Biologically Reasonable Phenomenological Approach to Investigation of Health Consequences of Dioxin-Containing Ecotoxicants

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Introduction

Analytical integration of the results of numerous experimental, epidemiological and biomedical investigations permits the specification of the following peculiarities of the toxicology of dioxin-like compounds (DLC), which limit the applicability of traditional paradigms, as well as common toxicological and reductionistic epidemiological approaches in risk assessment [1,2].

1). Realization of the hormone-like multifunctional and disregulative biological activity of DLC in target cells is mediated by ligand-dependent activation of the Ah-receptor system (AhRS). Fast primary molecular and biochemical effects, including activation of AhRS and proteinkynase phosphorylation, elevation of intracellular Ca2+ levels and changes in DNP configuration, are the most directly connected with the biologically effective doses of these compounds. Formation of subsequent "coordinated" cytological responses (activation of the Ah-locus genes transcription rates; changes in the pathways of growth factors, proliferation and differentiation) involves development of complex cascades of interconnected AhRS-dependent and independent events. The dependence of these processes upon the type and functional state of cells, experimental conditions and a large number of cell/tissue-specific and external effect-modifying factors (determinants of the AhRS components expression; AhR agonists/antagonists; interaction of the AhRS and systems of signal transduction for insulin, estrogens and growth factors; proteins and biochemical processes regulating activity of the AhRS on cytoplasmic, nuclear and posttranslational levels) results in various and variable cytological responses and their relations with a dose observed in different cells and conditions. In an organism, a multitude of target cells in different tissues and complex interactions of the AhRS-mediated, AhRS-independent and adaptive responses, along with critical dependence of DLC's toxicokinetics and toxycodynamics on many internal and external factors determine expressed polymorphism and variability of systemic responses and dose-response relationships in different species, individuals and exposure conditions.

2). Initial molecular and biochemical effects, as well as many cytological and systemic responses to DLC in humans and animals, are qualitatively similar and humans may be as sensitive, at least, to some effects of DLC as many susceptible species and lines of laboratory animals.

3). At present, it is becoming more evident that health consequences of dioxin-containing chemicals may include not only a limited number of certain rare diseases (soft tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease and some other rare types of cancer; chloracne and acute porphyria in genetically predisposed persons), but additionally the broad spectrum of more or less specific manifested or sub clinical, disadaptive and latent pathological conditions of different systems of an organism, physiologically significant and insignificant alterations in homeostasis, and changes in physical and mental development.

4). These health outcomes, as a rule, are not independent events and reflect different stages of systemic response development. Many of them can not be measured by existing methods or are not identified as effects. Pleiotropic activity of DLC and critical dependence of systemic responses formation on peculiarities of exposure (conditions, dose, composition) and large number of intrinsic biological (species, line, age, sex, genotype, metabolic and endocrine phenotypes), external (AhR agonists/antagonists; other chemicals and physiologically active substances; common risk factors), and temporal (duration of exposure/postexposure period) factors result in quantitative, qualitative and temporal variability of health outcomes and their relations with an exposure in different situations.

All these findings underlie the ambigious and frequently inconsistent results of different epidemiological studies, obtained even in the apparently similar situations of exposure to dioxin-containing chemicals. These findings also determine the relative character of conclusions about the health risks from exposure to DLC derived from implication of different procedures of formal modelling and extrapolations. Therefore, there is a need for the development of more biologically reasonable and health-based methodological approaches to risk assessment, enabling the evaluation of all possible health hazards associated with exposure to DLC in a "real life situation".

Biologically reasonable methodological requirements for evaluation of health hazards associated with exposure to dioxin-containing chemicals in a "real life situation"

Pleiotropic activity of DLC and critical dependence of effects development on the above mentioned effect-modifying factors permit specification of the following methodological requirements for the best estimation, in a causative sense, of exposure and identification of various possible health outcomes:

1) reconstruction of histories and scenarios of exposure to DLC as well as to other toxicokinetically and pathogenetically significant factors united in the dioxin-containing ecotoxicological factor (DEF) concept [1, 3-5];

2) epidemiological monitoring of a sufficiently broad range of symptoms and signs of pathological states of different systems of an organism and investigation of their responses to various external loadings (physical and mental exertion; biochemical, immunological and endocrinological tests; infectious agents) to reveal all possible health outcomes, including latent dysfunctions, dysregulative and disadaptive conditions [3-5,13];

3) appropriate investigation of a causality in accordance with the specific features of DLC's toxicology;

4) availability of sufficiently numerous groups of exposed and unexposed persons with similar biomedical parameters (race, age, sex) and history of influence of toxicokinetically and pathogenetically significant environmental factors to permit evaluation of biological and methodological variability, uncertainty of exposure, and effects estimates.

Investigation of health hazards associated with the influence of dioxin-containing ecotoxicological factor using "phenomenological models" of exposure and effects

Based on the above mentioned reasons, evaluation of exposure to DEF by the levels of DLC in available biomaterials and environmental samples seems to be insufficient for causal inference. Besides the high cost, this is due to: 1) DLC's toxicokinetics dependence on biological effects and toxicokinetic interactions of different congeners as well as on the peculiarities of exposure and a history of influence of many individual and environmental toxicokinetically significant factors, 2) the well-known limitations of the toxic equivalency factor concept, 3) the inability to determine biologically effective doses in toxicokinetically insignificant compartments of an organism, 4) the inability to evaluate important contribution of other ethiopathogenetically significant constituents of

the DEF [3-5]. All these require development of methods for determination of exposure measures, integrating direct and indirect characteristics of a history of DEF influence, and for evaluation of the exposure measures uncertainty. This problem may be resolved by multidimensional statistical investigation of associations between datasets of logically and toxicologically linked direct and indirect characteristics of different constituents of DEF bringing sufficient information about the most important features of exposure. This procedure allows representation of exposure in terms of its structured "phenomenological model" and definition of statistically homogenous "exposure risk groups" (ERG) with similar patterns of exposure to different components of DEF, ranked by characteristics of likely intensity of exposure to DLC. The allocation of exposed persons in the ERG may be considered as their statistically grounded individual measures of exposure to DLC on the background of comparable influence of other components of DEF in a given situation [4,5]. The ethiopathogenetical specificity of DEF in each exposure situation determines the importance, in a causal sense, informativity of such exposure measures only in the investigated conditions and determine the need to apply the "phenomenological approach" to compare exposure conditions in different situations [6]. "Phenomenological modelling" can also reveal the correlative characteristics of exposure and make possible their selection in accordance with the particular purposes of the research.

Another key issue of risk assessment is the identification of polymorphic health consequences associated with exposure to DEF. Taking into account the special features of DLC's toxicology, we may propose the following approaches to the investigation of causal inferences in epidemiological studies which have been substantiated by the results of investigation of the long-term health consequences (LTHC) of Agent Orange (AO) in Vietnam [3-6]. In particular, the possibility of LTHC development after a single acute impact of DLC and increased susceptibility to DLC at the perinatal stages of the ontogenesis require estimation of the strength of associations with characteristics of the most toxicologically significant periods of exposure. In Vietnam, the (eco)toxicological priority of past direct contacts with AO and of chronic exposure to DEF on the sprayed territories has been established by preliminary investigations of the ecotoxicological situation and confirmed by the results of subsequent epidemiological and clinical laboratory studies [5]. The specificity of associations for pleiotropic effects of DLC can be estimated by comparative investigation of "specific patterns of pathological manifestations" displayed in sub groups of persons stratified by strong separate influence of DEF and other common health risk factors. This approach has been successfully applied to prove specificity of the LTHC of AO in South and North Vietnamese peasants and to the identification of exposed persons in the entire populational sample using the Bayesian classification procedure [4]. Estimation of the consistency of associations, even with similar conditions of exposure to DLC may be complicated by different concomitant influence of effect-modifying or temporal factors. Predominating toxicological significance of acute exposure to AO and established similarity of "phenomenological models" of the exposure in large groups of exposed northern and southern Vietnamese peasants [5,6] allow explanation of significant consistency of LTHC manifestations that has been registered in these groups [7,8]. Statistical investigation of associations between all characteristics of pathological states of different systems of an organism in the selected ERGs allows definition of sub groups of persons with certain relatively discrete sets of polymorphic responses ("Pathologies Development Risk Groups", PDRG). Applicability of this procedure has been illustrated by definition of three kinds of main "symptom complexes" of the LTHC in the entire group of exposed Vietnamese peasants [6]. Implementation of this procedure and identification of the increased prevalences of prognostic epidemiological indicators of pathological states for each system of an organism in a group of exposed persons [8] appear to be a promising approach to investigation of the nosological specificity of expected syndromes. The results of experimental investigations have shown that estimation of the dose-response relationships for complex systemic effects of DLC is problematic, even in conditions of controlled exposure and with the use of biologically-based toxicodynamic modelling [2]. Thus, in epidemiological studies there is a sense only in the empirical ERG-based

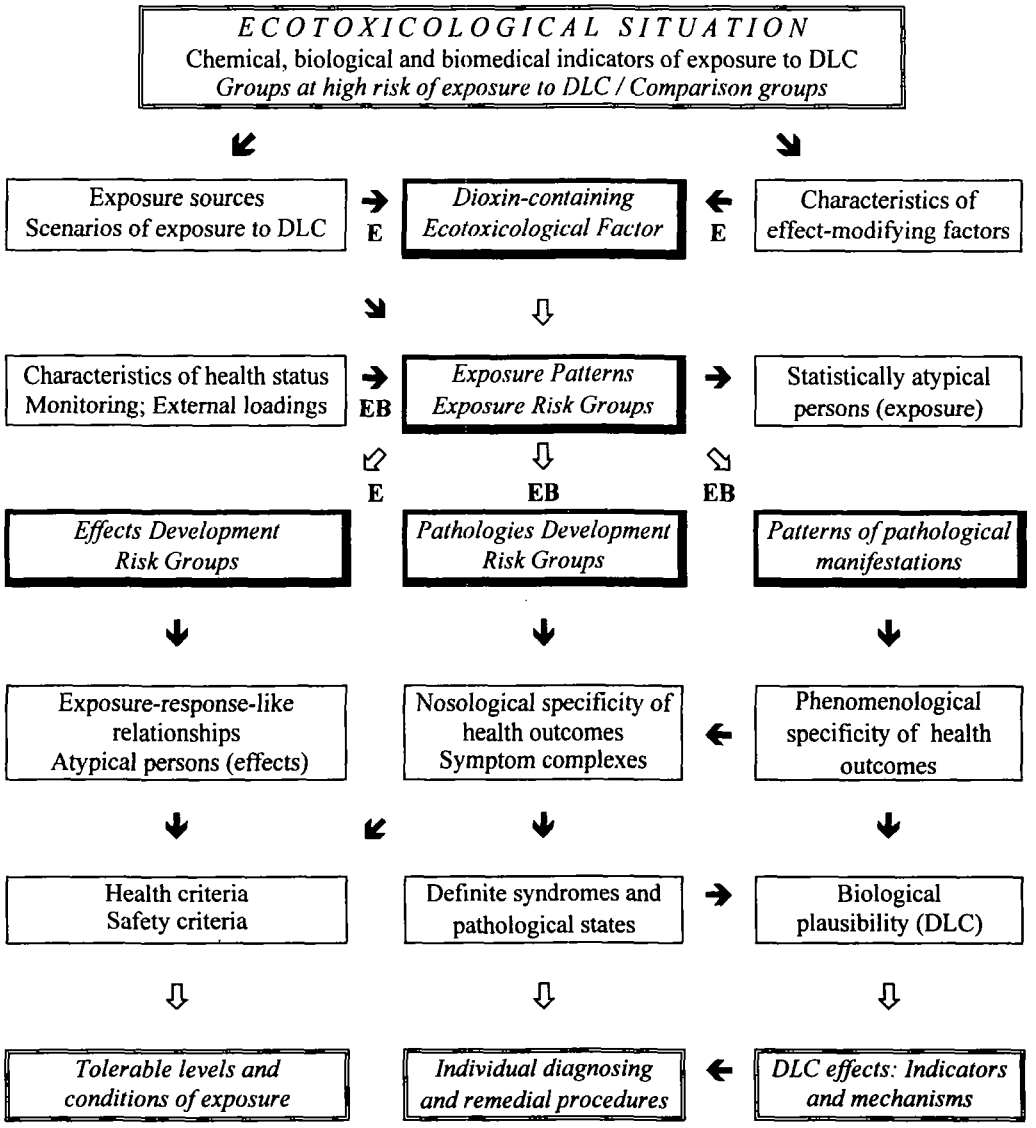
investigation of exposure-response-like relationships which are displayed only for a given time point, contingent and situation. By virtue of high polymorphism, individual variability and situational specificity of expected health outcomes, the integrated characteristics of deterioration of general health status may be the most acceptable for general comparative evaluation of the medical importance of different levels and conditions of exposure. We have carried out this approach based on the "instantaneous health continuum" concept [9] which allows the quantitative evaluation of general health status in terms of individual values of "Indices of Health Status" (IHS) representing fractions of pathological manifestations in the set of registered health characteristics, irrespective of their nature [4,7,8]. Determination of functional importance for each pathological manifestation in terms of the "qualitative scale of well-being" allows the calculation of another kind of IHS expressed in values of the "person-years of life in a state of well-being". This index reflects the social importance of different health outcomes [10] and has been successfuly applied to evaluation of general health damage associated with exposure to AO in Vietnam [11]. Due to the specific disregulative and disadaptive activity of DLC [12], decreased adaptability of different systems of an organism may serve as one more generalized biomedical indicator of health effects. The subjectivity of epidemiological information and high individual differences in the susceptibility to DLC result in high uncertainty of exposure-response-like estimates, obtained with probabilistic measures of exposure to DEF and epidemiological characteristics of health effects. This uncertainty can be evaluated by a statistically reasonable definition of the "Effects Development Risk Groups" (EDRG) consisting of persons with statistically similar structure and strength of associations between epidemiological characteristics of exposure and deterioration of health status [4]. The impossibility of reconstructing the complex webs of causation for DLC and identifying systemic alterations in the homeostasis using available molecular epidemiological parameters of the AHRS functioning and the procedures of toxycodynamic modelling necessitate development of special approaches to revealing such effects. Qualitative phenomenological similarity, at least, of some systemic responses to DLC in humans and animals opens a promising way to solution of this problem by establisihing the "phenomenological models of effects" and including the criterion of biological plausibility [6]. Implementation of this "phenomenological" approach implies revealing the special features of associations between biologically-linked components of a given system of an organism or between different physiologically-linked systems in a group of exposed persons and qualitative comparison of the phenomenology of established and expected effects. We have proved an applicability of this approach in a contingent of apparently healthy Vietnamese exposed to AO by identification of expected: 1) disadaptive functional changes in cardiovascular system and in the system ensuring vitamin A status of an organism, 2) discriminating characteristics of immunological status and porphyrin metabolism, and 3) peculiarities of antipirine metabolism and lymphocytes responsiveness to mitogens and CYP1A1 inducers [4,5,13].

Summarizing the above mentioned considerations and the results of our investigations we may conclude that the proposed concept of "biologically reasonable phenomenological modelling", based on multidimensional statistical investigations of associations between sufficiently informative number of logically, toxicologically or biologically interconnected direct and indirect characteristics of exposure and health status, seems to be a promising approach to evaluation of health hazards associated with exposure to DLC in a "real life situation ". Application of this procedure, also permits the identification of statistically atypical persons due to errors in the collection of epidemiological data, or to their distinctive individual susceptibility to the systemic effects of DEF [4,6]. This approach can be realized by successive implementation of stages presented in scheme 1.

Scheme 1.

Basic elements of an algorithm for investigation of health hazards associated with exposure to dioxin-containing ecotoxicants

E - epidemiological information: direct and indirect characteristics of exposure, indicators of pathological states for different systems of an organism;
B - biomedical information: characteristics of interrelations between biologically and physiologically linked components of certain systems of an organism.



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