for the population from Terra Nova Bay, which is located south (at 74°S) and therefore more subject to the effect of cold trap (Vetter et al., op. cit.) both data sets are compatible. This might be better explained by the possible nutritional stress of the individuals collected for the present work. Indeed, such a stress would notably remobilize and increase the concentrations of contaminants in the tissues (Burek et al., 2008). The qualitative profile of PCBs is shown in Fig. 1.

With regard to PCB distribution, A. gazella presents the proportionally heavier profiles, followed by L. weddellii and then by L. carcinophagus, in an analogous arrangement to the one found in Table 1. Because of the greater environmental persistence of the heavier congeners (Fuoco and Ceccarini, 2001), the biomagnification effect not only makes absolute values higher throughout a trophic web, but also makes qualitative profiles heavier. Previous data for A. gazella (Schiavone et al., 2009a,b) show a reasonably similar distribution, with the exception of octa-CBs, which prevailed then and represented less than 5% of the total PCBs in the present study.

In the liver, M. leonina showed a similar congener distribution to the one presented by Miranda-Filho et al. (2007), in which prevailed the hexa (51.2%), penta (17.8%) and hepta-CBs (15.9%); hexa-CBs also prevailed in the present work, however less significantly (32.8%), followed by hepta and tetra-CBs (Fig. 1).

3.2. Stable isotopes

Opportunistic sampling limits the number of individuals but at the same time offers the possibility of collecting several different tissues per individual. This presents a great advantage since different tissues have different turnover rates and their stable isotope values therefore represent different integration times. Stable isotope data are presented in Fig. 2.

Turnover of stable isotopes varies according to the protein metabolic rate, so the analyses of multiple tissues from one individual allow a more thorough approach by providing data over a range of timescales (Kurle and Worthy, 2002; Mendez-Fernandez et al., 2012). For nitrogen, according to the literature, liver has the fastest turnover rate, followed by skin and then muscle (Kurle and Worthy, 2001, 2002; Lesage et al., 2002). For example, the protein matter half-lives for several tissues of the northern fur seal, Callorhinus ursinus, goes from 1.9 to 6.7 days for the liver and from 12.5 to 83.3 days for the muscle (Kurle and Worthy, 2002). In the skin of the same species, this variable has been estimated as between 6.4 and 27.6 days (Kurle et al., 2001). In both of the previous studies, the authors stated that the turnover rate itself ranges from 2 to 3 times the half-life, thus it is possible to deduce, with some approximation, that liver, skin and muscle will reflect the diet from 4 to 20, 13 to 83 and 25 to 250 days, respectively, before sampling. This overlapping between liver and skin (13–20 days), and especially between skin and muscle (25–83 days), is a complicating factor, since the latter might include winter periods, when diet and distribution are less known than during the summer.

Zhao et al. (2004) reported isotopic data for the blood serum of four Antarctic seal species, two of which occur also in the present study (L. carcinophagus and L. weddellii). Since this matrix represents a period of the same amplitude as the liver (Lesage et al., 2002), it is reasonable to use them as equivalents for comparison. Results for δ¹⁵N show the highest values for L. weddellii, followed by A. gazella and then by M. leonina. According to dietary studies

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Lobodon carcinophagus</th>
<th>Arctocephalus gazella</th>
<th>Leptonychotes weddellii</th>
<th>Leptonychotes weddellii n = 1 from Yogui (2002)</th>
<th>Mirounga leonina n = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum HCHs (x,β,γ,δ)</td>
<td>0.223</td>
<td>3.21</td>
<td>2.59</td>
<td>–</td>
<td>1.41</td>
</tr>
<tr>
<td>HCB</td>
<td>7.23</td>
<td>4.72</td>
<td>5.77</td>
<td>2</td>
<td>7.48</td>
</tr>
<tr>
<td>δDrins (Aldrin, Endrin, Dieldrin and Isodrin)</td>
<td>18.4</td>
<td>82.4</td>
<td>18.5</td>
<td>–</td>
<td>6.88</td>
</tr>
<tr>
<td>ΣChlordanes (Heptachlor, epoxides, oxychlordane, x and γ-chlordane)</td>
<td>22.8</td>
<td>78.2</td>
<td>9.5</td>
<td>4</td>
<td>37.7</td>
</tr>
<tr>
<td>Endosulfan (III)</td>
<td>2.09</td>
<td>21.15</td>
<td>14.0</td>
<td>–</td>
<td>2.72</td>
</tr>
<tr>
<td>ΣDDTs (DDD, DDT and DDE in op’ and pp’ configurations)</td>
<td>14.4</td>
<td>168</td>
<td>131</td>
<td>460</td>
<td>98.7</td>
</tr>
<tr>
<td>Mirex</td>
<td>14.4</td>
<td>17.0</td>
<td>5.53</td>
<td>18</td>
<td>16.2</td>
</tr>
<tr>
<td>ΣPCBs</td>
<td>154</td>
<td>523</td>
<td>300</td>
<td>150</td>
<td>73.9</td>
</tr>
</tbody>
</table>
(Jefferson et al., 2008 and references therein), the importance of benthonic prey is higher for *M. leonina* than for *L. weddellii*, whilst for *A. gazella* the diet is highly seasonal and population dependent. It is worth mentioning that, similarly to what happens with organic pollutant concentrations, $\delta^{15}$N values also increase in individuals under nutritional stress (Lesage et al., 2002; Dehn et al., 2006), which might enlighten the understanding of the obtained results.

Data for *A. gazella* show a clear shift in the feeding area due to the large variation in $\delta^{13}$C (from $-21.6$ to $-25.0$; Fig. 2), presenting enriched values in skin tissue, the one with the intermediate turnover rate. This suggests a foraging area of lower latitude to be more likely and the increase of coastal/benthic prey in diet to be less likely, which would be reflected in $\delta^{15}$N as well, as shown by Dunton (2001). The results presented for liver, with faster turnover, are plausible with the site collection, whereas the results for muscle, with slower turnover, are plausible with the distribution presented by Jefferson et al. (2008) that reported males in winter even south of the consolidated pack ice. These authors reported the species to occur in some areas north of the Antarctic Convergence, which has a significant effect on $\delta^{13}$C (the colder the temperature, the higher the fractionation, e.g. Cherel et al., 2007). With regard to the $\delta^{15}$N fluctuation, Ciaputa and Sicinski (2006) reported variations in the diet of *A. gazella* with the decrease of krill consumption according to its distribution in certain years, but also because fur seals feed closer to the shore at the time of year they remain in the King George Island area. Consequently, *A. gazella* increase its fish consumption, which explains the higher $\delta^{15}$N in the tissue with the fastest turnover. In fur seals from Bouvet Island, which is also under the influence of the Antarctic Convergence, more variation in preying on benthonic organisms than pelagic ones has been reported (Jacob et al., 2006), which also enlightens the data set, since in the study area Dunton (2001) reported an average $\delta^{15}$N of $0.5\%e$ for a pelagic primary producer and $4\%e$ for a benthonic one. Thus one might conclude that generally, benthonic organisms will have a higher $\delta^{15}$N, which will be reflected in their consumers.

For *L. weddellii*, there seems to be no variation in feeding areas according to the results for $\delta^{13}$C analysis in the three tissues as little variation is observed (Fig. 2). Interestingly, temporal variation for this species has been shown at Mawson ($68^\circ 00’S 066^\circ 00’E$), with prey from upper trophic levels being less frequent during winter, as well as the total quantity of prey (Lake et al., 2003). These observations strongly support the fact that a nutritional stress is reflected by the increase in $\delta^{15}$N observed in skin samples. Taking all this information into account, muscle tissue has the lowest $\delta^{15}$N and the slowest turnover rate on average, which would indicate a period of consumption of lower trophic level prey and/or no nutritional stress. This is followed by a period of higher trophic level prey and/or nutritional stress (indicated by skin $\delta^{15}$N values, with the intermediate turnover rate), finally followed by another period of consumption of lower trophic level prey and/or no nutritional stress, indicated by $\delta^{15}$N values of the liver, the tissue with the fastest turnover rate and therefore the one which reflects diet closer to the time of collection. However, this hypothesis is impaired by the overlapping of the tissues’ turnover rates, as previously discussed.
With regard to *L. carinophagus*, the hypothesis of individuals having been collected under nutritional stress is evident for two reasons: firstly, because of the concentrations of organic pollutants, which were in several cases comparable to those from *L. weddellii*, while this species occupies an upper trophic position and is therefore more prone to biomagnification; secondly, because the values of $\delta^{15}N$ in the present work are from 3‰ to 4‰ higher than those found by Zhao et al. (2004). Since this species is highly adapted to the consumption of krill, which is largely the most frequent item in its diet (Berta et al., 2006), the possibility of a dietary shift capable of justifying such an increase is highly unlikely.

The interpretation of the elephant seal *M. leonina* results presents yet another complicating factor: a significant sexual segregation in diet and feeding strategies and consequently in isotopic analyses, as demonstrated by Lewis et al. (2006). Nevertheless, there seems to be no significant difference for $\delta^{15}N$ between the tissues (liver and muscle), which makes the hypothesis of nutritional stress unlikely. This is additionally confirmed by the extractable organic matter content of the liver (~85%), which might indicate a good health condition.

### 3.3. Statistical tests

The results for Spearman’s rank correlation for the whole data set are shown in Table 2.

Only three values were statistically significant, for two reasons: the reduced sampling number and the large fluctuations in data, due to the nutritional stress of some individuals as previously addressed. Correlation with $\delta^{15}N$ was negative for all the contaminants, except for Endosulfan, DDTs and mirex. This could be due to the fact that (1) these three contaminants are less subject to metabolism/excretion than the others, and (2) nutritional stress causes fluctuations proportionally higher in $\delta^{15}N$ than in the concentrations of organic pollutants. This second hypothesis is less likely to occur because of the preferential mobilization of lipids compared to proteins.

### 4. Conclusions

The data presented here, in spite of the limitations caused by small sampling numbers, characteristic of opportunistic sampling studies in remote environments, contribute to the scarce literature on POPs (especially on PBDEs) and SIA in Antarctic predators, and moreover in the correlation of the two data sets. Results showed stratification in concentrations of organic pollutants in accordance with ecological data, however stable isotope analyses provide a deeper insight into data fluctuations due to migrations, diet change and mainly nutritional stress, made evident by the different turnover rates of the three tissues present in the study, which reflects diet from more than 8 months to a couple of days before the collection. Correlation between $\delta^{15}N$ and organic pollutants for the majority of the compounds, especially (but not only) the lighter ones, suggests a considerable ability to metabolize or excrete the compounds. Additionally, important information is given about wintering periods, when several ecological parameters of the species are poorly known.

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