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Comparison of the Uptake and Distribution of Chromate in Rats and Mice

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ABSTRACT

The purpose of this study was to evaluate species differences in tissue accumulation of chromium. Rats and mice were orally exposed to Cr(VI) (potassium chromate) via drinking water (8 mg/d/kg body wt for 4 or 8 wk), or by ip injection (0.3 and 0.8 mg/d/kg for 4 or 14 d). Chromium concentrations were measured by atomic absorption spectrophotometry, and tissues were compared for exposure route and species differences. After oral exposure, irrespective of treatment duration, liver concentrations of chromium were three to four times higher in mice than rats, whereas kidney concentrations were about 50% lower. However, after ip injection, kidney and blood concentrations in rats were two- and four-fold higher, respectively. Both rats and mice showed high values of Cr concentration in the bone. After single ip injection of $Na_2{}^{51}CrO_4$, Cr concentrations were higher in the blood of rats than mice both after 24 and 72 h. Red blood cell concentrations of Cr were also greater in rats than mice by approximately threefold, whereas white blood cell Cr concentrations were higher in mice than rats. There was also a twofold greater binding of Cr/μmol of hemoglobin in rats compared to mice. These data indicate that species differences exist for Cr metabolism and that they differ with respect to the route of exposure. These results may be owing to species differences in the reduction of Cr and different binding of Cr to hemoglobin.

Index Entries: Cr distribution; species differences; toxicokinetics.

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INTRODUCTION

Mounting experimental evidence attests to the complicated biological effects of chromium compounds (*1,2*). The multiple-oxidation states of chromium as well as its complicated chemistry are at the root of this complexity. Hexavalent chromium (Cr) exists as an oxianion at physiological pH, and this form is actively transported into cells by the anionic transport system (*3*). In contrast, the other most stable form of Cr is trivalent and enters cells at approx 1000th the rate of the hexavalent form (*4*).

The trivalent form of Cr is also very inert to ligand substitution reaction, whereas hexavalent chromate is readily reduced by many intracellular reductants, such as microsomal enzymes, glutathione, and ascorbic acid (*5*), to intermediate oxidation states of Cr(V), Cr(IV), and finally to the stable trivalent form (*6,7*).

In studying the toxicity of chromate and in attempting to develop biomarkers for Cr exposure, animal models must be utilized. However, in view of the complexity of its chemistry, one would expect substantial species differences in chromate uptake and distribution. Which animal would best model the uptake and distribution of chromate in humans? A possible species difference between the rat and human that could cause a significant difference in chromate distribution involves the structure of the rat hemoglobin. Native rat hemoglobins were found to bind simetryn sulfoxide to an extent 40-fold greater than human hemoglobin (*8*). The β-chain cysteine-125 residue of the rat hemoglobin is 40 times more reactive than that of the human or the mouse for sulfur-seeking ligands, such as chromium. Since both rats and mice are commonly used in toxicological studies, these species were employed to study and compare the uptake and distribution of chromate to determine whether the binding of Cr to hemoglobin or other unknown factors produces significant species differences in Cr distribution. The results show that the differences in chromium distribution between rats and mice are remarkably small considering the differences in reactivity of hemoglobin.

MATERIALS AND METHODS

Animals

Male Fisher 344 rats were obtained from Charles River Breeding Laboratories (Kingston, NY) and arrived at the facility at 6 wk of age. Male mice (C57BI/6J) of the same age as rats were obtained from Jackson Laboratories (Bar Harbor, ME). Both rats and mice were quarantined for 2 wk, and observed for anomalous behavior and disease. Animals were then culled by weight, and randomly assigned to exposed and control groups. Rats were housed singly in wire-mesh cages. Mice were housed in plastic cages in groups of 5/cage.

Table 1
Experimental Scheme—Oral and Parenteral Exposure to K_2CrO_4 in Mice and Rats

Exposure			Dose	
Route	Species	Time	ppm Cr	mg/kg/d
Oral		4 wk	40	
	Mice			8 mg
		4 wk	130	
	Rats			8 mg
		8 wk	130	
IP	Mice	4 d		0.3 mg
	Rats	4 d		0.3 mg
	Mice	14 d		0.8 mg
	Rats	14 d		0.8 mg
IP		24 h	single ip	0.38 μg Cr/kg
	Mice, rats		dose of	
		72 h	$Na_2{}^{51}CrO_4$	1.07 μg Cr/kg

Animal Exposures

Animals were exposed to chromium in chronic and acute studies according to the experimental scheme shown in Table 1.

Chronic

Rats and mice were exposed to chromate (VI) as K_2CrO_4 (Baker Chemical Co., Phillipsburg, NJ) orally or by ip injection according to the experimental scheme shown in Table 1. For oral exposure, chromium was dissolved in distilled water and placed in standard rat water bottles. Control rats received distilled water. Intraperitoneal injections were administered using freshly prepared potassium chromate solution made in physiologic saline. Control animals received physiologic saline.

Acute

Rats and mice received a single ip dose of $Na_2{}^{51}CrO_4$ (154 μCi/kg; 0.38 μg Cr/kg for 24-h exposure and 430 μCi/kg; 1.07 μg Cr/kg for 72-h exposure). Animals were anesthetized using pentobarbital, and blood was drawn by cardiac puncture at time intervals of either 24 or 72 h following exposure. Blood was collected using 3.8% sodium citrate as an anticoagulant and was centrifugated at 1500 rpm for 10 min in order to separate plasma. Ammonium chloride (0.83%, pH 6.8–7.2) was added to the pellet to lyse red blood cells, and the samples were left at room temperature for 15 min. After red blood cell lysis, white blood cells were separated and washed three times with 0.83% ammonium chloride. Determination of ^{51}Cr count in blood, plasma, lysed red blood cells, and white blood cells was by γ scintillation counting (Pharmacia LKB Nuclear, Gaithersburg, MD). RBC count before lysis and WBC count after

separation were determined using a Coulter counter. Hemoglobin concentration was determined by alkaline hematin method (*9*) using a Beckman DU 50 spectrophotometer at a wavelength of 575 nm.

Chromium Analysis

Total chromium concentrations were measured in liver, kidney, spleen, femur, lung, heart, muscle, and blood by atomic absorption spectrophotometry after acid digestion following the procedure (*25*). Samples of tissue (up to 1.5 g) were digested with concentrated HNO_3 (instranalyzed, J. T. Baker, Inc., Phillipsburg, NJ) with small additions of 30% H_2O_2 in a Multi Blok heater (Baxter Scientific Products, Edison, NJ) using a maximum temperature of 140°C. After evaporation to near dryness, the residue was dissolved in 2% HNO_3, and the concentration of total chromium in the samples was measured with an atomic absorption spectrophotometer (Thermo Jarrell Ash Corporation, Franklin, MA) using a nitrous oxide/acetylene flame, a wavelength of 357.9 nm, and deuterium background correction.

Statistical Analysis

Chromium concentrations in various tissues, RBC, and WBC were compared in mice and rats using the *t*-test.

RESULTS

After ip injection, significant differences were observed in blood Cr concentrations of rats vs mice after short- (4-d) and long- (2-wk) term exposure (Fig. 1). Following 4 d of dosing at 0.3 mg/kg/d, blood Cr concentrations in rats were 8.3-fold higher than in mice receiving the same dose of Cr. After 2 wk of exposure to 0.8 mg/kg/d, concentrations were also higher in rats by approximately threefold.

These results were confirmed by studies using radiolabeled Cr. As shown in Figs. 2 and 3, Cr concentrations were higher in the blood of rats vs mice both 24 and 72 h after a single ip injection of ^{51}Cr. Red blood cell concentrations of Cr were also greater in rats than mice by approximately threefold. White blood cell Cr concentrations, however, were higher in mice than rats after both 24 and 72 h, suggesting that rapid accumulation of Cr by red blood cells in the rat may decrease the availability of Cr to other cells.

The amount of Cr bound per molecule of hemoglobin in rats vs mice was determined by measuring the hemoglobin content of red blood cell preparations. There was a twofold greater binding of Cr/μmol of hemoglobin in rats compared to mice after a single ip injection of 1.07 μg Cr/kg body wt (Fig. 4).

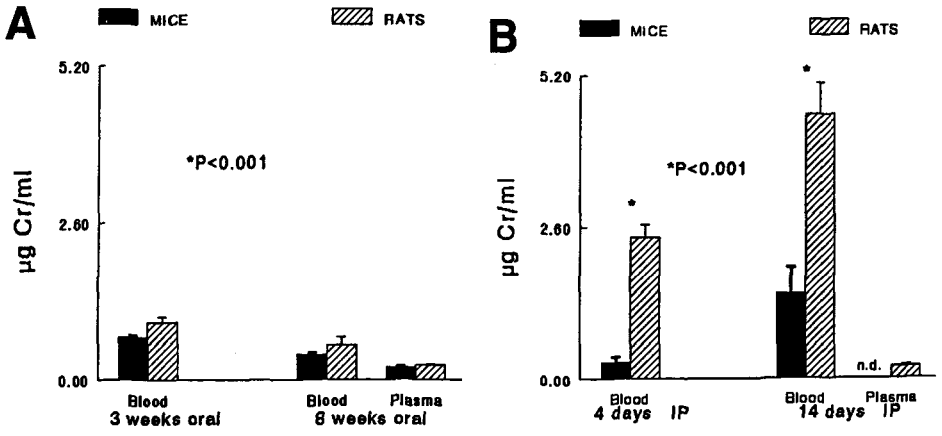

Fig. 1. Chromium concentration in the blood and plasma of mice and rats exposed to K_2CrO_4 A: orally (3 or 8 wk—8 mg/kg/d) or B: ip for 4 (0.3 mg/kg/d) or 14 d (0.8 mg/kg/d). Bars are the mean ± SE. *Values significantly different ($P < 0.001$) between mice and rats.

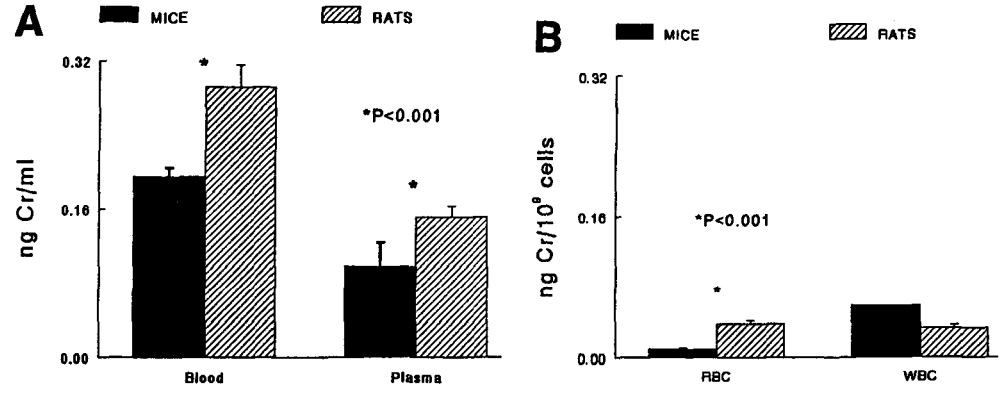

Fig. 2. Chromium concentration in the A: blood and plasma, and B: RBC and WBC of mice and rats 24 h after a single ip injection of 0.35 mg/kg $Na_2{}^{51}CrO_4$. Bars are the mean ± SE. *Values significantly different between mice and rats.

Species differences in tissue Cr accumulation were further examined to determine whether duration and route of exposure to Cr alter tissue uptake and accumulation of the metal. Organ concentrations of Cr in rats vs mice following chronic oral or ip exposure to potassium chromate are shown in Tables 2 and 3, respectively. Under both exposure regimens, Cr was concentrated primarily in the blood, liver, kidney, spleen, and bone (femur), and concentrations of Cr in these organs increased with time of

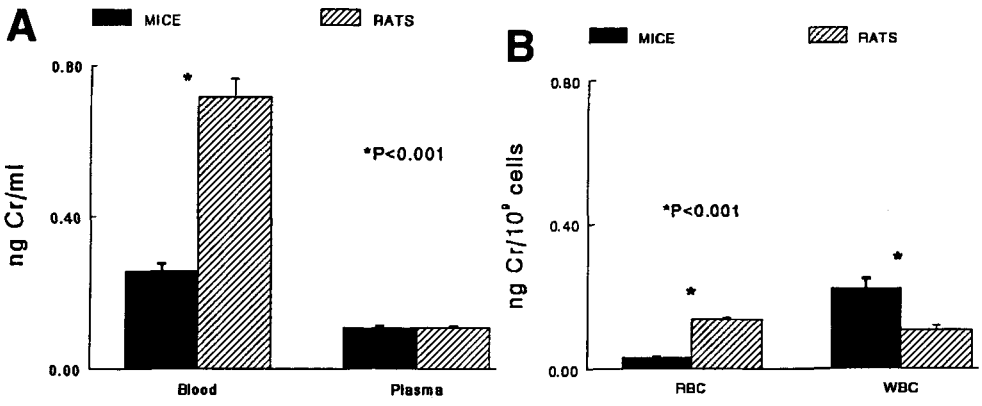

Fig. 3. Chromium concentration in A: blood and plasma, and B: RBC and WBC of mice and rats 72 h after single ip injection of 1.07 mg/kg $Na_2{}^{5}{}^{1}CrO_4$. Bars are the mean ± SE. *Values significantly different between mice and rats.

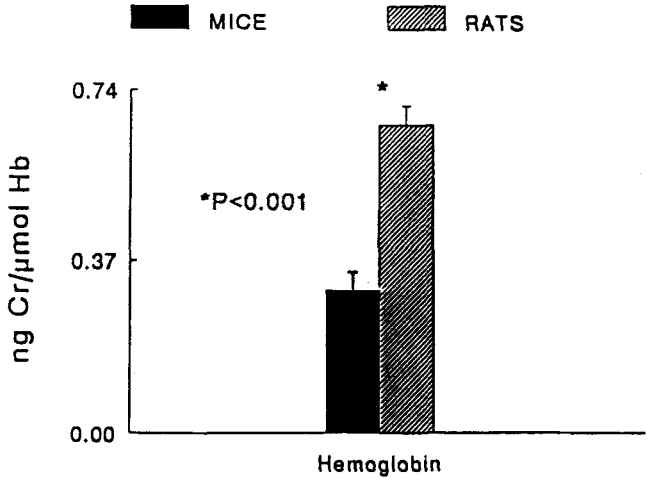

Fig. 4. Chromium binding in hemoglobin of mice and rats 72 h after a single ip injection of 1.07 mg/kg $Na_2{}^{51}CrO_4$. Bars are the mean ± SE. *Values significantly different between mice and rats.

exposure. Lung, heart, and muscle tissue concentrations were elevated above control values, but did not increase with time, averaging approx 0.5–1.0 ppm Cr throughout the exposure periods. Liver concentrations in mice reached as high as 13.8 ppm Cr after 8 wk of exposure to 40 ppm Cr, and in rats, kidney concentrations were highest after 8 wk at approx 9.5 ppm Cr. Species differences in the tissue accumulation of Cr after ip and oral exposure are highlighted in Figs. 4–6. Contrary to results obtained from the ip injection studies, there was no difference in blood Cr concen-

Table 2
Chromium Retention (μg Cr/g Wet Tissue; For Blood μg Cr/mL) after Oral Exposure to Potassium Chromate in Mice and Rats (8 mg/kg Body Wt/D)

	Control		4-Wk Exposure			8-Wk Exposure		
Mice								
Liver	50[c]	0.22 ± 0.14[a]	15[c]	10.92 ± 5.48		24[c]	13.83 ± 6.06	
Kidney	16	0.24 0.14	15	3.77 0.99		13	4.72 0.68	
Spleen	14	0.53 0.38	4	5.04 1.45		5	10.09 2.50	
Femur	9	0.90 0.48	4	7.43 1.03		5	12.55 2.99	
Lung	14	0.24 0.12	4	0.99 0.10		4	1.08 0.26	
Heart	10	0.32 0.15	4	0.80 0.23		5	1.02 0.20	
Muscle	13	0.32 0.23	4	1.12 0.37		5	0.60 0.25	
Blood	7	0.14 0.05	4	0.71 0.07		1	0.42 0.04	
Rats								
Liver	10[c]	0.19 ± 0.14	7[c]	3.32 ± 0.93	3.3[b]	8[c]	3.59 ± 0.73	3.9[b]
Kidney	11	0.34 0.20	7	8.62 2.40	0.4	8	9.49 4.38	0.5
Spleen	11	0.43 0.20	7	3.65 1.87	1.4	8	4.38 0.84	2.3
Femur	15	1.00 0.46	7	1.85 0.46	4.0	7	1.78 0.99	7.1
Lung	11	0.39 0.43	6	1.10 0.38	0.9	7	0.67 0.24	1.6
Heart	12	0.38 0.22	5	0.52 0.12	1.5	8	1.05 0.19	1.0
Muscle	13	0.24 0.14	4	0.19 0.10	5.9	7	0.17 0.10	3.5
Blood	9	0.19 0.17	7	0.73 0.15	1.0	7	0.58 0.13	0.7

[a]Arithmetic mean ± SD.
[b]Ratio mice/rats.
[c]Number of samples.

Table 3
Chromium Concentration (μg Cr/g Wet Tissue; For Blood μg Cr/mL) in Mice and Rats after Intraperitoneal Injection (4 D–0.3 mg/kg/D; 14 D–0.8 mg/kg/D) of Potassium Chromate

	Control		Ip 4 d			Ip 14 d		
Mice								
Liver	50[c]	0.22 ± 0.14[a]	11[c]	1.72 ± 0.54		8[c]	8.89 ± 1.24	
Kidney	16	0.24 0.14	10	1.59 0.28		10	11.77 2.37	
Spleen	14	0.53 0.38	4	1.78 0.76		4	6.92 2.11	
Femur	9	0.90 0.48	2	3.69 0.66		3	6.30 1.64	
Lung	14	0.24 0.12	5	0.61 0.35		4	2.89 0.72	
Heart	10	0.32 0.15	4	1.07 0.75		4	1.75 0.16	
Muscle	13	0.32 0.23	3	1.02 0.27		4	0.51 0.18	
Blood	7	0.14 0.05	4	0.29 0.09		2	1.46 0.45	
Rats								
Liver	10[c]	0.19 ± 0.14	4[c]	1.91 ± 0.28	1.1[b]	8[c]	6.00 ± 0.93	0.7[b]
Kidney	11	0.34 0.20	5	10.69 1.24	6.7	8	24.14 2.40	2.1
Spleen	14	0.43 0.20	4	2.94 1.50	1.7	7	15.26 1.87	2.2
Femur	15	1.00 0.46	4	2.55 0.67	1.2	8	6.53 0.95	1.0
Lung	11	0.39 0.43	4	1.27 0.32	2.1	8	3.99 0.80	1.4
Heart	12	0.38 0.22	5	1.49 0.14	1.4	8	3.13 0.88	1.8
Muscle	13	0.24 0.14	5	0.50 0.17	0.5	8	1.10 0.55	2.2
Blood	9	0.19 0.17	5	2.42 0.23	8.3	8	4.52 0.54	3.1

[a]Arithmetic mean ± SD.
[b]Ratio mice/rats.
[c]Number of samples.

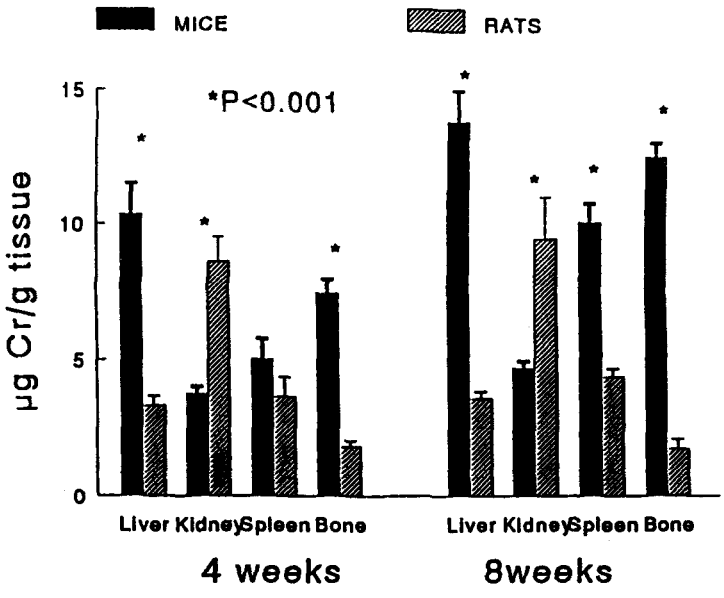

Fig. 5. Chromium organ concentration of mice and rats exposed orally to K_2CrO_4 for 4 or 8 wk (8 mg/kg/d). Bars are the mean ± SE. *Values significantly different between mice and rats.

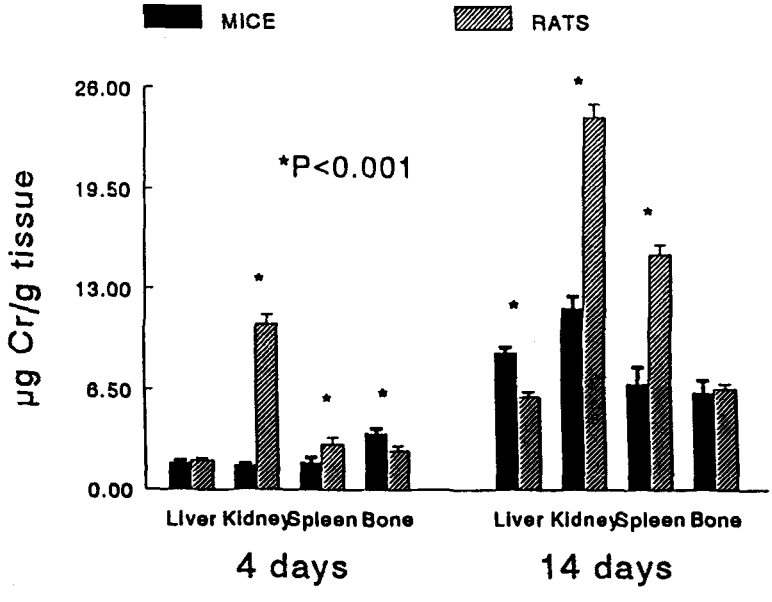

Fig. 6. Chromium organ concentration in mice and rats exposed ip to K_2CrO_4 for 4 (0.3 mg/kg/d) or 14 d (0.8 mg/kg/d). Bars are the mean ± SE. *Values significantly different between mice and rats.

trations in rats and mice orally exposed to Cr for 3 or 8 wk (Fig. 4). However, data from oral exposure studies showed that mice accumulated significantly more Cr in liver and bone compared to rats after both 4 and 8 wk of exposure (Fig. 5). Also, significant differences were apparent in kidney concentrations of Cr, which were higher in rats than mice after both 4 and 8 wk.

Organ concentration data from ip exposure studies indicated that liver concentrations of Cr were also higher in mice than rats after 2 wk of exposure ip (Fig. 6); however, the difference was much less than that observed after oral exposure (1.5-fold vs 3.9-fold after ip vs oral exposure, respectively), and there was no difference after only 4 d of exposure to 0.3 mg/kg/d. The concentration of Cr in the bone of rats and mice was also not significantly different after ip exposure, even though there was a striking difference in bone concentrations of Cr after oral exposure. Kidney Cr concentrations were approximately twofold higher in rats vs mice after ip injections, as they were after oral exposure, and spleen Cr concentrations were significantly greater in rats after ip injection.

DISCUSSION

These studies have demonstrated that Cr accumulates to a greater extent in the blood of rats vs mice after short-term exposure by ip injection. This increased accumulation appears to be the result of an increased sequestration of Cr by rat red blood cells (rbc), since the accumulation of Cr by (wbc) was lower in rats than mice. However, in view of the differences in the reactivity of hemoglobin toward sulfur-seeking ligands, the differences were less than expected.

The higher accumulation of Cr in rat (rbc) observed in this study after ip exposure was associated with a greater binding of Cr to the rat hemoglobin molecule. This finding was predicted based on known structural differences in rat hemoglobin compared with other mammalian species, but it was considerably less than expected. Rat erythrocytes, in contrast to those of the human and most other mammals, bind acetaldehyde (*10*) and show unique behavior toward thiol-oxidizing diazenes (*11*). Rat hemoglobin has also been found to bind simetryn sulfoxide to a 40-fold greater extent than human hemoglobin (*8*). These properties have been attributed to the presence of unusually reactive cysteine residues within the hemoglobin. Mouse and human hemoglobin, however, are quite similar with respect to the binding of sulfur-seeking ligands. Therefore, although rats are commonly used animal models in toxicology studies, one would think that they should be used with caution, particularly with sulfhydryl-binding compounds, such as metals. However, although there were differences in Cr binding to rat tissues, the differences observed between rats and mice were not remarkable and certainly less than predicted.

Chromium uptake across rbc membranes has been well characterized in whole blood and rbc preparations (*12,13*), and a high velocity of chromate influx into rbc has been demonstrated by Weigand and coworkers (*14*). Results regarding species differences in Cr transport and uptake into RBC are somewhat confusing, however. One in vitro study showed no significant difference in chromate transport rates in human vs rat RBCs (*14*), whereas greater uptake by human vs rat RBCs was found in another study (*15*). Wbc Cr uptake rates were greater in rats than humans, however (*15*).

A large number of studies determining the toxicokinetics of chromium have been performed (*16–21*); however, these have all used only a single animal species, usually the rat or the mouse, and only short-term exposures. Results from this study indicated that chronic oral exposure to chromate yielded some species differences in the tissue accumulation of chromium with time, and these differences were not the same as those observed following short-term ip exposures. Models of chromium metabolism must be developed using appropriate animals species and appropriate exposure regimens. Studies by Squibb et al. (*22*) have shown that tissue elimination rates for chromium following chronic oral exposure to the chromate are much longer than those predicted from single-dose pharmacokinetic studies (*18*).

Following oral exposure, considerably less Cr accumulated in tissues compared to the amount accumulated after ip exposure, indicating a poor absorption of chromate from the GI tract (*23*). This is consistent with studies that suggest that hexavalent Cr is readily reduced to the trivalent form in the GI tract (*24*) and that Cr(III) penetrates cells at a much slower rate than Cr(VI) (*4*). Some absorption from the GI tract did occur, however, in both rats and mice, and species differences observed in this study may be owing in part to differences in the reduction of Cr(VI) to Cr(III) in the two species. The finding that Cr concentrations in the mouse liver were so much higher than in the rat after oral dosing, but not after ip dosing suggests that more Cr(VI) may be absorbed in mice; however, it may be more rapidly reduced and consequently sequestered by the mouse liver as it enters via the portal blood.

Differences in the in vivo reduction of Cr(VI) to Cr(III) between mice and rats may also account for the fact that larger differences were not observed in the accumulation of Cr by red blood cells between these species, particularly after oral exposure. In view of the fact that the hemoglobin molecule is 40 times more reactive in rats compared to mice (*8*), other factors, such as reduction rates, might be involved in regulating the accumulation of Cr in rbc in vivo. After oral exposure, when the rate at which Cr(VI) enters the blood stream is much lower than after ip injection, the reducing components of the plasma may not be depleted as rapidly. This may account for the fact that there was no difference in blood Cr concentrations between the rats and mice after oral exposure.

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