Statistical Examination of the Urinary Porphyrins Excretion Profiles as Potential Indicators of Past Exposure to Agent Orange in Rural South Vietnamese.

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Specific systemic hormone-like mode of dioxin's toxic, dysregulatory and disadaptogenic action in the organism[1] necessitate the development of special approaches to reveal its subclinical medico-biological effects. At least up to the determination of dioxin effects and susceptibility biomarkers, these approaches should be based on comparative cohort studies of discriminating features of the homeostasis, homeokinesis and adaptational responses in the exposure risk groups (ERG) ranged according to the likely extent and peculiarities of exposure[2] and homogeneous regarding influence of toxicokinetically and pathogenetically significant factors. The ERG-associated ultrastructural and cell-populational changes in some tissues, shifts in distribution patterns for certain characteristics of the homeostasis and altered interrelationships between physiologically associated systems in the organism may serve as subclinical indicators of dioxin impact in the steady-state conditions. Special dynamic (kinetics of cell renewing and endogeneous compounds metabolism), loading (physiological, meta-bolic, endocrine or immunologic responses to physical, mental and stressory loadings, to physiologically active or immunotropic substances injection) and provocative (temporary activation of latent pathological states) tests must be performed as well as observations on special features of morbidity, physical and mental capacites must be analyzed in the ERG to reveal potential hidden health effects of dioxin.
High incidence of coproporphyrinuria among apparently healthy adult males was identified with history of their past direct contacts with Agent Orange (AO) using porphyrinuria classification approach in the preceding study[3]. A purpose of this study was to evaluate indicative significance of urinary porphyrins excretion profiles by statistical methods.
Four ERG consisting of healthy males (31-50) with similar socio-economic, nutritional,

medical and toxicological (with the except of AO and pesticides) status were thoroughly selected from the previously investigated contingent[3] using epidemiological and clinical laboratory data avaliable[2]. The results of statistical examination for a 24-h urine porphyrins excretion profiles[3] and some characteristics of liver function ("One-way ANOVA", Statgraphics v.3.0) in these ERG (N) are presented in the following table (average, 95% c.i.):

Parameters	1(11)	2(18)	3(14)	4(6)	p
MET1*	0	0	.647±.278	.363±.190	–
MET2*	0	0	.600±.292	.550±.214	–
Indir	–	+	+	+	–
Pest	≤5	≤5	≤5	≥100	–
W/H	.304	.293	.314	.286	ns
	.292÷.316	.283÷302	.304÷.325	.270÷.301	
AST	17.1	17.9	16.3	18.9	ns
	14.0÷20.2	15.7÷20.1	13.9÷18.7	15.3÷22.4	
ALT	14.6	12.0	12.2	11.0	ns
	11.2÷17.9	9.7÷14.4	9.6÷14.8	7.2÷14.8	
LDHx10^3	.273	.216	.191	.243	.037
	.247÷.299	.198÷.235	.170÷.211	.213÷.273	
BDHx10^3	.128	.106	.116	.140	ns
	.108÷.147	.092÷.119	.101÷.131	.118÷.162	
Chol	3.41	3.56	3.57	3.38	ns
	3.04÷3.77	3.28÷3.85	3.24÷3.90	2.86÷3.89	
Bilir	8.59	6.99	4.74	12.6	.002
	6.82÷10.36	5.32÷8.66	3.16÷6.32	10.4÷14.8	
APM	4.66	9.97	10.1	14.9	.039
	1.54÷7.78	7.15÷12.8	7.43÷12.8	11.1÷18.8	
TP	114.2	146.3	188.6	200.3	ns
	45.9÷182.5	92.9÷199.7	128.1÷249.2	107.8÷292.8	
Uro%	19.5	15.6	10.7	21.1	.021
	16.1÷22.9	13.0÷18.3	7.7÷13.7	16.4÷25.7	
7C%	2.67	2.19	1.80	3.70	.065
	1.79÷3.55	1.50÷2.88	1.01÷2.53	2.49÷4.88	
6C%	.47	.83	1.15	1.39	.053
	.15÷.79	.59÷1.08	.87÷1.44	.96÷1.83	
5C%	1.57	2.29	1.51	2.55	ns
	98÷2.16	1.83÷2.76	.99÷2.04	1.75÷3.35	

	1	2	3	4	p
C3%	57.3	52.6	63.3	46.5	.011
	52.6÷62.1	48.8÷56.3	59.2÷67.5	40.1÷52.9	
C1%	18.4	26.6	21.5	24.8	.019
	15.6÷21.3	24.2÷28.7	18.9÷24.0	20.9÷28.7	
TC%	75.8	79.0	84.8	71.3	.022
	71.7÷79.8	75.8÷82.2	81.2÷88.4	65.8÷76.8	
C3/C1	3.22	2.23	3.17	1.94	.001
	2.69÷3.75	1.81÷2.64	2.70÷3.64	1.23÷2.65	
TC/Uro	4.26	6.50	10.09	6.07	.006
	1.51÷6.71	4.59÷8.41	7.92÷12.3	2.76÷9.39	

Abbreviations: * Medical Equivalents of Toxodose for potential direct contacts[2], M±SD; Indir - potential indirect exposure from the environment; Pest - average yearly contacts with conventional pesticides without symptoms of intoxication in the ERG "1-3"; W/H - weight for height index (kg/cm); AST, ALT, LDH, BDH : serum aspartate- and alanine-aminotransferase, lactate- and γ– butyrate- dehydrogenase activities (u/L); Chol, Bilir : total plasma cholesterol and bilirubin (μM); APM - urinary antipyrine metabolites excretion rates (μg/24h); TP - total porphyrins excretion rates (μg/24h); Uro, 7C, 6C, 5C: uro - , 7 -, 6 -, 5 - carboxylic- porphyrins; C1 and C3 - I and III coproporphyrins; TC=C1+C3.

From these data it can be concluded that in the selected person (but not in the whole ERG) only alterations in porphyrins excretion and the increase in APM excretion rate are consistently associated with the increasing risk of AO exposure. A tendency to increased TP, 6C%, TC% and TC/Uro values with a concomitant decrease in Uro% reaching significant differences between the ERG "1" and "3" for Uro%, 6C%, TC% and TC/Uro on the background of elevated cyt P-450 activity is characteristic for the ERG "1-3" . Significantly increased Uro%, 7C% and decreased C3% and TC% values are characteristic of the ERG "4" in comparison with the ERG "3".

A set of integrated statistical methods consisting of "Correlation Analysis", "Principal Component Analysis", "Variables Projection" and "Pattern Recognition"[4] (Statex2, User's Guide, Moscow, 1992) was further employed to reveal the most indicative characteristics of urinary porphyrins excretion profiles in the ERG "1" and "3" and the ranges for these parameters which are critical for the ERG identification:

Indicative parameters	AO Exposure Risk Groups/ Recognition quality	
	1 (10/11)	3 (14/14)
TP	30.8<<148.2	148.2<<442.7
Uro%	11.2<<33.5	4.62<<19.7
7C%	ns	2.24<<8.99
6C%	.29<<1.03	.567<<3.10

5C%	.10<<1.39	ns
C1%	12.9<<24.0	ns
C3%	ns	57.2<<75.6

Calculated limits critical for ERG recognition are underlined.
These results are in accordance with the "normal" ranges for porphyrins excretion established in a preceding study[3] and confirm significance of elevated TP, 7C%, 6C%, C3% and decreased Uro% values for the ERG "3" recognition. Due to high incidence of past malarial disease among patients under the study (1/11, 5/18 and 9/14 in the ERG "1-3"; >3 years after the last case) a special investigation was performed to estimate potential influence of this confounding factor. No malaria-related differences for porphyrin excretion profiles were found using "Kruscall-Wallis Rank Test" in the ERG "2", "3". As a whole, these results allow to consider the existence of partial blockage for coproporphyrinogen III metabolism as potential indicator for the long -term consequences of dioxin exposure in a given contingent of S. Vietnamese. A concomitant pesticides influence (ERG "4") may result in partial inhibition of uroporphyrinogen decarboxylase activity (a tendency to increased 7C%, 6C% and 5C% values) and in the intrahepatic cholestasis development (decreased C3/C1 ratios).
Due to a great variability in porphyrin and creatinine excretion rates and to ignoring confounding factors a satisfactory ERG recognition was not achieved when a fresh urine samples randomly collected from 80, 68 and 41 persons (m, 31-60, "1-3" ERG) were examined. Enlarged sex/age/confounding factors - adjusted contigents of rural S. Vietnamese divided into a larger number of ERG must be tested in the latent porphyrinopathia - activating conditions to evaluate the "dose-response" - like relation-ships for porphyrin indicators and to elaborate a reliable method for currentidentification of dioxin -susceptible AO - exposed persons. A hospital based study employing supplementation with vit B_6, glycine and carbamazepine (a porphyrinogenic, cyt P-450 A-testing probe) is in progress now.

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