DEVELOPMENT OF ECOGENETIC ASPECTS FOR THE ASSAY OF LONG-TERM MEDICO-BIOLOGICAL CONSEQUENCES OF THE AGENT ORANGE EXPOSURE

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The problem of characterizing genetic and carcinogenic consequences of exposure with dioxin-like substances has been formed on the base of high biological activity and resistance of these chemicals, their continuous absorption into the organism and ability to migrate along the nutrient chains, as well as medical and biological consequences revealed on different structure levels[1]. These consequences may appear in acute exposure and in remoteness also. The necessity arose to develop the ecogenetic aspects in investigation the dioxin-like chemicals and the combination of locally formed ecotoxicological factors containing dioxin (DEF). It is important to produce the theoretical basis for studying the populations exposed to a new class of chemical influence (like DEF), as well as to adjust the possibilities of DEF influence on genetic apparatus and these influence realization peculiarities on the background of individual genetic features, organism's functional and morpho-physiological characteristics.

The following tasks have been put up to achieve this aim:

1 - comparative epidemiological, ecotoxicological and medico-genetical (that was subjected to somatic and germ cells reactions: cytogenetic analysis and reproduction function state)[2] characterization of population in regions with different level of contamination with DEF; 2 - production of basis for cohort group formation for the following comparative medico-genetic characterization of inhabited regions; 3 - examination of cytogenetic parameters and specificity of alterations of chromosome apparatus in people with different rate of DEF contacts; 4 - search of respond phenomenon for inherited and environmental components of sister chromatid exchanges in cells of people exposed to dioxin (South Vietnam population after Agent Orange spraying); 5 - elucidation of general phenomenon of the human organism cytogenetic response and the features of complex medico-biological syndrome of dioxin-containing chemicals influences long-term consequences.

After characterization of ecotoxicological situation in the selected region (Song Be Province, South Vietnam) the epidemiological data was collected on the first step - by house-to-house investigations of several villages with different level of Agent Orange contamination. Questionnaires have been obtained from 2235 persons (m, f, age 21-60) and analyzed according to data base computer programs)[1]. The selection of sample for the comparative cohort study of health status and reproduction function

analysis in the contaminated area (f, N=370) and in the control region (f, N=443) was done on the second step. The samples for the clinical and laboratory examination were selected also (m, N=53; f, N=100). The reproduction function analyses among selected cohorts[3] were carried out according to medico-genetical questionnaires (health status and obstetrics information). Genealogical data had been received for 3 generations of the family if possible. The questionnaires have been repeatedly collected for those who had been subjected for the clinico-laboratory examination.

The cytogenetic analysis of the cultivated blood cells has been carried out in groups of males (N=53, age 21-50) from 2 villages with different level of exposure to Agent Orange. Two samples has been shown[4] to be different according to genetic instability level. The detailed analysis of the cytogenetic parameters was presented[4,5] earlier. The cytogenetic analysis of exfoliated cells of buccal mucosa has been done in groups of females (N=100, average age 57.44 ± 8.88) that had been in reproductive age during spraying. The results of the last study are presented in this issue[6] and they prove the enhanced level of breakage in persons inhabiting the contaminated territory. The destructive alterations were even registered in the epithelial cells of buccal mucosa. This fact supports our previous assumption on the stem cells' alterations in different tissues as the result of exposure with dioxin[7].

The formation of the new ecotoxic factor with ecogenetic activity - DEF - has been shown on the base of the research work on the South Vietnam territory and the general biological phenomenon of homeostasis alterations in persons exposed to dioxin-containing chemicals[8]. DEF's ecogenetic activity is characterized by: - shifts in medico-genetic parameters, registered in cohorte groups with different level of DEF exposure[3]; - alterations in chromosome apparatus stability in cells of DEF-exposed people[4,5].

The reproduction function abnormalities are more frequent in families from the sprayed region (up to 31.1% of females with disturbances as compared to 20.3% in the referent village). In the families of young women the tendency of stillbirths and malformations increase could be seen. These were associated by the alterations in physiological maturity parameters. There were no such alterations in elder women, but the general weakness had been registered. The women in the contaminated region were much more subjected for diseases, and inflamatory first of all.

The genealogical analysis and the search of pathological signs in ascending and/or descending generations allowered to obtain the characteristics of the native subpopulation in the sprayed village (Binh My, Song Be Province) and those immigrated from the North provinces. The supposition on the inheritable features of the reproductive pathology had been denied on the base of the reserched data.

The results of cytogenetic studies on the somatic and germinative cells showed the alterations in the sprayed region on the level of chromosome material. That was obvious in the cultured peripheral blood lymphocytes where an increased level of environmental SCE had been registered as well as of rates of cells with high SCE numbers (HFC). The rates of cells with disturbed chromatic material was observed in mucous cells also.

The observed ecogenetic effects of the dioxin activity consequences correlate well with homeostasis alterations on different level (lymphocytic system[4], immunity parameters[5,9], dermatological morphometric changes[10], reproductive system[3], microsomal enzymes' activity[11], porphirine and vitamins' metabolism[12], etc.).

Thus, the basis is produced for the population ecogenetic investigations. The comparative investigation is foreseen to be aimed at ecogenetic characteristics of the long-term medical consequences of dioxin effects on populational (medico-genetical, genealogical analysis, toxicogenetic tests), organism (individual systems' analysis, capacity to work, adaptive reactions), cellular (blood formula, ultrastructural analysis, etc.), and chromosomal (aberrations, SCE, micronuclei) levels. The successiveness of the study includes the population total epidemiological assay, selection of contingent for medico-genetical analysis, genetico-toxicological and toxico-genetical investigations among the selected groups.

The phenomenon of the cytogenetic alterations had been revealed on application the special methodological techniques (differential analysis of the genetical and exogenous components of the parameter[4]) and loading tests (additional induction, e.g. wit mitomycin C [13,14]) and indicate the significantly higher sensitivity of the genetical apparatus of persons from DEF-contaminated area. They also permit to delineate persons with different level of chemical individual susceptibility.

Practical and scientific realization of these studies implies the possibility of forecasting the dioxin and/or DEF long-term consequences on the base of large population groups screening in chemically contaminated areas.

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Table 1. Representation of classes for distinguished parameters studied in two villges with different levels of dioxin contamination (Song Be Province, South Vietnam).

		INCREASING RANGES OF THE EFFECT'S SIGNIFICANCE																				
PARAMETERS	ERG	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	>20
sSCE (eSCEcl) [a]	I	•	•																			
	II		•	•	•	•																
	III	•	•	•	•	•																
HFC (eSCEcl) [a]	I	•	•																			
	II		•	•	•	•																
	III	•	•	•	•	•																
MN (Types 1,2,3,4) [b]	I	•	•	•	•	•	•	•														
	II, III	•	•	•	•	•	•	•	•	•	•	•	•									
Binucleated cells [b]	I	•	•	•	•																	
	II, III	•	•	•	•	•	•															
Cells with "Vacuolated chromatin" [b]	I	•	•	•	•	•	•	•	•	•	•											
	II, III	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Defected cells [b]	I	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•					
	II, III	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

ERG - exposure risk groups: I - controls, II - indirect exposure to dioxin, III - direct and indirect exposure to dioxin;
a - Classes for spontaneous SCE and HFC were delineated according to values of eSCE (environmental SCE);
b - Classes for micronucleus-test (MN-test) were delineated according to smoothed values of each parameter rate.