

THE LONG-TERM HEALTH CONSEQUENCES OF AGENT ORANGE IN VIETNAM

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This paper concerns the results of the 12-years prospective scientific and practical researches on the problem of the Long-Term Health Consequences (LTHC) of exposure to dioxins. The results were obtained while solving the tasks of revealing, characterizing and identification of the LTHC of the war chemicals application in Vietnam, and the most prominent of them is "Agent Orange" (AO) – the dioxin-contaminated phytotoxicant used by the US Army during the 2nd Indochina War [IOM, 1994].

Subjects of the study

In 1988 we got the opportunity to examine in dynamics the health state among the large contingents of the Vietnam rural population exposed to AO and to the specific exogenous harmful factor – the "dioxin-containing ecotoxicological factor" (DEF) – which was formed in the sprayed regions under the local specific natural, climatic and social conditions [Roumak, 1993; Roumak et al., 1996]. According to the international experts' conclusions the large-scale implementation of AO in the South Vietnam produced one of the most adequate model for solving the theoretical, scientific and practical problems in the "dioxins" human ecotoxicology and the LTHC [HIW, 1993; Schechter et al., 1995; Poznyakov et al., 1998].

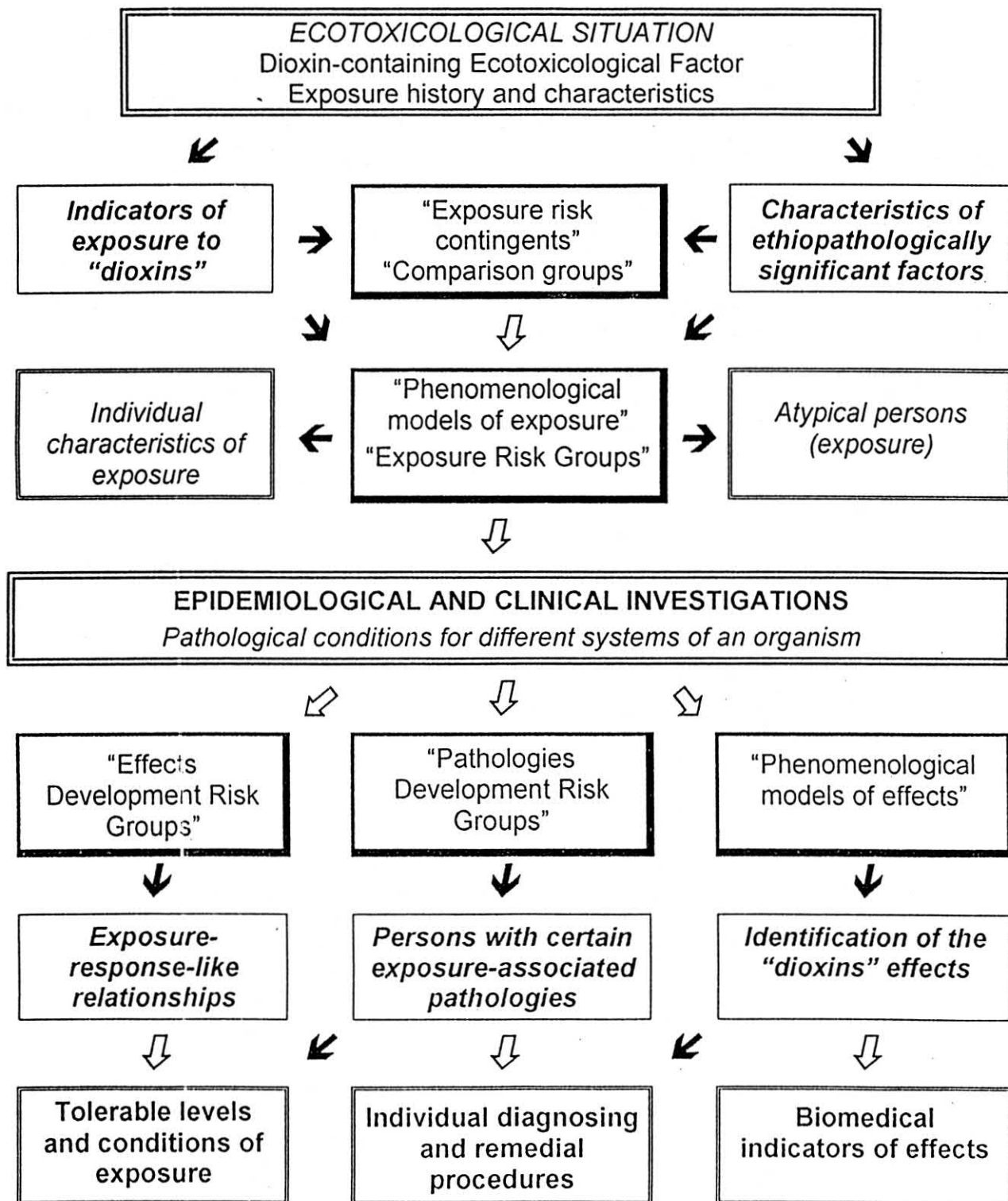
There were the US Air Forces data on periods, intensity and location of AO sprayings ("HERBS tape") at our disposal as well as the toxicological characteristics of acute human intoxication (collected by the Vietnamese and Russian toxicologists); and results of different foreign and our own chemical analytical studies [Schechter et al., 1995; Kluyev et al., 1997] on the dioxins' residual quantities in natural objects and in human bodies in the regions under investigation. All these allowed to identify the exposed persons, and to elaborate the methods for historic exposure reconstruction for the remote direct contacts with AO and/or the long-term chronic exposure to "dioxins"/DEF. From the methodological point of view, besides the high degree of the exposure, the constant and conservative life style

of rural Vietnamese contingents, their social and demographic stability, local food consumption, strong and relatively invariable influence of the common health risk factors, and low medical interference into the LTHC development strongly favored our studies. The major preference of the studied population is the possibility to select numerous contingents of unexposed and high exposed persons (even within one and the same village) with the similar life history and current life style, nutrition, general epidemiological, professional and social features, and contacts with other chemicals. All these conditions create favorable grounds for receiving the valuable scientific data on the LTHC of remote acute and chronic contacts with the dioxin-containing chlorophenoxyherbicides and environmental "dioxins" [Roumak, 1993; Roumak et al., 1996; Poznyakov et al., 1998].

The strategy of the medico-biological investigations was based on the population comparative study in the regions sprayed with the war chlorophenoxyherbicides [Roumak et al., 1998]. The population of the territories similar on all parameters, except for the AO spaying and/or current DEF, was used as the comparative groups. The groups of the military veterans from northern Vietnam served as an external comparison. A basis of the population characteristics was data obtained during the dynamic epidemiological surveys of more than 10000 individuals with various DE levels and conditions of exposure with AO and/or DEF, and subsequent clinical and laboratory examination of the representative samples.

Three main cohorts were investigated during the study:

- The native rural population of the southern and central Vietnam with the history of acute intoxication with "Agent Orange" during the war and the chronic dioxin exposure as the result of living on the contaminated territory;
- The native rural population of the southern and central Vietnam without direct contacts with "Agent Orange" during the war, but with the chronic dioxin exposure as the result of living on the contaminated territory;



Scheme 1: Algorithm for investigation of medico-biological consequences of exposure to dioxin-containing ecotoxins

- The Vietnamese war veterans, exposed to "Agent Orange" during the war, and currently living on the contaminated with "Agent Orange" and non-contaminated territories.

Study algorithm

Study algorithm presumed the step-by-step decreasing of the uncertainty of epidemiological characteristics of exposure and effects by successive increasing the homogeneity of the studied cohorts (selection of persons with typical values of the characteristics and their associations). The basic elements of the algorithm (scheme 1) permitted to study the direct and indirect causal relationships between the exposure parameters and different retrospective and current characteristics of the health state and homeostasis [Roumak. 1993; Poznyakov, 1997; Poznyakov et al., 1997 a, b].

In the following research work the "CM I" group was considered as the external comparison for the groups "BM II" ("DEF effects") and "BM III" ("AO/DEF effects"); "BM II" group – as the internal comparison for the "BM III" group ("AO effects"). "BH I" group was considered as the internal comparison for the "BH II" ("AO effects").

The next step of the study was to characterize and classify the likely extent and conditions of exposure to AO and DEF in the terms of the "Phenomenological models of exposure" and the "Exposure Risk Groups" (ERG). The individual exposure measures were obtained by allocation of subjects in one of the statistically defined homogenous "Exposure Risk Groups" (ERG). These groups were stratified by multidimensional analysis of the concrete values and interrelationships between individual epidemiological characteristics of the exposure for a given sample size and characteristics variability. The effect-modifying influences of different other specific local factors were revealed and considered by the common statistical methods for the stratification analysis and multifactor analysis of variances.

Further investigations were aimed at the examination of pathognomonic symptoms and signs of pathological conditions in different organism's systems. The syndromes of polytropic pathology, dysregulatory and desadaptive states were revealed and characterized using the epidemiological monitoring methods, profound systemic clinical and laboratory investigations, as well as by implementing special me-

dical and biochemical loading tests. Application of multidimensional statistical analysis for investigation of associations between the situationally, logically, toxicologically, biologically and physiologically linked characteristics of exposure and effects – the "Phenomenological approach" – allowed to define the "Effects Development Risk Groups" (EDRG), embracing the individuals with certain levels and associations of characteristics of responses to the concrete similar likely levels and conditions of exposure. The "Pathologies Development Risk Groups" (PDRG) were selected as well – that are the individuals with the different similar leading syndromes of polytropic pathology. So, the systemic health outcomes and alterations in the homeostasis were determined and described in the terms of the "Phenomenological models of effects" [Poznyakov, 1997; Poznyakov et al., 1997; Antonyuk and Poznyakov, 1997; Poznyakov et al., 1998; Roumak et al., 1998].

The study has begun from the selection and characterization of the "exposure risk contingents" that should be principally different by the history of contacts with AO and subsequent DEF influence, but should be comparable according to the history of other risk factors influences. The methodical procedure included the examination of chemical, ecological, epidemiological and biomedical indicators of the history of exposure not only to AO and DEF, but also to other possible local effect-modifying, confounding, and common risk factors. In our studies, five such contingents were selected among the inhabitants of the South and North Vietnam. All these groups were differentiated according to the history of direct contacts with AO, that could be supported by the "HERBS Tape" data; by documents of the local authorities; by analyzing the exposure situations and typical symptom complexes of the acute intoxication, and by increased prevalences of the residual signs of exposure-associated alterations on skin, mucous layers, and in the homeostasis. To account the DEF influence the stratification was made according to the history of residence on the territory sprayed with AO – that is Binh My village (AO – 50.7 l/ha; DEF – < 15 and ≥ 15 years of residence after sprayings). Unexposed peasants from the intact village Chanh My (40 km away of Binh My) with the same life style, nutrition, profession, socio-economic status, and the history of agricultural chemicals' applications and unexposed war veterans living together with the exposed veterans in the native village Bac Hong (North Vietnam)

Table 1

Selected contingents of Vietnamese peasants with different histories of exposure to Agent Orange (AO) and/or Dioxin-containing Ecotoxicological Factor (DEF)

Village (name)	Likely exposure		Residual "dioxins" levels *			Groups (name)
	AO	DEF	"1"	"2"	"3"	

South Vietnam, Song Be province

Chanh My	-	-	<5	0.6-6.4	1.2-6.0	"CM I"
Binh My	-	+	6.0-40	4.0-11	1.7-14.0	"BM II"
Binh My	+	+				"BM III"

North Vietnam, Dong Anh province

Bac Hong	-	-	Nd	<1.0	<1.0	"BH I"
Bac Hong	+	-				"BH II"

* "International Dioxin Toxic Equivalents" (1988-1990): "1" — blood plasma lipids (ppt); "2" — soil (ng/kg); "3" — meat, fish (ng/kg) [Schechter et al. 1995; Kluyev et al., 1997].

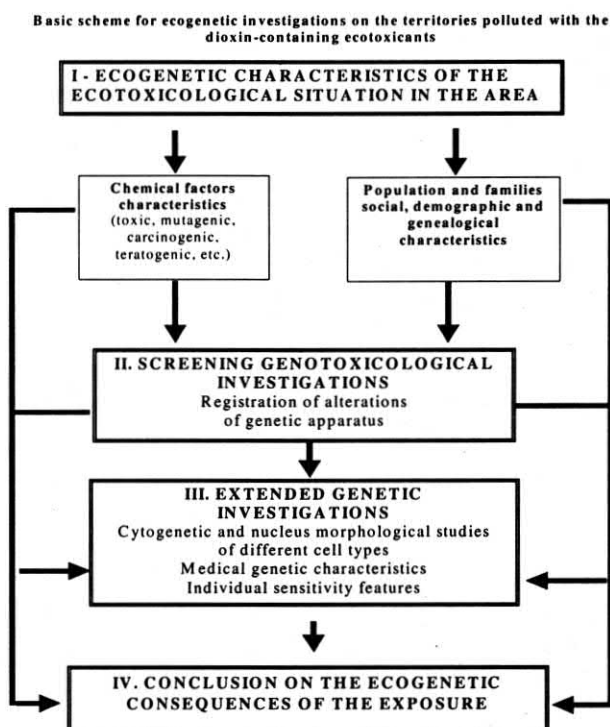
after the wartime were selected as control groups (Table 1).

In general, the realization of such procedures allows considering the variability and uncertainty of epidemiological characteristics of exposure, effects and their associations. The qualitative and quantitative characteristics of individual local exposure conditions, pleiotropic systemic health outcomes and exposure-response-like relationships could be obtained. Also, the statistically atypical individuals with the presumably improper results of examination, with different susceptibility and/or with atypical exposure conditions could be identified. The procedure for the EDRGs selection permits to characterize the real "exposure-responses" relationships for the health parameters under the study and to assess the real relative hazards of exposure different levels and conditions in every concrete situation. Also, this procedure determines the perspective for elaborating and implementing the specially aimed treatment and preventive measures. The comparison of expected and observed systemic effects allows to solve the tasks of identification of medical and biological consequences of DEF's dioxin component using the criterion of "biological plausibility", in addition to hypothesizing on the LTHC origination mechanisms; and searching for the most informative biomedical

Clinical and laboratory investigations were aimed at the accumulation of the additional objective information on the exposure and effects. These investigations included functional diagnostics of several systems' state. Those were:

- Cardio-vascular and respiratory systems; visual analyzer; characteristics of mental and physical working capabilities; characteristics of cellular and humoral immunity; blood serum biochemistry, state of microsomal system involved in xenobiotics metabolism in lymphocytes and liver (pharmacological probes);
- Some characteristics of endocrine system, peculiarities of vitamin A status and porphyrine metabolism; ultra structural morphometric investigations of epidermis in semi-thin skin samples; cytogenetic effects (different chromosomal aberrations) in lymphocytes and
- Oral cavity mucous exfoliated cells.

Deep medical and clinical laboratory examination of the selected typical and apparently healthy individuals from different ERGs (males, aged 31–50), implication of loading and provocative tests, and biomaterials sampling for the analysis were carried out in a hospital-based trial. The samples were proceeded and analyzed at the specialized laboratories



Scheme 2

at several RAS and RAMS institutes.

Practical use of this very algorithm supposes the need to adopt and modify the elements presented above for the concrete aims of every investigation, as well as supplying it with the additional blocks for solving the specific tasks. Mostly, it concerns the block of revealing, characterization and identification of the various expected effects. For instance, the different traits has the methodology for solving the problem of revealing and characterization of the multilevel ecogenetic consequences of the "dioxins" exposure – on the background of the genotype and phenotype peculiarities of the studied population, as well as local specific factors modulating influences on the analyzed effects (Scheme 2). The algorithm of the ecogenetic studies allows to characterize the ecogenetic consequences of the exposure on the cellular, sub cellular, and cell subpopulation levels; to access the modification factors (internal – genetic and phenetic, and external – environmental) influence on the functional alterations; to analyze the parameters of individual susceptibility to active factors, and an adaptive potential of organism. The application of this methodology in Vietnam presumed the multilevel step-by-step investigation of the following manifestations: 1) cytogenetic para-

meters were analyzed as the biological base for probable genetic effects; 2) systemic alterations in cellular sub populations were considered for revealing, characterization and identification of medical and genetic effects; 3) reproductive function disturbances were analyzed for estimation of medical and demographic significance of ecogenetic effects; 4) modifying role of "dioxins" was studied in altering the specificity of local environmental factors activity. All these points were settled by consolidation of the results of epidemiological, medicogenetic and laboratory examinations of the "dioxins" genetic hazards on the territory contaminated with the ecotoxins, particularly of the population demographic and genealogical studies, and the analytical summary of the results obtained during genotoxicological, cytomorphological and cytogenetic investigations [Oumnova et al., 1997; 1998; Golikov et al., 1998].

Results and discussion

The application of the promoted procedures to classify the remote direct exposure to all components of AO allowed to divide the contingents of "BM III" and "BM II" into the groups of low and high likely extent of the exposure – "BM III.2" > "BM III.1"; "BH 11.2" > "BH 11.1". The epidemiological study with applied external ("CM I") and internal ("BM II", "BH I") comparison groups allowed to reveal the consistent and statistically significant associations between the epidemiological measures of the direct AO exposure (and of the DEF exposure to a lesser extent), and the increases in frequency of the pathological manifestations for different organism's systems, and the significant deterioration of current general health status (integrated individual "Indices of Health Status", IHS) in all the age groups examined. An example of the associations for the IHS values is presented in the table 2.

In addition, the multidimensional statistical definition of the «phenomenological models of effects» allowed to reveal several relatively discrete groups of «symptom complexes» of the LTHC of AO and to identify the persons with such health outcomes in a group of exposed South Vietnamese [Poznyakov et al., 1997; Antonyuk and Poznyakov, 1997; Roumak et al., 1998].

Also, the epidemiological evidences of the hidden immunodeficient state were obtained by the increased prevalences of infectious pulmonary dise-

Table 2

Consistent significant associations between the epidemiological measures of exposure ("Exposure Risk Groups", ERGs) and deterioration of general health status in the most typical cohorts of South and North Vietnam peasants

Population	South Vietnam (Chanh My, Binh My, 1989; m, 31-50)				North Vietnam (Bak Hong, 1993; m, 40-60)		
ERGs:	CM I	BMII	BM III.1	BM III.2	BH I	BH II.1	BH II.2
N	291	194	51	29	208	42	32
DEF	-	+	+	++	-	-	-
AO	-	-	+	++	-	+	-H-
IHS+	.81-.85 .829 345.7	.72-.77 .746 264.7	.60-69 .644 194.7	.45-.59 .522 130.6	.64-.69 .657 160.0	.56-64 .604 130.2	.45-55 .503 71.8
Factors ^x	ERG (AO***, DEF***); M**, N**, S*				ERG (AO***); M*, N**, A*		

+ — Index of General Health Status: SS symptoms and signs / number of health characteristics under the study — 1 m 0 — deterioration; 95 % C.I., Averages, Average Ranks.

x — «Multifactor ANOVA» for the IGHS values with the factors: «ERG» — allocation in one of the statistically defined exposure risk groups, «M» — history of malarial diseases; "N" — inadequate protein nutrition; «S» — intensive smoking; "A" — high alcohol consumption.

*, **, *** — Main effects: $p < 0.05, 0.01, 0.001$; 2-Factor Interactions — «No» in both groups.

Table 3

Relative occurrence of diagnosed cases of acute pneumonia (P) and pulmonary tuberculosis (T) among the South Vietnam peasants with different histories of exposure to Agent Orange (AO) and Dioxin-containing Ecotoxicological Factor (DEF)

ERG	AO / DEF	Age, y	N, m+f	"P"	OR (P) ^x	"T"	OR (T) ^x
Phu Hoa	-/-	21-40	1440	12	-	1	-
		41-60	1177	17	-	1	-
		> 60	448	19	-	2	-
BM 1	-/+	21-40	782	7	1.07	1	1.37
		41-60	645	10	1.07	1	
		> 60	245	12	1.15	1	
BM 2	+/+	21-40	439	13	3.34*	0	4.98**
		41-60	338	28	5.74*	4	
		> 60	118	18	3.49*	4	

^x — M-H Odds Ratios for the "P" and combined "T" cases: BM II / Phu Hoa, BM III / BM II.

*, ** — 95 % C.I. excludes 1.0, Fisher exact 2-tailed value — $p < 0.05, < 0.01$.

ases among the exposed populations during the epidemic periods, that was further confirmed by systemic examination of clinical immunological parameters (table 3) [Poznyakov et al., 1995; Roumak et al., 1998].

As a whole, the results of the developed approach application and special investigation of the epidemiological criteria of causality brought up strong epidemiological evidences of the reality, relative specificity and medical significance of the LTHC of AO sprayings in Vietnam. Also, they supported the practicability and high reliability of the applied methodology for epidemiological revealing and characterizing the LTHC of dioxin-containing ecotoxins [Roumak, 1993; HIW, 1994; Poznyakov et al., 1994 a, b, 1996, 1997 a, b; Roumak et al., 1996, 1997, 1998].

These conclusions were confirmed by the results of further profound systemic clinical and laboratory investigations. The clinical significance of the LTHC effects had been evidenced by the results of the comparative clinical and laboratory examinations. For instance, the existence of associations was revealed between the exposure level increase and the increases in frequencies of persons with the hypertension and hypotension, functional heart abnormalities, tissue hypoxia development, increased levels of serum LDG and creatinekinase, erythropenia and haemoglobinemia. Such alterations were expressed mostly among the exposed individuals when the "dosed physical loading" (test PWC-170) was applied. In unexposed persons ("CM I") the physical ability was provided by the adequate cardiovascular system reaction to the physical exertion. At the same time, in persons with the LTHC the high level of physical ability was associated with the hyper reactive responses of the system – because of the higher myocardium tension. The same conclusions supporting the clinical informativity of the observed effects' epidemiological characteristics, were revealed by the clinical examination for the organs of gastroenteral system, immune (altered immune "portraits") and endocrine (increased prevalences of the hypoinsulinemia and hyperthyroxinemia) systems, visual analyzer, and dermal tissue (increased prevalences of the chloracne-like signs and altered keratinization) [Roumak, 1993, 1997; Poznyakov et al., 1994, 1997a; Antonyuk and Poznyakov, 1997; Roumak et al., 1997]. Thus, the integrated multilevel analysis of the data obtained during the epidemiological, clinical and laboratory

studies permitted to ascertain not only the presence and polytropic character of the LTHC, but their relative specificity as well. From our point of view, the epidemiological characteristics of the LTHC could be considered as the primary manifesting indicator of the LTHC existence in the exposed sub population.

Several evidences were obtained for self medical significance and effect modifying activity of the "dioxins" – as the main element of the local specific complex of the environmental factors – "DEF" – unfavorable influence. That are: – insignificant differences in pathological profiles among the groups, stratified only according to the duration of residence on the sprayed territory; – some qualitative similarity of the pathological profiles after the exposure to AO or DEF; – much more wide and expressed spectrum of the pathological states, associated with AO intoxication and following DEF exposure, and significant differences in the registered LTHC of malaria, protein malnutrition, and intensive smoking in groups of persons exposed and unexposed to DEF. The differences in diagnostic significance of these "dioxin"-related alterations should be mentioned for individuals with different exposure levels and conditions. The chances for dioxin-dependent pathology appearance among persons exposed to DEF depended also on other unfavorable influences (diet quality, smoking, contacts with pesticides, malaria in the past). The specific pathology profile was wider, more stable and much more manifested after the combined exposure to AO and DEF.

The following evidences for the informativity of the clinically and epidemiologically based exposure and effects measures, as well as objective information for the biological reliability of the observed LTHC, were received in the process of special clinical and laboratory studies. That concerns the widely known medical and biological indicators of dioxin exposure, and other special features of the homeostasis as well.

The biologically significant alterations in microsomal benzo-a-pyrene hydroxylase (as marker for dioxin-dependent form of the cyt P450) were revealed in isolated lymphocytes, and it was demonstrated that in the exposed persons the new atypical associations were formed between the indicators of the dioxin-sensitive hydroxylating and other activities of the cyt P450 in lymphocytes and liver [Roumak, 1993; Ostashevsky et al., 1994].

The cytogenetic analysis revealed the structural

and functional alterations in the nucleoprotein complexes of lymphocytes and epitheliocytes of the exposed persons. The levels of chromosomal aberrations were increased in some individuals, as well as frequencies of sister chromatid exchanges (SCE) and micronucleated cells. The individuals with polyploid (4n) lymphocytes were also more frequent, and the appearance of cells with the endoreduplication was observed. The presence of cells with the increased SCE numbers was higher also among the exposed individuals. The increased level of some chromosome aberrations possibly associated with the stem cells lesions was registered for the exfoliated mucous cells of the oral cavity [Oumnova, 1997; Oumnova et al., 1997].

The immunosuppression with the specific defense system functional decrease was also diagnosed. This was characterized by the leucocytosis development, by alterations in T-h and T-s frequencies correlation, by decrease in phagocyte index, by B-cells absolute number increases. The qualitatively new associations were observed between the functionally related characteristics of the immunity [Roumak, 1993; Oumnova et al., 1994; Poznyakov et al., 1995; Roumak et al., 1997, 1998].

The hemopoetic system very characteristic disturbances were seen also. Along with an increase in the exposure levels the frequencies arise of the individuals with the low erythrocytes numbers, with the decreased haemoglobin concentrations, with abnormally low serum iron. The erythropoetic disturbances were followed by the significant alterations in haem metabolism. The differences between the compared ERGs according to increased values of excreted porphyrins and coproporphyrin III, to decreased percentage of uro- and increased percentage of 7-carboxyporphyrins – were not only statistically significant, but had the evidently demonstrated causal associations with the exposure. The revealing of the local standard characteristics for the porphyrins excretion and the investigation of common liver clinical and biochemical functional parameters allowed to show the increased frequency of coproporphyrinuria among the exposed individuals and to demonstrate the chemical etiology of such state (Table 4) [HIW, 1994; Poznyakov et al., 1997a; Poznyakov, 1997; Roumak et al., 1998].

The systemic investigation of the vitamin A (vit A) status permitted to reveal two different forms of its alterations among Vietnamese peasants: the “common” type of alimentary hypo-vitaminosis A and the

chemically induced “hyposensitive hypovitaminosis A”. The latter is characterized by low sensitivity to the treatment with test and pharmacological doses of vit A and influences the LTHC pathogenesis in the exposed individuals (table 5) [Poznyakov et al., 1992, 1997a; HIW, 1994; Poznyakov, 1997; Roumak et al., 1998].

The desadaptive character of many effects observed should be mentioned particularly. This was diagnosed with the help of special provocative and loading tests and by studying the biological system reserve possibilities. For example, the tests for impaired dark adaptation and cardiovascular responses to physical exertion may be used as physiological indicators for the systemic desadaptive state among the exposed individuals. For the characteristics of lymphocyte microsomal system activities only one may be called the informative indicator for the LTHC. That is the “inducibility index” – the functional ability of the cyt P450 to react on the induction with 3-methyl-cholantrene. There were no blood retinol level increases after the vit A single administration to the significant part of those individuals with the revealed vit A hypovitaminosis and non-revealed liver functional state alterations. Such effect proves specific disregulation of the processes involved into the support of the vit A dynamic homeostasis in exposed persons. This may lead to development of the desadaptive states of the vit A status ensuring systems and to formation of the specific type of vit A deficiency [Roumak et al., 1994].

The similar peculiarities of reactions on the specific induction were seen for the lymphocyte cytogenetic parameters as well. In particular, the significance of the SCE frequency levels alterations (as indicators of exposure consequences) was increased in the series: genetically determined SCE < total SCE < environmentally determined SCE < SCE induced by mutagenic substance (mitomycin C, 0.1 mcg/ml). The consequent exhaustion by specific stresses of the cells’ reserve possibilities (CRP) to self-replication may explain such an effect. The higher levels of induced lesions in chromosomes (as compared to existent level of genetically and environmentally induced) provided the much more exhaustion of reserved possibilities needed for cell’s material repair and replication. An exhaustion of the CRP during the exposure to dioxin-containing environmental factors is able to provide the higher effectiveness and medical significance of the cyto-

Table 4

Prevalences of different types of the diagnosed and chemically-induced chronic hepatoporphyria (CHP) and of the cholestasis in different Exposure Risk Groups (ERG)
Established local diagnostic criteria

CHP types Urinary porphyrins	Coproporphyrinuria, "CPU"	CHP type A, "CHP A"	Intrahepatic cholestasis, "CHOL"
All, pmol/24 h	> 155.5	> 155.5	-
Uroporphyrins, %	< 20	< 40	-
Heptacarboxyl-, %	< 3	< 5	-
Coproporphyrins, %	> 68	> 40	-
Copro- / Uro-, ratio	> 5.8	> 5.8	-
Copro- III / I, ratio	1.6 << 4.5	1.6 << 4.5	< 1.6

Prevalences of the CHP and cholestasis

ERG (N)	CM I (31)	BM II (29)	BM III (42)	P*
"CPU", cases (%)	2 (6.4%)	5 (17.0%)	35 (83.3%)	< 0.001
"CHP A", cases (%)	0	0	2 (4.7%)	Ns
"CHOL", cases (%)	9 (29.0%)	4 (13.1%)	7 (16.7%)	Ns

* — Chi-square test; Fisher exact 1-tailed p-value.

Table 5

Prevalences of different diagnosed types of the hypovitaminosis A in different Exposure Risk Groups (ERG)
Established local diagnostic criteria

Types of the hypovitaminosis A	R ₀ * (mcg/ml)	(R ₅ - R ₀) / R ₅ * (%)	(R _A - R ₀) / R _A * (%)
"Common" ("A-")	0.22 « 0.65	> 18	> 15
"Hyposensitive" ("h A-")	< 0.41	≤ 15	≤ 15

* — Characteristics of the vit A status: R₀, R₅, R_A — plasma retinol levels: basal, 5 hours after administration of 1 mg of vit A, 2 weeks after administration of 30 mg of vit A; (R₅ - R₀) / R₅ — the "Relative Dose-Response Test", (R_A - R₀) / R_A — the vit A status restoration test.

Prevalences of the hypovitaminosis A types

ERG (N)	CM I (31)	BM II (29)	BM III (42)	P*
"A-", cases (%)	8 (25.9%)	10 (34.5%)	12 (28.6%)	Ns
"hA-", cases (%)	1 (3.2%)	2 (6.9%)	15 (35.7%)	0.005

* — Chi-square test; Fisher exact 1-tailed p-value.

Table 6

**Reproduction function disturbances in South Vietnam female peasants
from different Exposure Risk Groups**

Exposure Risk Groups	CM I ^a	BM II ^b	BM III ^c	P ^{ab}	P ^{bc}	P ^{ac}
Number of families studied	541	438	137	-	-	-
Congenital malformations	9	11	6	Ns	Ns	0.053
Spontaneous abortions, single	114	109	47	Ns	0.03	0.001
Spontaneous abortions, several	35	33	16	Ns	Ns	0.039
Stillbirths, single	30	36	17	Ns	Ns	0.005
Stillbirths, several	3	6	5	Ns	0.09	0.003

toxic and/or genotoxic (mutagenic) effects of other biologically active compounds.

As a result, the LTHC consisting of different clinical and sub clinical health outcomes may be manifested by increased susceptibility to different environmental factors, elevated levels of different developmental pathologies, increased prevalences of specific and non-specific diseases, disorders and dysfunctions of many systems, and, possibly, by the accelerated ageing. All these may cause the worsening of the medico-demographic situation at the ecotoxicologically unsafe regions.

For example, the medical genetic consequences of the "AO" and "DEF" exposure were characterized by disturbances in reproductive function and by systemic pathology of chromosomal apparatus [Oumnova et al., 1997; Golikov et al., 1998]. Reproductive system infringements in females included menstruation violations and non-regularity, delayed menarche, high frequency of genitalia inflammatory diseases, hormonal shifts, pregnancy and delivery pathology (table 6). Among the population of the polluted regions the reproduction function lesions were registered even at super small levels of dioxins. The possibility of weak health among infants was also supposed. The relative frequencies of pathologic reproductive outcomes were considerably amplified among females born after the war.

It is obvious that shifts in homeostasis of the exposed population and somatic pathology increase (Fig. 1 A) imply the higher sensitivity to exogenous factors (physical, chemical and biological). The cytological analysis of the inflammatory and infectious processes in female genitalia on the contaminated territory (B) demonstrated the high incidence of urogenital pathology – the frequencies of colpitis and

endocervicitis of the nonspecific etiology were higher in the exposed Binh My village (B). No increase in bacterial infections was observed in the same cohorts.

The cytogenetic and morphological analysis registered the alterations of chromosomal apparatus in different cell types of the exposed veterans and inhabitants of the dioxin-contaminated regions in the south of Vietnam - destabilization and dysfunction of chromosome material, chromatin fragmentation revealing the signs of cells apoptotic elimination. Fig. 2 A. shows the increase in percentage of cells with high frequency of environmentally determined sister chromatid exchanges (eSCE > 1.28 exchanges per cell) among cultured lymphocytes of exposed males. Fig. 2 B. shows the same tendency of increase in percentage of oral cavity epitheliocytes with different types of nucleus abnormalities – cells in apoptosis, binucleated cells, cells with nucleus defects. The statistically significant increase in cells with nucleus defects and micronucleated cells in oral mucous of females from three studied villages (Fig. 3) can serve as the next example of nucleus material instability in sprayed region as compared to non-sprayed. Presence of micronucleus structures, nucleus morphological defects (perforation, vacuolization, karyorhexis, pyknosis, etc.), and dysplastic changes of cellular structures testify to desadaptive and functional alterations of mucous, and insufficiency of its protective mechanisms. Such effects are supported by the increased occurrence of inflammation signs in mucous of the exposed individuals. The greater expressiveness of chronic processes could be observed, as well as the correlation of the cellular apparent effects with the immunodeficiency and other systemic somatic pathological signs and diseases [Roumak et al., 1998; Oumnova, Roumak, 1998].

A. Somatic pathology

B. Urogenital infections

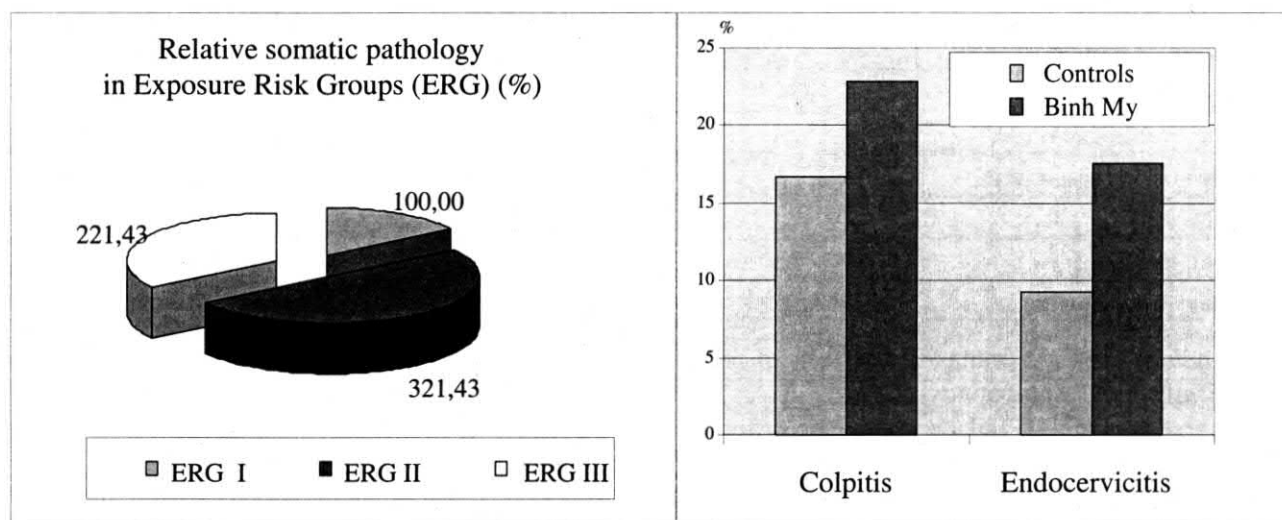
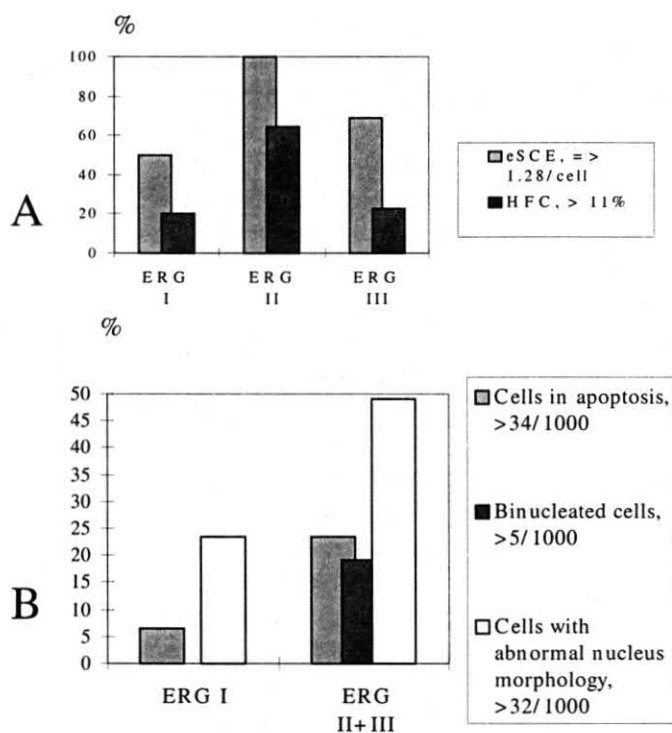


Fig. 1: Morbidity analysis among vietnamese females



Significance of subcellular and chromosome structures alterations in different Exposure Risk Groups (ERG)

ERG I – control
 ERG II – DEF exposure
 ERG III – AO + DEF

SCE – sister chromatid exchanges
 HFC – high SCE frequency cells

A – cultured lymphocytes
B – oral cavity epitheliocytes

Fig. 2: Cytogenetic and Nucleus Morphology Analysis

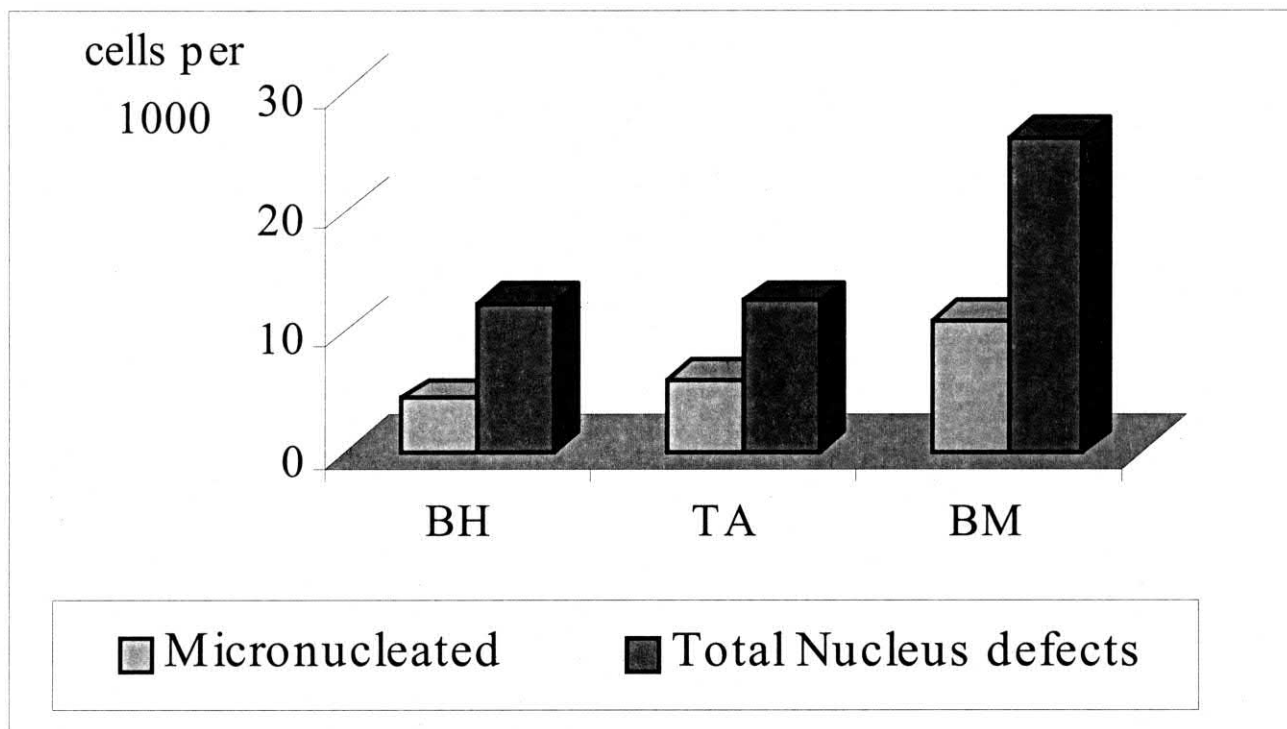


Fig. 3: Cytomorphological analysis of oral cavity epitheliocytes in three Vietnam villages

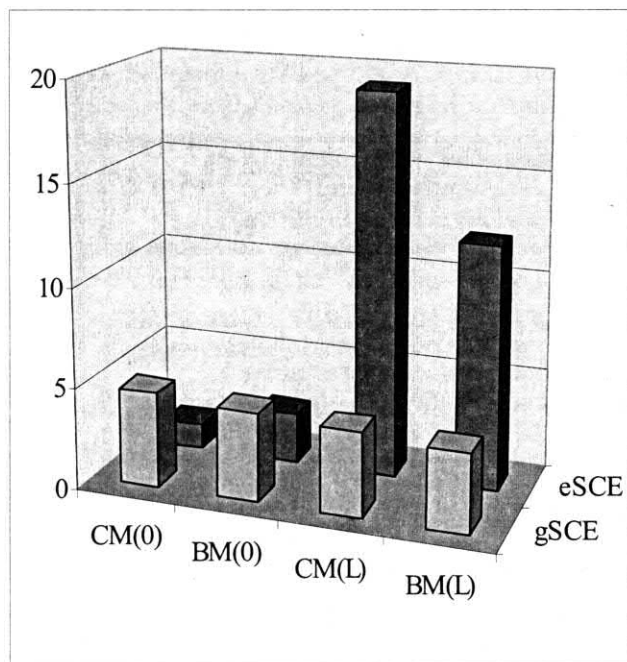
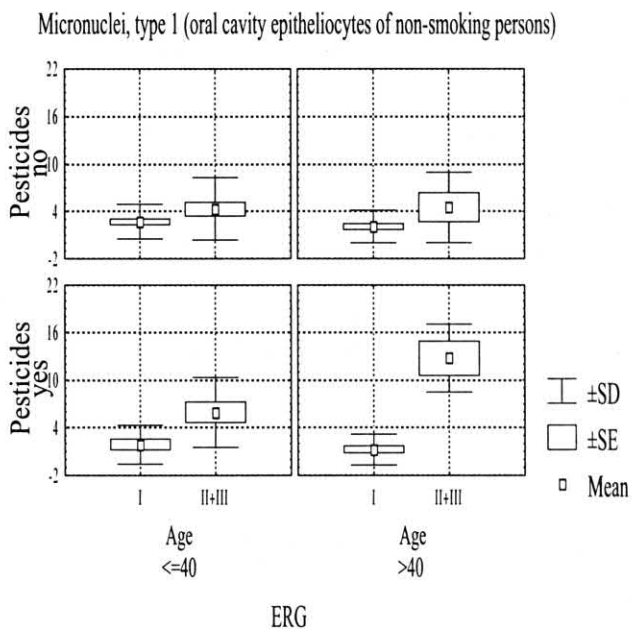


Fig. 4: Modification factors



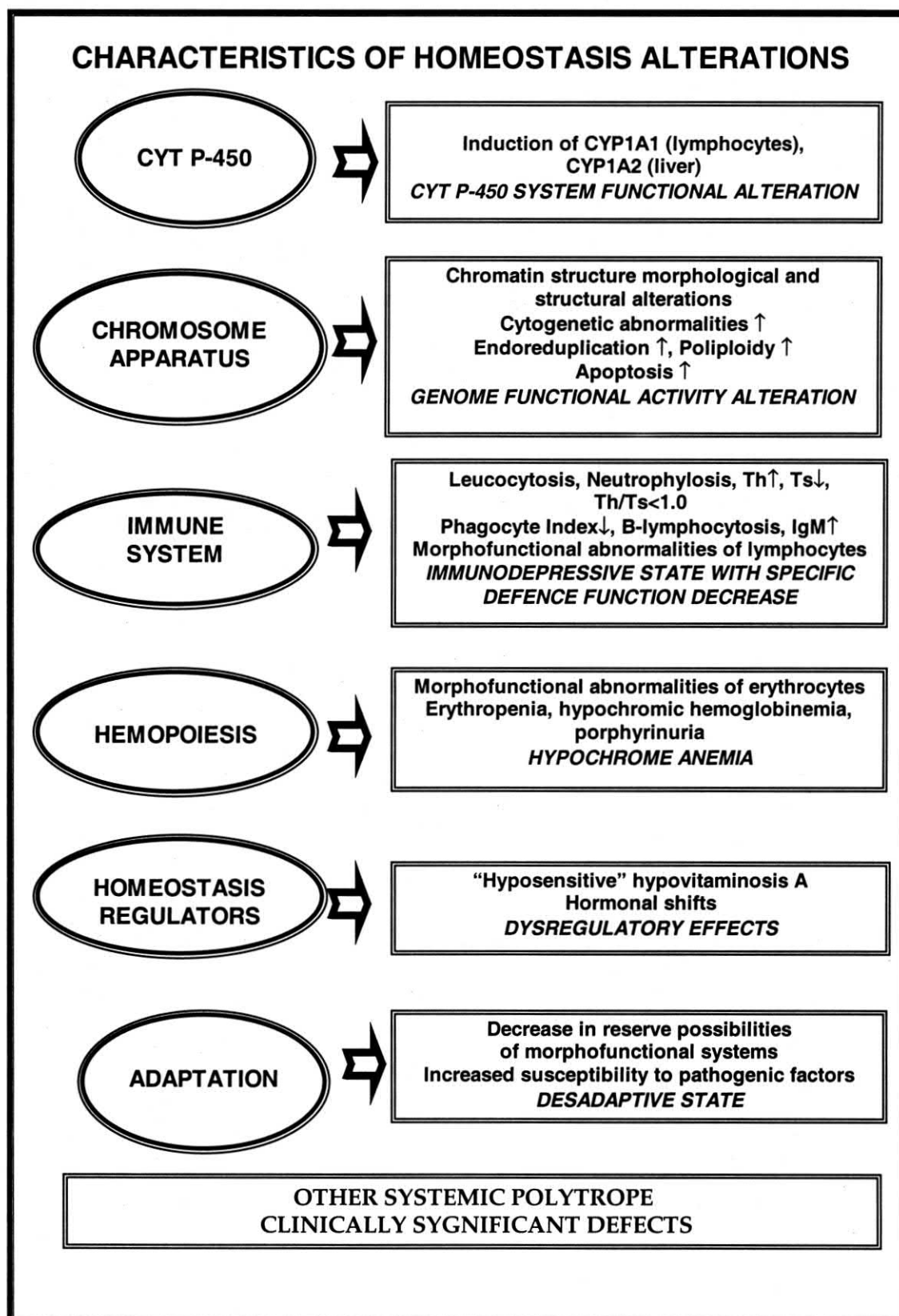


Fig. 5

The extreme variability of individual effects, and the LTHC's somatic manifestations particularly, assumes involvement of modifications generated in general biological mechanisms of biosystems maintenance – those required for life support, cell cycling, and cellular homeostasis preservation. It is obvious that confounding factors may modify the individual effects and adaptive reactions. For instance, the induction of cytogenetic lesions in cultured human lymphocytes by mutagenic substance - mitomycin C – demonstrated the decrease of cells functional activity in exposed persons (BM) as the result of cellular reserves exhaustion in reaction to mutagenic loading *in vitro* (Fig. 4 A). The pesticides influence on mucous cells *in vivo* could be demonstrated by the maximum increase of cells with small micronuclei (type 1) in persons aged above 40 years living on the sprayed territory (Fig. 4. B).

Thus, different functional and morphological changes of nucleus apparatus were observed in cells of persons with the LTHC. Such modifications include: destabilization and abnormal function of chromosome apparatus; chromatin fragmentation and cells apoptotic elimination;

nucleus degradation (dyskaryosis) and accumulation of cells with nucleus defects; association of chromosome apparatus alterations with the environmental exposure (AO, DEF, etc.). At present, higher alterations were registered in persons exposed to DEF as compared with direct AO exposure. All these proves the instability and rearrangements in genetic apparatus of the exposed individuals that make possible their higher susceptibility to modifying and risk factors, increases the opportunity of mutagenic and carcinogenic processes in ERGs. So far, the demographic situation in exposed population may be shifted by the individual desadaptive alterations in polymorphic reactions of the organism.

It is important to note the significance of the homeostasis alterations and polytropic effects in pathogenesis of the LTHC (Fig. 5). Dioxins persistence and accumulation in the environment and human body promote new intersystem relations producing qualitative functional and morphological changes. Considering the chromosome apparatus destabilization in the LTHC development, and modifications in its sensitivity to influence of other risk factors, the epigenetic mechanisms' participation in functional alterations of tissues and organs may be assumed. The study of associations between the ecogenetic consequences' features and

the LTHC systemic polytropic effects revealed the formation of new, variable multilevel characteristics of homeostasis, as well as appearance of functional alterations determined by the population genetic structure and the different degree of activity of anthropogenic factors under investigation.

As a whole, the investigation of the homeostatic peculiarities proved the significant reliability of the exposure assessment method, the presence and specificity of systemic alterations in line of the applied characteristics among the exposed groups. The existence of cellular populations' effects, associated with the AO and/or DEF exposure, permitted to suppose that the exposure may lead not only to the alterations in functional activity, intra- and intercellular cooperation of some differentiated cells, but also to the alterations in proliferation and differentiation processes on the stem cells' level. The biological plausibility of the revealed LTHC is supported by their close similarity with the effects observed in the experimental researches and among several contingents with high exposure to dioxin and/or dioxin-containing compounds (Yu-Cheng, Yu-Sho, Seveso, and workers of the chemical plants in Germany, USA, Russia, etc).

Conclusions

The integrated comparative, multilevel epidemiological, clinical and laboratory investigations of the population exposed to AO and/or inhabiting the territories contaminated with "dioxins" allowed to obtain the representative information on the exposure-associated alterations of the health status and homeostasis. The evidences of causative relationships between the observed health effects and developed exposure measures are revealed. The consequences of the exposure to "dioxins" are manifested by: - the increase in frequencies of individuals with the atypical characteristics of health status and homeostasis; - the ecogenetic effects; - the appearance of new specific medical and biological effects; - the formation of new stable associations between the functionally linked characteristics of homeostasis; - the complex of dysregulatory and desadaptive alterations.

On the clinical level the LTHC are characterized by the polytropic pathology of different organs and systems of an organism and by the reproduction function pathology. Several features are diagnosed:

an increase in activity of cyt P450 forms - indicator for "dioxins" exposure; immunodepressive state with the decrease in specific defense function; some endocrine shifts (hypoinsulinemia and hyperthyroxinemia); disturbances in erythropoiesis and haem biosynthesis; development of chemically induced chronic hepatoporphyrin and special type of the vit A hypovitaminosis; non-inheritable aberrations in the somatic cells' chromosomal apparatus; dyskaryotic changes; alterations in the keratinocytes' differentiation process. Dioxin-containing ecotoxins form the complex of the sub clinical desadaptive alterations in different organism's systems. Such feature permits to define the sub clinical components of the LTHC syndrome as the group indicators of the exposure medical consequences.

The revealed, characterised and identified complex of health alterations, associated with the exposure, lead to the loss of 1 to 1.4 years of life in a state of well-being for every 10 years of residing on the contaminated territory [Antonyuk, 1995].

The long-term medico-biological, ecological and analytical chemical examinations of changes in contaminated with dioxins biological systems in Vietnam allowed for the first time to conclude the following:

- The wide-scale pollution of environment with dioxin-containing ecotoxins (DE) can be considered as new and poorly studied version of technogenic accidents. Exposure consequences can be comparable to consequences of radioactive contaminations (by a level of acquired social and economic problems);- The 30-year period of nature and biosystems adaptation to Dioxin-containing Ecotoxins contamination is insufficient for the neutralization of such chemicals effects. The zones of dangerous pollution distribute to hundreds of kilometers from the centers of primary pollution. This results in numerous social and economic problems;

- Destruction of biosystems continues in the contaminated regions, and biodiversity is suppressed (up to ecosystem destroying);- People have numerous multi-level, polytropic and polymorphic lesions and pathologies, including nonreversible diseases and neoplasms, early ageing, medico-genetic abnormalities, infringements of reproduction function and other effects. The demographic shifts develop in human populations. Thus, many years of researches in Vietnam and observations of the sub populations suffered from phytotoxins application

or living on the sprayed territories demonstrated the existence of the Long-Term Health Consequences (LTHC) characterized by multi-level, polytropic, polymorphic systemic lesions in the vitally important organs and systems. The pathogenesis of lesions and desadaptive states in many respects are determined by an individual pattern and dioxin-containing ecotoxins combination with variety of factors formed in phylo- and ontogenesis. Dynamics, expressiveness and pathogenetic specificity of the LTHC manifestations allow considering such lesions as distinctive nosology - the "Dioxin-connected Disease".

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