CYTOGENETIC DAMAGES INDUCED *IN VIVO* IN HUMAN LYMPHOCYTES BY ENVIRONMENTAL CHEMICALS OR RADIATION

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ABSTRACT:

The importance of various environmental exposures has been evident in variation in cancer incidence and mortality. Benzene is considered to be a human carcinogen, is clastogenic to rodents and humans, and it affects the immune response. Workers in various industrial plants, are exposed to benzene and benzene related compounds as a result of various activities in which benzene is processed, generated or used. Major sources of environmental exposure to benzene related compounds, continue to be active and passive smoking, auto exhaust, and driving or riding in automobiles. Benzene is of a particular interest, not only because of its known toxicity, but also because this was to be the parent compound and a model for extensive programs of metabolism of a variety of aromatic chemicals. Ionizing radiation is an unavoidable physical agent that is presented in environment, and public opinion is well aware against radiation risk and strongly against it. The aim of the presentation was comparison between cytogenetic damages induced *in vivo* by environmental chemicals with those of radiation. Results from biomonitoring survey on genotoxicity in human blood cells of benzene and benzene related compounds were compared to damages detected in lymphocytes of persons who had been accidentally exposed to gamma radiation. In the groups, that had been occupationally or environmentally exposed to benzene related compound, total aberration frequencies, or percent of aberrant cells ranged between 0 - 0.16 aberrations/cell or 16% of aberrant cells respectively. A multivariate regression analysis confirmed: (i) a significant association between cytogenetic damage and exposure to benzene related compound, (ii) a possible association between cytogenetic damage and cancer, (iii) a significant influence of smoking habit. In 1996 few persons were suspected of accidental exposure to gamma radiation. To estimate the absorbed doses, lymphocytes from their blood have been analyzed for the presence of chromosomal aberrations. The frequency of dicentric and rings detected in lymphocytes of two persons confirmed an exposure to ionizing radiation. The estimates of absorbed doses were done on the base of dose response curves obtained previously. The doses exceeded ten times the annual permissible dose. The highest total aberration frequency measured was 0.14 aberrations/cell. Comparable levels of cytogenetic damage observed in the groups from environmental survey and from accidental exposure to radiation source confirmed that health hazard from radiation exposure in a public opinion is often overestimated in a contradiction to the everyday environmental hazard.

INTRODUCTION:

Individuals vary greatly in their likelihood of developing specific diseases and in their response to environmental hazards. A large number of interacting factors contribute to an individual's risk for disease; these factors include environmental exposures, genetic factors, diet, socioeconomic status, age, and gender. The term "biomarker" is a general term for specific

measurements of an interaction of a biological system and an environmental agent. Biomarkers of exposure measure an exogenous substance or its metabolite and its interaction with a biological molecule. Biomarkers of effect are measurable biochemical, physiological, behavior, or other alterations within an organism. Because in many instances, these biomarkers of effect are believed to represent events in a causal pathway to disease, their occurrence may be viewed as indicative of an acquired susceptibility for that disease [1].

Public opinion is strongly against the radiation and often ignores everyday risk from chemical pollutants. Chemical carcinogens can initiate the carcinogenic process by genotoxic and mutagenic mechanisms. Benzene is considered a human carcinogen, is clastogenic to rodents and humans, and it affects the immune response. The amount of benzene and the number of benzene related compounds in the environment are constantly increasing. According to L.Wallace [2] a major sources of exposure to benzene continue to be active and passive smoking, auto exhaust, and driving or riding in automobiles. The overwhelming source of benzene exposure for smokers was mainstream cigarette smoke. Smokers had on average benzene body burden about 6 to 10 times that of nonsmokers, and received about 90% of their benzene exposure from smoking. Roughly half the total benzene exposure in the United States was borne by smokers [2].

In the studies reported by A.Carrere and R.Crebelli [3] biomonitoring of human populations exposed to petroleum fuels with special consideration of the role of as genotoxic component were performed. Blood samples from Polish workers were also examined for cytogenetic effects [4], $p21^{ras}$ protein levels [5], and for any relationship to confounding factors (benzene related occupational exposure, smoking habit, sex, family cancer history and seasonal influence) [6-8]. There were also investigated cytogenetic damages in the blood from four people who were suspected of accidental exposure to gamma radiation. Unstable chromosome aberration frequencies (dicentrics and rings), in the lymphocytes of persons potentially exposed to radiation, were used to estimate absorbed doses [9]. The aim of this paper was to compare cytogenetic damage induced *in vivo* in human lymphocytes by environmental chemicals with the chromosomal damage observed in the blood of persons suspected of exposure to ionizing radiation.

MATERIALS AND METHODS:

Blood sampling *in vivo and in vitro:*

In the biomonitoring survey, carried out among the polish workers, following groups of people were investigated; twenty-four workers from petroleum plants, thirty-five unexposed controls, thirty-one prior to treatment lung cancer patients of a similar socio-economic status and from the same region of Poland. There were also investigated blood samples from four persons suspected of the accidental exposure to gamma radiation. Heparinized whole blood samples transported at 4°C to DREB in Kraków, where appropriate procedures for culturing as described previously have been applied (48 hour in the case of chromosome aberration analysis, and 72h for sister chromatid exchanges)[4]. Dose response curves for various LET radiation's were estimated from the lymphocytes of the healthy young male nonsmoking donor cultured according the same procedures as for the biomonitoring survey [4,10]. For the purpose of statistics 100 - 800 cells (in a confirmed first mitosis) for chromosome aberrations, and 50-100 cells in second mitosis for sister chromatid exchanges were analyzed. SPSS package was applied for the use of t-test, multivariate analysis of variance, and stepwise regression analysis.

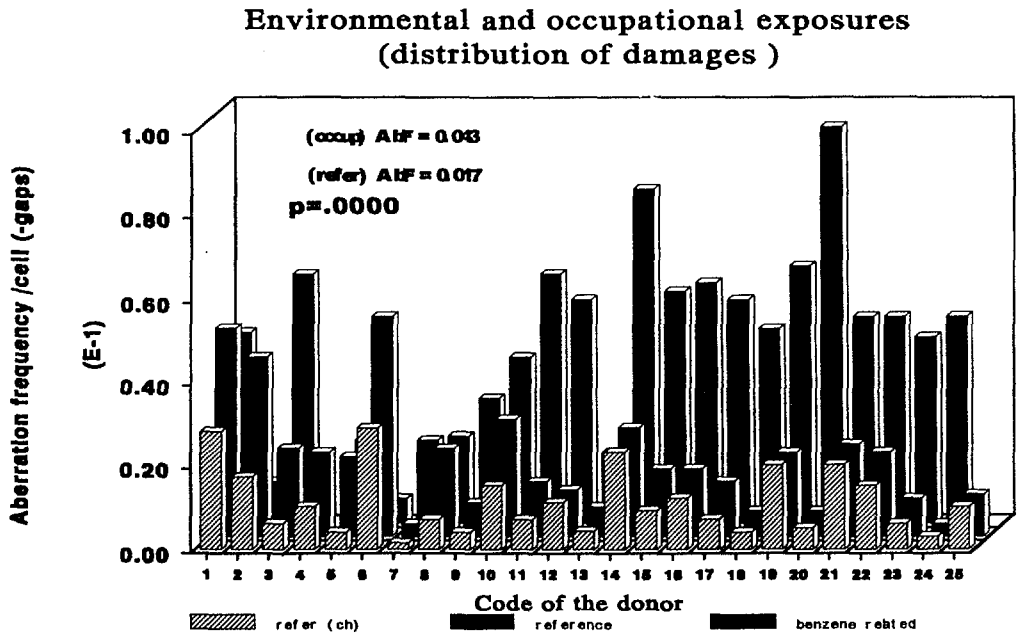

Fig. 1.
Distribution of cytogenetic damages detected in the blood of people occupationally exposed and unexposed to benzene-related compounds.

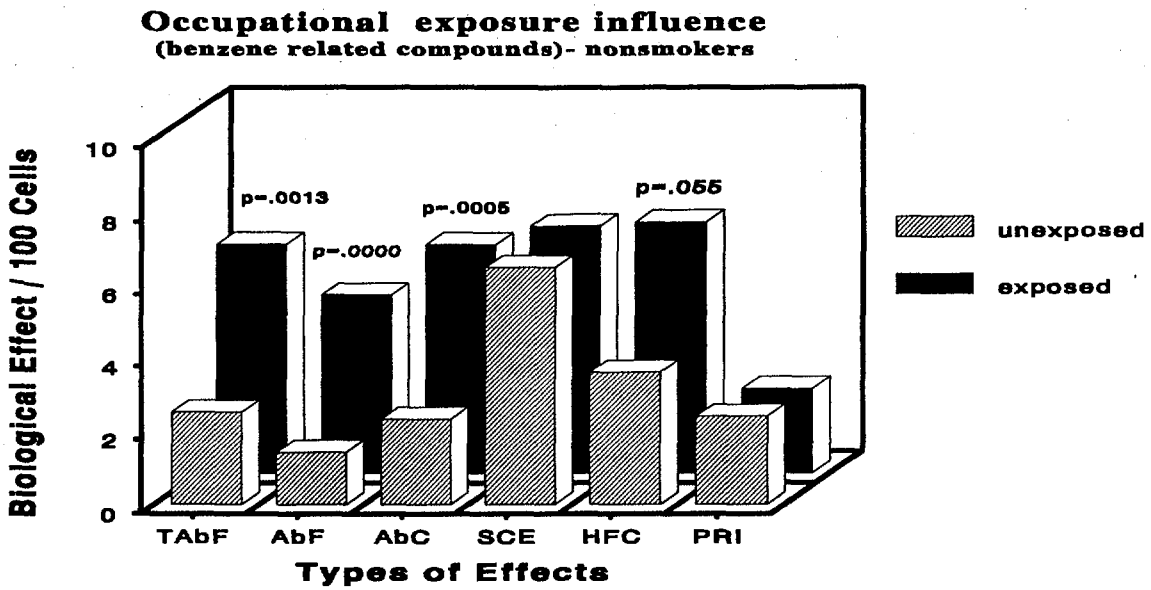

Fig.2
Influence of occupational exposure on biomarkers in a healthy and nonsmoking donors group (TabF/ AbF - aberration frequency including/excluding gaps respectively, AbC - percentage of aberrant cells, SCE - sister chromatid exchanges/cell, PRI - proliferating rate index, HFC - percent of cells with significantly elevated SCE levels).

RESULTS:

Biomonitoring survey results showed that majority of cytogenetic damages detected in the lymphocytes stopped at the first mitosis have consist of single or both chromatid gaps or breaks, minutes and acentric fragments. Figure 1 shows the distribution of cytogenetic damages detected in lymphocytes from blood samples of unexposed and occupationally exposed to benzene-related compounds people. On this figure is also shown, on the example of unexposed group, that impact to the aberration frequencies detected in a first mitosis that come from typical chromosome damage ((refer ch) - both chromatid involved in the damage) was rather high. Presented results showed a statistically significant difference between exposed and unexposed group.

There was a critical question whether the smoking impact could affect the final cytogenetic damage observed, as it was reported previously there was 78% of smoking persons among the studied group [7]. Figure 2 presents an influence of occupational exposure on biomarkers in a healthy and nonsmoking donors group. There is shown that in spite of decreased number of persons in the exposed and unexposed groups a statistically significant influence of occupation exposure on cytogenetic damage is observed.

Various biomarkers in lymphocytes of lung cancer patients prior to treatment had been studied previously [8]. Characteristics of patients and the influence of the confounding factors on mean values of biomarkers have been presented. As is seen from the results presented on Figure 3, all types of biomarkers under the study were significantly higher in lymphocytes of lung cancer patients than in healthy donors. Results presented in the Table 1 were obtained by the stepwise multivariate analysis of regression (in which an age was treated as a co-variance), that had been performed on the biomarkers levels measured in healthy donors and lung cancer patients' group. Those coefficients are confirming a significant causal association between cytogenetic damage, protein p21 ras level and cancer diagnosis.

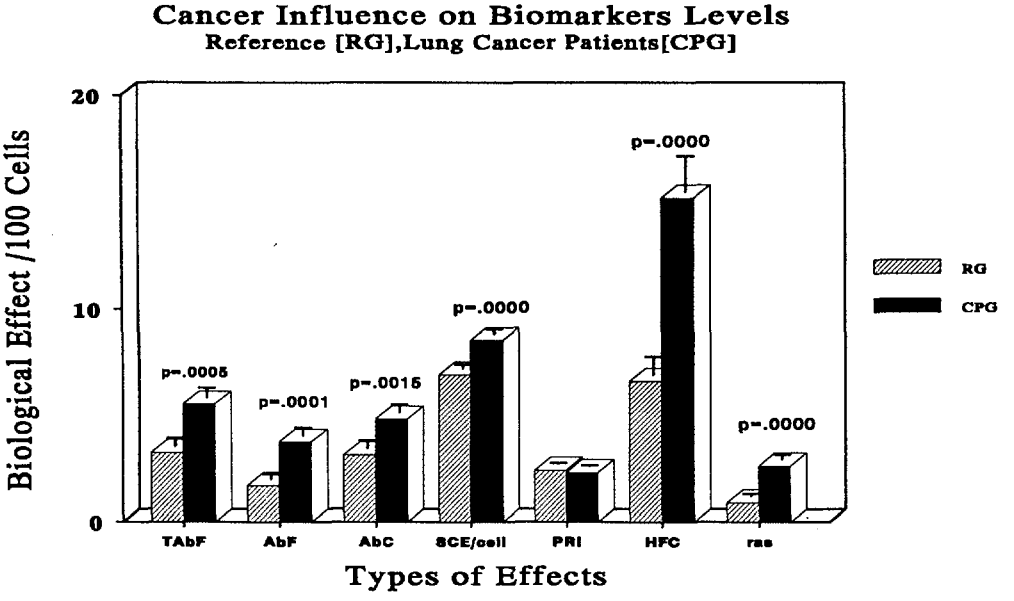

Fig. 3. Differences in biomarkers in cancer patients and healthy donors . (abreviations as on Fig.2)

Table 1

Statistically significant regression coefficients estimated by stepwise multivariate analysis of biomarkers in the cancer patients' group.

	TabF /cell	AbF /cell	AbC %	SCE /cell	RAS [μg]
Mean ± SD	.055 ±.036	.037 ±.029	4.8 ±2.9	8.5 ±1.8	2.56 ±1.56
Age	.001	-.002	-.002	-.04	.068
Sex	.102	.091	.089	3.4	-1.16
Smoking	.041	.037	.036	n	n
CIF	n	n	N	n	n
RAS	.0001	.0006	.0008	n	-
D3	n	.016	N	n	2.11
D2	n	n	.027	n	n
D5	n	-0.039	-.042	n	-2.54
R	.808	.809	.854	.494	.656
R^2	.653	.644	.724	.244	.430
F	24.96	18.16	27.33	8.540	10.76
p value	.0000	.0000	.0000	.0006	.0000

Abbreviations as in the Table 1, D1-D5 classification of lung cancer (WHO system), 1 - squamous cell epidermal cancer, 3 - adenocarcinoma, 2 - small cell carcinoma, 5 - adeno-squamous carcinoma, n = variables excluded by analysis. R - correlation coefficient; R^2 - variability explained by variables (factors) accepted; F = variance ratio.

Characteristics of donors and mean values of various biomarkers observed in persons suspected of the accidental exposure to ionizing radiation were presented previously [9]. Comparison of the distribution of the all types of chromosomal damages detected in the first mitosis of lymphocytes from environmental biomonitoring samples, and from the blood samples of people that were suspected of exposure to gamma radiation source, are shown on figure 4. There is seen that levels of the maximal values observed in all groups are comparable. Figure 5 shows dose response curves for a percent of aberrant cells induced in human lymphocytes after *in vitro* irradiation with various LET radiation. Dose response curve for X-ray radiation, evaluated under the same laboratory and culturing procedure conditions, was treated as an internal standard of genotoxic potency. Table 2 presents mean and maximal values of chromosomal and chromatid types of damages detected in the epidemiological survey groups.

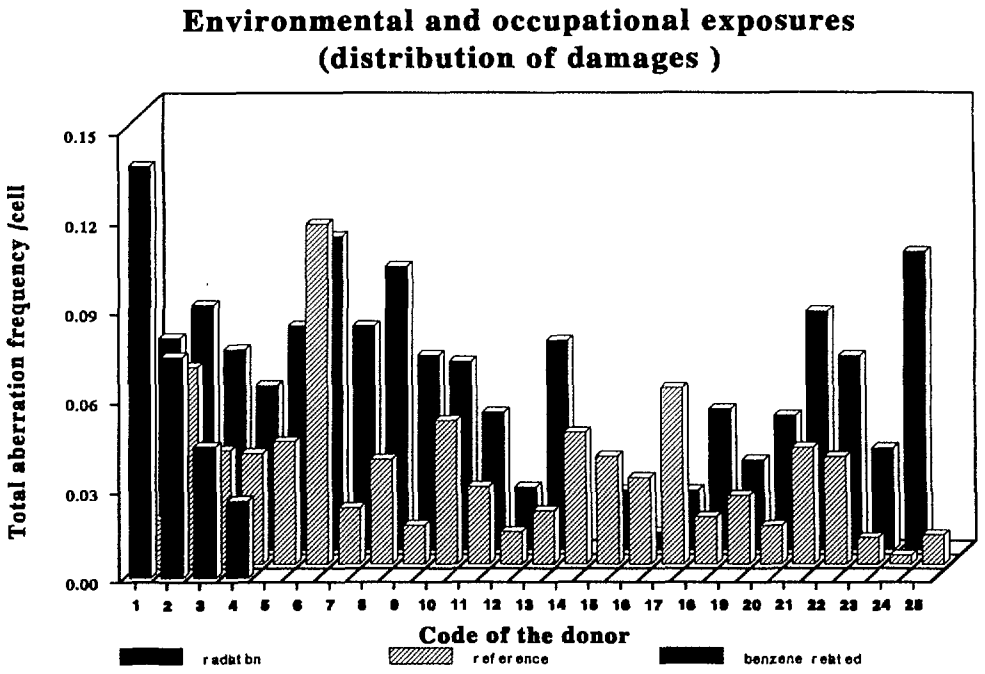

Fig. 4. Comparison of the distribution of the damage detected in the environmental biomonitoring survey and in exposed to radiation.

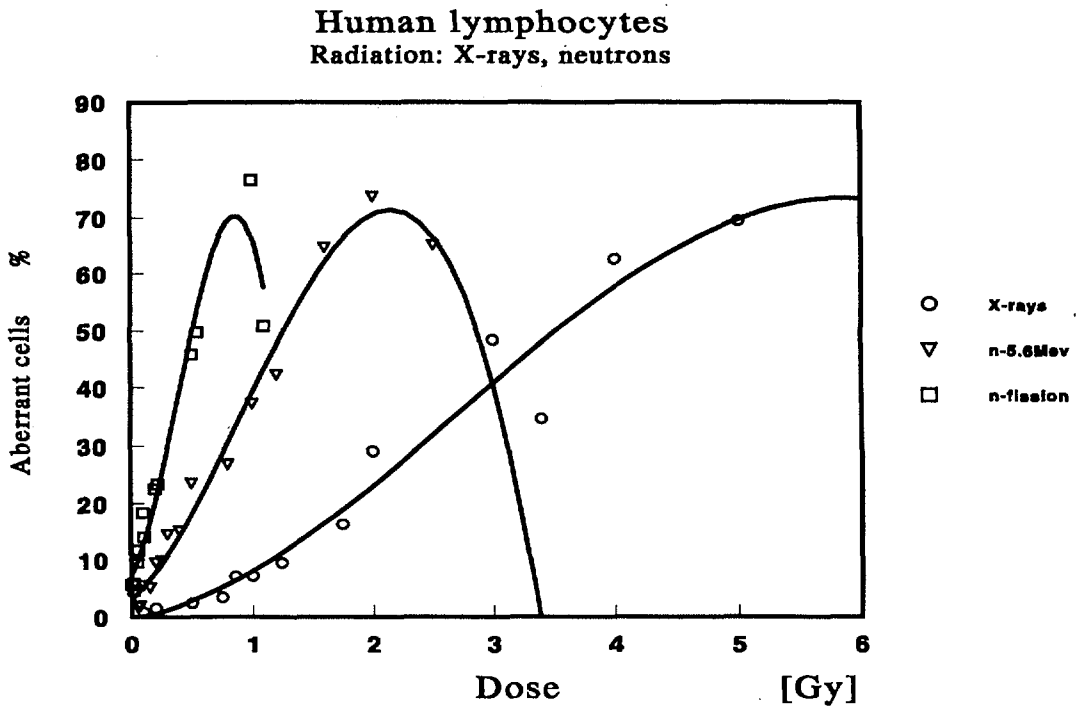

Fig. 5. Dose response curves for percent of aberrant cells induced in human lymphocytes after irradiation with various LET radiation.

Sv-equivalents in Table 2 present radiation doses (evaluated on the bases of the dose response curve shown on Fig. 5) that would induce in human lymphocytes biological effects equal to the maximal values of aberrant cells percentage (AbC) detected in the survey.

Table 2
Mean and maximal values of chromosomal and chromatid damages observed in the environmentally exposed groups, and Sv-equivalents estimated from the maximal AbC values

G	NoP	TAbF	TAbF max	AbF	AbF Max	AbC %	AbC Max	Sv-equiv
R	25	.032	.16	.017	.08	3.1	16	1.5
E	24	.056	.12	.043	.10	5.3	10	1.2
P	30	.055	.15	.037	.14	4.8	14	1.4

G groups: R-reference, E-occupationally exposed, P-lung cancer patients prior to treatment, NoP - No of persons investigated, TAbF, AbF - aberration frequency/cell (including or excluding gaps / cell), AbC - percentage of aberrant cells, Sv-Equiv; radiation dose that would induced in lymphocytes a similar % of aberrant cells.

DISCUSSION:

Group occupationally exposed to benzene and benzene related compounds had statistically significant increases in cytogenetic damages, and the percentage of aberrant cells. In the papers reported by D.Anderson et.all [7] and A.Cebulska-Wasilewska et.all [8] an influence from the confounding factors; exogenous (smoking, sampling season) and endogenous (age, gender, cancer reported in immediate family) was shown. Authors showed that smoking and sampling season affected significantly levels of aberration frequency (including and excluding gaps), percent of aberrant cells, sister chromatid exchanges (SCE), high frequency cells (HFC), and ras p21 proteins. All types of chromosome damages (including and excluding gaps), percent aberrant cells, SCE and ras p21 oncoproteins were statistically significantly higher in cancer patients than in the healthy donors. In some biomarkers was also seen an influence of gender. Stepwise multivariate analysis of regression performed on the biomarkers levels measured in healthy donors and cancer patients' group, confirmed a significant casual association between cytogenetic damage and exposure to benzene related compounds [8]. Results presented in this paper showed also a possible association between cytogenetic damage and cancer (Table 1). Previously reported [4,6] and presented in this paper results from the polish workers studies on genotoxicity of benzene and benzene related compounds are in a good agreement with reported by A.Forni [11] and B.T.Tuunca U.Egeli [12] cytogenetic benzene induced abnormalities.

Characteristics of donors and mean values of various biomarkers observed in persons

suspected of the accidental exposure to ionizing radiation were presented by Cebulska-Wasilewska et al [9]. The potentially absorbed doses were estimated on the base of the dose response curves for dicentric and rings obtained *in vitro,* and were lower than 1Gy. Absorbed radiation doses lower than 1Gy wouldn't imply any nonstochastic effects in somatic cells, although persons exposed were of medical concern. From the radiation-protection point of view, those findings would rather imply a much higher risk of stochastic effects (i.e. cancer).

In two recent, independent reports, increased rates of CA in peripheral lymphocytes were shown to be associated with later development of cancer. Thus, the analysis of CAs is presently regarded as the cytogenetic method of choice in studies of human exposure to genotoxic carcinogens. *In vivo* inducers of CAs in human include, among others, ionizing radiation, alkylating cytostatic, tobacco smoking, benzene, and styrene. Besides smoking, factors such as age, gender, and diagnostic and therapeutic X-rays are usually taken into account as possible confounders. *In vitro* challenging of lymphocytes from healthy individuals with genotoxins has revealed individual differences in CA response to some genotoxic agents. Therefore, the involvement of government in regulating the use of technologies is appropriate in those instances where public exploitation or harm could arise from access to inadequately tested products, promoted in the absence of adequate review. It is the scientist's role to pronounce the time when they deem products or technologies to have been adequately tested and hence safe, reliable, and accurate enough for public use. Society has to be involved in the decisions that define "acceptable levels of unintended effects" as a consequence of any new technology. It then becomes the burden of those in risk communication to ensure public understanding any risk associated with a new technology used for identifying susceptibilities or for building risk management policies around susceptibility issues [1].

Presented in this paper comparison demonstrates that cytogenetic damages observed in environmental survey were comparable to levels of the damages detected in the blood of people accidentally exposed to a radiation source. Dose equivalents to environmentally induced biological effects were high, much higher than annual permissible dose for occupational exposure to radiation, and higher than doses resulting from accidental radiation exposures. Our results bring a proof that particularly in the condition of Poland, health hazard from radiation exposure is overestimated in contradistinction to the environmental hazard. Such a comparison should be helpful for Polish decision-makers to give the highest rank to the protection action decreasing air pollution particularly from traffic and conventional energy sources. This comparison should also be helpful to Polish population to realize how smoking habit (unfortunately still very popular in Poland) affects the health risk.

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