Methods for Assessing the Effects of Mixtures of Chemicals
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Analysis of Cytogenetic Damage in Human Lymphocytes as a Biological Indicator of Mutagenic Effect

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1 INTRODUCTION

A large amount of data has now accumulated on the ability of chemical substances to induce mutations. According to rather controversial information, about 5% of environmental chemicals are characterized by potential mutagenic activity. At the same time, data on mutagenic activity of overall pollutants of air, water, and foodstuffs are being accumulated. All this indicates the possibility of increased contact of man with mutagens.

In order to assess genetic risk of environmental factors for man, two mutually complementary trends are being developed:

(1) Experimental evaluation of the danger (risk) of chemical pollution for man. The studies are mainly carried out with mammals and aim at obtaining information for regulating mutagens in human environment.
(2) Epidemiological studies of the mutagenic effects of chemical pollution. These studies give information on the mutagenic effects of some chemical substance, a group of compounds, or the total chemical contamination. The experiments performed in this context can be subdivided into three groups:
    (a) analysis of the mutagenic effect of chemical factors without determining the real danger in terms of disease occurrence, which is a consequence of generative and somatic mutations;
    (b) study of the connection between chemical exposure and the frequency of disorders in man, which are related to some extent to the effect of mutagens (spontaneous abortions, congenital developmental defects, stillborns, etc.); and
    (c) direct study of the influence of environmental factors on the frequency of hereditary diseases, or changes in the nature of the mutation, the dominant mutations, chromosome and genome mutations, etc.

In this paper the first group of studies, the genotoxic effect of environmental

factors on the people in contact with them, will be considered. The most valuable information will be obtained from these studies if there is a detailed characterization of environmental factors and an additional biological indication of the genotoxic effect of the factors. An evaluation of chemical exposure must, as far as possible, be completed for the exposed individual or group or an evaluation of the total chemical burden of the population should be completed.

The main methods for indicating the effect of genotoxic factors on man are analysis of chromosomal aberrations and sister chromatid exchange (SCE) in the lymphocytes of human peripheral blood.

2 ANALYSIS OF CHROMOSOMAL ABERRATIONS

2.1 General Questions

At present, the analysis of chromosomal aberrations in the lymphocytes of the people who have come into contact with a well-known or supposed mutagen because of therapeutic reasons, occupational conditions, or by chance, is being widely conducted. The merits of this approach are as follows: (a) the material is easily obtained, (b) the methods for cultivating and obtaining the preparations are well developed, and (c) knowledge of the spontaneous level of aberrations and factors affecting it is available. All this serves as the basis for using the method both for the purpose of biological dosimetry in the case of exposure to ionizing radiation, and for a biological indication of the genotoxic effect of chemical and biological factors.

2.2 Technical Factors Influencing the Level of Chromosomal Aberrations

Methods for cultivating lymphocytes, preparing metaphase chromosomes, the principles of analysis of aberrations and the classification of aberrations according to their types are described in a number of guides (Buckton and Evans, 1973; Zacharov *et al.*, 1982). Of the technical factors which must be taken into consideration in conducting the studies, the most important are the length of time of lymphocyte cultivation and the length of storage before cultivation.

Duration of cultivation is of great importance for the correct assessment of the rate of chromosomal aberration. The culture should be fixed in the first mitosis after stimulation. The experiments conducted by us allowed construction of an imitation model which described the dynamics of the first, second, and subsequent mitoses at different times of cultivation (Figure 1). The optimal duration of cultivation was 48–50 hours. Lately the phenomenon of differential painting of chromatids using BUdR (bromodeoxyuridine) has been suggested for distinguishing the first and second mitoses.

The influence of lymphocyte storage before cultivation on the yield of chromosomal aberrations is not well studied. Kagramanian (1981a,b) reports the

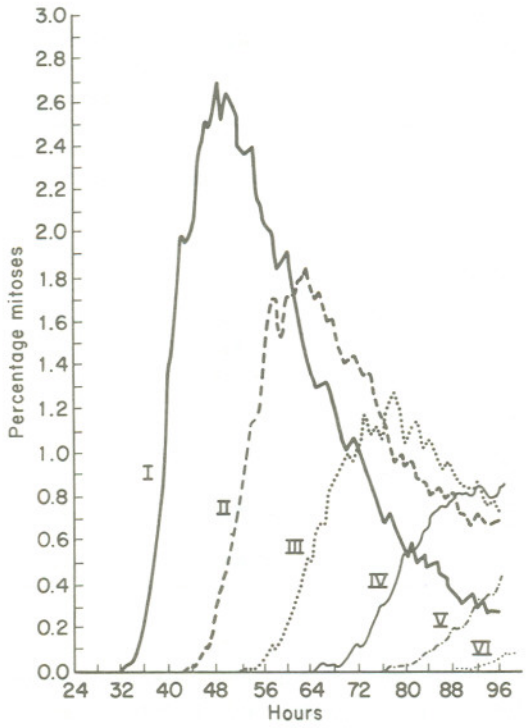

Figure 1 Results of the simulation experiment on the dynamics of mitoses in human lymphocyte cultures. Abscissa: cultivation time, hours. Ordinate: percentage of mitoses of the total number of cells. I, II, III, IV, V, VI: mitoses after PHA administration

absence of an effect on the chromosomal aberrations rate and SCE after 24- and 48-hour storage of lymphocytes at 5°C and 21°C.

Necessary for carrying out the research are a well-trained staff, simultaneous examination of treated and control groups, and, to exclude subjective errors, the analysis of coded slides.

2.3 Spontaneous Level of Chromosomal Aberrations in Human Lymphocytes

Knowledge of the spontaneous level of chromosomal aberrations is necessary for planning studies on the evaluation of the mutagenic effect of environmental factors. We have studied the rate of chromosomal aberrations in the lymphocytes of 360 people of each sex and of three age groups: newborns, those 20–30 years old, and those over 60 years. All donors were healthy, they had not had any viral diseases for the last three months, no professional contact with chemical or radiation factors was noted, and they had not been subjected to extensive X-ray examination. The data from the cytogenetic analysis are presented in Table 1.

Table 1 The spontaneous level of chromosomal aberrations in human lymphocytes. The cultivation time was 50–56 hours and there were 100 or 200 metaphases per donor

Age group	Sex	Number of persons	Number of metaphases	Metaphases with chromosome aberrations	Chromosome aberrations per 100 metaphases
Newborns	M	48	5400	1.37	1.38
	F	44	5000	1.12	1.12
	M + F	92	10400	1.25	1.26
20–30	M	140	18900	1.07	1.10
	F	72	10000	1.07	1.08
	M + F	212	28900	1.07	1.09
> 60	M	39	4600	1.20	1.24
	F	17	2500	1.00	1.00
	M + F	56	7100	1.13	1.15
Total	M	227	28900	1.15	1.18
	F	133	17500	1.07	1.08
	M + F	360	46400	1.12	1.14

The numbers of cells with chromosomal aberrations and the number of aberrations per 100 cells did not depend on the donor's age or sex, and averaged 1.12 and 1.14, respectively. A similar rate of aberrant metaphases is shown by Bochkov *et al.* (1972) (1.14% per 140 cultures, at 50–60 hours of cultivation), Sevankaev *et al.* (1974) (0.97% per 205 donors, at 50–55 hours of cultivation), and Ivanov *et al.* (1978) (1.17% per 105 donors, at 48–51 hours of cultivation). In a number of studies the spontaneous level of aberrant metaphases is higher and this is probably related to the different methods of accounting for fragments and gaps. For example, in the article by Whorton *et al.* (1979) the aberrant cell rate was 2.2% per 239 donors.

The number of types of chromosomal aberrations increases after exposure to ionizing radiation. The types of spontaneous chromosomal aberrations in the cultures of the donors after 50–56 hours of cultivation are shown in Table 2. Chromosomal and isochromatid fragments were included in the group of double fragments because of the complexity of their differentiation. Among the aberrations, fragments accounted for 91.7%, chromatid exchanges for 4%, and chromosomal exchanges for 4.3%. The number of dicentric chromosomes was 0.034 per 100 cells, which is in good agreement with the literature (Evans, 1975).

The distribution of individuals by the number of aberrant cells per 100 metaphases is presented in Table 3. Of all donors, 96.3% had between 0 and 3 aberrant metaphases. This distribution of individuals did not differ from the Poisson and binomial distribution.

Table 2 Types of chromosomal aberrations in lymphocytes of healthy donors after 50–56 hours of cultivation

Type of chromosomal aberrations	Number of aberrations	Aberrations per 100 metaphases[a]	Percentage of the total number of aberrations
Single fragments	336	0.724	63.5
Chromatid exchanges	17	0.037	3.2
Sister union	4	0.008	0.8
Double fragments	149	0.321	28.2
Dicentric chromosomes	16	0.034	3.0
Ring chromosomes	1	0.002	0.2
Atypical chromosomes	5	0.011	0.9
Acentric rings	1	0.002	0.2
Total	529	1.140	100.0

[a] Total 46400 metaphases in 360 donors.

Table 3 Distribution of persons according to the number of cells with chromosome aberrations per 100 metaphases per donor after 50–56 hours of cultivation

Number of cells with chromosome aberrations	Number of persons	Percentage of the total number of persons
0	121	33.6
1	121	33.6
2	74	20.6
3	31	8.6
4	10	2.8
5	1	0.3
6	2	0.6
Total	360	100.1

2.4 Statistical Planning of Research

The statistical planning of research implies the determination of the number of individuals in the groups and the number of cells analysed per each individual. A number of studies on this problem have already been conducted, in which evaluation is made of the necessary volume of samples, the number of cells per individual, error I and error II, and the level of aberrant metaphases in the control group (Bochkov *et al.*, 1974; Kuleshov and Šrám, 1982; Whorton *et al.*, 1979).

The total variance, D^2, characterizes the error of the mean value of a cytogenetic index in the population and is described by the following formula (Youden, 1951):

$$D^2 = \frac{W^2}{K} + \frac{G^2}{Km} \tag{1}$$

where K = the number of individuals under examination, m = the number of cells analysed per each individual, and W^2 and G^2 = the interindividual and intraindividual variance, respectively.

It is obvious in considering equation (1) that, since both components of the total variance are reduced proportionally to the number of individuals examined, the number of individuals is limiting. However, the strategy of 'one cell' gives rise to many objections. Firstly, in this case we practically completely lose the information on the population structure. Secondly, such a strategy is economically unprofitable since the cost of cultivating the samples is high. Introduction of the value criterion allows researchers to find a more optimal way of carrying out studies (Brownlee, 1965).

Assuming the value of the culture from one donor and the obtainment of the stained slide for cytogenetic analysis to be equal to C_1, the value of the analysis of one cell to be equal to C_2, and the total amount of the means allotted for research to be C in conventional units, it is necessary to minimize the total variance D^2 described by equation (1), taking into account that

$$KC_1 + KmC_2 \leqslant C \tag{2}$$

Using the last condition, we have

$$D^2 = \frac{W^2}{K} + \frac{G^2 C_2}{C - C_1 K} \tag{3}$$

To establish the value for K which would minimize the variance, we differentiated equation (3) with respect to K and equated the derivative to zero:

$$\frac{\mathrm{d}D^2}{\mathrm{d}K} = -\frac{W^2}{K^2} + \frac{G^2 C_1 C_2}{(C - C_1 K)^2} = 0 \tag{4}$$

Since $KmC_2 = C - KC_1$, we find from equation (4) that the optimal number of cells analysed in each individual, m, equals

$$m = \frac{G}{W}\sqrt{\frac{C_1}{C_2}} \tag{5}$$

The corresponding optimal number of individuals, K, is obtained by substituting the solution of equation (5) into equation (2).

In order to use equation (5) it is necessary to know, or to estimate in advance, the parameters G, W, C_1 and C_2. Nevertheless, the consideration of equation (5),

even in a general sense, can be useful. So, $m = 1$, in that the strategy of 'one cell from each individual' will be optimal only when intraindividual variance is equal to population variation (which is improbable) and when the value of the cultivation and slide preparation from one donor equals the value of analysis of one cell (which is impossible).

The above-mentioned algorithm for obtaining the optimal sample number while considering economic limitations can be used to determine the ratio of the number of individuals examined and the number of cells analysed in each individual. However, this algorithm provides no information on the statistical significance of the sample obtained.

To settle the problem of determining the minimum necessary samples that provide a given accuracy, additional *a priori* information is needed (Sachs, 1972; Shannon, 1976):

(1) What is the distribution of the response studied in the population and what is the distribution of the sample values?
(2) How does the variability of the population alter with a change in the mean of the target response?
(3) Which value of a shift in the population parameters is necessary to distinguish a change, i.e. what is the accuracy necessary to estimate population parameters?
(4) What is the level of risk which is allowable?

As a rule the solutions of the first two questions are independent problems in population mutagenesis and monitoring systems. As far as the other two questions are concerned, they can be settled by reasonable agreement.

When the answers to the above questions are obtained (naturally with certain assumptions), and the decision on the use of the model of normal distribution is taken, the calculation of the minimum necessary volume of the sample can be made using the formula:

$$n = 2\left[\frac{Z_{(1-\alpha/2)}D_1 + Z_{(1-\beta)}D_2}{\delta}\right]^2 \tag{6}$$

where n = the number of cells analysed in each group, α and β = error I and error II, respectively, δ = the value of a shift in absolute units, D_1 and D_2 = the standard deviation in the groups examined, and Z = the standard normal statistics for the chosen risk levels. If approximation with normal distribution is impossible, it is necessary, before using equation (6), to transform the values, characterizing the target response in a proper way. In this case the standard deviations (D_1 and D_2) and the value of a shift on a new scale acquire other values.

The number of the sample (n) obtained is the product of the number of individuals examined (K) by the number of cells (m) analysed in each of them. The optimal number of cells for an individual can be determined using equation (5), and the necessary number of individuals is obtained by dividing n by m.

The distribution of the individuals by the aberrant cell rate (p) is unknown. The cells of one individual, as determined experimentally (Yakovenko and Tarusina, 1976) as well as from general principles, are described by the binomial distribution, normalized by the transformation of 2 arcsin $\sqrt{p}$ (Scheffe, 1959). The values obtained from this transformation have a variance equal to 1 independent of p. Taking this into account, equation (6) for a given case can be modified to

$$n = \frac{(Z_{(1-\alpha/2)} + Z_{(1-\beta)})^2}{2(\arcsin\sqrt{p_1} - \arcsin\sqrt{p_2})^2} \tag{7}$$

where p_1 and p_2 = the supposed aberrant cells in the population studied and the values of the arcsines are in radians.

It can be seen from equation (7) that a change in n will be dependent on the range of p_1 and p_2. The spontaneous rate of aberrant cells averages near 0.01 per metaphase per donor (Table 1) and in the calculation below we considered cases where the spontaneous level was two times higher than this basic level. Assuming that it is possible to neglect the variance of the transformed values, and giving different values to the shift of the spontaneous level and risk levels α and β, by solving equation (7) one can obtain the minimum necessary number of cells that should be analysed in each of the populations examined. Results of the calculations are presented in Table 4.

Table 4 The number of cells necessary to be analysed in each group under study to establish the difference, Δ, in the rate of aberrant cells for the given α and β errors and for different rates of aberrant cells in the control group

The rate of aberrant metaphases in the control group (%)	Desirable increase in the rate of aberrant metaphases in comparison to the control (Δ%)	Desirable rate of aberrant metaphases in the experimental group (%)	$\alpha = 0.05$	
			$\beta = 0.1$	$\beta = 0.05$
0.5	25	0.625	75018	92758
	50	0.750	20680	25570
	100	1.000	6081	7519
1.0	25	1.25	37297	46117
	50	1.50	10275	12765
	100	2.00	3018	3732
2.0	25	2.50	18437	22797
	50	3.00	5073	6273
	100	4.00	1486	1838

The value of the variability in individuals of the spontaneous rate of aberrant cells is still unknown. However, it follows from the literature on individual sensitivities to chemical mutagens that the variation coefficient varies from 10% to 30% under exposure to different mutagens and remains approximately the same for various doses of a single mutagen (i.e. a change in the standard deviation is related to a change in the average by quasilinear dependence (Tarusina and Yakovenko, 1976; Yakovenko *et al.*, 1976)). Therefore after setting the variation coefficient at 15% for the main value, we considered the situations in which it was two times as low or two times as high. Using these variation coefficients one can calculate the value of W for each case presented in Table 4. The solution of the equation for the variance of binomial distribution can serve as an evaluation of G:

$$G^2 = p_1(1 - p_2) \tag{8}$$

Now, assuming that $\sqrt{C_1/C_2} = 5$, it is easy to calculate the optimal number of cells analysed in an individual and the number of individuals examined in each population using equation (5). These calculations are shown in Table 5.

In most studies the number of individuals varies greatly, while the number of cells per individual is usually 100. This often leads to a decreased sensitivity in the method.

Table 5 Number of individuals and metaphases per individual (underlined) needed to establish the difference (Δ) in the rate of aberrant metaphases in the studied group for $\alpha = 0.05$ and $\beta = 0.1$ or 0.05 and for different levels of aberrant metaphases in the control group

The rate of aberrant metaphases in the control group (%)	Desirable increase in the rate of aberrant metaphases in comparison to the control (Δ%)	$\beta = 0.1$		$\beta = 0.05$	
		Variation coefficient in the population (%)			
		15	30	15	30
		470	235	470	235
0.5	50	44	88	54	109
	100	13	26	16	32
		332	166	332	166
1.0	50	31	62	38	77
	100	9	18	11	22
		233	117	233	117
2.0	50	22	43	27	54
	100	6	13	8	16

2.5 Factors Affecting the Level of Chromosomal Aberrations in Human Lymphocytes

2.5.1 Individual Biological Peculiarities

It is noted in a number of articles that the age and sex of individuals influence both the spontaneous (Ivanov *et al.*, 1978; Sevankaev *et al.*, 1974) and the mutagen-induced level of chromosomal aberrations. Increased aberrations in lymphocytes are found during some diseases, malignant tumours, and with alcoholism and allergies. Much information has accumulated on the increased level of chromosomal aberrations in human lymphocytes during viral infections and vaccination. Analysis of the information shows that cytogenetic indices return to normal three months after the disease or vaccination is over. The effect of genotype on the level of cytogenetic disorders in the somatic cells has been studied on a large scale. However, accounting for genetic peculiarities in the examination of subjects is practically impossible.

2.5.2 Physical Factors

Professional, therapeutic, and diagnostic ionizing irradiation increases the level of chromosomal aberrations in human lymphocytes. The large amount of information and the specificity of the effect of ionizing radiation allows the use of the number of cells with chromosomal aberrations as a biological dosimeter. It is necessary to consider this factor when examining a group of people for mutagens. At present there are no systematized data on the effect of other physical factors on the level of chromosomal aberrations.

2.5.3 Chemical Factors

The influence of occupational and accidental exposure to chemicals, as well as to a number of drugs, on the level of cytogenetic disturbances in human somatic cells has been identified. It is necessary to consider this in selecting the groups for cytogenetic analysis.

3 ANALYSIS OF SISTER CHROMATID EXCHANGES

3.1 General Questions

The analysis of SCE is widely used for indicating the genotoxic effects of environmental factors. Though the nature of SCE is not completely clear, numerous experimental data indicate the possibility of using this index for the assessment of the mutagenic activity of chemical substances and that it is more sensitive than using chromosomal aberrations to study the effects of some

mutagens. At present, data are rapidly accumulating on spontaneous SCE levels in human lymphocytes and the effects of technical and environmental factors and individual biological peculiarities on spontaneous and induced SCE levels. This information can be used to develop the requirements for the selection of sample groups, and the statistical planning of research.

3.2 Spontaneous Level of SCE in Human Lymphocytes and the Factors Affecting It

When analysing the spontaneous levels of SCE in human lymphocytes it is necessary to consider that they are greatly affected by BUdR concentrations. Spontaneous SCE levels in the lymphocytes of healthy donors were studied by Morgan and Crossen (1977) and by Yakovenko and Platonova (1979) when the BUdR concentration in the culture was 10 mg/ml. In the first study, 50 donors 0–85 years old were examined and 20 metaphases for each individual were analysed. The range of individual fluctuations was 5.5–12.5 SCEs per cell with an average of 7.88 and a standard deviation of 1.35. In the second study, 50 donors 2–54 years old were examined and 50 metaphases per person were analysed. The range of individual values was 4.58–8.94 SCEs per cell, the average was 6.525 and the standard deviation was 0.956. The distribution of individuals by the mean number of SCEs per cell was described by a normal distribution. The influence of age on SCE levels was noted. This study shows that when using this method it is necessary to select groups similar in age. The most significant factors affecting SCE levels in human lymphocytes are chemical factors and smoking.

3.3 Statistical Planning of Research

The calculation of the number of individuals in the group and the number of metaphases per individual was made for the first time in the article by Yakovenko and Platonova (1979). This article contains the calculations of these values in which the approach stated in section 2.4 was used. As shown above, the distribution of individuals by the mean number of SCEs is well approximated, with the average equal to 6.5 SCEs per cell and the variation coefficient equal to approximately 15%. However, the number of SCEs per cell appears to vary widely. Little information is available on the variability of the spontaneous level. Therefore, we studied the situations in which the spontaneous SCE level was 1.5 times as low and 1.5 times as high as 6.5, supposing that in other populations the variation coefficient can change from 7.5% to 30%.

Although distribution of SCEs in cells deviates from the normal (Yakovenko and Platonova, 1979), this may be ignored in the first approximation since the variation coefficient is approximately 40% in this case, whereas its limiting value for a normal distribution is 33%.

If a normal distribution is assumed, equation (6) can be used to determine the number of cells to analyse in each group. The only difficulty appears in the estimation of the standard deviations D_1 and D_2. The square of each of these values equals the sum of $W^2 + G^2$, i.e. the sum of variances between individuals and between cells of one individual.

Given the parameters of the examined populations to be the average and the variation coefficient, the variance between individuals can be calculated since it is synonymously determined from the formula for computing the variation coefficient (V):

$$V = \frac{M}{W} 100 \tag{9}$$

where M = the mean number of SCEs per cell. As far as the estimation of the variance between cells of one individual is concerned, according to our observations it exceeds the average by a factor of approximately 1.5.

With these assumptions one can easily estimate D_1 and D_2. For example, if the spontaneous level is 6.5 and the variation coefficient is 30% in the first population, then

$$D_1 = \sqrt{[(6.5 \times 0.3)^2 + (6.5 \times 1.5)]} = 3.68$$

If we need to estimate an increase in the spontaneous level by 10%, i.e. $\delta = 0.65$, the mean rate of SCEs per cell will be equal to 7.15 in the second population

$$D_2 = \sqrt{[(7.15 \times 0.3)^2 + (7.15 \times 1.5)]} = 3.91$$

From the tables of normal distribution we have: $Z_{(1-\alpha/2)} = 1.96$ and $Z_{(1-\beta)} = 1.645$ for risk levels $\alpha = 0.05$ and $\beta = 0.05$. Substituting the values obtained in equation (6) we have $n = 882$ for each group. Bearing in mind that W in the control group is equal to $6.5 \times 0.3 = 1.96$ and $G = \sqrt{6.5 \times 1.5} = 3.12$, and supposing that $\sqrt{C_1/C_2} = 10$, we can determine by equation (5) that it is optimal in the given situation to analyse 16 cells in each individual, for each group $882/16 = 55.13 \approx 56$ individuals. The results of reports are shown in Table 6.

4 THE MAIN TRENDS OF RESEARCH ON THE USE OF CYTOGENETIC DAMAGE IN HUMAN LYMPHOCYTES AS A BIOLOGICAL INDICATOR OF THE GENOTOXIC EFFECT OF ENVIRONMENTAL CHEMICAL FACTORS

The problems of the use of the methods for evaluating cytogenetic disorders in lymphocytes in persons in contact with the known or supposed mutagens have been discussed in a number of papers (Kilian and Picciano, 1976; Kuleshov and Šrám, 1982; Šrám and Kuleshov, 1980). Three main trends of research can be singled out: (a) comparison of the levels of cytogenetic damage between groups of people living in places with different levels of environmental pollution;

Table 6 Number of individuals and metaphases per individual (underlined) needed to establish the difference (Δ) in the level of SCE in the study group for $\alpha = 0.05$ and $\beta = 0.1$ or 0.05, and for different levels of SCE in the control group

The level of SCE cells in the control group	Desirable increase (Δ%)	$\beta = 0.05$			$\beta = 0.1$		
		Variation coefficient in the population (%)					
		7.5	15	30	7.5	15	30
		79	39	20	79	39	20
4.3	10	13	26	61	18	37	86
	25	3	5	11	3	7	16
		64	32	16	64	32	16
6.5	10	11	22	56	15	31	79
	25	2	4	10	3	6	14
		52	26	13	52	26	13
9.8	10	9	19	52	12	27	74
	25	2	4	9	3	5	14

(b) analysis of chromosomal damage in people subjected to occupational exposure to known or supposed mutagens; and (c) analysis of cytogenetic disorders in people subjected to accidental exposure to mutagens (Bridges *et al.*, 1979).

The studies carried out in each of these trends have their own tasks and organizational and methodological peculiarities such as the selection of the groups of individuals, accounting for the level of cytogenetic damage, characterizing the environment, etc. By taking these factors into account the methods will become more sensitive and the results more accurate.

The information obtained is of great significance from the hygienic point of view. Examination of the occupational subgroups allows (a) establishment of the genotoxic effect of occupational factors and, in a number of cases, assessment of the dose–effect relationship, (b) determination of mutagenic agents and the construction of recommendations for their regulation using the data of experimental studies and the evaluation of the genotoxic effects of environmental factors, (c) the exercise of control of the effectiveness of the regulations, and (d) identification of groups of people at high risk.

5 STUDY OF MUTAGENIC EFFECTS IN PEOPLE EXPOSED TO PESTICIDES

This section contains a brief review of the literature on the study of the mutagenic effects of pesticides in people to illustrate the main trends in research.

Pesticides are a group of pollutants that can cause many long-term effects. Sufficient experimental material has been accumulated to relate some pesticides to the group of mutagens which are potentially dangerous genetically. According to literature data, of more than 400 pesticides 50% contain genetically active compounds.

Of great significance for the geneticohygienic evaluation of pesticides is information obtained from cytogenetic examination of their possible mutagenic effect on humans in the occupational groups that have a high exposure to pesticides. Unfortunately, such evaluations are very limited not only because of organizational and methodological difficulties, but also because of insufficient awareness of the degree of the possible genetic hazard of pesticides to people coming into contact with them.

Chromosomal aberrations were analysed in the lymphocytes of peripheral blood in people subjected to occupational exposure to 14 pesticides (Table 7). No cytogenetic effect was found in the workers in contact with benomyl, γ-HCH, DBCP, klorinol and buvinol. In the examination of the persons in contact with DDT and phospho-organic pesticides, a tendency towards an increase in the rate of chromosomal disorders was established; the examination of the people in contact with other pestcides shows a definite increase in the rate of aberrant metaphases in comparison with the control level.

A number of articles deal with the cytogenetic examination of the groups of agricultural workers and hothouse workers who come into contact with a complex of different pesticides. The examinations show that the level of chromosomal aberrations did not differ from the spontaneous level in only one group; the results from this group of experimental hothouse workers were apparently related to the efficiency of the sanitary-technical measures and methods of individual protection. In the other cases there was a statistically significant increase in the rate of chromosomal aberrations. It can be assumed with great probability that the cytogenetic effect observed in these occupational groups is caused by their contact with pesticides under occupational conditions.

It is demonstrated in most articles mentioned above that within each occupational group the induced effect is highly variable, which apparently results from the different sensitivities of the individuals to the effects of chemical mutagens.

It should be noted that the methods of the cytogenetic examinations of the occupational groups are not the same and differ by the following parameters: (a) the number of the people examined, which varies from 7 to 57; (b) the number of metaphases per individual, which varies from 25 (Yoder *et al.*, 1973) to 400 (Pilinskaya, 1974, 1976) and equals 100 in most papers; (c) lymphocyte cultivation time which varies from 48 to 72 hours; (d) the approach to the evaluation of the type of aberrations; (e) the form in which the results of the cytogenetic analysis are presented; and (f) the criteria used in the selection of a control. These differences greatly impede the comparative evaluation of the

Table 7 Results of cytogenetic study of persons in contact with pesticides

Generally accepted name[a]	Chemical name[a]	Number of persons	Results[b]	References
I. *Occupational contact with individual pesticide*				
1. Ziram	Zinc dimethyldithiocarbamate	9	+	Pilinskaya (1970)
2. Zineb	Zinc ethylenebis(dithiocarbamate)	27	+	Pilinskaya (1974, 1976)
3. Thiram	Tetramethylthiram disulphide	7	+	Kuriny and Pilinskaya (1976)
4. Benomyl	Methyl-1-(butylcarbamoyl)benzimidazol-2-yl carbamate	20	−	Ruzicska *et al.* (1975)
5. Pirimicarb	2-Dimethylamino-5, 6-dimethylpirimidin-4-yl dimethylcarbamate	11	+	Pilinskaya (1982)
6. DDT	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane	33	(+)	Rabello *et al.* (1975)
7. γ-HCH	$1\alpha,2\alpha,3\beta,4\alpha,5\alpha,6\beta$-Hexachlorocyclohexane	Not stated	−	Kiraly *et al.* (1979)
8. Terpene polychlorinates	Chlorinated mixed terpenes	10	+	Samoch (1981)
9. DBCP	1,2-Dibromo-3-chloropropane	18	−	Kapp *et al.* (1979)
10. Klorinol	2,4,5-Trichlorophenoxy-ethanol	36	−	Czeizel and Kiraly (1976)
11. Buvinol (klorinol + atrazine)	2,4,5-Trichlorophenoxy-ethanol + 2-chloro-4-ethyl-amino-6-isopropylamino-1,3,5-triazine	26	−	Cziezel and Kiraly (1976)
12. Trichlorphon	Dimethyl-2,2,2-trichloro-1-hydroxyethylphosphonate	17	(+)	Kiraly *et al.* (1979)
13. Phosmet	*O,O*-Dimethyl-*S*-(phthalimidomethyl)phosphorodithioate	25	(+)	Kiraly *et al.* (1979)
14. Diazinon	*O,O*-Diethyl-*O*-(2-isopropyl-6-methylpirimidin-4-yl)-phosphorothioate	34	(+)	Kiraly *et al.* (1979)
II. *Occupational contact with a complex of pesticides*				
1. Complex of phospho-organic pesticides (agricultural workers)		Not stated	+	Kiraly *et al.* (1980)
2. Complex of different pesticides (workers in hothouses)		11	−	Pilinskaya (1982)
3. Complex of different pesticides (agricultural workers)		42	(+)	Yoder *et al.* (1973)
		30	(+)	Georgieva (1976)
		14	+	Shabtai *et al.* (1979)
		25	+	Volnyanskaya and Vasilos (1981)
		57 (SCE)	(+)	Crossen and Morgan (1978)

Table 7 (*Cont'd*)

Generally accepted name[a]	Chemical name[a]	Number of persons	Results[b]	References
III. *Acute pesticide intoxication*				
1. Terpene polychlorinates	Chlorinated mixed terpenes	30	+	Samoch (1981)
2. Malathion	*S*-[1,2-Bis(ethoxycarbonyl)ethyl]-*O*,*O*-dimethylphosphorodithioate	14	+	Czeizel *et al.* (1973) Van Bao *et al.* (1974)
3. Parathion methyl	*O*,*O*-Dimethyl-*O*-(4-nitrophenyl) phosphorothioate	5	+	Czeizel *et al.* (1973) Van Bao *et al.* (1974)
4. Dichlorvos	2,2-Dichlorovinyldimethylphosphate	3	(+)	Czeizel *et al.* (1973) Van Bao *et al.* (1974)
5. Trichlorphon	Dimethyl-(2,2,2-trichloro-1-hydroxyethyl) phosphonate	5	+	Czeizel *et al.* (1973) Van Bao *et al.* (1974)
6. Diazinon	*O*,*O*-Diethyl-*O*-(2-isopropyl-6-methylpirimidin-4-yl) phosphorothioate	2	(+)	Czeizel *et al.* (1973) Van Bao *et al.* (1974)
7. Dimethoate	*O*,*O*-Dimethyl-5-(methylcarbamoylmethyl)-phosphorodithioate	2	(+)	Czeizel *et al.* (1973) Van Bao *et al.* (1974)
IV. *Non-occupational contact with pesticides*				
The population of South Viet Nam exposed to massive doses of herbicides and defoliants (2,4,5-T; dioxin)		58	+	Bach Quoc Tuyen *et al.* (1973)
		Not stated	(+)	Babbitt *et al.* (1972)

[a] The generally accepted and chemical names of pesticides are cited according to the *Pesticide Manual* (Martin and Worthing, 1974).

[b] + = significant increase in the level of cells with chromosomal aberrations,
(+) = tendency to, or increase in the level of cells with chromosomal aberrations,
− = no difference with control group.

results and indicate the necessity to develop common obligatory requirements for both the method of cytogenetic examinations of the occupational subgroups and the presentation of the results.

Of interest is the information collected by a number of authors who detected a pronounced cytogenetic effect in people subjected to accidental acute intoxication by some pesticides (Table 7). An attempt was made in these works to study the dynamics of mutagenic effect with time. In the case of intoxication with chlorinated terpenes, an increased level of chromosomal aberrations remained 10–12 months after the primary examination. With malathion, the cytogenetic effect was determined immediately after intoxication, and the effects of parathion-methyl and trichlorphon were detected one month following exposure. In this study, chromosomal aberration rates returned to the normal level in six months. The absence of the necessary samples in other studies makes the evaluation of the dynamics of mutagenic effect difficult.

Thus, in the course of the cytogenetic examination of occupational groups, the data confirm the possibility of mutagenicity in the somatic cells of man when exposed to pesticides.

Such information is of scientific and practical value in several aspects: it is necessary for the development of studies on comparative mutagenesis; it is of independent significance from the point of view of the occupational monitoring of the genetic hazard of pesticides; it allows identification of the groups at high risk; and it can serve as one of the serious signals of possible genetic hazard for the population under conditions of environmental pollution with mutagenic pesticides.

The overall contribution of pesticides to the mutation process in the population can be assessed in the course of the cytogenetic examination of the subpopulations, for which pesticides are a prevailing anthropogenic factor of environmental pollution. The studies carried out by Bach Quoc Tuyen *et al.* (1973) and Babbitt *et al.* (1972) indicate an increased level of chromosomal aberrations in the lymphocytes of the inhabitants of South Viet Nam who were exposed to massive doses of defoliants and herbicides. In order to clarify the role of pesticides in the mutation process it is advisable to perform cytogenetic examinations of representative groups of the population in the agricultural regions which greatly differ in the level of local pesticide pollution.

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