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Biological Assessment, Heamatological Study, and Environmental Detection of Eugenol

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Abstract : Eugenol is found in essential oils of many plants. It belongs to a class of naturally occurring phenolic monoterpenoids, chemically it is an allyl chain-substituted guaiacol. A study was conducted on the compound of Eugenol, which included different studies. The first study was the determination of eugenol in body fluid, which includes serum, saliva and urine has been found the highest concentration was in urine then serum and saliva. The second study was the hematological study. Complete blood count was accomplished on the volunteers alredy administrated with eugenol contained mouthwash the analysis was accomplished before and after the mouth wash use. The result observed a slightly negative results and was not that significant, which confirms the strict blood system to resist the toxicity of the compound or the fact that the time period of giving was short.The third study included a histological study on the effect of this compound on the tissues of the animal body by conducting experiments on albino male albino male mice. The side effects were found after 10 days of rat infusion, with yellow spots on the skin and hepatomegaly. The fourth study was the environmental analyses and the effect of this compound on the environment. The eugenol was stable and persistent in water and had found of 11 mg/L concentration in the main tank of wastewater for the dental hospital swage.
Keywords : Eugenol; Biological Assessment; Heamatological Study.

Introduction

Eugenol (E) (C10H12O2; 4-allyl-2-methoxy phenol) is a phenylpropene and an allyl chain-substituted guaiacol, having a density of 1.067 g/mL at 25ºC [1]. It is obtained as a component of aromatic plants such as

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clove, cinnamon, and ginger among others with remarkably unique biological activities [2]. Eugenol is a yellowish oily fluid has been widely used in foods, pharmaceuticals, cosmetics, and active packaging materials due to its potent antimicrobial and antioxidant properties [3], can be useful in increasing protection of Periodontal Ligament[4] Fibroblasts in oxidative injuries and have restorative and prosthodontic applications in dentistry [5]. This compound have recognized acaricidal activity, including activity on R. microplus [6].

A mouthwash is defined as a non-sterile aqueous solution used mostly for its deodorant, refreshing or antiseptic effect [7]. Mouthwashes or rinses are designed to reduce oral bacteria, remove food particles, temporary reduce bad breathe and provide a pleasant taste [8]. Determination of eugenol in mouthwash, study the biological assessment in body by determination level of enzyme in body fluid, enviromental study by determination of eugenol in wastewater of dental hospital, histopathological study of eugenol by the study the effect of eugenol in deferent organism of albino male albino male mice, hematological study by detection of variation of deferent blood cells of (RBC,WBC, PLATELETS) were the aims of this study[9].

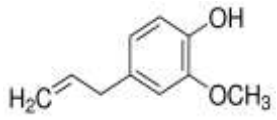

Fig.1 structure of Eugenol

2. Materials and methods

Cary 100 conc, Double beam with 1 cm3Quartz **UV-Vis Spectrophotometer** Australia Cell was used for spectrophotometry. ABS 220-4 4KERN **Digit Balance** Sohn GmbH Germany. VWR VS-C10 (50-60 Hz, 30 Watts)**Magnetic stirrers** VWRInternational Inc, American Stock Transfer & Trust Company, LLC USA . EA940 with stainless steel micro pH probe **pH meter** London Scientific (London, ON); calibration buffers of pH 4.00,7.00, and 10.00 were purchased from BDH Inc. 6*15 mL centrifuge tubes and a maximum speed of 6000 rpm, Corning LSE TM Compact (Tewksbury MA), USA was used to enhance the phase separation.

Eugenol water soluble,4-(Dimethylamino)benzoic acid, Potassium hexacyanoferrate(III), Potassium hydroxide, Ammonia, Potassium chloride, Methanol, Chloroform with high purity were purchased from sigma Aldrich, AlQiffaf Scientific Co. , Baghdad, Iraq. Dichloromethane, trichloromethane, chlorobenzene, and carbon tetrachloride, which were used as extraction solvents, were from omma scientific barau, Bghdad, Iraq. Analytical grade methanol, ethanol, acetone, and acetonitrile were used as dispersive solvent from sigma Aldrich, AlQiffaf Scientific Co. , Baghdad, Iraq. Solutions were prepared in double distillated pure water. All salts, which were of purity more than 98%, were purchased from AlQiffaf Scientific Co. , Baghdad, Iraq

2.1 Preparation of eugenol stock solution (500 mg/L)

A stock solution of eugenol were prepared by transfere 0.116 ml of 100 % eugenol to 500 ml of volumetric flask and filled by DW .

2.2 General Analytical procedure of eugenol

A series of five eugenol working standards (5, 10, 25, 40, and 60 mg/L) was prepared by transferring the appropriate volume of stock standard into 100-mL volumetric flasks and diluting with the same water as that used to prepare the stock standard. Four or five aliquots of each working standard were transferred into individual disposable cuvettes (Fisherbrand, plastic, 4.5 mL capacity, 10 mm lightpath, Fisher Scientific) and measured by UV-vis spectrophotometry (Genesys 2, Thermo Electron Corp, Madison, WI) at 279.0 nm. Before a set of working standard samples were measured, the spectrophotometer absorbance reading was set to zero by using an empty cuvette. Mean absorbances were plotted against eugenol concentrations, and linear standard curves were fit in SigmaPlot 11(SYSTAT 2008). The R2value, slope, and y-intercept values were determined from a standard curve generated for each set of working standards[10].

2.3 Determination of eugenol in human body fluid

Eugenol were determined in human body fluid samples which include serum, urine, saliva after 10 days mouth wash administration of three times a day mouth rinse by volunteer who already using the studied mouth wash (kin, zak, paradontax) . The volunteers were divided to three groups according to the mouthwash types.

2.4 Hematological study

Complete blood picture study were achieved for the volunteers of 10 days mouth wash administration of three times a day mouth rinse by volunteer who already using the studied mouth wash (kin, zak, paradontax). Control samples was measured for all volunteers before any experiment.

2.5 Histopathological study

Four groups of albino male albino male albino male mice, aged (5-6) weeks and weighing (20-25) g were obtained and kept in plastic cages in the animal house of Baghdad Research Center, university of Baghdad. Room temperature was 22±3 ℃ were collected for histopathological study three of them were dosed with 10, 100, 500 mg/L of eugenol for 10 Days and one group was a control with normal feeding of tab water. All the meals was at normal nutrition. All the albino male mice were heir shaving, killed using diethyl ether and vivisection to liver, spleen, intestine and heart sectioned. The organs were kept in formalin before lab investigation. The mice were left for 10 day for adaptation before the experiments beginning.

2.6 Environmental study

Samples were collected from main tank of dental hospital of college of dentistry. The main tank gathered the flow of medical wastewater from all sewage network from all clinics including the period, endo, exo dontic departments. The samples were transferred immediately for lab investigation related to eugenol determination

3. Result and Discussion

3.1 Determination of eugenol in human body fluid

The result of human body fluid of eugenol was shown in table (1) the maximum amount of eugenol was in urine and the lowest in serum that may due to the high excretion of eugenol by kidney to remove the further amount of eugenol in body.

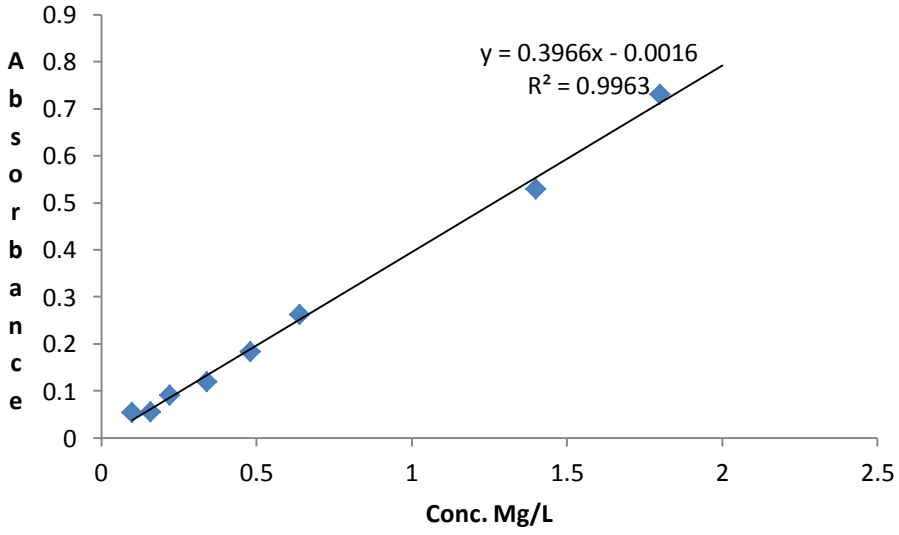

Fig.2 shown the calibration curve of determination of eugenol using the analytical procedure the conc. against the absorbance were plotted.

Table (1) determination of eugenol in body fluid

Volunteer	Type of mouthwash	Urine mg/L	Serum mg/L	Saliva mg/L
V1	Zak	0.372163	0.899143	0.828543
V2	Zak	0.377206	0.210792	0.525971
V3	Zak	0.389813	0.24357	0.31417
V4	Zak	0.679778	0.8235	0.528492
V5	Kin	0.488149	0.286435	0.33182
V6	Paradontax	0.291478	0.863843	0.508321
V7	Kin	0.409985	0.538578	0.589007

The results improve that the mouth was highly active in absorption of eugenol and it is found in serum more than saliva with short time while in contrast the amount of eugenol urine was high may due to the human ability to excrete the eugenol by urinary system.

3.2 Hematological study

The result of hematological study was shown in table (2) the effect of eugenol was slightly negative in different blood picture parameters.

Table (2) hematological study

Mouth wash	N %	N %	L %	L %	M %	M %	E %	E %	B %	B %
Zak	50	50	47	47	2	2	1	1	0	0
Zak	55	54	42	42	1	1	0	0	0	0
Zak	45	46	50	50	2	2	3	3	0	0
Zak	60	60	38	39	1	1	1	1	0	0
Kin	60	60	37	37	2	2	1	1	0	0
Paradontax	59	60	38	38	2	2	1	1	0	0
Kin	62	62	35	35	2	2	1	1	0	0

Mouth wash	Hb g./100ml	Hb g./100ml	PCV %	PCv %	WBC /cmm.	WBC /cmm.	Plat /cmm.	Plat /cmm.
Zak	16		50	49	7,900	7,880	125,000	200,000
Zak		16	51	49	9,100	9,100	230,000	230,000
Zak			42	23	8,800	8,800	280,000	280,000
Zak			36	36	11,200	11,200	220,000	220,000
Kin	11	12	35	37	7,100	7,100	187,000	187,000
paradontax	14	14	45	45	9,800	9,800	210,000	210,000
Kin	15	15	47	47	6,100	6,100	180,000	180,000

The results of complete blood count was not gave the positive response may due to the blood circulatory system is closed system and the effect cannot be expected within dose administration period of 10 days.

3.3 Histopathological study

Histopathological study was accomplished by feeding the albino male albino male mice with different concentrations of eugenol for 10 days period. The full procedure was detailed in experimental part.

Group 1 (10 mg/L)

The kidney showed sever necrosis of renal tubules with infiltration of MNCs in kidney parenchyma, in addition there is decrease in cellularity and vaculation in the glomerular tuft (Figure 3)

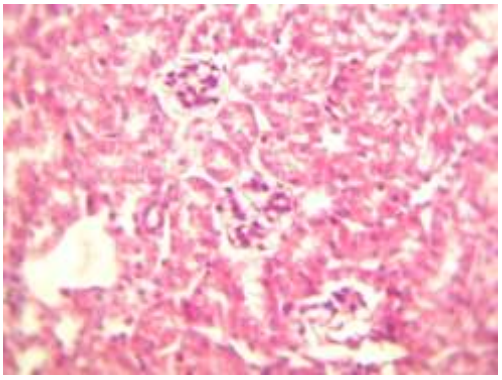

Fig. 3 The kidney showed decrease of cellularity and vaculation in the glomerular tuft (H & E; 400×).

The liver showed congestion of central veins with multiple micro abscess in the liver parenchyma, other sections showed multifocal aggregation of MNCs, while the portal areas showed infiltration of inflammatory cells mainly neutrophils and MNCs (Figure 4).

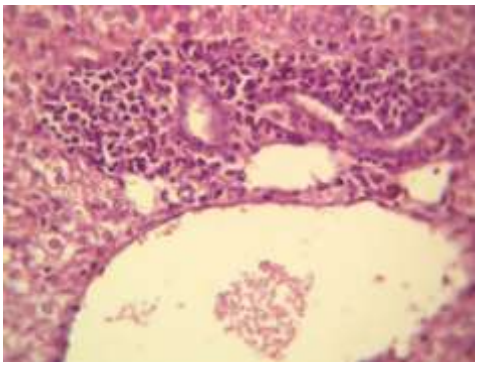

Figure 4: The liver showed that infiltration of inflammatory cells mainly neutrophils and MNCs (H & E; 400×).

Group 2 100 mg/L

The kidney showed degeneration and necrosis of the renal tubules, infiltration of inflammatory cells mainly MNCs in the interstitial tissue, many glomeruli showed shrinkage of the glomerular tuft with vacuoles in the cytoplasm of its cellular content (Figure 5).

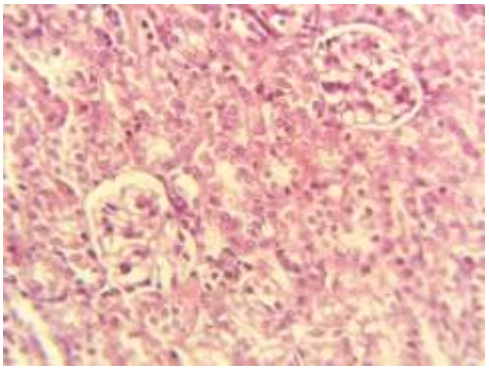

Figure 5: The kidney showed shrinkage of the glomerular tuft with vacuoles in the cytoplasm of its cellular content (H & E; 400×).

The liver showed severe necrosis and infiltration of neutrophils, and MNCs in the liver parenchyma with proliferation of kupffer cells, also there is congestion of sinusoids. The portal areas showed mild hyperplasia in the epithelial cells of the bile duct and infiltration of neutrophils, MNCs and Fibrous connective tissue (FCT) (Figure 6).

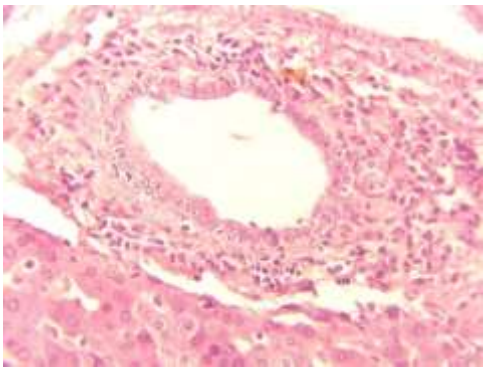

Fig. 6 The portal area of the liver showed infiltration of neutrophils, MNCs and Fibrous connective tissue (FCT) (H & E; 400×).

The spleen showed hyperplasia of the white pulp, while the red pulp showed congestion of blood vessels, proliferation of megakaryocytes and infiltration of neutrophils (Figure 7).

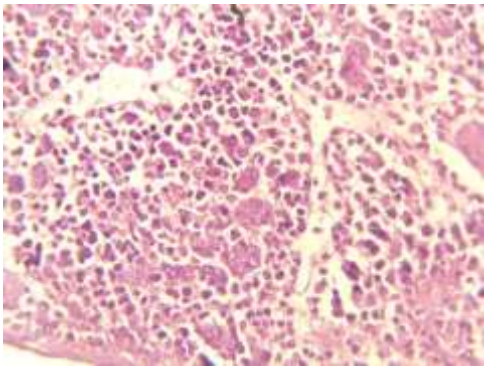

Fig. 7 The spleen showed that the red pulp revealed congestion of blood vessels, proliferation of megakaryocytes and infiltration of neutrophils (H & E; 400×).

The intestine in group 5 showed necrosis of the villi and infiltration of neutrophils, MNCs and plasma cells in the lamina propria and in the submucosa layer (Figure 8).

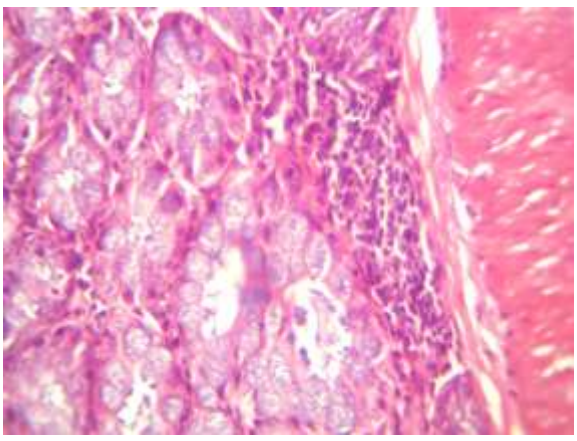

Fig. 8 The intestine infiltration of neutrophils, MNCs and plasma cells in the submucosa layer (H & E; 400×).

Group 3 (500 mg/L)

The kidney showed degenerative changes in epithelial cells lining the renal tubules with shrinkage of some glomerular tufts leading to increase in Bowman's space (Figure 9).

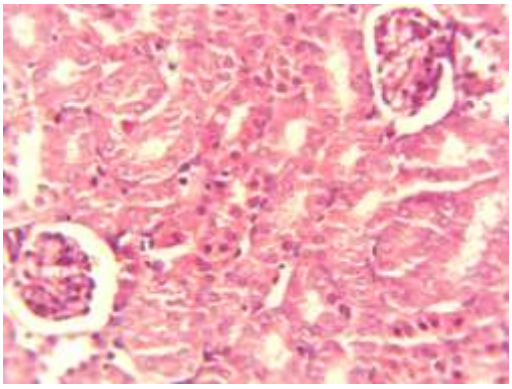

Fig. 9 The kidney showed degenerative changes in epithelial cells lining the renal tubules with shrinkage of some glomerular tufts leading to increase in Bowman's space (H & E; 400×).

In liver there is swelling of the hepatocytes due to vacular degeneration which lead to disorganization of the hepatic cords and narrowing or disappearance of the sinusoids, also the is focal aggregation of mononuclear cells (MNCs) adjacent to the central vein (Figure 2). In addition, there is cystic dilation of the central vein, while the portal areas showed congestion of blood vessels and infiltration of inflammatory cells mainly MNCs.

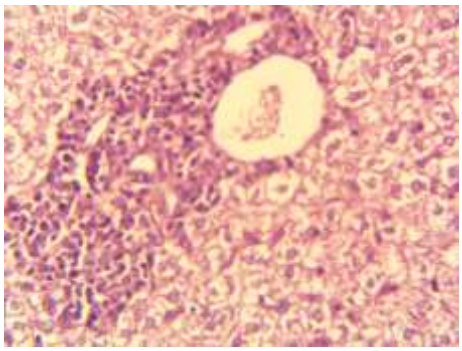

Fig.10 The liver showed vacular degeneration of the hepatocytes and focal aggregation of mononuclear cells adjacent to the central vein (H & E; 400×).

The spleen showed mild hyperplasia of the white pulp and proliferation of megakaryocytes in the red pulp (Figure 10)

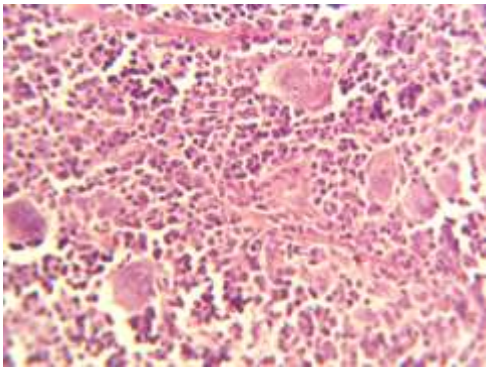

Fig. 11 The spleen showed proliferation of megakaryocytes in the red pulp (H & E; 400×)

The stomach show no clear lesion (Figure 12)

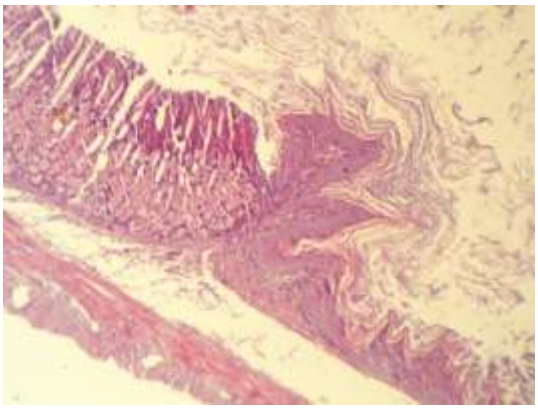

Fig. 12 The stomach showed normal histological section (H & E; 100×).

3.4 Environmental study

The eugenol was detected in the main tank of tank of dental hospital of college of dentistry. The main tank gathered the flow of medical wastewater from all sewage network from all clinics including the period, endo, exo dontic departments. The samples were transferred immediately for lab investigation related to eugenol determination.

Table 3 environmental study of eugenol

control	Non detected
Sample	11 mg/L

The result show in table (1-4) the concentration of eugenol was non detected in control sample (the hospital was in break during holyday and no any hand worker in the clinics but the concentration of eugenol was 11 mg/L in the next weak and the clinics was in work hand .

Conclusion

1. The concentration of eugenol in body fluid (saliva,serum,urine) is be founded at highest concentration in urine thus due the body recognized the eugenol as toxic substance and they can be excreted while in saliva was found in low concentration this might the eugenol are high absorbance by the mouth and found in serum also in deferent concentration.
2. The results revealed there is no that effect in hematological parameters may due to the short period of mouthwash administration.
3. Side effects were noticed such as yellow skin, hepatomegaly in non-microscopically investigation.
4. In Environmental study we found the eugenol is stable compound in water so we found in conc. about 11 ppm when collected the waste water of dental hospital.

Recommendation

1. The time period in using the medical samples should be not exceeded than 10 days to prevent the further side effect by long time using the mouthwash in addition to the doctor's prescription advice.
2. The concentration of eugenol should be controlled before pharmaceutical perceptions ‘and the concentration should be written clearly in the mouthwash ingredient depend on minimum inhibitory zone (MIC).
3. Waste water treatment is recommended for the medical wastewater related to the dental materials such as eugenol.

References

1. Bonilla, J.;Poloni, T.;Lourenço, R.V.;Sobral, P.J.A., Antioxidant potential of eugenol and ginger essential oils with gelatin/chitosan films, Food Bioscience, 2018,23,107-114.
2. Adefegha, S.A.;Okeke, B.M.;Oboh, G.;Ijomone, O.M.;Oyeleye, S.I., Modulatory effect of eugenol on arginase, nucleotidase, and adenosine deaminase activities of platelets in a carrageenan-induced arthritis rat model: A possible anti-arthritic mechanism of eugenol, Biomedicine & Pharmacotherapy, 2018,106,1616-1623.
3. Sakat, M.S.;Kilic, K.;Akdemir, F.N.E.;Yildirim, S.;Eser, G.;Kiziltunc, A., The effectiveness of eugenol against cisplatin-induced ototoxicity, Brazilian Journal of Otorhinolaryngology, 2018.
4. Boyle, P.;Koechlin, A.;Autier, P., Mouthwash use and the prevention of plaque, gingivitis and caries, Oral diseases, 2014,20 Suppl 1,1-68.
5. Masghati, S.;Ghoreishi, S.M., Supercritical CO2 extraction of cinnamaldehyde and eugenol from cinnamon bark: Optimization of operating conditions via response surface methodology, The Journal of Supercritical Fluids, 2018,140,62-71.
6. Novato, T.;Gomes, G.A.;Zeringóta, V.;Franco, C.T.;de Oliveira, D.R.;Melo, D.;de Carvalho, M.G.;Daemon, E.;de Oliveira Monteiro, C.M., In vitro assessment of the acaricidal activity of carvacrol, thymol, eugenol and their acetylated derivatives on Rhipicephalus microplus (Acari: Ixodidae), Veterinary Parasitology, 2018,260,1-4.
7. Becker, D.E., Drug therapy in dental practice: general principles. Part 1 - Pharmacokinetic considerations, Anesth Prog, 2006,53,140-146.
8. Storehagen, i.;Midha, N.O.o.S., DENTIFRICES AND MOUTHWASHES INGREDIENTS AND THEIR USE Det odontologiske fakultet, Universitetet i Oslo2003
9. http://www.mouthwashes.net.
10. Bowker, J.D., the Robustness of a Simple UV-vis SpectrophotometricMethod to Determine the Concentration of Eugenol in Water, DRUG RESEARCH INFORMATION BULLETIN, 2011,20,1-2.

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