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EPIDEMIOLOGICAL STUDIES HIGHLIGHTING THE RELATIONSHIP BETWEEN ENVIRONMENTAL POLLUTION WITH NITROGENOUS COMPOUNDS AND HEALTH OF CHILDREN

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Abstract. The authors present a synthesis of nine epidemiological studies performed in the interval 1988-2000 in areas with a long term-exposure to nitrogenous compounds, pollutants of environment (drinking water and food products). 1488 children (0-3 years) from five districts in eastern Romania were investigated for chronic effects. The selection of study areas has been made based on the incidence rate of infant methemoglobinemia in those districts. The chronic health effects were emphasized by some bioindicators of exposure and effect. The causal relationship has been proved especially by a higher frequency of cases with pathological methemoglobin levels in the exposed children (61.9%) in comparison with those unexposed ones (23.7%). The results were used as criteria for ranking the areas at risk in the East of Romania and also for risk communication..

Key words: environmental pollution, nitrogenous compounds, epidemiological studies, territories at risk, risk communication

Rezumat. Autorii prezintă o sinteză a 9 studii epidemiologice realizate în perioada 1988-2000 în teritorii cu expuneri îndelungate la substanţe azotoase, poluanţi ai mediului (apă de băut, aliment). S-au investigat efectele cronice la 1488 copii 0-3 ani în cinci judeţe din estul României. Selectarea teritoriilor pentru aceste studii s-a făcut pe baza incidenţei intoxicaţiilor acute la copiii 0-1 an din aceste judeţe. Efectele cronice au fost puse în evidenţă printr-o serie de bioindicatori de expunere şi de efect. S-a dovedit relaţia cauzală a acestora, îndeosebi printr-o frecvenţă mai mare a cazurilor cu valori patologice ale methemoglobinei la copiii expuşi (61,9%) în comparaţie cu cei neexpuşi (23,7%). Rezultatele au fost folosite drept criterii pentru ierarhizarea zonelor cu risc din estul României şi de asemenea, pentru comunicarea riscului.

Cuvinte cheie: poluare ambientală, substanţe azotoase, studii epidemiologice, bioindicatori, teritorii cu risc, comunicare de risc

INTRODUCTION

After the first case of nitrate acute intoxication reported by Comley in 1945, lots of studies, descriptions of case incidence, clinical aspects, etiology of diseases throughout the world and also in our country were carried out (1,2,3). The acute effect of brutal nitrate exposure is worldwide recognized and the already warned population avoids the use of high nitrate level water for infants. As consequence, decreasing tendency of severe and lethal cases have been recorded.

But, the chronic effects of nitrate in drinking water and food products become a problem of concern in the recent years. There were noticed a wide range of health effects, from

non-response to illness which, in its turn, vary from mild to severe clinical symptoms and death.
This health effects could be compared with the community-disease model (4).
To highlight the effects of environmental contaminants, the epidemiological approaches use both descriptive and analytical studies (5). The Hill-Evans decalog of causality and adequate biomarkers were used to find out the causal relationship between environmental agents and health (6,7,8).

STUDY DESIGN

The geographical area of our investigations extended into the whole eastern territory of Romania. During 1981-1988, the frequency, distribution and tendency of infant methemo-globinemia cases in eastern Romania, as well as the nitrite/nitrate levels in drinking water and food products have been investigated (9,10,11). The results revealed the following aspects:

- the incidence rate of infant methemoglobinemia varied between a lowest value of 1.5 up to a highest one of 7.0 cases$^{0}/_{00}$ in Vrancea district and Iaşi district, respectively;
- the frequency of inadequate nitrate levels in drinking water (over maximum allowable concentration) ranged between 44.6% of the total analysed water samples (Vrancea district) and 94.7% (Botoşani district);
- an additional intake of nitrate/nitrite from food products was also noticed.

Based on these findings, areas at risk were selected for epidemiological investigations.
In order to point out if the chronic exposure to environmental pollutants (nitrites and nitrates) have one's mark on infants (0-3 y age), nine epidemiological studies were carried out in the period 1988-2000 (table 1).

Table 1. Numbers of children investigated during 1988-2000

Study	No. of children										% of total examinated
	A	B	C	D	E	F	G	H	I	Total	
Exposed group	29	116	90	138	161	32	82	126	110	884	59.4
Unexposed group	20	126	126	114	56	72	23	33	34	604	40.6
Total	49	242	216	252	217	104	105	159	144	1488	100.0
% of total children in area	-	100.0	100.0	42.5	7.5	21.8	17.2	12.4	25.4		

- During 1988–1994 three studies (A÷C) were performed on 507 subjects. The aim of this study was to find out the health effects of high – levels– nitrite - drinking water of Bacău town, including the reversibility of them after the improvement of drinking water quality;

- During 1997–2000 other six studies (D÷I) on representative samples consisting of 981 subjects, extended the follow up of health effects in many other rural areas of high risk from eastern Romania (Vrancea, Botoşani, Iaşi, Galaţi).

RESULTS AND DISCUSSION

Table 2 data indicate the distribution of exposed children to different levels of nitrogenous compounds in well water (the studies D÷I; n = 649).

39.3% of subjects were moderately exposed to nitrate levels up to 150 mg/L whereas 41.8% of them were highly exposed to more then 150 mg/L. About 19% of children were exposed either only to nitrites or to a mix of nitrates + nitrites in drinking water.

Table 2. The distribution of the exposed children by nitrogenous compounds levels (%)

Nitrogenous compounds in drinking water (mg/L)		Percentage of exposed children
Nitrate	46 – 100 101 – 150 151 – 250 251 +	21.4 17.9 26.5 15.3
Nitrite	> 0.03	1.8
Nitrates and Nitrites	> 45 > 0.03	17.1

The following syndromes, symptoms or diseases have been found mentioned in medical records of children (table 3).

Table 3. The symptoms, syndromes or illnesses in the children' anamnesis (%)

	Exposed group	Unexposed group	χ^2 test
Anaemia	11.2	1.5	27.5; $p < 0.001$
Convulsions/cyanosis	1.2	0.6	
Acute methemoglobinemia	6.8	-	

There have been used the following exposure bioindicators as measured in blood samples: hemoglobin (12) and methemoglobin (12), antioxidant defense biomarkers such as reduced glutathione (13), glutathione peroxidase (14), lipid peroxides (15), urinary and salivary nitrates and nitrites levels (16,17). Main results are further presented.

Although the mean value of hemoglobin were not significantly different between the two groups, the frequency of cases with hemoglobin

levels close to and under the inferior cut-off of normal range (18) was higher in the exposed one comparing with the unexposed group (table 4).

Table 4. Number and frequency of children with hemoglobin levels close to and under the inferior cut-off of normal range

Age of children (y)	Exposed group		Unexposed group	
	no of subjects (92)	%	no of subjects (26)	%
under 1	45	39.8	14	24.1
1-2	33	18.0	6	6.5
2-3	9	5.9	4	4.7
over 3	5	3.7	2	4.1
% of sample	15.8*		8.0*	

* test χ^2 yates: 7.27, $p < 0.01$ (freedom degrees = 117)

Two features distinguished the exposed group: higher values of methemoglobin levels which decreased with age and higher frequency of these values notwithstanding age group (module 4.1-5 *vs* module 2.1-3 of unexposed group) (fig. 1 and 2).

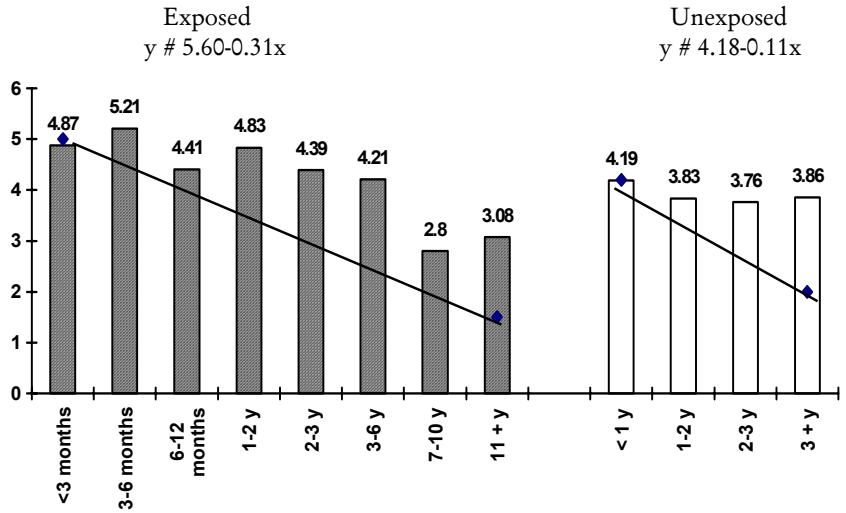


Fig. 1. Mean values of methemoglobin in exposed and unexposed children and their trend on children' age

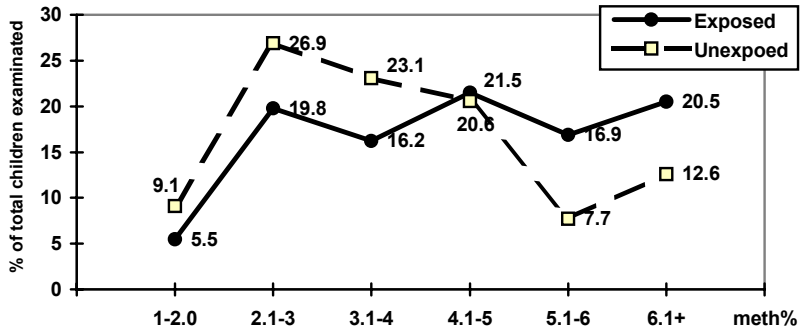


Fig. 2. The distribution of children according to the methemoglobin levels in two groups of children (% of total examinated children)

Pathological values of methemoglobin (over 4%), has been found statistically significant increased in some age groups: 1-2 y ($p<0.001$) and 2-3 y ($p<0.05$). In the whole, 58.7% of exposed children had pathological methemoglobin values, *vs* 41.9% of unexposed ones ($p<0.005$) (fig. 3).

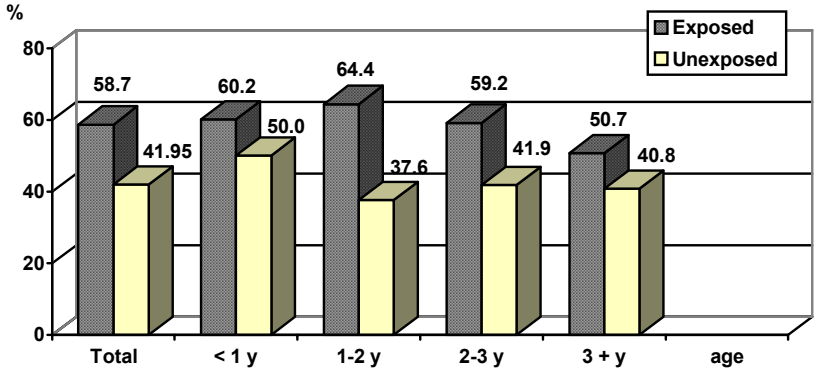


Fig. 3. Frequency of children with pathological values of methemoglobin (% of examinated children)

A symmetry between the increase of methemoglobin level and nitrate concentration in water samples was noticed (fig. 4).

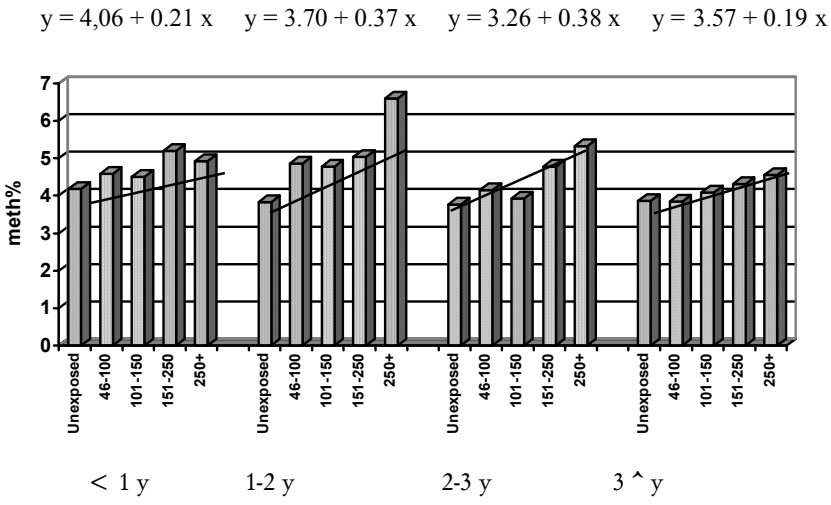


Fig. 4. Mean values of methemoglobin and the nitrate concentrations in water samples

Children from three districts: Botoşani, Iaşi and Galaţi were tested for bioindicators of oxidative and antioxidant effects and the results were published elsewhere (19). The biochemical disorders found in the exposed children group were associated to methemoglobinemia and related to the levels of exposure to nitrates in drinking water.

The mean values of urinary nitrites and nitrates were higher in the exposed group comparing with the unexposed group and their levels were proportional to nitrate levels in drinking water (fig. 5).

These findings were analysed through epidemiological methods. The analysis confirmed the hypothesis of existing relationship between health indicators and nitrogenous compounds exposure:

	RR	RA %
♦ frequency of anemia in anamnesis	1.47	31.97
♦ frequency of convulsions and cyanosis in anamnesis	1.21	17.3
♦ frequency of anemia based on hemoglobin level	1.19	15.97
♦ frequency of cases with high methemoglobin values	1.48	32.4

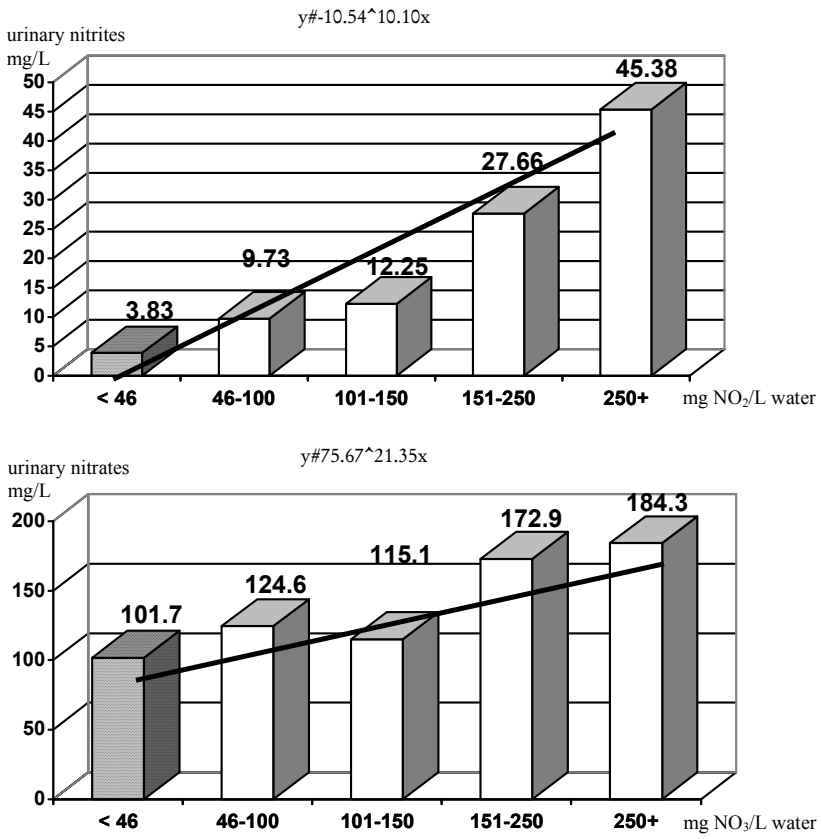


Fig. 5. Urinary nitrites and nitrates levels and the nitrate levels in drinking water

The results of these studies were used as criteria for ranking the territories at risk. Thus, the resulted ranking of area was as follows:

1) depending on the incidence rate of acute methemoglobinemia in the interval 1981-1988 (for 1000 of 0-1 year children):

Iaşi	7.0	Vaslui	3.3
Botoşani	6.7	Galaţi	2.9
Bacău	3.9	Neamţ	1.6
		Vrancea	1.5

2) depending of the frequency of drinking water samples with nitrate concentrations over MAC (%):

Botoşani 94.7 of a total of 1468 samples analysed in the period 1989-2000;
Iaşi 79.2 of a total of 159 samples analysed in 2000;
Galaţi 76.4 of a total of 144 samples analysed in 2000;
Bacău 64.3 of a total of 274 samples analysed in the period 1996-1997;
Neamţ 63.0 of a total of 2708 samples analysed in the period 1984-1998;
Vrancea 44.6 of a total of 74 samples analysed in 1989.

3) depending on the maximum levels of nitrates and nitrites in vegetables :

Galaţi 591.1 mg nitrates/kg
Botoşani 230.2
Vrancea 186.2
Iaşi 174.3

4) depending on the mean values of methemoglobin:

	< 1 year	1-2 years	2-3 years	3 years +
Bacău				
a) high risk area	6.46	6.43	6.13	
b) moderate risk area	3.75	3.45	3.17	
Iaşi	6.02	5.82	5.90	5.54
Galaţi	5.01	4.84	4.62	4.36
Vrancea	4.44	3.32	3.09	
Botoşani	3.00	3.18	2.68	

5) depending on the frequency of cases with methemoglobin levels over 4%:

Iaşi 95.7
Galaţi 75.9
Botoşani 39.0
Bacău 38.6
Vrancea 9.5 %

6) depending on RR values – for the frequency of cases with high levels of methemoglobin :

	RR	95 % CI
Iaşi	11.9	0.62 – 1.56*)
Galaţi	3.6	0.60 – 1.52*)
Vrancea	2.5	1.69 – 11.87
Botoşani	2.2	0.02 – 2.32
Bacău		
a) high risk area	2.03	1.47 – 3.37
b) moderate risk area	1.30	0.58 – 2.54

*) there were no significant differences between exposed and unexposed group of children from Iaşi and Galaţi districts.

The rural localities of *Iaşi, Galaţi* and *Botoşani* districts were considered the areas with highest risk. The final picture of investigation was the following:

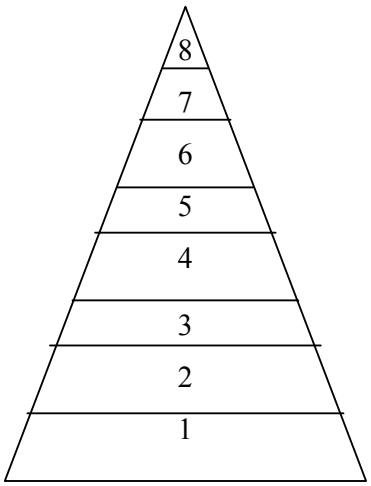
	Bacău urban	Bacău rural a	b	Vrancea	Botoşani	Galaţi	Iaşi	Total subjects
no. of 0-1 y children with acute intoxication (1981-1988)	32	43	45	21	32	16	16	205
no. of 0-3 y children investigated (1988-2000)	507	252	217	104	105	144	159	1488
Percentage of 0-3 y cases with methl.+	73.3	67.8	24.9	9.5	39.0	75.9	95.7	47.8
Total exposed								61.9
unexposed								23.7

The acute effects namely infant intoxications represent the fifth step of the pyramid of health effects in relationship with the environment (fig. 6). Due to their clinic symptoms, they are at once recognized and treated. The local measures consist in interdiction of consumption of the incriminated well water.

After 1 year age, it is supposed that the risk is overwhelmed and, consequently children also start to consume again drinking water with high level of nitrate. Our investigations noticed some effects of this chronic consumption.

Hematological, biochemical and bioenzimatic changes (reversible or not) were found in apparently healthy children but, these effects may be find, out by screening exams using adequate bioindicators (the pyramid forth step).

Measures of secondary prevention have to be used in this stage of health damaging but also measures of primary prevention to mitigate the risk are useful. In this respect, the risk communication and educational measures in population has been carried out and they have to be intensified.



1. general population;
2. population exposed;
3. unspecific effects: physiological, biochemical, immunological and morphological; detailed clinical investigations are required;
4. unspecific physiological, biochemical, immunological and morphological changes on the alert; screening are necessary to emphasize the liability to illness;
5. diseases in an incipient stage; to put into evidence the clinical symptoms in the population, active medical investigation are required;
6. advanced, disorder stage, hospitalisation;
7. disability;
8. death.

Fig. 6. The pyramid of health effects

CONCLUSIONS

1. 1488 children have been investigated throughout of North-Eastern Romania, for health effects of exposure to high levels of nitrite/nitrate in drinking water and food products.
2. The studied group of children suffered a chronic exposure to nitrogenous substances, especially through drinking water, but acute intoxication of the infant occurred also. The chronic subclinical effects exist as significant changes of hematological bioindicators.
3. A battery of bioindicators of exposure and effects has confirmed the relationship between environmental causal factor and health effects.
4. Epidemiological analysis indicated the levels of relation and attributable risks in these areas.
5. Rankings of area at risk based on these epidemiological evidences support the risk communication and educational measures in population.

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