



Investigation of Lead Concentrations in the Blood of Kohl Users from Dangora Community of Kano State, Nigeria

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ABSTRACT

The blood of 15 regular Kohl users and that of 10 non-Kohl users from Dangora community were analyzed for lead (Pb) concentrations. The work is to ascertain the possibility of absorption of Kohl through the eyes when used as cosmetic, to elevate the blood lead level. The lead concentrations in water used by the Dangora community were also analyzed. 15 registered and unexpired eye/ear products were also analyzed for the minimum body requirement of lead concentration to the eye. The blood lead concentration results indicate a mean Pb level for the Kohl users as $10.9 \pm 4.4 \mu\text{g}/\text{dl}$ and for non Kohl users as $5.2 \pm 3.9 \mu\text{g}/\text{dl}$. The Pb concentration in the well waters analyzed, were found to be highest at CGWW = 3342.6ppb and lowest at GTWW = 37.2ppb . The tap and bore-hole waters were observed to have an average of 57.4ppb lead concentration. The detected Pb concentrations in 14 of the eye/ear products range between 0.0266ppm and 0.1751ppm with a mean value of $0.0932 \pm 0.0142 \text{ppm}$ which is found to be significantly lower than the safety limit in cosmetics (10ppm)

Keywords: AAS, Blood Pb concentration, kohl, Lead Poison, Risk.

1. INTRODUCTION

Despite the fact that kohl has been in use since antiquity (Al-Hazzaa and Krahn, 1995), it was towards the end of the 21st century that kohl was realized to be a potential source of lead poisoning (Kapoor, 2007). This was discovered by pioneer studies that traced the death of an Asian child, among the Indian and Pakistani children residing in England, to the liberal use of kohl (Ali *et al.*, 1978; Betts *et al.*, 1973; Snodgrass *et al.*, 1973; Warley *et al.*, 1968;). Later reports (Hardy *et al.*, 2006; Al-Ashban *et al.*, 2004; Hardy *et al.*, 2002; Hardy *et al.*, 1998; Al-Kaff *et al.*, 1993; Al-Saleh *et al.*, 1993; Madany and Salim, 1992; Parry and Eaton, 1991; Tabbara and Burd, 1987; Rahman *et al.*, 1986; Abdulla, 1984; Fernando *et al.*, 1981; Shaltout *et al.*, 1981;) from kohl using countries conformed to the observation that kohl is a source of lead poison. The worst attributed the death incidence of 25% among children with encephalopathy to the liberal use of kohl. (Shaltout *et al.*, 1981). Research findings also reveal a correlation between blood Pb and symptoms of diseases. It was also demonstrated that the use of kohl elevates the concentration of blood Pb (Al-Ashban *et al.*, 2004; Al-Kawajah, 1992). The United States environmental Protection Agency (USEPA) identified that Pb level above $20 \mu\text{g}/\text{dl}$ in the body may cause health damage even if there are no symptoms.

Despite this evidence, other reporters argued that the absorption of lead through the eyes would not be possible; that kohl is not practically soluble in the aqueous medium of the eye and the positive intra ocular pressure further prevents its absorption or penetration. Therefore, the so called lead toxicity through the eye is unfounded (NHMH; 2007; Nir *et al.*; 1992). Hence the

need for further research along this direction for possible toxic effect of lead from the use of kohl.

Not only is kohl famous for cosmetic appeals but also for medicinal reasons (Al-Hazza and Kran, 1995). As a natural health product, kohl has many uses that vary among cultures including; use as an aid to blood cloth, and thus applied on the umbilical stump of newly born babies, circumcision after-care. Kohl as an eye infection cure, digestive aid, sun glare prevention, eyestrain reliever, and general anti-microbial treatment (Health Canada, 2006; Grimes H; 1997). A common practice among some Nigerian communities is that kohl powder thoroughly mixed with honey is taken on daily basis as a cure for asthma. Since the ancient time, kohl has been a major ingredient of formulae used in the traditional treatment of diseases. (Yaish *et al.*, 1993).

In Nigeria, the use of kohl as a cosmetic still remains in vogue as in Saudi Arabia (Al-Kawajah, 1992) as well as in other recognized kohl using countries (Kapoor, 2007). The popularity of kohl as cosmetic could further be appreciated from its availability even in European countries and the United State (Health Canada, 2009). It is hoped that the findings of this work will add to literature on kohl absorption studies for health, economic and legislation benefits.

2. EXPERIMENTAL

2.1 Materials and Methods

Blood and water samplings were carried out at Dangora town of Kiru Local Government Area of Kano State. Dangora is located

on latitude 11°31'N and longitude 80°0'E with a population of fifteen thousand people in accordance with the 2006 census.

In the selection of subjects for our analysis, their feeding frequency questionnaire (FFQ) was considered, demographic information questionnaire (DIQ); including smoking, alcohol consumption and age was used. The activity frequency questionnaire (AFQ) and medical information related to Pb induced diseases were applied. As a community with the same tradition and feeding habit, their age had to be considered. The selection was conditioned to make water intake the only variable parameter among the subjects.

The skin of each of the chosen subjects from the community was carefully cleaned with a piece of absorbent cotton (H₂O₂ bleached) that had been soaked in a disinfectant (methylated spirit), prior to blood sampling. Venous blood was collected using syringe into a 0.1ml of lithium Heparin as an anti-coagulant. The samples were then gently agitated after collection to ensure dissolution of the heparin. The samples were then stored in a refrigerator at 4°C before being prepared for analysis.

The sampling equipment (needles, syringes and blood tubes) were also checked for pb contamination, and surfaces of all glass-ware and tubes used were de-ionized before use.

2..2 Water Sources

Five water sources were identified as being patronized by the subjects viz: Dogon Pampo well water (DPWW), Gidan Taki well water (GTWW), Cikin Gari well water (CGWW), Bore Hole water (BHW) and Tap water (TPW). The waters were respectively collected separately into high density polythene containers (25ml) into which 1ml of concentrated nitric acid were added for preservation.

The water sampling was done during the rainy season (August, 2010) when maximum dilution of minerals is expected to occur.

2..3 Eye/ear Products

Different registered and unexpired products comprising 13 eyes/ear drops and 2 eye ointments were bought at random as normal consumer products. Eight (8) of them were manufactured in Nigeria and the remaining were imported products from France (1), Pakistan (3), China (1), and India (1).

2..4 Sample Preparation (Digestion)

2ml each of the blood and eye/ear drops were hydrolyzed by wet ashing (digestion) with 1ml concentrated nitric acid and 1ml perchloric acid (Riedel de Haen, Germany). This was afterward diluted to 25ml with de-ionized water in a volumetric flask. Blank samples were treated in the same way and appropriate corrections were made in the final calculation.

Ointments were digested by heating 0.7gm of each sample in 1ml of hydrogen peroxide, 1ml of concentrated nitric acid and 1ml of concentrated perchloric using a furnace.

The sample analyte/digest were made ready for Atomic Absorption Spectroscopy (AAS) metal determination analysis. Machine model Shimadzu AA 6800 was used for the analysis. The machine adopts the Zeeman's correction method for the background intensity. The instrument was calibrated with National Institute of Standards (NIST) standard reference material (SRM) 955a, after every 20 samples.

3. RESULTS AND DISCUSSION

Tables 1 and 2 shows the results of blood Pb concentrations of kohl users and non kohl users respectively. The Pb concentrations detected (Table 1) range from 3.5 $\mu\text{g}/\text{dl}$ to 21.2 $\mu\text{g}/\text{dl}$ with a mean of $10.93 \pm 4.4 \mu\text{g}/\text{dl}$ which is comparable to the 10 $\mu\text{g}/\text{dl}$, as the highest tolerable concentration of Pb in blood. Any value above this is defined as high (www.webmd.com/news/20060918/lead-on-blood-safe-levels-too-high). It is clear from the table 1 that 6/15 (40%) of kohl users have their Pb concentration in blood below the acceptable limit while 60% have the blood Pb concentration in excess of the defined limit. It has been reported that 99% of Americans fall below the threshold. However, an increased likelihood of death from heart attack or stroke has started to be seen in people with Pb blood levels greater than 2 $\mu\text{g}/\text{dl}$ (www.webmd.com/news/20060918/lead-on-blood-safe-levels-too-high).

In table 2, the concentration of Pb in blood of the non-kohl users ranges from 0.4 to 12.8 $\mu\text{g}/\text{dl}$ with no Pb detected in the blood of one of the subjects. The mean value $5.2 \pm 3.9 \mu\text{g}/\text{dl}$ was found to be statistically lower than the threshold limit (10 $\mu\text{g}/\text{dl}$; $p < 0.01$). It is thus clear that the Pb concentration in the blood of kohl users is significantly higher than for non kohl users, but with the concentration of Pb in their water highest for the two communities (DPW and CGWW; Fig.1). It is also evident (Fig.1) that where the observed Pb level in water used by the subjects is lower (BHW(E₁ and E₂)), the Pb level in blood of kohl users is observed to be much higher than that of all the non kohl users. Also a difference of more than 50% of Pb concentration in blood is observed between the regular kohl users and that of non-kohl users that uses the same source of water. It can therefore be concluded that Pb actually penetrates the eye to the blood stream and more probably through the conjunctiva.

Table.3 shows that there is no difference in Pb concentration between male and female kohl users as well as for male and female non kohl users. Hence, Pb uptake does not depend on sex. Age of the subjects that use kohl could not be observed (as was expected) to be a major factor in the accumulation of lead in their blood. In Table 1, a 14 year old has 11.6 $\mu\text{g}/\text{dl}$ concentration of Pb in his blood and less Pb concentration in his

source of water when compared with a 60 year old having 10.8 μ g/dl pb concentration in his blood but more lead concentration in his source of water.

On the other hand the Table 4 further shows that there could be a remarkable difference in blood Pb levels arising from water intake. This is also as depicted in Fig.1 with variations in Pb concentration in blood of Kohl users and non kohl users with common water sources. Among the four major sources of water analyzed for Pb concentration, Cikin Gari well water (CGWW) has the highest Pb concentration of 3342.6ppb (1/10th of value shown in Fig1). Water from Gidan Taki well (GTWW) has the least Pb concentration (37.2ppb). In the first instance none of the waters carry Pb below the acceptable limit of 10ppb (0.01 μ g/dl) of Pb in water. Rather statistical analysis (student's t-test) reveals the waters to carry Pb at a concentration considerably higher than the safety limit (10ppb; $p < 0.01$). Thus, the people of Dangora town could be at risk of Pb poisoning even from their source of water alone (Fig. 1)

The mean blood Pb concentration value ($11.20 \pm 3.62 \mu$ g/dl) of the six subjects patronizing CGWW water was found to be considerably higher than that of the other six people with a mean blood Pb concentration ($5.33 \pm 2.14 \mu$ g/dl; $P < 0.01$) patronizing GTWW water. This probably explains why the subject with code S7M (Table2) has an elevated Blood Pb concentration of 12.8 μ g/dl despite the fact that the subject does not use Kohl but has his source of water from CGWW (Fig.1 showing 1/10 of actual data value for water only) with observed highest Pb concentration (3342.6ppb) in the water.

Table 5 shows that Pb was detected in all the eye/ear products and ointments analyzed (all registered) except the product EED2 with serial number six. The detected values range from 0.0266 ppm (EED5; S/N 14) to 0.1751ppm (EO2; S/N 15) with a mean of 0.0932 ± 0.0142 ppm. From the fact that Pb was detected in 14/15 (93%) of the samples, with a mean value that is significantly lower than the established safety limit of Pb in cosmetics (0.2932 ppm), it could be concluded that Pb must be playing some important role as an ingredient in eye disease as well as a preventive and curative agent. Therefore, the presence of Pb in cosmetic products should not be the main source of concern, but the elevated Pb level in the cosmetic (kohl). The finding supports the idea of the kohl proponents to a greater extent that, application of kohl on the eye has some health

benefits except that the Pb level should be reduced to the levels of those carried by the registered products and within the defined laws.

The result further suggests that not only does Pb play a role as eye disease curing agent but also as an ear disease curing agent, therefore, the idea of the total condemnation of Pb in cosmetics by other researchers should be reconsidered.

On the other hand, the absence of Pb in EED2 suggests that there is an alternative to Pb in eye/ear drops and ointments. Hence eye/ear drops manufacturers should adopt the same formulae if proved more effective and safer; it has been reported that no Pb level in the body is safe (www.werbmd.com/news/200691/lead-in-blood-safe-levels-too-high).

4. CONCLUSION

In our attempt to resolve the possibility of kohl absorption through the eyes to elevate the blood Pb concentration, it was observed that application of kohl as cosmetic may enable Pb to penetrate the eyes' muscles into the blood. However, it was thought that the absorption is most probable through the conjunctiva. Hence, the need for further work to investigate the means of absorption of Pb through the cornea or the conjunctiva to the blood. We also realized that the source of water of Dangora community could be a source of Pb poisoning and this account for the elevated Pb of one of our subject despite the fact he does not use kohl. This may in addition to the use of kohl by some of the subjects, be the reason for the observed increase in Pb related diseases in the community.

The reasons of the ancient physicians to heavily depend on Pb as a major ingredient in the formulas employed in treatment of their patients (Yaish *et al.*, 1993) could therefore be inferred. Though the finding was in favour of the kohl proponent to a greater extent, the Pb level must be reduced to the acceptable values. Because all our subjects that uses kohl had more than 50% of their Pb concentration in blood above the Pb concentration in blood of non kohl users (Table 3) our finding agrees with the earlier reporters, and hence we also conclude that kohl cosmetic is a source of Pb poisoning.

Table 1: Results of analysis of Pb blood levels for 14 members of Dangora community that are regular Kohl Users with their gender, age and sources of water

S/N	Code	Sex	Age	Water source	Blood Pb Level μ g / dl
1.	S2M	M	60	DPWW	10.8
2.	S5M	M	45	DPWW	13.2
3.	S6M	F	38	DPWW	12.4
4.	SK11	F	41	TW	3.5
5.	SK12	F	14	TW	11.6

6.	SK13	F	26	TW	7.6
7.	SK 1	F	21	GTWW	5.2
8.	SK 4	M	30	GTWW	8.4
9.	SK 6	F	27	GTWW	10.0
10.	S2	M	35	CGWW	14.4
11.	S4	F	50	CGWW	9.2
12.	S5	F	53	CGWW	15.2
13.	SL1	F	30	BH	21.2
14.	S8M	F	25	BH	13.6

Mean value (\bar{x}) = 10.93 $\mu\text{g} / \text{dl}$

Standard deviation (SD) = 4.40 $\mu\text{g} / \text{dl}$

DPWW – Dogon pampo well water = 266.8ppb

GTWW – Gidan taki well water = 37.2ppb

CGWW – Cikin gari well water = 3342.6ppb

BHW – Bore hole water = 57.4ppb

TW – Tap water = 57.4ppb

No Pb is detected in the distil water used for sample preparation

Table 2: Results of analysis Pb blood levels of 10 members of Dangora community that are Non Kohl Users with their gender, age and sources of water

S/N	Code	Sex	Age	Water source	Blood Pb Level $\mu\text{g} / \text{dl}$
1.	SK2	M	22	GTWW	3.2
2.	SK3	M	26	GTWW	4.0
3.	S1	F	24	GTWW	3.6
4.	S3	F	21	TW	6.0
5.	S3M	M	30	TW	ND
6.	S4M	M	17	TW	1.6
7.	S7M	M	24	CGWW	12.8
8.	S9M	M	35	CGWW	5.6
9.	ST10	F	33	BH	9.6
10.	S1M	F	20	BH	0.4

$\bar{x} = 5.2 \mu\text{g} / \text{dl}$

SD = 3.9 $\mu\text{g} / \text{dl}$

ND = Not detected

Table 3; Result of statistical analysis for the mean standard error and the t-test for the significance of Pb levels in the blood of Dangora community and water sources

Group	No. of samples	mean blood pb ($\mu\text{g} / \text{dl}$)	t-cal	t-critical	Decision $\alpha = 0.01\%$
Users	15	10.93 \pm 4.40	3.62	2.50	Significant
Non users	10	4.68 \pm 4.03			
Male users	5	11.70 \pm 4.68	0.11	3.01	Insignificant
Female users	10	10.95 \pm 5.13			
Male non users	6	4.53 \pm 4.49	0.21	3.36	Insignificant
Female non users	4	4.90 \pm 3.88			

users of CGWW	6	11.20 ± 3.62	5.39	3.17	Significant
Users of GTWW	6	5.33 ± 2.14			

Table 4: Results of Calculated Pb concentrations in Blood due to effect of Kohl cosmetics usage

Source of water	Samples(Kohl users/no-kohl users)	Pb in water source $\mu\text{g} / \text{dl}$	Pb in blood of kohl users $\mu\text{g} / \text{dl}$	Pb in blood of non-kohl users (as background) $\mu\text{g} / \text{dl}$	Pb in blood from kohl $\mu\text{g} / \text{dl}$
DPWW	A1(S5M/-)	26.68	13.2	-	13.2
	A2(S6M/-)	26.68	12.4	-	12.4
	A3(S2M/-)	26.68	10.8	-	10.8
TW	B1(SK12/S3)	5.74	11.6	6	5.6
	B2(SK13/S4M)	5.74	7.6	1.6	6
	B3(SK11/S3M)	5.74	3.5	-	3.5
GTWW	C1(SK6/SK3)	3.72	10	4	6
	C2(SK4/S1)	3.72	8.4	3.6	4.8
	C3(SK1/SK2)	3.72	5.2	3.2	2
CGWW	D1(S5/S7M)	334.26	15.2	12.8	2.4
	D2(S2/S9M)	334.26	14.4	5.6	8.8
	D3(S4/-)	334.26	9.2	-	9.2
BH	E1(SL1/ST10)	5.74	21.2	9.6	11.6
	E2(S8M/S1M)	5.74	13.6	0.4	13.2

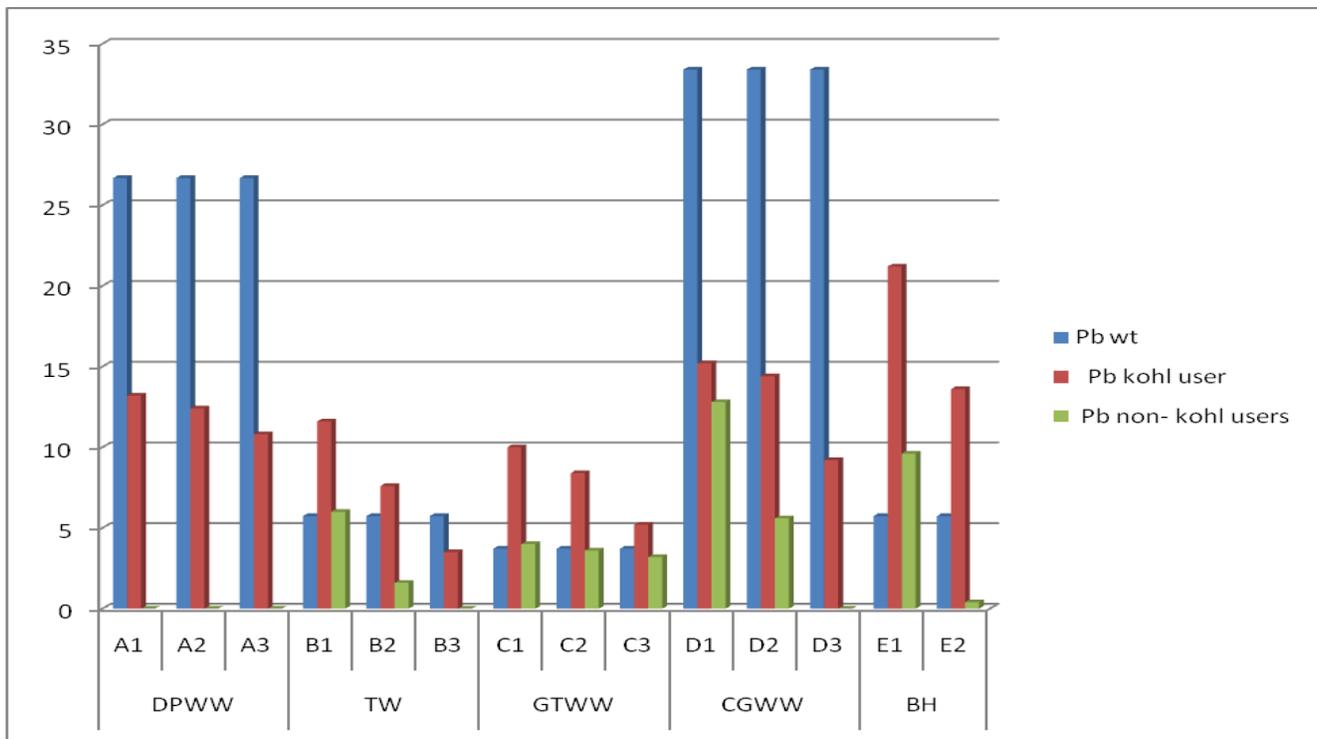


Fig. 1 Plot of Variation of Pb in Blood of Kohl users, Non Kohl Users using same water source

Table5: Results of the analysis of Pb levels in Eye and ear Drops/Ointment to validate the Pb requirement for eye treatment

S/N	Product's name	Country/Reg No	Mtg Date	Exp Date	Detected Pb (ppm)
1.	ED1 (Eye Drops)	Pakistan 017894	2/2010	1/2012	0.1385±0.0121
2.	EED1 (Eye/ear)	Nigeria 04-4861	2/2009	1/2014	0.0852±0.0031
3.	ED2 (eye drop)	Nigeria 04-0528	9/2009	9/2011	0.0746 ±0.0032
4.	ED3 (eye)	Nigeria 04-0529	4/2009	4/2011	0,0479 ±0.0012
5.	ED4 (eye)	Nigeria 04-5588	7/2009	6/2012	0.1438 ±0.0211
6.	EED2 (eye/ear)	India 04-8741	09/2008	08/2011	ND
7.	ED5 (eye)	France 4-060962	-	05/2012	0.1704 ± 0.0322
8.	EED3 (eye/ear)	Pakistan MH/DRUGS/682	10/2009	09/2012	0.1172 ± 0.0211
9.	EED4(eye/ear)	Nigeria 04-5094	02/10	01/13	0.0373 ± 0.0300
10.	ED6 (eye)	Pakistan 0030	07/09	06/11	0,0676 ± 0.0062
11.	EO1(eye ointment)	Nigeria 04-1184	7/2008	06/2011	0.0852 ± 0.0051
12.	ED7 (eye drops)	Pakistan 04-9990	7/2009	7/2011	0.1278 ± 0.0786
13.	ED8 (eye drops)	Nigeria 04-7787	09/09	08/12	0.1278 ± 0.0148
14.	EED5 (eye/ear)	Nigeria 04-5665	10/07	09/10	0.0266 ± 0.0072
15.	EO2(eye ointment)	China 04-7999	01/2009	01/2012	0.1751 ± 0.0440

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REFERENCES

- [1]. Al-Ashban R. M., Aslam, M. Shah A. H., (2004). Kohl (surma): A toxic traditional eye cosmetic study in Saudi Arabia. *Public health*; 118(4) 292 -8.
- [2]. Ali, A. R, Snales, ORC Aslam, M. (1978) Surma and Lead Poisoning. *Br Med J*; 2: 915
- [3]. Abdulla, M. A. (1984) Lead poisoning among children in Saudi Arabia. *Ann Trop. Pediatr.* 87:67-70.
- [4]. Al-Saleh I. A., Fellows, C., Delves, T., Taylor, A. (1993). Identification of Sources of Lead Exposure to among children in Arrar, Saudi Arabia. *Ann Clin Biochem* 30: 142-5
- [5]. Al-Hazaa, S. A. and Krahn, P. M. (1995). Kohl: a hazardous eyeliner. *Int. Ophthalmology*; 19(2): 83 – 8.
- [6]. Al-Kaff, A., Al-Rajhi, A., Tabbara, K., El-Tazigi, A. (1993) Kohl – The traditional Eye liner. Use and Analysis. *Ann Saudi Med.* 13: 26-30
- [7]. Al-Kawajah A. M. (1992). Al kohl used in Saudi Arabia: Extent and possible lead toxicity. *Trop Geograph. Med*; 44 (4): 373 – 7.
- [8]. Betts, P. R., Astley, R. Raine D. N. (1973) Lead Intoxication in Children in Birmingham. *Br. Med J*; 1 (850); 402–6.
- [9]. Fernando, N. F., Healy, M. A., Aslam, M. (1981) Lead poisoning and Traditional practices – the consequences for world health: A study in Kuwait, *Public Health* (London):95: 250 – 60.
- [10]. Grimes, H. (1977) Specific Working Paper on Lead. Proceedings of the International Workshop at Luxembourg. April (18-22): p 191 - 201
- [11]. Hardy A. D. Sutherland H. H. and Vaishnav R. (2002). A study of the composition of some eye cosmetic (Kohls) used in the united Arab emirates, *J. Ethnopharmacol*; 80(2-3): 137-145.
- [12]. Hardy, A. D., Vaishnav R., Al-Kharusi S. S., Sutherland H. H. and Worthing M. A. (1998). Composition of eye cosmetics (Kohl) used in Oman *J. Ethnopharmacol*; 60 (3): 223 – 34.
- [13]. Hardy, A. D., Walton R. I., Myers K. A. and Vaishnav R. (2006). *Availability and chemical composition of traditional eye cosmetics (“Kohls”) used in the United Arab Emirate of Dubai*, sharjah, Ajman, Umm Al-Quwain, Ras Al-Khaimah, and Fukairah. *J. cosmetic Sci.* 57(2) 107-125.
- [14]. Health Canada (2006). Consumer information – Health concerns about lead in traditional Kohl.

http://www.bc-sc.gc.ca/cps-spc/person/cosmet/kohl_info-kohl-eng.php

- [15]. Health Canada (2009). Consumer Product safety. Draft Guidance on Heavy Metal Impurities in cosmetics. File:cafe001/documents/draftguidance on heavy metals impurities in cosmetics.htm
- [16]. Kapoor, V. P. (2007). Kohl and Poisoning: *International Society of Environmental Botanist*, 13(3) <http://isebindia.com/05/08/07-07-html>
- [17]. Madany, I. M. and Salim, A. M. (1992). Lead levels in some eye cosmetics used in Bahrain: *Journal of environmental science and health*; 27 (6) 1541 – 1547.
- [18]. New York City Department of Health and Mental Hygiene (NHMH), 2007. <http://forum.chatdd.com/information-center/20119-kohl-kajal-surma.html>
- [19]. Nir, A. Tamir A. Zelmik A. and Iancu T. C. (1992). Is eye cosmetic a source of lead poisoning? *Israel J. med*, 28 (7): 417 – 21
- [20]. Parry C. and Eaton J. (1991) Kohl: A lead hazardous eyeliner from the third world, *Env. Health Persp*, 94: 121 – 3.
- [21]. Rahman, H., Al-Khayat, A. Menton, N. (1986) Lead Poisoning in Infancy: Unusual cases in the UAE. *Ann Trop. Pediatr*: 6:213-7
- [22]. Shaltout A. Yaish S. A. and Fernando N. (1981). Lead Poisoning Encephalopathy in infants in Kuwait. *Ann Trop. Pediatr*, 1:209 – 15
- [23]. Snodgrass, G. J., Ziderman, D.A, Gulati V., Richards, J. (1973) Letter: Cosmetic Plumbism. *Br. Med J*; 4 (886): 230
- [24]. Tabbara K. F. and Burd E. M. (1987). Microbial Content of Kohl. *Ann Saudi Med*; 7 (3): 177 – 9.
- [25]. Warley, M. A. Blackledge, P., O’Gorman, P. (1968). Lead poisoning from eye Cosmetic. *Brit. Med J*; 1 (584): 117.
- [26]. Yaish, N. H., Niazi, G., Al-Soby, A. (1993) Lead Poisoning among Saudi Children. *Ann. Saudi Med*; 3(5):395-401