

# Dioxin '97, Indianapolis, Indiana, USA

## Abnormalities of a nuclear material in two types of human cells analyzed thirty years after application of the Agent Orange

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### Abstract

Among inhabitants of South Vietnam village sprayed in the past with Agent Orange (AO) statistically significant increase is observed in frequency of cells bearing anomalies of nucleus material. The amplification of damages is defined in cells of different origin (epitheliocytes, erythrocytes). It is displayed by formation of micronucleus structures, other chromatin and nucleus membrane defects, and is observed even among rather young people - in age group up to 35 years. From our point of view, systemic character of observable abnormalities of nuclear material and their stable character testify to the existence of primary damages in stem cells. The nuclei's aberrations, obvious even in remote period after AO effect, can promote not only dysregulatory shifts, but also could result in carcinogenic processes manifested in various tissues.

### Introduction

The significant differences in frequency of cells with different micronuclei's types were shown<sup>1)</sup> while examining the pathologic changes of nucleus in exfoliated buccal cells of females from two villages of Song Be province (Southern Vietnam) with different levels of dioxin-containing ecotoxigants. It was displayed by gaining the number of cells with abnormalities of nucleus in contaminated region, and was observed among females of younger age as compared to those in control region. To check the assumption on the environmental factors' influence on such shifts usually observable in elder persons we have conducted additional researches in groups of younger females from the same villages. Modifications of nuclear structures were analyzed not only in the mucous cells, but also in peripheral blood erythrocytes. The researches should answer the question on systemic character of observable changes, and solve a number of methodological problems also.

### Materials and Methods

In Binh My village, exposed to Agent Orange during the 2-nd Indochina war, 50 women (age group 30-58, average age  $38.24 \pm 8.16$ ) were examined. In the control area 50 women (age group 30-54, average age  $38.46 \pm 8.27$ ) were examined in Tan An village, matching by most epidemiological parameters. After clinical examination the samples were taken from each person. The individual slides were prepared of the buccal cavity mucous cells and of peripheral blood erythrocytes. Slide preparations stained according to Giemsa were processed<sup>2)</sup> and analyzed: 1000 cells - for mucous exfoliated cells, and 2000 - for erythrocytes. All cells with different types of abnormal nuclear structures were counted separately. Statistical procedure was conducted with use of the software packages "Statgraphics, v.3" and "Statistica, v.4.3".

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## Results and Discussion

The analysis of nuclear structures in exfoliated buccal cells has demonstrated the presence of the same types of nucleus abnormalities described earlier<sup>1)</sup>. The results of the analysis of damaged cells are submitted in table 1. The frequency of cells with nuclear material defects has appeared significantly greater in contaminated region as well as in the previous study. So, it confirms our conclusions about stable character of alterations, arising in stem cells of the sprayed village's inhabitants.

Table 1. Frequency of buccal cells with various types of nucleus' damages among females from two villages with different degree of contamination with dioxin-containing ecotoxics (South Vietnam)

Parameters	Control region	Sprayed region	p*
Persons surveyed	50	50	
Cells analyzed	50000	50000	
Defected cells	616	1282	0.0000
Cells with micronuclei:			
Type 1	144	216	0.0002
Type 2	31	66	0.0004
Type 3	81	80	ns
Type 4	42	67	0.02
Sum of micronucleated cells	298	545	0.0000
Cells with fragmented chromatin	55	120	0.0000
Binucleated cells	161	229	0.006
Cells with punched nucleus	102	388	0.0000

\* - Analysis by methods of Non-parametric statistics

The microscopic analysis of erythrocytes has revealed smaller variety of nucleus defects' types. By analogy to damages of nuclei in epitheliocytes<sup>1)</sup> the following types of cells were registered - cells with 1 micronucleus (types 1, 2 and 4) and cells with several micronuclei (type 3). The last type met rather seldom among persons from both villages. At the same time, the comparison of damaged cells' frequencies in erythrocytes from different risk groups has appeared a little bit less significant then those for the abnormalities in buccal cells (table 2).

A group of women born and living on the sprayed territory was examined separately in Binh My village (N=23, average age  $31,48 \pm 1,20$ ) as marked increase of abnormalities was observed among younger persons. In our opinion, just these persons could undergo the greater effect from dioxin, because this substance could have been obtained by their organism by transplacental penetration as well as with breast milk. Accordingly on the territory of the control region the comparison group (N=24, average age  $31,58 \pm 1,72$ ) was selected. The frequency of cells with nuclear damages has appeared augmented among the natives of the sprayed area - 2.58 % against 1.76 %. The results of the analysis of separate types of damages are shown in table 3. The received data confirm the significance of the observed increase of damages in chromatin and nuclear structures. We consider, that the organisms of these females continue to execute influence of DEF - the dioxin-containing local specific factor, formed on the background of the last AO effect and of current influences of the other environmental factors<sup>3,4)</sup>.

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Table 2. Frequency of blood cells with various types of nucleus damages among females from two Southern Vietnam villages with different level of ecotoxics' contamination

Parameters	Control region	Sprayed region	p*
Persons surveyed	50	48	
Cells analyzed	100000	96000	
Cells with micronuclei:			
Type 1	86	56	0.023
Type 2	106	201	0.0000
Type 3	11	22	0.042
Type 4	36	30	0.567
Sum of micronucleated cells	239	309	0.0005

\* - Analysis by methods of Non-parametric statistics

Table 3. Frequency of damaged cells in mucous of persons born on the sprayed territory

Parameters	Control region	Sprayed region	p*
Persons surveyed	24	23	
Cells analyzed	24000	23000	
Defected cells	423	593	0.0000
Cells with micronuclei:			
Type 1	66	124	0.0000
Type 2	18	37	0.0061
Type 3	58	38	ns
Type 4	32	27	ns
Sum of micronucleated cells	174	226	0.0021
Cells with fragmented chromatin	81	111	0.012
Binucleated cells	40	48	ns
Cells with punched nucleus	88	131	0.0002
Cells with perforated	40	77	0.0002

\* - Analysis by methods of Non-parametric statistics

The spectrum of observed damages of a nuclear material was rather close among the inhabitants of both villages (fig.1). At the same time, the distribution of separate types of damages can testify to probability of growth of the contribution of small-sized chromosome fragments to the total number of defective cells observed among the inhabitants of the Binh My village.

## Conclusions

The received results testify to the appearance of the stable nuclear damages in different human tissues after the Orange Agent spraying and the DEF current influence on the inhabitants of contaminated regions. Thus, our conclusion is supported on the probability of occurrence of aberrations in stem cells of various tissues, which can be saved at further cells' differentiation. Such damages may result in anomalous functioning of various systems and organs, and promote general dizadaptation. They also could cause faults in differentiation and proliferation of tissues, and even promote carcinogenic processes in persons, living on the contaminated territory.

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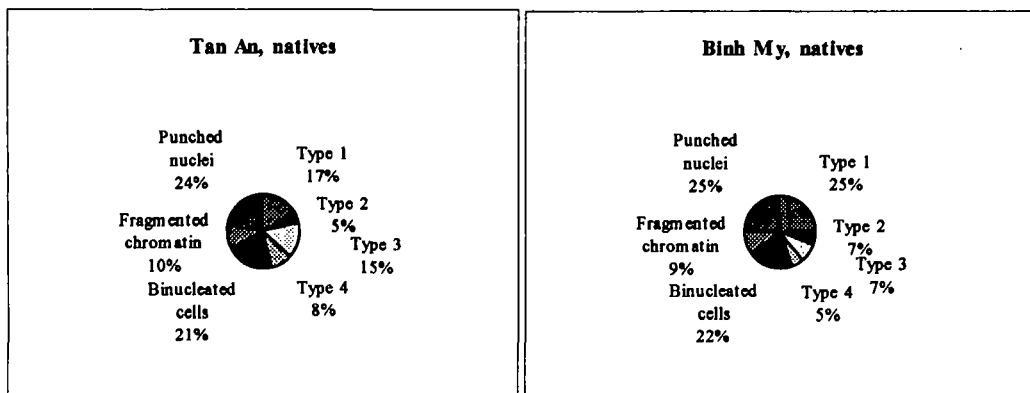


Fig. 1. Distribution of types of nucleus' damages in buccal cells among natives of the two villages in the Song Be province

## Literature Cited

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