KNOWLEDGE OF METHEMOGLOBINE BIOMARKER OF NITROGEN SUBSTANCES

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Abstract
Intoxications with nitrates are monitored by using a specific biomarker, methemoglobin (MeHb). Methemoglobin (MeHb) is hemoglobin (Hb) containing oxidized iron (rather than oxygenate). Normal hemoglobin contains four iron atoms in the ferrous state (Fe + 2). If, iron ions lose an electron and are oxidized to ferric (Fe + 3), it can no longer bind oxygen. Therefore, methemoglobin reduces the oxygen transport capacity and reduces the release of oxygen into the tissues. Observations took place over a period of 4 years; the average of the recorded cases was 9.5 per year. The mean age of children (months) was 2.2, the mean methemoglobin (%) being 32.6. The lethal threshold of nitrates and nitrites varies according to age, rapid intake of food and water. Nitrates and nitrites are primarily used as fertilizers for cultivated plants, but also have negative effects on infants through the disease called "cyanosis", namely the blue color of the skin, especially the lips. The data show that the use of methemoglobin as a biomarker is welcome.

Keywords: Biomarker, methemoglobin, nitrates, children toxicity

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1. INTRODUCTION

Nitrates and nitrites are primarily used as fertilizers for cultivated plants, but also have negative effects on infants through the disease called "cyanosis", namely the blue color of the skin, especially the lips. The phenomenon occurs more frequently where mothers of children who feed on cow's milk grazing nitrate-rich grass and have been drinking water with high nitrate and nitrite content (Fischbach, 2009; Wallach, 2001; Aspevall et al., 2001). In calves, nitrate and nitrite intoxication is manifested by bruising Snout and weakness. Animal death occurs after ingestion of lethal amounts of nitrates, which happens when animals are grazed or fed in feed with nitrate-rich feed. Sometimes the ingested nitrate content is in relatively low amounts. However, these quantities are harmful because the productive performance of the animals is affected. Nitrites are much more toxic than nitrates. When they are less than nitrates, they have a stronger and faster toxic action but when nitrates enter the action of reducing bacteria in the intestinal tract, they are converted into nitrites. Nitrates lead to gastro-enteritis and diarrhea, and conversion is rapid. The acute toxicity of nitrates should be based on a concrete situation, an analysis of how ingestion of food, its quality, and drinking water on its content nitrates and nitrites. The most effective nitrate and nitrite poisoning approach is the brown color of blood and tissues. After that, it is possible to approximate the substance that has been ingested and caused the intoxication of the animals.
Sources of contamination of humans with nitrites and nitrates are numerous such as drinking water, water used for irrigation, substances used for fertilization, fruits, vegetables, preserved meat, meat preparations (U.S. Agency for Toxic Substances and Diseases Registry. 2001; Sanchez-Echaniz, J., et al. 2001; Reinik, M., et al. 2005;).

2. MATERIALS AND METHODS

Co-oximetry is the main method for the diagnosis of methemoglobinemia. Generally, this measurement is made of processed arterial blood in a device specifically designed to be able to measure the proportions of multiple components of several hemoglobin fragments using multiple wavelength spectrophotometry. The co-oximeter is able to measure the

concentration of different types of blood hemoglobin by spectrophotometry using different wavelengths. This technology is based on the Lambert-Beer law, which correlates the concentration of a dissolved substance to the intensity of light transmitted through a solution. Measuring a greater number of wavelengths allows the instrument to distinguish between them and the carboxy-hemoglobin, -COHb, methemoglobin-metHb and other hemoglobin fragments (Laborator Synevo, 2010).

3. RESULTS AND DISCUSSION

Lethal doses show the following, after the first symptoms, when seizures occur, in about 1-3 hours the animals die (Table 1).
Pregnant cows surviving in induced methemoglobin may be aborted. Animal species have a variable sensitivity to ingested nitrites, due to differences in the physiology and anatomy of their intestinal tract. In sheep and cattle, the reduction of nitrates to nitrites occurs in the rumen. In the rumen of these animal species, ammonium ions are directly absorbed into the bloodstream. If this transformation occurs in the gastrointestinal system, it is caused by microorganisms.
The lethal threshold of nitrates and nitrites varies according to age, rapid intake of food and water. Synthetic data are presented in Table 2 as follows:

- Deadly doses of nitrates: horns: 169 mg/g administered orally in aqueous solutions, 230 mg DL50 consumed in feed; turkey chicken: 500 mg / kg DL30 and 1200 mg (DL90) in drinking water.
- Nitrate doses producing toxicity symptoms: calves: 71.6 mg / kg in rapidly ingested feed.
- Deadly doses of nitrite: horns: 20 mg / kg, DL50 applied in plenty of drinking water; sheep: 90 mg / kg DL50 intraperitoneally administered, 64 mg / kg rapidly applied intra arterially; swine: 12-20 mg / kg administered orally, 8-12 mg / kg applied subcutaneously, 21.35 mg / kg DL100, applied by stomach tube, 15-18 mg / kg DL50 applied to the stomach tube; mice: 15-18 mg / kg DL50 applied to the stomach tube; dogs: 12 - 30 mg / kg administered orally.
- Nitrate doses producing toxicity symptoms: sheep: 24.5 mg / kg administered intravenously; Pig: 12.2 - 19 mg / kg applied through the stomach tube; chickens or turkeys: 200 mg in drinking water; mouse: 31.6 (2.29 mM NaNO2 / kg), DL50, intraperitoneally applied (Laboratory Corporation of America, 2010).

Table no. 1 Comparative diagnoses of poisoning of animals with different chemical

Toxic factor	Color	Pathophysiological mechanism	Treatment
Chlorate of Na(Ca)	Brown	Methemoglobinemia	Methylene Blue.
Gases of silos	Light brown light	Low methemoglobinemia	Idem. Calcium gluconal
Cyanamide	Red like the cherry	Anti-cytochrome. Tissues that can not use oxygen	Nitrite thiosulphate.
Carbon dioxide	Dark blue	Oxygen has been substituted. Cyanosis	Oxygen, fresh air.
Carbon monoxide	Bright red	Carbon monoxide. Stable methemoglobinemia complex.	Oxygen + 5% thiamine solution. Fresh air.

Table no.2 Lethal doses of nitrates and nitrites

Specification	mg / kg live body weight	
Nitrat	NO^-_3	- N
Adult peaple	80 – 300	18 – 68
Horn	75 – 140	75 – 140
Ships	- 380	- 70
Nitrite of sodium ($NaNO_2$)	NO^-_2	-
Adult peaple	110 – 165	22 – 33
Home rabbits	60 – 90	16 – 18
Piglets	- 90	- 18
Ships	- 170	- 34

Table no. 3 Normal values of methemoglobin

Direction 98/83 / EC Annex IB	Guide OMS 2005	Minister health Canada	The Agency Protection a environment US	National Legislation
0,5mg/l	3mg/l (ef. acute) 0,2mg/l (ef. chronic)	3,2mg/l	3,3mg/l	0,5 mg/l

Table no.4. Clinical symptoms based on hemoglobinemia

1-2%	Under10%	10-20%	20-30%	30-50%	50-70%
normal	Has no clinical symptoms	Skin discolouration of mucous membranes	Anxiety, headache, dyspnea	Fatigue, confusion, dizziness, tachypnea, palpitations	Coma, convulsions, arrhythmia and acidosis

Methemoglobin (MeHb) is hemoglobin (Hb) containing oxidized iron (rather than oxygenate). Normal hemoglobin contains four iron atoms in the ferrous state (Fe + 2) . If iron ions lose an electron and are oxidized to ferric (Fe + 3), it can no longer bind oxygen. Therefore, methemoglobin reduces the oxygen transport capacity and reduces the release of oxygen into the tissues. According to the various organizations in the world normal values of methemoglobin are shown in the Table 3.

The highest risk of methaemoglobinemia in children is the high level of nitrates present in water consumed by them (Table 4). The toxicity of nitrates is due to the fact that they are converted to nitrates in the presence of bacteria found in the intestines. Hence, they reach the bloodstream and convert hemoglobin to methemoglobin. The peroxide-induced increase in methaemoglobin is due to the fact that it does not carry oxygen through the blood, resulting in anoxia-induced cyanosis (Wallach, 2001).

The Table 5 shows that the age of children who have methemoglobinemia ranged from 1 to 4 months. If iron ions lose an electron and are oxidized to ferric (Fe + 3), it can no longer bind oxygen. If even one of the iron atoms is oxidized, the release of oxygen bound to other iron atoms in the hemoglobin molecule is affected. Therefore, methemoglobin reduces the oxygen transport capacity and reduces the release of oxygen into the tissues. It also affects the transport of carbon dioxide from the tissues to the lungs for disposal. Lack of oxygen in the tissues leads to blue color of the skin in the area of the nose of the ears and in more severe cases and to the peripheral tissues. Also the air pollution with nitrogen oxide lowers the level of hemoglobin leading to anemia especially in the case of the elderly (Honda et al., 2017).

Table no. 5 Indicators for methemoglobinemia cases

Statistical indicators	Year				Average
	2012	2013	2014	2015	
Case of methaemoglobinemia	8	10	11	9	9,5
Age of children (months)	1.5-4	2-3	1-3	1-3	2.2
Methemoglobina (%)-average	34.6	27.8	32.6	35,4	32.6
Methemoglobina (%)- maximum	60	42	58	55	53.7
Methemoglobina (%)- minimum	21	15	15	15	16,5

4. CONCLUSIONS

The most convincing reason for using biomarkers in assessing environmental risk is that they can give information on the effects of pollutants. Thus, the use of biomarkers in biomonitoring is complementary to the most commonly used monitoring, those involving the determination or prediction of residual levels.

The first point in any of the evaluation processes is to decide what needs to be assessed. This may be obvious but surprisingly seldom the objectives are well defined, as is the case with many programs to monitor the levels of toxic chemicals.

Methemoglobinemia occurs due to the presence in drinking water of nitrates in an amount exceeding the maximum admissible value of 50 mg / L, which may be a useful biomarker of the nitrogenous substances ingested by living predisposed organisms. It can be defined as biomarkers, any of the biological responses to an individual chemical substance or a removal of the body from the normal state of the organism. Biological responses at organizational levels superior to individual levels (population, community and ecosystem) - are considered bio indicators.

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6. REFERENCES

[1] Frances Fischbach. Urine Studies. In A Manual of Laboratory and Diagnostic Tests. Lippincott, Williams & Wilkins, USA, 8 Ed., 2009, 199-210.
[2] Jacques Wallach. Urina. In Interpretarea testelor de diagnostic. Editura Stiintelor Medicale, Romania, 7 ed., 2001, 121-150.
[3] Kouri T et al. European Urinalysis Guidelines. Scan J Clin Lab Invest, Vol.60, Supplement 231, 2000.
[4] O. Aspevall, H. Hallander, V. Gant, T. Kouri, European guidelines for urinalysis: a collaborative document produced by European clinical microbiologists and clinical chemists under ECLM in collaboration with ESCMID, Volume 7, Issue 4, 2001, p. 173–178.
[5] U.S. Agency for Toxic Substances and Diseases Registry. 2001. "Case Studies in Environmental Medicine: Nitrate/Nitrite Toxicity."
[6] Reinik, M., et al. 2005. "Nitrites, nitrates and N-nitrosoamines in Estonian cured meat products: intake by Estonian children and adolescents." Food Addit.Contam 22(11):1098-1105.
[7] Sanchez-Echaniz, J., et al. 2001. "Methemoglobinemia and consumption of vegetables in infants." Pediatrics 107(5):1024-1028.
[8] Laborator Synevo. Referinte specifice tehnologiei de lucru utilizate 2010. Ref Type: Catalog.
[9] Laboratory Corporation of America. Directory of Services and Interpretive Guide. Urinalysis, Routine. www.labcorp.com.2010.
[10] Lothar Thomas. Kidney and urinary tract. In Clinical Laboratory Diagnostics. 1998; 362-364.
[11] V.Dumitrascu, D.Grecu, S.Giju, C.Flangea, V. Daliborca. Examenul de urina – de la arbitrar la standard. In Revista Romana de Medicina de Laborator, Vol. 1, Nr.1, decembrie 2005, 9-19.
[12] DIRECTIVE 98/83/EC on the quality of water intended for human consumption
[13] U.S. EPA, Toxicity and Exposure Assessment for Children’s Health, 2007.
[14] Trenton Honda, Vivian C. Pun, Justin Manjourides, Helen Suh, Anemia prevalence and hemoglobin levels are associated with long-term exposure to air pollution in an older population, Environment International, 101, 2017, p. 125-132.