ENVIRONMENTAL DEFENSE FUND, EARTHJUSTICE, ENVIRONMENTAL
WORKING GROUP, CENTER FOR ENVIRONMENTAL HEALTH, HEALTHY HOMES
COLLABORATIVE, HEALTH JUSTICE PROJECT OF LOYOLA UNIVERSITY
CHICAGO SCHOOL OF LAW, BREAST CANCER FUND, IMPROVING KIDS'
ENVIRONMENT, CONSUMERS UNION, NATURAL RESOURCES DEFENSE
COUNCIL, CONSUMER FEDERATION OF AMERICA, LEARNING DISABILITIES
ASSOCIATION OF AMERICA, MARICEL MAFFINI, AND HOWARD MIELKE

February 4, 2017

Dr. Dennis Keefe
Director of the Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
5001 Campus Drive
College Park, MD 20740

Re: Color additive petition (CAP) 7C0309 pursuant to 21 USC § 379e seeking to
        delete 21 CFR § 73.2396 regarding the use of lead acetate to color hair on the
        scalp because its continued use is not safe.

Dear Dr. Keefe:

With this color additive petition (CAP), designated by the Food and Drug Administration
(FDA) as CAP 7C0309, the undersigned organizations and individuals ask the agency to
take prompt action prohibiting the use of lead acetate as a hair dye. There is no doubt that
lead is a potent neurotoxin with no safe level of exposure [1]. It is also reasonably
anticipated to be a human carcinogen [2]. The continued use of lead acetate as a hair
colorant used in the home will inevitably result in dangerous lead exposure to the user
and others in the household, including children and pregnant women who are most
vulnerable to permanent harm. FDA must take steps as soon as possible to bring the
unsafe use of lead acetate as a hair colorant to an end.

A color additive is only safe when there is "convincing evidence that establishes with
reasonable certainty that no harm will result from the intended use of the color additive."[1]
The statute also deems a color additive unsafe if it is ingested and found to induce cancer
in man or animal.[2] If the color additive is not ingested, the color additive is unsafe if "it is
found by the Secretary to induce cancer in man or animal" using tests that "are
appropriate for the evaluation of the safety of additives for such use, or after other
relevant exposure of man or animal to such additive."[3]

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[1] 21 CFR § 70.1(i).
[2] 21 U.S.C. § 379e((b)(5)(B) and 21 CFR § 70.50(a).
[3] 21 U.S.C. § 379e((b)(5)(B). See also 21 CFR § 70.50(b).

In 1980, the Food and Drug Administration (FDA) approved lead acetate as a repeated use hair dye at 21 CFR § 73.2396.[4] Although the agency found that 0.5 microgram (µg) of lead was absorbed with each use, it found that the levels were insignificant. It also dismissed evidence that lead acetate induced cancer in animals and approved its use because it assumed the carcinogen would not be ingested from the repeated use on the scalp.

Unfortunately, time has demonstrated that **the health risks of lead acetate exposure from hair dye are real.**

Health Canada's weight-of-the-evidence evaluation of lead acetate hair dyes made the point clear when it said:

> "The results showed that relatively small incremental exposures, such as those which would occur with regular use of hair dyes containing lead acetate, could result in the accumulation of potentially harmful body burdens of lead." [3]

> "Use of lead acetate dyes would add to the cumulative population exposure for lead, which has already been found to be within the range of potential effects for some end-points and sensitive sub-populations. Given the conclusions of the report, companies were asked to remove lead acetate-containing hair dyes from the market. Since January 2008 lead acetate hair dyes have not been available for sale in Canada." [3]

The risk became clear in 2014 when a case study was published where a person experienced tingling and then numbness in his feet and hands after applying the lead acetate hair dye to his beard for 7 months. [4] While the use on the beard is contrary to label instructions, it is not surprising that someone seeking to hide the grey hair on the scalp would also use it on the beard without noticing the limit in the instructions. The person's blood lead levels were 17.3 µg/dL, fourteen times the contemporary average adult's level. The levels gradually dropped to within normal limits over six months after use of the hair dye stopped. The peripheral neuropathy symptoms were gone in a year.

In January, 2015 an entry in FDA's CFSAN Adverse Event Reporting System[5] describes a 66-year old man that presented "hypotrichosis, metal poisoning, blood pressure increased." The suspect, in FDA parlance, was the progressive hair coloring Grecian Formula cream. Although there is no additional information and FDA advises to be cautious with the interpretation of the events reported, the symptoms and the suspected cosmetic are a good match. We found three additional reports on the same product.

For these reasons, we submit this color additive petition and ask FDA to remove its approval. Below, we provide our rationale for this claim.

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[4] 45 *Federal Register* 72112, October 31, 1980.
[5] US Food and Drug Administration CFSAN Adverse Event Reporting System (CAERS). See http://www.fda.gov/Food/ComplianceEnforcement/ucm494015.htm

I.   Safety standard for color additives

Pursuant to 21 U.S.C. § 379e(b)(4), FDA shall not list a color additive "unless the data establish that such use, under the conditions of use specified in the regulations, will be safe." The agency's rules at 21 CFR § 70.3(i), define safe to mean "there is convincing evidence that establishes with reasonable certainty that no harm will result from the intended use of the color additive."

Section 21 U.S.C. § 379e(b)(5)(A) requires the agency to consider four factors when determining safety:
- Probable consumption of, or other relevant exposure from, the additive because of its use.
- Cumulative effect of the additive in the diet taking into account the same or chemical or pharmacologically-related substance or substances in the diet.
- Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of color additive, are generally recognized as appropriate for the use of animal experimentation date. FDA uses a default safety factor of 100 from the maximum no-effect level for the most susceptible experimental animal species tested per 21 CFR § 70.40.
- Availability of any needed practicable methods of analysis.

In addition, the statute at 21 U.S.C. § 379e(b)(5)(B) establishes what FDA refers to in its regulations as the "cancer clause." This clause requires that FDA deem a color additive to be unsafe
- "for any use which will or may result in ingestion of all or part of such additive, if the additive is found by the Secretary to induce cancer when ingested by man or animal, or if it is found by the Secretary, after tests which are appropriate for the evaluation of the safety of additives for use in food, to induce cancer in man or animal." and
- "for any use which will not result in ingestion of any part of such additive, if, after tests which are appropriate for the evaluation of the safety of additives for such use, or after other relevant exposure of man or animal to such additive, it is found by the Secretary to induce cancer in man or animal."

FDA has promulgated a regulation defining how it apply the cancer clause at 21 CFR § 70.50. This regulation treats the clause differently if the color additive may be ingested (paragraph (a)) or will not be ingested (paragraph (b)). It does not specifically address color additives in cosmetics intended for use on the skin that are likely to be incidentally ingested as a result of the use.

II. Summary of FDA's 1980 decision approving lead acetate as a color additive

In 1980, FDA approved lead acetate as a repeated use hair dye at 21 CFR § 73.2396.[6] In previous notices in the *Federal Register,* it stated the agency had found the use to be safe for health effects other than cancer.[7] In the final notice promulgating the rule, it resolved the remaining question regarding the cancer risk and the application of the cancer clause.

Regarding the cancer clause, the agency concluded that lead acetate was found to induce cancer in animals. FDA acknowledged that the statute "makes an animal ingestion study demonstrating carcinogenicity an absolute bar to the approval of a petition of an ingested color additive,"[8] and concluded that, if ingested, lead acetate was barred because it was found to induce cancer in animals.

However, FDA interpreted the absolute ban on the cancer clause so that it did not apply. The agency appeared to assume that the lead acetate in the hair dye would not be ingested from the repeated use on the scalp. FDA did not state a basis for this assumption.

The agency also found that the "scientific data submitted to FDA concerning the issue of whether lead is a human carcinogen are not sufficient in substantiating a direct correlation between lead exposure and human carcinogenicity."[9] It then concluded that it "cannot find that the animal feeding studies [showing lead acetate induced cancer] are either 'appropriate' or 'relevant' for making the safety determination for lead acetate hair dyes."[10] As a result, it interpreted the statute to give it more flexibility in assessing the cancer risk.

FDA also concluded that the amount of skin absorption was minimal compared to the absorption of lead from environmental exposures common at the time. While the agency acknowledged that 0.5 microgram of lead acetate was indeed absorbed through the skin per application, it found that the "increase of absorbed lead from hair dyes over the normal 'background' levels of lead does not augment the existing risk of acute or chronic toxicity, including cancer, in any clearly discernible, much less significant manner."[11]

Based on the 0.5 microgram of lead absorption per use and the product use of approximately twice per week, FDA estimated an average of 0.3 microgram of lead would be absorbed through the user's skin per day. We could not find a clear explanation for FDA's calculation. **The agency deemed this level insignificant** because adults were

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[6] 45 *Federal Register* 72112, October 31, 1980.
[7] 43 *Federal Register* 8793, March 3, 1978.
[8] 45 *Federal Register* 72115, October 31, 1980.
[9] *Id.*
[10] *Id.*
[11] *Id.*

likely to absorb 35 micrograms of lead per day from a daily exposure of 100-500 micrograms of lead,[12] and had average blood lead levels of 17 micrograms per deciliter.[13]

FDA's 1980 rule puts two limits on the use. First, the product could only be used for the scalp and not the beard or moustache.[14] Second, the maximum concentration of lead acetate in the final product has to be less that 0.6 percent or 6000 ppm.[15] To put this into context, three years earlier, the Consumer Product Safety Commission (CPSC) banned the sale of household paint containing more than 600 ppm.[16]

To warn product makers who buy the concentrated lead acetate and formulate into the hair dye, FDA required the lead acetate label to say "Wash thoroughly if the product comes into contact with the skin"[17] To warn hair dye users, the product label must say:

> "Caution: Contains lead acetate. For external use only. Keep this product out of children's reach. Do not use on cut or abraded scalp. If skin irritation develops, discontinue use. Do not use to color mustaches, eyelashes, eyebrows, or hair on parts of the body other than the scalp. Do not get in eyes. Follow instructions carefully and wash hands thoroughly after use."[18]

FDA sets no limits on the frequency of use, the amount used, or who can use it.[19] The agency also does not recommend wearing gloves. The warning makes no mention of lead acetate being a carcinogen, a neurotoxin, or otherwise toxic.

III.   Toxicological evidence since 1980 shows there is no safe level of exposure to lead compounds

Since FDA made its decision in 1980, the toxicological evidence demonstrating the hazards posed by lead has strengthened and the scientific community has found that the risk is more significant than FDA recognized. As a result, there is no longer convincing evidence establishing a reasonable certainty of no harm from the use of lead acetate.

III.A.   Evidence with respect to lead acetate as a carcinogen

The year after FDA's decision, the National Toxicology Program (NTP) designated lead acetate and lead phosphate as "*reasonably anticipated to be human carcinogens* based on

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[12] 45 *Federal Register* 72113, October 31, 1980.

[13] *Id.* at 72114.

[14] 45 *Federal Register* 72117, October 31, 1980. See also 21 CFR § 73.2396(c)(2).

[15] *Id*, at 72117. 21 CFR § 73.2396(c)(1).

[16] Consumer Protection Safety Commission (CPSC). CPSC Announces Final Ban on Lead-Containing Paint, Release #77-096, 1977, http://www.cpsc.gov/en/Recalls/1977/CPSC-Announces-Final-Ban-On-Lead-Containing-Paint/.

[17] 21 CFR § 73.2396(d)(1).

[18] 21 CFR § 73.2396(d)(2).

[19] Such as use by pregnant or lactating women, a group at special risk as discussed more completely later. While unlikely to be used on children, there is no age restriction provided. We believe children are likely to be exposed indirectly, however, as described in the next section.

sufficient evidence of carcinogenicity in experimental animals." [2] In 2004, NTP expanded the designation to lead and lead compounds as a class based "on limited evidence of carcinogenicity from studies in humans and sufficient evidence of carcinogenicity from studies in experimental animals." [2]

Petitioners maintain that this conclusion by the agency designated by Congress to evaluate the carcinogenicity of chemicals indicates that the scientific evidence substantiating a direct correlation between lead exposure and human carcinogenicity is now sufficiently strong for FDA to conclude that lead acetate is unsafe pursuant to the cancer clause at 21 U.S.C. § 379e(b)(5)(B).

Additional studies have been published in recent years including occupational exposures, animal and *in vitro* studies. See Appendix 4 for literature search.

Kašuba and colleagues [5] showed that lead-exposed workers had increased markers of genotoxicity demonstrated by comet assay, DNA diffusion and micronuclei in peripheral blood lymphocytes. Similar results were found in animals by Cavas [6], Alghazal et al. [7], Xu et al [8] and Celik and colleagues [9].

It is also apparent the scientific community agrees that lead acetate induces genotoxicity, DNA damage, and alters DNA repair because the chemical is used as positive controls in studies related to investigate the protective effects of other substances. Animal studies by Alcaraz-Contreras et al [10], Abdou and Hassan [11], Sharma et al [12], Martinez-Alfaro et al [13], Abdel Moneim et al [14], Nava-Hernandez et al [15], Li et al [16], Wang et al [17], Zhang et al [18], Wang et al [19], El-Ashmawy et al [20] and El-Ashmawy et al [21]. Others have showed similar effects in studies using cells *in vitro*. [22-28]

III.B. *Evidence of health effects other than cancer*

Since FDA made its decision in 1980, the evidence has become overwhelming that lead acetate:
- has adverse health effects across multiple systems at extremely low levels; [1]
- is a potent neurotoxin with no safe level of exposure for children; [29] and
- is particularly harmful to pregnant women. [32]

The toxic effects are observed regardless of the route of exposure, namely ingestion, inhalation, or dermal. These studies have led public health authorities and regulators to reduce the amount of lead considered "normal" in blood, as well as reduce allowable limits of lead in products and environmental media. As described below, multiple agencies of the United States government have found there to be no safe level of lead exposure, especially in children.

In 2012, the NTP released the "NTP Monograph on Health Effects of Low-Level Lead" reviewing the state of science around impacts of lead on adults and children at levels below 10 and below 5 µg/dL, two standards commonly referenced for lead exposure. [1]

Amongst adults, the NTP found sufficient evidence for increased blood pressure and risk of hypertension, and increased incidence of essential tremor at blood lead levels below 10 µg/dL. They further identified sufficient evidence of decreased kidney function in all adults and reduced fetal growth rate amongst pregnant women with blood lead levels below 5 µg/dL. Limited evidence was also identified for number of other neurologic, cardiovascular, and reproductive health concerns amongst adults with blood lead levels below 10 µg/dL.

In response to this increasing evidence of harm, the National Institute for Occupational Safety and Health (NIOSH) lowered the adult blood lead reference level to 5 µg/dL in 2015, and recommended public health authorities manage cases with levels at or above 5 µg/dL.[30] This change was reflected in the Centers for Disease Control and Prevention (CDC)'s National Notifiable Diseases Surveillance System (NNDSS) and the Council of State & Territorial Epidemiologist's (CSTE) case definition of adult elevated blood lead level. CSTE further noted, "Recent evidence suggests that chronic low-level lead exposure has adverse health effects in adults and no blood lead threshold level for these effects has been identified."[31]

The risks posed by lead to pregnant and lactating women are particularly significant. Following a review of the science by its expert advisory panel, the CDC released guidance on the identification and management of lead exposure in pregnant and lactating women in 2010. While recommending public health follow-up action for such women with a blood lead level of 5 µg/dL or greater, the CDC made clear that there was no basis for determining a "safe" level of lead. It said that "Because there is no apparent threshold below which adverse effects of lead do not occur, CDC has not identified an allowable exposure level, level of concern, or any other bright line intended to connote a safe or unsafe level of exposure for either mother or fetus." [32]

Amongst children, studies have broadly identified cognitive and neurologic impacts with extremely low levels of lead exposure with no identifiable threshold. The 2012 NTP Monograph found sufficient evidence for lower IQ scores, decreased academic achievement, and increased incidence of attention-related behaviors and problem behaviors at blood lead levels below 5 µg/dL. [1] Sufficient evidence was also reported for delayed puberty, reduced postnatal growth, and decreased hearing at levels below 10 µg/dL. Limited evidence for other health impacts in children were also noted.

In 2012, the CDC moved away from a childhood blood level of concern, above which children should receive services to address their exposures, and towards a population-based reference level to target children with the highest levels. In doing so, CDC specifically noted that there is no safe level of exposure to lead for children. [33] The CDC's Advisory Committee on Childhood Lead Poisoning Prevention, in its recommendation leading to the elimination of the level of concern, noted that, "[b]ecause no measurable level of blood lead is known to be without deleterious effects, and because once engendered, the effects appear to be irreversible in the absence of any other interventions, public health, environmental and housing policies should encourage prevention of all exposures to lead." [34]

The Environmental Protection Agency (EPA) has reached similar conclusions as to the health risks caused by lead in adults and children, and also found that there is no evidence of a threshold for harmful effects in children. The agency's 2013 Integrated Science Assessment for Lead found causal relationships between lead exposure and cognitive function impairments, impulsivity and hyperactivity, and delayed pubertal onset in children, as well as causal relationships between lead and hypertension, coronary heart disease, blood disorders, and male reproductive function in adults. [35] In support of the lack of a safe level of lead exposure amongst children, the agency also noted that, "…it is clear that Pb exposure in childhood presents a risk; further, there is no evidence of a threshold below which there are no harmful effects on cognition from Pb exposure." [35]

EPA's Integrated Risk Information System (IRIS) program has also provided evaluations of the toxicity of inorganic lead compounds. The agency first explored creating an Oral Reference Dose (RfD), an estimate of "…a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime," in 1985.[36] The agency decided an RfD value was simply inappropriate because, in part, "[i]t appears that some of these effects, particularly changes in the levels of certain blood enzymes and in aspects of children's neurobehavioral development, may occur at blood lead levels so low as to be essentially without a threshold."[36,] The agency reviewed this position in 2004 and opted not to change it. [36] As a result of this and similar findings, EPA set the maximum contaminant level goal (MCLG) for lead in drinking water as zero.[20]

Petitioners maintain that FDA should agree with the conclusions of its sister federal agencies who have considered the issue that there is no safe level of exposure to lead. Using FDA's terminology in 21 CFR § 70.40, the "maximum no-effect level" of lead acetate in humans is zero. No safety factor is sufficiently large to be generally recognized as appropriate. Therefore, the use of lead acetate as a color additive is unsafe because there is no longer convincing evidence of a reasonable certainty of no harm.

IV. FDA's 1980 decision rested primarily on a single industry study

FDA's decision to list lead acetate rested largely on a skin absorption study funded by the manufacturer of a lead acetate containing hair dye. In the late 1970s, FDA decided that there was insufficient scientific evidence as to the extent which lead acetate is absorbed through the scalp, and challenged the industry to provide evidence.[21] Combe, Inc., the manufacturer of Grecian Formula, responded with a proposed study to utilize radioactive tracers to measure dermal absorption of lead acetate, and the FDA extended the provisional listing of lead acetate multiple times to allow the study to be conducted and to consider the findings.[22] Only after receiving and reviewing this study, and no other

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[20] 40 CFR § 141.501(b)

[21] As described by the agency in summarizing the procedural history at 45 *Federal Register* 72113, October 31, 1980.

[22] 45 *Federal Register* 72114, October 31, 1980

additional studies on skin absorption, did the agency make the determination to permanently list lead acetate. While the study may have used advanced techniques at the time, the post-1980 evidence makes clear that this industry-funded study had serious flaws, and many of its findings have since been contradicted.

The study, as utilized by the FDA, had not been published in a peer reviewed journal, and was simply cited by the Agency as, "Goldberg, A. and M.R. Moore, 'Lead Absorption Study for Combe, Incorporated, August 1978,' report submitted to FDA, September 1978."[23] The study was later published in *Food and Cosmetics Toxicology* with M.R. Moore as the first author. [37]

Moore, et al.[24], in short, placed four different samples of lead acetate spiked with radioactive lead-203 on the foreheads of eight men[25], and measured the amount of lead-203 in the blood and excreted in urine over 24 hours, as well as the amount present in the "whole-body" through a count collected through a ring of apparatuses around the calves at 12 and 24 hours after the exposure. The "whole-body" count was calibrated using an intravenous administration of a known amount of lead-203 chloride. Using primarily the whole-body count, Moore, et al., calculated the amount of lead acetate absorbed and applied this percentage to the amount of lead they believed to reach the scalp of the user of a hair dye, resulting in about 0.4 µg absorbed per application.[26]

Especially in light of more recent studies which show more extensive absorption described in detail in the next section, there were a number of limitations and flaws in the Moore, et al., paper. Most notably, in drawing their conclusions, they discarded the scenarios which resulted in the highest exposure. One of the four samples of lead acetate was placed on "scratched" skin – skin that had a sterile needle dragged across it lightly enough NOT to draw blood. This slightly damaged skin resulted in an average of two to three times as much absorption as the average across intact skin. Moore, et al, note in their discussion that results from the "wet" and "cream" applications may be artificially lower because of a loss of the lead acetate to the cover over it, meaning it wasn't in contact with the skin. However, all the results of the higher "scratched" cells were excluded from the final analysis, while all the lower "wet" and "cream" tests were included.

Moore, et al., also excluded all of the results of the 24-hour "whole-body" count, relying on the 12-hour data after deciding that the increased absorption from 12 to 24 hours reflected, in part, "mechanical damage" to the skin from washing the test material off after 12 hours. One could argue that a thorough washing is not necessarily an unrealistic activity, in addition to the fact (admitted by Moore, et al.) that additional absorption and

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[23] 45 *Federal Register* 72116, October 31, 1980

[24] As the unpublished version provided to the FDA is not available to the petitioners, we must base our understanding of the study from the published version.

[25] Given the clear documented toxicity of lead acetate, it is extremely unlikely that any reputable IRB would ever approve this study design today, a fact that in and of itself should speak to the need to eliminate its listing as a color additive.

[26] It is unclear to the petitioners how or why this is slightly different than the 0.5 µg referenced by the agency in its documents.

transport may have occurred between the 12 and 24 hour measurements. The 24 hour non-scratch average absorption was just over two times greater than the average from the 12 hour non-scratch average.

Had Moore, et al., utilized the "worst case scenario" from their data, a rational approach when evaluating safety, and used the 24-hour measurement on scratched skin, the average absorption value would have been nearly 4.5 times higher than they reported. Even a simple averaging of all the "whole-body" scans would have resulted in a value twice as high as their selective approach yielded.

Inherent in their calculation of the "whole-body" count is also an assumption on the transport and distribution of lead compounds. Moore, et al, calibrated the count on the basis of the transport and distribution through the body and to the calf of an IV solution of a known quantity of lead-203 chloride. This fundamentally assumes the transport and distribution of lead acetate through the skin to follow a similar path. However, more recent studies, discussed in the next section, at best, call this assumption into question, and it is unclear how this may have caused Moore, et al.'s calculation of "whole-body" count to actually accurately represent the amount of lead absorbed and present in the entire body. They may have only measured a portion of the lead absorbed if it was not proportionately transferred to the blood and/or tissue in the calf area in the way the lead chloride injection was.

Even if one accepts the absorption rates calculated by Moore, et al, as accurate, there is absolutely no data or even source to support Moore, et al.'s calculations for applying these figures to actual products. Moore, et al., in their discussion simply state, "Since 6 ml of cosmetic is normally applied, of which 0.18 ml of will reach the scalp, 612 μg of lead will reach the scalp for each application and become available for absorption." This 612μg of lead times the .058% average absorption results in the 0.355μg of lead absorbed. There is no reference or calculation for how they determined that 6 ml constitutes a normal use, nor how they determined that that 97% of the product applied never makes it to the skin. Currently, the label directions for Liquid Grecian Formula, a leading lead acetate based hair dye, make no reference to the amount to use, leaving it to the user to find the amount necessary to make their hair slightly damp.[27] It is quite logical that the amount actually used would depend greatly on the amount of hair. Similarly, the amount reaching the scalp would also, logically, vary greatly on hair length and application method. A small change in the assumption on the amount of the product reaching the scalp vastly changes the calculation on the amount of lead absorbed. If only 94% remained in the hair or elsewhere than the scalp instead of 97%, the quantity of lead absorbed from the same application doubles. This calculation also excludes the lead that may be absorbed through the skin of the hands while applying the product to the hair.[28]

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[27] https://grecian-formula.com/pdf/GrecianFormula_Instructions.pdf

[28] The current instructions for Grecian Formula specifically call for the user to pour the product into one's palms and then apply to the hair. In the frequently asked questions attached to instructions, it answers, "Do I need to wear plastic gloves?" with, "No. No gloves, caps or tests." https://grecian-formula.com/pdf/GrecianFormula_Instructions.pdf

There is no evidence that the amount of lead acetate reaching the scalp is based on peer-reviewed or even sound science, nor is there any documentation that the FDA considered the accuracy of these assumptions or considered the additional absorption from the skin contact of the product with the hands.

V.   Exposure evidence since 1980 shows that skin absorption of lead acetate may be more significant than FDA considered

Several studies have been published since 1980 demonstrating the capacity of the skin to absorb lead is more significant than FDA estimated in 1980. The studies varied from occupational exposures to in vivo (human and animal) and in vitro (using human or animal skin) testing.

Eight years after the approval of lead acetate, researchers showed that lead could be absorbed through the skin and rapidly distributed throughout the body. [38] A simple experiment of applying a dose of 6 mg of lead on the left arm and measuring the levels of the chemical in the right arm sweat was illuminating for two main reasons:

- *Lead transport was very quick*: The elevated lead levels were found within 2 hours of starting the application. The levels of lead in sweat also continue to raise and peaked at 48 hours after the start of the experiment.
- *The blood lead levels were not increased:* Although lead in sweat and saliva increased, no measurable increment was observed in blood which was assumed to be the carrier for the lead. The authors concluded that "lead must be transported in plasma and rapidly concentrated into the extracellular fluid pool of sweat and saliva without significant uptake by erythrocytes." They also stated that this type of transportation differs from that of lead that enters the body through ingestion.

The findings from the 1988 publication by Florence et al. were corroborated by many other articles that also show lead that penetrates the skin reaches multiple organs. [39-42] '' Lead skin penetration was confirmed in *in vitro* assays using animal or human skin and measuring how much lead moved through it. [43] This type of experiment allowed for assessing different exposure scenarios such as the effects of skin abrasion or personal care product (e.g. hand soaps) on the absorption of lead. Using human skin, it was found that hand soap containing sodium lauryl sulfate and sodium laureth sulfate, both very common ingredients, increased lead skin penetration by 8-fold when the skin was intact compared to non-cleansed samples. [44] Damaged skin also increased lead penetration nine times in the absence of soap and four times after the soap was applied.

Scratches in hands and scalp are not uncommon and sulfates mentioned above are very common ingredients not only in soaps but also in shampoo. And it is fair to assume that greater amounts of lead will penetrate the skin under normal conditions of use of the lead acetate-containing hair dye. These conditions were not explicitly taken into consideration at the time of FDA approval of lead acetate. The likely underestimation of the amount of lead entering the body through dermal conditions makes it even more important and

urgent to remove the approval of lead acetate as color additive to hair dye. This exposure is completely unnecessary and its contribution to the body burden of lead may be greater than anticipated.

VI. Overall exposure to lead have dropped since 1980 so FDA's conclusion that the exposure was insignificant is no longer valid

At the time FDA approved the use of lead acetate as color additive in hair dyes it concluded that the "increase of absorbed lead from hair dyes over the normal 'background' levels of lead does not augment the existing risk of acute or chronic toxicity, including cancer, in any clearly discernible, much less significant manner."[44]

FDA also estimated that an average of 0.3 microgram of lead would be absorbed through the user's skin per day and deemed this level insignificant because adults at that time were likely to absorb 35 micrograms from a daily exposure of 100-500 micrograms of lead.[29] The average blood lead level was also high, 17 µg/dL.[30]

Since that decision in 1980, both exposures and blood lead levels have dropped dramatically as a result of Congressional action to limit lead in consumer products and reduce exposure to the legacy of lead uses. As reported by the Agency for Toxic Substances and Disease Registry's Toxicological Profile for Lead, the National Human Exposure Assessment Survey published in 1999 found the adult mean daily exposure to lead was approximately 36 micrograms, [45] an approximately 3 to 14 times reduction from the exposures referred to by FDA.

These reduced exposures have resulted in reduced blood lead levels. The National Health and Nutrition Examination Survey (NHANES) found the mean blood lead levels amongst adults in the United States in 2009-10 to be 1.2 µg/dL. [30] Although this level is 14 times lower than in 1980, it is not a level without potential health effects as we mentioned is Section III.

Given the substantial reductions in other exposures, even by FDA's standards in 1980, the levels of skin absorbed lead are no longer insignificant. Whereas the 0.3 microgram daily average absorption for a dye user was assumed to represent less than 1% of their total absorption, the same amount today would likely represent 12%.[31] In addition, the scientific evidence is that any amount of lead is significant. Intentional exposures such as from hair dyes can no longer be considered safe under the safety standard for color additives described in Section I given the increased knowledge of hazards associated with lead.

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[29] 45 *Federal Register* 72113, October 31, 1980.
[30] *Id.* at 72114.
[31] Presuming the amount of lead typically absorbed today reflects the similar 14 times reduction in blood lead levels and similar 14 times reduction in estimated exposures.

In addition, two cases indicate that the use of lead acetate as a color additive poses real risks.

- In 2014, a case study reported on peripheral neuropathy from use of lead acetate as a color additive. A man in the United States applied lead acetate hair dye to his hair and beard for seven months. Apparently, he did not notice, understand, or follow the label instructions limiting use to the scalp. His doctor alertly made the connection after he experienced tingling and then numbness in his feet and hands. [46] His blood lead levels were 17.3 µg/dL, fourteen times higher than the mean levels seen in US adults. The levels gradually dropped to within "normal limits" over six months after the man stopped using the hair dye. The peripheral neuropathy symptoms were gone in a year. It is likely that many other lead acetate hair dye users have made the same mistake and their doctor never made the connection or published a study describing the clinical case.

- In January, 2015 an entry in FDA's CFSAN Adverse Event Reporting System [47] describes a 66-year old man that presented "hypotrichosis, metal poisoning, blood pressure increased." The suspect, in FDA parlance, was the progressive hair coloring Grecian Formula cream. High blood pressure is one of the adverse health effects NTP reported is associated with lead exposure in adults. Although there is no additional information and FDA advises to be cautious with the interpretation of the events reported, the symptoms and the suspected cosmetic are a good match. We found three additional reports on the same product.

VII.   Post-1980 evidence indicates that lead acetate is likely to be ingested from typical use

In its 1980 decision, FDA found that lead acetate would not be ingested as a result of its use as a color additive in a hair dye.[32] It cited no studies to back up the conclusion.

In 1997, Mielke, et al. documented that lead was likely to be ingested from various brands of lead acetate hair dyes when used according to manufacturer's instructions. [48] They showed that 153 to 689 micrograms of lead coated the user's hands after applying products containing lead acetate, and 26 to 79 micrograms of lead remained on the hands even after washing them. They also tested surfaces touched by the user or the user's hair in the process of application, including the comb used in the hair, the handle of the blow dryer, the outside of the hair dye container, the faucet that was touched while washing hands after use, and a telephone receiver touched before the hands were washed. All these surfaces were found to have lead with values up to 2,804 micrograms of lead per square foot. Considering the frequent application of the hair dye, the contamination of household objects shared by members of the family, including children, ensures lead is a constant presence that will contaminate people's hands and make ingestion likely.

For comparison, in 2001, the EPA said that more than 40 micrograms of lead per square foot on the floor posed a hazard to children.[33] In 2009, EPA acknowledged that this level

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[32] 45 *Federal Register* 72115, October 31, 1980.
[33] 40 CFR § 745.65(b).

needed to be updated in light of recent science showing that those levels posed an excessive risk.[34] No user of lead acetate hair dye imagines that everyday objects in his home will be contaminated with lead at levels 70 times higher than the standard for allowable lead-based paint dust on the floor.

The Mielke, et al. study also measured the lead that could still be removed from dry hair following application of lead acetate dyes. Simply passing a dry hand through the hair resulted in as much as 286 micrograms of lead on the hand. Passing a wet wipe through the dry hair five times after treatment with the dye collected 601 micrograms of lead on the wipe.

Given the lead remaining on a users' hands, even after washing, and the lead contaminating other objects following use, it is difficult to see how they would not end up transferring the lead to their mouth or food. Others, including children, using the contaminated objects would also be exposed. The frequency of hand-to-mouth activity has been extensively studied. The EPA, in its Exposure Factors Handbook [49], provides estimates ranging from an average of 20 times per hour for one year old children (and a 95th percentile of 63 times per hour) to 7 times per hour for six to ten-year old children (with a 95th percentile of 21 times per hour).

The evidence is clear that the lead remaining on the hair and on objects poses a risk of transfer to both the user and quite likely their partner or child who may touch it. In such cases, the user, partner, or child would likely not be aware of the contamination and have no reason to wash their hands. They too will almost certainly ingest some lead as a result. Given the evidence presented by Mielke, et al., and the simple logic of how these products are used in practice demonstrates that lead ingestion as a result is not just possible, but is likely.[35] As FDA acknowledged in 1980, if the lead acetate used as a hair dye MAY be ingested, the use is absolutely barred.[36]

VIII. Canada and Europe found the use of lead acetate as a color additive to be unsafe

Health and safety regulators in other countries have taken action to prohibit lead acetate broadly in cosmetics, including its use as a hair dye or color additive, on the basis of its toxicity and documented skin absorption.

Canadian statute prohibits the sale of any cosmetic that "(a) has in or on it any substance that may cause injury to the health of the user when the cosmetic is used (i) according to the directions on the label or accompanying the cosmetic, or (ii) for such purposes and by

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[34] EPA accepted a petition to start a proceeding to revise the lead dust standard based on evidence presented. The petition and EPA's response are available at https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/tsca-section-21-petition-requesting-epa-lower-lead-dust.
[35] Note that Mielke did not allow actual ingestion given the risks posed by lead.
[36] 21 U.S.C. § 379e((b)(5)(B) and 21 CFR § 70.50(a). See also 45 *Federal Register* 72117, October 31, 1980

such methods of use as are customary or usual therefor."[37] Based on this provision, Health Canada maintains a "Cosmetic Ingredient Hotlist" of ingredients restricted or prohibited from use in cosmetic products for sale in Canada. In May of 2005, lead acetate, previously restricted, was prohibited entirely. Health Canada noted, "This ingredient is no longer restricted but prohibited for use in cosmetic products based on data indicating skin absorption and possible links to carcinogenicity and reproductive toxicity." [50] In subsequent media interviews, Health Canada made clear that this prohibition included the use of lead acetate in hair dyes.[51]

Lead acetate is also prohibited from use in cosmetic products, including hair dyes, within the European Union. "Lead and Lead Compounds" are "Annex II" ingredients with a strict prohibition under Regulation (EC) No 1223/2009 governing cosmetic safety.[38] Prior to revisions of the regulation, in 2004, the European Commission's Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) was asked to review the safety of lead acetate in hair dye based on safety data provided by industry.[39] SCCNFP determined that there was, essentially, no need to conduct a review as lead acetate had already been classified by the EU as "toxic to reproduction, category 1; R61. 'May cause harm to the unborn child.' Therefore, SCCNFP is of the opinion that lead acetate is covered by its existing opinions and should not be intentionally added to cosmetic products." [52]

Alternatives to lead acetate are widely available. Manufacturers of well known "progressive" dyes using lead acetate, have altered their formulas to utilize other chemicals, such as bismuth citrate, to meet the Canadian and European standards or customer concerns about lead.[40] More traditional permanent dyes, and a range of non-metallic gradual dyes for hair coloring are available, as readily identified by a web search for "hair coloring."

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[37] Section 16(a), Food and Drugs Act, Revised Statutes of Canada (1985, c. F-27). Retrieved from http://laws.justice.gc.ca/eng/acts/F-27/page-2.html#h-7.

[38] Regulation (EC) No 1223/2009 as amended. Accessible at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009R1223.

[39] SCCNFP noted that, following a general opinion on carcinogenic, mutagenic or toxic for reproduction (CMR) ingredients, it was asked to review lead acetate in light of data provided by COLIPA (a trade association, now known as Cosmetics Europe) and Combe International (a manufacturer of a lead acetate containing hair dye).

[40] Youthair specifically markets the fact that their bismuth citrate based "LEAD FREE Youthair® Crème" does not contain lead acetate. http://www.youthair.com/youthair-products/lead-free-youthair/.

IX.   Request for Fee Waiver

Pursuant to 21 CFR 70.19(q), petitioners request a waiver of the color additive petition fees and deposit requirements. The petitioners are non-profit organizations and individuals who submit this petition because it is in the public interest to protect public health by reducing exposure to lead. They have no financial interests in lead acetate or any of the alternatives that may benefit from removing this color additive from the market.

To avoid delays in processing, we are enclosing a check for $1600 to cover the petition fee should the request for waiver not be granted. We understand that the agency will not cash the check until a final decision is made on that request be denied.

X.   Summary

With this color additive petition, we ask that FDA strike 21 CFR § 73.2396 because evidence since FDA made its decision in 1980 demonstrates lead acetate is readily absorbed by the skin, quickly transported to various organs including the brain, and is likely to be ingested as a result of use as a hair dye. Additionally, multiple science-based organizations and federal agencies have stated that there is no safe level of lead exposure. Therefore, petitioners conclude that there is no longer convincing evidence that establishes with reasonable certainty that no harm will result from the intended use of the color additive as required by law.

Appendix 1 provides additional details on the petition required by 21 CFR Part 172; Appendix 2 supplies relevant scientific evidence on the toxicity and skin absorption of lead acetate. Appendix 3 is an excerpt of the 2007 summary of health effects and risks from dermal exposure by the ATSDR. Appendix 4 provides reports on the carcinogenicity of the lead acetate since the 2002 review by NTP.

This letter and all the following appendices, constitute our complete color additive petition.[41] We have enclosed three copies per 21 CFR § 71.1. This petition contains no confidential information, so we ask that FDA include it in the docket for any regulatory action it takes so the public can assess the information.

If you have questions or comments, please contact Tom Neltner, our agent on this petition, at tneltner@edf.org or 317-442-3973, and copy Eve Gartner at egartner@earthjustice.org, and Dr. Maricel Maffini at drmvma@gmail.com on all responses.

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[41] Please note that this is NOT a citizen petition.

Sincerely,

[signature: Tom Neltner]

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Appendices:

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Appendix 1
Responses to elements required by 21 CFR § 71.1

Per 21 CFR § 71.1, we provide responses to the requested elements of a color additive petition with one element per page.

Identity, Composition, Properties, and Specifications of Color Additive
The identity of the color additive is described in 21 CFR § 73.2396(a) and (b) reprinted below:

(a) *Identity.* The color additive lead acetate is the trihydrate of lead (2+) salt of acetic acid. The color additive has the chemical formula Pb (OOCCH3)2·3H2O).

(b) *Specifications.* Lead acetate shall conform to the following specifications and shall be free from impurities other than those named to the extent that such impurities may be avoided by good manufacturing practice:

Water-insoluble matter, not more than 0.02 percent. pH (30 percent solution weight to volume at 25 °C), not less than 4.7 and not more than 5.8.
Arsenic (as As), not more than 3 parts per million.
Lead acetate, not less than 99 percent.
Mercury (as Hg), not more than 1 part per million.

Amount of Color Additive Proposed for Use
We are asking FDA to remove its approval for the use of lead acetate as a color additive, effectively banning the use since all color additives must be specifically approved in its regulations.

Enforcement Methods
We are asking FDA to remove its approval for the use of lead acetate as a color additive, effectively banning the use since all color additives must be specifically approved in its regulations. FDA could enforce it by testing hair dyes for the presence of lead. If found, it could conduct additional investigation.

Estimation of Color Additive Exposure
We are asking FDA to remove its approval for the use of lead acetate as a color additive, effectively banning the use since all color additives must be specifically approved in its regulations.

We did not conduct an updated exposure assessment. Based on the available data, there is insufficient information upon which to conduct an assessment. We maintain that we have demonstrated the exposure assessment conducted by FDA in 1980 significantly underestimated the risk in two ways:
- FDA relied on studies that used blood lead levels as the biomarker of exposure. Subsequent studies showed that lead acetate is absorbed and transported to various organs but no increase in blood lead levels were recorded.

- FDA only considered the risk of skin absorption and did not consider the risk of incidental ingestion by the user and others who may be exposed due to widespread contamination of the room where the dye use takes place. Subsequent studies show that the user and others are likely to be exposed via ingestion as a result.

Full reports of Investigations Made with Respect to the Safety of the Color Additive
See Appendix 2 and 3.

Batch Certification
We are asking FDA to remove its approval for the use of lead acetate as a color additive, effectively banning the use since all color additives must be specifically approved in its regulations. No batch certification is needed.

Full information on each proposed change to the original regulation
Delete 21 CFR § 73.2396.

Environmental component
This color additive petition is categorically excluded from the need to prepare an Environmental Assessment under 21 CFR § 25.32(m) as an "action to prohibit or otherwise restrict or reduce the use of a substance in food, food packaging, or cosmetics." Approval of the petition will reduce the use of lead in a cosmetic. Therefore, it complies with the categorical exclusion criteria. We have identified no extraordinary circumstances as defined at 21 CFR § 25.21 for the action requested in this petition which would require the submission of an Environmental Assessment.

Where hair dyes manufacturers need substitutes, the color additives approved by FDA in its regulations provide many alternatives. While some of the alternative color additives were approved by FDA before the National Environmental Policy Act was adopted and have not been reassessed by the agency for their current risk, we did not identify a potential for serious harm to the environment or protected species from the marginal increase in production or use of these alternatives.

Appendix 2
Review of Studies Regarding Skin Absorption of Lead Acetate since FDA's 1980 Decision
(Sorted by date – newest to oldest)

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
Toxicology of lead (2016 by American Academy of Pediatrics)	Literature review / policy statement	Not applicable	Not applicable	Not applicable	Lead and lead compounds	All	Blood lead concentrations have decreased dramatically in US children abstract over the past 4 decades, but too many children still live in housing with deteriorated lead-based paint and are at risk for lead exposure with resulting lead-associated cognitive impairment and behavioral problems. Evidence continues to accrue that commonly encountered blood lead concentrations, even those below 5 μg/dL (50 ppb), impair cognition; there is no identified threshold or safe level of lead in blood. From 2007 to 2010, approximately 2.6% of preschool children in the United States had a blood lead concentration ≥5 μg/dL (≥50 ppb), which represents about 535,000 US children 1 to 5 years of age. Evidence-based guidance is available for managing increased lead exposure in children, and reducing sources of lead in the environment, including lead in housing, soil, water, and consumer products, has been shown to be cost-beneficial. Primary prevention should be the focus of policy on childhood lead toxicity.	[53] American Academy of Pediatrics, Prevention of Childhood Lead Toxicity, 2016. http://pediatrics.aappublications.org/content/early/2016/06/16/peds.2016-1493

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
Exposure assessment (2015 by NHANES)	Biomonitoring	Not applicable	Not applicable	Not applicable	Lead and lead compounds	Blood lead levels (also urinary lead)	People (1 year and older) - 1999-2000 – mean 1.66 µg/dL, 95th 5.00 µg/dL - 2001-2002 – mean 1.45 µg/dL, 95th 4.50 µg/dL - 2003-2004 – mean 1.43 µg/dL, 95th 4.20 µg/dL - 2005-2006 – mean 1.29 µg/dL, 95th 3.91 µg/dL - 2007-2008 – mean 1.27 µg/dL, 95th 3.70 µg/dL - 2009-2010 – mean 1.12 µg/dL, 95th 3.34 µg/dL Adults (20 years and older) - 1999-2000 – mean 1.75 µg/dL, 95th 5.00 µg/dL - 2001-2002 – mean 1.56 µg/dL, 95th 4.50 µg/dL - 2003-2004 – mean 1.52 µg/dL, 95th 4.30 µg/dL - 2005-2006 – mean 1.41 µg/dL, 95th 4.12 µg/dL - 2007-2008 – mean 1.38 µg/dL, 95th 3.90 µg/dL - 2009-2010 – mean 1.23 µg/dL, 95th 3.57 µg/dL	[54] CDC, National Report on Human Exposure to Environmental Chemicals – Updated Tables, 2015. http://www.cdc.gov/exposurereport/.
Exposure assessment (2014 by Deeb et al.)	Clinical study	Dermal (hair and beard)	Hair dye product	Approximately 8 months	Lead acetate	Blood, electromyography	Blood lead level was 17.3 µg/dL, fourteen times higher than the mean US adult population exposure. Electromyography suggested polyneuropathy. Levels gradually decreased to within normal limits over 6 months after hair dye use stopped. Patient's symptoms completely resolved with normal electromyography within 1 year.	[46] Deeb et al. J Clin Neuromusc Dis (2014). 15, 161-163. https://www.ncbi.nlm.nih.gov/pubmed/?term=J+Clin+Neuromusc+Dis+(2014).+15%2C+161-163
Weight-of-the-evidence evaluation (NTP in 2012	Literature review	Not applicable	Not applicable	Not applicable	Lead and lead compounds	Not applicable	Overall, the NTP concludes that there is sufficient evidence that blood Pb levels <10 µg/dL and <5 µg/dL are associated with adverse health effects in children and adults. In adults, there is sufficient evidence that blood Pb levels <5 µg/dL are associated with decreased renal function and that blood Pb levels <10 µg/dL are associated with	[1] National Toxicology Program (2012). https://ntp.niehs.nih.gov/ntp/ohat/lead/final/

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
							increased blood pressure and hypertension. There is sufficient evidence that maternal blood Pb levels <5 μg/dL are associated with reduced fetal growth and limited evidence that maternal blood Pb levels <10 μg/dL are associated with increased spontaneous abortion and preterm birth. There is sufficient evidence that blood Pb levels <10 μg/dL, and limited evidence that blood Pb levels <5 μg/dL, are associated with essential tremor in adults. There is also limited evidence for an association between blood Pb <10 μg/dL and increased cardiovascular-related mortality, decreased auditory function, the neurodegenerative disease amyotrophic lateral sclerosis (ALS), and decreases in specific measures of cognitive function in older adults. The NTP conclusions of associations between blood Pb levels <10 μg/dL in adults and health effects cannot completely eliminate the potential contributing effects of early-life blood Pb levels	monographhealtheffectslowlevellead_newissn_508.pdf
Dermal penetration (2010 by Pan et al.) (*In vitro* part)	*In vitro*, nude mouse skin (intact and without stratum corneum).	Dermal	120 mM	10 hours	Lead acetate and lead nitrate	Skin, buffer containing lead nitrate or lead acetate that permeated across the skin.	Uptake values of 11.10 and 14.38 μg/mg of lead acetate and lead nitrate, respectively, were found within the skin. Uptake increased 2.3 to 2.5-fold in the stratum corneum-stripped skin. Less lead accumulated in the skin with lead was present in simulated sweat compared to distilled water.	[42] Pan et al. Toxicology Letters (2010) 197, 19-28. https://www.ncbi.nlm.nih.gov/pubmed/?term=Toxicology+Letters+(2010)+197%2C+19-28

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
Dermal penetration (2010 by Pan et al.) (*In vivo* part)	*In vivo*, nude mouse.	Dermal	120 mM	120 hours	Lead acetate and lead nitrate	Skin, blood, liver, and kidney.	No lead was detected in plasma after 120 hours of exposure. Lead was found in skin, liver and kidney. Lead acetate had greater skin deposition than lead nitrate. Lead acetate concentration in the skin was 4.5 µg/mg, in the liver 0.0003 µg/mg and in the kidney 0.015 µg/mg. Lead nitrate concentrations in liver and kidney were slightly higher.	[42] Pan et al. Toxicology Letters (2010) 197, 19-28. https://www.ncbi.nlm.nih.gov/pubmed/?term=Toxicology+Letters+(2010)+197%2C+19-28
Exposure assessment (2009 by NHANES)	Literature review (biomonitoring levels are updated in the 2015)	Not applicable	Not applicable	Not applicable	Lead and lead compounds	Blood lead levels (also urinary lead)	In occupationally exposed adults, subtle or nonspecific neurocognitive effects have been reported at BLLs as low as 20-30 µg/dL (Mantere et al., 1984; Schwartz et al., 2001), with overt encephalopathy, seizures, and peripheral neuropathy generally occurring at much higher levels (e.g., higher than 100-200 µg/dL). BLLs higher than 40 µg/dL can result in proximal tubular dysfunction and decreased glomerular filtration rate leading to interstitial and peritubular fibrosis when high body burdens persist. Low level environmental lead exposure may be associated with small decrements in renal function (Kim et al., 1996; Muntner et al., 2003; Payton et al., 1994). Results of studies of adults with either occupational or environmental lead exposure have shown consistent associations between increased BLLs and increased blood pressure (Nash et al., 2003; Schwartz, 1995; Staessen et al., 1995) and associations between increased bone lead concentrations and blood pressure (Hu et al., 1996; Korrick et al., 1999).	[55] CDC, National Report on Human Exposure to Environmental Chemicals, 2009. http://www.cdc.gov/exposurereport/.

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
							High dose occupational lead exposure, usually with BLLs greater than 40 mg/dL, may alter sperm morphology, reduce sperm count, and decrease fertility (Alexander et al., 1996; Telisman et al., 2000). At low environmental exposures, lead in women may be associated with hypertension during pregnancy, premature delivery, and spontaneous abortion (Baghurst et al., 1987; Bellinger 2005; Borja-Aburto et al., 1999).	
Weight-of-evidence evaluation (2008 by Health Canada)	Literature review	Dermal	Lead acetate in hair dyes	Not specified	Lead acetate	Not specified	Relatively small incremental exposure, such as those which would occur with regular use of hair dyes containing lead acetate, could result in the accumulation of potentially harmful body burdens of lead.	[3] Abstract # 3.18 by Koniecki and Healey from the Book of Abstracts. 2008 Health Canada Science Forum http://publications.gc.ca/site/eng/371511/publication.html
Toxicology assessment (2007 by ATSDR)	Literature review	Not applicable	Not applicable	Not applicable	Lead and lead compounds	Not applicable	See Appendix 3 for summary of health effects and of dermal absorption.	[45] ATSDR, Toxicological Profile for Lead, 2007. https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
Dermal penetration (2006 by Filon et al.)	*In vitro*, human skin	Dermal	5 mg per square centimeter in 2 mL synthetic sweat	30 minutes and 24 hours	Lead oxide	Buffer (receiving fluid) containing lead oxide that permeated across the skin and skins.	Lead oxide can pass through intact human skin with a median amount at 24 hour of 2.9 ng/cm^2; lead penetration increased 9-fold when the skin was abraded. When skin was exposed for only 30 min and then cleaned with soap, lead has been found to penetrate completely through the fullness of the skin. Median 19.7 ng/cm^2 and 7.1 ng/cm^2 for soaps 1 and 2, respectively. Lead intact skin content after 24 h was 321.3, 19.7 and 10.5 ng/cm^2 for not washed, washed with soap 1 and washed with soap 2, respectively. Increase in blood lead levels was estimated assuming three exposure scenarios: hands; hands and arms; and hands, arms, head and neck. For 250 days a year and a 5 mg lead oxide/cm^2 exposure the median steady-state increase in blood lead due to uptake through the unwashed skin was 0.7, 2.5 and 3.6 µg/dL for each of the three scenarios. If the skin is washed with soap 1, the median lead blood levels are higher: 5.4; 20.2 and 29.2 µg/dL.	[44] Filon et al. J Occup Environ Med (2006). 48, 692-699. https://www.ncbi.nlm.nih.gov/pubmed/?term=J+Occup+Environ+Med+(2006).+48%2C+692-699%22
Dermal penetration (2002 by Sun) (Human part)	*In vivo*, human	Dermal	Occupational: lead battery workers.	Occupational: 5 days	Lead stearate, lead sulfate, lead oxide, lead powder.	Skin stripping from exposed and unexposed areas Blood	Using Stauber et al. estimate that 29% of lead nitrate was absorbed from the skin in 24 hour, total skin absorption within 24 hour application to the human skin were calculated as follows: lead stearate: 5.7%; lead sulfate: 4.8%; lead oxide: 4.6%; lead powder: 1.9%. Using information from the skin stripping, the estimated range of absorption efficiency in 8 hour of work exposure was 0.13 µg to 2.27 µg depending on the ratio of lead sulfate, oxide and powder on the skin surface.	[39] Sun et al. Am Industrial Hygiene Assn. (2002) 63, 641-646. https://www.ncbi.nlm.nih.gov/pubmed/?term=Am+Industrial+Hygiene+Assn.+(2002)+63%2C+641-646

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
Dermal penetration (2002 by Sun) (Rat part)	*In vivo*, human	Dermal	100 mg	12 days	lead stearate, lead sulfate, lead oxide, lead powder, and lead naphthenate	Urine	Lead naphthenate was the earliest to appear in urine and produced the highest concentration, followed by nitrate, stearate, sulfate, oxide and lead powder. Based on lead in urine and assuming lead nitrate absorption is 100%, the percentage of lead absorption for stearate, sulfate, oxide and powder is 19.8, 16.7, 15.7, and 6.5, respectively.	[39] Sun et al. Am Industrial Hygiene Assn. (2002) 63, 641-646. https://www.ncbi.nlm.nih.gov/pubmed/?term=Am+Industrial+Hygiene+Assn.+(2002)+63%2C+641-646
Dermal penetration (1998 by Florence et al.)	*In vivo*, mouse	Dermal	6.4 mg of lead	2 hour to 1 week	Lead acetate and lead nitrate	Urine, feces, blood, organs (skin and muscle remote from exposure patch, patch muscle, pancreas, spleen, kidney, liver, mid-gut, cecum, bone, brain, heart)	A total analysis of the organs, feces and urine showed that, of the 6.4 mg Pb applied to the skin, 26 µg or 0.4% was absorbed through the skin and enter the circulatory system in 21 hour. Skin absorption of lead increased with time and, in general, reach maximum organ concentration 24-48 h depending on the organ. There was no significant increase in blood lead concentration.	[41] Florence et al. J. Nutrit. & Environ. Med. (1998) 8, 19-23. http://www.tandfonline.com/doi/abs/10.1080/13590849862267
Exposure assessment (1997 by Mielke et al)	Epidemiology	Dermal	Hair dye products	One use	Lead acetate	Amount on hand, comb, faucet handle, product container,	*Hand* - before application = < 1 to 3 µg/hand - after application = 153-689 µg/hand - after washing = 26-79 µg/hand - dry hand passing through dry hair = 69-286 µg/hand	[48] Mielke, et al., Journal of the American Pharmaceutical Association, Vol. NS37,

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
						blow dryer handle	- wet hand passing through dry hair = 23-86 μg/hand *Other* - Comb – 289-1,804 μg/ft^2 - Faucet handle – 120-417 μg/ft^2 - Product container- 153-2,804 μg/ft^2 - Telephone - 674 μg/ft^2 - Blow dryer - 628 μg/ft^2	No. 1, pages 85-89. January/February 1997. http://www.japha.org/article/S0003-0465(15)32422-8/abstract
Dermal penetration (1994 by Stauber et al.)	*In vivo*, human	Dermal	5 mg lead enriched with ^{204}Pb	24 and 48 hours	Lead acetate, lead nitrate	Sweat, whole blood, plasma and urine	Lead, as nitrate or acetate, was rapidly absorbed through the skin and detectable in sweat, blood and urine within 6 hour of skin application. Of the 5 mg of lead applied to the skin in one experiment, 1.3 mg was absorbed into or through the skin in 24 hour. This is 29% absorption. Over 24 hour, the average increase in ^{204}Pb concentration in blood was 0.12 μg/L which is the equivalent of 0.2% of the skin-absorbed ^{204}Pb. After 24 hour, much of the lead absorbed may have been retained in the epidermis and not yet absorbed into blood capillaries. 0.006% of the absorbed ^{204}Pb was excreted in urine; this is the equivalent of 6% of the ^{204}Pb increase in blood.	[40] Stauber et al., Sci. Total Environ. 145 (1994) 55-70. https://www.ncbi.nlm.nih.gov/pubmed/?term=Sci.+Total+Environ.+145+(1994)+55-70
Dermal penetration (1990 by Bress and Bidanset) (*In vitro* part)	*In vitro*, guinea pig and human skin	Dermal (excised skin from autopsy)	10 mg to 1.3 cm^2 of skin	24 hours	Lead acetate (others also studied)	Amount diffused through skin into saline	Human @37ºC = 5 μg ±0.9 Guinea pig @ 37ºC = 3 μg ±0.2 @ 23ºC = 5 μg ±0.5	[43] Brees and Bidanset, Vet Hum Toxicol 33 (1991) 212-214. http://europepmc.org/abstract/med/1858297

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
Dermal penetration (1990 by Bress and Bidanset) (*In vivo* part)	*In vivo*, guinea pig	Dermal – (shaved back skin)	300 mg/kg-bw on 2 cm^2 of skin	Daily for 7 days	Lead acetate (others also studied)	Amount in blood, brain, liver and kidney	Blood = 0.47 µg ±0.03 Liver = 0.65 µg ±0.05 Brain = 0.65 µg ±0.04 Kidney = 0.82 µg ±0.08	[43] Brees and Bidanset, Vet Hum Toxicol 33 (1991) 212-214. http://europepmc.org/abstract/med/1858297
Dermal penetration (Lilley, Florence, Stauber 1988)	*In vivo*, human	Dermal	6.2 mg lead	24 hours	Lead nitrate	Sweat, saliva, whole blood, urine	Lead in sweat collected from the opposite arm where exposure occurred increased from 25 µg Pb/L to 261 and 370 µg Pb/L at 21 and 48 hours, respectively. Lead in sweat and saliva increases rapidly by 7.7 and 3.5 fold, respectively after 3 hours of exposure. Blood and urine lead levels did not increase significantly. Authors did not estimate percentage of lead absorbed by the skin.	[56] Lilley et al. Sci Total Environ. 76 (1988) 267 278. https://www.ncbi.nlm.nih.gov/pubmed/?term=The+Sci+Total+Environ.+76+(1988)+267+278
Dermal penetration (1980 by Moore et al.)	*In vivo*, human	Dermal	0.1 mL of a 6 mmol lead acetate/L hydroalcoholic solution containing ^{203}Pb 0.1 mL of cream containing 9 mmol	12 hour/ exposure 5 different exposures/ person Six months, total	Lead acetate	Blood, forehead and whole body radioactivity, urine	Approximately 1% (up to 7% in one case) of the applied dose remained in the forehead following wash-off. Mean uptake of ^{203}Pb activity as a percentage of the dose in 1) total blood volume: 0.023 ± 0.021% 2) whole body count: 0.058 ± 0.081%. The total absorption of 0.337 µg of lead in the whole body or 0.3% of the applied dose was estimated using the following assumptions: 6 ml of cosmetic is applied of which 0.18 ml reach the	[37] Moore et al. Food Cosmet Toxicol (1980). 18, 399-405. http://www.sciencedirect.com/science/article/pii/0015626480901972

Study type	Model used	Route of exposure	Concentration of exposure	Duration of exposure	Lead compound tested	Biomarkers of exposure	Findings and conclusions	Reference and hyperlink
			lead/kg, also spiked with ^{203}Pb				scalp corresponding to 612 μg of lead available for absorption each time.	

Appendix 3
Excerpts of Key Sections from Toxicological Profile for Lead by the Agency for Toxic Substances and Disease Registry in 2007 [45]

2.2 SUMMARY OF HEALTH EFFECTS

An enormous amount of information is available on the health effects of lead on human health. In fact, the toxic effects of lead have been known for centuries, but the discovery in the past few decades that levels of exposure resulting in relatively low levels of lead in blood (e.g., <20 μg/dL) are associated with adverse effects in the developing organism is a matter of great concern. Most of the information gathered in modern times regarding lead toxicity comes from studies of workers from a variety of industries and from studies of adults and children in the general population. The most sensitive targets for lead toxicity are the developing nervous system, the hematological and cardiovascular systems, and the kidney. However, due to the multi-modes of action of lead in biological systems, lead could potentially affect any system or organs in the body.

Studies of lead workers suggest that long-term exposure to lead may be associated with increased mortality due to cerebrovascular disease. The same was found in a study of adults from the general population who were hospitalized for lead poisoning during childhood. Population studies suggest that there is a significant association between bone-lead levels and elevated blood pressure. Blood lead levels (PbBs) also have been associated with small elevations in blood pressure. Between the two biomarkers, bone lead appears to be the better predictor. Lead also affects kidney functions; glomerular filtration rate appears to be the function affected at the lowest PbBs. Decreased glomerular filtration rate has been consistently observed in populations with mean PbB <20 μg/dL and two studies have reported effects at PbB <10 μg/dL. Lead may alter glomerular filtration rate by several mechanisms.

Lead has long been known to alter the hematological system by inhibiting the activities of several enzymes involved in heme biosynthesis. Particularly sensitive to lead action is δ-aminolevulinic acid dehydratase (ALAD). Inhibition of ALAD activity occurs over a wide range of PbBs beginning at <10 μg/dL. The anemia induced by lead is primarily the result of both inhibition of heme synthesis and shortening of erythrocyte lifespan, but lead also can induce inappropriate production of the hormone erythropoietin leading to inadequate maturation of red cell progenitors, which can contribute to the anemia.

A recent study in children 8–10 years of age suggested that lead accelerates skeletal maturation, which might predispose to osteoporosis in later life. Lead also has been associated with increased occurrence of dental caries in children and periodontal bone loss, which is consistent with delayed mineralization in teeth observed in studies in animals. Current mean PbBs in these cohorts were <5 μg/dL; however, the cross-sectional nature of the studies precluded assessment of the exposure history.

Changes in circulating levels of thyroid hormones, particularly serum thyroxine (T4) and thyroid stimulating hormone (TSH), generally occurred in workers having mean PbB ≥40–60 μg/dL. Altered serum levels of reproductive hormones, particularly follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone have been observed at PbB ≥30–40 μg/dL. Lead also has been shown to decrease circulating levels of the active form of vitamin D, 1,25-

dihydroxyvitamin D, in children with moderate to high PbB (30–60 μg/dL), but not in children with low to moderate PbB (average lifetime PbB between 4.9 and 23.6 μg/dL, geometric mean, 9.8 μg/dL). Normal levels of vitamin D are important for maintaining calcium homeostasis. Altered immune parameters have been described in lead workers with PbB in the range of 30–70 μg/dL. Reported effects included changes in some T-cell subpopulations, response to T-cell mitogens, and reduced chemotaxis of polymorphonuclear leukocytes. Several studies of children reported significant associations between PbB and increases in serum IgE levels. IgE is the primary mediator for type-I hypersensitivity and is involved in various allergic diseases such as asthma. These findings in children along with results from studies in rodents exposed *in utero* have led some to suggest that lead may be a risk factor for childhood asthma, although a recent relatively large study (4,634 children) found that PbB was less a predictor of asthma than was race.

Exposure to high amounts of lead resulting in PbBs of 100–120 μg/dL in adults or 70–100 μg/dL in children produce encephalopathy, a general term that describes various diseases that affect brain function. Symptoms develop following prolonged exposure and include dullness, irritability, poor attention span, epigastric pain, constipation, vomiting, convulsions, coma, and death. Lead poisoning in children can leave residual cognitive deficits that can be still detected in adulthood. Neurobehavioral effects including malaise, forgetfulness, irritability, lethargy, headache, fatigue, impotence, decreased libido, dizziness, weakness, and paresthesia have been reported in lead workers with PbBs in the range of 40–80 μg/dL. Also, PbBs between 40 and 80 μg/dL have been associated with neuropsychological effects in lead workers. A recent study of lead workers reported that higher tibia lead was associated with increased prevalence and severity of white matter lesions, as assessed by brain MRI. Studies of older populations with current mean PbBs <10 μg/dL have reported associations between PbB and/or bone lead and poorer performance in neurobehavioral tests. Lead also has been shown to affect nerve conduction velocity and postural balance in workers with PbB in the range of 30–60 μg/dL. Alterations of somatosensory evoked potentials also have been reported in lead workers with mean PbBs in the range of 30–50 μg/dL.

As previously mentioned, one of the major concerns regarding lead toxicity is the cognitive and neurobehavioral deficits that are observed in children exposed to lead. Prospective studies have provided the greatest amount of information. Analyses of these and other studies suggest that an IQ decline of 1–5 points is associated with an increase in PbB of 10 μg/dL. Of special interest and concern are the results of recent studies that have reported neurobehavioral deficits in children associated with PbBs <10 μg/dL and an apparent lack of threshold down to even the lowest PbBs recorded in these studies. Lead also has caused neurobehavioral alterations in developing animals, and at PbBs similar to those reported in children. Studies in animals, particularly in monkeys, have provided key information for the interpretation of a cognitive basis for IQ changes. Studies of children also have shown associations between PbB and growth, delayed sexual maturation in girls, and decreased erythropoietin production.

Some studies of humans occupationally or environmentally exposed to lead have observed associations between PbB and abortion and preterm delivery in women and alterations in sperm and decreased fertility in men. On the other hand, there are several studies that found no significant association between lead exposure and these end points. At least for the effects in males, the threshold PbB appears to be in the range of 30–40 μg/dL. Studies have shown that lead can affect the association of protamines with DNA in sperm cells from exposed males. Lead does so by competing or reducing zinc in protamine P2 *in vivo*, which would leave sperm chromatin and DNA open to damage from other exposures.

In vitro mutagenicity studies in microorganisms have yielded mostly negative results for lead, but lead is a clastogenic agent, as shown by the induction of chromosomal aberrations, micronuclei and by sisterchromatid exchanges in peripheral blood cells from lead workers. Studies of cancer in lead workers have been inconclusive. A meta-analysis of eight major occupational studies on cancer mortality or incidence in workers with high lead exposure concluded that there is some limited evidence of increased risk of lung cancer and stomach cancer, although there might have been confounding with arsenic exposure in the study with highest relative risk of lung cancer. The results also showed a weak evidence for an association with kidney cancer and gliomas. In the only study of the general population available, there was suggestive evidence for an increased risk of cancer mortality in women, but not men, with a threshold PbB of 24 μg/dL. This study used data from the Second National Health and Nutrition Survey (NHANES II) Mortality Study. Lead has produced primarily renal tumors in rodents by a mechanism not yet elucidated. Some nongenotoxic mechanisms that have been proposed for lead-induced cancer include inhibition of DNA synthesis and repair, alterations in cell-to-cell communication, and oxidative damage.

The Department of Health and Human Services (DHHS) has determined that lead and lead compounds are reasonably anticipated to be human carcinogens based on limited evidence from studies in humans and sufficient evidence from animal studies. The EPA has determined that lead is a probable human carcinogen based on sufficient evidence from studies in animals and inadequate evidence in humans. The International Agency for Research on Cancer (IARC) has determined that inorganic lead is probably carcinogenic to humans based on sufficient evidence from studies in animals and limited evidence of carcinogenicity from studies in humans. IARC also determined that organic lead compounds are not classifiable as to their carcinogenicity in humans based on inadequate evidence from studies in humans and animals.

Neurodevelopmental Effects. Lead can impair cognitive function in children and adults, but children are more vulnerable than adults. The increased vulnerability is due in part to the relative importance of exposure pathways (i.e., dust-to-hand-mouth) and differences in toxicokinetics (i.e., absorption of ingested lead). Although the inhalation and oral routes are the main routes of exposure for both adults and children, children are more likely to have contact with contaminated surfaces due to playing on the ground and to hand-to-mouth activities. Furthermore, children absorb a larger fraction of ingested lead than adults. However, perhaps more important is the fact that the developing nervous system is especially susceptible to lead toxicity. During brain development, lead interferes with the trimming and pruning of synapses, migration of neurons, and neuron/glia interactions. Alterations of any of these processes may result in failure to establish appropriate connections between structures and eventually in permanently altered functions. Because different brain areas mature at different times, the final outcome of the exposure to lead during development (i.e., *in utero* vs. pediatric exposure) will vary depending on the time of exposure. This has been demonstrated in studies in animals. The time of exposure-specific response appears to have contributed to the failure to identify a "behavioral signature" of lead exposure in children. Other factors that may affect individual vulnerability are certain genetic polymorphisms, such as that for the vitamin D receptor, the lead-binding enzyme ALAD, or the APOE genotype. One important additional factor shown to influence the toxicity of lead is the characteristics of the child's rearing environment, a modifying factor. It has been argued that effect modification is a property of a true association and should be distinguished from confounding. Effect modification can explain inconsistencies in findings, and if it exists, failure to address it will lead to an error in inference. For example, if social class is an effect modifier of the association between PbB and IQ, and differs between two cohorts, the strength of the association based on these two studies will necessarily be different.

Despite the many factors that can potentially work against finding agreement among studies, the preponderance of the evidence indicates that lead exposure is associated with decrements in cognitive function. Meta-analyses conducted on cross-sectional studies or a combination of cross-sectional and prospective studies suggest that an IQ decline of 1–5 points is associated with an increase in PbB of 10 μg/dL. Most importantly, no threshold for the effects of lead on IQ has been identified. This has been confirmed by a series of recent studies in children that found significant inverse associations between cognitive function and PbBs <10 μg/dL. Moreover, these and other studies have shown that the slope of the lead effects on cognitive variables is steeper (the effect is greater) at lower than at higher PbBs (supralinear dose-response relationship). However, there is not complete agreement on the interpretation of the lack of linearity in the dose-response relationship among the scientific community. Some have argued, based on a theoretical statistical analysis, that the supra-linear slope is a required outcome of correlations between data distributions where one is log-normally distributed and the other is normally distributed. Perhaps the strongest evidence for nonlinearity is provided by an international pooled analysis of seven prospective studies (details in Section 3.2.4). After testing several models, these investigators determined that the shape of the dose-response was nonlinear insofar as the quadratic and cubic terms for concurrent PbB were statistically significant (p<0.001, p=0.003, respectively). Additional support for the steeper slope at low PbB was provided by plotting the individual effects estimates for each of the seven cohorts, adjusted for the same covariates. The plot showed that the studies with the lowest mean PbBs had a steeper slope compared with studies with higher PbBs. Yet further evidence for nonlinearity was presented when the data were divided at two cut-points *a priori* (maximal PbB above and below 10 μg/dL and above and below 7.5 μg/dL). The investigators then fit separate linear models to the data in each of those ranges and compared the PbB coefficients for the concurrent PbB index. The stratified analyses showed that the effects estimate for children with maximal PbB <7.5 μg/dL was significantly greater (*p*=0.015) than those with a maximal PbB ≥7.5 μg/dL. Similar results were seen at the cut-off point of 10 μg/dL. A reanalysis of the pooled studies found that a log-linear relationship between PbB and IQ was a better fit within the ranges of PbBs in the studies than was a linear relationship (*p*<0.009). Collectively, the results of the pooled analysis and of additional studies provide suggestive evidence of lead effects on cognitive functions in children at PbBs <10 μg/dL and, possibly as low as 5 μg/dL. It should be stressed, however, that the effects of lead on IQ and other neurobehavioral scores are very small compared with the effects of other factors such as parents' IQ, but is also important to stress that lead exposure, unlike most of those other factors, is highly preventable.

The other aspect that has been questioned regarding the nonlinear shape of the dose-response relationship is the apparent lack of a biological mechanism that could produce this result, and this clearly represents a data need. To explain the nonlinear shape of the dose-response, it was proposed that "the initial damage caused by lead may reflect the disruption of different biological mechanisms than the more severe effects of high exposures that result in encephalopathy or frank mental disability. This might explain why, within the range of exposures not producing overt clinical effects, an increase in PbB beyond a certain level might cause little *additional* impairment in children's cognitive function."

While measurements of IQ are convenient in that they allow comparison across populations of different demographic and cultural characteristics, and help define the extent of the public health issue, they only partially advance our understanding of the problem of lead-induced behavioral toxicity. It is important to elucidate the underlying basis of the deficits in IQ as well as the behavioral mechanisms that account for them. It was noted that "the answers are critical not only to further define neurobiological mechanisms associated with learning deficits, but also to determine behavioral or neurochemical therapeutic approaches to alleviate them." Studies in

animals have provided answers to some of these questions. Studies in animals have great utility because the possibility of confounding is reduced with the controlled experimental design and genetic factors. In addition, they address specific domains of cognitive function and allow determination of critical periods of exposure. Results of behavioral tests performed primarily in rats and monkeys exposed to lead have suggested that the impaired performance is the result, at least in part, of a combination of distractibility, inability to inhibit inappropriate responding, and perseveration in behaviors that are no longer appropriate. Evaluation of children exposed to lead with different subscales of IQ tests in conjunction with assessments of behavior on teacher's rating scales on young school-age children suggest that increased distractibility, impulsivity, short attention span, and inability to follow simple and complex sequences of directions are associated with increased lead body burden. The similarity between neurobehavioral effects in lead-exposed children and in animals, and the fact that the deficits are observed at similar PbBs should stimulate continued research to elucidate the biochemical and morphological substrates that underlie specific behaviors.

Although the decrement of IQ points in children associated with lead exposure is generally small, lead neurotoxicity may have major implications for public health when exposure is considered in terms of large populations and its preventable nature. One study quantified the economic benefits from projected improvements in worker productivity resulting from the reduction in children's exposure to lead in the United States since 1976. Based on data from NHANES (a study designed to provide national estimates of the health and nutritional status of the U.S. civilian noninstitutionalized population aged 2 months and older) and meta-analyses, it was estimated that mean PbBs declined 15.1 µg/dL between 1976 and 1999 and that IQ scores increased between 0.185 and 0.323 points for each 1 µg/dL blood lead concentration. It was further estimated that each IQ point raises worker's productivity by 1.76–2.38%, and that the economic benefit for each year's cohort of 3.8 million 2-year-old children ranges from $110 to $319 billion. In another study, using an environmentally attributable fraction model, it was estimated that the present value of economic losses in the United States attributable to lead exposure in amounts to $43.4 billion per year in each annual birth cohort. More recently, one study estimated that mild mental retardation and cardiovascular outcomes resulting from exposure to lead amounts to almost 1% of the global burden of disease, with the highest burden in developing regions.

A related and important issue is whether lead-lowering interventions, such as with chelators, are paralleled by improvement in health outcomes reportedly altered by lead. In one study, improvement in cognitive functions was related to decreases in blood lead but not to chelation treatment. In a multi-center study of 780 children, chelation therapy lowered blood lead by a mean of 4.5 µg/dL during the 6 months after initiation of treatment, but it did not improve scores on tests of cognition, behavior, or neuropsychological function in children with PbB below 45 µg/dL. Re-analysis of these data showed that improvement in test scores was associated with greater falls in PbB only in the placebo group. A further evaluation of this cohort showed that chelation therapy lowered blood lead, but produced no benefits in cognitive, behavioral, or neuromotor end points. The conclusion of this series of studies reached by the investigators was that chelation therapy is not indicated in children with moderate blood lead levels. Thus, it appears that lead abatement must remain the primary approach in the public health management of lead poisoning.

Cardiovascular/Renal Effects. Although lead has been shown to produce various cardiovascular and renal effects in animals, end points of greatest concern for humans at low exposures and low PbB are elevations in systemic blood pressure and decrements in glomerular filtration rate. These effects may be mechanistically related and, furthermore, can be confounders and covariables in

epidemiological studies. Decrements in glomerular filtration rate may contribute to elevations in blood pressure, and elevated blood pressure may predispose people to glomerular disease.

Effects on Blood Pressure. Numerous covariables and confounders affect studies of associations between PbB and blood pressure, including, age, body mass, race, smoking, alcohol consumption, ongoing or family history of cardiovascular/renal disease, and various dietary factors. Varying approaches and breadth of inclusion of these may account for some of the disparity of results that have been reported. Including confounders in a regression model will attenuate the apparent association between lead exposure and the measured health outcome. Measurement error may also be an important factor. Blood pressure estimates based on multiple measurements or, preferably, 24-hour ambulatory measurements, are more reproducible than single measurements. Few studies have employed such techniques and, when used, have not found significant associations between PbB and blood pressure.

An additional limitation of blood lead studies, in general, is that PbB may not provide the ideal biomarker for long-term exposure to target tissues that contribute a hypertensive effect of lead. Bone lead appears to be a better predictor of lead-induced elevations in blood pressure than PbB. In a recent prospective analysis of the Normative Aging Study, higher tibial lead levels, but not PbBs, were associated with higher systolic blood pressure and abnormalities in electrocardiographic conduction.

Chronic lead exposure increases blood pressure in rats through diverse mechanisms that include alterations in neurohumoral control of peripheral vascular resistance, heart rate, and cardiac output (see Section 3.4.2). Studies conducted in animal models provide strong evidence for the plausibility of lead elevating blood pressure in humans. Meta-analyses of the epidemiological findings have found a persistent trend in the data that supports a relatively weak, but significant association. Quantitatively, this association amounts to an increase in systolic blood pressure of approximately 1 mmHg with each doubling of PbB. The results of more recent epidemiology studies indicate that the lead contribution to elevated blood pressure is more pronounced in middle age than at younger ages. A longitudinal study of males, mean age 67 years, found positive associations between systolic blood pressure and bone lead concentrations, and increased risk of hypertension in association with increased bone lead concentration. Based on this study, an increase in patella bone lead from the midpoint of the lowest quintile (12.0 μg/g) to the highest quintile (53.0 μg/g) was associated with a 1.71-fold increase in hypertension risk (rate-ratio, 95%; confidence interval [CI], 1.08–2.71). A case-control study of women, ages >55 years, found increased risk of hypertension in association with increased bone lead concentration. In this study, an increase in patella bone lead from 6 to 31 μg/g was associated with a 1.86-fold (odds ratio [OR], 95%; CI, 1.09–3.19) increase in risk of hypertension. A large-scale cross-sectional analysis of the NHANES III data on males and females, age 40–59 years, found increasing risk for hypertension in association with increasing PbB, with higher risks in postmenopausal women than in premenopausal women. Risks of diastolic hypertension for pre- and postmenopausal women, combined, who were in the highest blood lead quartile (mean, 6.4 μg/dL; range, 3.0–31.1) was predicted to be 3.4-fold higher (OR, 95%; CI, 1.3– 8.7) than that of women in the lowest quartile (mean, 1 μg/dL; range, 0.5–1.6); corresponding risks for postmenopausal women were 8.1 times greater (OR, 95%; CI, 2.6–24.7) (highest vs. lowest quartile). The results of two analyses of the NHANES III data on adult subjects provides evidence for an association between increasing PbB and increasing blood pressure that is more pronounced in blacks than whites. Lead exposures during infancy and childhood (reflected in PbB) have been associated with increased blood pressure and altered responses to acute pressor stresses in childhood. Lead poisoning in childhood has also been associated with hypertension during adulthood in the absence of clinically significant renal disease and discernable elevations in PbB.

Effects in Renal Glomerular Filtration. Classic lead nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis, and interstitial fibrosis and related functional deficits, including enzymuria, low- and high-molecular weight proteinuria, impaired transport of organic anions and glucose, and depressed glomerular filtration rate. In humans, the overall dose-effect pattern suggests an increasing severity of nephrotoxicity associated with increasing PbB, with effects on glomerular filtration evident at PbBs below 10 µg/dL, enzymuria and proteinuria becoming evident above 30 µg/dL, and severe deficits in function and pathological changes occurring in association with PbB exceeding 50 µg/dL. Thus, the renal effects of greatest concern, at low exposures (i.e., low PbB), are on glomerular filtration.

The results of epidemiological studies of general populations have shown a significant effect of age on the relationship between glomerular filtration rate (assessed from creatinine clearance of serum creatinine concentration) and PbB (see Section 3.2.2. Renal Effects). Furthermore, as noted previously, hypertension can be both a confounder in studies of associations between lead exposure and creatinine clearance as well as a covariable with lead exposure. Another important complication in the assessment of associations between lead exposure and adverse effects on glomerular filtration is the potential confounding effect of decrements in glomerular filtration rate and increased lead body burden. Lead exposure has also been associated with increases in glomerular filtration rate. This may represent a benign outcome or a potentially adverse hyperfiltration, which may contribute to subsequent adverse renal effects. Increases in glomerular filtration rate have been observed in the early phases of development of chronic renal injury in rats. When age and other covariables that might contribute to glomerular disease are factored into the dose-response analysis, decreased glomerular filtration rate has been consistently observed in populations that have average PbBs <20 µg/dL, with some studies finding effects at PbBs <10 µg/dL (see Section 3.2.2, Table 3-4). Two studies provide evidence for an effect at lead concentrations below 10 µg/dL. A longitudinal study found a significant relationship between increasing serum creatinine concentration and increasing PbB below 10 µg/dL. A cross-sectional analysis of data from the NHANES III found increased risk of chronic renal disease (defined as severely depressed glomerular filtration rate) in association with PbB <6 µg/dL. The confounding and covariable effects of hypertension are also relevant to the interpretation of the regression coefficients reported in these studies. Given the evidence for an association between lead exposure and hypertension, and that decrements in glomerular filtration rate can be a contributor to hypertension, it is possible that the reported hypertension-adjusted regression coefficients may underestimate the actual slope of the PbB relationship with serum concentration of creatinine or creatinine clearance.

Hematological Effects. The adverse hematological effects of lead are mainly the result of its perturbation of the heme biosynthesis pathway. The activity of ALAD, an enzyme occurring early in the heme synthesis pathway, is negatively correlated with PbBs between 5 and 95 µg/dL. Although inhibition of ALAD occurs at very low exposure levels, there is some controversy as to the toxicological significance of a depression in ALAD activity in the absence of a detectable effect on hemoglobin levels. Nevertheless, because the impairment of heme synthesis has a far-ranging impact not limited to the hemopoietic system, there is concern that developing organisms might be particularly susceptible.

A potential consequence of the inhibition of heme synthesis is a decreased formation of mixed function oxidases in the liver resulting in impaired metabolism of endogenous compounds, as well as impaired detoxification of xenobiotics. Mitochondrial cytochrome oxidase is another heme-requiring protein that could be affected by heme synthesis inhibition. In addition, tryptophan pyrrolase, a hepatic heme-requiring enzyme system, is inhibited via the reduction in

the free hepatic heme pool. This could ultimately lead to increased levels of the neurotransmitter serotonin in the brain and increased aberran neurotransmission in serotonergic pathways. Inhibition of heme synthesis also results in increased levels of δ-aminolevulinic acid (ALA), which has a structure similar to that of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), and therefore, interferes with GABA neurotransmission. Finally, a prospective study of children with moderate PbB (25–40 µg/dL) and hemoglobin levels within normal limits found that serum erythropoietin (EPO) was positively associated with PbB at ages 4.5 and 6.5 years, but the magnitude of the association gradually declined from 4.5 to 12 years. EPO is a glycoprotein hormone produced in the kidney that regulates both steady-state and accelerated erythrocyte production. This suggested that in nonanemic children with moderate PbB, hyperproduction of EPO is necessary to maintain normal hemoglobin concentrations. The decline in slope with age suggested that the compensatory mechanism gradually begins to fail due to direct lead-induced inhibition of EPO production or indirectly through toxic effects of lead on the kidney. Inhibition of EPO production may contribute to lead-induced anemia. Anemia occurs at PbBs of ≥20 µg/dL.

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3.3.1.3 Dermal Exposure

Inorganic Lead. Dermal absorption of inorganic lead compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure; however, few studies have provided quantitative estimates of dermal absorption of inorganic lead in humans, and the quantitative significance of the dermal absorption pathway as a contributor to lead body burden in humans remains an uncertainty. Lead was detected in the upper layers of the stratum corneum of lead-battery workers, prior to their shifts and after cleaning of the skin surface (Sun et al. 2002), suggesting adherence and/or possible dermal penetration of lead. Following skin application of [203]Pb-labeled lead acetate in cosmetic preparations (0.12 mg Pb in 0.1 mL or 0.18 mg Pb in 0.1 g of a cream) to eight male volunteers for 12 hours, absorption was ≤0.3%, based on whole-body, urine and blood [203]Pb measurements, and was predicted to be 0.06% during normal use of such preparations (Moore et al. 1980). Most of the absorption took place within 12 hours of exposure. Lead also appears to be absorbed across human skin when applied to the skin as lead nitrate; however, quantitative estimates of absorption have not been reported. Lead (4.4 mg, as lead nitrate) was applied (vehicle or solvent not reported) to an occluded filter placed on the forearm of an adult subject for 24 hours, after which, the patch was removed, the site cover and the forearm were rinsed with water, and total lead was quantified in the cover material and rinse (Stauber et al. 1994). The amount of lead recovered from the cover material and rinse was 3.1 mg (70% of the applied dose). Based on this recovery measurement, 1.3 mg (30%) of the applied dose remained either in the skin or had been absorbed in 24 hours; the amount that remained in or on the skin and the fate of this lead (e.g., exfoliation) was not determined. Exfoliation has been implicated as an important pathway of elimination of other metals from skin (e.g., inorganic mercury; Hursh et al. 1989). Lead concentrations in sweat collected from the right arm increased 4-fold following the application of lead to the left arm, indicating that some lead had been absorbed (amounts of sweat collected or total lead recovered in sweat were not reported). In similar experiments with three subjects, measurements of [203]Pb in blood, sweat and urine, made over a 24-hour period following dermal exposures to 5 mg Pb as [203]Pb nitrate or acetate, accounted for <1% of the applied (or adsorbed) dose. This study also reported that absorption of lead could not be detected from measurements of lead in sweat following dermal exposure to lead as lead carbonate.

Information on relative dermal permeability of inorganic and organic lead salts of lead comes from studies of *in vitro* preparations of excised skin; the rank ordering of penetration rates through excised human skin were: lead nuolate (lead linoleic and oleic acid complex) > lead naphthanate > lead acetate > lead oxide (nondetectable) (Bress and Bidanset 1991).

Studies conducted in animals provide additional evidence that dermal absorption of inorganic lead is substantially lower than absorption from the inhalation or oral route. In a comparative study of dermal absorption of inorganic and organic salts of lead conducted in rats, approximately 100 mg of lead was applied in an occluded patch to the shaved backs of rats. Based on urinary lead measurements made prior to and for 12 days following exposure, lead compounds could be ranked according to the relative amounts absorbed (i.e., percent of dose recovered in urine; calculated by ATSDR): lead naphthalene (0.17%), lead nitrate (0.03%), lead stearate (0.006%), lead sulfate (0.006%), lead oxide (0.005%), and metal lead powder (0.002%). This rank order (i.e., lead naphthalene>lead oxide) is consistent with a rank ordering of penetration rates of inorganic and organic lead salts through excised skin from humans and guinea pigs: lead nuolate (lead linoleic and oleic acid complex) > lead naphthanate > lead acetate > lead oxide (nondetectable) (Bress and Bidanset 1991).

Following application of lead acetate to the shaved clipped skin of rats, the concentration of lead in the kidneys was found to be higher relative to controls, suggesting that absorption of lead had occurred (Laug and Kunze 1948). This study also observed that dermal absorption of lead from lead arsenate was significantly less than from lead acetate, and that mechanical injury to the skin significantly increased the dermal penetration of lead.

Organic Lead. Relative to inorganic lead and organic lead salts, tetraalkyl lead compounds have been shown to be rapidly and extensively absorbed through the skin of rabbits and rats (Kehoe and Thamann 1931; Laug and Kunze 1948). A 0.75-mL amount of tetraethyl lead, which was allowed to spread uniformly over an area of 25 cm^2 on the abdominal skin of rabbits, resulted in 10.6 mg of lead in the carcass at 0.5 hours and 4.41 mg at 6 hours (Kehoe and Thamann 1931). Tetraethyl lead was reported to be absorbed by the skin of rats to a much greater extent than lead acetate, lead oleate, and lead arsenate (Laug and Kunze 1948). Evidence for higher dermal permeability of organic lead compounds compared to inorganic organic salts of lead also comes from *in vitro* studies conducted with excised skin. The rank order of absorption rates through excised skin from humans and guinea pigs was as follows: tetrabutyl lead > lead nuolate (lead linoleic and oleic acid complex) > lead naphthanate > lead acetate > lead oxide (nondetectable) (Bress and Bidanset 1991).

Appendix 4
Reports on the carcinogenicity of the lead acetate

Goal: To support color additive petition to FDA to ban lead acetate based on carcinogenicity.

Search terms: (lead acetate) AND (Cancer OR carcinogenesis OR carcinogenic OR mutagenicity OR mutagenic OR genotoxicity OR gene toxicity OR DNA damage OR DNA adducts OR neoplasm OR carcinogen OR carcinogenicity OR tumor OR leukemia)

Dates: January 1, 2004 to August 16, 2016. Petitioners used 2004 as a starting point for its literature search because the National Toxicology Program (NTP) conducted a thorough assessment of the carcinogenicity of lead and lead compounds, including lead acetate, in 2003. [42,43] Since the petition is based on carcinogenicity, no other endpoints were evaluated.

Source: PubMed

Findings: 146 articles

Relevant articles: 1 human, 16 in vivo, 7 in vitro studies

A) <u>Relevant human studies</u>

Int Arch Occup Environ Health. 2012 Oct;85(7):807-18. doi: 10.1007/s00420-011-0726-4. Epub 2011 Dec 9.

Evaluation of genotoxic effects of lead in pottery-glaze workers using micronucleus assay, alkaline comet assay and DNA diffusion assay.

Kašuba V, Rozgaj R, Milić M, Zeljezić D, Kopjar N, Pizent A, Kljaković-Gašpić Z, Jazbec A.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/22160131

PURPOSE: We investigated genotoxic effects of occupational exposure to lead acetate in pottery-glaze ceramic workers.

METHODS: The study was carried out in 30 exposed workers and 30 matched controls, to whom several biochemical parameters-the blood lead (B-Pb; range: exposed, 41.68-

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[42] NTP, 13th Report on Carcinogens, Lead and Lead Compounds, 2014. See
https://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html and
https://ntp.niehs.nih.gov/ntp/roc/content/profiles/lead.pdf.
[43] NTP, Scientific Review of Lead and Lead Compounds, 2004. See
http://ntp.niehs.nih.gov/pubhealth/roc/listings/l/lead/summary/index.html.

404.77; controls, 12-52) and cadmium (B-Cd) level, the activity of delta-aminolevulinic acid dehydratase (ALAD), erythrocyte protoporphyrin (EP), the level of vitamin B(12) and folate in serum-were measured. The genotoxic effects were evaluated by the alkaline comet assay, the DNA diffusion assay and micronucleus test in peripheral blood lymphocytes.

RESULTS: Subjects exposed to lead had significantly higher B-Pb level and, consequently, increased values of tail intensity (TI), frequency of apoptotic and necrotic cells, and frequency of micronuclei (MN). In contrast, their activity of ALAD, the level of vitamin B(12) and folate in serum were significantly lower compared to controls. Poisson regression analysis showed a significant correlation of profession, duration of exposure, smoking, level of cadmium in blood, ALAD and EP with primary DNA damage. A majority of primary damage repairs in a short period after exposure to a genotoxic agent. In addition, the influence of gender and level of vitamin B(12) and folate in serum MN frequency in exposed group was observed.

CONCLUSIONS: In this study, DNA diffusion and micronucleus test showed higher influence of tested parameters to DNA damage. The results indicate a need for concomitant use of at least two different biomarkers of exposure when estimating a genetic risk of lead exposure.

B) <u>Relevant in vivo studies</u>

Silymarin and dimercaptosuccinic acid ameliorate lead-induced nephrotoxicity and genotoxicity in rats.

Alcaraz-Contreras Y, Mendoza-Lozano RP, Martínez-Alcaraz ER, Martínez-Alfaro M2, Gallegos-Corona MA, Ramírez-Morales MA, Vázquez-Guevara MA.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/26078282

We studied the effect of silymarin and dimercaptosuccinic acid (DMSA), a chelating agent that was administered individually or in combination against lead (Pb) toxicity in rats. Wistar rats (200 ± 20) were randomly divided into five groups. Group A served as a control. Groups B-E were exposed to 2000 ppm of lead acetate in drinking water for 8 weeks. Group B served as a positive control. Group C received silymarin (100 mg kg(-1) orally) for 8 weeks. Group D received DMSA (75 mg kg(-1) orally) once daily for the last 5 days of treatment. Group E received DMSA and silymarin as groups C and D, respectively. The effect of Pb was evaluated and accordingly the treatments on blood lead levels (BLLs), renal system, and genotoxic effects were calculated using comet assay. The BLLs were significantly increased following the exposition of lead acetate. The

administration of silymarin and DMSA provided reduction in BLLs. Silymarin and DMSA provided significant protection on the genotoxic effect of Pb. The toxic effect of Pb on kidneys was also studied. Our data suggest that silymarin and DMSA improve the renal histopathological lesions.

Protective role of omega-3 polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats.

Abdou HM, Hassan MA.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/25045676

The present study was conducted to investigate the protective role of Omega-3 polyunsaturated fatty acids against lead acetate-induced toxicity in liver and kidney of female rats. Animals were divided into four equal groups; group 1 served as control while groups 2 and 3 were treated orally with Omega-3 fatty acids at doses of 125 and 260 mg/kg body weight, respectively, for 10 days. These groups were also injected with lead acetate (25 mg/kg body weight) during the last 5 days. Group 4 was treated only with lead acetate for 5 days and served as positive control group. Lead acetate increased oxidative stress through an elevation in MDA associated with depletion in antioxidant enzymes activities in the tissues. Moreover, the elevation of serum enzymes activities (ALT, AST, ALP, and LDH) and the levels of urea and creatinine were estimated but total proteins were decreased. Also, lead acetate-treatment induced hyperlipidemia via increasing of lipid profiles associated with decline in HDL-c level. Significant changes of Hb, PCV, RBCs, PLT, and WBCs in group 4 were recorded. The biochemical alterations of lead acetate were confirmed by histopathological changes and DNA damage. The administration of Omega-3 provided significant protection against lead acetate toxicity.

Toxic effects of lead exposure in rats: involvement of oxidative stress, genotoxic effect, and the beneficial role of N-acetylcysteine supplemented with selenium.

Sharma S, Raghuvanshi BP, Shukla S.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/24579807

This study was carried out to investigate the in vivo protective role of N-acetylcysteine (NAC) per se, along with selenium (Se), against lead-induced hepatic, nephritic-oxidative, and neuronal-oxidative damage in rats. Lead acetate at a dose of 50 mg/kg body weight administered intraperitoneally for 3 days was preferred as the source of lead. Various oxidative stress markers such as reduced glutathione content, lipid peroxidation, superoxide dismutase, and catalase were measured to determine the degree of oxidative

damage and healing due to NAC (50 mg/kg body weight administered orally) and Se (0.5 mg/kg body weight administered orally) and were studied along with the activities of enzymes such as transaminases (aspartate aminotransferase/alanine aminotransferase), δ-aminolevulinic acid dehydratase, δ-aminolevulinic acid synthase, and acetyl cholinesterase activity. The genotoxic effect of lead also was studied in terms of DNA damage using comet assay. The effect of lead was studied in blood biochemical variables such as cholesterol, triglycerides, urea, uric acid, and creatinine. Our data suggest that supplementation of Se with NAC can improve the lead-induced biochemical oxidative stress in blood and tissue, the burden of lead on the body, and molecular alterations by recoupment in mean DNA damage.

Effect of melatonin administration on DNA damage and repair responses in lymphocytes of rats subchronically exposed to lead.

Martínez-Alfaro M, Hernández-Cortés D, Wrobel K, Cruz-Jiménez G, Rivera-Leyva JC, Piña-Zentella RM, Cárabez Trejo A.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/22142833

Lead exposure induces DNA damage, oxidative stress, and apoptosis, and alters DNA repair. We investigated the effects of melatonin co-administered to rats during exposure to lead. Three doses of lead acetate (10, 50 and 100mg/kg/day) were administered to rats during a 6-week period. Lymphocytes were analyzed. Lead exposure decreased glutathione (GSH) levels in blood, and at doses of 100mg/kg/day and 50mg/kg/day without melatonin, caused high levels of DNA damage, induced apoptosis, and altered DNA repair. Melatonin co-treatment did not attenuate the effects of lead at 100mg/kg/day, indicating that the effect of melatonin on GSH reduction is not sufficient to reduce the genotoxic effects of lead at this high dose. After 6 weeks of treatment, decreased weight gain was observed in high lead-dose groups (100mg/kg/day), with or without melatonin, and in medium-dose groups (50mg/kg/day) with melatonin, compared with the control group. The protective action of melatonin against lead toxicity is dependent on the dose of lead. Further pharmacological studies are needed to determine whether melatonin acts via melatonin membrane receptors on lymphocytes.

Effects of flaxseed oil on lead acetate-induced neurotoxicity in rats.

Abdel Moneim AE, Dkhil MA, Al-Quraishy S.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/21479539

It is well known that chronic exposure to lead (Pb(+2)) alters a variety of behavioral tasks in rats and mice. Here, we investigated the effect of flaxseed oil (1,000 mg/kg) on lead acetate (20 mg/kg)-induced brain oxidative stress and neurotoxicity in rats. The levels of Pb(+2), lipid peroxidation, nitric oxide (NO), and reduced glutathione (GSH) and the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) were determined in adult male albino rats. The level of Pb(+2) was markedly elevated in brain and blood of rats. This leads to enhancement of lipid peroxidation and NO production in brain with concomitant reduction in GSH, CAT, SOD, GR, GST, and GPx activities. These findings were associated with DNA fragmentation. In addition, lead acetate induced brain injury as indicated by histopathological changes of the brain. Treatment of rats with flaxseed oil resulted in marked improvement in most of the studied parameters as well as histopathological features. These findings suggest to the conclusion that flaxseed oil significantly decreased the adverse harmful effects of lead acetate exposure on the brain as well as Pb(+2)-induced oxidative stress.

Lead-, cadmium-, and arsenic-induced DNA damage in rat germinal cells.

Nava-Hernández MP, Hauad-Marroquín LA, Bassol-Mayagoitia S, García-Arenas G, Mercado-Hernández R, Echávarri-Guzmán MA, Cerda-Flores RM.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/19388847

Toxic agents can interfere with the male reproductive system at many targets. One of the major unresolved questions concerning male infertility is identification of its molecular origins. Clinical and animal studies indicate that abnormalities of spermatogenesis result from exposure to three toxic metals (lead acetate, cadmium chloride, and arsenic trioxide), but the effects on primary spermatocyte DNA of the male rat after chronic exposure to these metals have not been identified. The aims of this study were to analyze, in three independent experiments, the DNA damage induced by lead (Pb), cadmium (Cd), and arsenic (As) in rat germinal cells during three time periods, and to determine the relationship between DNA damage and blood Pb, blood Cd, and urine As levels. For lead acetate and cadmium chloride experiments, blood was collected by cardiac puncture, while for arsenic trioxide a 24-h urine sample was collected. Afterward, the animals were sacrificed by decapitation. Pachytene spermatocytes from rat testes were purified by

trypsin digestion followed by centrifugal elutriation. After establishment of cell purity and viability, DNA damage (tail length) was measured employing a single cell gel/comet assay. Significant DNA damage was found in primary spermatocytes from rats with chronic exposure (13 weeks) to toxic metals. In conclusion, these findings indicate that exposure to toxic metals affects primary spermatocyte DNA and are suggestive of possible direct testicular toxicity.

Hydrogen peroxide reduces lead-induced oxidative stress to mouse brain and liver.

Li RG, Li TT, Hao L, Xu X, Na J.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/18974911

Lead (Pb) intoxication may initiate many disorders in human and animals. This study investigates the role of exogenous hydrogen peroxide (H(2)O(2)) in inducing mouse tolerance to Pb exposure. Results showed that the simultaneous application of 1.2 microg H(2)O(2) per kg body weight efficiently protected mice against the Pb-caused injury, as revealed by decreased growth suppression caused by the Pb stress, increased antioxidative enzyme activity, reduced lipid peroxidation, and the protective effect on the nuclear DNA integrity. To our knowledge, this is the first finding of this sort.

Induction of micronuclei in rat bone marrow after chronic exposure to lead acetate trihydrate.

Alghazal MA, Sutiaková I, Kovalkovicová N, Legáth J, Falis M, Pistl J, Sabo R, Benová K, Sabová L, Váczi P.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/19106125

Lead increasingly contributes to pollution of the environment and may play a role in the development of adverse effects in the human and animal body. Data concerning its mutagenic, clastogenic, and carcinogenic properties have been conflicting. In this study, we evaluated the frequency of micronuclei in bone marrow erythrocytes of rats treated with lead acetate trihydrate. Outbred Wistar rats were exposed to a daily dose of 100 mg/L drinking water for 125 days. The mean value of the total number of micronuclei observed in polychromatic erythrocytes of female rats was significantly higher than that found in the control group (13.375 +/- 2.722 against 9.625 +/- 3.204 micronuclei/1000 cells; P = 0.024 in ANOVA). In exposed female animals, no significant reduction of the ratio of polychromatic to normochromatic erythrocytes was observed (0.990 +/- 0.228 against 1.208 +/- 0.195; P = 0.060 in ANOVA). The effects of lead acetate trihydrate in

male rats are both cytotoxic and genotoxic because of a decrease in ratio of polychromatic to normochromatic erythrocytes (0.715 +/- 0.431 against 1.343 +/- 0.306; P = 0.023, ANOVA followed by Tukey test) and an increase in frequency of micronucleated polychromatic erythrocytes (24.167 +/- 7.859 against 4.0 +/- 4.528 micronuclei/1000 cells; P < or = 0.001, ANOVA followed by Tukey test), respectively.

Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice.

Xu J, Lian LJ, Wu C, Wang XF, Fu WY, Xu LH.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/18226849

Oxidative stress is considered as a possible molecular mechanism involved in lead toxicity. This study was carried out to investigate whether lead acetate could induce oxidative stress in mice, and the following damages as well. Lead acetate was given orally to mice for 4 weeks at doses of 0, 10, 50, 100mg/kg body weight every other day, respectively. Production of reactive oxygen species (ROS) and malondialdehyde (MDA) were measured as indicators of oxidative stress. DNA damage in peripheral blood lymphocytes was determined by comet assay. Ultrastructure alteration was detected using transmission electron microscopy. The alterations of p53, Bax, and Bcl-2 expression were determined by western blotting. The results showed that lead acetate significantly increased the levels of ROS and MDA in mice. Meanwhile, severe DNA damage and ultrastructure alterations were obviously observed. In addition, p53 and Bax expressions increased and the imbalance of Bax/Bcl-2 occurred. Therefore, it strongly suggests that lead may induce oxidative stress and change the expressions of apoptosis-related proteins in mouse liver.

In vivo genotoxicity of mercury chloride and lead acetate: Micronucleus test on acridine orange stained fish cells.

Cavaş T.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/17889980

The genotoxic effects of mercury chloride and lead acetate were evaluated in vivo using the micronucleus (MN) assay on acridine-orange (AO) stained peripheral blood erythrocytes, gill and fin epithelial cells of Carassius auratus auratus. Fish were exposed to three different concentrations of mercury chloride (MC) (1 microg/, 5 microg/L and 10 microg/L) and lead acetate (LA) (10 microg/L, 50 microg/L and 100 microg/L) for 2, 4

and 6 days. A single dose of 5 mg/L cyclophosphamide was used as a positive control. In addition to micronuclei, nuclear buds (NBs) were assessed in the erythrocytes. The ratio of polychromatic and normochromatic erythrocytes (PCE/NCE) in peripheral blood was also evaluated to assess cytotoxicity. MN frequencies in all three tissues were elevated in fish exposed to both LA and MC. However, NBs showed different sensitivity to metal treatments. MN frequencies in both control and treated fish were highest in gill cells and generally lower in erythrocytes and fin cells. PCE/NCE rations decreased in relation to MC and LA treatments. The results of this study indicate that LA and MC have genotoxic and cytotoxic damage in fish and confirmed that AO staining is a suitable technique for in vivo MN test in fish.

Effect of ascorbic Acid and thiamine supplementation at different concentrations on lead toxicity in liver.

Wang C, Liang J, Zhang C, Bi Y, Shi X, Shi Q.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/17878260

OBJECTIVE: To investigate the effect of ascorbic acid [vitamin C (VC)] on liver damage parameters in the lead-exposed mice, when given in combination with thiamine [vitamin B1 (VB(1))] at different concentrations.

METHODS: Sixty-six male mice were randomly assigned into 11 groups (n = 6). Mice in Group I were supplied with only the tap water as the drinking water; mice in Group II were provided with a tap water containing 0.2% lead acetate; mice in Group III-XI were given different dose of VC (140, 420, 1260 mg kg(-1) bw) and VB(1) (10, 30, 90 mg kg(-1) bw) according to 3 x 3 factorial design by oral gavages, along with ingestion of 0.2% lead acetate. After 42 test days, DNA damage of liver cells was assessed using single-cell gel electrophoresis. The apoptosis rate of liver cells was determined by flow cytometry. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in blood and the level of reduced glutathione (GSH) in liver cells were measured based on individual biochemical reactions.

RESULTS: Compared with the Group I, sub-chronic lead ingestion (Group II) resulted in a significant decrease of Hb, GSH-P(X), SOD in blood and GSH level in liver cells; lead exposure induced also a significant increase in DNA damage and apoptosis of liver cells (P < 0.05). Supplementation with VC and VB(1), however, reversed these effects. The best effective combination was VC (420 mg kg(-1) bw) and VB(1) (10 mg kg(-1) bw), followed by the combination of VC (420 mg kg(-1) bw) and VB(1) (30 mg kg(-1) bw). But no reversion was shown in the combination of the highest combination of VC (1260 mg kg(-1)) and VB(1) (90 mg kg(-1)).

CONCLUSIONS: These findings strongly indicated that combination of VC and VB(1) can lessen the damage to liver cells from oxidative stress induce by lead, but the antioxidant effects are dependent on their concentrations.

[Impacts of combined supplementation with ascorbic acid and thiamine on certain biochemical and morphologic indexes of testes in mice treated by lead].

[Article in Chinese]

Zhang Y, Wang CH, Liang JC, Zhang C, Wang CQ.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/17290753

OBJECTIVE: To investigate the impacts of the combined administration of ascorbic acid and thiamine on certain biochemical and morphologic indexes of testes in mice exposed to lead.

METHOD: s 75 male mice were divided into control groups, groups received with 0.2% lead acetate and groups treated by the same lead acetate dose in combination with ascorbic acid and thiamine (subdivided into: low, middle and high-dose) ad libitum with 15 mice in each. 5 mice in each group were sacrificed at 2, 4 and 6 weeks respectively, and then testes were separated from mice. To evaluate the lead toxicity in testis, the levels of TGFbeta1 and Caspase-3 were detected by immunohistochemistry, apoptotic cell was determined by terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick end labeling (TUNEL); DNA damage of germ cells was assessed by single cell gel electrophoresis (SCGE or Comet assay).

RESULTS: The levels of TGFbeta1 and Caspase-3, apoptotic index were significantly higher in groups given by lead than those in control groups ($P < 0.05$). After intervention of low and middle-dose vitamins, DNA damage and the number of apoptotic cell in testis were obviously lower than those of groups exposed to lead ($P < 0.05$). Also, the impaired tissues were markedly ameliorated under light microscope. Groups administrated with vitamins at the highest dose, however, promoted testicular cell apoptosis via elevated expression of TGFP, and Caspase-3, percentage of tail cell and tail length were reduced significantly.

CONCLUSION: Thiamine and ascorbic acid could antagonize the action of certain toxicity of testes in mice treated by lead acetate.

Impacts of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in testis.

Wang C, Zhang Y, Liang J, Shan G, Wang Y, Shi Q.

BACKGROUND: Lead is a ubiquitous environmental and industrial pollutant that may have toxic effects on the male reproductive system. We explored the mechanism and examine the impacts of combined administration of ascorbic acid and thiamine at different levels on apoptosis in the testes of lead-exposed mice.

METHODS: Seventy-five male mice were randomly divided into 5 groups (15 mice/group): control, lead-treated and vitamin-treated group (low, middle and high dose) with ascorbic acid (140, 420, 1260 mg/kg) and thiamine (10, 30, 90 mg/kg) by oral gavage daily. All lead-exposed animals received 0.2% lead acetate in drinking water. DNA damage of testicular cells was assessed by SCGE; the levels of TGFbeta1 and caspase-3 were detected by immunohistochemistry; apoptotic cell was determined by TUNEL.

RESULTS: Compared with control group, the expressions of TGFbeta1 and caspase-3, apoptotic index (AI) and DNA damage were increased significantly in lead-exposed group ($P<0.05$). After intervention of low and middle doses vitamin, the incidence of DNA damage and the number of apoptotic cells in testis were obviously lower than the lead-exposed group ($P<0.05$) and the impaired tissues were ameliorative. However, simultaneous supplementation of ascorbic acid and thiamine at the highest dose promoted testicular cell apoptosis via increased expressions of TGFbeta1 and caspase-3.

CONCLUSIONS: The combination treatment with thiamine and ascorbic acid at lower doses effectively inhibited testicular cells from apoptosis by lead acetate, but higher doses could aggravate the testicular lesion.

Protection by turmeric and myrrh against liver oxidative damage and genotoxicity induced by lead acetate in mice.

El-Ashmawy IM, Ashry KM, El-Nahas AF, Salama OM.

The effects of lead acetate in the diet (0.5% w/w) on reduced GSH, activity of phase II metabolizing enzyme glutathione S-transferase (GST), lipid peroxidation in liver homogenate and bone marrow chromosomes of mice simultaneously supplemented with powdered turmeric and myrrh for 8 weeks were investigated. Five groups of Swiss male albino mice, each of 30 mice, the first group received a basal diet and served as negative control, the second group received basal diet supplemented with lead acetate only and served as positive control. The other three groups received basal diet supplemented with lead acetate and 1% or 5% turmeric powder and 1% myrrh powder, respectively. Results

revealed a significant decrease in the amount of GSH in all treated groups compared with negative control. Also, the activity of GSH S-transferase was significantly decreased in positive control compared with other groups. However, co-administration of the protective plants resulted in a significant increase in the activity of GST compared with both positive and negative control groups. Furthermore, lipid peroxidation was significantly increased in positive control alone, while co-treatment with the protective plants resulted in reduction in the level of lipid peroxidation by 31% and 49% in mice receiving 1% and 5% turmeric powder respectively and 45% in 1% myrrh treated when compared with their respective positive control group. Lead genotoxicity was confirmed through significant reduction in the number of dividing cells, increased total number of aberrant cells and increased frequency of chromosomal aberrations. Simultaneous treatment with these plants significantly reduced the genotoxicity induced by lead administration and the powerful protection was observed with 5% powdered turmeric. It may be concluded that turmeric and myrrh are useful herbal remedies, especially for controlling oxidative damages and genotoxicity induced by lead acetate intoxication.

The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate.

Celik A, Ogenler O, Cömelekoglu U.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/16135535

The data concerning the mutagenic, clastogenic and carcinogenic properties of inorganic lead compounds have been conflicting. Here, we evaluated the frequency of micronuclei in the peripheral blood of female rats treated with three different lead acetate doses. Outbred female Wistar rats were treated by gavage once per week for 10 weeks with cumulative doses of 140, 250 and 500 mg/kg body weight (body wt) of lead acetate. Mitomycin C (MMC) 2 mg/kg body wt was used as a positive control. The aim of the present study was to investigate the possible cytotoxic and genotoxic effects of lead acetate on peripheral blood reticulocytes using the micronucleus test following chronic exposure. The results show the effects of lead acetate in peripheral blood reticulocytes. These effects are both cytotoxic and genotoxic because of a decrease in the number of polychromatic erythrocytes in the peripheral blood and an increase in frequency of micronucleated reticulocytes, respectively.

Protective effect of volatile oil, alcoholic and aqueous extracts of Origanum majorana on lead acetate toxicity in mice.

El-Ashmawy IM, el-Nahas AF, Salama OM.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/16176560

Natural dietary antioxidants are extensively studied for their ability to protect cells from miscellaneous damages. Origanum majorana L., Lamiaceae, is a potent antioxidant. The effect of administration of O. majorana (volatile oil, alcoholic and aqueous extracts) on oral administration of lead acetate in the diet of mice at concentration 0.5% (W/W) for one month were studied by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine, histopathological changes of the liver and kidney and genotoxicity including, rate of micronucleus and chromosomal aberrations in bone marrow cells. Mice were treated with the 3 different forms of O. majorana, one month before and maintained with lead acetate administration. The 3 forms of O. majorana induced a significant decrease in serum activities of transaminases (AST & ALT), ALP, urea and creatinine and improved the liver and kidney histology in comparison with lead acetate treated group. Alcoholic extracts of O. majorana significantly reduced the rate of micronucleus, number of aberrant cells and different kinds of chromosomal aberrations. Volatile oil extract significantly reduced the rate of micronucleus and chromosomal fragments. Aqueous extract and volatile oil also of O. majorana significantly reduced number of gaps, ring chromosome and stickiness. It could be concluded that O. majorana plays an important role in ameliorating liver and kidney functions and genotoxicity induced by lead toxicity.

C) <u>Relevant in vitro studies</u>

Combined exposure to nano-silica and lead induced potentiation of oxidative stress and DNA damage in human lung epithelial cells.

Lu CF, Yuan XY, Li LZ, Zhou W, Zhao J, Wang YM, Peng SQ.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/26432026

Growing evidence has confirmed that exposure to ambient particulate matters (PM) is associated with increased morbidity and mortality of cardiovascular and pulmonary diseases. Ambient PM is a complex mixture of particles and air pollutants. Harmful effects of PM are specifically associated with ultrafine particles (UFPs) that can adsorb

high concentrations of toxic air pollutants and are easily inhaled into the lungs. However, combined effects of UFPs and air pollutants on human health remain unclear. In the present study, we elucidated the combined toxicity of silica nanoparticles (nano-SiO2), a typical UFP, and lead acetate (Pb), a typical air pollutant. Lung adenocarcinoma A549 cells were exposed to nano-SiO2 and Pb alone or their combination, and their combined toxicity was investigated by focusing on cellular oxidative stress and DNA damage. Factorial analyses were performed to determine the potential interactions between nano-SiO2 and Pb. Our results showed that exposure of A549 cells to a modest cytotoxic concentration of Pb alone induced oxidative stress, as evidenced by elevated reactive oxygen species generation and lipid peroxidation, and reduced glutathione content and superoxide dismutase and glutathione peroxidase activities. In addition, exposure of A549 cells to Pb alone induced DNA damage, as evaluated by alkaline comet assay. Exposure of A549 cells to non-cytotoxic concentration of nano-SiO2 did not induce cellular oxidative stress and DNA damage. However, exposure to the combination of nano-SiO2 and Pb potentiated oxidative stress and DNA damage in A549 cells. Factorial analyses indicated that the potentiation of combined toxicity of nano-SiO2 and Pb was induced by additive or synergistic interactions.

[Effect of CCM3 gene defect on lead-induced cell genotoxicity in mouse embryonic fibroblasts].

[Article in Chinese]

Su X, Xing X, Lai G, Sun Y, Zhao Z, Chen J, Shen B, Liu X, He Y.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/26268874

OBJECTIVE: To investigate the effect of CCM3 gene defection on lead induced cell genotoxicity in mouse embryonic fibroblasts.

METHODS: C57 female mice were mated with CCM3 gene heterozygous male mice. E13.5 embryos were taken to isolate primary mouse embryonic fibroblasts. After genotyping, wild type and heterozygous cells were treated with different doses of lead acetate. Cell viability, genotoxicity and protein expression were detected by MTS assay, CB micronucleus method and Western blot, respectively.

RESULTS: Mouse embryonic fibroblasts with lead acetate treatment for 24 h, wild-type cells 100.00 µmol/L lead acetate-treated group (69.16±1.36) and the control group (100.00±2.33) compared to cells decreased by 30%, CCM3 heterozygous type cell 100.00 µmol/L lead acetate-treated group (87.16±5.50) and the control group (100.00±2.06) compared to cells decreased by 13%, the difference was statistically significant (F values were 98.59, 82.63, P<0.001). Lead acetate treatment after 48 h, wild-type cells 100.00 µmol/L lead acetate-treated group (51.99±5.62) and the control

group (100.00±3.11) compared to cells decreased by 50%, heterozygous type cells 100.00 µmol/L lead acetate treatment group (66.33±4.06) and the control group (100.00±5.72) compared to cells decreased by 35%, the differences were statistically significant (F values were 82.63, 36.86, P < 0.001). The results of CBMN test showed that with increased dose, micronucleus cell rate of two genotypes showed an increasing trend, in the wild-type cells, the micronucleus cell rate (/1 000) for the control group, 29.6±2.2, 6.25 µmol/L dose group 47.3±6.6, 25 µmol/L dose group 55.5±9.1, 100.00 µmol/L dose group 66.8±3.5; heterozygous cells micronucleus cell rate (/1 000) for the control group, 35.3±5.6, 6.25 µmol/L dose of 50.0±8.3, 25.00 µmol/L dose group 57.0±8.5, 100.00 µmol/L dose group 58.8±2.1. Micronucleus cell rates (/1 000) were significant differences, in 100.00 µmol/L dose groups of two genotypes. Western blot results showed that wild-type cells CCM3 expression 100.00 µmol/L lead acetate-treated group (0.70±0.03) was 1.32 times higher than the control group (0.53±0.07), heterozygous cells CCM3 expression 100.00 µmol/L lead acetate-treated group (0.48±0.02) was 1.77 times higher than control group that of 0.27±0.04, there was statistically significant difference (F values were 14.77, 25.74, P < 0.001); wild-type cells γ-H2AX expression 100.00 µmol/L lead acetate-treated group (0.69±0.03) was 1.06 times higher than the control group (0.65±0.07), heterozygous cells γ-H2AX expression 100.00 µmol/L lead acetate-treated group (0.99±0.04) was 1.55 times higher than the control group CCM3 expression levels (0.64±0.06), there was statistically significant difference (wild-type cells: F = 7.08, P = 0.012, heterozygous type cell: F = 13.49, P = 0.002).

CONCLUSION: CCM3 gene may play a role in lead-induced genetic toxicity of mouse embryonic fibroblasts, CCM3 gene-lead interactions effects on mouse embryonic fibroblasts cell toxicity.

Probit analysis of comparative assays on toxicities of lead chloride and lead acetate to in vitro cultured human umbilical cord blood lymphocytes.

Patnaik R, Padhy RN.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/27486358

This work describes that cytotoxicity of lead chloride and lead acetate to in vitro cultured lymphocytes from human umbilical cord blood, using four monitoring methods namely, trypan blue staining, acridine orange/ethidium bromide staining, 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyl tetrazolium bromide (MTT) and neutral red uptake assays; lead genotoxicity to lymphocytes was monitored by comet assay. The MIC value in each method was invariably 300 mg/L for PbCl2. Lethal concentration25 (LC25) values were almost in an agreeable range: 691.83 to 831.76 mg/L; LC50 values in each method were almost in the range: 1174.9 to 1348.9 mg/L; LC100 values were in the range: 3000 to

3300 mg/L, for lead chloride. Similarly, The MIC value in each method were invariably 150 mg/L; LC25 values were almost in the range: 295.12 to 371.53 mg/L; LC50 values were in the range: 501.18 to 588.84 mg/L; LC100 value was 1500 mg/L in all assays, for lead acetate. The comet assay also indicated that the LC100 values were 3300 mg/L lead chloride and 1500 mg/L lead acetate. Thus, both cytotoxicity and genotoxicity were recorded at 3300 mg/L lead chloride and 1500 mg/L lead acetate with lymphocytes.

Induction of oxidative stress by low doses of lead in human hepatic cell line WRL-68.

Hernández-Franco P, Silva M, Valverde M, Rojas E.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/21503682

Even though the molecular mechanisms by which lead induces toxicity and cancer have been intensely studied for many years, its carcinogenic mechanisms are not well understood yet. Several possible mechanisms have been examined to gain understanding on the carcinogenic properties of lead, which include mitogenesis, alteration of gene expression, and oxidative damage, among others. The aim of the present study was to explore the induction of oxidative damage at low lead concentrations using human embryonic hepatic cells WRL-68. Our results showed induction of reactive oxygen species, changes in the superoxide dismutase and catalase activity, as well as an induction of lipid peroxidation and DNA damage. However, after 5 weeks of exposure, these alterations returned to their basal levels. These results taking together indicate that at low concentrations, lead is able to establish an oxidative stress scenario; however under optimal antioxidant defense the oxidative scenario could be abolished through an adaptative process.

Protective effect of C(60) -methionine derivate on lead-exposed human SH-SY5Y neuroblastoma cells.

Chen T, Li YY, Zhang JL, Xu B, Lin Y, Wang CX, Guan WC, Wang YJ, Xu SQ.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/20878908

Oxidative stress has been considered as one of the possible mechanisms leading to the neurotoxicity of lead. One of the effective ways to prevent cellular damage after lead exposure is using antioxidants. In this paper, a novel C(60) -methionine derivate (FMD), a fullerene molecule modified with methionine, was synthesized. The protective effect of FMD on lead-exposed human SH-SY5Y neuroblastoma cells was investigated. In this research, after incubating with 500 μm Pb acetate alone for 72 h, the cells had undergone

a series of biological changes including viability loss, apoptotic death, the depletion of glutathione (GSH), the peroxidation of membrane lipid and DNA damage. Pretreatment with FMD before lead exposure could improve cell survival, increase the GSH level, reduce malondialdehyde content and attenuate DNA damage without obvious toxicity. In addition, the protective effects of FMD were proven to be greater than those of other two C(60) -amino acid derivates, β-alanine C(60) derivate and cystine C(60) derivate, which have been confirmed in our previous work to be able to protect rat pheochromocytoma PC12 cells from hydrogen dioxide-induced oxidative injuries. These observations suggest that FMD may serve as a potential antioxidative and neuroprotective agent in the prevention of lead intoxication.

Activation of protein kinase Calpha signaling prevents cytotoxicity and mutagenicity following lead acetate in CL3 human lung cancer cells.

Wang CY, Lin YW, Yang JL.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/18590793

Protein kinase C (PKC) family of serine/threonine protein kinases is sensitive signaling transducers in response to lead acetate (Pb) that could transmit phosphorylation cascade for proliferation and de-differentiation of neural cells. However, little is known as to the impact of PKC on Pb genotoxicity. Here we investigate whether Pb activates the conventional/classical subfamily of PKC (cPKC) signaling to affect cytotoxicity and mutagenicity in CL3 human non-small-cell lung adenocarcinoma cells. Pb specifically promoted membrane localization of the alpha isoform of PKC in CL3 cells. Pb also elicited Raf-1 activation as measured by the induction of phospho-Raf-1S338 and the dissociation from the Raf-1 kinase inhibitor protein. Inhibition of cPKC activity using Gö6976 or depletion of PKCalpha by introducing specific small interfering RNA blocked the induction of phospho-Raf-1S338, phospho-MKK1/2 and phospho-ERK1/2 in cells exposed to Pb. Intriguingly, declining PKCalpha enhanced the Pb cytotoxicity and revealed the Pb mutagenicity at the hprt gene. The results suggest that PKCalpha is obligatory for activation of the Raf-1-MKK1/2-ERK1/2 signaling module and plays a defensive role against cytotoxicity and mutagenicity following Pb exposure. Results obtained in this study also support our previous report showing that ERK1/2 activity is involved in preventing Pb genotoxicity.

Lead-induced apoptosis in PC 12 cells: involvement of p53, Bcl-2 family and caspase-3.

Xu J1, Ji LD, Xu LH.

Abstract http://www.ncbi.nlm.nih.gov/pubmed/16887300

It has been reported that lead could induce apoptosis in a variety of cell types. Although mitochondrion is regarded as the most pertinent pathway in mediating apoptosis, the exact mechanisms of lead-induced apoptosis are still largely unknown. Furthermore, there is little information about expressions and regulations of Bax, Bcl-2, and p53 in lead-induced apoptosis, which are critical regulators of mitochondrial stability. The present study was undertaken to determine whether lead could induce DNA damage and apoptosis in PC 12 cells, and the involvement of Bax, Bcl-2, p53, and caspase-3 in this process. The results showed that lead could induce DNA damage and apoptosis in PC 12 cells, accompanying with upregulation of Bax and downregulation of Bcl-2. Additionally, the expression of p53 increased, and caspase-3 was activated. Therefore, it suggests that lead can induce activation of p53 by DNA damage, which may lead to imbalance of Bax/Bcl-2 and mitochondrial dysfunction. Subsequently, after activation of caspase-3, lead-induced apoptosis occurres.