第一页为封面页

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论文题目：The release of antimony in bottled beverages and health risk assessment

第二页为创新性申明

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第三页此处开始为论文的主体部分……

论文题目 The release of antimony in bottled beverages and health risk assessment

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论文摘要 Antimony (Sb) trioxide is commonly used as catalyst for polyethylene terephthalate (PET) plastic production. Antimony may release from PET bottles under improper storage conditions, particularly at high temperature. In this study, PET-bottled beverages (n = 33), covering six categories (carbonated, juice, tea, sport, protein, and coffee beverages) were used to study the Sb release from PET plastics. After incubation at 70oC for 3 days, Sb concentrations in beverages increased from <DL–2.10 ng/mL (average: 0.36 ng/mL) to <DL–15.9 ng/mL (average: 1.43 ng/mL), and 21% of beverages had Sb levels exceeding the regulation limit of 2 ng/mL in drinking water. The Sb released in carbonated beverages was significantly ($p<0.05$) higher than those in other beverages due to the lower pH values in carbonated beverages. The Sb oral relative bioavailability (RBA) in beverages was measured using a mouse model, which ranged from 0% (coffee) to 59.8 ± 4.27% (tea). The chronic daily intakes (CDI) of Sb through beverage consumption were calculated based on both Sb concentrations and RBA in beverages. The contributions to CDI values from each beverage category varied when considering the Sb RBA, indicating that the health risk assessment can be refined by measuring contaminant bioavailability. Results in the current study highlighted the necessity to strengthen the supervision of PET-bottled beverage transportation and storage safety in the future.

关键词 Antimony (Sb), PET bottled beverages, Bioavailability, Risk assessment

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1. Introduction

1.1 Bottled beverages

Over past years, the world population have increased the consumption of beverages at a rate of 3.6 % per year. In 2009, the total market for beverages reached to

approximately 1.6 trillion liters worldwide, and the main beverage categories are shown in Figure 1.1. One survey on primary and middle school students of Beijing (China) in 2013 showed that more than 12% of participants drunk beverages every day, and over one-third of participants consumed beverages 1–3 times a week.[1] Non-carbonated sugary beverages were the highest consumption, accounting for 30.0%, followed by fruit juice (15.6%), and carbonated beverages (14.6%).[1] According to China national standard (GB/T 10789-2015), the classification of Chinese beverages is shown in Table 1.1.

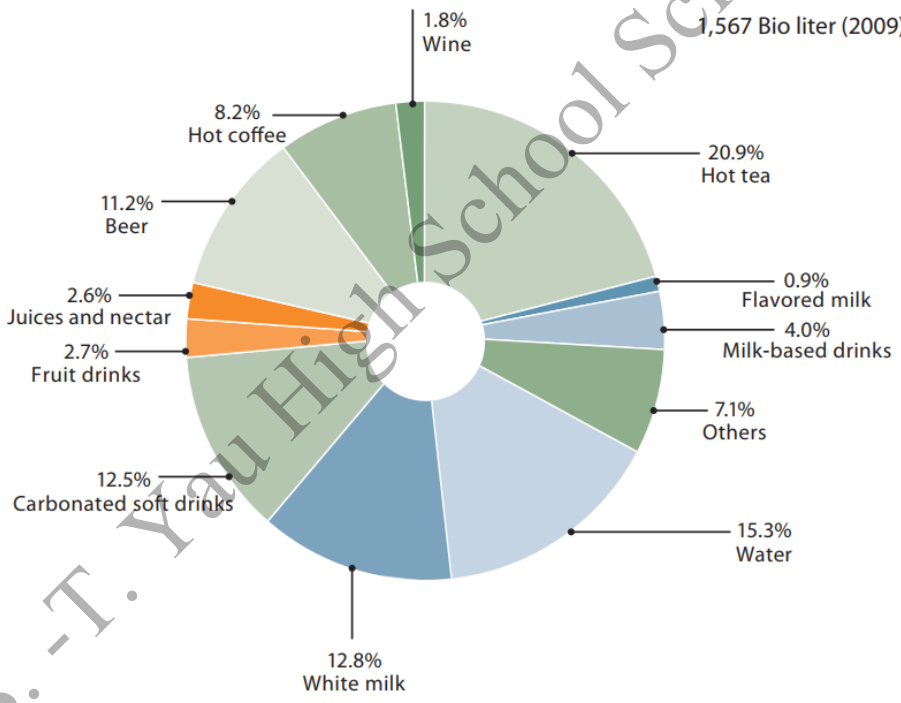


Figure 1.1. The main beverage categories worldwide in 2009 prepared by Markestrat based on data from Euromonitor.

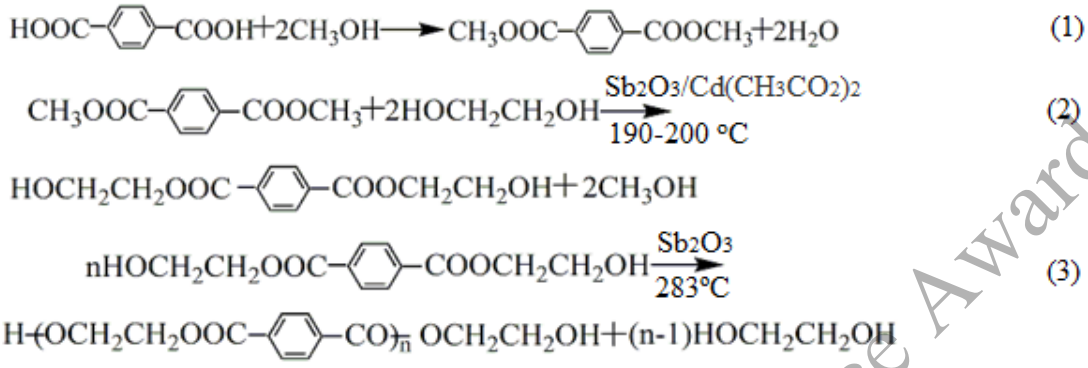
Table 1.1 Beverage category according to China national standard (GB/T10789-2015)

Categories	Notes
Juices	fruit juice, fruit juice concentrates and fruit squash
Protein	milk beverages, plant-protein beverages and compound protein beverages
Tea	pure tea, tea concentrates, juice tea beverages and milk tea beverages

Coffee	beverages made of coffee beans or coffee products
Bottled Water	mineral and pure drinking water
Solid	solid drink products that can be dissolved in water for brewing
Carbonated Soft	beverages with dissolved carbon dioxide based on food additives
Flavored Water	water that has flavors added (sugar, sweeteners or acidulating agent) to enhance the taste, including tea beverages and fruit beverages
Functional Water	sports beverages, nutritional beverages, energy beverages and electrolyte beverages
Others	

Due to its advantages of high chemical stability and low diffusion coefficient, polyethylene terephthalate (PET) is used as packaging material for over 90% of beverage bottle.[2] PET is a long chain polymer produced by Terephthalic Acid (PTA) and Ethylene Glycol (EG). The synthesis of PET requires the participation of catalysts, which mainly include antimony (Sb), titanium, germanium, cobalt, and other metal salts. Among them, antimony trioxide (Sb_2O_3) is used as catalyst in the synthesis of over 90% of PET plastic packaging bottles, as it has favorable catalytic activity, no color and few side reactions.[3] Traditional PET generation methods include the following steps: 1) methyl esterification: PTA and excess methanol were first esterified into dimethyl terephthalate. After evaporation of water and low-boiling substances (such as excess methanol and methyl benzoate) and distillation, pure dimethyl terephthalate can be obtained. 2) transesterification: dimethyl terephthalate was transesterified with EG (molar ratio, 1:2.4) to form polyester oligomers at 190–200 ^oC with cadmium acetate and Sb_2O_3 as catalysts. The methanol was then distilled to make the transesterification sufficient. 3) final polycondensation reaction: at a temperature higher than the melting point of polyester, Sb_2O_3 is used as a catalyst to make polycondensation or transesterification. With reduced pressure and high temperature, the by-product EG is

continuously distilled to gradually increase the degree of polymerization. The chemical equations for each step are listed below:



Due to use of Sb_2O_3 during bottle production, the Sb level in PET bottles was reported to be up to 190–350 mg/kg.[4-5] Although PET bottles all meet food-related standards before they leave the factory, the aging of PET plastic bottles, caused by poor storage and transportation conditions (such as high temperature, direct sunlight), may lead to the release of harmful chemicals from PET plastic into the beverages. For example, bisphenol A, plasticizer and Sb were frequently detected in beverages stored in PET bottles.[3,6-10] The leaching concentrations of Sb were the highest among all these contaminants, and the Sb level in beverages stored at 80°C for 7 days was up to 14.4 ng/mL, which was far higher than the safe level of Sb in drinking water (5 ng/mL) regulated by China.[3] Therefore, the potential health risks of Sb in PET bottled beverages are of a great concern.

1.2 Release of Sb in PET bottled beverages and the influence factors

Recently, there were some reports on the release of Sb from PET plastic bottles. The factors affecting Sb release from PET bottle include storage conditions (temperature, light intensity, and storage time), properties of bottled water/beverage

(pH value, carbon dioxide content, and salinity), and characteristics of PET bottle (bottle color, thickness, and contact surface area). Temperature was reported to be the most important factor affecting the release of Sb from PET bottles. High temperature can accelerate the aging rate of PET plastics, promoting the Sb release.[11] Westerhoff et al found that the concentration of Sb in water reached 6 ng/mL after PET bottled water was stored at 60°C for 176 days, while it took only 2.3 days to reach the comparable Sb concentration at 80°C.[3] When the time was extended to 7 days, the Sb concentration was high up to 14.4 ng/mL, which was far higher than the safe level of Sb in drinking water (5 ng/mL) prescribed by China. For the bottled water stored at 70 °C for 7 days, the Sb concentration in bottled water reached to 38.5 ± 7.68 ng/mL (median), which was 5.60 times of that at 25°C.[2] Keresztes et al. found that the Sb levels in bottled water increased with time and eventually tended to equilibrium.[12] A Sb diffusion model in PET bottles was established to predict Sb release and found that Sb concentration in PET bottled water could reach 6 ng/mL when stored at 70°C for 3 years.[13]

The properties of bottled water/beverage (such as pH, salinity, etc.) can also influence the migration of Sb from PET bottles. Reimann et al.[14] found that a lower pH promoted Sb release, and a greater Sb release was observed in sparkling water with pH value of 4.9-5.3 compared to pure water with pH of 6.3–8.1. Mineral concentration in PET bottled water also affected Sb release, for example, Westerhoff et al. observed a positive correlation between Sb release and magnesium and calcium concentrations in PET bottled water.[3] However, previous studies mainly focused on bottled drinking water, and few studies have considered the PET bottled beverages. In addition, the

constitutes in beverages (such as carbohydrate, organic acids and proteins) are usually complicated than those in bottled water, which may further promote the Sb leaching process.[12,15,16]

1.3 Toxicity of Antimony (Sb)

Antimony is a non-ductile, hard and brittle metal with a silver-white luster. It can conduct electricity and heat, and belongs to heavy metal with a melt point of 630.5°C, a boil point of 1750 °C, a density of 6.68 g/cm^3 and an atomic weight of 121.8. Antimony is similar to the same groups of arsenic and bismuth, and the electron structure of the secondary shell is 18 electron configuration. There exist four main valence states of Sb, including 3, 0, + 3 and + 5. Among them, Sb(III) and Sb(V) are the most common inorganic Sb in the natural environment.

Antimony in the environment can enter the organism through respiration, skin contact, and dietary intake. In organisms, Sb, combined with sulfhydryl (-SH) and other functional groups, can disrupt enzyme activity and break the intracellular ion balance, causing metabolic disorders.[17] A study on chronic exposure to Sb (III) in water showed the cytotoxicity and damage to the thyroid, liver, kidney and other organs of rats.[18] Moreover, the inorganic compounds of Sb were reported to be more toxic than its organic compounds, and Sb(III) toxicity can be 10 times higher than that of Sb(V). [17,19] Animal studies have found that Sb_2O_3 can cause hypertension, myocardial degeneration and even lung cancer.[20] In addition, exposure of pregnant women to Sb_2O_3 can lead to premature birth, miscarriage, and may adversely affect intellectual development of the infants. Considering the toxicity, Sb has been listed as one of the priority pollutants by

US Environmental Protection Agency and European Union. Considering the adverse effect of Sb and the potential release from PET bottles into beverages, it is important to assess the health risk for Sb exposure through beverage drinking.

1.4 *In vivo* bioavailability

Traditional human exposure and health risk assessment was based on total contaminant concentration, without considering bioavailability, which may produce overestimation. [21] After ingestion, contaminants may be released from the matrix into gastrointestinal fluids and absorbed into the systemic circulation. Contaminants entering the blood circulation are regarded as the bioavailable fraction, which may be determined using *in vivo* experiments.[22] *In vivo* methods utilizing swine, monkey and mouse animal models have been used to measure contaminant bioavailability.[23-25] Studies have proved that swine are ideal animal models due to their similar physiological processes of intestinal absorption as that of children.[26] However, the swine model is expensive, and time-consuming (about one year one cycle), which limit its wide application in risk assessment. In addition, the physiological parameters of monkey gastrointestinal tract are similar to those of humans, whereas, high cost and ethical challenge largely restrict the application of monkey model in determination of contaminant bioavailability.[27] Therefore, mice are the most common models to determine the bioavailability of contaminants because of their relatively smaller mass and volume, lower cost, and simpler experimental operations. Feeding and gavage are the main pathways to measure bioavailability of contaminants *in vivo*.

According to different experimental methods, bioavailability was divided into

absolute bioavailability (ABA) and relative bioavailability (RBA).[28] ABA determination requires contaminant exposure to animals by intravenous injection. RBA refers to the ratio of the contaminants absorbed by organisms in the experimental and the reference groups, where contaminants from test media and reference substances are exposed to animals through oral or other exposure pathways.[29] Some organs or tissues with continuous accumulation of contaminants are used as biological endpoints for bioavailability tests. For example, Bradham selected mouse urine as a biological endpoint to determine arsenic RBA in soil samples from 9 mining areas.[30] The liver, kidney and thigh bone of mice were also used as biological endpoints to measure the RBA of heavy metals such as lead and cadmium. The RBA determined by comparing contaminant accumulation in biological endpoints following exposure to contaminated samples and reference samples is described in the following equation:

$$RBA=\frac{Organ_{sample}}{Organ_{refernce}}\times\frac{Dose_{reference}}{Dose_{sample}}\times 100\% \quad (1)$$

Where $Organ_{sample}$ and $Organ_{reference}$ are contaminant mass accumulated in biological endpoints following exposure to contaminated samples and reference samples; $Dose_{sample}$ and $Dose_{reference}$ are the dosing levels of contaminants in the contaminated sample and reference samples. In this work, Sb RBA was measured for beverages using a mouse model and the equation mentioned above. Antimony potassium tartrate ($C_4H_4KO_7Sb$, APT) was used as Sb reference compound. The measured Sb RBA was then used to refine the exposure assessment and health risk through exposure to Sb in beverages.

1.5 The research target of this work

Totally 33 PET-bottled beverages, including six categories (namely carbonated, tea, fruit juice, protein, sport, and coffee beverages), were purchased from local markets, Nanjing, China. The Sb leaching from PET bottles was investigated by incubating beverages at 70°C for 3 days, which simulated the high-temperature condition in car trunk in summer. Questionnaires were designed and assigned to collect information about beverage consumption among middle and high school students, Nanjing, China. Risk assessment of Sb exposure through drinking beverages was conducted based on the Sb leaching concentrations measured under high temperature and the information collected from questionnaires. During the risk assessment, Sb relative bioavailability (RBA) in beverages, which was measured by *in vivo* mouse model, was considered to refine the exposure scenario.

2. Materials and methods

2.1 Sample collection and preparation.

PET-bottled beverages (n = 33) produced in China were purchased from local markets in Nanjing. There were 6 categories of beverages, including carbonated (n=5), fruit juice (n=7), tea (n=6), sport (n=5), protein (n=5), and coffee (n=5). Details are shown in Table 2.1. For the leaching experiment, all the PET-bottled beverages were stored at 70°C for 3 days in an incubator (Jinghong Experimental Equipment Co. LTD,

Table 2.1 Characteristics of PET bottles used for beverages

ID	Category	Bottle color	Bottle volume (mL)	Bottle mass(g)	T_{Sb}(mg/kg)
1	Carbonated	Transparent	380	26.1	208
2	Carbonated	Transparent	550	20.0	188
3	Carbonated	Transparent	560	17.2	188

4	Carbonated	Transparent	300	22.6	195
5	Carbonated	Transparent	300	22.6	171
6	Juice	Transparent	450	20.3	153
7	Juice	Transparent	450	20.3	174
8	Juice	Transparent	450	20.3	192
9	Juice	Transparent	500	23.6	58.0
10	Juice	Transparent	500	25.1	161
11	Juice	Transparent	330	22.8	156
12	Juice	Transparent	330	26.6	175
13	Tea	Transparent	500	22.5	181
14	Tea	Transparent	300	22.8	189
15	Tea	Transparent	500	23.0	148
16	Tea	Transparent	500	30.0	159
17	Tea	Transparent	500	29.7	257
18	Tea	Transparent	500	23.4	141
19	Sports	Transparent	400	31.0	84.0
20	Sports	Transparent	600	34.5	174
21	Sports	Transparent	600	34.1	155
22	Sports	Transparent	600	25.0	215
23	Sports	Transparent	500	32.8	113
24	Coffee	Transparent	500	27.1	207
25	Coffee	Transparent	450	24.0	229
26	Coffee	Transparent	268	19.0	193
27	Coffee	Transparent	480	22.2	181
28	Coffee	Transparent	440	22.4	200
29	Protein	Transparent	450	26.2	129
30	Protein	Transparent	330	20.7	162
31	Protein	Milky white	400	27.4	0.27
32	Protein	Transparent	500	26.8	238
33	Protein	Semitransparent	100	5.70	0.16

Shanghai, China) without light. The incubation conditions were selected to simulate storage in car trunk at hot summer. The Sb concentrations in beverages before (Sb_0) and after (Sb_3) 3 day incubation were measured. In addition, the Sb total contents in PET bottles (T_{sb}) were also analyzed.

The total concentrations of Sb in both PET bottles (T_{Sb}) and beverages were determined by inductively coupled plasma mass spectrometry (ICP-MS, NexION300X, Perkin Elmer, USA) after digestion according to Fan et al. PET bottles were washed with Milli-Q water three times.[2] After drying, bottles were cut into 2×2 mm^2 pieces by acid-cleaned ceramic scissors. Briefly, 0.5 g of PET pieces or 10 mL of beverages were mixed with 8 mL of HNO_3 and digested on a hotplate (LabTech, EG35B, China) at 250°C for 4 h. Then 2 mL of H_2O_2 was added for another 2 h digestion. The Sb in PET bottle was completely digested since only white powder residue remained in the digested solution. Finally, digestion solutions were diluted to 50 mL with Milli-Q water, filtered through a 0.45 μm membrane filter (Anpel, Shanghai, China). Indium as internal standard was added before ICP-MS analysis. The extraction process of Sb is shown in Figure 2.1.

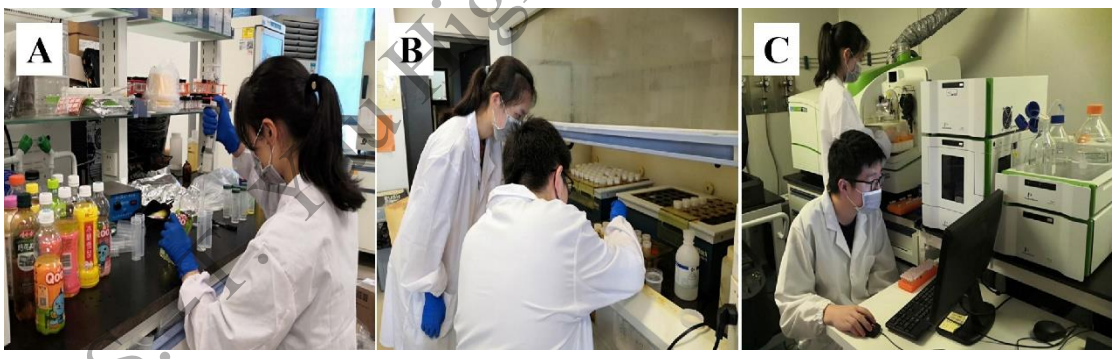


Figure 2.1 Extraction process of Sb: (A) pipetting samples; (B) digestion on a hotplate; (C) determination of Sb by ICP-MS.

2.2 Determination of Sb relative bioavailability using a mouse model.

Due to the time-consuming and ethnic issue associated with *in vivo* tests, only 8 beverage samples were selected to determine Sb relative bioavailability (RBA) using a mouse model. The 8 samples covered a range of nutrient contents and beverage

categories. Female Balb/c mice weighing 20-22 g were acclimated for 1 week under standard animal house conditions (12 h light/dark cycle, 25 ± 2 °C, and 50 ± 5% humidity) and supplied with Milli-Q water and clean mouse chow ad libitum. Initially, a dose-response study was performed with antimony potassium tartrate ($C_4H_4KO_7Sb$, APT)-spiked water. Briefly, mice were gavaged daily with 0.5 mL of aqueous solution containing APT at dosing concentrations (0.4, 3.4, 8, and 20 mg/L) for 7 days, which were corresponding to 10, 85, 200, 500 μg/kg body weight (bw). Poon et al., found that the accumulation of Sb in liver was significantly higher than that in kidney, followed by the brain, adipose tissue and serum using a rat model,[31] therefore, the livers were collected after 7 day exposure and freeze-dried for further analysis.

The Sb concentrations in selected beverages ranged from 0.24 to 15.9 ng/mL, which was too low to accurately assess Sb RBA in a mouse bioassay. Therefore, APT was spiked into the 8 samples to reach Sb concentration of 0.4 mg/L. Aliquots of 1 mL of Sb-spiked beverages or 0.5 mL of APT-spiked water solution were gavaged to mice daily for 7 days, after which mice were sacrificed and livers were collected. Liver samples were freeze-dried and digested with 1:1 HNO_3/H_2O and 30% H_2O_2 (USEPA Method 3050B). Sb RBA was determined by comparing Sb liver accumulation following exposure to beverages and APT-spiked water (as reference) as described in the following equation:

$$\text{Sb-RBA (\%)} = \left(\frac{\text{Liver Sb}_{\text{beverage}}}{\text{Liver Sb}_{\text{APT}}} \times \frac{\text{Sb dose}_{\text{APT}}}{\text{Sb dose}_{\text{beverage}}}\right) \times 100\% \quad (2)$$

where liver $Sb_{beverage}$ and liver Sb_{APT} are Sb (μg) accumulated in mouse livers after 7-d gavage with Sb spiked beverages and APT-spiked water, Sb $dose_{beverage}$ and Sb

$dose_{ATP}$ are the Sb mass (μg) in beverages and APT gavaged to mice.

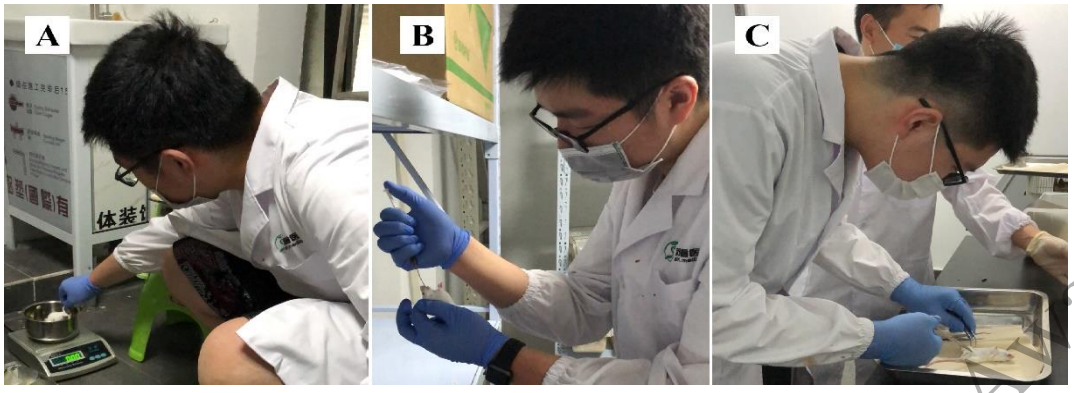


Figure 2.2 Determination of Sb relative bioavailability using mouse model: (A) mouse weighing; (B) the gavage process; (C) liver collecting

2.3 Risk assessment

The chronic daily intake of Sb through beverage consumption for middle/high school students was calculated according to the following equations (Eq.3 and 4).

$$CDI_{total} = \frac{C \times AI}{BW} \quad (3)$$

$$CDI_{RBA} = \frac{C \times AI \times RBA}{BW} \quad (4)$$

where CDI_{total} is the total Sb intake (ng/kg/day); C is the total Sb concentration in beverages after incubation at 70 ºC for 3 days (ng/mL); AI is the daily average intake of beverages (mL/day), BW is the body weight (kg); CDI_{RBA} is Sb intake considering RBA (ng/kg/day).

The self-designed questionnaire was used to collect information used for risk assessment, including daily beverage intake, beverage type, age, gender, and body weight. A total of 267 volunteers aged 13-18 years old were randomly recruited in Nanjing, China. For all the volunteers, the average weight is 59.5 kg. Among them,

there are 113 males with the average weight of 67.5 kg and 154 females with the average weight of 53.5 kg. Details on beverage intake is listed in Table 2.2.

Table 2.2 Beverage intake (mL/d) collected from questionnaires

Beverage type	Female	Male	All volunteers
Carbonated	73.5	132	98.0
Juice	69.3	77.4	72.8
Tea	122	125	123
Sports	39.9	75.2	54.8
Coffee	104	110	107
Protein	116	86.9	104

2.4 Quality assurance and statistical analysis.

The certified water reference material GSB07-1376-2001 from Chinese National Standard Reference Center was used for quality control. The accuracy of the HNO_3/H_2O_2 digestion method was confirmed by comparing Sb concentration in the reference material after digestion (25.0 ± 1.24 ng/mL) with the certified value of 22.0 ± 1.10 ng/mL. Four replicates were adopted in *in vivo* mouse assay. Indium was used as internal standard to quantify the Sb concentration. A standard solution with moderate levels (1 ng/mL) was analyzed with each batch of 10 samples to confirm the stability of detector response during instrumental analysis. The Sb levels in procedural blanks were below the limit of detection and the relative standard deviation of duplicate samples was less than 10%.

All results are shown as the mean ± standard deviation (mean ± SD). One-way analysis of variance (ANOVA) by SPSS (version 17.0) was used to examine the differences among groups, and statistical significance level was set at $p<0.05$. Linear relationships were established by SigmaPlot (version 12.5).

3. Results and discussion

3.1 Basic properties of beverage samples

Concentrations of Sb in the bottle (mg/kg), pH, and major nutrient contents of 33 PET bottled beverages are shown in Table 3.1. Nutrient contents were obtained according to information on the beverage containers. The carbonated, juice, tea and sports beverages contained no fat and protein, except 13# and 16# belonging to milk tea. Most beverages contain carbohydrate ranging from 0-26.0 g/100mL and Na ranging from 0-100 mg/100mL. All beverages tend to be acidic to neutral with pH of 2.43-7.47. There was no obvious difference for the pH value before (pHt_0) and after (pHt_3) high-temperature incubation.

3.2 Release of Sb from PET bottles into beverages under high temperature incubation

Temperature was reported to be one of the dominate factors affecting the release of Sb from PET bottles.[2,3,13] The temperatures in cars, trucks or warehouses can exceed 70 °C in summer sometimes. To simulate the release of Sb from PET bottles in the worst-case scenario, the 33 PET bottled beverages were incubated for 3 days at 70 °C in an incubator. In addition, the total concentrations of Sb in PET bottles were also measured, which ranged from 0.16 to 257 mg/kg (Table 2.1). This was similar to the Sb

Table 3.1 Characteristics and constituents of PET-bottled beverages

ID	Category	pH (t_0)	pH (t_3)	Protein (g/100mL)	Fat (g/100mL)	Carbohydrate (g/100mL)	Na (mg/100mL)
1	Carbonated	3.57	3.30	0.00	0.00	0.00	0.00
2	Carbonated	2.92	2.92	0.00	0.00	3.20	0.00
3	Carbonated	3.48	3.67	0.00	0.00	0.00	28.0
4	Carbonated	2.99	2.99	0.00	0.00	11.2	16.0
5	Carbonated	2.43	2.42	0.00	0.00	10.6	12.0
6	Juice	2.74	2.72	0.00	0.00	11.3	5.00
7	Juice	3.29	3.27	0.00	0.00	10.2	17.0
8	Juice	3.16	3.15	0.00	0.00	12.2	17.0
9	Juice	3.90	3.86	0.00	0.00	10.8	29.0
10	Juice	3.04	3.03	0.00	0.00	10.1	27.0
11	Juice	3.20	3.20	0.00	0.00	11.2	16.0
12	Juice	3.29	3.33	0.00	0.00	7.70	0
13	Tea	6.80	6.58	0.60	0.60	7.10	39.0
14	Tea	5.87	5.41	0.00	0.00	26.0	4.50
15	Tea	3.18	3.15	0.00	0.00	9.70	29.0
16	Tea	6.71	6.02	0.60	1.50	9.00	60.0
17	Tea	3.26	3.24	0.00	0.00	8.00	12.0
18	Tea	5.32	4.75	0.00	0.00	2.00	14.0
19	Sports	3.49	3.47	0.00	0.00	11.0	43.0
20	Sports	3.17	3.16	0.00	0.00	6.00	45.0
21	Sports	3.21	3.2	0.00	0.00	11.5	30.0
22	Sports	3.73	3.71	0.00	0.00	4.00	37.0
23	Sports	3.84	3.84	0.00	0.00	5.50	75.0
24	Coffee	6.13	6.10	0.70	1.10	9.10	28.0
25	Coffee	6.77	6.53	0.70	1.10	9.00	65.0
26	Coffee	6.75	6.59	1.60	2.20	7.90	68.0
27	Coffee	6.60	6.42	0.60	0.60	6.00	31.0
28	Coffee	6.63	6.34	0.00	0.00	7.30	57.0
29	Protein	4.11	4.14	6.90	75.0	1.10	0.60
30	Protein	6.60	6.19	0.50	1.80	6.00	26.0
31	Protein	3.60	3.57	1.10	0.00	14.6	22.0
32	Protein	4.19	4.23	1.00	0.90	6.50	100
33	Protein	3.81	3.77	1.30	0.00	8.30	27.0

concentrations of 73.0-290 mg/kg in PET bottles collected from in China,[2] Hungary,[32] Mexico[33] and Japan[13]. For example, in the PET bottles from Mexico, the Sb levels were reported to be 73.0–111 mg/kg.[33] Rung et al. found the concentration of Sb in PET bottles of Japan was 0.1–216.5 mg/kg.[13]

The Sb concentrations in each beverage before and after 3 days incubation at 70°C are shown in Table 3.2. Before incubation, The Sb concentrations (Sb_0) in beverages ranged from <DL to 2.10±0.64 ng/mL, with the median of <DL ng/mL (average:0.36 ng/mL). Following 3 day incubation at 70°C, the Sb levels (Sb_3) increased to <DL–15.9±0.79 ng/mL with the median of 0.69 ng/mL (average:1.43 ng/mL). These results are comparable with previous studies where temperature promoted the Sb release from PET bottles. For example, Westerhoff et al. found that the concentration of Sb in PET bottled water increased from 0.5 ng/mL to 9.7 ng/mL after storage at 80 °C for 48 hours.[3] In the study on bottled ultrapure water, the concentration of Sb increased to 3.5 ng/mL after storage at 60 °C for 10 days.[34] Compared with the drinking water, Sb concentrations in PET bottled beverages increased at a higher extent (1.0–71.5 folds), which may be due to the complex components of beverages.

Among all the 33 beverages, the Sb concentration in 1 sample after 3 days incubation was 15.9±0.79 ng/mL, which was much higher than Sb drinking water standards in the U.S. (6 ng/mL) and China (5 ng/mL). In addition, 21% of the 33 beverages contained Sb levels exceeding the 2 ng/mL regulated by Japanese Sb drinking water standard. This demonstrated that poor storage conditions (e.g., high temperature) can promote the release of Sb from PET bottles, leading to health risk for people. Therefore, it is necessary to strengthen the regulation for proper transportation and storage for the PET-bottled beverage in the future.

Table 3.2 Concentration of Sb in PET bottled beverages

ID	Sb_0 (ng/mL)	Sb_3 (ng/mL)	ID	Sb_0 (ng/mL)	Sb_3 (ng/mL)

1	0.17±0.08	1.61±0.21	18	<DL	<DL
2	1.21±0.11	15.9±0.79	19	0.14±0.00	1.10±0.03
3	<DL	0.24±0.01	20	1.24±0.25	1.26±0.09
4	0.74±0.02	1.97±0.05	21	0.23±0.04	0.83±0.00
5	2.10±0.64	3.48±0.07	22	<DL	<DL
6	<DL	<DL	23	<DL	<DL
7	<DL	<DL	24	<DL	0.42±0.13
8	<DL	<DL	25	<DL	0.63±0.01
9	0.61±0.46	2.47±0.01	26	<DL	0.25±0.39
10	<DL	0.18±0.01	27	<DL	<DL
11	<DL	<DL	28	<DL	<DL
12	<DL	1.74±0.05	29	1.34±0.44	1.40±0.04
13	0.81±1.09	1.81±0.15	30	0.29±0.03	0.69±0.01
14	<DL	<DL	31	<DL	2.26±0.21
15	0.02±0.38	1.67±0.05	32	0.65±0.26	2.46±0.14
16	1.70±1.05	2.18±0.87	33	<DL	<DL
17	0.74±0.02	2.60±0.05	average	0.36	1.43

DL: detection limit.

To further explore the Sb release in various beverages, 33 samples are divided into six categories. The Sb concentrations (Sb_3) and the released Sb concentrations (ΔSb, ng/mL, $\Delta Sb=Sb_3-Sb_0$) in each category of beverages are shown in Figure 3.1. The average concentrations of Sb in carbonated beverages was significantly higher than those in other beverages ($p<0.05$). The relatively higher ΔSb levels in carbonated beverages may be attributed to the lower pH values. The pH values of carbonated beverages (2.43-3.57, average=3.08) were lower than those of other beverages (2.74-6.80, average=4.51, Table 3.1). It was reported that the protonation effect of Sb was enhanced at low pH, which promoted the Sb release from PET bottles.[35] Keresztes et al

also found that Sb migration rate was significantly higher in bubble water with a pH of 4.94-5.27 than in non-bubble water with a pH of 6.40-8.12.[12]

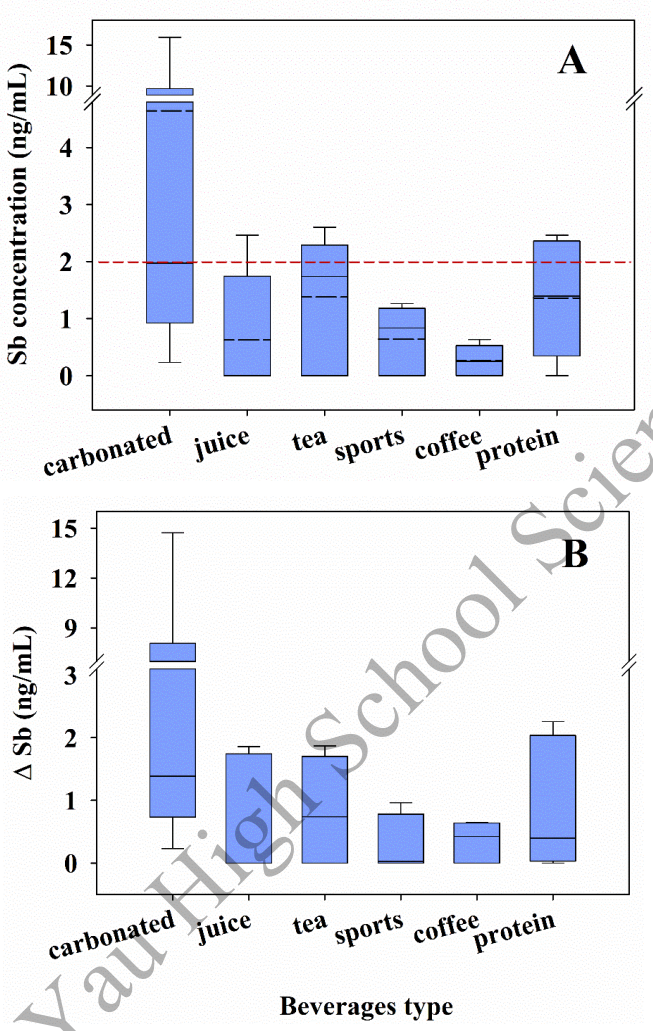


Figure 3.1 Sb concentrations in beverages after 3 day incubation at 70ºC (A), and released Sb into beverages (ΔSb) after 3 day incubation(B). The red line indicated the most strict drinking water Sb standard (2 μg/L), which is from Japan.

3.3 Risk assessment

3.3.1 Relative bioavailability of Sb (Sb RBA) in PET bottled beverages.

The Sb RBA in PET bottled beverages may be one of the most important constraints for accurate exposure assessment. Therefore, in this study the Sb RBA were

measured based on mouse model. Prior to assessing Sb-RBA in beverages, Sb-spiked Milli-Q water (0.4, 3.4, 8, and 20 mg/L) was daily gavaged to mice over a 7 day period, which were equivalent to 10, 85, 200, 500 μg/kg bw/day. Following exposure, the Sb accumulation amount in livers was measured and the dose- response was established accordingly. As shown in Figure 3.2A, a strong linear correlation was observed between Sb accumulation in livers and Sb dose level ($r = 0.93$, $p<0.001$), suggesting the feasibility of using mouse liver as a biological end point for Sb RBA measurement.

Eight beverages covering six types with various constituent concentrations were selected to measure the Sb RBA. Sb RBA ranged from 0 (26#) to 59.8 ± 4.27% (3#), with the average value of 19.0 % (Figure 3.2B). For each beverage category, the Sb RBA values were in the order of 55.0% (tea)>32.0% (carbonated)>9.81% (fruit juices)>9.04 % (protein)>4.54 % (sport)>0% (coffee). To our knowledge, other *in vivo* studies exploring the Sb RBA in beverages are not available; however, limited studies have assessed Sb RBA in soil, showing that Sb RBA using swine liver as biological endpoint after exposure with contaminated soil were 1-6%, and much lower than the results for beverages of this work.[26] The relatively low Sb RBA in soil may due to the strong binding affinities of Sb with soil constituents such as sulphides, iron oxy-hydroxides, and refractory soil constituents.[26]

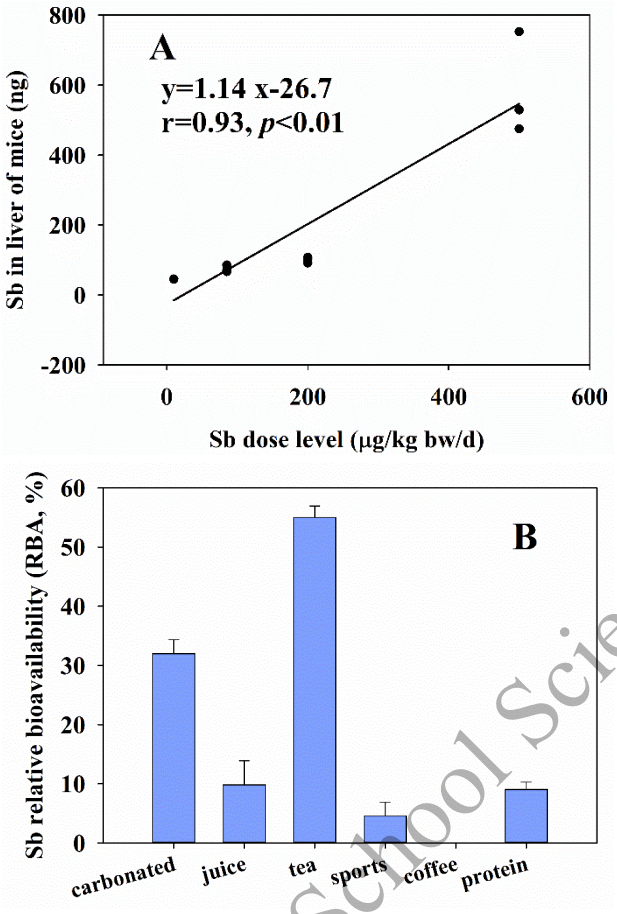


Figure 3.2 Linear response of Sb accumulation in mouse liver with Sb dose level following gavage of Sb potassium tartaric for 7 days (A); Sb RBA in 8 Sb spiked beverages (B).

3.3.2 Chronic daily intake and health risk assessment

To evaluate Sb exposure level through drinking poorly-stored PET bottled beverages, the chronic Sb daily intake (CDI) for teenagers (13-18 years old) were calculated according to equation 3 and 4, and the results are shown in Figure 3.3. For females, the mean CDI values of carbonated beverages were 6.38 ng/kg/day, followed in the order of tea (3.13 ng/kg/day) > protein (2.96 ng/kg/day) > juice (0.81 ng/kg/day) > coffee (0.51 ng/kg/day) > sports (0.48 ng/kg/day). For males, the mean CDI values of

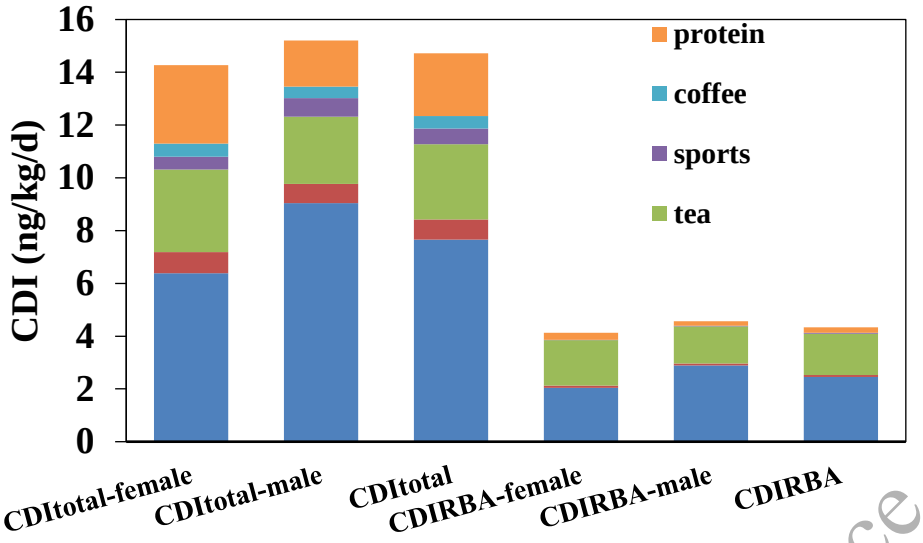


Figure 3.3 CDI_{total} (Sb chronic daily intake based on total Sb concentration in PET bottled beverages) and CDI_{RBA} (with consideration of Sb RBA) through beverage consumption for female, male and the both genders.

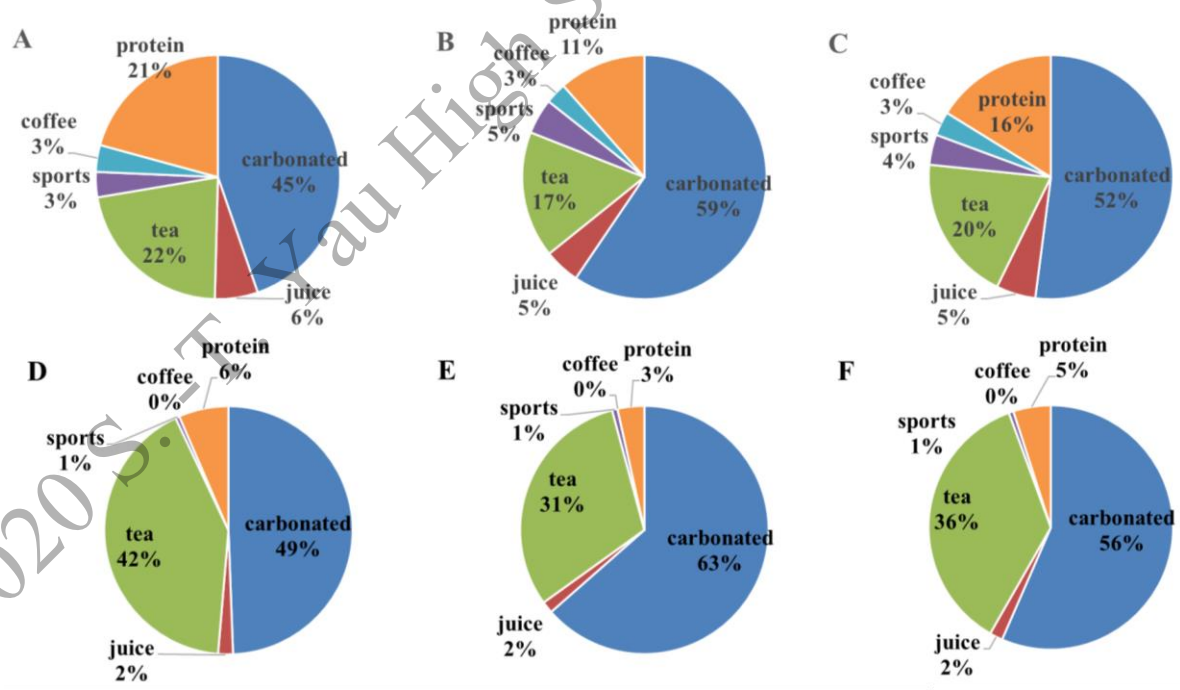


Figure 3.4 The contribution of different beverage categories to CDI_{total} for females (A), males (B), both the genders (C); and to CDI_{RBA} for females (D), males (E), both the genders (F).

Sb from carbonated (9.05 ng/kg/day) and sports (0.71 ng/kg/day) beverages were higher than those for females, while lower mean CDI were observed for tea (2.55 ng/kg/day), protein (1.75 ng/kg/day), coffee (0.43 ng/kg/day) beverages when compared with females. Generally, the CDIs of Sb from all the beverages by females (14.3 ng/kg/day) were comparable to that for the males (15.2 ng/kg/day), and both the genders (14.7 ng/kg/day). Among all the 6 categories, carbonated beverage was the predominant contributor to Sb CDIs, accounting for 52%, followed in the decreased order by tea 20%, protein 16%, juice 5%, sports 4% and coffee 3% (Figure 3.4). Carbonated, tea, and protein beverages were the three major contributors, and accounted for 88% of the total CDI_{total} (88%). Similar composition profiles were also observed for females and males, although the percentage of carbonated beverages increased from 45% for females to 59% for males, which can be explained the higher intake of carbonated beverages from males (132 mL/day) than females (73.5 mL/day, Table 2.2).

When Sb RBA was taken into consideration, the CDI values sharply decreased to 4.13, 4.56, and 4.34 ng/kg/day for the females, males, and both the genders, respectively. The contribution from tea to CDI_{RBA} increased from 17-22% to 31-42%, but the contribution of protein beverages decreased from 11-21% to 3-6%. For all the groups, the order of CDI_{RBA} contributor changed to carbonated > tea > protein > juice> sports > coffee, for example, the percentages of each beverage category for all the teenagers were 56%, 36%, 5%, 2%, 1% and 0%, respectively. The dominant contributors changed to carbonated and tea beverages instead of carbonated, tea, and protein beverages if without considering RBA, which accounted for 91-94% of CDI_{RBA}. The difference of

contribution profiles from various beverage categories observed between CDI_{total} and CDI_{RBA} further suggested that it is important to consider bioavailability for an accurate assessment of exposure risk. The essential role of Sb RBA for risk assessment was demonstrated for beverages, and the same conditions may be expected for other food stuffs.

The CDI values based on both Sb total concentrations and RBA in beverages were below the USEPA regulated CDI (400 ng/kg/day), indicating that exposure to Sb in improperly stored PET bottle beverages in this study did not produce considerable health risks. However, consumption of PET-bottled beverages is only one pathway for human exposure to Sb, and there are many other pathways such as dietary, air inhalation, and drinking water. The Sb exposure and health risk should be evaluated by considering all the pathways besides the consumption of PET-bottled beverages in the future.

4. Conclusion

This study investigated the release of Sb in PET bottled beverages under high temperature storage and determined the chronic daily Sb intake from beverages. The high temperature can promote the Sb release from the PET bottles. Following incubation at 70 °C for 3 days, Sb levels in 21% of beverages were higher than standard for Sb in drinking water (2 ng/mL) regulated by Japan, which is the most strict regulation so far. This suggested the potential health risks from consumption of poorly stored PET bottled beverages. Although the CDI_{total} values here were below the USEPA regulated CDI, the potential risks of long-term exposure to Sb still cannot be ignored if considering all the pathways for human exposure to Sb. We believe that the PET bottles

used for beverages should meet all food-related standards. However, the high Sb levels released from PET bottled beverages under high temperature storage in this study highlighted the necessity to strengthen the supervision of beverage transportation and storage safet

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此页开始为简历部分

如果有必要，最后可以列出团队成员和指导老师的简历。

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许亮亮， 2006 年研究生毕业于南京大学化学化工学院高分子系，获硕士学位。南京外国语学校化学教师，江苏省“333 高层次人才培养工程”培养对象。2014 年获得“南京市优秀青年教师”称号。2012 年获得南京市高中化学优质课评比一等奖；2013 年获得江苏省化学优质课评比一等奖；2014 年获得全国高中化学优质课评比一等奖。2017 年 4 月获江苏省第六届化学创新实验大赛一等奖。在《化学教育 》、《化学教学》等全国中文核心期刊上发表论文 15 篇。2015 年主持江苏省教研室省级重点课题《基于技术素养视角培养学生实验创新能力的实践研究》，2018 年结题。2017 年获得江苏省教学成果一等奖。2018 年获得全国教学成果一等奖。