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Review article

Kohl and surma eye cosmetics as significant sources of lead (Pb) exposure

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Summary:

Kohl (surma) is a traditional eye cosmetic used in the Middle East, India, Pakistan, and parts of Africa that often contains high levels of lead (Pb), a developmental neurotoxicant. Many researchers have called for stricter governmental regulations of kohl trade and quality control and improved public education regarding its hazards and some governments have adopted import controls and educational campaigns to alert users to the hazards of using Pb-containing kohl. However, users remain unaware of the hazards of kohl usage, and some authorities minimize its potential danger. In this review, we summarize available data from the peer-reviewed literature on prevalence and attitudes regarding kohl use, Pb content of kohl samples from many sources, potential routes of entry of Pb into the body from kohl, and epidemiologic evidence that kohl is a source of Pb exposure in infants and women. Chemical analyses show that kohl has a wide range of formulae, with some containing PbS as the principal ingredient and others based on carbon and often Pb-free. Ocular, dermal, or gastrointestinal routes of entry of Pb into the body from kohl had been insufficiently studied to rule any of them out. The preponderance of epidemiologic evidence supports the conclusion that Pb-based kohl is associated with increased PbB in women who use kohl and their children.

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Introduction:

Kohl or surma is a traditional eye cosmetic used in the Middle East, India, Pakistan, and parts of Africa that often contains high levels of lead (Pb). For simplicity, in this paper we will use the terms kohl and surma interchangeably. Kohl is also used by émigrés from these regions in Europe, the United States, and elsewhere in the world. Unlike mascara, which is applied to the epidermal surface of the eyelids, kohl is applied to the palpebral conjunctiva. In traditional Middle Eastern medicine, kohl is also applied to the umbilical stump of newborn babies as an astringent and to the eyes of infants and small children to improve eye health. Its usage is a centuries-old tradition with esthetic, religious, and medicinal significance [1-4]. Nevertheless, the use of kohl is potentially harmful because a large proportion of preparations contain high amounts of Pb. Many studies provide strong evidence that Pb-containing kohl is a significant source of Pb exposure to women and children and therefore several countries have put in place government regulations concerning its importation and labeling, as well as public education to eliminate its use. Pb toxicity is an increasingly recognized public health concern in the Middle East, India, and Pakistan [5-14].

Pb is a ubiquitous environmental contaminant to which humans are exposed throughout life from dust, air, and water [15]. Extensive investigations of Pb-induced toxicity for the past 40 years have established that Pb is toxic to the nervous and neuroendocrine systems of fetuses, infants, pre-adolescents, and aging adults [16-19] and that Pb causes renal and cardiovascular toxicity [20]. The human brain is most sensitive to Pb exposure during the first two years of life. This age corresponds to physiologic immaturity of the brain and gut that increase Pb accumulation and to behaviors, such as mouthing and crawling, that increase exposures [21]. In the United States (US), the blood lead level (BPb) of concern in children which triggers environmental exposure evaluation, education of parents, and PbB monitoring is 10 µg/dl or higher (U.S. Centers for Disease Control, 1991). In children, reduced IQ scores, reduced attention span, and increased aggression are associated with PbBs above 10 µg/dl [22, 18, 23, 16, 24-25]. Furthermore, delayed pubertal development in girls has been reported at 3 µg/dl [17]. In addition to its developmental neurotoxicity, Pb is also implicated in the etiology or pathology of protein folding disorders of the nervous system, including Alzheimer 's Disease (AD), Parkinson's Disease, and cataracts [26].

Epidemiologic studies of Pb toxicity are still few in the Middle East, but public health authorities and researchers are beginning to investigate this area. For example, elevated PbB is associated with cognitive deficits in school girls in Egypt [27], in agreement with findings in the US [22] and a pooled international analysis [28]. In addition, elevated PbB is associated with the delayed onset of puberty in both males and females in Egypt [13], which is in agreement with studies in Russia, South Africa, and the United States [29, 17, 30-31], as well as with experimental rodent studies [32]. Moreover, elevated PbB in post-menopausal Saudi Arabian women is associated with high blood pressure [7], indicating that the risk of Pb toxicity is not limited to children.

The general adult population is exposed to Pb primarily through food and water, though workers in some construction, mining, and manufacturing industries may be at risk for occupational exposure. Major potential exposure pathways for infants and young children are food, air, water, and dust or soil [33]. Two other potential sources of exposure are traditional medicine [34] and cosmetics such as kohl [35], and these are important because they are voluntary, avoidable exposures. Many studies have demonstrated that Pb crosses the placenta and that maternal PbB is directly correlated with umbilical cord PbB [36-38]. Therefore, the mother's body burden of Pb is partially transported to the fetus.

Many researchers in the Middle East have called for stricter governmental regulations of kohl trade, quality control of its manufacture and labeling, and improved public education regarding its hazards [4, 3, 39]. Kohl is unapproved for use in the US by the Food and Drug Administration

(U.S. Food and Drug Administration, 2006) and is subject to import alerts, such as Import Alert # 53-15 "Detention Without Physical Examination of Eye Area Cosmetics Containing Kohl, Kajal, or Surma," which was published on March 27, 2012. The United Kingdom (UK), Canada, France, and other countries similarly ban the use of kohl or warn consumers against its use. The Saudi Food and Drug Safety Authority also advises consumers as follows: "Avoid color additives that are not approved for use in the area of the eye, such as 'permanent' eyelash tints and kohl. Be especially careful to keep kohl away from children, since reports have linked it to lead poisoning." (Saudi Food and Drug Authority, 2012) .

Why then, should this topic be of interest for global and local health? The concern is that users remain unaware of the hazards of kohl usage, and that some authorities minimize its potential danger. In particular, Mahmood et al., [40] claims that "the relation between Kohl and the toxicity of increased blood lead concentration upon its application to eyes as reported elsewhere is likely to be more of theoretical nature than a practical health hazard." These investigators do not dispute that Pb is toxic, particularly to children, that kohl usually contains high levels of Pb, and that Pb may enter the body orally. However, they argue that 1) no evidence has been reported that Pb enters the body through the conjunctiva, 2) while children may ingest kohl from their hands after rubbing their eyes, adults likely do not, and 3) previous studies in which elevated blood Pb levels were associated with kohl exposure are flawed, and studies which show no association between kohl exposure and elevated PbB are ignored.

This review will summarize studies on the chemical analyses of kohl from various sources, the plausibility of Pb exposure from the traditional use of kohl as a cosmetic and astringent, and the effects of kohl use on PbB in women and children. We will also discuss attitudes and beliefs regarding kohl usage. We will review work conducted in Bahrain, Egypt, India, Iraq, Israel, Kuwait, Morocco, Oman, Pakistan, Qatar, Saudi Arabia, and the United Arab Emirates (UAE), as well as studies in Europe and the United States of kohl-users who emigrated from these countries and carried the tradition to their new homes.

Lead sulfide as a major component of kohl:

There is confusion in both the terminology used for various eye cosmetics in different regions and cultures and in the historical composition of these compounds. In countries of the Arabian Peninsula, kohl refers to several preparations, including black kohl, which is largely composed of a fine powder of the lead sulfide (PbS) ore galena [4]. In Northern India, the galena-derived preparation is called surma and contains Pb, but the eye cosmetic Kajjal (a.k.a. Kaajal or Kajjali) is prepared from carbon soot and is Pb-free [41]. Other modern variations in the composition of the black eyeliner termed kohl or kahal also exist. This problem in terminology immediately points to the lack of standardization in the formulation, packaging, labeling, and selling of such products.

Many studies have reported the chemical content of kohl and surma, particular Pb contents. These studies in general show that kohl has a wide range of formulae, some under brand names and some unlabeled or prepared at home. Some preparations contain PbS as the principal ingredient and others are Pb-free. Thirty years ago, Sweha [42] described Arabian kohl as a product made by burning aromatic resin, frankincense, and almond shells. Mahmood et al., [40] describes modern kohl (surma) used in Pakistan as ground kohl stone (which is the mineral galena or PbS) mixed with other ingredients such as zinc oxide, silver leaves, gold leaves, ground rubies or emeralds, ground coral or pearls, and herbs.

The focus of this review is PbS in kohl and surma, though other components may also contribute to its toxicity. Before turning the discussion to Pb, we will mention two examples of other toxic components of kohl. In 2011, a case of cadmium (Cd) toxicity characterized by severe corneal edema and faint scarring from kohl eye makeup was reported in a Saudi woman [43]. The kohl she used, which was purchased from a traditional herbalist outside the city of Riyadh, was found to contain toxic levels of both Pb and Cd. In addition, 16 polyaromatic hydrocarbons (PAH), including the potent carcinogen benzo(a)pyrene (BAP), have been found in carbon-based kohl samples from Pakistan. The median value of BAP was 197.47 μg/ g of kohl sample [44].

Analyses of Pb in kohl began in 1968 as part of clinical work-ups of children treated for plumbism in the UK. In these cases, Pb content of the kohl to which the patients were exposed was high, as anticipated. Subsequent analyses by quantitative elemental analysis, usually atomic absorption spectroscopy (AAS), of kohl or surma from many sources have shown that their composition varies, and that many, though not all, samples contain high amounts of Pb. In one of the first surveys of Pb content in kohl, Fernando et al. [2] measured 13 samples obtained in Kuwait, 9 of them submitted by parents whose children had plumbism, and found that 8 were at least 81% Pb (w/w) and only two had low Pb levels. Parry and Eaton [45] sampled 22 kohl specimens purchased in Morocco, Mauritania, the UK, and the US, some originating from Saudi Arabia, Pakistan, and India, and found that 9 had < 0.6% and 7 had >50% Pb. Haq and Khan [46] measured the Pb content of 40 samples of surma locally produced in Islamabad or purchased abroad by Hajis from Mecca; 50% contained Pb in proportions ranging from 0.03% to 81.37% and the rest had no Pb. Madany and Akhter (1992)[47] analyzed 21 kohl samples used by children in Bahrain and found comparatively low Pb levels ranging from 0.007-15.6% Pb. Al-Hazzaa and Krahn (1995)[48] examined 21 samples of kohl purchased in Saudi Arabia and originating in Saudi Arabia, elsewhere in the Middle East, and India. Of these specimens, 10 had Pb levels >84%, 7 were Pb-free, and 4 contained 2.9-34.1% Pb. Five contained at least 60% carbon, and of these 2 contained no Pb. One sample contained 7.8% antimony (Sb), the highest amount reported in any study. These samples were home-made powders (6), commercial preparations (9), and natural kohl stones ground into a powder (6), the last of which would be expected to be galena (PbS). Lekouch et al., (2001) [49] measured Pb in 10 samples of kohl purchased in Morocco and originating in Yemen, Saudi Arabia, Algeria, and Morocco and found >54% Pb in all of them and >75% Pb in 6 of them. Al-Ashban et al. (2004)[4] analyzed 107 kohl samples from different regions of Saudi Arabia. Pb was detected in 53% of the samples ranging from trace amounts to 52% w/w. Of 27 labeled and branded samples tested, 12 had high Pb levels (25-52%), whereas 15 had low Pb levels (0.004-0.649%).

In addition to the above quantitative analyses, Hardy and colleagues reported qualitative analysis of kohl or surma composition by X-ray powder diffraction (XRPD) and scanning electron microscopy (SEM), which provides information on speciation of the metals and on organic components. In their studies, the majority of Omani, Saudi-Arabian, Egyptian and Indian kohls sampled contained at least 70% PbS in their major phase. However, in the minor phase the chemicals differed considerably among the kohl specimens. In the first such study, a total of 47 kohl samples used in Oman were analyzed [50]. Of 18 Omani-made kohls, the major component of 5 was PbS, whereas 12 were based on amorphous carbon and 1 on hematite. Specimens from other countries contained one of the following as the major component: PbS, minium (Pb_3O_4), amorphous carbon, magnetite (Fe_3O_4), zincite (ZnO), calcite ($CaCO_3$), or sassolite (H_3BO_3). Hardy et al. (2002) [51] next analyzed 23 kohl samples from the UAE, none made locally. The main component of 11 samples was PbS, and of the other 12 was one of the following: amorphous carbon, zincite (ZnO), sassolite (H_3BO_3) or calcite/aragonite ($CaCO_3$). Hardy et al. (2004) [52] also analyzed 18 kohl specimens purchased in Egypt and found that the main component of 6 samples (4 originating from Egypt and 2 from India) was PbS. The main component of the other 10 was one of the following: amorphous carbon, iron oxides, quartz, sassolite (H_3BO_3), zincite (ZnO), or talc ($Mg_3Si_4O_{10}(OH)_2$). Similar results were obtained from samples from Yen and Qatar [53].

Jallad and Hedderich (2005)[54] introduced a new method of analyzing kohl by confocal Raman microscopy and tested this method on 3 samples of kohl from UAE markets. The advantage of this method is that it is non-destructive to the sample, in contrast to AAS, XRPD, and

SEM. Of the 3 samples examined, sample 1 (Indian-made) was primarily PbS and sample 2 (Indian-made) was both PbS and $PbCO_3$. Pb content was confirmed by AAS and was >85% w/w. Sample 3 (Pakistani-made) was carbon-based and contained no Pb. This method might be considered for quality control by manufacturers and inspectors of kohl before sale to consumers.

Though many studies that have reported the Pb content of kohl from several countries over the last 3 decades, no clear picture emerges to suggest that the amount of Pb in a kohl sample is related to its region of origin. Indeed, Al-Saleh et al. (2005) [55] conducted a statistical analysis of their large study and found that Pb was equally present in kohl samples obtained from all regions of Saudi Arabia. We speculate that the final formulation of kohl available in the market is probably highly dependent on local sellers, who may obtain their base component from various unstandardized sources and may further alter the formulation through the addition of their own ingredients. As will be seen in our later discussion of epidemiologic studies, variability in the composition of kohl presents challenges in interpreting the results of some epidemiologic studies, particularly those in which the Pb content of the kohl used by the subjects studied was not measured.

Plausibility of lead entry into the blood from kohl:

Entry of Pb into the body is typically by ingestion or inhalation of particulates, though exposure through the skin or eye is possible, as will be discussed in this section. Kohl is typically applied to the conjunctiva, as well as to the umbilical stump and, in some cultures, to the circumcision wound of infants [4]. Mahmood et al., (2009) [40] argue that Pb from kohl cannot enter the body through the conjunctiva or skin based on selected studies which they considered in their review. PbS is nearly insoluble in aqueous solutions, though it may form a colloid if the particle size is small. We will reconsider these studies as well as all others that we found in our search of the literature in order to assess the evidence that Pb can enter the body from kohl either through the eyes, transdermally, or by ingestion.

Four studies have addressed to possibility of Pb entering the body and bloodstream from kohl applied to the eyes. The rabbit was used as an experimental model in 3 studies, receiving ocular exposure to surma in a manner similar to child exposure. These studies produced conflicting results, as shown in Table 1. In the first study [56], a small number of rabbits was treated with surma (dose not given) of a fairly large particle size (90 ± 20 μm) 1-3 times per day for 15 days. According to Healy et al. (1982)[56], the majority of specimens obtained in UK have a mean particle size of 100 μm, but Kuwaiti samples have a mean diameter of about 30 μm. The mean diameter of various Pb compounds (including Pb chromate and Pb octoate paint films) is known to affect gut absorption of Pb in rats, with absorption increasing 5-fold when particle size is reduced from 196 to 6 μm, and this factor may also be relevant to eye exposure [57]. The treated rabbits showed no difference from control in Pb levels in either the aqueous humour or blood, and both levels were relatively low. In the second study [58], no difference was found between rabbits treated with 0.5 -1.0 mg surma 1-3 times per days for 60 days and rabbits not treated (particle size was not mentioned). However, both surma-treated and untreated groups of rabbits had elevated PbB at all three time points (about 4 times as high as controls in the previous study), indicating that the controls were also exposed to Pb or the samples were contaminated. This study is therefore not interpretable. In contrast, the third study [59] found that Pb content was significantly elevated in rabbits whose eyes were treated once per day with 100 mg kohl for 15 days. The dose was larger than in the second study and particle size was not mentioned. Also, in the third study the rabbits' eyes were kept closed overnight, but the means of closure was not described. If it involved punctures or abrasions to the skin, it may have influenced Pb uptake.

Pb exposure and blood sampling regimen (reference)	Results	Comments
7 rabbits, 1 used as control, 3 treated in right eye once daily with surma (86% Pb w/w, 90 ± 20 μm particle size), 3 treated 3x daily. Metal applicator rod was dipped in surma and streaked across eyeball as used by mothers on their children. Blood drawn from ear vein after 15 days and aqueous humour sampled by paracentesis, both for Pb analysis (Healy et al., 1982)	No differences in Pb levels. Pb level in aqueous humor was < 2.07 μg/dl. PbB was 6.22 μg/dl	The number of rabbits per group was small. The particle size of surma tested was larger than has been reported The authors concluded that transcorneal transport of Pb from surma did not affect PbB.
24 1.0-1.5 kg rabbits were divided into 4 groups. Group I received no treatment. Groups II , III, and IV were given about 0.5-1.0 mg surma 1, 2, or 3 times per day, respectively, for 60 days. Blood was drawn from the ear vein at intervals of 15 days for Pb analysis. Surma used in these studies contained 69% Pb as PbS (Khalid et al., 1992)	PbB of controls was 25.5 ± 4.1 μg/dl after 15 days and 24.7 ± 3.1 μg/dl ml after 60 days. PbB in surma treated rabbits ranged between 26.4±1.3 μg/dl and 30.4 ± 3.4 μg/dl. These values did not differ significantly from controls.	Breed and sex of rabbits was not given. Method of applying surma to the eyes was not given. PbB levels were elevated in controls, suggesting exposure to Pb from other sources or cross-contamination of samples.
7 1.5-2 kg rabbits were treated with kohl as follows: 100 mg was instilled into the conjunctival sac of each eye and the eyelids were kept closed overnight to simulate human use; paws were taped to prevent licking them after rubbing off the kohl; treatment was repeated daily for 2 weeks; blood was drawn on day 15 from ear vein for Pb analysis. 7 control rabbits were handled in the same way except for kohl treatment (Abdulaziz et al., 1992)	PbB content was significantly higher ($p<0.0001$) in rabbits with kohl (68.59 ± 7.67 μg/dl) than in rabbits without kohl (1.28 ± 0.02 μg/dl)	Pb level of the kohl used was not given. Breed and sex of rabbits was not given. Method of keeping eyelids closed was not given. If it caused dermal wounds, such as suture holes, this may have been a route for Pb absorption into the blood.
Karachi, Pakistan, quasi-experimental design (i.e. clinical trial without randomization or blinding), 62 volunteers residing at the Pakistan Council of Scientific & Industrial Research campus: 23 children 2-11 years old and 39 "adults" 12-55 years old. Subjects served as their own controls, with PbB sampled after 0 (control), 30, 60, and 90 days of at least daily application of surma to the conjunctival surface (Khalid et al., 1995)	PbB in children showed no significant change over the 90 day course of surma use. Mean values were 0.88 to 0.94 μmol/l (18.23 to 19.48 μg/dl). Similarly, older subjects showed no significant change over the 90 day course of surma use. Mean values were 0.77 to 0.81 μmol/l (15.95 to 16.78 μg/dl).	The authors concluded that surma had no effect. However, surma use by subjects prior to the trial was not reported, and their baseline PbB was elevated. Also, the Pb content of the surma provided (Mohammad Hashim Tajir Surma) was not measured. Because of these 2 omissions, no valid associations between surma use and PbB can be drawn. In addition, the use of child "volunteers" raises ethical questions.

Table 1. Effect of experimental kohl eye treatment on blood lead levels in rabbits and humans* (*PbB levels were determined by atomic absorption spectroscopy.)

The fourth study of surma uptake from application to the eyes was conducted with human volunteers aged 2 months to 55 years. This study showed no difference in PbB between subjects with or without surma use and no change in PbB during the 90 day course of the experiment. Unfortunately, prior history of surma use by the subjects was not reported and PbB in both groups was above normal. More importantly, the Pb content of the surma used in the experiment was not measured, and it might have been Pb-free or low-Pb.

No firm conclusions can be drawn from the above studies about a possible ocular route of Pb exposure from kohl because of differing results and experimental weaknesses. In the study that showed elevation of PbB in rabbits whose eyes were treated with surma, the route of entry was not determined. Entry of Pb into the body through the lacrimal duct and subsequent swallowing seems unlikely, although one unique case was reported in which an archeological site worker in Pompeii who used a kohl pencil experienced a black secretion from her nose. The Pb content of the kohl was not determined as it was irrelevant to the case. The nasal secretion resulted from an unusually wide lacrimal duct that may have been caused by the lodging of a small insect in the patient's nose [60].

The penetration of kohl through skin has not been studied experimentally. We therefore examined available evidence about other Pb compounds. These studies show that Pb in ointments does not penetrate human skin[61], whereas soluble Pb appears to enter sweat glands of humans [62]. Several studies have analyzed Pb absorbance in vivo and in vitro using organic (e.g. lead acetate, $Pb(CH_3COO)_2$)) and inorganic lead (e.g. lead nitrate $Pb(NO_3)_2$, lead oxide PbO, and PbS). Table 2 shows a summary of these studies with their major outcomes. Although these studies used different methods for Pb analysis, different subjects, and different exposure times, they all approximately reached the same conclusion that Pb applied to the skin can be absorbed, although to different extents according to the exposure regimen and solubility of the Pb compound. Relevant to the question of kohl ingestion were findings in humans and rats that PbS applied to the skin could elevate PbB [63].

A case study from 1949 on plumbism from the use of Pb dressings on wounds offers another interesting possibility for Pb entry though the skin [64]. An adult female patient had exfoliative dermatitis that was treated with the application of Pb dressings to the entire body surface. The dressings included 2% plumbi subacet. fort and other ingredients and were applied intermittently for 7 months. This Pb solution is known as Goulard's Extract or subacetate of lead, an astringent composed of Pb acetate and Pb oxide that was used until the early 20th century. The patient developed chronic Pb poisoning. The authors concluded that the patient absorbed Pb through her damaged skin. This case is significant when considering kohl, in that kohl may be used by mothers to pack the umbilical stump of newborns. In addition, some Middle Eastern tribes pack the circumcision wound with kohl [4]. It therefore presents a potentially important route for Pb entry into an infant's body from kohl.

Gastrointestinal absorption of Pb by animals fed kohl or surma has not been tested, to our knowledge, let alone in immature animals exposed to kohl chronically under conditions that mimic exposure of infants and young children. Moreover, the bioavailability of ingested Pb from PbS has not been studied to any great intent. The 3 available studies are summarized in Table 3. Two feeding studies with rats [65-66] both show that rats absorb Pb into body tissues when fed PbS, though in both cases the absorption level was about 10% that of Pb acetate. A small study with 5 men also shows that PbS is absorbed when ingested, and is greater by a factor of 3 to 4 after a brief fast [67].

Experimental subjects or models	Pb exposure regimen	Pb analysis technique	Application	Measurements	Results	Reference
Volunteer healthy male adult	20 mg Pb powder ($Pb(NO_3)_2$ on left arm	Anodic Stripping Voltametry (ASV)	6hr every day for 4 day	Lead in sweat	Inorganic lead is absorbed through skin	Lilley et al., 1988
Rats	PbS PbO Pb powder Pb stearate	Graphite furnace atomic absorption spectrometry	100mg Pb on a linen cloth attached to the rat's shaved back	Urine samples every 2 days of exposure	Pb excreted in urine(ng/48 h) is significantly increased in Pb-treated groups at 12 days: 146.0 + 6.4 ng (SD) for lead stearate, 123.1 ± 7.2 ng for PbS, 115.9 ± 5.3 ng for PbO, 47.8 ± 6.9 ng for Pb powder, and 10.3 ng for the control, indicating significant skin absorption through skin.	Sun et al., 2002
Pb battery workers	PbS PbO Pb powder Pb stearate	Graphite furnace atomic absorption spectrometry	Exposed during their work	Skin strips collected daily before starting work.	Inorganic lead is absorbed through skin and skin protection should be carefully considered	Sun et al., 2002
Human abdominal skin from hospitals	PbO	Electrothermal atomic absorption spectrometry	5mg PbO per square centimeter of normal and damaged skin for 30min and 24hrs	Pb in normal and damaged skin	Dermal Pb uptake may contribute to PbB. PbO can pass throught he skin with mean penetration of 2.9 ng/cm2. Cleansing the skin with soap increases penetration.	Filon et al., 2006

Table 2. Absorption of lead (Pb) by or through skin

Experimental subjects or models	Pb exposure regimen	Pb analysis technique	Application	Measurements	Results	Reference
Two human volunteers (adult males)	Stable 204Pb isotope, $Pb(NO_3)_2$, and $Pb(CH_3COO)_2$	Thermal ionizing mass spectrometry, Inductively coupled plasma mass spectrometry , ASV	6 hrs : Forearm of subject	Sweat, urine and blood sample	Pb is absorbed through skin and detectable in sweat, urine, and blood within 6 hrs of skin application	Stauber et al., 1994
In vivo skin from female nude mice	$Pb(NO_3)_2$, and $Pb(CH_3COO)_2$	Graphite furnace atomic absorption spectrometry	120h topical application	Lead in skin, liver and kidney	Both Pb acetate and Pb nitrate are absorbed through the skin. Skin deposition of Pb is greater for Pb acetate, but liver and kidney deposition are the same for both compounds.	Pan et al., 2010
33 Volunteers age 18-65	Plumbum Metallicum ointment	Atomic absorption Spectrometry and Inductively coupled plasma mass spectrometry	Application every 3- 4 days for 0, 1, 2, 4,8 weeks	Blood, Urine and scalp hair	No lead absorption detected	Gorter et al., 2005

Table 2. Absorption of lead (Pb) by or through skin

Experimental subjects or models	Pb exposure regimen	Pb analysis technique	Application	Measurements	Results	Reference
Five Humans	$Pb(NO_3)_2$, PbS and Pb cysteine	Thermal ionizing mass spectrometry	Every day for 20 days for 4 subjects, 10 days for 1 subject	Pb ingested with food and fecal excretion	All forms of Pb are absorbed. Pb absorption is higher in the absence of food	Rabinowitz et al., 1980
Male Fisher rats	PbS, PbO , $Pb(CH_3COO)_2$,or Pb, particle size ≤38 μm at doses of 0, 10, 30, or 100 ppm		Pb added to diet for 30 days	Pb levels in blood, bone, and kidney	Pb from all Pb treatments is bioavailable, though bioavailability is greater for PbO and $Pb(CH_3COO)_2$ than for PbS or Pb ore. The highest PbB in 100 ppm groups is about 80 μg/dl in rats fed PbO or $Pb(CH_3COO)_2$ and about 10 μg/dl in rats fed PbS or Pb ore. Bone and kidney levels show similar patterns of Pb accumulation, i.e., higher accumulation from the soluble Pb salts.	Dieter et al., 1993
Male Fisher rats	$Pb(CH_3COO)_2$, PbS, Pb-contaminated soil	Inductively coupled plasma mass spectrometry	Rats fed 17, 42, or 127 ppm Pb, through diet and assayed at on 7, 15 and 44	Blood, Bone, liver and kidney tissue samples	Bone and tissue Pb levels increase in a dose-dependent manner for all Pb treatments; bioavailability of PbS (Pb in body/Pb in diet is about 10% of that for Pb acetate at 44 days.	Freeman et al., 1996

Table 3. Absorption of ingested lead (Pb)

Researchers who have studied Pb exposure from kohl postulate that the major route of entry is by ingestion [56]. Several scenarios are plausible. Children may rub their eyes and lick their contaminated hands. A nursing infant may ingest kohl from its mother's contaminated areola if her hands are contaminated with kohl when she places the baby at her breast, as has been suggested in the case of mothers who use sindur, another Pb-containing cosmetic [68]. Adults or children whose hands are contaminated with kohl may also ingest kohl if they eat without utensils. In addition, users may lick kohl on the applicator when applying it to their eyes. Kohl is typically stored in a special jar and applied with a metal applicator rod (the miroued) designed for the purpose [1, 48, 69]. As kohl is a fine powder, a user may lick the contaminated metal applicator to moisten the powder so that it applies more easily to the eyes ([49]; and RB, personal observations). The prevalence of such practices has not been surveyed to date, but remains anecdotal.

Analytical chemistry may provide a clearer understanding of the environmental sources of Pb body burdens by kohl users. For example, a comparison of 206Pb:207Pb isotopic ratios in the blood or feces of kohl users, their kohl source, and other sources of Pb in the environment may be able to discriminate the major source of Pb exposure. Al-Saleh et al., (1993) [70] performed this type of study by inductively coupled plasma-mass spectrometry on blood from Saudi children who had elevated PbB and determined that their likely sources of Pb exposure were cosmetics and traditional remedies, rather than Pb in gasoline.

Kohl and surma as epidemiologic determinants of blood lead levels:

We have attempted to collect and review all studies in peer reviewed journals that examine kohl use as a potential factor associated with elevated PbB levels or Pb intoxication in the Middle East, India, Pakistan, Europe, and the US. We found 26 studies dating from 1968 to 2012. Twenty of these papers, including 7 case reports and case series, report an association between kohl use and Pb exposure (Table 4) and 6 studies find no association (Table 5). Short commentaries are offered in the tables for each study that mention unique features or critique the methodology. The preponderance of evidence supports the conclusion that Pb-based kohl is associated with increased PbB in women who use kohl and their children.

The first reported cases of plumbism associated with the use of Pb-containing kohl or surma appeared in the late 1960's in London hospitals among the infants and young children of Indian origin. In most cases, a history of surma use in the children's eyes and high PbS content of the surma used was reported. The weakness of case reports is that they usually describe an unusual or novel occurrence and the patients are not adequately representative of the general population. The value of these reports is that they alerted physicians to a source of Pb exposure novel to them and prompted a government campaign in the UK and later in other countries of public education about the potential hazards of Pb-containing cosmetics. These case reports have been succeeded by a number of cross-sectional and a few cohort studies, as well as additional case reports that highlight new findings of clinical significance. Very few longitudinal or prospective studies have been reported to date.

Location, study design, subjects (reference)	Findings	Comments
London, UK, case report, 3 year old Indian boy, living in England for 2 years, admitted to hospital with symptoms of Pb encephalopathy (Warley et al., 1968)	PbB was 178 µg/dl; PbB of mother was 65 µg/dl and of 5-year-old sibling was 72 µg/dl; PbB of father and 3 older siblings at school was < 10 µg/dl; paint, cooking utensils, and food in the home were eliminated as Pb sources. Pb source used by all 3 affected patients was surma applied to the eyelids and conjunctivae, which contained 80% PbS.	UK's educational campaign against the use of Pb-containing surma began as a result of this poisoning (Aslam and Aslam, 1996)
London, UK, case series,12 children from 5 Indian families seen in the previous 6 months in a London hospital (Snodgrass et al., 1968)	PbB was >36 µg/dl in 10 children and 61-69 µg/dl in 3 children, who were immediately treated for plumbism. None had a history of pica. Checks of some homes revealed no potential sources of Pb. Samples of eye cosmetics were obtained in 3 instances and found to contain 80-85% PbS w/v.	The authors mentioned that the application of Pb cosmetics to the eyes of newborns was observed in the hospital's newborn nursery and seemed to be common among Punjabi patients. Selection processes for the subjects was not defined.
Nottingham, UK, case-control study, 62 Asian children who needed blood drawn for management of other medical conditions; 37 were known to be surma users (21 males and 16 females; 29 Punjabi) and 25 were thought to be non-users (9 males and 16 females; 21 Punjabi) (Ali et al., 1978)	PbB was significantly higher ($p<0.001$) in surma users (34.37 ± 14.16 vs. 20.41 ± 8.75 µg/dl). 5 of 37 (13.5%) of surma users had PbB ≥ 49.7 µg/dl, though none had evidence of Pb toxicity.	29 surma samples were obtained from the patients' families and examined for Pb content. 23 (grey surma and black surma) contained at least 75% Pb as PbS; 6 (white surma) contained no Pb.
Bradford, UK, case-control study 117, Asian children including 45 surma users and 49 White children used as controls; the children were seen at Bradford Children's Hospital during 6 months in 1978 and needed blood drawn for management of other medical conditions; ages were about 0-6 years old; the Asian children's families were predominantly from Mirpur and Punjab (58%), village dwellers before coming to the UK (70%), and Muslim (88%) (Green et al., 1978)	PbB in Asian surma-users (20 male, 25 female; 24.04 ± 10.98 SD µg/dl) was significantly higher ($p<0.001$) than Asian non-surma users (39 male, 33 female; 17.82 ± 5.59 µg/dl) and White controls (29 male, 20 female; 17.82 ± 7.67 µg/dl). 8 children, all Asian surma users, had PbB > 35 µg/dl.	28 surma samples were obtained from the patients' families and examined for Pb content. 13 contained at least 80% Pb; 7 contained 50-80% Pb, 6 contained 20-50% Pb, and 2 had only trace Pb levels. Several samples contained camphor, menthol, or ashed herbal compounds to induce lacrimation.

Table 4. Epidemiologic studies associating use of kohl (surma) with Pb exposure
*In these studies, exposures were self-reported through questionnaires. Pb was usually measured by graphite furnace or flame atomic absorption spectroscopy (AAS).

Location, study design, subjects (reference)	Findings	Comments
Kuwait, case studies, 4 infants admitted to Al Adan Hospital for convulsions, irritability, or other symptoms consistent with Pb intoxication: 1) 4 month old Syrian Bedouin male, 2) 28 day old premature Lebanese non-Bedouin male, 3) 16 day old Syrian non-Bedouin male, and 4) 4.5 month old Kuwait Bedouin female (Fernando et al., 1981)	PbB in the infants was apparently 21-30 µg/dl (data were not clearly presented in the paper); kohl was used on the eyes of infants 2, 3, and 4 and by the mothers of infants 1, 2 and 4 on their own eyes. All four kohl samples contained at least 85% Pb. The mother who did not use kohl on herself applied it to her infant's eyes and his umbilical stump. The infant not directly treated with kohl may have been exposed to Pb through breast milk.	The high sensitivity of newborns to Pb was highlighted by this study. The authors also mentioned their failure to effectively teach the 4th mother that Pb-containing kohl and Pb-free carbon-based kohl could be distinguished simply by their densities in water, as she continued to use Pb-based kohl during her next pregnancy.
Kuwait, case study, 20 patients ages 1-18 months with Pb encephalopathy (9 Bedouin, 4 Kuwaiti, 2 Saudi Arabian, 3 Syrian, 1 Iraqi; 13 males, 7 females) (Shaltout et al., 1981)	19 children had PbB of 60-257 µg/dl (one was not measured); 5 died before or during chelation treatment. Source of Pb in 11 was kohl, identified because some infants had kohl applied to their eyes and some lactating mothers used kohl	18 infants were breastfed, eliminating contaminated food or water but not breast milk as Pb source; inhalation of Pb fumes from tribal practice of heating pure Pb and immersing it in water to generate fumes was implicated in 4 cases
Dammam, Saudi Arabia, case-control study, 32 volunteers aged 10-45 years, including 10 controls who denied kohl use and 22 regular kohl users (Alkawajah et al., 1992)	PbB was ($p<0.0001$) in kohl users (20.51 ± 2.49 µg/dl) than in non-users (5.39 ± 1.24 µg/dl)	Pb content of 15 kohl samples was measured, 10 randomly collected from the markets of Dammam. Riyadh and Jeddah. The other five were home-made samples provided by volunteers. Not all samples had a high Pb content, though Pb was 87% in some home-made samples. The authors did not match kohl samples with the subjects who provided them in their presentation of results.
Two rural villages in Mount Carmel District, Israel, community-based cross-sectional study targeting all infants brought to clinic for routine anemia screening from January to May 1990; infants aged 6-16 months included 24 kohl users (kohl used on infant's eyes every 1-3 days since birth) and 30 non-kohl users (not receiving kohl in the 2 months preceding blood sampling) (Nir et al., 1992)	PbB was significantly higher ($p<0.001$) in kohl users (11.2 ± 5.8 SD µg/dl) than in non-users (4.3 ± 2.7 µg/dl. 12% of the infants receiving kohl had PbB > 20 µg/dl. Within the non-user group, kohl use by the mother or other household member significantly increased PbB of the infant (5.2 ± 2.6, n=18 vs. 2.8 ± 2.2, n=12).	Pb was measured in 7 kohl samples brought by the mothers and contained 17.3 – 79.5% Pb w/w.

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Location, study design, subjects (reference)	Findings	Comments
California, US, retrospective chart review, subset of 175 children for whom PbB results were available from among 1200 children 8 months to 6 years of age who received fluoride prescription in a county hospital clinic from October 1991 to February 1994 (Sprinkle et al., 1995)	A retrospective telephone questionnaire was administered to parents of children "from ethnic groups who use eye cosmetics." PbB was significantly higher (p=0.03) in eye cosmetic users than non-users: 12.9 vs. 4.3 µg/dl.	Sample size was small: 18 Pakistani and Indian children were in the subset of 175. The authors apparently grouped those with unknown or no kohl usage in the non-users group. Kohl samples from two families were measured and contained 16.4% and 70% Pb.
Arar, Saudi Arabia, community survey 108 children ages 1 month to 12 years attending an outpatient clinic of Arar central Hospital in a 6 week period; 25 had Pb/hemoglobin ratios exceeding 1.5 µg Pb/g Hb and 21 available were followed up with clinical exams and 2 questionnaires. Pb/gHb was used instead of PbB because anemia can influence PbB (Al-Saleh et al., 1996)	The prevalence of elevated µg Pb/g Hb was high (25/108, 23%). Mean PbB of the 21 children was 23.03 ± 7.59 µg/dl. Many family members also had elevated PbB. Sources of Pb exposure from soil, water, and dust were systematically ruled out by environmental sampling. Kohl was applied on 17 of 21 children to eyes or umbilicus.	Arar was largely a nomadic Bedouin community before 1950 and the people remain attached to traditional cultural practices. Kohl use was underreported in a pre-interview survey, but reported use was higher in a post-interview survey. In this population, kohl was the major contributor to elevated PbB in children.
Nashville, Tennessee, US, case report, 11 month old child seen on routine screening (Jones et al., 1999)	PbB was 43 µg/dl. No obvious source of Pb exposure was identified. Parents revealed that they applied surma brought from India to child's eyes daily for 5 months. The surma sample was 25% Pb by weight. 8 weeks after surma application was discontinued, the child's PbB was 23 µg/dl.	The authors cautioned medical practitioners in the US to consider unusual sources of Pb poisoning, given that common sources (paint dust, soil, and water) are being eliminated though screening, education, and abatement.
Nagpur, India, community-based cross-sectional study, 297 children aged 6 months to 6 years representing demographic cross-section of city; study targeted all children born from May 1989 – May 1995 and still resident in January 1996 (Patel et al., 2001)	Mean PbB was 18.4 ± 16.5 µg/dl; prevalence of elevated PbB (> 10 µg/dl) was 67%. Significant risk factors for elevated PbB were Hindu religion, higher caste, painted house, parental occupation, and daily use of surma. 18% of children used surma and 81% of the users had elevated PbB. The percentage of Pb poisoning attributable to surma use in this population was 15.2%, and the etiologic fraction (proportion of disease that could be prevented by eliminating exposure to Pb in the exposed population) was 61.5%.	Study was carefully constructed to avoid population sampling bias; environmental sampling also conducted by questionnaire to identify risk factors for elevated PbB

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Location, study design, subjects (reference)	Findings	Comments
Brussels, Belgium, case study, 22 year old Moroccan woman who had lived in Belgium one year, presenting with abdominal pain, behavioral evidence of encephalopathy, and other signs of Pb poisoning (Bruyneel et al, 2002)	Initial PbB was 490 µg/dl, which was reduced after 6 months of chelation therapy to 49 µg/dl. The source of Pb exposure was concluded to be Moroccan kohl applied daily to her conjunctivae for many years. The woman's 3 month old daughter also had elevated PbB (46 µg/dl).	The patient's Moroccan kohl sample contained 73% Pb. This case was presented again by De Caluwé (2009) together with an excellent historical review of Pb-poisoning from kohl usage and recommendations for educational campaigns in French-speaking countries to eliminate the use of Pb-containing kohl cosmetics.
Saudi Arabia, case-control study, 20 adult regular kohl users and 10 age-matched adult non-users (Al-Ashban et al, 2004)	PbB was significantly higher ($p<0.0001$) in kohl users (17.65 ± 2.29 SEM) than non-users (0.9 ± 0.43 µg/dl)	The study also surveyed 1250 kohl users, non-users and sellers in 5 regions of country for kohl use attitudes and beliefs. The study measured Pb, Al, and Sb in 107 kohl samples (see text).
Karachi, Pakistan, cross-sectional study, 565 mother-infant pairs randomly selected from births at two tertiary care hospitals in Karachi during January to August 2005; participation rate was 95.6% (Janjua et al., 2008)	The arithmetic mean of PbB of neonates was 10.8 ± 5.7 SD µg/dl, range 1.8 to 48.9µg/dl. Cord PbB of women took < 58.5 mg of elemental iron supplement per day during pregnancy was 10.0µg/dl; compared to 8.4µg/dl in women who had higher iron intake. Women who used surma daily had higher cord PbB (11.5 µg/dl) than those who used it less frequently (9.4µg/dl).	Pb levels in surma used by the subjects were not measured. This is the first systematic study of umbilical cord PbB in Pakistan.
Al-Kharj area of Saudi Arabia, longitudinal cross sectional study; original sample was 653 children born at King Khalid Hospital between March and July 2004; 66 children were enrolled in three groups, low PbB (lowest 10th percentile of population), intermediate PbB, and high PbB (highest 10th percentile). Children were followed up every 6 months, but were quickly lost from the study due to non-participation (119 at 0 months dwindling to 43 at 24 months) (Al-Saleh et al., 2009)	Initial mean cord PbB was low (2.21 ± 1.69 µg/dl), with 1% having PbB > 10 µg/dl. PbB rose as the infants grew older. An extensive survey of characteristic of mothers and infants to identify risk factors for Pb exposure and early cognitive development showed that the following variables were negatively associated with the Psychomotor Development Index at 24 months: prenatal Pb exposure, applying henna to the mother's feet during pregnancy, mother's education, number of gestational weeks, head circumference, sibling rank, applying kohl to the infant's eyes when born, and sleeping problems.	Pb levels in kohl were not measured. The goal was to assess the effect of prenatal and postnatal Pb exposure on early cognitive development. Results were inconclusive because of bias introduced by a high drop-out rate, especially in the low PbB group. However, an idea of prevalence of kohl use can be drawn from some of the data: in the low Pb group (n=74), 89% of mothers applied kohl to their eyes during pregnancy, 10% to the infant's umbilicus at birth, and 30% to the infant's eyes at birth. The rates in the high Pb group (n=45) were 91%, 16%, and 27%, respectively.

Table 4. Epidemiologic studies associating use of kohl (surma) with Pb exposure

*In these studies, exposures were self-reported through questionnaires. Pb was usually measured by graphite furnace or flame atomic absorption spectroscopy (AAS).

Location, study design, subjects (reference)	Findings	Comments
Basrah, Southern Iraq, prospective cross-sectional study,602 randomly selected individuals attending 17 Primary Health Centers in the center of Basrah from 1 July to 31 December 2002. Age range was 9-79 years (Al Naama et al., 2010)	PbB of children ≤ 15 years old (n=26) was significantly higher ($p<0.05$) than older subjects (12.62 ± 3.85μg/dl vs. 11.20 ± 3.4 μg/dl). Risk factors for elevated PbB were male sex, regional location of dwelling, higher education, smoking, occupational exposure to Pb, and (among females) use of kohl. Prevalence of kohl usage in females was 25.6%; mean PbB was significantly higher in users than non-users (13.91 ± 4.42 μg/dl vs. 9.88 ± 2.68 μg/dl, $p<0.0001$). Drinking water sources, amount of milk consumed, and exposure to paint were not significantly correlated with PbB.	Kohl was not analyzed for Pb content. This study was among the first reported Pb survey from Iraq, and the number of children in it was small. However, it identifies a need for further studies of Pb exposure in children.
Al-Kharj area of Saudi Arabia, cross sectional study, 1578 women who delivered at the Al-Kharj Hospital between 2005 and 2006; Pb, Cd, and Hg were measured in maternal blood and placental tissues; participation rate was 99.9% (Al-Saleh et al., 2011)	Mean maternal PbB at delivery was 2.897 ± 1.851μg/dl (range 0.073-25.955). Mean cord PbB was 2.551 ± 2.592 μg/dl (range 0.154-56.511). 14 mothers (0.89%) and 13 newborns (0.83%) had PbB > 10 μg/dl. The Mann–Whitney U statistical test showed that mothers' PbB was significantly ($p<0.05$) increased by no history of outside employment, passive smoking, taking vitamins during pregnancy, having dental amalgam fillings, applying henna to the hair and hands, using kohl on their eyes, and eating seafood. Placental Pb levels were significantly increased by mothers' use of dye or henna on their hair or kohl on their eyes.	The study collected information on about 30 demographic and lifestyle characteristics of the subjects. It revealed that mean PbB in child-bearing Saudi women in the Al-Kharj region has decreased by half since a study reported in 1995 (Al-Saleh et al., 1995), perhaps owing to the removal of Pb from gasoline in Saudi Arabia in 2001, but that non-occupational heavy metal exposure (to Pb, Cd, and Hg) is a matter of concern. The prevalence of kohl usage was 65% (1026/1575), though metal analysis of the kohl used was not performed in this study.

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*In these studies, exposures were self-reported through questionnaires. Pb was usually measured by graphite furnace or flame atomic absorption spectroscopy (AAS).

Location, study design, subjects (reference)	Findings	Comments
Nagpur, India, hospital-based cross-sectional study, 100 breast-fed and 100 non-breast-fed infants with normal development and no major illnesses aged 4-9 months attending a Nagpur clinic between April 2000 and April 2001 (Patel et al., 2011)	Mean PbB was 10.148 ± 9.128 μg/dl; prevalence of elevated PbB (>10 μg/dl) was 38.2%. Of 20 risk factors analyzed, including occupational exposure of father, caste, mother's education, feeding method, per capita income, and others, the major risk factors for elevated PbB (as shown by multivariate analysis) were house paint removal done in last 12 months, use of surma on the infant, and maternal use of sindur (cosmetic vermillion powder used by Hindu women that may contain high amounts of Pb).	Study was intended to determine the effect of breast-feeding vs. non-breast-feeding on PbB. No effect of feeding practice was found. Environmental sampling was conducted by questionnaire to identify risk factors for elevated PbB.
Marseille, France, case study, 39 year old woman exposed 20 years earlier to Pb acetate as a worker analyzing wine; she had no other occupational exposure to Pb; her PbB was analyzed twice about 20 years later as a follow-up and increased between 2009 and 2011 (Kervegant et al., 2012)	PbB was 24 μg/dl in 2009 and 28 μg/dl in 2011 with no known occupational exposure to lead since the exposure 20 years prior. Kohl was suspected as the source of Pb exposure, as the woman had applied kohl to hers eyes daily since the age of 20. Her kohl contained 80% Pb and traces of arsenic and mercury.	The kohl used by the patient at the time of analysis was purchased from a shop in Marseilles, although it is banned in France.

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Location, study design, subjects (reference)	Findings	Comments
Glasgow, Scotland, case-control study, 217 Asian children from 64 families, aged 4 months to 18 years, studied at a special clinic in 1979; divided into surma users, occasional users, and non-users (Attenburrow et al., 1980)	PbB was not significantly higher in the 17 regular surma users (16.56 ± 5.91 µg/dl) than in 178 non-users (15.75 ± 6.23 µg/dl). It was not clear whether 22 occasional users were included in the analysis.	The investigators concluded that, "Surma remains a theoretical rather than a practical health hazard." Recruitment and selection of the study population were not described. Pb content of 7 surma samples was 5-30% w/w, which is lower than that found in other studies. Mean PbB was ≥ 10 µg/dl (the intervention level for children in the US) for both surma users and non-users, indicating other sources of Pb exposure in the environment that might have masked Pb exposure from surma.
New Delhi, India, case control study, 253 children 0.2 – 15 years: 82 controls (defined as asymptomatic for Pb poisoning) 88 with pica, 16 with pica who used surma, 21 without pica who used surma, and 46 with suspected Pb poisoning (Gogte et al., 1991)	PbB levels of the 5 groups (µg/dl) were: 9.6 ± 6.8 (control), 23.0 ± 13.82 (pica), 30.8 ± 18.7 (pica and surma), 11.6 ± 8.4 (surma, no pica), and 22.5 ± 26.9 (suspected Pb poisoned). Those with pica and surma use had higher PbB than those with pica alone, but it was not statistically significant. However, the proportion of children with PbB > 40 µg/dl was 5/16 (31%) in the pica + surma group, compared to 14/88 (16%) in the pica group.	Surma samples were not tested for Pb content. However, surma may have contributed to elevated PbB in children exhibiting pica, but the statistical power of the study was small and its contribution was not significant.
Karachi, Pakistan, cross-sectional survey, 345 children (53 girls and 66 boys) 0-12 years old systematically sampled from patients who had blood drawn at The Aga Khan University Hospital between June 1997 and August 1997; participation rate was 119/345 (34.5%) (Khan et al., 2001)	Children were divided into those exposed or not exposed to potential sources of Pb (paint, renovation, adults with Pb exposure, utensils, pica, surma, and folk medicine). Mean PbB of all the children was 7.9± 4.5 SD µg/dl. The prevalence of surma use was 18/119 (15.1%). Children exposed to Pb via paint (n=25), renovation (n=37), and pica (n=14) but not the other factors had significantly higher Pb levels ($p<0.05$) than unexposed children, with means from 9.0 to 11 µg/dl. Surma was a borderline risk factor ($p<0.07$) for elevated PbB: PbB for unexposed children was 7.5 µg/dl , compared to 9.5 µg/dl for exposed children.	The authors concluded that surma is an important source of Pb exposure, although the association between elevated PbB and surma use was of borderline statistical significance. The sample size may have been too small to detect differences between surma-exposed and unexposed children. Also, Pb levels of the surma used were not measured and may have included non-Pb-based surma.

Table 5. Epidemiologic studies showing no association between use of kohl (surma) and Pb exposure*

*In these studies, exposures were self-reported through questionnaires. Pb was usually measured by graphite furnace or flame atomic absorption spectroscopy (AAS).

Location, study design, subjects (reference)	Findings	Comments
Karachi, Pakistan, geographically stratified cross sectional study, 430 children aged 36-60 months from 5 regions: the city center (Sadar), two suburbs (North Karachi and Malir), a rural community (Gadap), and Baba Island near Karachi harbor; response rate was 93% (400 children) (Rahbar et al., 2002)	Mean PbB was 15.6 µg/dl; prevalence of elevated PbB (>10 µg/dl) was 80.5%. Children exposed to surma 2 or more times per week had significantly higher ($p<0.001$) median PbB levels than others (15.48 vs. 12.66 µg/dl). However, when adjusted for 10 other variables a multivariate model, the effect of surma was not significant. 5 independent factors associated with elevated PbB were: father's education and his exposure to Pb in the workplace, child's habit of eating food from vendors, child's hand-to-mouth activity, use of metal cooking utensils, and west-facing house.	Surma samples were not tested for Pb content. However, more than half the children in the study used surma. Given this finding, the higher median PbB level of surma users than non-users, and the association of surma use with elevated PbB by other investigators, the authors recommended that mothers be discouraged from applying surma to their children's eyes.
Rural village and a lower middle-class urban district of Giza, Egypt 164 children aged 9-60 months (Boselia et al., 2004)	Mean PbB was 12.96 µg/dl; prevalence of elevated PbB (>15 µg/dl) was 46% of rural children and 20% of urban children; younger children had significantly higher PbB than older children. Of 14 risk factors analyzed, including parents' occupations and level of education, use of kohl, and various housing data, both expected and unexpected correlations emerged by multivariate analysis. Children unexpectedly had significantly higher PbB if they lived on low traffic streets and if their mothers were employed outside the home. As expected, PbB was also higher if they slept on the floor and if streets were unpaved. Use of kohl was not a significant risk factor for elevated PbB.	Kohl samples were not tested for Pb content. Mean PbB levels were ≥ 10 µg/dl in all four groups of children studied (9-36 and 37-60 months of age, rural or urban-dwelling). Kohl use was reported for 71 of 164 children (43.3%). These results suggest that if kohl was associated with elevated PbB, its contribution to overall Pb exposure may have been masked or overshadowed by other sources of Pb exposure in the environment.

Table 5. Epidemiologic studies showing no association between use of kohl (surma) and Pb exposure*

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Location, study design, subjects (reference)	Findings	Comments
Lucknow, India, 500 pregnant women enrolled from 70 randomly selected designated municipal slums out of 203 in Lucknow, cross-sectional study; women were registered at their local "aganwandi" center for prenatal care from June 1994 to July 1995 (Awasthi et al., 1996)	Mean PbB was 14.3±7.9 (SD), distributed as follows: 180 (36.2%) had PbB <10 μg/dl, 223 (44.6%) had PbB of 11-20 μg/dl, and 96 (19.2%) had PbB > 20 μg/dl, 25 of whom had PbB > 30 μg/dl. No factors were found that influenced PbB except higher parity and living near heavy vehicular traffic. The reported use of surma was negatively associated with increasing PbB, and the reported presence of paint in the home showed a trend in the same direction, both unexpected findings.	The investigators were surprised by negative findings for surma use and speculated that the Pb content of local surma may be negligibly low (it was not measured), that the women's poverty may have caused them to use surma sparingly, or that the women may have misreported the use of surma. They recommended more detailed studies of surma in India.

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The major difference between the 20 studies and case reports showing an association between kohl use and Pb exposure (Table 4) compared to the 6 showing a weak or no association (Table 5) is that most (12/20) of the former group of studies included chemical analysis of the kohl used by the subjects and only one of the latter group did so. The authors of half the studies showing weak or no associations recognized identified limitations of their surveys and therefore recommended that kohl still be considered an important source of Pb exposure. Weaknesses of most of the studies are small sample size, attrition of the members of the study population, and sampling bias from the use of convenience samples. For example, sampling bias may have occurred in studies that included hospital births but excluded home births, as the populations of women involved may have differed regarding cultural practices or health awareness. In addition, the studies were likely to have information bias, particularly from imperfect recall or underreporting by parents about kohl practices. Details are sketchy in most of the studies regarding avoidance of sampling bias, confounding variables, or recall bias.

Other information has also emerged from these studies. Kohl or surma users from the Middle East, India, and Pakistan who have emigrated to Europe or the US continue their traditional use of the cosmetic [71-74]. Government regulations on kohl importation and public education on the hazards of Pb-containing kohl use have not yet eliminated poisoning incidents among the émigrés.

Despite concerns about existing epidemiologic studies which render our understanding of the association of kohl usage with PbB levels imperfect, what emerges is a strong case that Pb-containing kohl presents a real and present risk for elevated PbB in both children and adults. Investigators in many countries have called for greater governmental regulation of the composition of kohl or surma and more widespread education of the public about the hazards of its use. Nevertheless, the use of kohl continues, because of strong cultural traditions.

Attitudes and beliefs regarding kohl usage:

The centuries-long history of kohl use and its religious and medicinal significance have been adequately presented elsewhere [42, 40] and therefore will be only briefly discussed. Its use has a long tradition that is recorded in the archeological record of the Middle or New Kingdom of ancient Egypt, c. 2040 to c. 1070 B.C. [75] and in religious and historic texts of Islam. Ancient Egyptians used kohl to enhance beauty, protect their eyes from the sun and from infection, and ward off the "evil eye." In Islam, the Hadith of the Prophet Mohammad (Peace be upon Him) states: "Use kuhl made of ithmid on the eye; it brightens the eyesight, and strengthens and increases the growth of the eye lashes." (Hadith 1). Some texts imply that kohl used centuries ago was an antimony (Sb)-based cosmetic [4]. However, only one ancient Egyptian kohl sample of 18 studied was been found to contain Sb [75]. Moreover, two studies of more than 100 modern kohl samples have found only low or trace amounts of Sb [45, 4]. No early Islam sample has yet been analyzed. According to the Hardy et al. (2006)[75], "The reason for the occasional statement that antimony/antimony (tri)sulphide/stibnite was used as an eye cosmetic in ancient Egypt is mainly one of philology. The ancient Egyptian word for eye-paint in general and the black form in particular was "msdmt" (mesdemet) and it became "cthm" (stem) in Coptic, then "stimmi" in Greek and finally "stibium" in (Roman) Latin. This last word was later used for the element antimony, and stibnite for its sulphide ore."

Does kohl have medicinal properties? There is evidence that black eye makeup used by ancient Egyptians had potential antibiotic properties [76]. Chemical analysis of 52 samples from ancient Egyptian makeup containers preserved in the Louvre museum in Paris shows that they contain 4 Pb-based chemicals: naturally occurring galena (PbS) and cerussite ($PbCO_3$), as well as man-made laurionite (Pb(OH)Cl) and phosgenite ($Pb_2Cl_2CO_3$). The two chloride compounds are of interest because they might generate a small concentration of free Pb ions (Pb^{2+}) in an environment of lacrimal fluid. By means of platinized carbon fiber microelectrodes, the investigators were able to measure enhanced nitric oxide (NO) production by cultured keratinocytes treated with 0.2 and 0.4 μM Pb acetate in an aqueous solution. NO stimulates macrophages to attack bacteria, and therefore Pb2+ in the eye has theoretical benefit. However, the presence or absence of laurionite in modern formulations of kohl also has not been reported. Therefore, we do not know whether modern Pb-based kohls have NO-generating capacity and its potential antibacterial properties. Furthermore, this finding contradicts previous reports that Pb inhibits NO production in vitro by mouse macrophages [77-78].

Does Pb-based kohl have a place in the 21st century pharmacopeia? To our knowledge, no modern research has been conducted to address the therapeutic value of Pb-based kohl. It has not been tested in either animal or human studies in a controlled experiment to determine whether it protects the eyes from either sunlight or infectious agents. Likewise, public health surveys have not been conducted to compare the eye health of kohl-users vs. non-users. Mothers traditionally apply kohl to infants and children to protect them from the "evil eye" [51], but no surveys have been reported to compare the psychological well-being of kohl users vs. non-users. The idea that kohl can ward off the "evil eye" may be a particularly strong motivation for using it in Bedouin tribes, which view the evil eye as "one of the most dangerous forces that can interfere with their lives" [79]. Given the absence of medical evidence and the presence of strong tradition, the modern use of kohl appears to be deeply rooted in cultural attitudes and beliefs regarding its value.

A number of small surveys have been conducted by clinicians and epidemiologists to discern the extent of kohl use and the reasons for its use. In a case control study in the UK that included 45 Asian children whose mothers treated them with surma, investigators learned that 19 parents used it to clean the eyes, 14 purely for cosmetic reasons, and 2 for religious reasons. The remaining parents were uncertain why they used surma but believed it was good for the eyes [80]. In two other early studies in the UK, Healy et al., (1986) [81] found that 15 of 46 (32.6%) of Asian women interviewed in Nottingham used surma, and Bhopal (1986)[82] found that 12 of 65 (11.7%) of Asian women in Glasgow used surma. In Oman, Vaishnav (2001) [83] found that 72.7% of the population she surveyed used kohl, though the sample size was not given.

Alkhawajah et al., (1992) [59] interviewed a random sample of 500 employees, students, and patients at King Faisal University and King Fahd Hospital, mean age 34.0 ± 7.1 years, including 382 females and 118 males, 485 of whom were Saudi citizens. The prevalence of kohl usage was 233/500 (47%), 59% among women and 5% in men. Kohl was used as an eye cosmetic (66%), children's eye and umbilical stump remedy (26%), and both for children's eyes and umbilical stump (8%). Forty-five percent who used it as an eye cosmetic used it daily. Most users (94%) obtained it ready-made from markets, while the rest made their own.

One small survey of attitudes has also been reported about kohl users in the US. Mojdehi and Gurtner (1996) [84] interviewed 40 women in Alexandria, Virginia (85% from the Middle East, India, and Pakistan, and 15% from Africa), 33 of whom has a personal history of kohl use. Of these women, 12 used kohl on their infants, both girls and boys. The reasons given for kohl use were cosmetic value for women, cleaning the eyes, reducing soreness or redness of the eye, and increasing visual acuity. Some mothers also mentioned traditional value and religious significance to kohl use.

Al-Ashban et al. (2004) [4] surveyed 1250 kohl users, non-users and sellers in 5 regions of Saudi Arabia about their attitudes and beliefs regarding kohl use. The survey revealed that kohl use was common as a cosmetic, eye treatment, or treatment for the umbilical stump. Most of the respondents believed that kohl was safe to use as an eye cosmetic and 81% of kohl sellers believed it valuable for treating eye ailments. Families of about half of kohl sellers used kohl themselves, and the percentage of kohl sales was similar between city-dwellers and villagers. Kohl users commonly applied it to their children.

Ahmad et al., (2006) [85] surveyed 160 male and female students aged 9 to 12 years and their 16 teachers in 2 government and 2 private schools in the Abbottabad district, northern Pakistan, to assess their knowledge and attitudes about eye health. Kohl use was identified by the investigators as a practice that should be of concern to health educators. Only 1 of the 16 teachers would advise students to avoid the use of kohl to keep their eyes healthy, and 23/160 students indicated they would use kohl as one of several actions to keep their eyes healthy.

Mohta (2010) [86] interviewed 100 consecutive children under 12 years of age attending a tertiary care pediatric hospital in northern India (48 girls, 52 boys, most <5 years old, 65% Hindus, 30% Muslims, and 5% other religions) to assess their use of "Kajal". The prevalence of use was 86%. The survey found that 64% of the mothers had at least a class 12th level education, 90% of the mothers applied Kajal simply on the advice of their elders, and >50% of parents did not know the advantage of applying 'Kajal'. Reasons cited for using Kajal were that it increases the size of the eyes, improves eyesight, and protects the eyes against diseases. About 80% of parents surveyed made their Kajal at home, most commonly a soot-based mixture. This small study is somewhat reassuring that at least in this group of respondents, the cosmetic used by most of them was carbon-based, rather than Pb-based.

From the above surveys carried out since 1979 in the UK, Saudi Arabia, and other countries, the use of kohl by mothers on themselves and their young children seems common among Muslim and Hindu populations. The shortcomings of these surveys are small sample size, absence of demographic data in many of them, lack of information on the duration and frequency of kohl usage, and lack of information on the source and composition of the kohl used. In particular, no distinction was usually made to determine the comparative prevalence of use of Pb-based vs. carbon-based formations, the latter of which would be benign with regard to Pb toxicity. Nevertheless, the studies are in general agreement that kohl use is common in the populations sampled and that users do not recognize the hazards of Pb-based kohl use, especially in children.

Conclusions and future directions:

The preponderance of evidence indicates that the use of kohl as an eye cosmetic or umbilical stump astringent is a significant risk factor for elevated PbB levels in both children and adults and that this risk is not "more of theoretical nature than a practical health hazard," as claimed in one recent review [40]. The majority of investigators who are studying Pb exposure and Pb toxicity in the Middle East and elsewhere have called for greater public awareness of the dangers of Pb-containing kohl usage and for government regulation of its distribution and manufacture, so that kohl that is purchased by consumers does not contain Pb. The major conclusions and suggestions for further research from this review are as follows:

- Definitive studies on Pb bioavailability from kohl via ocular, dermal, or gastrointestinal routes have not been carried out and reported to date, but epidemiologic studies clearly indicate that kohl users have higher PbB.
- Some kohl samples are made up of other ingredients, such as carbon, and (unless other toxic chemicals are present in them) should provide a safer cosmetic than galena-based kohls.
- No epidemiological study has controlled for the type of kohl used by subjects (Pb-free of Pb-containing) and its association with elevated PbB. Despite this lack of data, most studies conclude that kohl use increases the risk of elevated PbB in infants and children, suggesting that much of the kohl used contains Pb.
- In heavily contaminated environments, other sources of Pb may be of greater

importance than kohl, such as paint chips and roadway dust. However, it is important to emphasize that, in contrast to environmental exposures through dust and paint chips, exposure to Pb though kohl use is avoidable. Whereas roads and housing are difficult and expensive to remediate from Pb contamination, kohl can readily be formulated without Pb.

- As society becomes more globalized, the use of kohl has spread to countries beyond the Muslim and Hindu countries in which it originated, requiring health care providers to learn about its safety. Scientists have a responsibility to discover facts about the hazards of kohl use through well-designed studies, and public health officials have a duty to protect the public from potential health risks for which there is a reasonable expectation of harm.

Competing interests:

The authors have no competing interests.

Authors' contributions:

The authors all contributed to the preparation of this review.

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