ELECTROPHILIC REACTIVITY AS A MEASURE OF GENOTOXIC POTENCY

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The mutagenic effectiveness in forward mutation systems of a number of monofunctional alkylating agents with large differences in absolute reactivity and with different s-values (Swain and Scott, 1953) have been compared in the low-dose region of the dose-response curves. It was shown that the mutation frequency, \( R \), at equal dose, \( D^* \) (\( D \) is the time integral of the concentration of the alkylating agent, \( RX \)), was proportional to the second order rate constant, \( k_{n=2} \), for reaction with some nucleophilic center with a low nucleophilic strength (\( n=2 \) in the Swain-Scott scale, see Fig. 1) (Hussain and Ehrenberg, 1975; Osterman-Golkar, 1975; Ehrenberg, 1980; Hussain, 1981)

\[
R = \text{const} \cdot k_{n=2} \cdot D
\]  

(1)

The degree of alkylation, \( [RY]/[Y^-] \), of a nucleophilic center, \( Y^- \), is proportional to the dose of the alkyling agent according to the equation

\[
[RY]/[Y^-] = k_Y \cdot D
\]  

(2)

*Abbreviations and symbols used: \( L_i \), level (concentration); \( D_i \), dose \( (D = \int L(t)dt) \); Indices in \( L_i, D_i \) refer to system of steps from environmental level/dose \( (i=1) \) to biological response \( (i=6) \); \( i=2 \) absorbed amount of a chemical given e.g. in mg/kg b.w. (pharmacological dose); \( i=3 \) dose of an electrophilic compound in tissues; \( i=4 \) dose of an electrophilic compound in immediate environment of target molecules (DNA-dose); \( i=5 \) level of potentially mutagenic lesions in DNA (molecular dose); \( k_{i(i+1)} \), transfer coefficient between consecutive steps in this system (Cf. Osterman-Golkar this journal and Ehrenberg et al., 1981).
This means that the mutation frequency is proportional to the degree of alkylation of nucleophilic centers of the nucleophilic strength n=2 independent of the chemical used (see Fig. 2).

Nucleophilic sites of DNA, critical or non-critical, are alkylated at random—with rates > 0, determined by the nucleophilic strengths of these groups, by the reactivity and the s-value of the alkylating agent, in several cases also by steric factors, etc. (cf. Ehrenberg and Osterman-Golkar, 1980). Therefore reactions at sites of the reactivity n=2 occur along with reactions at all other reactive sites, and vice versa.

It follows, that the demonstration of alkylating activity of RX in DNA or its immediate environment (or outside the cell nucleus if it can be assumed that k_{34} > 0), is a sufficient criterion on risk of genetic damage.

It is probably valid for all other types of electrophilic agents as well (cf. Table II of Ehrenberg and Osterman-Golkar, 1980), that, if they can at all penetrate membranes, they will give rise to critical as well as non-critical chemical changes in the DNA.

Miller and Miller (1971) have claimed that all carcinogens (i.e. cancer initiators), which according to the same authors are (in general) also mutagenic, are electrophilically reactive (in the expression):

\[
\text{cancer initiators} \rightarrow \text{electrophilic reagents}
\]

It follows from the random distribution of reactions with different nucleophilic sites in the DNA that the conversion of expression (3) is also valid. The implication is that a demonstration of electrophilic reactivity \textit{in vivo} is a sufficient criterion on genetic risk, including cancer risk.

The conversion of (3) concerns the qualitative risk identification. If somebody would like to show that this conversion is not valid in a specific case a knowledge of quantitative parameters determining the rate of reaction with critical sites of DNA is required. The absence of a biological effect (i.e. response = 0) in a test for mutagenicity or carcinogenicity can, for statistical reasons, never prove the absence of genotoxic activity. Therefore, the proof for non-validity of the conversion (3) has to be based on the demonstration that the mutation frequency (etc) is significantly lower than the expected frequency (R_{\text{expect}}) or a certain fraction, a, thereof. On the analogy of corresponding calculations for