北京大学 PEKING UNIVERSITY

New Evidence of Health Effect Based on Chromate Exposure Assessment

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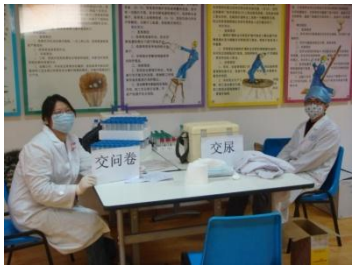
Peking University



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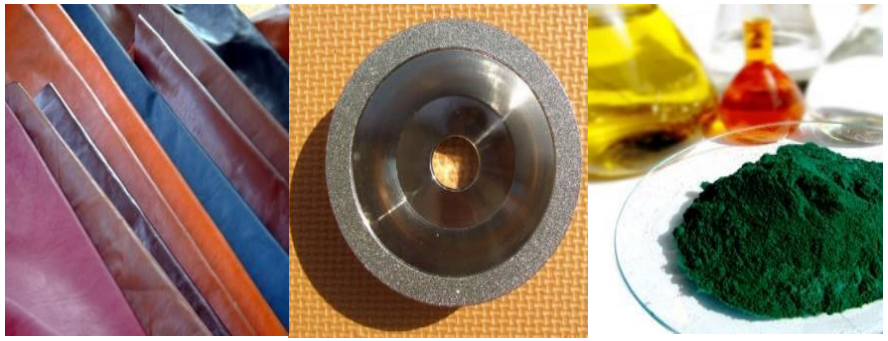
Environmental pollution of Cr compounds



widely application of chromium compounds

<u>Manufacture workers</u>

<u>Chromium residue</u>

中华人民共和国生态环境部
Ministry of Ecology and Environment of the People's Republic of China

关于征求《优先控制化学品名录（第一批）（征求意见稿）》意见的函

List of Priority Controlled Chemicals

PC012	二氯甲烷	75-09-2
PC013	镉及镉化合物	7440-43-9 (镉)
PC014	铬及铬化合物	7440-47-3 (铬)

chromium and its compounds

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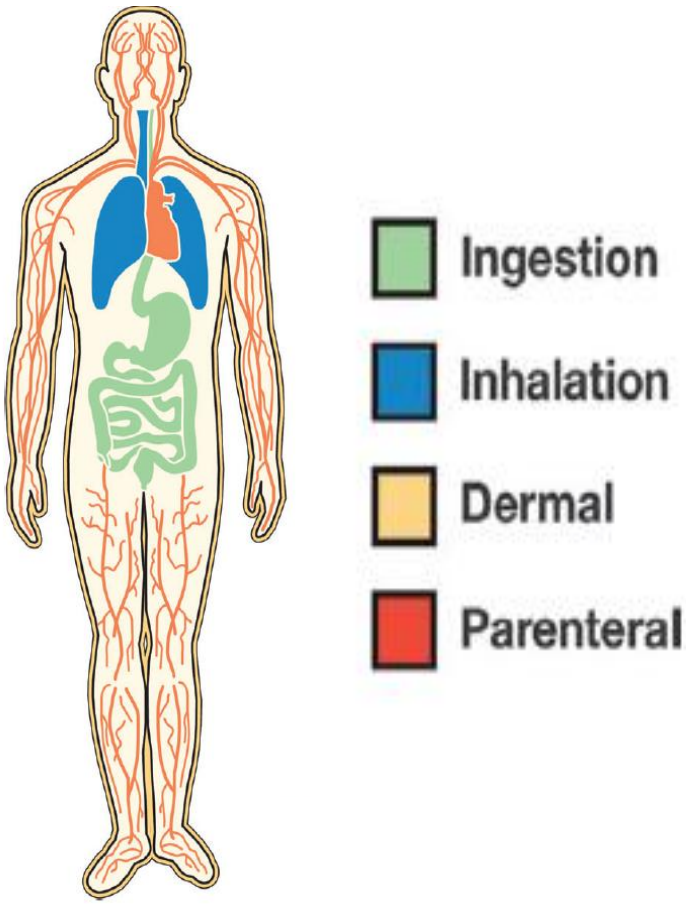
Routes of exposure

- inhalation, skin, ingestion

- multi-system disorders involving the skin , mucous membrane, liver, renal,and immune system...

- carcinogen: lung cancer
  digestive cancer



Scheme of our systematic study

environmental exposure assessment

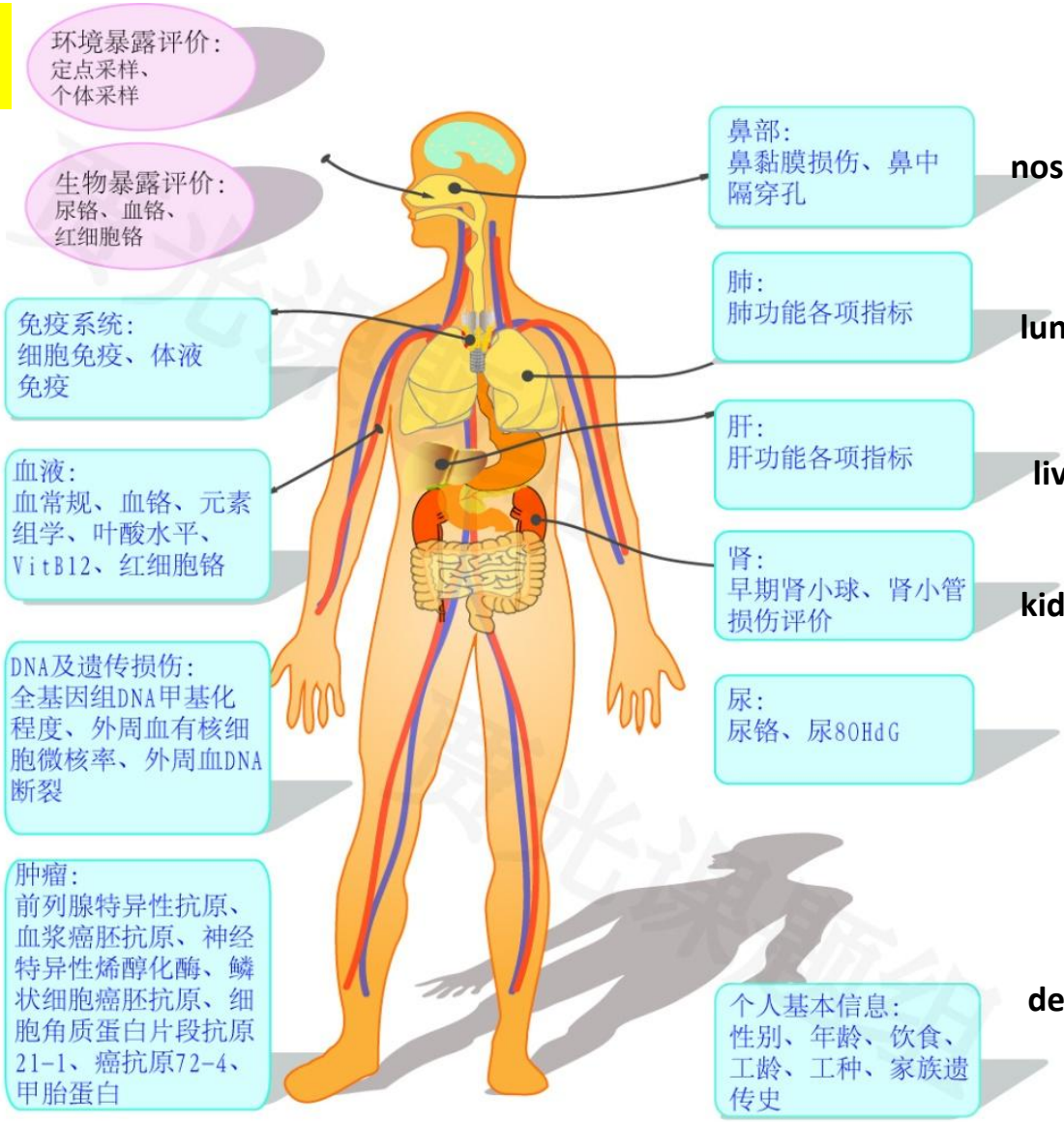
biological exposure assessment

immunological function

blood routine

genetic damage (micronucleus test & 8-OHdG)

tumor specific antigen



nose examination

lung function

liver function

kidney function

demographic, lifestyle information from questionnaires

Design based on occupational workers

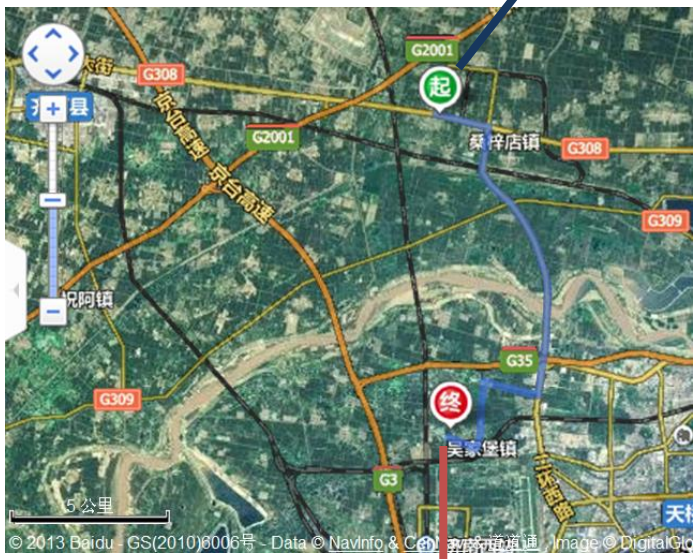
Biological sample bank

- ✓ 3692 person*year followed;
- ✓ 1.4 ×10^4 samples were frozen including blood and urine ;

2006 ····· 2010 ····· Now

Field Site Introduction

chromate manufacture plant



Control group:
Local residents without chromate exposure

- ✓**aged between 25-50;**
- ✓**at least one year employment in this factory and 3 months in the same work site;**

Both groups are

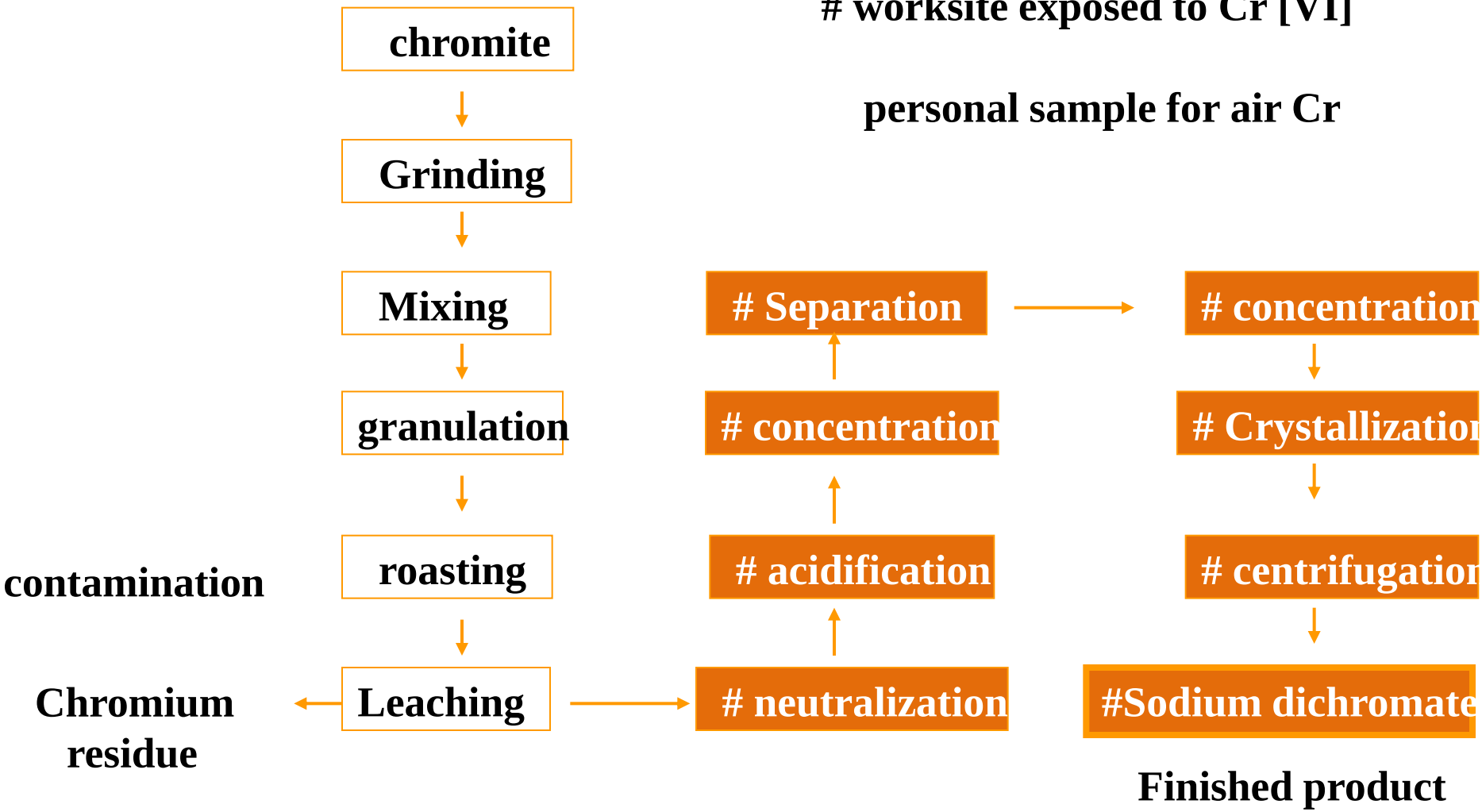
- **male blue collar workers or famers**
- **with similar education, social status and geographical location**

The distance between the control and exposed group is about 16 km

Air Exposure Assessment

worksite exposed to Cr [VI]

personal sample for air Cr



Main flowchart for sodium dichromate manufacture process

Questionnaire

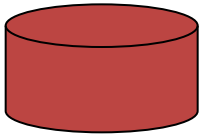
Confounding factor

①occupational history ② personal medical history③ medication used in 4 weeks before the study ④body weight and height⑤hair dye, ⑥house decoration⑦ radiation exposure ⑧individual protection ⑨smoking status and alcohol intake.

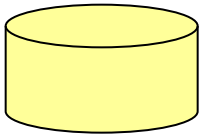
Personal sampling

Questionaire

Biological Exposure Assessment

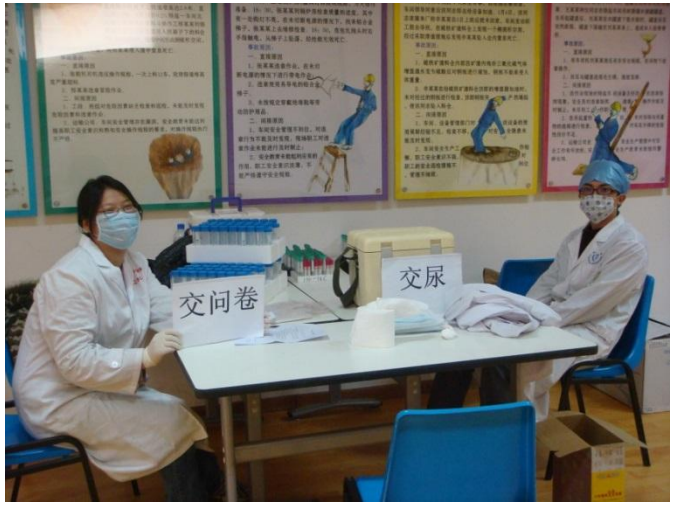


Cr in whole blood, WB-Cr



Cr in urine, U-Cr

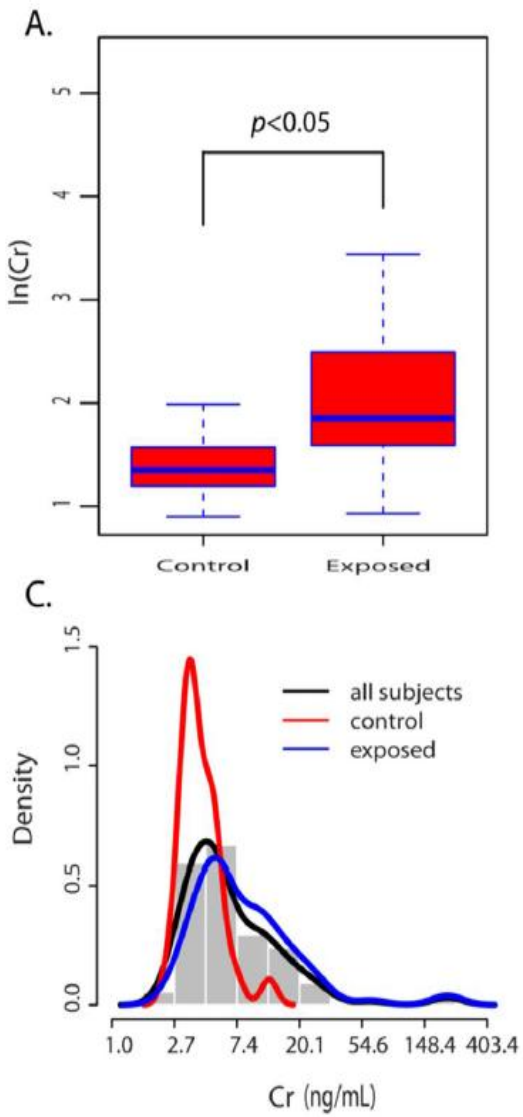
Detected by ICP-MS

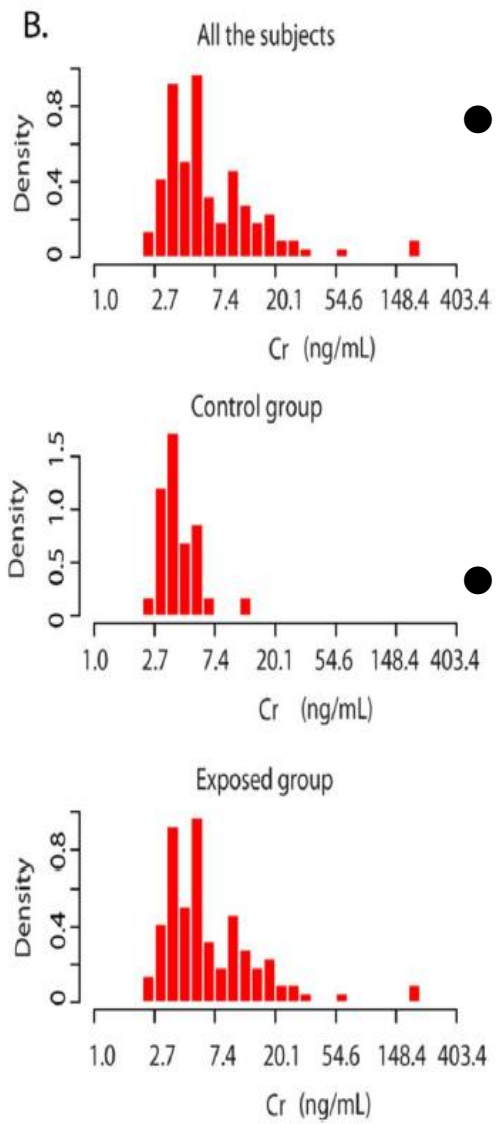


Exposed samples were collected after five consecutive working days;

The control ones were after completing the questionnaires;

Environmental and biological exposure assessment

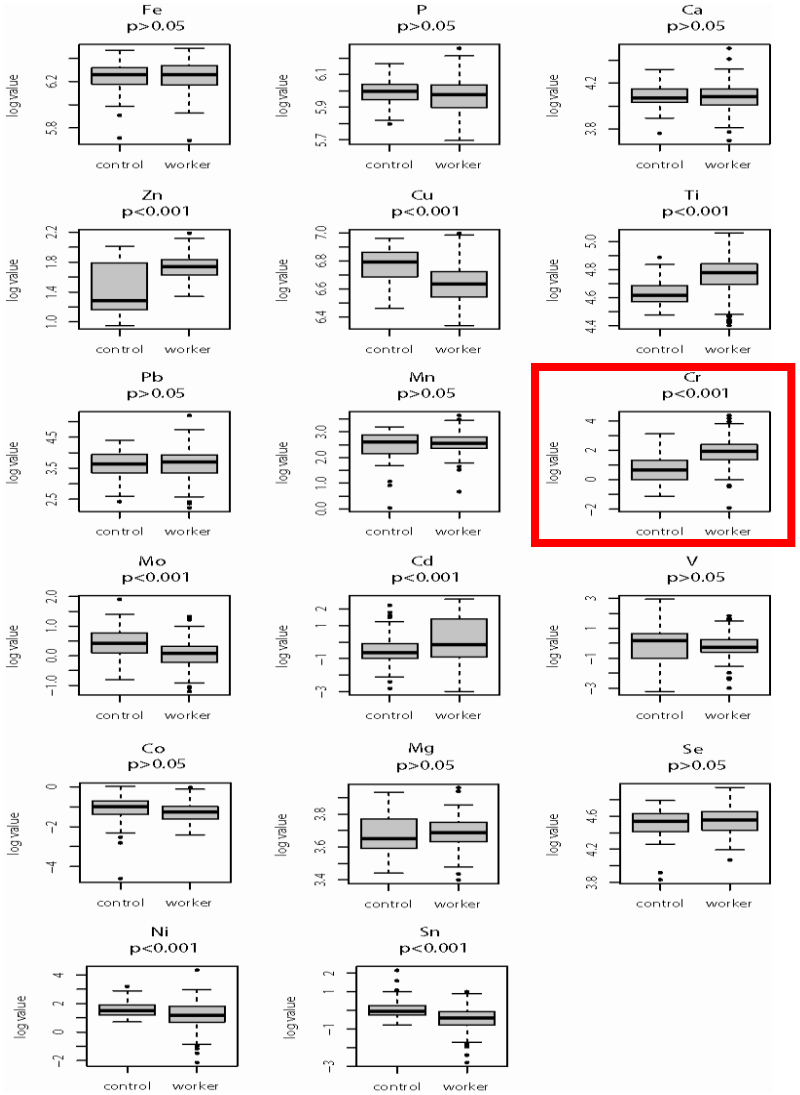




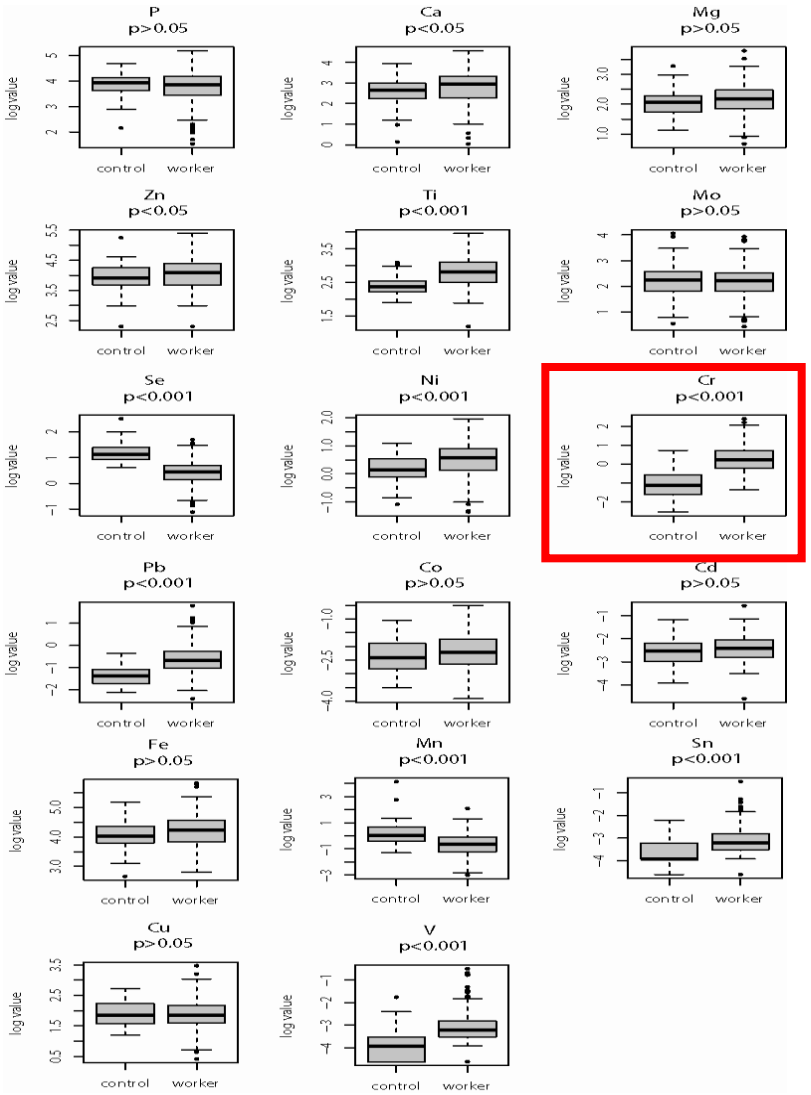
- The chromium concentrations in air under all working titles are below the standard limit of 50 mg/m^3, as recommended by the Government .recommended exposure limit

- The level of Cr in peripheral blood from the chromate-exposed group ((8.5±2.3) μg/L) was higher than control group ((4.4±1.4) μg/L). Significantly.

Multiple elements analysis

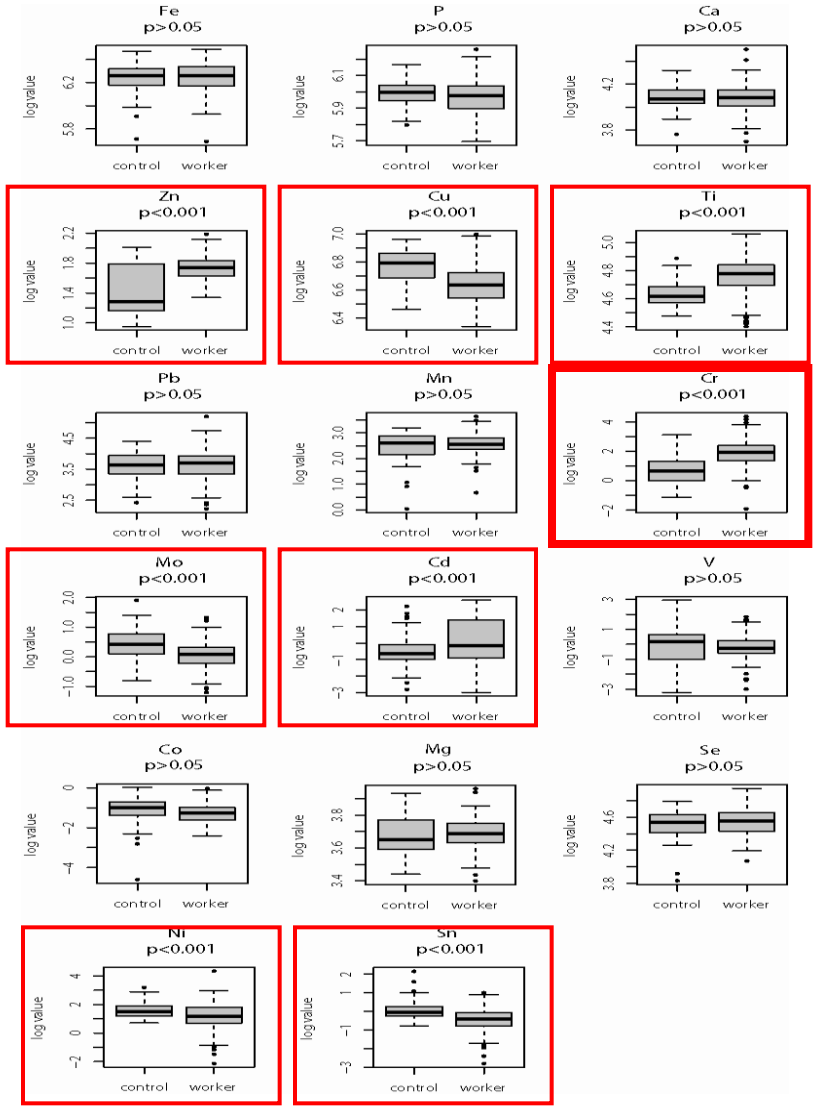


blood metals comparison between controls and exposed

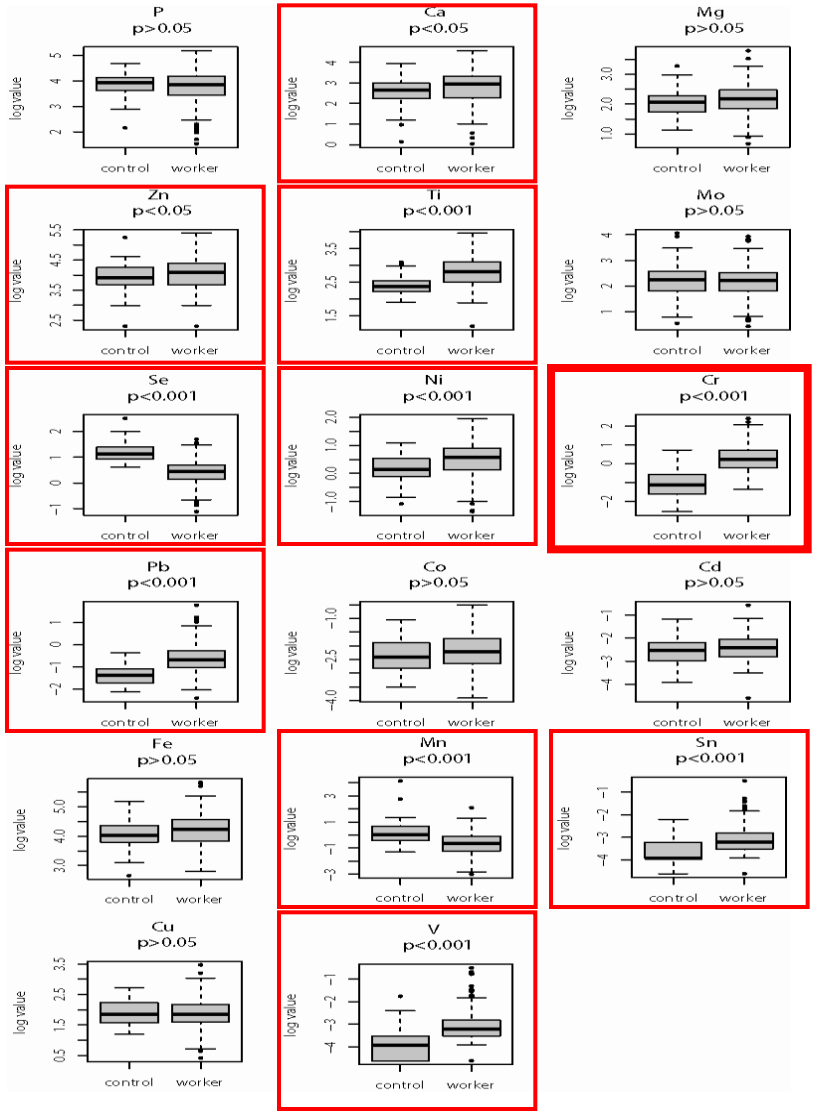


urine metals comparison between controls and exposed

Multiple elements analysis

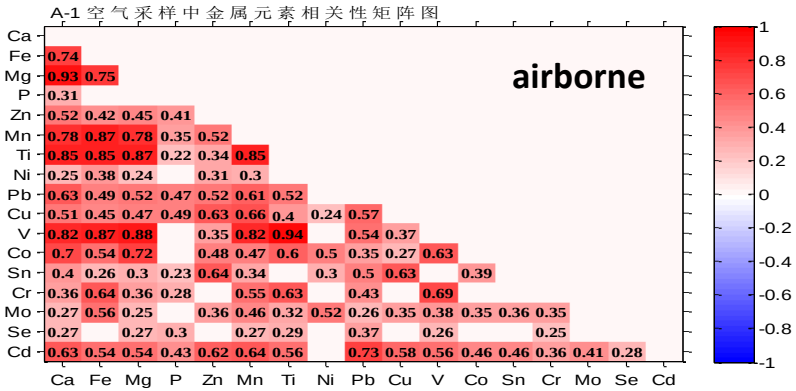


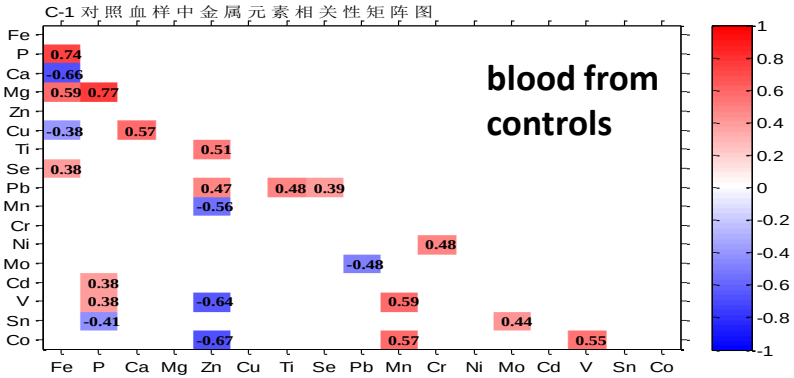
blood metals comparison between controls and exposed

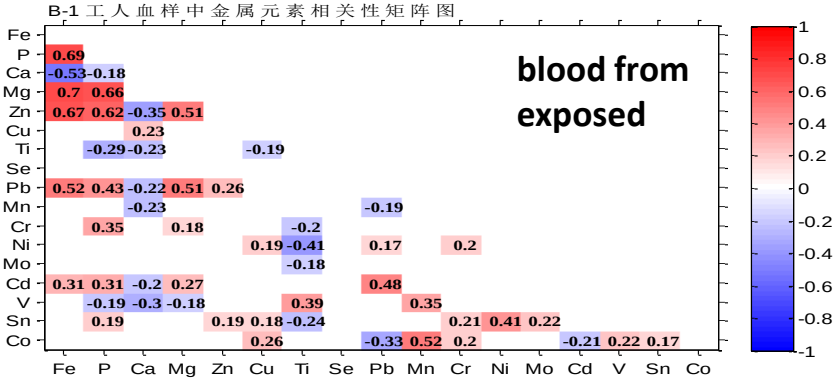


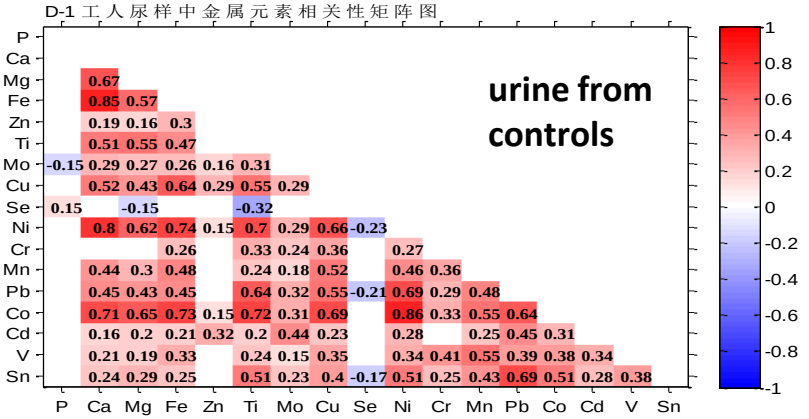
urine metals comparison between controls and exposed

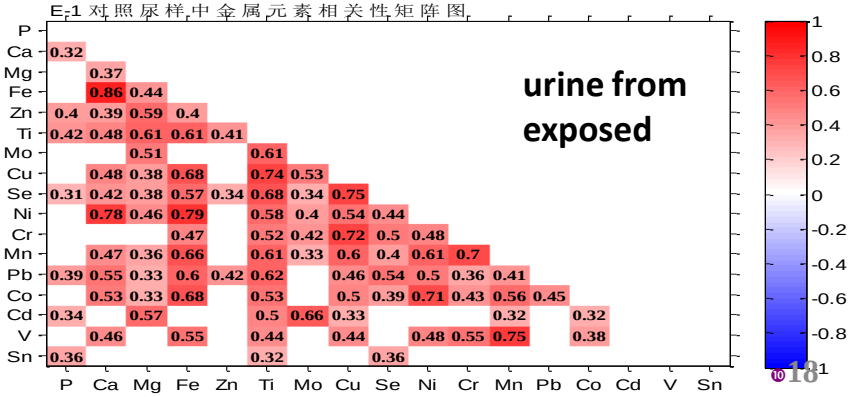
Multiple heavy elements co-exposure





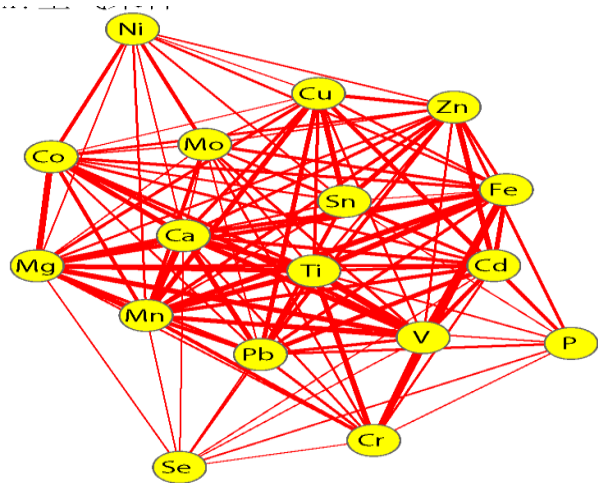




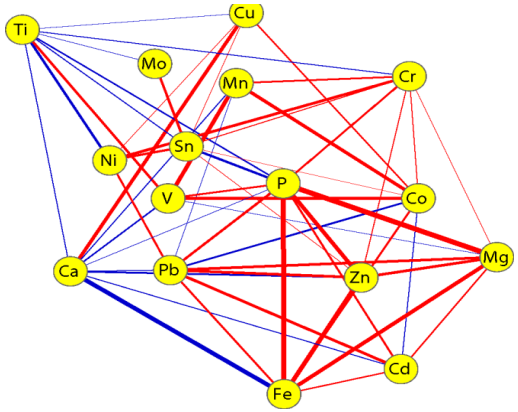


relationship among multiple elements

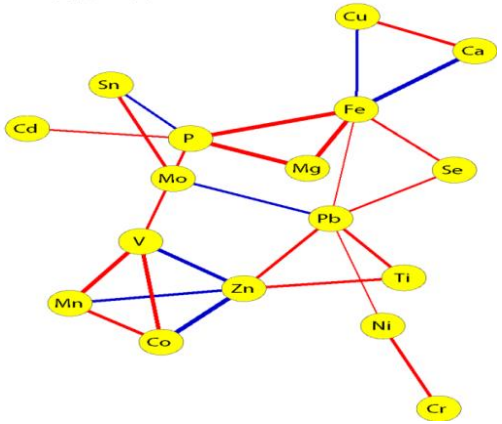
+Mn, Co, Mg and Zn
-Ti



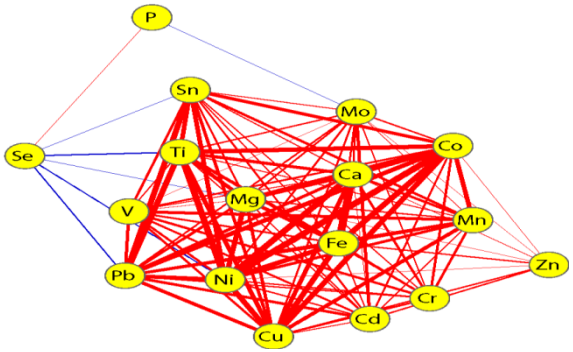
airborne



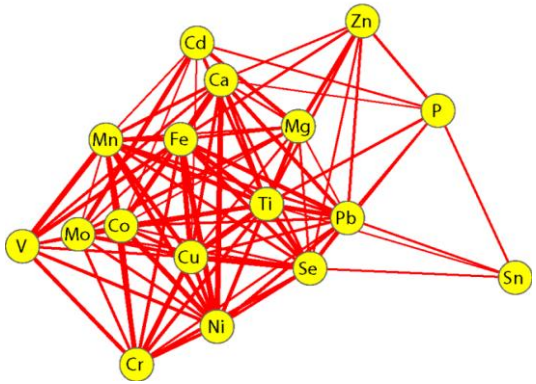
blood from exposed



blood from controls



urine from exposed



urine from controls

Animal model

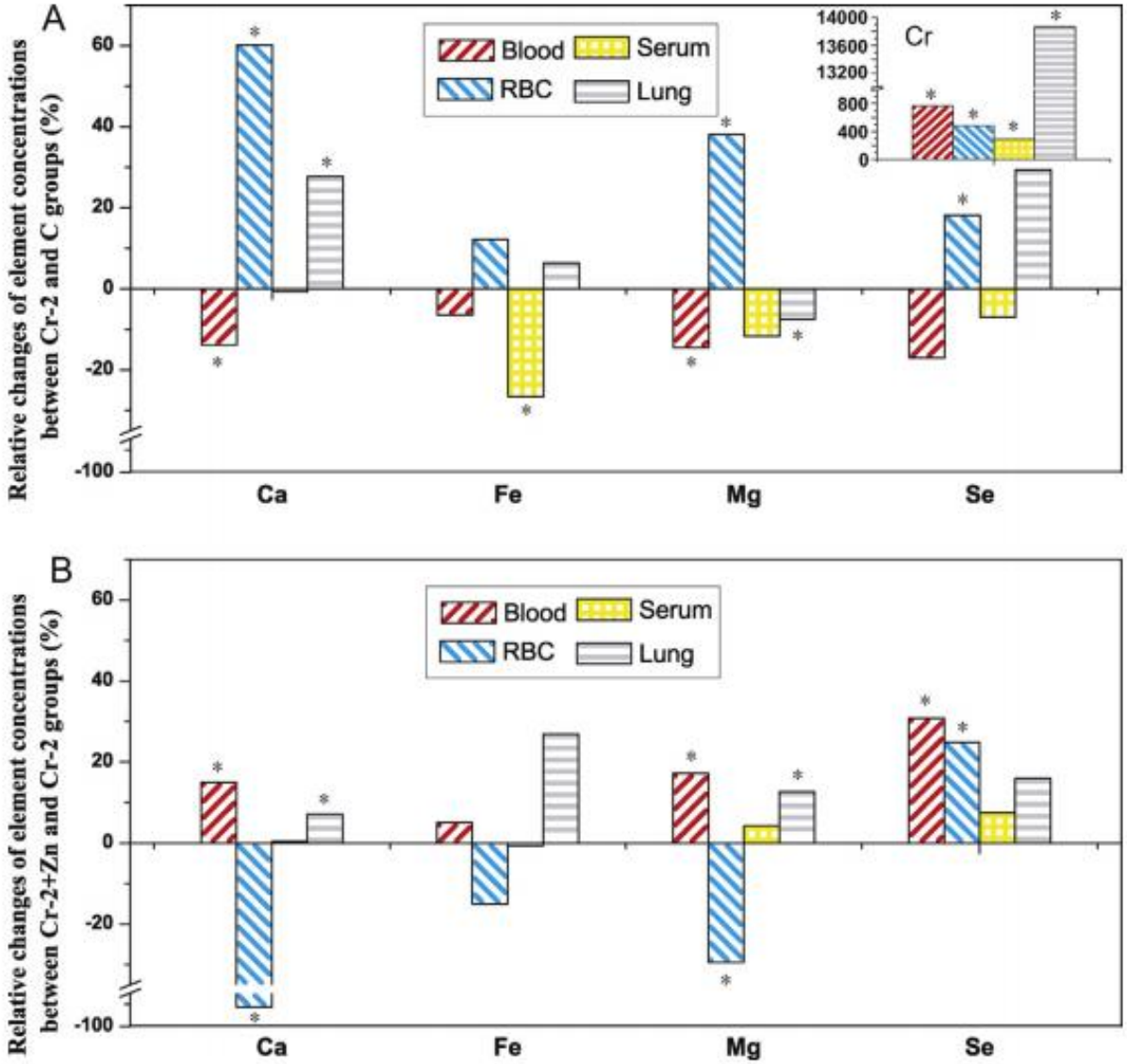


Fig. 2. Changes of element concentration in tissues and organs of Sprague-Dawley rats after high dose of Cr(VI) treatment (A) and Zn intervention (B). Abbreviation index, C, untreated control; Cr-2, high dose of Cr(VI) (0.630 mg Cr/kg); Cr-2 + Zn, high dose of Cr(VI) with Zn (10 mg Zn/kg). In (A), the relative change of Cr, Ca, Fe, Mg, and Se concentrations between Cr-2 group and C group was calculated by (mean of Cr-2 group − mean of C group)/mean of C group × 100%; In Fig. 2B, the relative change of Ca, Fe, Mg, and Se concentrations between Cr-2 + Zn group and Cr-2 group was calculated by: (mean of Cr-2 + Zn group − mean of Cr-2 group)/mean of Cr-2 group × 100%. Asterisks on boxes indicated $p < 0.05$.

This part tips

- ✓ **Multiple heavy metals co-exist in chromate manufacture process. We should pay attention when considering adverse effects caused by Cr.**

- ✓ **From the current data, Mn, Co, Mg and Zn show positive relationship with Cr. Ti shows negative relationship with Cr in Cr exposed group.**

- ✓ **Animal study is helpful in our understanding the possible human health effects.**

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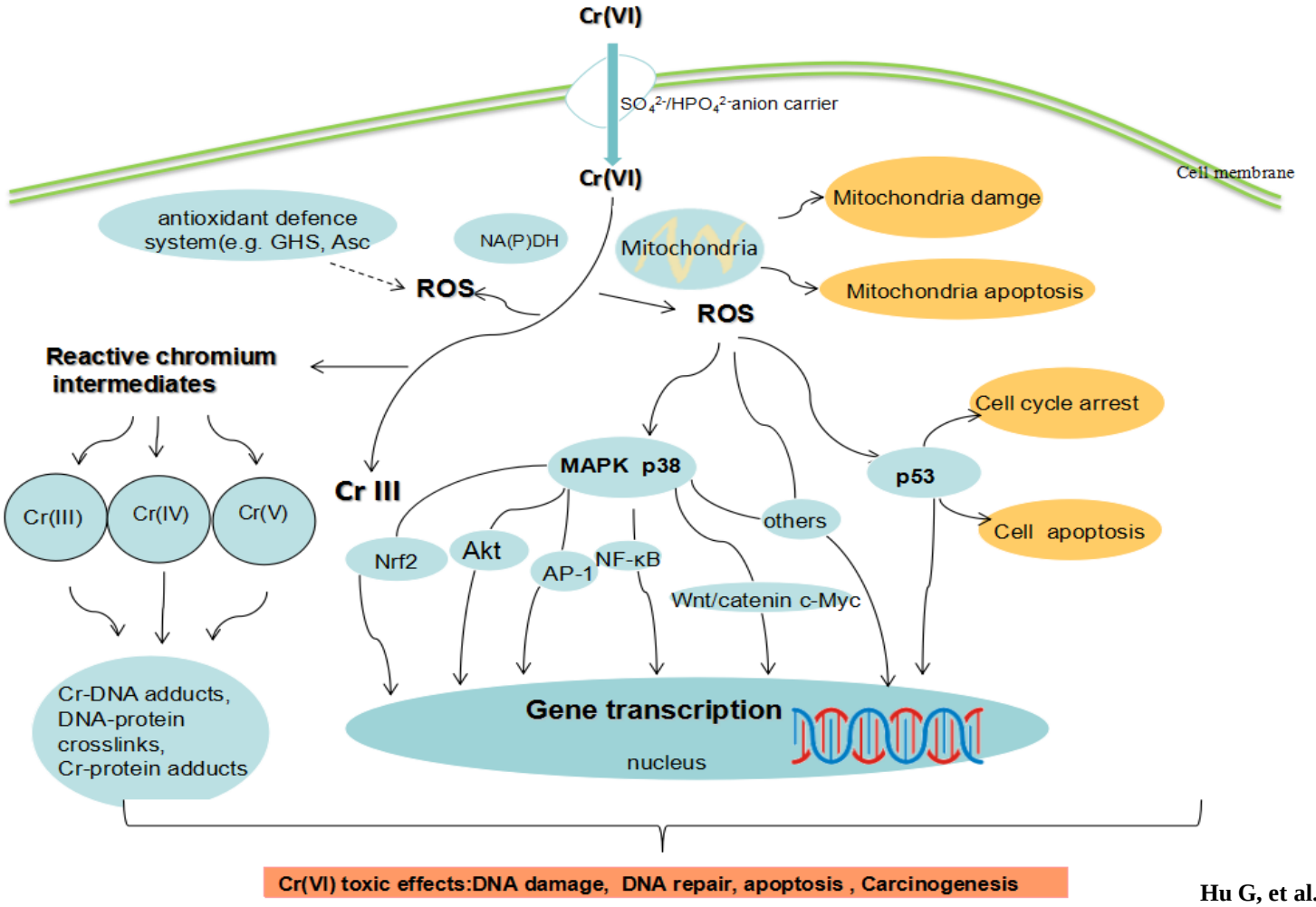
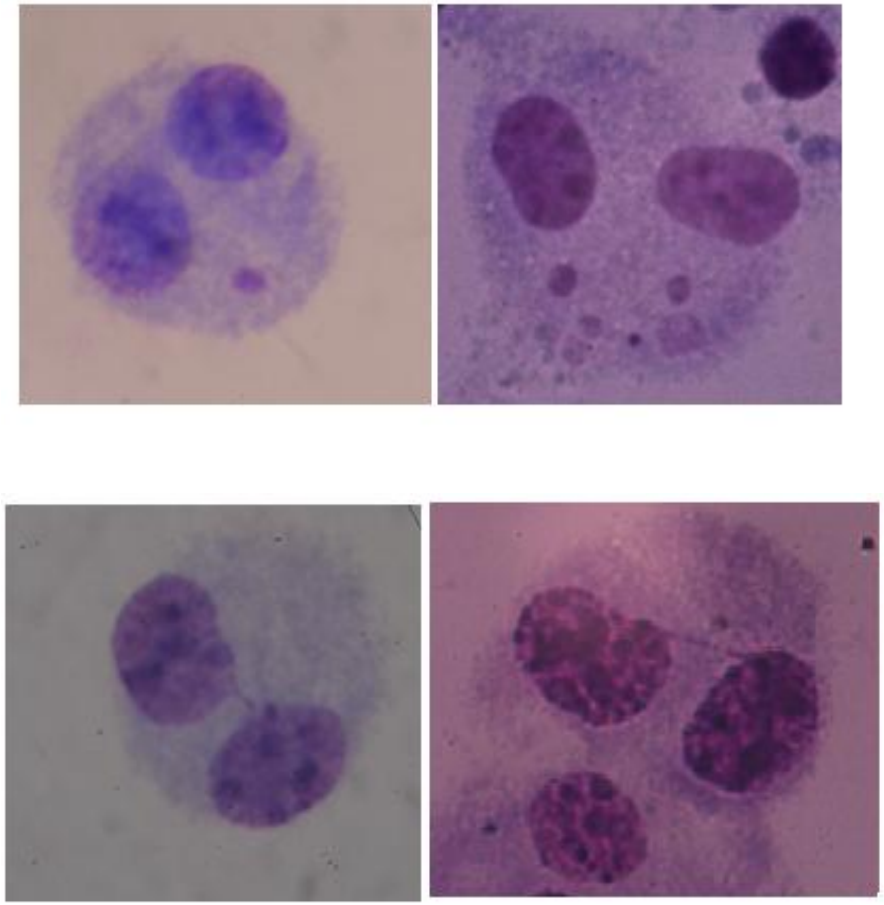
DNA damage induced by Cr(VI) exposure

- Cr(VI) closely resembles SO_4^{2-}/HPO_4^{2-} and can cross the cell membrane via an anion carrier.

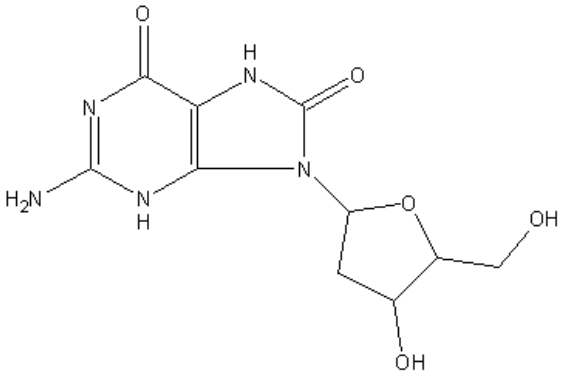


Hu G, et al. ROS 2017

genetic damage: cytokinesis-block micronucleus(CBMN) and 8-OhdG



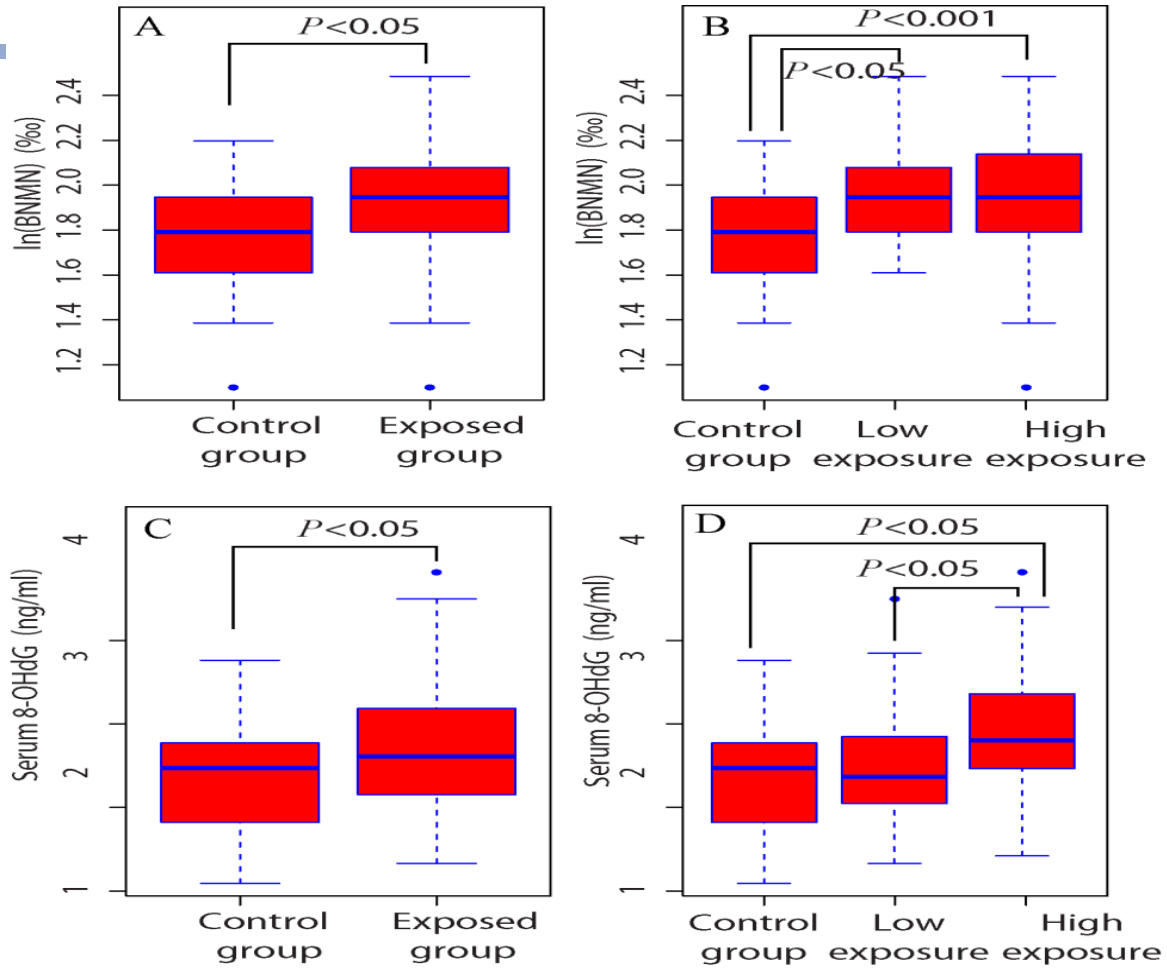
(Fenech's Protocol (2007)



micronucleus

8-OhdG

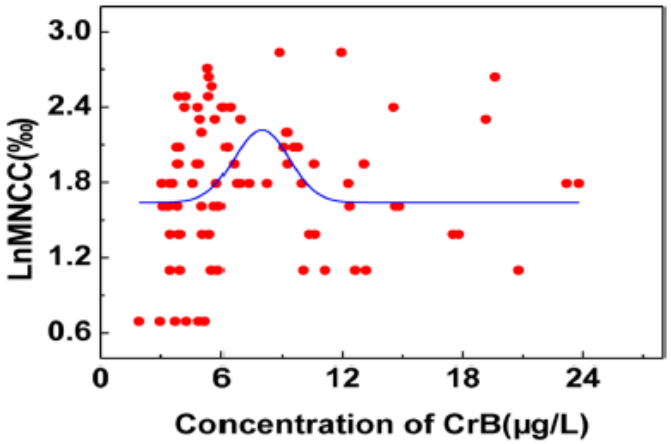
Genetic damage



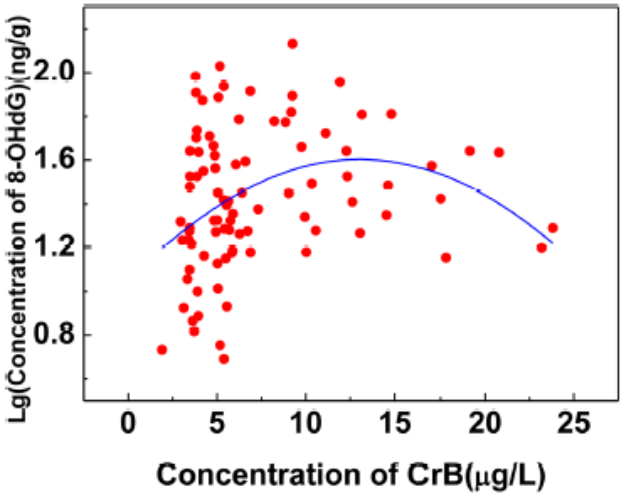
genetic damage among different groups

CBMN: control group (2.7±5.3)‰ ; exposure group (4.8±6.4)‰
8-OHdG: control (2.3±0.6) μg/L ; exposure group (2.8±1.3) μg/L

Correlation between genetic damage and CrB



correlation between LnCBMN and CrB

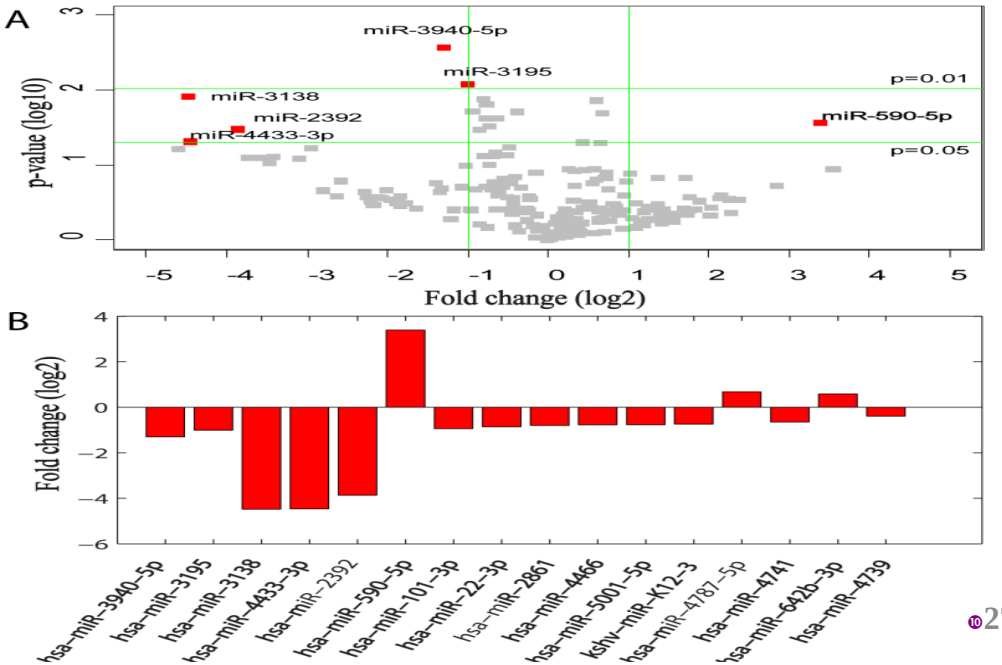


correlation between Ln8-OHdG and CrB

CBMN and 8-OHdG reflect the degree of genetic damage only when CrB levels are lower than 9.10 and 10.50 μg/L, respectively

epigenetic mechanism under genetic damage: different expressed miRNA

miRNA 名字	*P* 值*	Fold change (log2)	变化趋势
hsa-miR-3940-5p*	**0.0085**	**1.30**	下调
hsa-miR-3195*	**0.0027**	**1.01**	下调
hsa-miR-3138*	**0.0121**	**4.48**	下调
hsa-miR-4433-3p*	**0.0489**	**4.46**	下调
hsa-miR-2392*	**0.0335**	**3.86**	下调
hsa-miR-590-5p*	**0.0271**	**3.40**	上调
hsa-miR-101-3p	0.0203	0.94	下调
hsa-miR-22-3p	0.0138	0.86	下调
hsa-miR-2861	0.0195	0.80	下调
hsa-miR-4466	0.0237	0.77	下调
hsa-miR-5001-5p	0.0304	0.77	下调
kshv-miR-K12-3	0.0157	0.74	下调
hsa-miR-4787-5p	0.0240	0.68	上调
hsa-miR-4741	0.0133	0.65	下调
hsa-miR-642b-3p	0.0337	0.59	上调
hsa-miR-4739	0.0191	0.39	下调

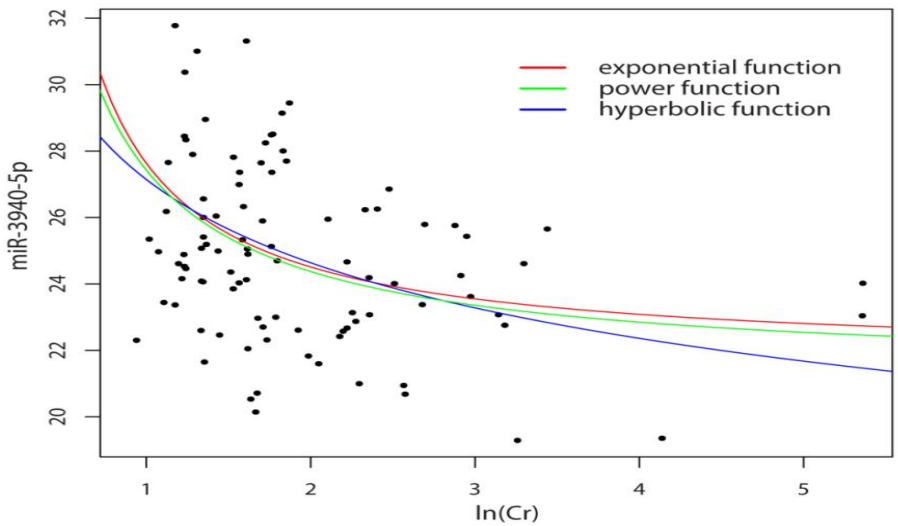


Validation of different expression of miRNA by real time PCR

miRNA	comparison of different expression			partial correlation coefficient with CrB		
	Exposure (n=87)	Control (n=30)	*P*	r (n=117)	95%CI	*p*
miR-3940-5p	24.21±2.10	25.59±2.23	0.015*	-0.33	(-0.50,-0.14)	0.001*
miR-3138	15.51±1.54	15.69±1.25	0.531	-0.04	(-0.23,0.15)	0.670
miR-2392	19.33±2.26	19.12±2.52	0.694	-0.09	(-0.28,0.10)	0.335
miR-590-5p	10.22±1.42	10.97±1.52	0.025*	-0.04	(-0.23,0.16)	0.711
miR-4433-3p	13.84±0.71	13.81±0.81	0.829	0.07	(-0.13,0.26)	0.512

variable	miR-3940-5p (n=117)			
	β	95%CI	*p*	β_{std}
CrB	-1.05	(-1.69,-0.40)	0.002*	-0.32
Work years	0.02	(-0.08,0.12)	0.680	0.04
Gender (male)	0.06	(-1.29,1.40)	0.932	0.01
age	0.02	(-0.05,0.09)	0.625	0.05
Smoking (yes)	1.42	(0.17,2.68)	0.027*	0.23
Drinking(yes)	-1.00	(-2.29,0.28)	0.123	-0.18
BMI	-0.19	(-0.37,-0.01)	0.056	-0.21

Relationship of miR-3940-5p expression level with blood Cr level



exposure-response relationship:

$y=26.3*\ln x^{-0.10}$

Function	expression	parameter
Exponential function	$Y=\alpha *e^{\beta/x}$	α =22.5*;β=0.17*
Power function	$Y=\alpha *x^{-\beta}$	α =26.3*;β=0.10*
Hyperbolic function	$Y=\alpha +\beta/x$	α =22.3*;β=4.35*

Estimated parameter $p<0.05$

Regression analysis of miR-3940-5p and genetic damage

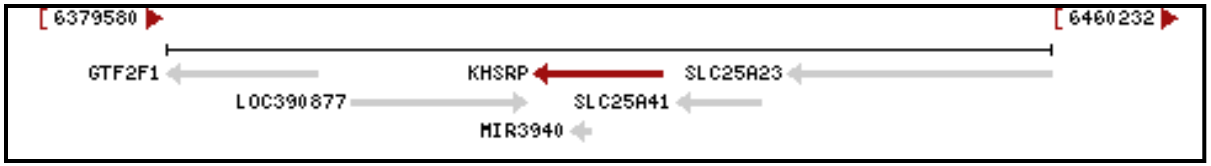
group	n	CBMN(‰)			Serum 8-OhdG		
		β_{std} [a]	95%CI	*P*	β_{std} [a]	95%CI	*P*
All subjects	117	-0.03	(-0.16,0.23)	0.761	-0.09	(-0.72,0.30)	0.411
Exposure with low & high Cr level	87	0.18	(0.08,0.37)	0.015*	-0.13	(-0.37,0.12)	0.312
Control & exposure with low	74	-0.01	(-0.26,0.26)	0.997	0.06	(-0.20,0.33)	0.633

[a] Poisson regression adjusted by working years, gender, age, smoking, drinking and BMI;

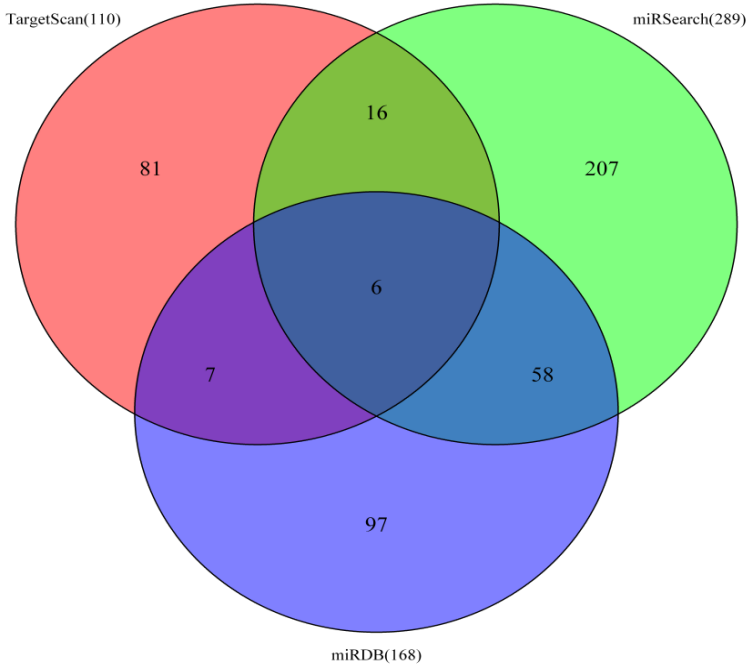
[b] Multiple linear regression adjusted by working years, gender, age, smoking, drinking and BMI;

Bio- information analysis for screened miR-3940-5p

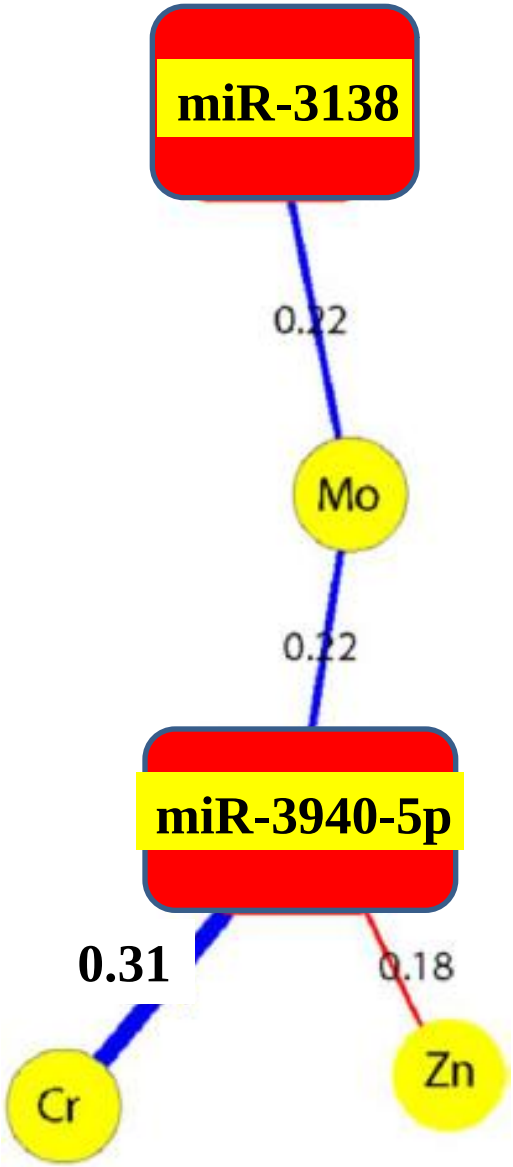
- **miR-3940-5p host gene: KHSRP**



- **miR-3940-5p targets genes: TargetScan(110), miRSearch(289), miRDB(168)**



Metal element associated with miR-3940-5p level

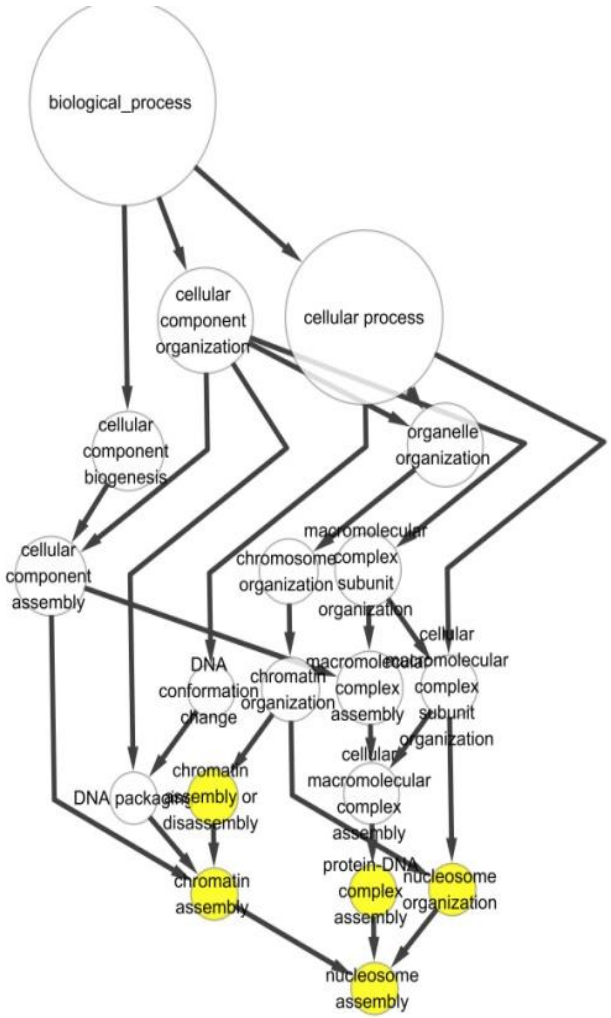


Molecular biological analysis shows that :

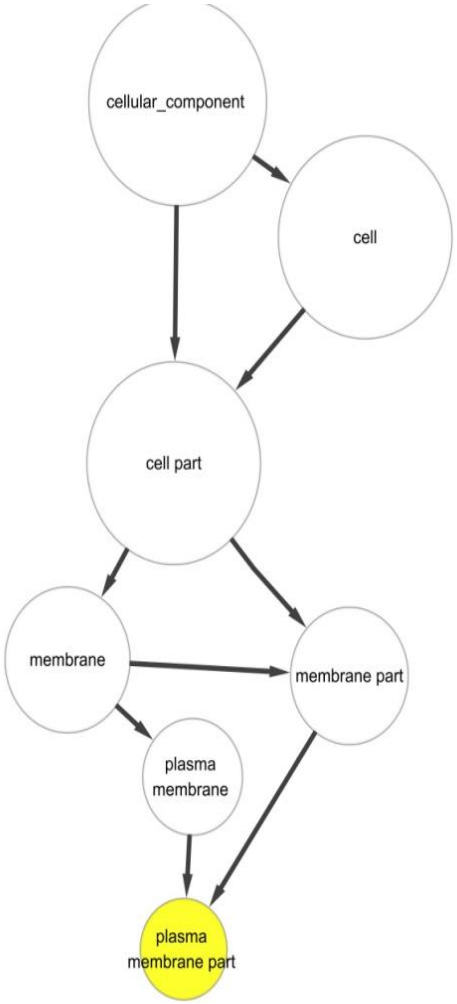
- ✓ **the gene could directly affect metal response element (MRE),**
- ✓ **indirectly regulate transcription factor binding sites (TFBS)**

miR-3940-5p targets genes: GO analysis

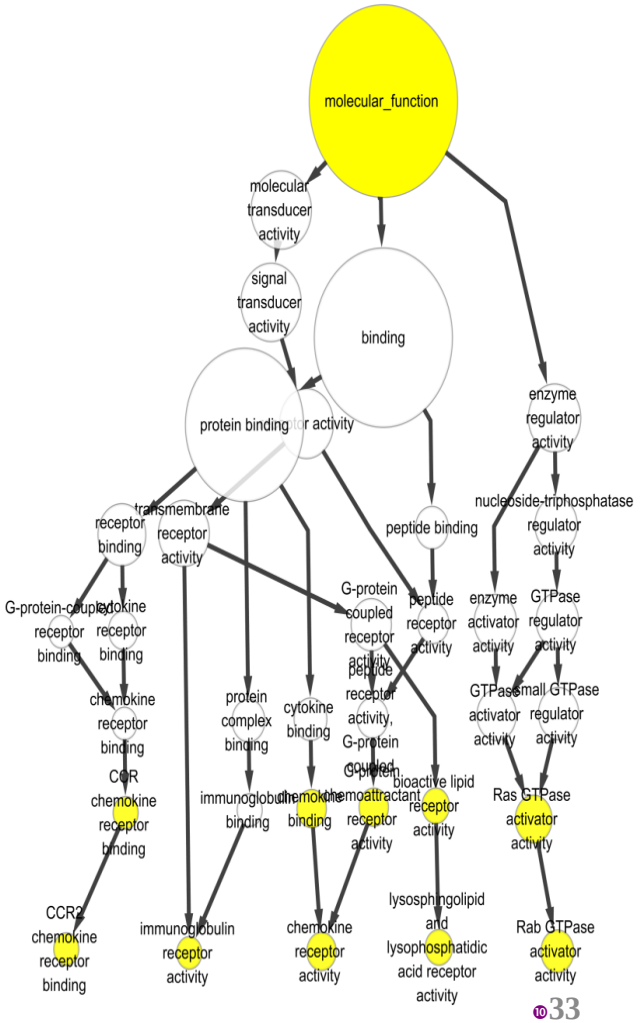
Biological process



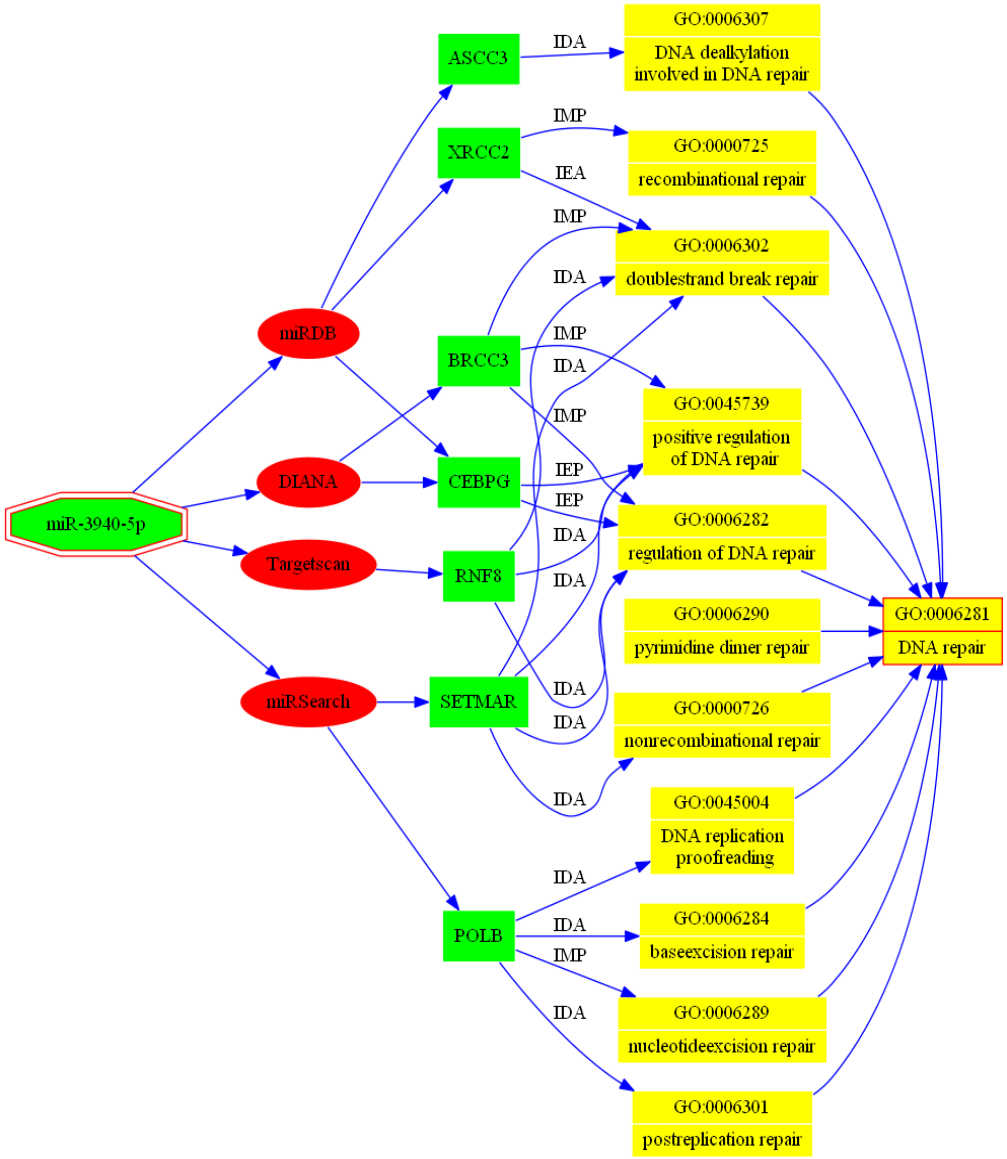
Cellular components



Molecular function



GO analysis: miR-3940-5p targeted genes related to genetic damage repair



POLB
ASCC3
BRCC3
XRCC2

Regression analysis in chromate exposed group

protein	miR-3940-5p (n=87)			CBMN (‰) (n=87)			Serum 8-OhdG (ng/ml) (n=87)		
	β_{std}[a]	95%CI	*P*	β_{std}[b]	95%CI	*P*	β_{std}[a]	95%CI	*P*
POLB	-0.06	(-0.28,0.16)	0.598	-0.04	(-0.23,0.16)	0.717	-0.13	(-0.36,0.11)	0.289
ASCC3	-0.01	(-0.23,0.22)	0.942	0.00	(-0.19,0.19)	0.988	-0.11	(-0.35,0.12)	0.339
BRCC3	-0.24	(-0.46,0.00)	0.047	0.11	(-0.10,0.30)	0.315	-0.01	(-0.26,0.23)	0.927
XRCC2	-0.31	(-0.58,-0.08)	0.010	-0.13	(-0.33,-0.01)	0.027	0.14	(-0.10,0.38)	0.249

a Multiple linear regression adjusted by working years, gender, age, smoking, drinking and BMI;
B Poisson regression adjusted by working years, gender, age, smoking, drinking and BMI;

second part tips

- **Cr(VI) exposure increases micronuclei frequency in a nonlinear relationship. Micronuclei frequency does not continue to increase under a certain high level of Cr(VI) exposure. Serum 8-OHdG seems to be less sensitive than micronuclei frequency.**

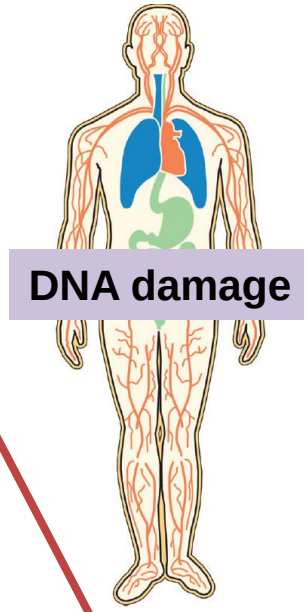
- **miRNA exert some roles in Cr(VI)-induced DNA damage and repair.**

Summary

New evidences for chromate hazard

Kidney damage

- NAG
- cystatin C
- microalbumin
- homocystein
- β-2-microglobulin
- BNMN Frequencies
- oxidative DNA damage
- prostate Specific Antigen
- Cr, Cd, Mg, Mo, Fe, Se, Zn



Epigenetic modification and regulation

- global DNA methylation
- Vitamin B-12
- folate
- Ca

Exposure assessment

- Environmental exposure
  - Air-born Cr
- Biological exposure
  - Whole blood Cr
  - Urine Cr

Thank you

NSFC grants support

My group and colleagues

Volunteers participated in this project