

Disposition of lead (Pb) in brain of rats following oral exposure to lipstick

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ABSTRACT

Information about the health risks that might be associated with lipstick consumption effects is scarce in the literature. The present work investigated the bioaccumulation of lead (Pb) in brain of rats originated from lipstick sample. First, Lead contents were determined in 12 different brands of lipsticks. Lead was detected in all the studied samples. The average lead content in 14 lipsticks samples was 12.2 PPM wet wt. Then, one brand was selected for feeding to the rats and amount of oral exposing in the three doses was calculated. Sixty rats were used for the experiment. Animals were divided into 4 groups of 15 animals each. While 1 group served as control group, the remaining 3 groups were exposed to lipstick through oral gavage for 12 weeks. Results show that, exposure to the lipstick cause significantly disposition of lead in the brain of rats.

Keywords: Lipstick ; Lead ; Rat ; Exposure, Brain.

INTRODUCTION

Lead toxicity remains a topic of current debate and concern because of its adverse effects on several organ systems [1]. One of the primary and important target for lead is the nervous system. Lead even at low levels, may penetrate into the brain by altering the functional competence of the blood–brain barrier [2] The brain is the organ most sensitive to lead exposure [3]. There is apparently no lower threshold to the dose-response relationship (unlike other heavy metals such as mercury [4]. In addition, it is well established that lead can cross the placenta during pregnancy and has been associated with intrauterine fetal death, premature delivery and low birth weight [5].

There are numerous reports and research papers on other potential sources for lead exposure that are hidden and need to be addressed. These include ethnic folk remedies and cosmetics, Mexican terra cotta pottery, toys and certain imported candies and spices [6-15]. Scientists and health care workers are aware of the long-term health effects of lead exposure. Pregnant women and young children are

particularly vulnerable to lead exposure. The use of lead contaminated lipstick by pregnant or /and lactating women could expose the fetus and infants to the risk of lead poisoning [16]. Recently, the Campaign for Safe Cosmetics in the United States raised another concern about the presence of lead in lipsticks. They found that more than half of the tested 33 brand-name red lipsticks (61%) contained detectable lead in the range of 0.03–0.65 PPM [12]. There have been various reports in the media and on the internet discussing whether lipsticks with lead levels ranging from 0.03 to 0.65 PPM reported by the CSC in 2007 should be of concern. The claims that these levels are well below the FDA limit for lead as impurities in color additives (20 PPM) [17]. Reports about lead in lipsticks are not new and in the 1990s, the FDA checked a similar claim from a commercial testing laboratory and found no action was necessary because the laboratory used an unvalidated and inappropriate testing method [16]. Lead in lipsticks represents a very minor source of lead exposure compared to other sources because the amount of lipstick that one applies daily is

actually very small in comparison the amount of water, food or air one takes. Nonetheless, one should not exclude the fact that lead accumulates in the body over time and repetitive lead-containing lipstick application can lead to significant exposure levels. However, the consequences of these products can only be properly verified by conducting population risk assessment exposure study[18].

In the present study, lead content was determined in different brands of lipstick samples which were collected from various stores in Iran market in order to check their lead level. Then, one brand was selected for feeding to the rats and amount of oral exposing in the three doses was calculated. The lipstick, which was dissolved in vegetable oil, feed to rats in three doses daily. Finally the concentration of lead determined in brains of rats after 12 weeks.

MATERIALS AND METHODS

Instrumentation

Lead analysis was performed using a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS), Varian AA-240 with a hollow cathode lamp and a deuterium lamp for background correction, coupled to GTA-120 electrothermal atomizer and a programmable sample dispenser (Varian Techtron PTY. Ltd., Australia). The optimized heating programs followed for the analysis of lead were that described by the instrument manufacturer. Lead was analyzed by mixing one volume of digest (usually 3 μ l) with an equal volume of 1% (w/v) ammonium dihydrogen phosphate modifier.

Samples and reagents

All 12-brand lipsticks were purchased in retail stores. Each brand has a manufacturing LOT number, which represents either one, or more color. We collected 4 different colors of lipsticks. Based on the brand's label, country of origin was specified. Lipsticks were imported from five different countries (China, Taiwan, USA, Korea and Germany). One brand was of unknown origin. Three batches per each brand of lipstick with the same LOT number were selected. Lipstick were dissolved in vegetable oil (Sunflower oil of Nina, manufacture by Frico Company, Iran).

30% hydrogen peroxide (H_2O_2), nitric acid and ammonium dihydrogen phosphate modifier were obtained from Merck (Darmstadt, Germany)

Determination of lead in lipstick

Lipstick of one lot of each brand was analyzed. From each lot, the total contents of three lipsticks were combined, and then lipstick was homogenized with stainless steel cutters. A weighed sample of 0.2 g lipstick was placed into a Teflon vessel and reacted with 4 ml concentrated nitric acid, left at room temperature for 4 h then placed in the oven overnight at 85 °C. After digestion, the sample was allowed to cool to room temperature. Furthermore, after adding 1 ml of 30% hydrogen peroxide, the sample solutions were heated at 85 °C for another hour. The clear supernatant was transferred to polypropylene tubes and diluted to 10 ml with deionized water. Lead content was expressed as part per million wet weight (PPM wet wt.) each analysis replicates three times.

Determination of oral exposure to lipstick by ingesting

The lipstick was applied by 10 women and weight loss of it was calculated for one time usage. 9.8 mg was the average of weight loss of the lipstick after applying by women with mean body weights of 60 ± 10 kg.

Three concentrations of lipstick were dissolved in vegetable oil. So 0.16, 0.32 and 0.48 mg lipstick/kg body weight were fed daily (equal of one, two and three, times applying of lipstick). 0.1 mL was the gavage volume.

Animals and treatment

Sixty male rats (Pasteur Institute, Karaj, Iran) with body weights 230 ± 10 g, were used for the experiment. They were housed in an animal room with a normal controlled temperature (20 ± 2 °C), constant humidity (60%) and exposed to a 12-h dark-light cycle. They were allowed 21 days to acclimatize before the commencement of lipstick exposure. Sixteen animals were divided into 4 groups. While 1 group served as control group, the remaining 3 groups were exposed to lipstick through oral gavage for 12 weeks. Control animals received vegetable oil for the same period of time. The animals were allowed free access to food and their respective distilled drinking water. Body weight and

water consumption were recorded daily. Water consumption was measured by weighing the canister and body weight was determined. At the end of lipstick exposure, rats were euthanized under anesthesia by exsanguination. Brain was immediately removed and weighed; finally, digested Samples were analyzed for the lead.

Determination of lead in brains

Lead concentrations in digested brain samples were analyzed by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS). Briefly, samples were dried under a HEPA filtered-air hood, weighed, and then digested with 6 mL nitric acid (69–71% GR grade, HNO₃) at 120 °C. Samples were allowed to cool, and then digested samples were transferred into polypropylene tubes for storage until analyses. All glass ware was acid-cleaned with 7.5 N HNO₃ for 2 days and rinsed with MilliQ water before use. Finally, digested Samples were analyzed for the lead. Sample aliquots spiked with known quantities of lead were used for quality control.

Statistical evaluation

Because the raw Pb data were not normally distributed, they were log-transformed to achieve normal distribution. Pb data in the table 2 shows the original values. Lognormal values were used for statistical tests. Results are expressed as mean ± SE. Test for normal distribution (Shapiro–Wilk test) and one-way analysis of variance (ANOVA) followed

by Duncan's test were used to analyze the results with $p < 0.05$ considered significant. The data were evaluated using the SPSS for Windows (16.0).

RESULTS

Limit of detection (LOD) of Pb was 25 µg/L by GF-AAS. Table 1 lists the results of lead analysis in the 12 brands of lipsticks. The mean value of lead in lipstick samples was 93.8 PPM wet wt.

The average is significantly skewed by the China lipstick sample (LOT No. 09) with a lead content of 1154 ppm wet wt. it was removed and the average was determined for the remaining 13 samples, the average is 12.24 ppm wet wt.

The closest lipstick sample to this average is the china orange (Lot No. 53) and it was chosen for oral exposure to the rats.

Deposition of Lead in the brain

The present study aimed to link lipstick exposure via oral consumption at 3 doses to brain concentrations of lead. Since that study used a dosing regimen similar to 12 weeks used here, any estimates of brain concentrations of lead should be revised upward. Lead concentrations in brain of control and lipstick-exposed animals tabulated in table 2.

Lead concentrations in the lead-exposed animals were significantly different from controls ($p < 0.05$) and obtained results, showed increases in Pb as a function of exposure dose, as expected.

Table 1. Lead contents (PPM wet wt.) in various lipstick samples.

Brand	Country of origin	LOT NO.	Color	Lead content (PPM wet wt.)
1	China	10	Bright rose	20.1 ± 1.1
		09	Red brown	1154.2 ± 7.3
2	USA	23	Shimmering pink	1.2 ± 0.2
3	China	53	Orange	18.5 ± 0.6
4	China	140	Brown	19.1 ± 1.4
5	Unknown	128	Pink	62.3 ± 2.5
6	Korea	10	Orange	21.2 ± 1.7
7	USA	906	Pink	5.1 ± 0.8
8	China	14	Rose	2.3 ± 0.5
		17	Bright rose	1.6 ± 0.3
9	Taiwan	33	Light pink	0.5 ± 0.2
10	China	23	Dark rose	0.7 ± 0.2
11	China	10	Shimmering rose	0.5 ± 0.1
12	Germany	07	Orange	6.8 ± 1.4

Table 2. Lead concentrations in brain of control and lead-exposed animals. (0.1 ml of each solution was gavaged)

Group	Gavages dose of Lipstick mg/kg/day	Mean of lead concentration (mg/kg wet weight)	Duncan Grouping ^a
Control	0	0.022 ± 0.001	A
G1	0.16	0.299 ± 0.013	B
G2	0.32	0.387 ± 0.020	C
G3	0.48	0.448 ± 0.023	D

^aMeans with the same letter are not significantly different.

Table 3. Mean weight of body rats after of lipstick exposure and mean of brains weight (g)

Group	Mean Body Weight before exposure (g)	Mean Body Weight after exposure (g)	Duncan Grouping ^a	Mean brain Weight (g)	Duncan Grouping ^a
Control	232 ± 11	234 ± 6	A	1.675 ± 0.019	B
G1	226 ± 14	224 ± 12	B	1.770 ± 0.014	A, B
G2	229 ± 12	217 ± 13	B	1.825 ± 0.034	A
G3	233 ± 9	206 ± 13	C	1.716 ± 0.064	A, B

^aMeans with the same letter are not significantly different.

Accumulation was dose-dependent. With the second lipstick dose, there was a 29% increase in brain lead concentration, whereas the third lead doses, it increased by 16%.

In order to exclude the possibility that any biochemical and/or behavioral effect of lead would be due to under nutrition associated with exposure to the lipstick, body and brain weights were assessed. Table 3 summarizes average weight of body rats after of lipstick exposure and mean of brains weight. The body weight of the animals (230 ± 10) was altered by treatment as rats, which expose to different doses of lipstick have a significant decrease in weight.

Accumulation was dose-dependent. With the second lipstick dose, there was a 29% increase in brain lead concentration, whereas the third lead doses, it increased by 16%.

DISCUSSION

Many research groups have revealed that lead could induce apoptosis in a number of experimental systems including rat brain [19], rat/mouse retinal rod cells [20, 21] cerebellar neurons [22] and PC12 neuronal cells [23]. The toxicity of lead is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage,

duration of exposure, frequency of intake, absorption rate, mechanism and efficiency of excretion [24]. The mechanism for these effects remains unknown, although several studies have identified the ability of Pb^{2+} to interact with intracellular proteins, particularly Ca^{2+} -binding proteins. For example, Pb^{2+} can bind Syt-1 with 1000-fold higher affinity than Ca^{2+} , which may prevent detection of Ca^{2+} signaling essential to neurotransmission [25].

There are many molecular targets for the action of lead. Lead has great affinity for sulfhydryl groups of endogenous biomolecules, which may contribute to its toxicity. It has been proposed that the toxicity of lead may be due, at least in part, to the inhibition of the sulfhydryl-containing enzyme aminolevulinic acid dehydratase (ALA-D). ALA-D activity in erythrocytes is currently the most sensitive indicator of human exposure to lead [26].

Although the toxic effects of high levels of lead have been well documented for centuries, of great concern is the relative recent discovery that low levels of blood lead ($BPb < 10 \mu g dL^{-1}$) are associated with adverse effects in the developing organism. In 1991, Centers for Disease Control and Prevention (CDC) in the US declared that a BPb level of $10 \mu g dL^{-1}$ should prompt public

health actions, while concurrently recognizing that although useful as a risk management tool, $10 \mu\text{g dL}^{-1}$ BPb should not be interpreted as a threshold for toxicity. Indeed, no threshold has yet been identified. Subsequently, low-level exposure to lead during early childhood was shown to be inversely associated with neuropsychological development through the first 7 years of life. In 2007, the CDC summarized the findings of a review of clinical interpretation and management of BLLs $< 10 \mu\text{g dL}^{-1}$ conducted by CDC's Advisory Committee on Childhood Lead Poisoning Prevention and concluded that research conducted since 1991 has strengthened the evidence that children's physical and mental development can be affected at BLLs $< 10 \mu\text{g dL}^{-1}$ [27]. Also, the results of present study indicates that our regimen of lipstick induced gross physical alterations in rats. There was no exact formula for selected lipsticks. Since the lipstick was mixture of different compounds and chemicals, it was not appropriate to conclude that the reduced animal body weight was a simply result of Pb exposure. Less increase for lead in third dose could be due to weight loss of rats in this group after treatment and toxic effects of lipstick [28]. In addition to the primary sources of lead exposure that we are likely expose to, there seems to be many recent studies and reports

revealing the presence of lead in numerous other products such as toys, jewelry, herbal remedies, candy etc. that put the vulnerable population at risk of lead poisoning [17].

To be exposed to lead from cosmetics raise another concern. Consumers are exposed to these toxins by absorbing them through their skin (for example in hand lotion), or oral exposure (by ingesting lipstick while eating, for example).

Lead was detected in all tested lipstick samples in the range of 0.48–1154 PPM wet wt. The overall results indicate that lead in most of lipsticks is below lead limit as impurities. 20 PPM is the US FDA's lead limit as impurities for the lipstick. In addition, results show that exposure to the lipstick cause disposition of lead in the brain of rats and since that study used a dosing regimen similar to 12 weeks used here, any estimates of brain concentrations of lead should be revised upward. These might put consumers at the risk of lead poisoning. In conclusion, our findings call for an immediate mandatory regular testing program to check lead and other toxic metals in lipsticks and other cosmetic products imported to Iran in order to curtail their excess and safeguard consumer health.

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