

# High Levels of Carcinogenic Polycyclic Aromatic Hydrocarbons in Mate Drinks

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## Abstract

**Background:** Drinking mate has been associated with cancers of the esophagus, oropharynx, larynx, lung, kidney, and bladder. We conducted this study to determine whether drinking mate could lead to substantial exposure to polycyclic aromatic hydrocarbons (PAH), including known carcinogens, such as benzo[*a*]pyrene. **Methods:** The concentrations of 21 individual PAHs were measured in dry leaves of eight commercial brands of yerba mate and in infusions made with hot (80°C) or cold (5°C) water. Measurements were done using gas chromatography/mass spectrometry, with deuterated PAHs as the surrogates. Infusions were made by adding water to the leaves, removing the resulting infusion after 5 min, and then adding more water to the remaining leaves. This process was repeated 12 times for each infusion temperature.

**Results:** The total concentrations of the 21 PAHs in different brands of yerba mate ranged from 536 to 2,906 ng/g dry leaves. Benzo[*a*]pyrene concentrations ranged from 8.03 to 53.3 ng/g dry leaves. For the mate infusions prepared using hot water and brand 1, 37% (1,092 of 2,906 ng) of the total measured PAHs and 50% (25.1 of 50 ng) of the benzo[*a*]pyrene content were released into the 12 infusions. Similar results were obtained for other hot and cold infusions.

**Conclusion:** Very high concentrations of carcinogenic PAHs were found in yerba mate leaves and in hot and cold mate infusions. Our results support the hypothesis that the carcinogenicity of mate may be related to its PAH content. (Cancer Epidemiol Biomarkers Prev 2008;17(5):1262–8)

## Introduction

Esophageal squamous cell carcinoma (ESCC) is a common cause of cancer death in some parts of South America, including southern Brazil, northeastern Argentina, Uruguay, and Paraguay (1). Drinking mate, an aqueous infusion of the herb *Ilex paraguariensis* (also known as yerba mate or erva mate), is considered a main risk factor for ESCC in these areas of South America (2). Daily consumption of large volumes of mate is common in these areas (3), and an association between mate drinking and ESCC has been found in most epidemiologic studies (2, 4–10).

The carcinogenic mechanism of mate is not entirely clear. Mate is usually prepared in a hollowed gourd by mixing the roasted leaves and stems of yerba mate with hot or cold water. Repeated thermal injury, resulting from drinking hot mate, has been suggested as a potential carcinogenic mechanism. However, data on the association between drinking temperature and ESCC

risk are inconsistent. Some studies (8, 10) have reported that only the temperature at which mate was drunk was associated with ESCC risk, and amount of mate was inconsequential. Other studies (2, 6), on the other hand, have reported that both high temperature and high volume of mate consumption were important and were independently associated with risk. Yet another study (7) reported that duration and amount of mate consumption were associated with cancer risk, but temperature was not.

Another potential mechanism for carcinogenicity of mate is its polycyclic aromatic hydrocarbon (PAH) content. PAHs are found in tobacco smoke and other burned organic material, and the IARC has classified some PAHs, such as benzo[*a*]pyrene, as carcinogenic to humans and experimental animals (11). The large leaves of yerba mate expose this plant to deposition of gaseous or particle-bound PAHs. More importantly, during production, leaves and stems are usually exposed to combustion products from burning gas, wood, or oil, and some of the roasted material may turn into ash, resulting in further PAH accumulation in the processed leaves. A recent study in Rio Grande do Sul, Brazil, showed that urine 1-hydroxypyrene glucuronide (1-OHPG), a stable metabolite of pyrene, a common PAH, was significantly higher in mate drinkers than in nondrinkers (12). In this study, there was a dose-response relationship between mate consumption and urine 1-OHPG, and drinking mate increased urine 1-OHPG approximately as much as smoking tobacco (12).

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The PAH content of dried leaves of yerba mate has been measured previously (13, 14). However, to our knowledge, no previous study has done a comprehensive examination of the PAH content of hot and cold mate infusions. We conducted this study to measure multiple PAHs in processed and dried yerba mate leaves, and in hot and cold infusions prepared from these leaves, and to estimate a range of PAH ingestion that could be expected from typical amounts of mate consumption.

## Materials and Methods

The concentrations of 21 individual PAHs were measured in dry leaves of eight commonly used commercial brands of yerba mate and in hot and cold infusions prepared from two commonly used brands. All assays were done in the Analytical Chemistry Division, National Institute of Standards and Technology, using previously validated methods (15, 16).

**Materials.** The eight commercial brands were purchased in Santa Maria, Rio Grande do Sul, Brazil. Standard reference materials (SRM), including SRM 2260a Aromatic Hydrocarbons in Toluene, SRM 2269 Perdeuterated PAH-I Solution in Hexane/Toluene, SRM 2270 Perdeuterated PAH-II Solution in Hexane/Toluene, Candidate SRM 3254 Green Tea Leaves (*Camelia sinensis*), and SRM 1649a Urban Dust, were obtained from the Standard Reference Materials Group at National Institute of Standards and Technology. All solvents were high-performance liquid chromatography grade.

**Sample Extraction from the Leaves.** Three subsamples (~2 g, exact mass known) of leaves from each brand of yerba mate and from SRM 3254 and one subsample (~0.3 g, exact mass known) from one bottle of SRM 1649a were put into pressurized fluid extraction cells containing hydromatrix (Isco) and were mixed with the hydromatrix. Hydromatrix was then used to fill the void space of the cells. A solution prepared by diluting SRM 2269 and SRM 2270 was added to each extraction vessel for use as the internal standards. The extractions used the following variables: solvent: dichloromethane; heat: preheat cell at 100°C for 1 min and extract at 100°C for 5 min; pressure: 13.8 MPa (2,000 psi); static cycles: three at 5 min each; flush: 90% volume; and purge: 100 s.

Because some fine leaf particles came through with the extract, the entire extract (~15 mL) was eluted through a silica plus Sep Pak (Waters) that was preconditioned with 20 mL of 20% (volume fraction) dichloromethane in hexane. The extract was then eluted from the Sep Pak using 20 mL of the same solvent mixture. The eluents from the Sep Pak were concentrated to ~0.5 mL under a stream of nitrogen gas using an automated evaporation system before gas chromatography/mass spectrometry analysis.

**Sample Extraction from the Mate Infusions.** Following analysis of the eight commercial brands of yerba mate, two commonly used brands (brands 1 and 2) were chosen for the infusion study. Mate infusions are usually made by filling three-fourths of a hollowed gourd with dry leaves and adding hot or cold water to the gourd. The infusion is typically drunk over ~5 min, and then more water is added to the remaining leaves in the

gourd. The laboratory experiments were designed to mimic the real-life use of mate.

Hot and cold infusions were prepared by mixing three subsamples (~5 g, exact mass known) of leaves with water at 80°C or 5°C, respectively. A solution prepared by diluting SRM 2269 and SRM 2270 was added to each infusion sample for use as the internal standards. Water (~30 mL; at the appropriate temperature) was added to the leaves in a 50 mL capped centrifuge tube, mixed, and allowed to sit for 5 min. The resulting infusion was removed after 5 min (without filtration) and put in a clean 50 mL capped tube. Fresh water at the appropriate temperature was then added to the leaves and the infusion was again removed after an additional 5 min. The process of adding water at the appropriate temperature and removing the infusion after 5 min was repeated for 60 min, with the infusions saved for analysis after 5 (as mentioned above), 15, 30, and 60 min from the initial contact of the leaves with water.

To back extract the PAHs from the infusions, 3 mL toluene was added to each infusion, and the mixture was vortexed for 30 s and left to sit overnight. The following day, the samples were centrifuged; the toluene layer was transferred to another vessel; and an additional 3 mL toluene was added to the infusion. The samples were again vortexed for 30 s, left to sit for 10 min, and then centrifuged. The toluene layers were combined, and this process was repeated once. The toluene was then concentrated to ~1 mL under a stream of nitrogen gas using an automated evaporation system and transferred to an autosampler vial. A known amount of *cis*-chlordane in hexane was then added to each autosampler vial before gas chromatography/mass spectrometry analysis for quantification.

**Gas Chromatography/Mass Spectrometry Analysis.** The PAHs of interest were quantified using gas chromatography/mass spectrometry. The column used for the gas chromatography/mass spectrometry analysis was a 0.25 mm × 30 m fused silica capillary column containing a 5% phenylmethyl-substituted polysiloxane phase, 0.25 μm film thickness. The column oven temperature was held isothermally at 60 °C for 1 min, temperature programmed at 40°C/min to 120°C for 15 min, and then temperature programmed at 4°C/min to 270°C for 30 min. The injection port was maintained at 250°C, and the transfer line was maintained at 280°C. All injections were done in the splitless mode (1 μL) with helium as a carrier gas at a constant flow rate of 1.2 mL/min. The split valve was opened at 1 min to 30 mL/min.

A five-point calibration curve was determined for each analyte of interest in the dry leaves by linear regression of response data from gravimetrically diluted solutions of SRM 2260a mixed with the internal standard solution (prepared from SRM 2269 and 2270). The five calibration solutions, as well as the blank extract (hydromatrix with internal standards spiked), were taken through the entire procedure (extraction, cleanup, and analysis). The response curves (not forced through the origin) were used for calculation of the concentrations in the leaves.

A five-point calibration curve was determined for each analyte of interest in the infusion samples by linear regression of response data from gravimetrically diluted solutions of SRM 2260a mixed with the internal standard

solution (prepared from SRM 2269 and 2270) and with the *cis*-chlordane solution added before analysis. The *cis*-chlordane was used for response calculations because the deuterated PAHs were extracted throughout the 60-min contact time with the hot and cold water. The five calibration solutions as well as a blank sample (internal standards added to water) were taken through the entire procedure (addition of hot or cold water, back extraction into toluene, and analysis). The response curves (not forced through the origin) were used for calculation of the concentrations in the infusion samples.

**Statistical Methods.** Each PAH measurement was repeated in the three subsamples. Means, SDs, and relative SDs (coefficients of variation) were calculated for these three subsamples. Pearson correlation coefficients were used to evaluate pairwise correlations between distributions of PAH concentrations in different brands of yerba mate, green tea, and urban dust. PAHs released into the infusions were measured (per g dry yerba mate) in the infusions collected at 5, 15, 30, and 60 min. For each individual PAH, we then fitted straight lines between consecutive measured values to estimate the amounts of the PAH released in the other unmeasured infusions (collected at 10, 20, 25, 35, 40, 45, 50, and 55 min), and we calculated an estimate of the total amount of each PAH released over the full 60 min.

All statistical analyses were done using Stata Software version 10 (Stata). Two-sided *P* values < 0.05 were considered as significant.

**Quality Control.** The concentrations of the PAHs determined in SRM 1649a Urban Dust used as a control sample during this study agreed well with the certified and reference concentrations from the Certificate of Analysis (Pearson correlation coefficient = 0.99). The relative SD for the individual PAHs measured in yerba mate samples ranged from 1% to 15%.

## Results

Table 1 shows mean mass fractions of each of the 21 PAHs in the eight commercial brands of yerba mate leaves and, for comparison, in a sample of Candidate SRM 3254 Green Tea Leaves. There were very high concentrations of PAHs in all yerba mate brands (Table 1). The total mass fraction of PAHs ranged from 536 ng/g in brand 5 to 2,906 ng/g in brand 1. For six of the brands (brands 1-4 and 6-7), the total PAH mass fraction was >1,900 ng/g. Benzo[*a*]pyrene mass fraction ranged from 8.03 ng/g in brand 5 to 53.3 ng/g in brand 6. In all six brands with high total PAH mass fractions, benzo[*a*]pyrene mass fractions were >35 ng/g. Compared to yerba mate leaves, green tea leaves had substantially lower mass fractions of all of the measured PAHs. The total mass fraction of the 21 PAHs in the green tea samples was 266 ng/g, and the mass fraction of benzo[*a*]pyrene was below the detection limit of 5 ng/g.

The mass fractions of individual PAHs showed similar distributions in the eight yerba mate brands. For example, pyrene mass fractions were high and perylene mass fractions were low in almost all brands. This resulted in very strong statistically significant pairwise correlations of the distribution of PAH mass fractions between each two brands, with most correlation coefficients being >0.96 and all *P* values < 0.01 (Table 2). Brand 5, however, showed only moderate correlations with other brands. In contrast, there were no statistically significant correlations between individual PAH mass fractions in yerba mate samples and those in green tea or urban dust. The correlation coefficients between PAHs in brand 1 leaves and green tea and urban dust were -0.28 (*P* = 0.56) and 0.00 (*P* = 0.99), respectively.

Table 3 shows the PAHs released (in ng/g dry leaves) into the four measured mate infusions made from brand 1. The infusions were removed every 5 min, and

**Table 1. Mean mass fractions (ng/g) of selected PAHs in eight brands of yerba mate leaves and Candidate SRM 3254 Green Tea Leaves**

|  | Brand 1 | Brand 2 | Brand 3 | Brand 4 | Brand 5 | Brand 6 | Brand 7 | Brand 8 | Green tea |
|--|---------|---------|---------|---------|---------|---------|---------|---------|-----------|
| Naphthalene                                  | 81.6    | 80.8    | 57.3    | 74.0    | 56.2    | 79.2    | 57.5    | 59.3    | 48.1      |
| Fluorene                                     | 17.4    | 18.5    | 20.4    | 16.1    | 12.0    | 18.2    | 22.7    | 15.7    | 12.7      |
| Phenanthrene                                 | 636     | 533     | 461     | 458     | 21.8    | 513     | 459     | 272     | 102       |
| Anthracene                                   | 72.0    | 57.4    | 70.9    | 55.1    | 25.7    | 60.0    | 69.5    | 32.8    | 4.22      |
| Fluoranthene                                 | 506     | 448     | 322     | 360     | 111     | 445     | 335     | 165     | 47.4      |
| Pyrene                                       | 535     | 498     | 318     | 387     | 111     | 518     | 337     | 181     | 27.7      |
| Cyclopenta[ <i>cd</i> ]pyrene                | 244     | 193     | 108     | 130     | 26.5    | 207     | 125     | 61.6    | —*        |
| Benzo[ <i>ghi</i> ]fluoranthene              | 94.6    | 83.7    | 62.7    | 67.6    | 16.0    | 91.8    | 56.8    | 29.0    | 4.24      |
| Benzo[ <i>c</i> ]phenanthrene                | 19.2    | 21.4    | 27.8    | 25.7    | 8.26    | 23.0    | 30.2    | 9.19    | —         |
| Benzo[ <i>a</i> ]anthracene                  | 84.8    | 79.5    | 88.7    | 99.9    | 24.5    | 84.9    | 88.4    | 31.9    | 4.24      |
| Chrysene + triphenylene                      | 154     | 131     | 141     | 169     | 42.3    | 143     | 140     | 51.2    | 15.9      |
| Benzo[ <i>b</i> + <i>j</i> ]fluoranthene     | 66.9    | 55.9    | 52.2    | 76.3    | 13.3    | 66.3    | 55.9    | 21.4    | —         |
| Benzo[ <i>k</i> ]fluoranthene                | 15.6    | 14.4    | 11.9    | 15.5    | 3.44    | 16.7    | 14.4    | 6.72    | —         |
| Benzo[ <i>a</i> ]fluoranthene                | 12.0    | 10.3    | 10.9    | 11.4    | 2.68    | 10.9    | 13.3    | 4.07    | —         |
| Benzo[ <i>e</i> ]pyrene                      | 72.9    | 30.8    | 24.0    | 47.5    | 6.53    | 39.2    | 24.9    | 10.3    | —         |
| Benzo[ <i>a</i> ]pyrene                      | 50.0    | 43.7    | 35.7    | 52.5    | 8.03    | 53.3    | 36.5    | 17.3    | —         |
| Perylene                                     | 5.02    | 5.56    | 2.83    | 5.38    | 2.67    | 5.35    | 4.39    | 1.56    | —         |
| Indeno[ <i>a</i> ,2,3- <i>cd</i> ]-pyrene    | 73.0    | 74.8    | 43.2    | 56.5    | 4.70    | 74.8    | 45.3    | 20.2    | —         |
| Benzo[ <i>ghi</i> ]perylene                  | 116     | 116     | 60.7    | 90.9    | 21.4    | 129     | 62.9    | 35.9    | —         |
| Dibenzo[ <i>a,h</i> + <i>a,c</i> ]anthracene | 20.8    | 20.0    | 16.7    | 17.3    | 17.6    | 22.4    | 17.6    | 17.6    | —         |
| Coronene                                     | 29.8    | 41.5    | —       | 26.7    | —       | 50.1    | —       | —       | —         |
| Total  | 2,906   | 2,557   | 1,936   | 2,242   | 536     | 2,651   | 1,996   | 1,044   | 266       |

NOTE: Mean mass fractions represent means of three subsamples assayed for each brand. The relative SDs for the three replicates are <15%.

\*Blank cells indicate that the mass fraction of the measured PAH was below the limit of detection.

**Table 2. Pairwise correlations of distributions of PAHs in different brands of yerba mate leaves**

|         | Brand 1 | Brand 2 | Brand 3 | Brand 4 | Brand 5 | Brand 6 | Brand 7 | Brand 8 |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Brand 1 | 1.00    |         |         |         |         |         |         |         |
| Brand 2 | 0.99    | 1.00    |         |         |         |         |         |         |
| Brand 3 | 0.98    | 0.98    | 1.00    |         |         |         |         |         |
| Brand 4 | 0.99    | 0.99    | 0.99    | 1.00    |         |         |         |         |
| Brand 5 | 0.71    | 0.74    | 0.66    | 0.72    | 1.00    |         |         |         |
| Brand 6 | 0.99    | 0.99    | 0.97    | 0.99    | 0.76    | 1.00    |         |         |
| Brand 7 | 0.99    | 0.98    | 0.99    | 0.99    | 0.68    | 0.98    | 1.00    |         |
| Brand 8 | 0.98    | 0.97    | 0.99    | 0.97    | 0.64    | 0.96    | 0.99    | 1.00    |

NOTE: All *P* values associated with the correlation coefficients are <0.01.

new water was added to the leaves. This process was repeated for 1 h, so there were a total of 12 samples of mate infusions. The PAHs released are shown for hot and cold mate samples removed at 5, 15, 30, and 60 min, which correspond to the 1st, 3rd, 6th, and 12th infusions, respectively. For hot mate, the totals of the 21 PAHs released into these four infusions were 182, 152, 60, and 46 ng/g dry leaves, respectively. The estimated total PAHs released into the 12 infusions was 1,092 ng/g. Benzo[*a*]pyrene release declined from 4.56 ng/g in the 1st infusion to 1.02 ng/g in the 12th infusion, with an estimated total of 25.1 ng/g released into all 12 infusions. For cold mate, the totals of the 21 PAHs released were 194, 97, 61, and 55 ng/g in the four measured infusions, respectively, with an estimated total release of 999 ng/g. Benzo[*a*]pyrene release declined from 4.39 in the 1st infusion to 1.50 ng/g in the 12th infusion, with an estimated total release of 28.1 ng/g.

Results for the infusions made from brand 2 are shown in Table 4. The amounts of PAHs (ng/g dry leaves) released into the infusions of this beverage were similar to those found for brand 1.

## Discussion

The results of our study show very high PAH mass fractions in all brands of the measured yerba mate leaves. There were large amounts of known carcinogens (IARC group 1), such as benzo[*a*]pyrene; probable carcinogens (IARC group 2A), such as cyclopenta[*cd*]pyrene; possible carcinogens (IARC group 2B), such as benzo[*b+j*]fluoranthene; and several other PAHs currently unclassifiable with regards to their carcinogenicity to humans (11). These high mass fractions may result from deposition of gaseous or particle-bound PAHs on growing leaves and/or exposure to combustion products or formation of ash during the processing of the leaves. The two previous studies that have measured PAHs in yerba mate leaves have found even higher amounts than we have. Schlemitz and Pfannhauser (13) reported that benzo[*a*]pyrene mass fractions were 542 ng/g in roasted leaves and 225 ng/g in green leaves. Ruschenburg (14) reported the presence of large quantities (24-461 ng/g) of benzo[*a*]pyrene in eight commercial samples of yerba mate leaves.

**Table 3. PAHs released into hot and cold mate infusions made from brand 1 (ng/g dry leaves)**

|                                    | Hot*         |              |              |                            |                              | Cold*        |              |              |                            |                              |
|------------------------------------|--------------|--------------|--------------|----------------------------|------------------------------|--------------|--------------|--------------|----------------------------|------------------------------|
|                                    | 1st infusion | 3rd infusion | 6th infusion | 12th infusion <sup>†</sup> | Estimated total <sup>‡</sup> | 1st infusion | 3rd infusion | 6th infusion | 12th infusion <sup>†</sup> | Estimated total <sup>‡</sup> |
| Naphthalene                        | 11.1         | 8.19         | 7.15         | 5.82                       | 89.7                         | 8.88         | 7.18         | 5.74         | 5.98                       | 78.0                         |
| Fluorene                           | 1.25         | 1.08         | 0.532        | 0.261                      | 7.88                         | 1.14         | 0.636        | 0.660        | <1                         | 8.02                         |
| Phenanthrene                       | 55.2         | 49.9         | 19.0         | 15.8                       | 348                          | 53.0         | 26.5         | 18.5         | 17.1                       | 289                          |
| Anthracene                         | 2.31         | 2.06         | 0.473        | 0.356                      | 12.0                         | 2.08         | 0.925        | 0.680        | 0.576                      | 10.5                         |
| Fluoranthene                       | 29.2         | 22.5         | 7.71         | 5.13                       | 153                          | 37.5         | 13.9         | 7.98         | 7.11                       | 152                          |
| Pyrene                             | 28.5         | 23.2         | 6.65         | 5.44                       | 150                          | 32.3         | 13.7         | 7.78         | 6.59                       | 141                          |
| Cyclopenta[ <i>cd</i> ]pyrene      | 18.1         | 13.6         | <4           | <3                         | 75.4                         | 20.8         | 10.5         | <3           | <3                         | 69.5                         |
| Benzo[ <i>ghi</i> ]fluoranthene    | 2.72         | 2.07         | <2           | <2                         | 17.3                         | 3.65         | <2           | <2           | <2                         | 16.0                         |
| Benzo[ <i>c</i> ]phenanthrene      | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |
| Benz[ <i>a</i> ]anthracene         | 2.61         | 2.11         | 1.01         | 0.796                      | 16.5                         | 2.75         | 1.32         | 0.935        | 0.751                      | 14.3                         |
| Chrysene + triphenylene            | 5.21         | 4.67         | 1.98         | 2.00                       | 35.4                         | 6.39         | 3.13         | 2.10         | 2.03                       | 34.0                         |
| Benzo[ <i>b+j</i> ]fluoranthene    | 3.63         | 3.23         | 1.80         | 1.02                       | 25.2                         | 4.25         | 2.51         | 1.08         | 0.935                      | 20.8                         |
| Benzo[ <i>k</i> ]fluoranthene      | 1.47         | 1.11         | 0.619        | 0.397                      | 9.16                         | 1.35         | 0.821        | 0.653        | 0.556                      | 8.96                         |
| Benzo[ <i>a</i> ]fluoranthene      | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |
| Benzo[ <i>e</i> ]pyrene            | 3.75         | 3.60         | 1.84         | 0.954                      | 26.2                         | 4.10         | 2.88         | 1.92         | 1.60                       | 27.6                         |
| Benzo[ <i>a</i> ]pyrene            | 4.56         | 3.64         | 1.25         | 1.02                       | 25.1                         | 4.39         | 2.76         | 2.07         | 1.50                       | 28.1                         |
| Perylene                           | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |
| Indeno[ <i>a,2,3-cd</i> ]-pyrene   | 2.76         | 2.25         | <2           | <2                         | 17.8                         | 2.30         | 1.23         | <2           | <2                         | 14.5                         |
| Benzo[ <i>ghi</i> ]perylene        | 4.19         | 3.59         | <2           | <2                         | 23.3                         | 4.08         | 3.12         | 2.16         | <2                         | 27.1                         |
| Dibenz[ <i>a,h+a,c</i> ]anthracene | 1.47         | 1.09         | <2           | <2                         | 12.9                         | 1.01         | <2           | <2           | <2                         | 12.0                         |
| Coronene                           | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |

\*Hot and cold mate infusions were prepared with 80°C and 5°C water, respectively.

<sup>†</sup>The infusion made with yerba mate was removed and fresh water at the appropriate temperature was added to the leaves every 5 min. The results are shown for infusions 1, 3, 6, and 12, which correspond to the infusions removed at 5, 15, 30, and 60 min.

<sup>‡</sup>The estimated totals were calculated by fitting regression lines between the four measurements.

Because most PAHs are hydrophobic, their release into the mate infusions has been generally thought to be low. Our results argue against this conclusion. Although small percentages of the PAH content were released in the first infusion, substantial portions were released into the total of 12 infusions, a situation that is more similar to what happens in daily life. For hot mate made from brand 1 leaves, for example, only 6% of total PAHs (182 of 2,906 ng) was released into the first infusion, but 37% (1,092 of 2,906 ng) was released in the 12 infusions. Similarly, 9% of the benzo[*a*]pyrene content (4.39 of 50 ng) was released into the first infusion, but 50% (25.1 of 50 ng) was released into all infusions. Interestingly, the percentages of released PAHs were very similar in the hot and cold infusions. Ruschenburg (14) made mate tea by mixing 15 g leaves with 1 L water and reported 0.02 to 0.12 µg/L benzo[*a*]pyrene in the infusion. Therefore, ~2% to 6% of the yerba mate benzo[*a*]pyrene content was found in the infusion. In a more recent study, Lin et al. (17) reported that 3% to 8% of the total PAH content of black tea was released into tea liquor. The results from these studies are similar to our findings in the first infusion, in which we found 6% of the total PAH content and 9% of the benzo[*a*]pyrene released into the mate infusion.

Our results also suggest that the benzo[*a*]pyrene intake associated with drinking mate may be comparable with that associated with smoking cigarettes. The reported per capita annual consumption of yerba mate leaves is 3 to 5 kg in southern Brazil (18), 5 kg in Argentina (19), and 8 kg in Paraguay (20). Consuming 3 kg/y is equivalent to using ~8 g yerba mate each day. Our results, which show a release rate of ~25 ng benzo[*a*]pyrene/g processed yerba mate leaves, suggest that drinking 12 infusions made from 8 g leaves will

mean an intake of ~200 ng benzo[*a*]pyrene. The benzo[*a*]pyrene content of the smoke from one cigarette is ~10 ng (21), so smoking a pack of 20 cigarettes will also mean an intake of ~200 ng of this chemical. A direct comparison of the benzo[*a*]pyrene or other PAH intake through tobacco smoking and drinking mate may be inaccurate because of differences in routes of absorption and metabolism. However, our findings are consistent with the previous study by Fagundes et al., which found that drinking mate increased urine 1-OHPG levels approximately as much as tobacco smoking did (12).

PAHs may be involved in the etiology of ESCC. Tobacco smoke, which includes PAHs, is strongly associated with ESCC (22, 23). Animal studies have shown a dose-response association between benzo[*a*]pyrene food levels and the incidence of esophageal cancer in mice (24). In Linxian, China, where the highest rates of ESCC are observed (25), previous studies have shown histopathologic evidence consistent with high exposure to PAHs in ESCC cases (26), presence of high levels of carcinogenic PAHs in staple foods (27), high concentrations of 1-OHPG in urine samples (28), and evidence of nuclear PAH-DNA adducts in esophageal tissue samples (29). In Golestan Province, Iran, another area with very high rates of ESCC (25), studies have also shown high levels of urine 1-OHPG, consistent with widespread exposure to PAHs (30). These findings, along with the results from our study, suggest that PAH exposure may be one reason for the consistent association observed between mate intake and ESCC.

In 1991, IARC categorized mate, *in toto*, as not classifiable with regards to human carcinogenicity but classified hot mate as a probable (group 2A) carcinogen to humans (31), suggesting that they judged that repeated thermal injury was probably the mechanism

**Table 4. PAHs released into hot and cold mate infusions made from brand 2 (ng/g dry leaves)**

|                                    | Hot*         |              |              |                            |                              | Cold*        |              |              |                            |                              |
|------------------------------------|--------------|--------------|--------------|----------------------------|------------------------------|--------------|--------------|--------------|----------------------------|------------------------------|
|                                    | 1st infusion | 3rd infusion | 6th infusion | 12th infusion <sup>†</sup> | Estimated total <sup>‡</sup> | 1st infusion | 3rd infusion | 6th infusion | 12th infusion <sup>†</sup> | Estimated total <sup>‡</sup> |
| Naphthalene                        | 10.4         | 7.37         | 6.06         | 4.33                       | 76.5                         | 8.70         | 7.68         | 5.68         | 4.20                       | 72.5                         |
| Fluorene                           | 1.83         | 1.18         | 0.750        | 0.334                      | 10.2                         | 1.82         | 1.16         | 0.628        | 0.445                      | 10.0                         |
| Phenanthrene                       | 58.0         | 32.0         | 25.0         | 10.0                       | 314                          | 60.4         | 29.5         | 23.5         | 16.1                       | 326                          |
| Anthracene                         | 2.39         | 1.24         | 0.576        | 0.215                      | 10.0                         | 2.45         | 1.22         | 0.769        | 0.473                      | 11.8                         |
| Fluoranthene                       | 31.0         | 16.4         | 12.2         | 5.48                       | 162                          | 32.7         | 16.7         | 12.2         | 8.91                       | 177                          |
| Pyrene                             | 30.4         | 17.0         | 11.2         | 4.08                       | 153                          | 34.3         | 17.5         | 12.6         | 7.49                       | 178                          |
| Cyclopenta[ <i>cd</i> ]pyrene      | 17.8         | 12.4         | <4           | <3                         | 72.0                         | 18.0         | 10.5         | <3           | <3                         | 65.3                         |
| Benzo[ <i>ghi</i> ]fluoranthene    | 3.38         | 2.06         | <2           | <2                         | 18.2                         | 3.88         | 2.04         | <2           | <2                         | 18.9                         |
| Benzo[ <i>c</i> ]phenanthrene      | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |
| Benz[ <i>a</i> ]anthracene         | 3.40         | 2.18         | 1.32         | 0.455                      | 18.1                         | 3.99         | 1.58         | 1.78         | 1.06                       | 21.7                         |
| Chrysene + triphenylene            | 7.74         | 5.07         | 2.56         | 1.23                       | 40.1                         | 8.52         | 4.03         | 3.40         | 2.34                       | 46.3                         |
| Benzo[ <i>b+j</i> ]fluoranthene    | 4.27         | 2.66         | 1.36         | 0.593                      | 21.2                         | 5.22         | 2.75         | 2.05         | 1.45                       | 29.0                         |
| Benzo[ <i>k</i> ]fluoranthene      | 1.87         | 0.742        | 0.614        | <2                         | 10.9                         | 1.55         | 1.08         | 0.920        | 0.749                      | 11.8                         |
| Benzo[ <i>a</i> ]fluoranthene      | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |
| Benzo[ <i>e</i> ]pyrene            | 3.90         | 2.01         | 1.54         | 0.377                      | 19.1                         | 3.45         | 2.32         | 1.56         | 1.31                       | 22.3                         |
| Benzo[ <i>a</i> ]pyrene            | 4.99         | 2.50         | 1.93         | 0.422                      | 23.9                         | 4.10         | 2.71         | 2.03         | 1.38                       | 26.9                         |
| Perylene                           | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |
| Indeno[ <i>a,2,3-cd</i> ]-pyrene   | 3.73         | 2.09         | 1.45         | <2                         | 20.1                         | 3.06         | 1.65         | 1.03         | <2                         | 16.9                         |
| Benzo[ <i>ghi</i> ]perylene        | 5.58         | 3.08         | 1.41         | 0.869                      | 25.5                         | 4.68         | 3.99         | 3.04         | 2.11                       | 38.1                         |
| Dibenz[ <i>a,h+a,c</i> ]anthracene | 1.49         | 1.18         | <2           | <2                         | 13.2                         | 1.50         | 0.869        | <2           | <2                         | 12.4                         |
| Coronene                           | <2           | <2           | <2           | <2                         | 12.0                         | <2           | <2           | <2           | <2                         | 12.0                         |

\*Hot and cold mate infusions were prepared with 80°C and 5°C water, respectively.

<sup>†</sup>The infusion made with yerba mate was removed and fresh water at the appropriate temperature was added to the leaves every 5 min. The results are shown for infusions 1, 3, 6, and 12, which correspond to the infusions removed at 5, 15, 30, and 60 min.

<sup>‡</sup>The estimated totals were calculated by fitting regression lines between the four measurements.

of carcinogenicity. Since then, however, additional articles have been published, which have shown an increased risk of ESCC associated with mate drinking independent of the temperature at which the beverage was consumed (2, 6). Also, a recent IARC review reevaluated carcinogenicity of PAHs and found more evidence for carcinogenicity of several of them (11). For example, benzo[*a*]pyrene, which was formerly classified as a probable carcinogen, is now categorized as a known carcinogen to humans. The more recent associations between mate and cancer, the recent IARC reevaluation of PAH carcinogenicity, and the results our study all provide evidence that mate itself is likely to be carcinogenic.

Other lines of evidence are also consistent with the hypotheses that mate is carcinogenic and that its carcinogenicity is, at least in part, due to the presence of mutagenic chemicals, such as PAHs. Fonseca et al. have reported that extracts of unprocessed yerba mate are mutagenic in bacterial assays and cause chromosomal aberrations in human peripheral lymphocytes treated *ex vivo* (32). In addition to ESCC, several other tobacco related cancers, such as cancers of the lung (33), larynx (34), oral cavity and oropharynx (35), kidney (36), and bladder (37, 38), have been significantly associated with mate drinking after adjustment for smoking. Interestingly, squamous cell and small cell lung cancers were more strongly associated with mate drinking than adenocarcinomas of the lung, a pattern that parallels the association of these histologic types of lung cancer with smoking (39, 40).

PAHs contaminating mate may increase cancer risk by local and/or systemic exposure. The esophagus expresses aryl hydrocarbon receptors that bind PAHs (41) and the xenobiotic metabolizing enzymes necessary for PAH activation (42, 43), so the esophagus can activate PAHs locally, without systemic absorption and passage through the liver. On the other hand, the association of mate drinking with increased risk for cancers of the larynx, kidney, and bladder suggests that the effect of mate is not solely local and suggests that absorbed contaminants, acting systemically, are also important.

Previous studies have attributed the carcinogenicity of mate to thermal injury, chemical components in the mate beverage, or both (2, 4-10). We found similar amounts of carcinogenic and total PAHs in mate infusions prepared with hot (80°C) and cold (5°C) water, suggesting that exposure to PAHs should be independent of the temperature at which the mate is prepared and drunk. This does not mean that the temperature of mate is unimportant to esophageal pathology or even carcinogenesis; it only means that the temperature of mate is probably not important to any component of carcinogenicity attributable to mate-related PAH exposure.

If further studies prove that the PAH content of mate is a main factor responsible for its carcinogenicity, then certain public health measures can be considered. Variation in the PAH content among the different brands measured in this study, such as the difference shown between brands 5 and 8 and the other brands, shows that it is possible to produce mate with a lower PAH content. One potential way to reduce the PAH content of mate is to modify processing methods. Studies have shown that the major source of PAHs in black tea is the air in the drying houses; the concentration of PAHs in the air

inside the drying houses is up to 100 times higher than in the air outside the drying houses (45), and the PAH content of processed black tea is >70-fold higher than that of fresh tea leaves (45).

The strengths of this study include measuring 21 PAHs in eight commonly used brands of yerba mate, strong quality control in the PAH measurements, and estimation of PAH intake in a way that mimics real-life consumption of mate. One potential limitation is that measurements were done on only eight brands of yerba mate. However, the PAH measurements were high in almost all brands, and inter-brand correlations for the distribution of individual PAHs were high. Therefore, it is probable that the results are relatively generalizable.

In summary, we found very high mass fractions of total and carcinogenic PAHs in processed dry yerba mate leaves and in both hot and cold mate infusions. Our results support the hypothesis that the carcinogenicity of mate may be related to its PAH content.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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