Letter to the Editor

Phthalates in Commercial Chinese Rice Wines: Concentrations and the Cumulative Risk Assessment to Adult Males in Shanghai*

HUANG Yue[1,2], LU Wen Wei[2], CHEN Bo[1], YOU Jie[1], WU Min[1], and LI Shu Guang[1,#]

The concentrations of 16 phthalates in 164 commercial Chinese rice wines (CRW) were detected by GC-MS, and consumption data on CRW in different packaging types was investigated from 634 adult males in Shanghai using a food frequency questionnaire. Based on the principles of probabilistic modelling and cumulative risk assessment, the exposure and health risk of phthalates from CRW to adult males in Shanghai was evaluated. DMP, DEP, DIBP, DnBP, BBP, and DEHP were detected in the samples, the range of detection frequency of individual phthalates varied from 6.10% for BBP to 15.24% for DIBP, and the detected concentrations were 51.06-200.34 ng/mL. All the respondents consumed CRW, 90.69% of them consumed CRW 0.01-49.9 mL/d, the minimum value of the average daily intake of CRW was 6.25 mL/d, the median was 13.72 mL/d and the maximum was 300 mL/d. The median exposure level of the 6 detected Phthalates to adult males in Shanghai were 6.58-7.10 ng/(d·kg), and the maximum exposure level were 137.38-540.47 ng/(d·kg). The cumulative exposure health risk index (*HI*) based on the median and maximum exposure level of the 6 Phthalates (DMP, DEP, DIBP, DnBP, BBP, and DEHP) were 0.001147 and 0.063396, both were far less than 1. In conclusion, CRW were generally consumed by the adult males in Shanghai, although multiple phthalates were detected in commercial CRW, health risk of such exposure levels from commercial CRW to the target adult males in Shanghai was very low.

Phthalates are ubiquitous environmental pollutants because of their widespread manufacture, use, and disposal as well as their high concentration in and ability to migrate from plastics, and they may enter the human body via ingestion, inhalation, and dermal absorption. Concerns over health effects of phthalates as a group, particularly on reproduction, have drawn attention from both the advocacy groups and lawmakers, especially given the fact that phthalate exposure is ubiquitous in the general population[1]. Foods are the major source of human exposure to phthalates, thus it is essential to monitor levels of phthalates in various foods to provide data for human exposure assessment. During the past decades, data on phthalates in many kinds of foodstuffs and packaging materials all around the word have emerged[2], while the research focused on the occurrence and profiles of phthalates in alcoholic beverage is relatively limited.

Chinese rice wines (CRW) are fermented alcohol beverages made from rice with various distiller's microorganism, which are widely consumed by the general public in China. Considering the widespread use, environmental persistence, abundant presence in many plastic materials (including packaging, pumps, tubing), there exists the potential risk of phthalates contamination during CRW making procedure, nevertheless, there is not any previous report, to the authors knowledge, on their concentrations in commercial CRW.

In this study the presence and concentrations of phthalates in commercial CRW were described for the first time. 164 samples were obtained from randomly sampling in commercial CRW in local supermarkets, which were in different brands and different packaging types, among the samples 16 were in urn or altar, 42 were in glass bottle, 60 were in plastic bucket and 46 were in plastic bag. The presence and concentrations of the 16 phthalates were determined by GC-MS according to the method in the national standard of the People's Republic of China GB/T21911-2008 *Determination of Phthalates in Food*[3]. The basic information for the standard and retention times of the determined 16 phthalates were shown in Table 1.

doi: 10.3967/bes2014.119

*This study was supported by Natural Science Foundation of China (81202208 and 81172673), Morningstar research projects in Shanghai Business School (A-0201-00-050-106).

1. Department of Nutrition and Food Hygiene, Key Laboratory of Public Health Safety of Ministry of Education, School of Public Health, Fudan University, Shanghai 200032, China; 2. Department of Tourism and Food Science, Shanghai Business School, Shanghai 200235, China

The analytical instrument was GC-MS (Agilent Technologies 7890A-5975C) with a capillary column (HP-5MS; 30 m × 0.25 mm i.d.; 0.25 μm film thickness). The limits of quantification (LOQ) of the 16 phthalates was 50.00 ng/mL. The average recoveries of 16 target compounds spiked into sample matrixes were 84.74%-112.19%, relative standard deviation (RSD) were 2.61%-7.50%. Determination of all the samples were under quality control.

The local residents especially the adult males in Shanghai have been an important part of CRW key consumers for many years, and it can be observed that drinking CRW in autumn and winter and using CRW while cooking are daily life habits of many local residents. However the exact consumption data covered drinking and using while cooking, considering CRW in different package types is vacant. The consumption data of CRW in different packaging types on drinking and using while cooking of the objects were investigated using a food frequency questionnaire, involving the socioeconomic status of respondents, CRW consumption data on drinking and using while cooking, handed out face to face through a household survey. The target population were the adult male residents from 18 to 80 years old in Shanghai, 720 objects were selected using multi-stage stratified random sampling method from the population in 219 streets or towns belongs to the 17 districts in Shanghai. The study was undertaken with the permission of the local authority and the Ethics Committee of School of Public Health, Fudan University, China. Informed consent was obtained from each subject. In October 2013 to February 2014, the CRW consumption data in the past three months of the respondents were investigated, according to average daily consumption of drinking and using while cooking in all kinds of packaging types, the average daily intake of CRW were obtained as total.

On the basis of the data on the concentration of phthalates in commercial CRW and the CRW consumption in the population, exposure of the 16 phthalates from CRW in adult males in Shanghai was estimated by *Monte- Carlo* simulation. The basic calculation for estimating individual phthalates exposure through CRW in different packaging types to the adult males in Shanghai was given in Equation 1.

$$E_{i,j}=C_jT_{i,j}/W \quad (1)$$

In which the index i refers to 16 individual phthalates, j refers to 4 different packaging types; $E_{i,j}$ refers to the exposure dose to the people of the i phthalate through the CRW in the j packaging type [ng/(d·kg)]; C_j refers to the intake of CRW in the j packaging type (mL/d); $T_{i,j}$ refers to the concentration of the i phthalate in CRW in the the j packaging type (ng/mL); W refers to the average body mass of the population (kg), 65 kg was used in this study.

Table 1. Basic Information for the Standard and Retention Time of the Determined 16 Phthalates

NO.	Phthalates (Abbreviation)	CAS NO.	Purity (%)	Retention Time (min)
1	Dimethyl phthalate (DMP)	131-11-3	99.0	7.69
2	Diethyl phthalate (DEP)	84-66-2	99.5	8.55
3	Diisobutyl phthalate (DIBP)	84-69-5	99.5	10.26
4	Di-n-butyl phthalate (DnBP)	84-74-2	99.8	10.93
5	Dimethoxyethyl phthalate (DMEP)	117-82-8	99.5	11.31
6	Bis(4-methyl-2-pentyl)phthalate (BMPP)	146-50-9	99.0	12.01
7	Bis(2-ethoxyethyl) phthalate (DEEP)	605-54-9	99.0	12.32
8	Di-n-pentyl phthalate-d4 (DPP)	131-18-0	99.2	12.68
9	Di-n-hexyl phthalate (DHXP)	84-75-3	98.2	14.82
10	Benzyl Butyl Phthalate (BBP)	89-68-7	98.9	14.93
11	Bis(2-n-butoxyethyl) phthalate (DBEP)	117-83-9	98.5	16.39
12	Dicyclohexyl phthalate (DCHP)	84-61-7	99.5	17.04
13	Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	99.5	17.28
14	Diphenyl phthalate (DPhP)	84-62-8	98.1	17.38
15	Dioctyl phthalate (DNOP)	117-84-0	98.5	19.68
16	Dinonyl phthalate (DNP)	84-76-4	98.7	22.19

$T_{i,j}$ were specified as a discrete distribution, value and weight were set according to the detected results. C_j were described as the most appropriate distribution selected by goodness-of-fit analyses. All values below LOD were set at LOD/2 before calculations were carried out. On the basis of these distributions a *Monte-Carlo* simulation was performed using the software package *@Risk* (Palisade corp., *vs.* 6.2 professional) together with *MS-Excel* (version 2010. Microsoft Corporation). The *Monte-Carlo* simulation included 10,000 iterations and was performed 1000 times, applying random starting values for each run. This 1000-fold repetition results in a range of estimates which allows for the description of the uncertainty due to the limited number of iterations reflected by the median and maximum-bands of the respective estimates.

Then health risk associated with phthalates intake of the target population through CRW was evaluated by applying cumulative exposure risk assessment method. To quantitatively assess the human risk of chemicals, the ratio between the actual level of exposure and an acceptable level of exposure is traditionally used. This ratio is sometimes referred to as the hazard quotient (*HQ*) and a *HQ* with a value of greater than one thus indicates a risk. In cumulative risk assessment individual *HQ*s can be added to produce the hazard index (*HI*) (Equation 2).

$$HI=\sum_{i=1,\ j=1}^{m,\ n} HQ_{i,j}=\sum_{i=1,\ j=1}^{m,\ n}(E_{i,j}/RfD_i) \quad (2)$$

In which *HI* is the hazard index; *HQ* is the Hazard Quotient calculated based on the exposure to the target population including *m* kinds of phthalates (the index *i* starting from 1) through CRW in *n* packaging types (the index *j* starting from 1); single $HQ_{i,j}$ is the ratio between the actual level of exposure ($E_{i,j}$) and an reference dose of the *i* phthalate (RfD_i). By definition, the *HI* must be less than 1 to indicate that no harm would result from dietary exposure to the analytes in the lifetime of any consumer[4].

Results showed that DMP, DEP, DIBP, DnBP, BBP, and DEHP were detected in the samples, whereas the rest 10 phthalates including DMEP, BMPP, DEEP, DPP, DHXP, DBEP, DCHP, DPhP, DNOP, and DNP were not detected in all the 164 CRW samples analyzed. The detection rates of the 6 detected phthalates were not high, the order from high to low was 15.24% (25) for DIBP, 14.63% (24) for DMP, 13.41% (22) for DnBP, 13.41% (22) for DEHP, 6.71% (11) for DEP, and 6.10% (10) for BBP. The detection rates were different among the CRW in different packing types, all phthalates were not detected among the samples in urns or altars, 4 phthalates (DEP, DIBP, DnBP, and DEHP) were detected among the samples in glass bottle, and 6 phthalates were all detected among the samples in plastic buckets and bags. As the detected frequencies of phthalates in each packaging type were not as many as enough, the detected values presented a non-normal distribution, therefore the median and quartile range were used to describe the values distribution, $T_{i,j}$ refers to the concentration of the *i* phthalate in CRW in the the *j* packaging type (ng/mL) and the detected concentrations were 51.06-200.34 ng/mL (Table 2).

634 valid questionnaires were obtained in the survey, respondents in 31-60 years old accounted for 70.60%; their education level were primarily university/college or high school/technical secondary school (76.66%); family per capita income were mainly in 3000-8000￥ (64.67%); monthly expenses for alcohol were generally less than 300￥(87.38 %) and the professions and living counties distribution were widely dispersed. 521 respondents (80.60%) drank CRW more or less in different package types, in which 452 respondents (71.29%) drank 0.01-49.9 mL/d and only 40 respondents (9.31%) drank more than 50 mL/d. 589 respondents (92.90%) consumed CRW through diet with CRW, the intake ranged from 0.63 mL/d to 33.33 mL/d. In the average CRW daily intake of adult males in Shanghai (C_j), the minimum, median and maximum value were 6.25 mL/d, 13.72 mL/d, and 300 mL/d respectively. 575 persons (90.69%) consumed CRW 0.01-49.9 mL/d, only 40 persons (6.31%) consumed 50.0-99.9 mL/d, and 19 persons (3.00%) consumed 100-300 mL/d.

The exposure dose of the 6 detected phthalates to the target people ($E_{i,j}$) were obtained from probabilistic modeling (Table 3). In overall consideration of CRW in the four different packaging types, the median exposure level of each phthalate were 6.58-7.10 ng/(d·kg), the maximum exposure level were 137.38-540.47 ng/(d·kg).

U.S. EPA Reference Dose (*RfD*) for Chronic Oral Exposure of DEP, DnBP, BBP, and DEHP were 0.80 mg/(d·kg), 0.10 mg/(d·kg), 0.20 mg/(d·kg), and 0.02 mg/(d·kg) respectively[1]. In addition, tolerable daily intake(*TDI*) suggested by the European food safety commission(EFSA) of DnBP was 0.01 mg/(d·kg)[5], which is smaller. This paper adopted 0.01 mg/(d·kg) as the risk assessment *RfD* of DnBP.

There are no authoritative international *RfD* of DMP and DIBP at present, 1.07 mg/(d·kg) was used as *RfD* of DMP, based on the value 107 mg/(d·kg), the No Observed Adverse Effect Level(LOAEL) of general toxicity of Sprague Dawley rat[6], divided by the safety factor of 100 times, and ADI for the general population of 0.14 mg/(d·kg)[7] was used as the risk assessment *RfD* of DIBP.

*HQ*s of DMP, DEP, DIBP, DnBP, BBP, and DEHP were calculated according to their corresponding $E_{i,j}$ and *RfD* respectively, *HQ*s were summed to produce the hazard index (*HI*). *HI* were 0.001147 (1/872) and 0.063396 (1/16) based on the median and maximum exposure level, both were far less than 1, which indicated there were little health risk from combined expose to the 6 phthalates, although several phthalates could be detected in some CRW samples (Table 3).

Table 2. Detection Results of 16 Phthalates in 164 CRW Samples

CRW in Different Packaging Types	No.	Phthalates	Detection Number	Detection Ratio(%)	Concentration of Phthalates (T_{ij}) (ng/mL)			
					Minimum (P0)	Median (M)	Maximum (P100)	Quartile range(Q)
Urn Altar (*n*=16)	1	DMP	0	0.00	-	-	-	-
	2	DEP	0	0.00	-	-	-	-
	3	DIBP	0	0.00	-	-	-	-
	4	DnBP	0	0.00	-	-	-	-
	5	BBP	0	0.00	-	-	-	-
	6	DEHP	0	0.00	-	-	-	-
Glass Bottles (*n*=42)	1	DMP	1	2.38	112.91	/	/	/
	2	DEP	0	0.00	-	-	-	-
	3	DIBP	2	4.76	68.87	/	72.33	/
	4	DnBP	1	2.38	104.44	/	/	/
	5	BBP	0	0.00	-	-	-	-
	6	DEHP	2	4.76	85.25	/	85.25	/
Plastic Buckets (*n*=60)	1	DMP	13	21.67	51.95	83.52	149.86	54.77
	2	DEP	5	8.33	63.42	70.53	78.57	12.23
	3	DIBP	12	20.00	63.81	72.80	83.90	8.77
	4	DnBP	11	18.33	56.03	72.16	166.81	58.00
	5	BBP	7	11.67	63.45	73.27	166.52	65.85
	6	DEHP	11	18.33	51.06	74.90	87.59	18.53
Plastic Bags (*n*=46)	1	DMP	10	21.74	52.22	87.87	174.32	60.86
	2	DEP	6	13.04	60.29	68.23	87.16	19.04
	3	DIBP	11	23.91	62.76	72.55	82.81	8.84
	4	DnBP	10	21.74	66.32	106.72	200.34	59.89
	5	BBP	3	6.52	67.78	69.75	83.41	15.62
	6	DEHP	9	19.57	59.50	74.99	88.51	16.42
Total (*n*=164)	1	DMP	24	14.63	51.95	85.63	174.32	54.92
	2	DEP	11	6.71	60.29	70.25	87.16	15.16
	3	DIBP	25	15.24	62.76	72.38	83.90	8.44
	4	DnBP	22	13.41	56.03	97.81	200.34	53.40
	5	BBP	10	6.10	63.45	71.68	166.52	29.96
	6	DEHP	22	13.41	51.06	76.15	88.51	15.37

Note. '-' not detected; '/' not calculated.

Table 3. Exposure and Hazard Quotient of Phthalates from CRW in Adult Males in Shanghai

Phthalates	Median Exposure Level of Phthalates from CRW in different Packaging Types($E_{i,j}$) [ng/(d·kg)]					Hazard Quotient (*HQ*)(10^{-4})	Maximum Exposure Level of Phthalates from CRW in different Packaging Types($E_{i,j}$) [ng/(d·kg)]					Hazard Quotient (*HQ*)(10^{-4})
	Urn altar	Glass bottle	Plastic Bucket	Plastic Bag	Total		Urn altar	Glass bottle	Plastic Bucket	Plastic Bag	Total	
DMP	0.85	2.57	2.39	1.29	7.10	0.07	13.60	94.69	304.31	48.13	460.73	4.31
DEP	0.85	2.50	2.06	1.16	6.58	0.08	14.20	33.82	59.84	29.52	137.38	1.72
DIBP	0.85	2.61	2.26	1.28	7.00	0.50	11.54	95.78	63.73	38.83	209.88	14.99
DnBP	0.85	2.57	2.27	1.31	7.01	7.01	12.97	112.380	106.51	88.29	320.15	320.15
BBP	0.85	2.50	2.18	1.10	6.64	0.33	11.58	41.00	353.13	46.35	452.06	22.60
DEHP	0.85	2.61	2.28	1.23	6.97	3.49	11.62	85.10	399.57	44.18	540.47	270.24
Total (*HI*)	-	-	-	-	-	11.47	-	-	-	-	-	633.96

The maximum permitted specific migration limit (SML) for DnBP, BBP, and DEHP in EU is 0.3 mg/kg, 30 mg/kg, and 1.5 mg/kg respectively[8]. The maximum permitted SML for DnBP, DEHP in China is 0.3 mg/kg and 1.5 mg/kg respectively[9]. In the 164 CRW samples, the detected concentrations of the six phthalates were 51.06-200.34 ng/mL, the value of DnBP, BBP, and DEHP does not exceed the standards of China and EU. Literature showed that the plasticizer often could be detected in Chinese liquor, such as DnBP and DEHP, which mainly could be explained by the widely use of plastics in the storage and transportation process[10]. The sources of phthalates of CRW may also be primarily related with the two process of storage and transportation, while the alcohol content in CRW is commonly 8%-20% (volume percent), the dissolution of phthalates migration quantity is relatively limited.

All values below LOD were set at LOD/2 before calculations were carried out, this calculation tended to overestimate, because the detection rate of majority of phthalates in CRW were relatively low. On the basis of overestimated, cumulative risk assessment of the 6 detected phthalates were performed, *HI* obtained were far less than 1, which indicated the health risk of phthalates exposure from CRW to adult males in Shanghai is relatively small.

#Correspondence should be addressed to Prof. LI Shu Guang, Tel: 86-21-54237260, Fax: 86-21-54237260, E-mail: leeshuguang@fudan.edu.cn

Biographical note of the first author: HUANG Yue, female, born in 1981, PhD candidate of Fudan University in medical science, majoring in Nutrition and Food safety.

Received: May 19, 2014;
Accepted: August 4, 2014

REFERENCES

1. U.S. EPA. Phthalates, teach chemical summary. Available at http://www.epa.gov/teach/chem_summ/phthalates_summar.pdf. [2012-03-30].
2. Fierens T, Servaes K, Van Holderbeke M, et al. Analysis of phthalates in food products and packaging materials sold on the Belgian market. Food Chem Toxicol, 2012; 50, 2575-83.
3. China National Standardization Management Committee. Determination of Phthalates in Food. In GB/T21911-2008, Beijing: Standards Press of China, 2008. (In Chinese)
4. National Research Council of Academies. Phthalates and cumulative risk assessment: the task ahead. The National Academies Press: Washington DC, 2008; p68-99.
5. EFSA. Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to di-butylphthalate (DnBP) for use in food contact materials: question no. EFSA-Q-2003-192. EFSA J, 2005; a242, 1-2.
6. Kent RC. Toxicity Review for Diisobutyl Phthalate. In U.S. Consumer Product Safety Commission: 2013. Available at http://www.cpsc.gov/PageFiles/125773/dibp.pdf. [2003-8-1].
7. Bell FP, Patt CS, Brundage B, et al. Studies on lipid biosynthesis and cholesterol content of liver and serum lipoproteins in rats fed various phthalate esters. Lipids, 1978; 13, 66-74.
8. THE EUROPEAN COMMISSION. On plastic materials and articles intended to come into contact with food. In (EU) No 10/2011, 2011-05-01.
9. China National Standardization Management Committee. Hygienic standards for uses of additives in food containers and packaging materials. In GB9685-2008, Beijing: Standards Press of China, 2008. (In Chinese)
10. WANG L, TIAN MH, LIN QL, et al. Detection method and control strategy of plasticizer in Chinese liquor. Sci Technol Food Ind, 2013; 34, 384-7. (In Chinese)