INTEGRATION OF PHARMACOKINETICS WITH MULTISTAGE CARCINOGENESIS MODELING IN CANCER RISK ASSESSMENT*

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1. INTRODUCTION

It is of public health concern to assess the risk posed by the presence of carcinogens in our environment. Unfortunately the only tools available until now, epidemiology and animal experiments, suffer severe drawbacks: The former is not considered as powerful enough, the latter are not immediately applicable to humans. Obviously there is a need for inter-dose and inter-species extrapolation procedures in the field of risk assessment.

Inter-dose extrapolation procedures should incorporate major features of the carcinogenic process that cause non-linearity in the dose-effect relationship:

- non-linear metabolic processes involved in the transformation of the applied dose into an effective dose (e.g. as measured by DNA-adduct concentrations at the target site),
- non-linearity of the relationship between the effective dose and the probability of cancer appearance, implied by the multistage nature of carcinogenesis.

Inter-species extrapolation requires the identification of the specific parameters (organ volumes, blood flows, metabolic rates, enzymatic equipment...) explaining the variation of animal susceptibility to carcinogens, and their integration into a coordinated scheme.

We present here a stratified physiological model that integrates all the preceeding requirements. Preliminary results obtained for vinyl chloride (V.C.) in the rat demonstrate the feasability and the interest of this type of approach.

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2. DESCRIPTION OF THE MODEL

The model is developed at three levels successively linked together:

- Level 1: Distribution of the compounds in the organism,
- Level 2: Metabolic transformations,
- Level 3: Interaction of metabolites with cellular processes of transformation, division, death, differentiation.

At each level a sub-model has been established. The first two are pharmacokinetic models. They allow the derivation of an intra-cellular effective dose of toxicant from the applied dose. They account for inter-species differences in the fate of compounds within the organisms, and of non-linear relationships between effective and applied doses. The third sub-model is a stochastic treatment of cellular behavior where the probabilities of cancerous transformation are functions of the effective doses. A probabilistic approach is the only one able to give a risk estimate, 'risk' being strictly equivalent to 'probability' from a scientific point of view. We shall now describe in more detail those sub-models.

2.1. Distribution level

At this level we use a compartmental model (Fig. 1) describing the distribution of any substance within a mammalian organism. Each one of the 11 compartments has a physiological meaning. Several parameters characterize those compartments: Volume, blood flow, diffusion flows. For each animal species the set of parameter values must be found in the literature or determined by appropriate experiments or simulations.

The transport of substances between the compartments is described by a system of differential equations (one per compartment and per substance). For details of formulation see GERLOWSKI and JAIN (1983). This type of approach has been successfully applied to inter-species extrapolation problems by LUTZ (1984), and by RAMSEY and ANDERSEN (1984).

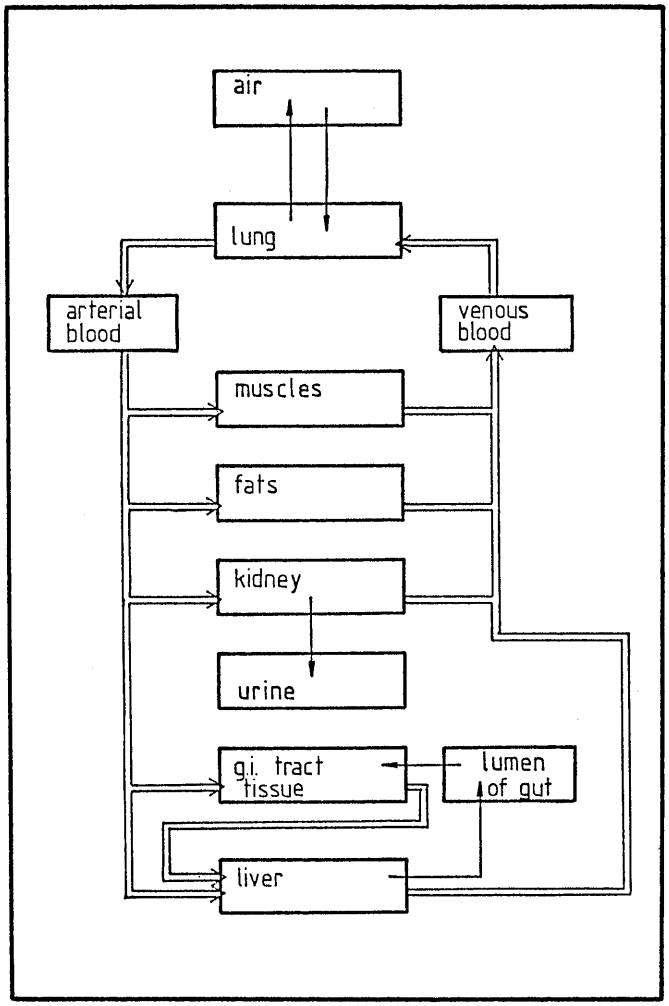

Figure 1: Compartmental model describing the distribution of substances within a mammalian organism (level 1 of the general model).

2.2. Transformation level

Within each compartment of the preceeding model (with the exeption of air, lumen and urine) the absorbed compound can be metabolized (Fig. 2). The scheme adopted is rather general and includes the major processes known to have an influence on carcinogenesis: Direct fixation of the parent compound on DNA or proteins, fixation after metabolic activation, detoxifications and repair of genetic lesions. The model allows these reactions to be saturable.

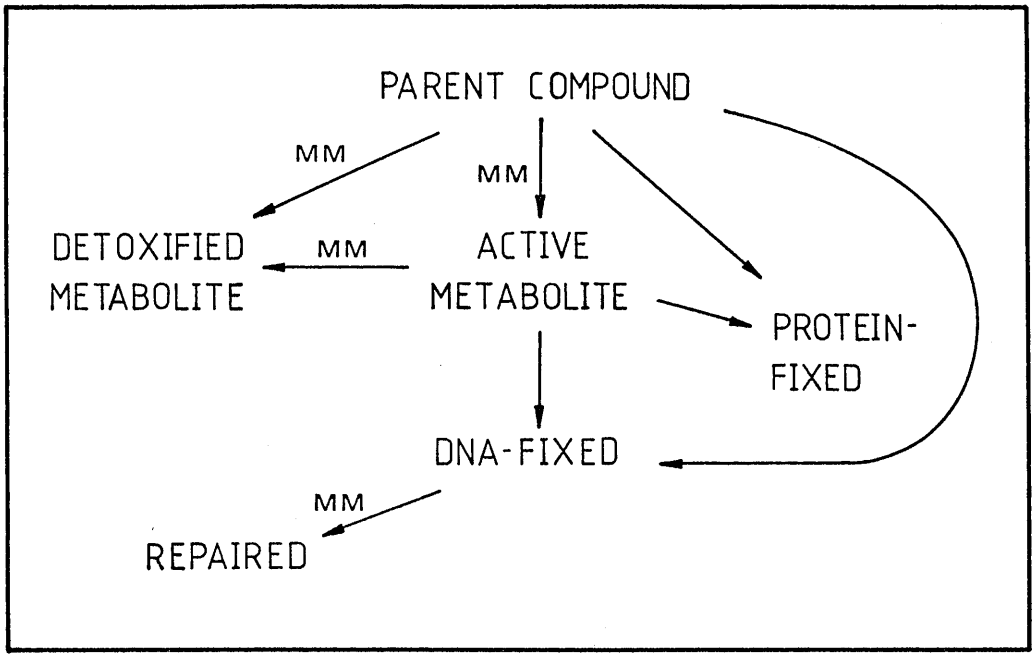

Figure 2: Kinetic model of metabolization (level 2). The parent compound and detoxified metabolite can diffuse in the whole body. M.M. indicates saturable enzymatic processes described by the Michaelis-Menten equation.

The corresponding differential equations are coupled with those of the preceeding level and solved simultaneously by numerical integration. Estimates of the kinetic constants can be found in the literature or obtained by in vitro experiments. Each organ has its own set of parameters values; Once they are fixed the time course of the concentration for each metabolic intermediate can be computed. Among these intermediates the DNA-adducts can be considered as an effective dose of carcinogen (at least for initiators). A similar modeling of metabolization has been performed by GEHRING and BLAU (1977).

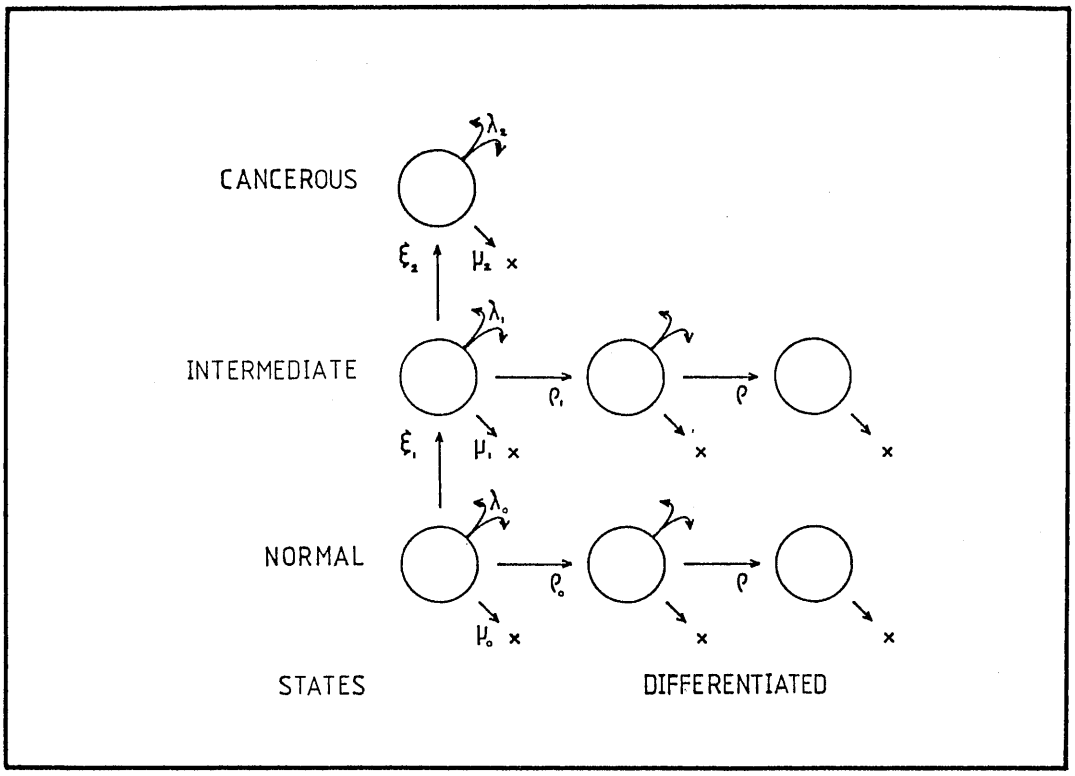

Figure 3: Stochastic model of cellular behavior.
λ_i: Probability of division per unit of time;
μ_i: Probability of death; ρ_i : Probability of differentiation; ξ_i : Probability of transformation

2.3. Cellular behavior level

All the cells of an organ are considered to be equivalent and independent. These cells can divide, die, differentiate or suffer transformation (bringing them closer to a cancerous stage). The probabilities that these events occur are time and environment dependent. Figure 3 present schematically the different states of the cells. Two stages have been considered: Intermediate cells and fully cancerous cells.

Mathematical treatment of the random behavior of cells in a population leads to a set of partial differential equations for probability generating functions (see CHIANG, 1980 for a general treatment of this type of problem). It is then possible to compute by numerical integration the time course of the number of cells in each state (given initial conditions, parameters values and effective doses of carcinogen), the associated variance and, finally, the instantaneous probability of having one or more cancerous cells in the population. This last probability can be considered as a cancer risk for the given organ; it is time and dose dependent.

3. PRELIMINARY RESULTS

Experiments on V.C. (MALTONI et al., 1980) in rats demonstrate a strong relation between the air concentration of the compound and the incidence of hepatic angiosarcoma for exposed animals (Fig. 4). It is difficult, if not impossible, to adjust to these results a dose-response curve which would have a physiological basis.

By a simulation of the exposure of rats to V.C. over 30 minutes, at the concentration tested by MALTONI et al., we computed the corresponding mean genetic damage. Figure 5 presents the results of this computation. An important non-linearity is observed in the relationship between the air concentration of V.C. (applied dose) and the number of DNA-adducts formed per gram of DNA (effective dose). A saturation of enzymatic activation occurs at concentration above 200 ppm of V.C.

Adduct formation is higher in the liver than in other organs, just as the liver is a preferential tumor site in rats exposed to V.C.

Figure 6 has been obtained by plotting the observed hepatic angiosarcoma incidence against the computed genetic damage, for a given concentration of V.C. (the mean genetic damage due to repeated 4 hours exposure is assumed proportional to the dammage from a single 30 minutes exposure). It is now possible to adjust the result of a physiological multistage model to this relationship. We computed the probability of appearance of cancerous cells in the liver by adjusting model parameters and making the transformation probabilities proportional to the genetic damage. The curve obtained is much better fitted to the results than, for example, a straight line (the residual variance is 67.8 in the case of the regression line and 19.4 for the fitted curve). Notice that the last point of the experimental results (for 10000 ppm) is below

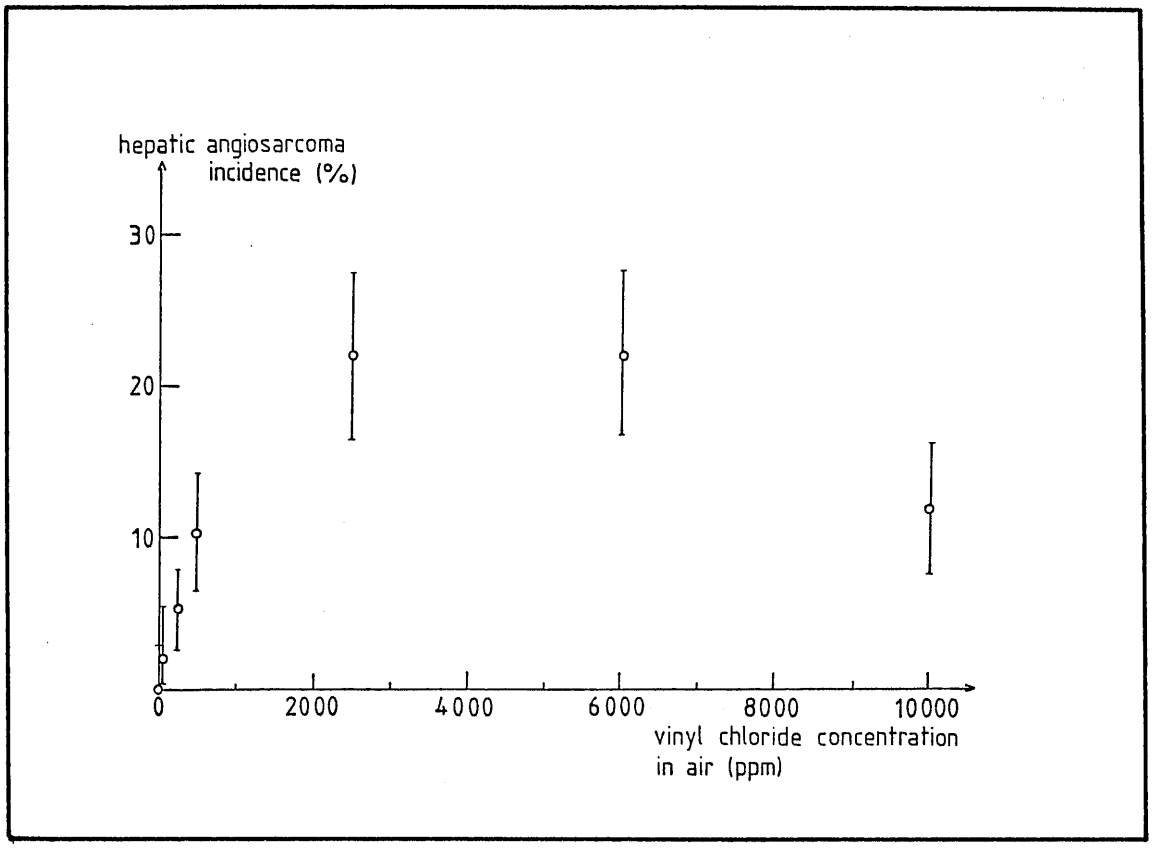

Figure 4: Hepatic angiosarcoma incidence in rats exposed to vinyl chloride in the air (results of MALTONI, after 135 weeks, exposition 52 weeks, 4 hours per day, 5 days per weeks) (± estimated s.d.).

computed curve, but this point corresponds to a V.C. concentration at which the longevity of the animals was reduced by general toxicity.

It is now possible to perform an improved dose extrapolation: For example, what is the risk of angiosarcoma associated with an exposure to 10 ppm of V.C. in the air? According to our simulation 10 ppm correspond to a mean genetic damage of 0.016 nM/g.DNA (in the experimental conditions of MALTONI et al.), and then a risk of 0.08%. MALTONI et al. finds, in an independent experiment, an incidence of 0.8% (with confidence limits of 3.9% and 0.04%). Our estimate falls within the range of probable values. A linear regression would have given an incidence of 6.9%, out of the confidence limits of the experimental result, overestimating the risk.

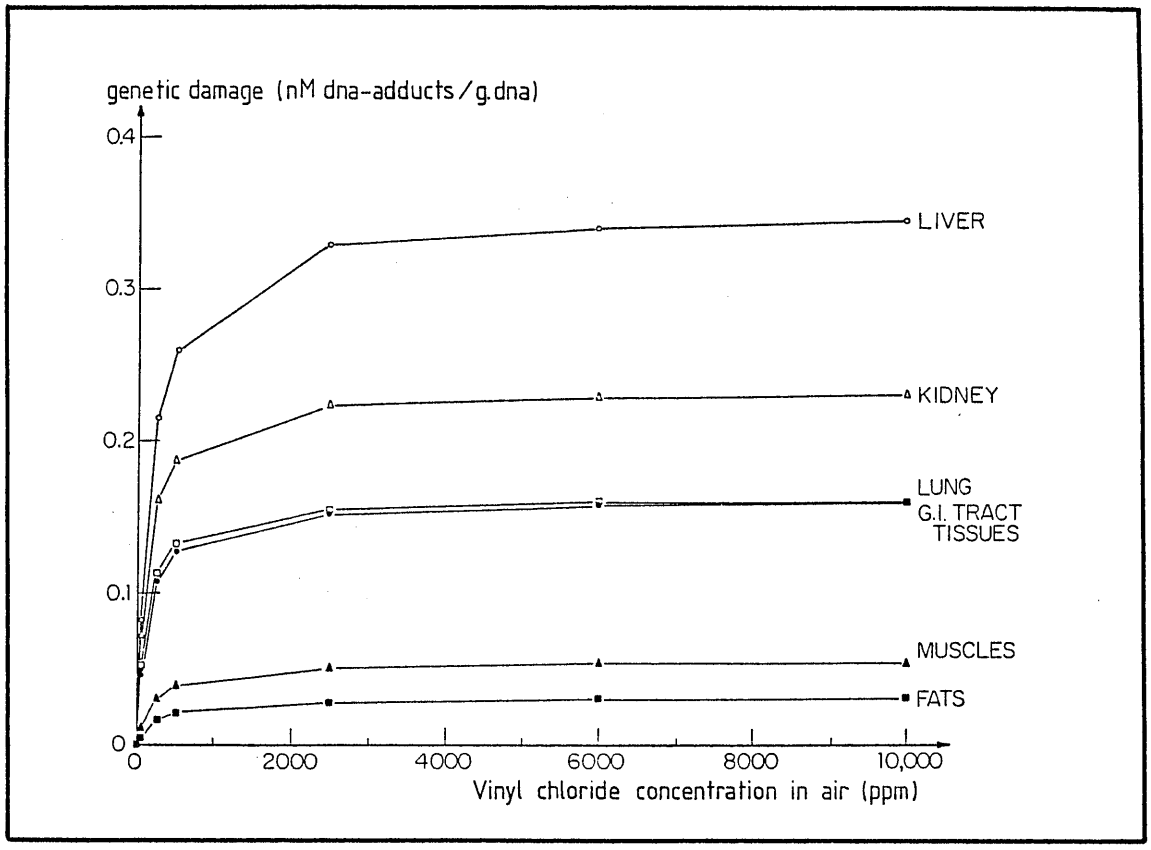

Figure 5: Genetic damages computed by simulation of rat exposure to vinyl chloride in air during 30 mn.

4. CONCLUSION

The main intent of this paper was to demonstrate the usefulness of the integration of pharmacokinetics with a multistage model of carcinogenesis. Non-linearities of the observed dose-response curve for V.C.-induced angiosarcoma are almost completly accounted for by this approach. This allows a better inter-dose extrapolation in spite of the uncertainty that affects our parameter estimates. The accuracy of the results relies heavily on the quality of such estimates and we emphasize the need for better determinations of physiological parameters (mainly blood flows, blood volumes, enzymatic activities in different organs and species) as well as cellular kinetic data (growth and death rates at different stages, differentiation or transformation rates).

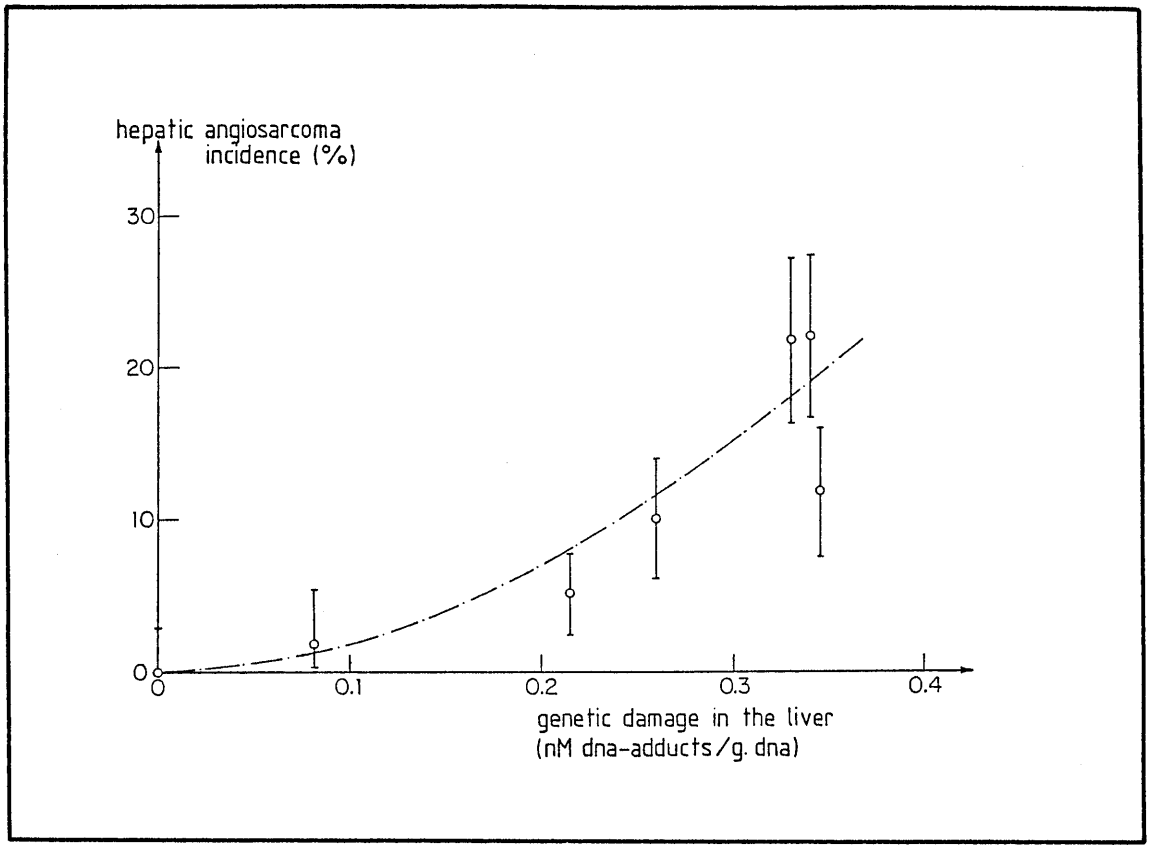

Figure 6: Observed hepatic angiosarcoma incidence in rats versus computed genetic damage in the liver at corresponding air concentrations. The dotted line shows the results of a simulation by the stochastic multi-stage model.

This model requires a more extensive validation: for example oral administration should be simulated, and we have not yet assessed its performance for inter-species extrapolation; This will be the next step of the study.

Beside cancer risk assessment for already commercialized compounds this type of model can be used for a priori carcinogenicity estimation of newly synthetized substances. Such an approach might also serve as conceptual guideline for planning research on chemical carcinogenesis mechanism.

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