Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Methods for Quantitative Cancer Risk Estimation Currently Used in the USSR

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ABSTRACT

Approaches for cancer risk assessment currently used in the USSR are described. The proposed methods are based on (1) threshold concept, (2) non-threshold concept and (3) application of probit-analysis.

The method based on the threshold concept is commonly used in the USSR. It was applied in the development of the maximum acceptable concentrations (MACs) adopted in the USSR for benzo[*a*]pyrene in atmospheric air, natural waters, soil and occupational environment. The method is based on experimental determination of the minimum effective and maximum non-effective carcinogenic dose and a mathematical model involving non-effective experimental dose and the latency period that is longer than the expected natural life-span. Its application is illustrated by the calculation of the maximum acceptable concentration of benzo(a)pyrene in atmospheric air.

1 INTRODUCTION

The recognition of the aetiological role of chemical agents in the development of malignant neoplasms is of great importance for the preventive measures that restrict human exposure to carcinogenic substances. The experience gained in occupational and environmental toxicology shows that regulatory action is an essential part of preventive action. There has been a long discussion in the Soviet literature about the regulation of carcinogenic substances because cancer risk associated with exposure to chemicals is not acceptable. The USSR standards for carcinogenic substances are set at such exposure levels that tumours are not expected to develop during the natural lifetime of individuals.

There is a unanimous agreement in the USSR that environmental carcinogens have to be regulated, and the first hygienic standards have been established for benzo[*a*]pyrene in ambient air (0.1 $\mu g/100\,m^3$), natural waters (0.005 $\mu g/l$), soil (20 $\mu g/kg$) and air in occupational environments (0.15 $\mu g/m^3$). Nevertheless, the approaches to cancer risk estimation and setting of maximum acceptable

concentrations (MACs) continue to be discussed in scientific literature (Janyševa, 1972; Šabad *et al.*, 1973; Sidorenko *et al.*, 1974; Janyševa *et al.*, 1977; Ilyin and Knižnikov, 1979; and others). Particular attention is being paid to the choice of experimental model and its relevance to human populations, latent periods and the selection of safety factors. Different attitudes regarding the existence of thresholds for carcinogenic substances have guided various approaches to cancer risk estimation.

The present chapter summarizes the views of Soviet investigators on this problem.

2 METHODOLOGICAL APPROACHES TO CANCER RISK ESTIMATION IN THE USSR

2.1 Cancer Risk Estimation Based on Non-threshold Concept

The non-threshold concept has been proposed as the basis of cancer risk estimation by several investigators (Knižnikov, 1975; Ilyin and Knižnikov, 1979). They argued as follows:

(1) In principle, there is no difference in the process by which ionizing radiation and chemical carcinogens transform normal cells into malignant tumour cells. Tumours are monoclonal, i.e. develop from a single cell. Therefore, a single interaction between the cell and a molecule of a chemical carcinogen may induce cell transformation that will eventually result in a malignant growth. Thus, theoretically, any quantity of a substance may be associated with tumour development.
(2) Both ionizing radiation and chemical carcinogens can induce the development of neoplasms, and there are no neoplasms that are specific either for ionizing radiation or for chemical carcinogens.
(3) The relationship between dose and response is similar for all carcinogenic agents.

According to these authors, the proposed acceptable cancer risk is 1.10^{-6}. The acceptable dose of a chemical carcinogen is estimated from the equation:

$$AD_c = \frac{MND_{100} \cdot Z}{f} \quad (1)$$

where:

AD_c = acceptable dose of a carcinogen;
MND_{100} = the maximum non-effective dose determined in a group of 100 animals;
Z = coefficient of extrapolation from animal data to man, which takes into account the sensitivity of experimental animals. For random bred animals, Z is in the range from 0.1 to 10 (radiation sensitivity of man is taken as 1);

$f = 10^{-4}$ = coefficient of acceptable risk reduction when the extrapolation is made from animals to man. If the experiment has been carried out with fewer than 100 animals, this value should be changed accordingly.

2.2 Cancer Risk Estimation from Effective Dose Calculated by Probit-analysis

Other investigators (Kurljandskij and Nevzorova, 1978) proposed to apply effective doses (ED_{16}, ED_{50}, and ED_{84}) calculated by probit-analysis. By fitting a straight line to experimental points in the high-dose region, it is possible to estimate cancer risk at low doses. The maximum non-effective dose is the dose at which the upper limit of the dose–response line intersects the lower limit of the incidence rate of spontaneous tumours in the control group.

According to these authors, the advantage of this method is that it avoids testing for carcinogenicity at low doses.

2.3 Cancer Risk Estimation Based on Threshold Concept

The method based on the existence of threshold for carcinogenic action of chemicals has been accepted by most investigators in the USSR (Sidorenko *et al.*, 1974; Janyševa *et al.*, 1977; Sanockij and Fomenko, 1979; and others). According to these authors, the existence of thresholds for carcinogenic action of chemicals is indicated by the following facts:

(1) As for other adverse effects of chemicals, the cancer incidence and the time-to-occurrence depend on the dose. With decreasing dose, the incidence decreases and the time-to-occurrence increases. The difference between carcinogenesis and other pathological processes lies only in different mechanisms and in a different degree of risk. The occurrence of cancer depends on the dose and on the state of resistance of the organism.

(2) Several compounds which occur in the organism naturally and which are essential for normal function of the organism if present in an appropriate dose range (for example, some hormones) may cause qualitatively different, adverse effects, including cancer when administered in a higher dose.

(3) There are data showing that carcinogenesis is not an entirely irreversible process. A healthy organism has a powerful system of adaptive and compensatory/defensive responses which may make the action of potentially carcinogenic agents harmless, and assist in the normalization of disturbed biochemical processes and recuperation of injured structures and functions (Kaveckij, 1962; Šabad, 1967, 1979; Kaveckij and Suklinov, 1979; Sanockij and Fomenko, 1979). Even the appearance of a transformed cell does not necessarily mean that a tumour will occur; tumour development is resisted by cellular repair processes and immune defence mechanisms (Timofeevskij,

1947; Kaveckij, 1962; Kaveckij and Suklinov, 1979). The regression of skin papilloma and of pretumour changes in organs of experimental animals (Šabad, 1967, 1979; Janyševa *et al.*, 1977) is an experimental verification of DNA repair that takes place after the exposure to carcinogens has been discontinued (Falk, 1980); this has also been confirmed by the disappearance of neoplastic changes in the bronchi of some persons who stopped smoking (Doll, 1963).

(4) During the last few years, new data on the dependence of carcinogenesis on the dose, time and the mode of intake have been obtained (Janyševa *et al.*, 1979, 1980). It has been found that at high dose rates 'critical levels' of carcinogens can be reached, and that further penetration into the organism is ineffective both as regards the frequency and the time of tumour occurrence (Table 1). The subsequent intake of the substance seems to affect more the tumour progression than its occurrence.

Identification of the dose that has maximal carcinogenic impact has led to the notion of the minimum dose that can induce a single neoplasm. Determination of the total amount of benzo[*a*]pyrene (BP) in mouse forestomach tissue that precedes the appearance of the first tumour, confirmed this assumption (Janyševa *et al.*, 1979). As shown in Table 2, similar amounts of BP are deposited in the

Table 1 The effects of fractionation of peroral administration of benzo(a)pyrene (BP) on the development of forestomach tumours

Mice strain	Duration of exposure	Number of administrations	BP dose (mg)		Incidence of forestomach tumours				
			Single dose	Total dose	Malignant	Benign	Total	t_i	t_{50}
$CC_{57}Br$	5	10	0.5	5.0	25.0	42.5	67.5	4	12
$CC_{57}Br$	17	35	0.5	17.5	54.2	15.8	70.0	4	12

t_i = time of occurrence of the first tumours (months); t_{50} = time of occurrence of 50% of tumours (months).

Table 2 Accumulation of benzo[*a*]pyrene (BP) in the forestomach tissue of C_{57} BI mice until the occurrence of first tumours

BP dose			Total amount administered	Time of occurrence of first tumours (months)
Single dose (mg)	Forestomach deposition (%)	Total forestomach deposition (mg)		
0.5	9.7	0.40	8	4
0.1	28.8	0.37	13	6
0.02	66.0	0.30	23	11

forestomach tissue up to the time of occurrence of the first tumour; these amounts are independent of the total dose administered and the fractionation of the dose.

The effectiveness of dose fractionation is often used as an argument for the non-existence of the threshold for chemical carcinogens. However, this applies to high and medium doses (Payne and Hueper, 1960; Turusov, 1971; Saffiotti *et al.*, 1972a,b; Sellakumar *et al.*, 1973; Janyševa *et al.*, 1977; and others). This phenomenon, shown in Table 2, is probably due to the fact that a smaller quantity of the carcinogen is deposited in the target organ when the absorption is fractionated. Slower accumulation of the dose extends the latent period (Table 3).

A reduction of tumorigenic effect down to a 'zero' effect (Payne and Hueper, 1960; Janyševa *et al.*, 1977) was observed when small doses had been fractionated, indicating the possibility of detoxication and the existence of a threshold. The level of the threshold has a probabilistic character and depends on several exogenous and endogenous modifying factors (such as sex, age, mode of action).

In view of these considerations, many oncologists in the USSR believe that the most important practical tasks at present are (1) to develop reliable methodological approaches for identifying doses of carcinogens which do not cause harmful effects during the natural life-span, and (2) to find appropriate ways of extrapolating experimental animal data to human populations.

Solutions to these questions could be found in two stages. The first stage is the experimental study of carcinogenic action. At this stage, it is of particular importance to determine in lifetime studies with experimental animals the maximum effective, minimum effective, and maximum ineffective dose. The administration of the substance should be as similar as possible to human exposure under real conditions. The assessment of carcinogenic action should be based on the registration of all induced and spontaneous tumours and of their latency time. This will enable the identification of cancers induced by exposure to a specific chemical. The second stage is mathematical modelling. Based on experimental dose–response relationships, the dose at which no carcinogenic effect is expected in experimental animals is estimated.

This procedure can be illustrated by the estimation of the maximum acceptable

Table 3 Carcinogenic effects of benzo[*a*]pyrene (BP) in C_{57} BI mice as a function of the fractionation and duration of peroral administration

Administered BP dose (mg)		Number of administrations	BP dose deposited in forestomach tissue		Incidence of forestomach tumours	Time of tumour occurrence (months)	
Single	Total		Single	Total	%	t_i	t_{50}
0.5	5.0	10	0.049	0.49	60.0	3	12
0.1	3.5	35	0.029	1.01	75.7	6	14

t_i = time of occurrence of first tumours; t_{50} = time of occurrence of 50% of tumours.

concentration of benzo(a)pyrene in atmospheric air (Yanysheva and Antomonov, 1976; Yanysheva *et al.*, 1979). A logarithmic function was fitted to the experimental data obtained after administering six different doses of benzo(a) pyrene in fractions of 1/10 of the total dose, either alone (Table 4) or together with several other substances (Table 5). The logarithmic function had the following form:

$$Y = 10 \ln \frac{x_n}{x} + 1 \qquad (2)$$

where:

Y = the fraction of animals with tumours (%);
x_n = doses inducing tumours (mg);
x = maximum non-effective dose (mg).

Using equation (2), it was possible to estimate the expected frequency of

Table 4 Occurrence of lung tumours in rats after ten intratracheal administrations of various doses of benzo[*a*]pyrene

Benzo[*a*]pyrene dose (mg)	Animals* with tumours (%)	Animals with malignant tumours (%)	Time of appearance of the first tumour (months)	Maximum life-span (months)
25.0	80.0	42.5	12	28
2.5	42.8	28.5	17	31
0.5	28.2	15.7	19	34
0.1	14.4	0	27	37
0.02	0	0	—	—
0.005	0	0	—	—
Controls	0	o	—	—

* Based on the number of animals surviving 10 months.

Table 5 Occurrence of lung tumours in rats after intratracheal administration of benzo[*a*]pyrene (BP) together with other substances

Dose (mg)	BP + india ink	BP + dust	BP + tween 60	BP + dibenzo [*a*,*h*] anthracene	BP + benzo[*g*,*h*,*i*] perylene, pyrene antracene, chrysene
0.1	14.4	18.7	26.3	14.2	0
0.02	0	0	0	0	0
Control	0	0	0	0	0

tumours at low doses. The results of these calculations (Table 6) show, for example, that the expected frequency of cancer is 6.9 % at a benzo[*a*]pyrene dose of 0.02 mg.

Similar results have been obtained by using an exponential model for dose–response relationships:

$$Y = 100\,(1 - e^{-ax}) \tag{3}$$

where:

Y = fraction of animals with tumours (%);
x = dose (mg);
a = constant.

This model made it possible to estimate the upper limits of low-dose risk using $a = 1.555$, determined at a dose of 25 mg. Thus, the upper limit of tumour risk at a 0.02 mg dose level was 3 % and the lower limit 0.023 %.

The above considerations imply that doses which are not effective in one experiment could induce neoplasms in another experiment, for example, if the number of animals in the test group is increased.

In order to obtain a more realistic estimate of cancer risk in humans, the dose–latency time relationship has been used. The time (T) when the first tumour occurs at a low dose of benzo[*a*]pyrene can be estimated from the equation:

$$T = \frac{a}{d} + b \tag{4}$$

where a and b are constants ($a = 1.121$ and $b = 16.79$).

Cancers induced by a dose of 0.05 mg of benzo(a)pyrene will become manifest approximately in the 38th month, which is the natural life-span of the rat; the latency times of tumours induced by 0.02 mg and 0.01 mg of benzo[*a*]pyrene are about 68 and 119 months, respectively, and exceed the natural life-span of animals.

Table 6 Calculated lung tumour risk for rats after intratracheal administration of various doses of benzo[*a*]pyrene

Benzo[*a*]pyrene (mg)	Animals with tumours (%)
0.1	17.9
0.02	6.9
0.005	2.23
0.002	0.95
0.0005	0.24

Consequently, the dose of 0.02 mg can be used for estimating the maximum acceptable concentration. However, one must bear in mind that the experimental results are obtained with a small homogenous group of healthy animals whereas human populations are large and heterogenous, and include children and unhealthy individuals; this may increase the probability of cancer induction. In order to take this into account, the dose chosen for extrapolation from animals to man should be reduced by one order of magnitude, i.e., a safety factor should be applied. It should be noted that the reduction of dose not only decreases the risk but also increases the time-to-occurrence (latency time). Depending on experimental conditions, a safety factor ranging from 10 to 100 is used in the USSR (Janyševa *et al.*, 1977; Šabad, 1979).

The following equation is proposed for converting the maximum ineffective dose into the maximum acceptable concentration in ambient air:

$$c = \frac{x \cdot p}{B \cdot k \cdot V} \tag{5}$$

where:

c = maximum acceptable concentration (mg/m^3);
x = maximum ineffective dose of a carcinogen (mg/kg);
B = the average weight of experimental animals;
k = safety factor;
V = estimated volume of air inhaled by a human individual during his lifetime.

3 CONCLUSIONS

Models for estimating cancer risk described in section 2.3 have been used in the USSR for establishing environmental health standards for benzo(a)pyrene. The approach outlined in the paper requires further development both as regards mathematical models for extrapolation to human populations, and the experimental and epidemiological data needed to improve our knowledge of dose–time–response relationships. The final criterion for the validity of mathematical concepts and models used are the practical results they provide.

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