

The experience of investigations on ecotoxicologic pressure on rural population of South Vietnamese in the region sprayed with Agent Orange

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1. Introduction

On the base of the progress in understanding the mechanisms of the biological activity of dioxin and dioxin-like ligands for the Ah-receptor it may be supposed that polyfunctional, dysregulatory and disadaptive hormone-like activity of these chemicals is manifested in humans not only by the well-known rare diseases but also by the qualitative and quantitative variable spectrum of dysfunctions of the main organism's systems. The formation, expression and medical significance of such premorbidial and often hidden states depend on the exposure peculiarities and on the great amount of the external and internal factors, and on the factor of time (the Long-Term Medical Consequences, LTMC). The massive sprayings of Agent Orange (AO) in Vietnam led to the acute intoxication of about 2 millions of Vietnamese (due to direct contacts), to the long-lasting contamination of the environment with dioxin, and to irreversible alterations in tropical ecosystems resulting in changes in spectrum of different health risk factors in sprayed regions. The following methodological issues set the possibility and adequacy for the development and approbation of our approach to investigation of the LTMC of the AO massive sprayings in Vietnam: 1) high intensity of past direct exposure to AO followed by prominent toxic responses from organs of contact and organism, 2) durable chronic exposure to dioxin from the environment and possible influence of other health risk factors on the chemically affected territories; 3) availability of the sufficiently large, comparable, non migrating contingents of heavy exposed and unexposed rural Vietnamese; conservative mode of their life; relatively invariable influence of common risk factors; poor medical aid; high morbidity and physical exertion; sub adequate provision with certain nutrients and other conditions favoring development and manifestation of the LTMC 1-3).

2. Object

The study of history (the data on main chemicals possibly being absorbed by the environmental objects during the life-time of the Vietnamese population) and characteristics of human contacts with different chemicals up to the moment of our investigations in the village Binh My (Song Be province, AO - 1966-1967; ≈ 50.000 l/sq. Km) indicated (eco)toxicological priority of past direct contacts with AO and subsequent chronic exposure to dioxin from the environment in the induction of the LTMC on the background of place-specific factors produced by the AO applications (Dioxin-containing Ecotoxicologic Factor, DEF). This fact permitted to consider the population under analysis as the contingent being at high risk for the dioxin-containing chemicals exposure and LTMC development and to specify three following population groups with different conditions of likely exposure: 1) persons with the history of direct contacts with AO during the war-time and residence on sprayed territory afterwards (Binh My III, BM III); 2) persons, settled on the sprayed territory after the war-time, and exposed to DEF for different

HUM II

time intervals (BM II); 3) population of the Chanh My village (40 km away) with no spraying history but with the same social and demographic structures, and the history of agricultural chemicals' applications (the external referent group, Chanh My I, CM I) 1,2).

3. Exposure assessment

According to the conclusions made by the CDC, WHAO Working group and Office of Technology Assessment in 1986, the elevated levels of dioxin in serum are often considered the best indicators of exposure to dioxin-containing chemicals. However, there are many sufficient evidences obtained in animal and human studies indicating significant limitations in this parameter application for reconstruction of toxicokinetically and pathogenetically significant history of overall exposure to dioxin-contaminated herbicides. It is enough to mention high individual variability in dioxin accumulation and metabolism; the dioxin toxicokinetics dependence on the history of individual metabolic changes associated with certain medical conditions; the reality of specific long-lasting health consequences of short-term acute chemical intoxications; poor informativity of dioxin residuals concerning a history of exposure to other chemicals 4).

The prominent symptoms and signs of the acute intoxication with AO had been registered in up to 90% of exposed local population in Vietnam. That is why, at the base of AO exposure assessment method we have put the well remembered and reproduced by different Vietnamese population contingents batteries of self-reported symptoms and signs of toxic responses of the organs of contact and organism. Thus the mostly appreciated method was designed for epidemiologic examination of large population groups and evaluation of overall past direct exposure to herbicides. To evaluate the possible role of chronic exposure to DEF and an influence of common health risk and effect-modifying factors the data were registered on the history of residence on the sprayed territory as well as the most prominent individual socio-demographic, medical, and (eco)toxicological characteristics (Fig.1) 1,2,5,6).

4. Epidemiological and biomedical investigations

To consider all the possible health outcomes, the pathologic states for different organism systems have been analyzed during cross-sectional epidemiologic study (1988-1989; questionnaires, primary medical examination; m, f, 21-60; N=2232; 31.6-86.2% of the village population) using the standardized and adapted to the Vietnam conditions battery of symptoms and signs developed in Russia for hygienic health screening. Validity of epidemiological information was improved by the exclusion of possible interests of the studied persons, the application of structured questionnaire enabling identification of contradictory statements, the special training of the personnel, the use of documents of the local People's Committees and the HERBS tape data, the primary medical investigations, and the selective repeated field and clinical examinations. The individual indices of pathologic conditions for different systems of the organism (IPC, fraction of medical problems in corresponding sets of diagnostic characteristics) and indices of health status based on the concept of "Instantaneous total health continuum" (IHS, total sum of problems/ total number of characteristics) and on the procedure of Kaplan M.R. (1988) ("person-years of well-being life") were calculated to evaluate alterations in the subjective health 1,5-8). To investigate the hidden alterations in the immunoresistence, the specialized epidemiological investigation on distribution of diagnosed cases of acute pneumonia and tuberculosis was carried out during the epidemic period in the Binh My village and in the closely located intact Phu Hoa village (1992, 21-60; m, f, N=2617 and 2204) 9).

During the complex clinical and laboratory matched investigations the selected typical representatives of the defined Exposure Risk Groups (ERG, fig. 1) were examined (1990, m, 31-50, N=107-215) to evaluate the validity and biological plausibility of epidemiological

observations and to reveal sub clinical alterations in the homeostasis associated with the exposure. This study included general medical examination and physiological (anthropometry; spirometry; step-test PWC₁₇₀; (rheo)-ECG; sensor-motor reactions; dark adaptation), general clinical and special (CYPIA1/2: lymphocytes, benz(a)pyrene hydroxylase; antipirine test; vitamin A and porphyrins metabolism, insulin, thyroxin) biochemical, clinical immunological, dermatological (chloracne consequences, morphometry), and cytogenetical (chromosome aberrations and SCE in lymphocytes, micronuclei and nucleus morphology in buccal mucosa epitheliocytes) investigations. Statistical investigations of the associations between biologically linked biomedical characteristics and application of certain physical, biochemical and cytogenetic loadings were performed to identify the local reference ranges and possible hidden dysregulatory and disadaptive states on different levels 1,2,10,11).

5. Results

The validity of the developed approach to evaluation of past direct exposure to AO is supported by the number of following findings. The reproducibility of associations between biomedical characteristics of the exposure (ERG: MET1/ MET2) and between characteristics of the exposure and effects (Effects Development Risk Groups, EDRG: MET1/MET2/IPC,IHS) has been established in different contingents studied (peasants of the Binh My village and exposed war veterans living in the village Bac Hong in Northern Vietnam) 7,8). The specificity of associations has been found between the employed exposure measures and several objective biomedical characteristics (fig. 2). Specific patterns of pathologic manifestations associated with separate influence of different health risk factors has been identified using epidemiologic information. So, successful identification of persons exposed to AO in the village Binh My has been achieved using the AO-specific pathologic profile and the Bayesian's classification procedure 5,6). In general, the results of integrated epidemiological and clinical laboratory investigations revealed the reality of strong, specific and consistent associations between certain characteristics of health status and the developed measures of exposure to AO and, to a lesser extent, to DEF. The effects include: 1) significant but differently expressed deterioration of health status in all age/sex-adjusted subgroups; 2) the increased prevalence of epidemiologic indicators of pathologic states of different organism systems consistent with the number of clinical and laboratory observations; 3) the increased susceptibility to the infectious respiratory diseases consistent with specific alterations in certain characteristics of the immune status; and 4) the number of dysregulatory and disadaptive effects on cellular and organism's levels 1,2,5-12).

The developed approach for investigation of the LTMC of the AO spraying in Vietnam includes the construction of the statistically typical profiles for a number of biologically and logically associated exposure characteristics, effects and their correlations. From the methodological point of view this approach has the following advantages: the selection of the statistically homogeneous groups ranked according to the determined relations between the exposure characteristics (ERG) and the characteristics of exposure and effects (EDRG); the classification of individuals in terms of their membership in ERG and EDRG; and determination of the pathologic states profiles for the studied characteristics of health status and separate risk factors. This approach allows: 1) to carry out the massive epidemiological investigations with the statistical verification of the data obtained; 2) to obtain the statistically grounded measures for the past direct AO exposure; 3) to identify the statistically untypical persons with the possibly different susceptibility in relation to the development of primary toxic responses of their organism (MET1/MET2) and different effects expressed in the LTMC (MET1/MET2/IPC, IHS); 4) to evaluate the significance of different exposure components and to receive the exposure-response-like associations for the different LTMC characteristics. The statistically based selection and thorough examination of typical representatives in different ERG and

EDRG sub groups permit to evaluate the validity of classification criteria used and to correct them according to more adequate medical and biological information as well as to chemical and analytical data available at present. The introduced definition of DEF permits to investigate significance and effect-modifying activity of integrated exposure to various place-specific physical, chemical, biogenic and life-style-related factors which are beyond the scope of the investigation.

6. References

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Fig. 1. Investigated types and characteristics of exposure

Past direct contacts with Agent Orange

Type	Multiple toxic exposure to 2,4-D, 2,4,5-T and dioxin
Available information	<ol style="list-style-type: none"> 1. Retrospective characteristics of exposure conditions (detailed description). 2. Standardized and toxicologically grounded set of symptoms and signs for toxic responses of the organs of contact and organism: 1) immediate acute responses (1-3 days postexposure) for the inhalative, eye-irritating, skin resorbitive and enteral components, and relevant systemic and vegetative responses (individual indices $MET1 = \sum$ of effects/number of characteristics); 2) subsequent systemic responses (3-6 months postexposure): relevant systemic, vegetative and skin responses (individual indices $MET2 = \sum$ of effects/number of characteristics).
Validation	<ol style="list-style-type: none"> 1. Plausibility and reproducibility of information, consistency with the US "HERBS tape" data (the computerized analog "Atlas", USSR). 2. Typical patterns of associations between exposure characteristics. 3. Biomedical and chemical analytical markers in specified Exposure Risk Groups.
Classification	<ol style="list-style-type: none"> 1. The "exposed/unexposed" approach. 2. The likely degree of exposure: a membership in the statistically definable homogenous Exposure Risk Groups with certain toxicologically grounded associations between exposure characteristics.

Dioxin-containing Ecotoxicologic Factor

Type	Integrated chronic exposure to environmental dioxin and place-specific physical, biogenic, anthropogenic and social-demographic factors on the sprayed territory.
Available information	<ol style="list-style-type: none"> 1. A history of residence on the sprayed territory.
Validation	<ol style="list-style-type: none"> 1. Reproducibility of information, documents of the local People's Committees. 2. The US "HERBS tape" data (the computerized analog "Atlas", USSR). 3. Residual levels and patterns of PCDD/Fs in the environment. 4. Ecological markers.
Classification	<ol style="list-style-type: none"> 1. Sub groups with different duration and periods of residence in the sprayed areas.

Common health risk factors, confounders and effect-modifying factors

Registered characteristics	Nationality, age/sex, social-economic status, nutrition, smoking, alcohol consumption, drugs application, pesticides and other chemicals application (types, frequency and mode of application, history of intoxications), a history of malarial diseases, tuberculosis, and hepatitis.
Classification	<ol style="list-style-type: none"> 1. Selection of persons with the untypical strong influence of the factors. 2. Statistical definition of associations between characteristics under the study, characteristics of exposure to Agent Orange and Dioxin-containing Ecotoxicologic Factor and health status ("MANOVA", stratified analysis). 3. Statistical investigation of biomedical significance and specificity of health outcomes associated with separate influence of risk factors (stratification, specific patterns of sub clinical pathological manifestations).

Fig.2. Increased prevalence of objective biomedical indicators of the long-term health consequences of Agent Orange in the defined Exposure Risk Groups

(the apparently healthy and HbSAg-free typical representatives of the Exposure Risk Groups: m, 31-50, typical associations between characteristics of exposure and health status within each group, the absence of significant influence of common health risk factors, similar results of general clinical biochemical examination, diagnosed cases/N; χ^2 -test, Fisher exact 1-tailed p-values, Kruskal-Wallis Rank Test)

Exposure Risk Groups	CM I ^a	BM II ^B	BM III ^C	p ^{AB}	p ^{BC}	p ^{AC}
AO	no	no	yes			
DEF	no	yes	yes			

Functional changes in cardio-respiratory system (test-PWC₁₇₀, 100 and 150 w)

Collapsible condition	1/32	3/37	7/37	ns	ns	0.044
Poor restitution (100 w)	1/32	10/37	15/37	0.02	ns	0.001
Tired out (150 w)	5/32	12/37	15/37	ns	ns	0.044

Lymphocyte test, Benz/a/pyren hydroxylase, inducibility index (II)

High II (>5.6)	0/32	nd	6/54	nd	nd	0.054
Low II (<1.2)	9/32	nd	1/54	nd	nd	0.002

Antipirine test*, excretion rate (%), median

4-OH-Antipirine	35.0	43.2	53.0	ns	ns	0.020
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* Correlating metabolites excretion rates and lymphocytic BPHase activity in the ERG BM III

Chloracne-derived skin lesions (CSL)

CSL	2/202	4/207	25/141	ns	0.000	0.000
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Coproporphyrinuria (CPU)* and "hyposensitive" hypovitaminosis A (hypoA)**

CPU	2/31	5/29	35/42	ns	0.000	0.000
hypoA	1/31	2/29	15/42	ns	0.002	0.001

*,** Normal ranges of liver functional state biochemical indicators in the ERGs. Estimated local normal ranges for porphyrins excretion, plasma retinol levels and their responses to test doses of vit A (RDR-test) are close to reference values of the europeoids. ** Decreased responses to test and pharmacological doses of vitA in persons with an ordinary type of hypovitaminosis A (approx. 50% of the population group).

Cytogenetic parameters in cultured lymphocytes (A) and in buccal epitheliocytes (B)

A) eSCE* => 1.28/lymphocyte	5/10	17/17	11/16	0.003	0.02	ns
A) % of HFC**, > 11%	2/10	11/17	3/16	0.031	0.008	ns
B) fragmented chromatin, > 34/1000	3/47	11/47		0.021		
B) two large nuclei, > 5 / 1000	0/47	9/47		0.0013		
B) abnormal nuclei morphol., > 32 / 1000	11/47	23/47		0.018		

*- eSCE - environmentally dependent SCE; ** - High SCEs Frequency cells