Ecogenetic Consequences of the Agent Orange and Dioxin-Containing Ecotoxicological Factor (DEF) Exposure

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Principally new problem is discussed - the ecogenetic aspects of the long-term medical consequences produced by the dioxin-containing ecotoxicants in Vietnam. This includes the tasks of identification, examination and characterization of the Agent Orange (AO) long-term consequences on the genetic structures of different people as well as possible genetic destabilization outcome in the form of reproduction system disfunctions.

Model examination was carried out as part of the complex JRVTC investigation in the regions with different level of AO spraying [1]. It was based on the multilevel approach and was carried out among the properly selected and characterized exposure risk groups (ERG, Table 1). The conclusion on the ecogenetic consequences of the dioxin-containing ecotoxicological factor (DEF) influence was grounded on the specially designed scheme of the analysis (Fig. 1). The scheme includes genetical description of the ecotoxicological situation on the territory, population demographical and genealogical studies, summary of the results obtained during genotoxicological and toxicogenetical investigations [2,3].

Table 1. Exposure Risk Groups (ERG) characterization according to medical equivalent of toxic dose (MET, for primary and secondary system reactions) and index of health state (IHS)

Parameter	ERG I	ERG II	ERG III	
AO	-	-	+	+
DEF	-	+	+	+
MET1	-	-	0.06-0.53	0.53-0.82
MET2	-	-	0.10-0.70	0.50-0.90
IHS	0.806-0.852 **0.829**	0.717-0.774 **0.746**	0.598-0.689 **0.644**	0.454-0.589 **0.522**

The reproductive function pathology was examined to reveal the medical and demographical significance of the AO long-term medical consequences [1,4]. An increase in pathological reproductive events could be seen in the contaminated region, as well as an extension of somatic disturbances (Fig. 2, [4,5]). The higher probability of abnormalities was found in families of persons, born during the war-time and soon after the war. The fragility of women's health in the sprayed area was expressed by deterioration of several features: the percentage decrease of those with the normal menstrual cycle; the somatic pathology and gynecological pathology increase;

the frequent chronicle inflammatory diseases, etc. Analysis of health deterioration involvement in reproductive dysfunctions revealed an association between the exposure level and an impact of IHS decrease (Fig.3).

Cytogenetical and nuclei's morphology analyses of different cell types were carried out to estimate the significance of alterations on the subcellular and chromosome levels [3,6-8]. The shifts were demonstrated in analyzed parameters of peripheral blood lymphocytes [2,7] and epitheliocytes (Table 2) of different ERGs representatives. The role of chromosome lesions in the immunodeficiency observed [9] on the sprayed territory is supported by the correlation of cytogenetical and immunological markers' values [10]. Systemic character of the realisation of dioxin's bioactivity was indicated as well by different cell types morphological and functional modifications [3,11,12].

The functional modifications of genetic apparatus lead to recombination enhancement only as manifested by SCE increase [2]. The exposure factor chronic influence induces DNA fragmentation and micronuclei appearance. The exhaustion of cell's reserves leading to abnormal function and replication produce fragility of DNA, lesions, endoreduplication and/or polyploidy. The outcome of such processes could be cells' elimination, abnormalities fixation or maligrization [1,11].

Thus, the ecogenetic consequences should be recognized as part of the homeostasis multi-level alterations. This is proved by the strong correlation of the cytogenetic parameters and the shifts in lymphocytic system, immune status, reproductive function, microsomal enzymes' activity, porphyrines and vitamins' metabolism, dermal structures' morphology [1,11]. Cluster analysis methods demonstrated the shifts in the associations of the cytogenetical parameters and the integrated IHS in different ERGs (Fig. 4). The close relation was shown between the IHS and the genetically based cellular features. So, the development of new levels in homeostatic functional correlation can be considered in residents of region sprayed with AO.

In general, the ecogenetic activity of AO and/or DEF can be characterized by multilevel aberrations. Such abnormalities could be seen in reproductive function medical and genetic manifestations; in general functional fragility of an organism, with probability of functional weakness of newborns. This assumption is supported by the alterations in chromosome apparatus on the level of structure destabilization and abnormal functional activity, as well as chromatin fragmentation during the early step of cell apoptotic elimination.

1. Roumak V., Poznyakov S.P., Oumnova N., Sofronov G.A. //Organohalogen Compounds. -1996.-V.30.-85-90.
2. Oumnova N.V., Roumak V.S., H.T.Kim Chi, N.Q.An //Organohalogen Compounds. -1994. -V.21. -383-388.
3. Oumnova N.V., Roumak V.S., N.Q.An, Sofronov G.A. //Organohalogen Compounds. -1995.-25. -155-159.
4. H.T.Kim Chi, Oumnova N.V., Nguyen Quok An, Roumak V.S. In: "Herbicides in War: the long-term effects on man and nature". Abst. book //Hanoi, Vietnam. - 1993. - 215-218.
5. Oumnova N., H.T.Kim Chi, Roumak V. //Organohalogen. Compounds. - 1996. - V.30. - 57
6. Zhuleova L.Yu., Oumnova N.V., H.T.Kim Chi, H.A.Tuyet, Roumak V.S. //Organohalogen Compounds. - 1995. - 25. - PP. 125-130.
7. Oumnova N.V., Zhuleova L.Yu., H.T.Kim Chi, Roumak V.S. //Organohalogen Compounds.-1995.-25.-131-136.
8. Zhuleova L.Yu., Oumnova N.V., Roumak V.S. Russ. J. Genetics. 1996, N12. - 1482-1485.
9. Kozhevnicova G., Kuzmin I., Roumak V., Karaulov A. //Organohalogen Compounds. - 1991. - 117.
10. Oumnova N.V., An N.Q., Roumak V.S. //Organohalogen Compounds. - 1994. - v. 21. - 373-377.
11. Oumnova N.V. Ecogenetic consequences of the dioxin-containing ecotoxicants exposure (thesis) //S.-Petersburg. – 1997. – 337 P.
12. Zhuleova L., Oumnova N., H.T.Kim Chi, H.A.Tuyet, Roumak V. //Organohalogen Compounds.-1997.- v.33.-520-523.
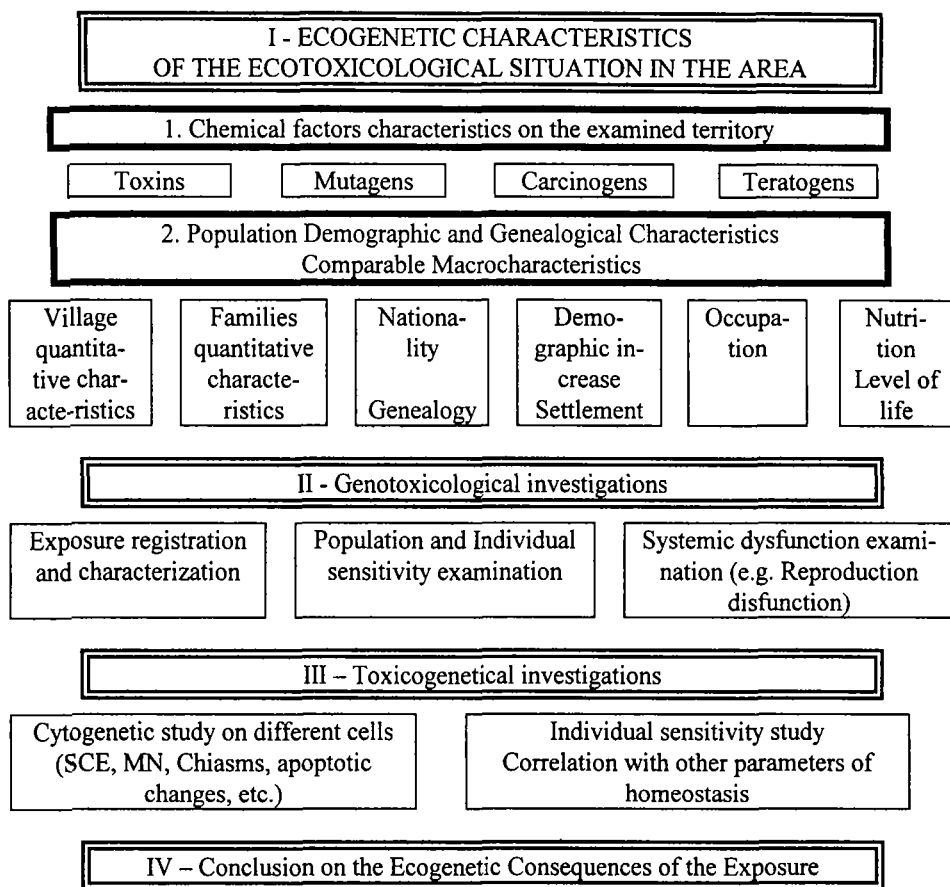
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│                I - ECOGENETIC CHARACTERISTICS                  │
│   OF THE ECOTOXICOLOGICAL SITUATION IN THE AREA                │
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┌──────────────────────────────────────────────────────────────┐
│   1. Chemical factors characteristics on the examined territory │
└──────────────────────────────────────────────────────────────┘
┌──────────┐  ┌───────────┐  ┌────────────┐  ┌────────────┐
│  Toxins  │  │ Mutagens  │  │ Carcinogens│  │ Teratogens │
└──────────┘  └───────────┘  └────────────┘  └────────────┘
┌──────────────────────────────────────────────────────────────┐
│   2. Population Demographic and Genealogical Characteristics   │
│              Comparable Macrocharacteristics                   │
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Village quantitative characteristics	Families quantitative characteristics	Nationality Genealogy	Demographic increase Settlement	Occupation	Nutrition Level of life

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│            II - Genotoxicological investigations               │
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Exposure registration and characterization	Population and Individual sensitivity examination	Systemic dysfunction examination (e.g. Reproduction disfunction)

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│            III – Toxicogenetical investigations                │
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Cytogenetic study on different cells (SCE, MN, Chiasms, apoptotic changes, etc.)	Individual sensitivity study Correlation with other parameters of homeostasis

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│  IV – Conclusion on the Ecogenetic Consequences of the Exposure │
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Fig. 1. Scheme of the JRVTC genetic investigations on the South Vietnam territory sprayed with dioxin-containing ecotoxicants (1989-1995)

Table 2. Frequency of persons with high values of cytogenetic parameters of oral cavity mucous epitheliocytes in different Exposure Risk Groups (age 30-56)

Parameters	Exposure Risk Groups			p#		
	BH I * a	I b	II+III c	p^{ab}	p^{bc}	p^{ac}
Micronucleated cells, > 6 / 1000	10/48	17/50	36/50	0.11	0.000	0.000
Cells with destroyed nucleus, > 0 / 1000	5/48	2/50	18/50	0.20	0.000	0.000

* - BH I - control group from Bach Hong village (North Vietnam);
 - I, II, III - exposure risk groups (South Vietnam)
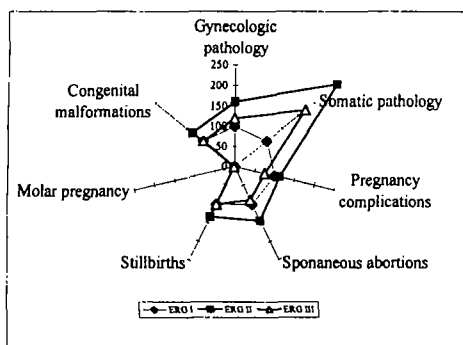# - non-parametric statistics (chi- square)

Fig. 2. Relative frequency of reproductive pathology (per 1 woman) in different Exposure Risk Groups (ERG). Frequencies for the control region present 100% level
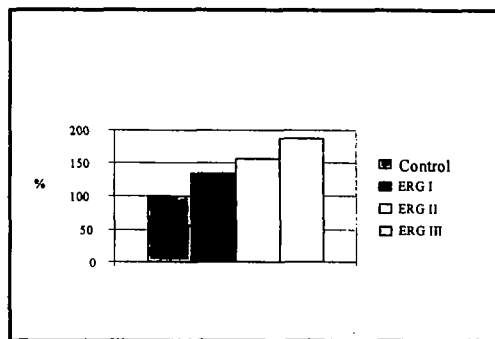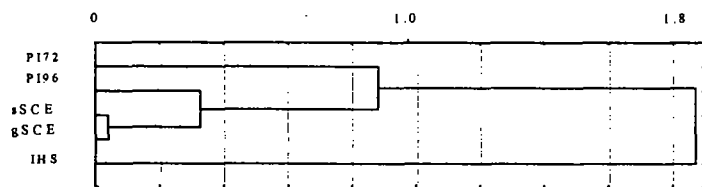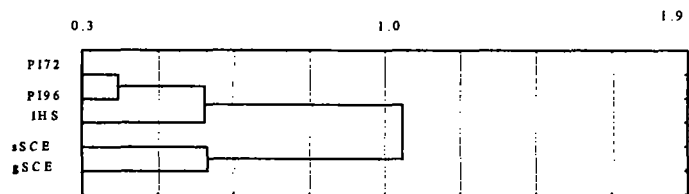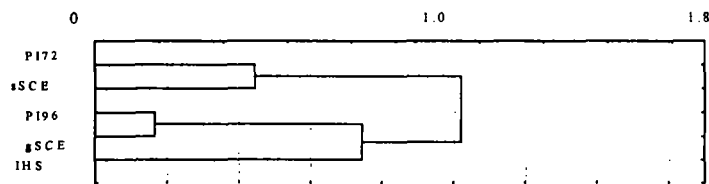
Fig. 3. Role of general health decrease in reproductive pathology in ERGs (age 31-50). IHS value for the control persons without reproductive pathology represents 100% level

A. ERG I

B. ERG II

C. ERG III

Fig. 4. Results of Pearson's claster analysis of IHS associations with cellular activity in Exposure Risk Groups

PI – proliferation index in 72- and 96-h whole blood cultures; sSCE – spontaneous sister chromatid exchanges (72 h); gSCE – genetical SCE (96 h); IHS – index of health status