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Bioavailability of Lead and Chromium from Encapsulated Pigment Materials

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Bioavailability is a major factor in determining toxicity of compounds taken into a human or animal. If an ingested compound is unable to cross the wall of the gut and enter into the body, then (except in very specialized cases) its capacity to cause systemic toxicity is small. Therefore, bioavailability is an important factor in determining the true exposure level for use in risk assessment models.

The results of a study of relative oral bioavailability of lead and chromium from pigment materials in both natural and encapsulated forms are reported. The levels of lead and chromium were measured In the blood after 2 and 4 weeks of treatment and after a further 2 weeks of recovery. Blood levels were used as an index of metal uptake into the body. Blood was chosen because of its role in metal transport, and its accessibility, allowing repeated measurements to be made on the same animals. After the recovery period, both blood levels and tissue levels in the kidneys were measured.

MATERIALS AND METHODS

Three materials were examined. One was a lead carbonate pigment (78 wt% lead) utilized as a positive control. The second was a lead chromate pigment called "Chrome Yellow", a lead chromate - lead sulfate solid solution pigment (60.8 wt% lead, 14 wt% chromate). The third compound was "Krolor Yellow", a silica coated "Chrome Yellow" pigment (46.5 wt% lead, 10.8 wt% chromium). In this formulation, silica encapsulates the pigment particles in order to reduce release of potentially toxic lead and chromate. All pigments were supplied by Cookson Pigments, Inc. Commercial talc was utilized as a negative control.

Young Sprague-Dawley rats, 10 per sex per group initially, were treated by gavage (oral intubation) daily with pigment materials

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suspended in corn oil. Animals were treated daily, 5 days a week for 4 weeks, and then allowed a recovery period of two weeks before final sacrifice. Rats received 1 ml of pigment suspensions per 100g of body weight. The concentration of the suspensions varied in order to treat each rat with the identical amount of lead. Each rat treated with the test compounds received daily doses of 150 mg Pb per kg body weight. This dose was chosen from the effect levels reported in the literature (Kennedy et al., 1976; Christofano et al. 1976). The rats were provided with ad lib food (Purina Rodent Chow) and water and housed in wire mesh cages. Body weights were recorded periodically and signs of toxicity assessed daily.

On days 14 and 28, rats were anesthetized with ether and bled through glass tubes from the ophthalmic orbital sinus (right side on day 14, left on day 28). Up to 1.5 ml of blood was taken and placed in heparinized plastic tubes sealed with plastic tops. No metal was involved in the blood collection. Blood tubes were collected on ice and frozen at -60°C until analyzed (within two weeks). On day 42 (at end of a 2 week recovery period), rats were anaesthetized, blood was taken from abdominal vessels, and both kidneys were removed and frozen in plastic containers.

Blood was extracted to concentrate lead and chromium in a standard method utilized for chelation-treatment patients (Zinterhofer et al., 1971). Whole blood was completely hemolyzed by 10% Triton X-100 (since most of blood lead is in the erythrocytes) and lead was extracted from the hemolyzed blood into reagent grade methyl isobutyl ketone (this results in higher sensitivity, minimization of viscosity variations and ionic interferences, and removal of insoluble impurities) as the ammonium pyrrolidine dithiocarbamate chelate. Tissue samples were digested in reagent grade fuming nitric acid (15.9%), extracted, and quantitatively diluted prior to analysis.

Lead and chromium levels were determined using a Perkin-Elmer 3030 atomic absorption spectrophotometer (AAS) after blood or tissue preparation and extraction. Chelated, extracted samples were analyzed either by acetylene-air flame AAS or by platform stabilized graphite furnace AAS (atomic adsorption method details may be obtained from USEPA, 1986). All AAS methods were carried out using 4 point standard curves with recalibration at least every 10 samples.

RESULTS AND DISCUSSION

Animals treated with the talc control (commercial talc) had low levels of lead in the blood (1.0 to 85 ug/l, overall; Table 1). Encapsulated pigment produced relatively low levels of lead in the blood after 2 and 4 weeks of treatment (203 $\pm$ 48.4 ug/l, and 35.2 $\pm$ 18.0 ug/l, respectively [mean $\pm$ standard error]). Treatment of rats with non-encapsulated pigment resulted in considerably higher levels of lead in the blood (714 $\pm$ 213 ug/l and 479 $\pm$ 220 ug/l at 2 and 4 weeks, respectively). Lead carbonate produced the highest

lead levels in the blood of treated animals (1056 ± 313 ug/l and 1432 ± 122 ug/l at 2 and 4 weeks, respectively).

After 2 weeks of recovery, without further dosing, the lead concentrations in blood from rats treated with non-encapsulated pigment or lead carbonate remained elevated above levels observed for blood from encapsulated pigment and talc treated animals. Lead carbonate treatment resulted in significantly higher blood lead levels than non-encapsulated pigment treatment.

Table 1. Levels of lead (ug/l) in blood of treated rats.

Time Point	Treatment			
	Talc	Encap	Non-Encap	Lead Carb
2 Weeks				
Overall	84.8 ± 16.8	203 ± 48.4	714 ± 213	1056 ± 313
Male	70.0 ± 19.1	225 ± 53.5	666 ± 167	1614 ± 618
Female	98.1 ± 27.2	179 ± 87.7	787 ± 500	561 ± 88.4
4 Weeks				
Overall	1.0 ± 1.0[a]	35.3 ± 18.0	479 ± 220	1432 ± 122
Male	2.3 ± 6.1	9.3 ± 6.1	781 ± 411	1706 ± 161
Female	ND	87.3 ± 45.1	178 ± 115	1219 ± 147
Recovery				
Overall	33.4 ± 11.5	111.6 ± 74.1	731 ± 133	1077 ± 90
Male	25.6 ± 9.9	114.0 ± 25.8	905 ± 239	1334 ± 101
Female	42.0 ± 22.2	108.8 ± 30.6	535 ± 52.1	789 ± 63

Mean ± standard error. Encap = encapsulated lead chromate pigment, "Krolor Yellow". Non-Encap = non-encapsulate lead chromate pigment, "Chrome Yellow". Lead Carb = lead carbonate pigment. a = mean, standard error. Statistics were calculated by computer program (SASS, Carey, NC). Statistic Analysis: No differences due to sex were significant. A two way ANOVA comparing treatment and time showed no significant differences between different time points (F value = 0.04), while treatments were significantly different (F value = 28.27). A Student-Newman-Keuls analysis demonstrated no statistically significant differences between talc control and encapsulated lead chromate pigment treatments, while non-encapsulated lead chromate pigment and lead carbonate differed from each other and from both encapsulated lead chromate pigment and talc treatments.

No chromium could be detected in blood from any treatment groups (10 ug/l detection limit).

Since the kidney is a major excretory organ that tends to concentrate metals, and can be said to "integrate exposure", this tissue was examined at the terminal sacrifice, after the 2 week recovery period.

Tables 2 and 3 present the kidney lead and chromium levels in treated rats. These data indicated that the administration of encapsulated pigment resulted in a small but not statistically significant increase in kidney lead over control, but no change in kidney chromium. Non-encapsulated pigment treatment resulted in approximately a 50 fold increase in lead and a 2-3 fold increase in chromium in the kidney. Lead carbonate treatment resulted in a 100x increase in tissue lead and no change in tissue chromium.

Lead from the silica encapsulated lead chromate pigment has considerably less oral bioavailability than unencapsulated lead chromate pigment or lead carbonate (3x, and 5x, respectively). Chromium content in kidneys from encapsulated lead chromate pigment or lead carbonate (which was chromium free) groups were the same as control, while the kidney lead levels were elevated. Comparisons between treatments containing chromium are difficult since the levels of chromium administered differed between treatment groups, whose dosages were balanced for lead content. It is concluded that lead is less bioavailable to rats orally from silica encapsulated lead chromate based pigment than from non-encapsulated pigment.

Table 2. Levels of lead (ug/l) in kidney of treated rats at terminal sacrifice.

Sex	Treatment			
	Talc	Encap	Non-Encap	Lead Carb
Overall	56.9 ± 11.5	342 ± 40.5	2890 ± 459	5442 ± 461
Male	50.3 ± 11.7	355 ± 53	3025 ± 798	4633 ± 784
Female	64.1 ± 21.2	327 ± 77	2737 ± 445	6169 ± 440

Mean ± standard error. Encap = encapsulated lead chromate pigment, "Krolor Yellow". Non-Encap = non-encapsulated lead chromate pigment, "Chrome Yellow". Lead Carb = lead carbonate pigment. Statistical Analysis: A two way ANOVA demonstrated no statistically significant difference by sex (F value = 0.85), while treatments differed. One or two way analysis of variance showed statistically significant differences (F value = 58.6) between non-encapsulated lead chromate pigment and lead carbonate treatments, which were also different from both talc and encapsulated lead chromate pigment treatments. Encapsulated lead chromate pigment treatment did not differ from talc treatment.

In rats treated with talc, or lead chromate compounds, less lead was observed in the blood samples obtained at the 4th week of the experiment than was observed at 2 weeks or after the recovery. A comprehensive review of the study eliminated methodological or analytical errors as the explanation for the observation. A possible explanation is that the rats themselves activated

protective mechanisms to reduce circulating lead and compartmentalized the lead into sequestering reservoirs.

Table 3. Levels of chromium (ug/l) in kidney of treated rats.

Sex	Treatment			
	Talc	Encap	Non-Encap	Lead Carb
Female	155 + 10.7	169 + 9.4	615 + 202	152 + 10.9

Mean ± standard error. Encap = encapsulated lead chromate pigment, "Krolor Yellow". Non-Encap = non-encapsulated lead chromate pigment, "Chrome Yellow". Lead Carb = lead carbonate pigment. Statistical Analysis: ANOVA demonstrated that treatment with non-encapsulated lead chromate pigment differed from the other treatments (F value = 1.46).

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