



## Heavy metal concentrations in hair of newly imported China-origin rhesus macaques (*Macaca mulatta*)

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Macaque monkeys are good sentinel to humans for environmental pollutions because their similarities in genetic and physiological characteristics. So, their reference values about exposures to heavy metals are required for proper data interpretation. Here, we report several heavy metals concentrations in the hair of rhesus monkeys which are widely used in biomedical research. The hair of 28 imported rhesus monkeys from an animal farm in southwest China were examined for the presence of eight heavy metals (Arsenic, Beryllium, Cadmium, Chromium, Iron, Lead, Mercury, and Selenium). The analyzed data in parts per million (ppm) for hair concentrations of heavy metals in rhesus monkeys were as follow: As ( $0.654 \pm 0.331$ ), Be ( $0.005 \pm 0.003$ ), Cd ( $0.034 \pm 0.022$ ), Cr ( $11.329 \pm 4.259$ ), Fe ( $87.106 \pm 30.114$ ), Pb ( $0.656 \pm 0.613$ ), Hg ( $0.916 \pm 0.619$ ), and Se ( $3.200 \pm 0.735$ ). The concentrations of Be, Cr, and As showed significant higher in females than in males ( $P < 0.05$ ). We present here the reference values of several heavy metals in healthy China-origin rhesus monkeys. These data may provide valuable information for veterinarians and investigators using rhesus monkeys in experimental studies.

**Key words:** Biomedical research, China-origin rhesus monkeys, heavy metals, hair

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Heavy metals play a key role in physiological functions and tissue structural elements in the body. Interestingly, small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity. Humans and animals may be exposed to various heavy metals through contaminated food, water, and house dust as well as through industrial activities that result in the generation of air and soil pollution and agricultural chemicals [1,2]. Although stringent measures and controls have been conducted

during recent decades, high levels of these pollutants still persist in the soil or sediments, and it makes chronic environmental exposure of the populations living in those areas via food chain [3].

Macaque monkeys share important immunological and physiological similarities with humans, particularly in the way in which they respond to toxic exposures [4-7]. This makes macaque monkeys potentially valuable as sentinels for toxic exposures and predictors of physiologic responses to chemicals in humans. Over the last three decades, many Asian countries have undergone rapid

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industrial development. Among them, China has experienced especially rapid economic growth which has resulted in high levels of heavy metal contamination in recent years [8, 9]. At present, many institutes receive an annual supply of captive nonhuman primates from China and Southeast Asia, and the number of institutes to do so has been increasing.

Therefore, we hypothesized that rhesus monkeys reared in China may have been exposed to various heavy metals through environmental pollution such as contaminated food, water, and soil. Many studies focusing on the health status of macaque monkeys in a specific Asian country, such as Japan, Nepal, and Singapore [10-12] have been reported. However, there was no report on the heavy metal exposure of Chinese rhesus monkeys. In this study, we measured the concentration of heavy metals from the hair and provided reference values for Chinese rhesus monkeys, a species commonly used in biomedical research.

## Materials and Methods

### Subjects

Twenty-eight rhesus monkeys, imported in 2006 from Guangxi Grandforest Scientific Primate Company, Dayiling Ping Nan County, China were used in this study. This colony consisted of 23 females weighting  $3.7 \pm 0.67$  kg, aged 4y 0 $\pm$ 6 m and 5 males weighting  $4.0 \pm 0.69$  kg, aged 4y 1 $\pm$ 4 m (Table 1). In China farm, these monkeys have been raised in the outdoor housing facility with shelter and were provided vegetables (e.g., sweet potato), biscuits, and bread which were made in their facility. After being imported from China, a quarantine process of 1 month was concluded with the subjects in good general condition. The monkeys were maintained in single-housed cages and had daily access to food (PS DIET<sup>®</sup>, Oriental Yeast Co. Ltd, JAPAN, fresh fruit, and vegetables) and unlimited access to water. Their room was maintained at  $24 \pm 4^\circ\text{C}$  and a relative humidity of  $50 \pm 10\%$ , with an artificial light-dark cycle of 12:12 (7:00 AM onset) and with 13-18 air changes per hour. All animals used in this study were cared for in strict accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals."

### Hair specimen collection and preparation

The monkeys were captured and chemically restrained with a 5 mg/kg dose of ketamine HCl prior to hair

**Table 1.** Information of experiment animals

Subjects	Sex	Number	Body weight (kg)	Age (yy/mm)
Rhesus	Male	5	$4.0 \pm 0.69$	4y 1m $\pm$ 4m
( <i>Macaca mulatta</i> )	Female	23	$3.7 \pm 0.67$	4y 0m $\pm$ 6m

specimen collection. Hair is a good biomonitoring tool for heavy metal assessment, because it integrates the accumulation and concentration of heavy metals over time [13]. So, hair specimens were taken, with the investigator using latex gloves and surgical scissors, by clipping hair as close to the skin as possible from the flank and femoral regions of each animal. Full-length hair specimens were collected from all animals sampled. Hair collection was conducted in May to June between annual molting periods. All hair specimens (1 g) were stored in sealed plastic bags until they were processed for analysis.

### Analysis of heavy metals

A Microwave Digestion System (MLS 1200 MEGA, Milestone Co., Italy) and an Inductively Coupled Plasma Mass Spectrometer (ELAN 6000, Perkin-Elmer Co., USA) were used in this study. Hair samples of 0.5 g were put in a vessel and mixed with 65% HNO<sub>3</sub> 8 mL and 30% H<sub>2</sub>O<sub>2</sub> 2 mL and digested by pressurization and heating for 20 minutes in the microwave digestion system. After cooling to room temperature, the samples were analyzed by ICPMS.

### Statistical analysis

Data are presented as mean $\pm$ SD. Differences between sexes were calculated using the Student T-test. Any difference with  $P < 0.05$  was considered to be statistically significant.

## Results

The mean values and standard errors for heavy metal concentrations of Arsenic (As), Beryllium (Be), Cadmium (Cd), Chromium (Cr), Iron (Fe), Lead (Pb), Mercury (Hg), and Selenium (Se) in the hair of monkeys are presented in Table 2. The mean values obtained in ppm were as follows: As (0.654), Be (0.005), Cd (0.034), Cr (11.349), Fe (87.106), Pb (0.656), Hg (0.916), and Se (3.200). There were statistically significant differences in Be, Cr, and As concentrations between males and females. Be concentrations were significantly higher in

**Table 2.** Gender comparison of heavy metal concentrations in hair of Chinese rhesus monkeys (ppm)

Elements	Female (n=23)	Male (n=5)	Total (n=28)
Arsenic (As)	0.690±0.353*	0.488±0.113	0.654±0.331
Beryllium (Be)	0.006±0.003**	0.003±0.001	0.005±0.003
Cadmium (Cd)	0.032±0.020	0.040±0.033	0.034±0.022
Chromium (Cr)	11.888±4.460*	8.757±1.729	11.329±4.259
Iron (Fe)	90.325±31.077	72.300±21.816	87.106±30.114
Lead (Pb)	0.659±0.663	0.644±0.341	0.656±0.613
Mercury (Hg)	0.943±0.671	0.793±0.291	0.916±0.619
Selenium (Se)	3.194±0.770	3.229±0.623	3.200±0.735

\* $P < 0.05$ ; \*\* $P < 0.01$  (Mean±SD)

females (0.006±0.003 ppm) compared to males (0.003±0.001 ppm) ( $P < 0.01$ ). Cr concentrations in hair were also significantly higher in females (11.888±4.460 ppm) compared to males (8.757±1.729 ppm) ( $P < 0.05$ ). As concentrations in hair were also significantly higher in females (0.690±0.353 ppm) compared to males (0.488±0.113 ppm) ( $P < 0.05$ ).

## Discussion

Our monkeys were imported from a breeding center that includes outdoor housing facilities in southwest China. Thus, it was suspected that they have sufficient opportunities for natural exposure to air, soil, and water pollution. In this study, we measured heavy metal concentrations in the hair of newly imported rhesus monkeys from southwest China.

Mercury and lead are potentially significant environmental toxicants. Mercury has gained worldwide attention due to its high toxicity, bio-accumulation, and difficulty to control, because it is readily released into the atmosphere from both natural and anthropogenic sources [14-18]. Exposure to methylmercury, a toxic form usually absorbed by ingestion, is absorbed more readily and excreted more slowly than other forms of mercury. More than half of the world's emissions of mercury occur in Asia [19]. Especially, China has been regarded as one of the largest anthropogenic sources of heavy metals such as mercury [20]. In recent years, more attention has been given to Hg emissions in southwest China, which is known as the main habitat of most macaques [20]. Mercury concentration (ranging from 0.123 to 2.188 ppm) in the hair of our monkeys was very higher than that of free-ranging rhesus monkeys in Nepal (ranging from 0.043 to 0.594 ppm) [10]. The high levels of Hg detected in our study suggest that the rhesus

monkeys were exposed to contaminated atmosphere around the farm or contaminated vegetables and water. Generally, Hg has been shown to damage the immune and nervous systems, and cause birth defects [21] but our monkeys have not shown typical clinical signs related with Hg.

Common sources of lead include dust containing paint chips or lead released into the atmosphere from industrial or automotive emissions such as leaded gasoline. Acute exposure to lead is known to damage the nervous, renal, circulatory, hepatic, and reproductive systems. These consequences are well recognized and often clinically obvious in humans and animals with acute exposure [22]. However, the effects of lower levels of Pb exposure are more subtle, but may be significant. According to recent reports from Nepal and Singapore [10,12], the lead concentration in the hair of cynomolgus and rhesus monkeys were 2.51 and 6.0 ppm, respectively. These levels are remarkable higher than that of our Chinese rhesus monkeys. In the other words, it may represent that the Chinese rhesus monkeys was less exposed to the lead.

In our results, the heavy metal concentrations detected in the hair of Chinese rhesus monkeys were not enough levels which affect the monkeys clinically. Although, we just report some heavy metal concentration in these monkeys, further studies about continuous monitoring, adverse effects, and comparison between blood and hair on heavy metal concentration are needed.

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