Supporting Information for

"Dietary intake and human milk residues of hexachlorocyclohexane isomers in two Chinese cities"

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This material includes 10 pages, 9 tables and 1 figure.

Characteristics of the subjects. The characteristics of the mothers who donated their breast milk are listed in Table S1 and S2. There were a total of 76 lactating women among which 40 from Beijing and 36 from Shenyang. The parameters listed include age, body weight and height, body mass index (BMI, weight/height^2), place of residence (rural or urban), occupation, the number of children breastfed and human milk sampling time (number of the days from the first breastfeeding to the sampling). Infant's birth weights are also listed. The parameters collected but not listed are migration history, years living in the cities, smoking, drinking, disease and exercise, spouse's age and occupation, etc. No significant influences were found by unlisted parameters on human milk HCH levels.

Table S1. Descriptive characteristics of the human milk donors from Beijing.

No.	Age	Height	Weight	BMI	Child birth weight	Residence	Occupation	No of Children	period between childbirth and sampling
		cm	kg	kg/m^2	g				day
1	23	160	70	27.3	3050	rural	vendor	1	2
2	27	160	67.5	26.4	3400	urban	vendor	2	20
3	25	155	64	26.6	2700	urban	tailor	1	2
4	29	159	75	29.7	3350	urban	childcare	1	75
5	34	160	88	34.4	3100	urban	teacher	1	14
6	29	152	72.5	31.4	3300	urban	secretary	1	15
7	36	158	82	32.8	3400	urban	housewife	2	3
8	31	160	62	24.2	3150	urban	vendor	2	45
9	25	173	85	28.4	4300	urban	accountant	1	4
10	28	168	70	24.8	3350	urban	sells men	2	5
11	26	151	67	29.4	3750	urban	housewife	1	3
12	33	163	65	24.5	3400	urban	tailor	2	1
13	26	162	75	28.6	3600	urban	vendor	1	10
14	27	160	86.5	33.8	3600	urban	vendor	2	not available
15	27	172	71	24.0	2950	rural	worker	1	5
16	36	156	64	26.3	2800	urban	electrician	1	7
17	30	155	82.5	34.3	3150	urban	nurse	1	12
18	24	163	60	22.6	3600	rural	vendor	1	4
19	33	158	65	26.0	3650	urban	shopkeeper	2	4
20	31	165	66	24.2	3400	urban	sells men	1	6
21	23	167	72	25.8	3150	rural	vendor	1	8
22	28	162	60	22.9	3300	urban	office worker	1	2
23	28	170	71	24.6	4050	urban	office worker	1	7
24	23	159	64	25.3	3000	rural	farmer	1	2
25	27	165	80	29.4	3800	rural	worker	1	11
26	33	166	58	21.0	2600	rural	vendor	2	3
27	27	155	70	29.1	3150	urban	nurse	1	10
28	33	165	70	25.7	3600	urban	vendor	2	3
29	28	162	73	27.8	3300	urban	subway driver	1	6
30	27	168	85	30.1	3500	urban	sells men	1	3
31	24	170	84	29.1	3950	urban	vendor	1	5
32	28	157	72.5	29.4	2700	urban	office worker	1	12
33	28	168	86.5	30.7	3900	urban	housewife	1	12
34	33	163	94.5	35.6	3050	urban	cooker	1	12
35	28	168	83	29.4	4350	rural	vendor	2	26
36	25	168	75	26.6	3700	urban	hotel clerk	1	2
37	33	153	66	28.2	3950	urban	vendor	2	10
38	31	160	65	25.4	3350	urban	hotel clerk	1	4
39	34	156	66	27.1	3000	urban	shop assistant	2	13
40	28	160	58	22.7	3000	rural	tailor	2	3

Table S2. Descriptive characteristics of the human milk donors from Shenyang.

No.	Age	Height	Weight	BMI	Child birth weight	Residence	Occupation	No of Children	Period between childbirth and sampling
		cm	kg	kg/m^2	g				day
1	29	158	88	35.1	3200	urban	teacher	1	13
2	25	163	76	28.6	4100	urban	accountant	1	2
3	30	168	84	29.8	3450	urban	office worker	1	2
4	24	160	55	21.5	2850	urban	housewife	1	20
5	29	161	70	27.0	3350	urban	secretary	1	3
6	28	158	72	28.8	3600	urban	office worker	1	22
7	22	162	81	30.9	3700	urban	not available	1	13
8	26	162	74	28.2	3750	urban	office worker	1	7
9	27	164	88.5	30.7	3150	urban	not available	1	13
10	25	165	91.5	33.6	3800	urban	not available	1	13
11	32	160	75	29.3	3050	urban	office worker	1	18
12	29	158	70	28.0	3400	urban	sells men	1	4
13	25	161	65	25.1	3600	urban	secretary	1	2
14	25	161	88	33.9	3600	urban	not available	1	13
15	30	162	81.5	31.1	3000	urban	hotel clerk	1	10
16	32	168	91	32.2	3750	rural	not available	1	13
17	31	170	89.5	31.0	3457	urban	free lance	1	13
18	22	162	73.5	28.0	3700	urban	office worker	1	2
19	21	154	80	34.0	3650	rural	vendor	1	15
20	25	165	90	33.1	3500	urban	free lance	1	5
21	29	164	71.5	26.6	4000	urban	office worker	1	10
22	28	158	65.5	26.2	3300	urban	worker	1	9
23	26	163	72.5	27.3	3200	urban	manager	1	13
24	28	162	86.5	33.0	3200	urban	not available	1	13
25	24	170	90	31.1	3000	urban	nurse	1	10
26	24	160	69	27.0	3200	urban	housewife	1	7
27	24	168	64	22.7	3300	urban	office worker	1	17
28	35	162	82	31.2	3457	rural	farmer	2	13
29	31	160	73	28.5	3800	urban	vendor	1	10
30	27	162	86	32.8	3000	urban	not available	1	13
31	30	160	77.5	30.3	4400	urban	secretary	1	22
32	39	164	80	29.7	3000	urban	office worker	1	14
33	29	168	84	29.8	3750	urban	worker	1	5
34	25	158	69.5	27.8	3800	urban	not available	1	13
35	25	168	74	26.2	3000	urban	secretary	1	14
36	29	155	89	37.0	3400	urban	worker	1	13

Food consumption. The average body weight adjusted consumptions of major foodstuffs in fresh weight per person per day are presented in Table S3 by the two studied groups from Beijing and Shenyang, respectively. The consumption data was derived by multiplying the frequency of consumption of each food item by the portion weight of that food, both provided by the breast milk donors. These food items were selected based on the dietary pattern of Chinese population (*1*). The questionnaires were standardized but were not able to be validated.

Table S3. Average per capita daily consumptions per body weight of major foods by the two groups as means and standard deviations, g (f.w.) /d. kg (body weight)

City	Apple	Banana	Pear	Grape	Orange	Wheat	Rice
Beijing	2.5 ± 2.3	1.3 ± 1.2	1.7 ± 1.9	0.97 ± 1.1	0.96 ± 1.2	0.74 ± 0.45	2.1 ± 0.88
Shenyang	1.5 ± 1.1	1.1 ± 1.4	1.1 ± 1.0	0.85 ± 1.1	0.97 ± 1.0	0.61 ± 0.32	2.3 ± 1.1

City	Chinese cabbage	Cabbage	Spinach	Cucumber	Carrot	Green pepper	Eggplant	Lettuce	Potato	Bean
Beijing	0.80 ± 0.94	0.46 ± 0.68	1.0 ± 1.4	0.81 ± 1.3	0.68 ± 0.82	0.48 ± 0.69	0.67 ± 1.1	0.38 ± 0.63	1.1 ± 1.3	0.81 ± 1.2
Shenyang	0.88 ± 0.80	0.17 ± 0.30	0.28 ± 0.30	0.53 ± 0.49	0.35 ± 0.43	0.26 ± 0.34	0.58 ± 0.47	0.19 ± 0.22	0.78 ± 0.83	0.56 ± 0.46

City	Grass carp	Crucian	Carp	Bighead	Beef	Chicken	Mutton	Pork
Beijing	0.10 ± 0.16	0.22 ± 0.28	0.11 ± 0.15	0.059 ± 0.12	0.36 ± 0.52	0.43 ± 0.50	0.23 ± 0.39	0.62 ± 0.60
Shenyang	0.10 ± 0.38	0.15 ± 0.39	0.06 ± 0.08	0.060 ± 0.18	0.27 ± 0.29	0.51 ± 0.70	0.22 ± 0.28	0.74 ± 0.60

City	Eggs	Milk	Oil
Beijing	1.0 ± 0.71	2.7 ± 2.5	0.24 ± 0.029
Shenyang	0.73 ± 0.43	1.1 ± 0.97	0.22 ± 0.027

Detection limits and recoveries. The detection limits for HCHs in various categories of samples are tabulated in Table S4 and the average recoveries of the determination procedure for various foodstuffs based on standard spiking are listed in Table S5. Zeros were assigned to those below the detection limits for calculation of means and standard deviations. For the geometric means, those below the detection limits were omitted.

Table S4. Detection limits for various categories of samples, ng/g (f.w.).

Sample	α-HCH	β-HCH	γ-HCH	δ-HCH
Human milk, milk and eggs	0.013	0.027	0.046	0.015
Vegetables and fruits	0.002	0.004	0.006	0.002
Cereals	0.007	0.015	0.027	0.008
Fish and meat	0.005	0.012	0.019	0.007

Table S5. Recoveries for various samples based on the spiked sample measurement as Mean±S.D.(range) %.

Sample	α-HCH	β-HCH	γ-HCH	δ-HCH
Human milk, milk and eggs	87 ± 3 (84-88)	88 ± 4 (84-91)	86 ± 4 (82-89)	88 ± 2 (85-90)
Vegetables, fruits and cereals	85 ± 5 (78-89)	89 ± 6 (82-93)	81 ± 4 (76-84)	87 ± 6 (79-90)
Fish and meat	105 ± 16 (86-119)	103 ± 7 (99-111)	102 ± 18 (81-113)	89 ± 16 (71-102)

Food concentrations. The measured concentrations of HCHs in foodstuffs are listed in Table S6 (Beijing) and S7 (Shenyang) for the two cities, respectively. Relatively high concentrations of HCHs were found in flour samples from Shenyang. The flour in the market of Shenyang was imported from Shandong and Henan, while those in the market of Beijing were from Henan and local producers. The measured concentrations of HCHs from Shandong were several times higher than those from other places and further effort is needed to investigate their sources.

Table S6. Concentrations of HCHs in various foodstuffs from Beijing as means and standard deviations as ng/ml for milk and oil and ng/g (f.w.) for the other samples.

Category	Food	Sample size	α-HCH	β-HCH	γ-HCH	δ-HCH
Fruits	apple	2	0.070 ± 0.003	0.073 ± 0.002	0.073 ± 0.004	0.017 ± 0.001
	banana	1	0.005	N.D	0.005	0.004
	pear	1	0.232	0.061	0.062	0.022
	grape	1	0.087	0.110	0.108	0.048
	orange	1	0.006	0.010	0.031	N.D
Vegetables	Chinese cabbage	4	0.011 ± 0.002	0.012 ± 0.013	0.040 ± 0.037	0.001
	cabbage	2	0.011 ± 0.016	ND	ND	ND
	spinach	2	0.017 ± 0.001	0.022 ± 0.007	0.118 ± 0.103	0.012 ± 0.003
	cucumber	2	0.036	0.016 ± 0.002	0.587	0.032
	carrot	2	0.021 ± 0.008	0.192 ± 0.013	0.072 ± 0.019	0.005 ± 0.007
	green pepper	2	0.013	0.014 ± 0.001	0.063 ± 0.003	0.009 ± 0.003
	eggplant	2	0.034	0.050 ± 0.044	0.568	0.034
	lettuce	2	0.031 ± 0.005	0.030 ± 0.013	0.046 ± 0.001	0.013 ± 0.005
	potato	2	0.020 ± 0.001	0.019	0.034 ± 0.005	0.014 ± 0.004
	bean	3	0.005 ± 0.002	ND	0.012 ± 0.006	0.003 ± 0.001
Cereals	flour	12	0.263 ± 0.076	0.068 ± 0.048	0.114 ± 0.049	0.097 ± 0.029
	rice	13	0.027 ± 0.008	0.018 ± 0.032	0.049 ± 0.026	0.037 ± 0.034
Fish	grass carp	11	0.059 ± 0.024	0.108 ± 0.072	0.051 ± 0.018	0.023 ± 0.014
	crucian	11	0.441 ± 0.333	1.325 ± 1.479	0.250 ± 0.265	0.141 ±0.103
	carp	10	0.281 ± 0.212	0.239 ± 0.134	0.094 ± 0.046	0.043 ± 0.034
	bighead	11	0.059 ± 0.043	0.178 ± 0.188	0.119 ± 0.105	0.078 ± 0.135
Meat	beef	2	0.030 ± 0.016	0.219 ± 0.042	0.051 ± 0.007	0.002
	chicken	5	0.017 ± 0.002	0.032 ± 0.012	0.031 ± 0.004	0.002
	mutton	2	0.055 ± 0.007	0.737 ± 0.025	0.092 ± 0.051	0.002
	pork	5	0.102 ± 0.013	0.529 ± 0.098	0.201 ± 0.160	0.002 ± 0.001
Eggs	eggs	6	0.069 ± 0.029	0.481 ± 0.790	0.119 ± 0.142	0.165 ± 0.094
Milk	milk	5	0.310 ± 0.163	1.341 ± 1.111	0.154 ± 0.144	0.247 ± 0.132
Oil	oil	14	1.495 ± 0.930	1.617 ± 2.441	2.027 ± 0.954	2.623 ± 2.210

Note: 1) ND - not detectable; 2) For those without replicates due to technical reason (lost during analysis or outliers of individual isomers), standard deviations were not available; 3) The same brands of oil from same producers (Fulinmen and Luhua) were the most popular products in the market of both cities and the samples were combined for analysis.

Table S7. Concentrations of HCHs in various foodstuffs from Shenyang as means and standard deviations as ng/ml for milk and oil and ng/g (f.w.) for the other samples.

Category	Food	Sample size	α-HCH	β-HCH	γ-HCH	δ-HCH
Fruits	apple	2	0.184 ± 0.053	0.043 ± 0.061	0.085 ± 0.011	0.043 ± 0.023
	banana	1	0.007	N.D	N.D	N.D
	pear	1	0.052	0.052	0.020	0.015
	grape	1	0.038	0.108	0.059	0.087
	orange	1	0.004	0.000	0.000	0.004
Vegetables	Chinese cabbage	3	0.015 ± 0.006	0.155 ± 0.018	0.070 ± 0.006	N.D
	cabbage	2	N.D	0.193 ± 0.030	0.047 ± 0.010	N.D
	spinach	1	0.113	0.921	0.140	0.054
	cucumber	3	0.039 ± 0.007	0.009 ± 0.002	0.007	0.008 ± 0.002
	carrot	3	0.041 ± 0.008	0.217 ± 0.010	0.027 ± 0.001	0.015 ± 0.001
	green pepper	2	0.041 ± 0.023	0.017 ± 0.004	0.057	0.051 ± 0.002
	eggplant	2	0.013 ± 0.001	0.067 ± 0.001	0.013 ± 0.001	0.003
	lettuce	2	0.242 ± 0.024	5.311 ± 0.172	0.171 ± 0.012	0.112 ± 0.007
	potato	2	0.068 ± 0.012	0.088 ± 0.021	0.037 ± 0.006	0.016 ± 0.001
	bean	2	0.043 ± 0.008	0.063 ± 0.018	0.023 ± 0.004	0.005 ± 0.001
Cereals	flour	13	2.713 ± 2.064	0.194 ± 0.119	0.117 ± 0.030	0.147 ± 0.044
	rice	13	0.093 ± 0.029	0.043 ± 0.056	0.049 ± 0.026	0.082 ± 0.019
Fish	grass carp	10	0.248 ± 0.123	0.833 ± 0.407	0.206 ± 0.074	0.287 ± 0.140
	crucian	13	0.252 ± 0.034	1.050 ± 0.215	0.208 ± 0.030	0.190 ± 0.037
	carp	10	0.876 ± 0.405	5.429 ± 1.635	0.430 ± 0.166	0.756 ± 0.337
	bighead	9	0.765 ± 0.250	1.655 ± 0.450	0.424 ± 0.108	0.523 ± 0.176
Meat	beef	2	0.207 ± 0.050	1.804 ± 0.004	0.146 ± 0.022	N.D
	chicken	4	0.117 ± 0.027	0.126 ± 0.088	0.126 ± 0.024	N.D
	mutton	2	0.122 ± 0.002	2.034 ± 0.202	0.093 ± 0.027	N.D
	pork	5	0.109 ± 0.032	0.068 ± 0.143	0.149 ± 0.059	N.D
Eggs	eggs	6	0.125 ± 0.062	0.303 ± 0.118	N.D	N.D
Milk	milk	6	0.281 ± 0.061	1.686 ± 0.929	0.155 ± 0.148	0.126 ± 0.020
Oil	oil	14	1.495 ± 0.930	1.617 ± 2.441	2.027 ± 0.954	2.623 ± 2.210

Note: 1) ND - not detectable; 2) For those without replicates due to technical reason (lost during analysis or outliers of individual isomers), standard deviations were not available; 3) The same brands of oil from same producers (Fulinmen and Luhua) were the most popular products in the market of both cities and the samples were combined for analysis.

Table S8. Daily dietary intakes (means, standard deviations and medians) of HCHs by the mothers from Beijing and Shenyang, ng/kg·day.

Population		α-HCH	β-HCH	γ-HCH	δ-HCH	Σ-HCH
Beijing	mean±SD	2.58 ± 1.00	5.90 ± 3.65	2.99 ± 1.42	1.89 ± 0.63	13.4 ± 6.0
n=40	M	2.32	4.68	2.77	1.69	10.9
Shenyang	mean±SD	3.62 ± 1.21	6.10 ± 2.07	1.55 ± 0.410	1.35 ± 0.350	12.6 ± 3.70
n=36	M	3.60	6.60	1.47	1.28	13.0

Regression statistics for the linear model. The ln-transformed human milk ΣHCH of the two populations could be predicted based on simple linear model using the dietary intakes as the independent variable. The regression statistics are listed in Table S9.

Table S9. Regression statistics for the linear regression model for predicting human milk ΣHCH.

Population	Intercept	S.E. of Intercept	Slope	S.E. of Slope	p of Intercept	p of slope
Beijing	4.12	0.193	0.049	0.0071	5.15E-22	6.89E-8
Shenyang	3.77	0.311	0.150	0.0237	6.90E-14	3.34E-7

Prediction of HCHs in the human milk. It was found that a non-linear model can predict the levels of HCHs in the human milk well. Both BMI and the dietary intake were used as the independent variables. The empirical models derived are as follows:

C_m (human milk) = $2.10\times10^{-5}\ I_d^{\,4.58}$ + 5.73 BWI - 2.55 (Beijing) $R^2 = 0.89$,

C_m (human milk) = $2.4\ I_d^{\,1.9}$ + 26.5 BWI - 735.8 (Shenyang) $R^2 = 0.58$,

where C_m and I_d were the residuals of HCHs in the human milk and the dietary intake, respectively. Due to the ill conditioned matrix, the standard errors could not be calculated.

The results of the Monte Carlo simulation on the breastfed risk. The result is presented in Figure S1 as cumulative frequencies of daily intakes of HCHs by breastfed infants. Normal distributions of body weight and milk consumption and log-normally distributed concentrations of β-HCH were assumed. The average diet of breastfed infants was set at 800 ± 160 g milk per day (*2*).

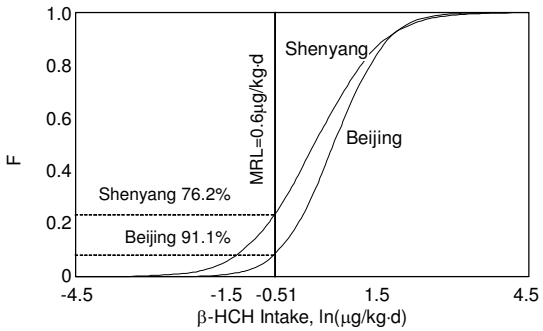

Figure S1. Cumulative frequencies of the estimated daily intake of β-HCH by infant through breast feeding over a range from -4.5 ln(μg/kg·day) to 4.5 ln(μg/kg·day) for Beijing and Shenyang, respectively. The results were derived from a Monte Carlo simulation using the data collected from Beijing and Shenyang during a period of 2005-2007. The results were assessed based on Minimal Risk Level (MRL) of 0.6 μg/kg·day issued by US Agency for Toxic Substances and Disease Registry.

References

(1) Zhai, F. Y.; Yang, X. G. 2006. Report on Nutrient and Health of Chinese Population in 2002, II Diet Pattern and Nutrient Intake. Beijing: People's Health Press.

(2) Xing, Y.; Zai, Z. How much you know about breast feeding. available: 2008, http://blog.sina.com.cn/s/blog_4a7bafdd010090i7.html (Accessed April 28, 2008).