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DOSE-RESPONSE ANALYSIS AND BIOLOGICALLY-BASED RISK ASSESSMENT FOR INITIATOR AND PROMOTER CARCINOGENS

SUMMARY REPORT OF NATO/CCMS STUDY

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INTRODUCTION

This is a summary of the detailed report of the Pilot Study on the Dose-Response Analysis and Biologically-Based Risk Assessment for Initiator and Promoter Carcinogens. The extensive report is planned to be published by Plenum Publishing Company, to which it has been made available on informatic support according to the specific requests. This document also includes a proposal for further activity in this field.

The Pilot Study on the Dose-Response Analysis and Biologically-Based Risk Assessment for Initiatior and Promoter Carcinogens was proposed by the Italian delegation in 1990. The pilot study was then accepted by NATO/CCMS, and the detailed scientific programme was defined during the first meeting, held in Rome, April 22–24, 1991. Participating countries were France, Germany, Greece, Italy, the Netherlands, Portugal and USA. In 1994, Belgium was also involved. Since 1991, annual or bi-annual meetings have been held in Germany (University of Bremen), Greece (at care of National Research Centre "Demokritos"), the Netherlands (National Institute for Environment and Health, RIVM, Bilthoven), Portugal (Lisboa, National Institute of Health), USA (at US EPA, 3 meetings), up to the end of 1995, mostly supported by NATO/CCMS grants or fellowships, and organized by Pilot Study participants.

One of the scopes of the Pilot Study was the proposal of a carcinogenic risk assessment procedure which derives the specific form of theoretical and mathematical models of carcinogenesis by considering the relevant biological processes and using the whole set of objective data available, and may also consent the verification of hypotheses. The Pilot Study Report consists of a comprehensive manual, that includes both theoretical and practical information. It has been presented to Plenum Publishing Company.

The main topics treated were:

- Basic Assumptions in Carcinogenic Risk Assessment
- Biological Basis
- Data Sources
- The Use of Biomarkers in Risk Assessment
- Multistage Models
- Biologically-Based Models, including Pharmacokinetic Modelling
- Statistical Considerations
- Case-Studies and Practical Applications

Each of these arguments has been treated with a multidisciplinary approach by several authors, experts in different fields. The Pilot Study activity has been characterized by a highly collaborative atmosphere, which was essential for a deep and detailed analysis of a problem on which different points of view, methodological approaches and regulations exist in the various member countries.

BACKGROUND AND OBJECTIVES

The multistage nature of the carcinogenic process has been recognized since 40 years ago, in particular due to the pioneer research by Armitage and Doll, who proposed a multistage model which was consistent with the increase of tumor incidence with age and the changes induced by carcinogenic agents on the age-incidence curves. Both epidemiological and animal experiment data provided support to the multistage theory of cancer. Successive developments indicated that two different processes, initiation and promotion, were involved in cancer induction. Experimental studies clearly indicated that the exposure to an "initiator", followed by the exposure to a "promoter" could induce high incidence of cancer in experimental animals, while this was not true when the sequence of these exposures was inverted. The recent development of the multistage and initiation-promotion theories resulted in the basic model of carcinogenesis, originally developed in its mathematical form by Moolgavkar and coworkers. This model, usually denoted as the two-mutation clonal expansion model of carcinogenesis, considers the "initiation", "promotion" and "progression" processes. Within this framework, initiation is regarded as the mutation event which transforms a normal, susceptible cell into an "intermediate", precancerous cell ("initiated cell"); promotion is considered to represent the process that leads to the proliferation of one initiated cell and to the development of a clone of initiated cells; lastly, progression is assumed to represent the mutational event that leads to the transformation of an initiated cell into a cancerous cell, which has the capability of proliferating into a detectable tumor. The two-mutation clonal expansion theory derived its basis from both epidemiological and clinical observations, and experimental data. In 1971 Knudson showed that a two-mutation recessive oncogenesis model could appropriately describe and explain the characteristics of retinoblastoma childhood cancer, including the inherited and sporadic form. The identification of tumor suppressor genes, as the Rb (retinoblastoma) gene and p53 gene, and proto-oncogenes, as K-ras, has provided further ground to the theory, showing that specific DNA

"targets" were involved in carcinogenic processes, for which, however, a recessive oncogenesis model, as the one described above, could not be considered as a general one. Moreover, recent experimental work has suggested that more than two mutational steps may be involved in several cancer processes (e.g., colorectal carcinoma). "Biologically-Based Models" for cancer risk assessment are aimed at formally describing mechanisms as the ones above mentioned.

A first objective of the Pilot Study was to provide theoretical and methodological contributions to improve "Biologically-Based Modelling". It is worthwhile noticing that the basic principles, on which the "Biologically-Based Modelling" for cancer risk assessment is founded, allow to extend or build model structure in order to take into account all the relevant biological aspects and experimental data. "Biologically-Based Models" represent a class of models, and not only a single specific model, even if the two-mutation clonal expansion models continue to represent the basic paradigm. An important feature of "Biologically-Based Models" is that their parameters are aimed at representing specific biological parameters, that may be directly measured or estimated from experimental data: this may consent hypothesis generation and testing, and provide some contribution to a better understanding of the examined processes. The modelling procedure proposed in this Pilot Study allows to take into account epidemiological observations, experimental results, dose-response relationships and in vitro data, and may be extended to include cell kinetics, adduct formation and DNA repair, as well as other relevant processes and data. Moreover, together with toxicodynamic modelling, aimed at describing the mechanisms involved in the carcinogenic process, the Pilot Study has also considered toxicokinetic modelling, aimed at translating the external dose into its fraction, or into its metabolite fraction effectively active at the target organ.

Together with the objective of providing a theoretical contribution to cancerogenic risk assessment, the Pilot Study was also aimed at proposing a practical method. This proposal has taken into account that different methods and approaches are adopted in this field by member countries for carcinogenic risk regulation, creating some difficulties in the method of standardization. It appeared that a flexible and biologically-founded strategy in modelling could overcome many of these difficulties, also allowing to analyze the problem through an interdisciplinary approach, jointly considering the various aspects and making easier the integration of formal mathematical modelling into expert judgement.

Resume of Accomplishments

The topics mentioned in the Introduction of this Summary Report also represent the chapters of the extended report, which is in the form of a comprehensive manual, including the scientific and historical background of the topics examined, the detailed examination of the relevant parameters and processes, the theoretical basis of "Biologically-Based Models" for cancer risk assessment, a presentation of formal models, including the mathematical details necessary to build up models in a computer system, a detailed analysis of statistical aspects in particular connected with model fitting and model parameter estimation, a number of case studies and informative cases, providing practical examples of the proposed procedures, and lastly, some conclusive remarks and recommendations.

The review of the procedures adopted in member countries for cancer risk assessment and regulation has pointed out significant differences, more concerning the theoretical and methodological approaches, rather than the final risk evaluation and risk regulation practical criteria. While a formally defined and standardized procedure is adopted in the USA (essentially founded on a mathematical model-based linear extrapolation procedure for low doses), this is not the case for most member countries. However, a significant percentage of European countries uses methods that are based on the no-threshold linearity hypothesis for low-dose dose-risk relationship, at least for genotoxic carcinogens, that appear not to be much different from the ones adopted in the USA, at least from the point of view of final results. In other national contexts, a case-by-case approach is adopted, that makes large use of expert judgement, without a specific reference to a formal procedure. The distinction between genotoxic and non-genotoxic carcinogens is generally considered of most importance in cancer risk regulation. Harmonization of risk assessment methods in member countries appears to be a rather complex but certainly possible goal. The "Biologically-Based Modelling" for cancerogenic risk assessment may offer a useful tool for this purpose. In fact, a wide consensus exists on the theoretical basis on which it is founded; moreover, owing to its capability to include different biological parameters it may consent to take into account evaluations derived from a multidisciplinary approach.

Lastly, the analysis of the recent proposal of using dose-response analysis also for non-carcinogenic risk assessment, through the "Benchmark Dose" (BD) approach, which makes reference to the lower confidence limit of the dose corresponding to a specific response within or close to experimental points (e.g., 10%, 5% or 1%), that is assumed as a substitute of the No-Adverse-Effect-Level (NOAEL), indicates that harmonization of non-carcinogenic and carcinogenic risk assessments is possible. In practise, the "BD" approach may be used for identifying the dose-response starting point for low-dose linear extrapolation (obtaining results comparable to the ones obtained from the classical "Linearized Multistage" procedure), or the dose-response point to which "Uncertainty" or "Safety" factor may be applied. Besides these simple considerations, it may be in general observed that current developments of non-carcinogenic risk assessment are in the direction of "Biologically-Based Modelling", or, in any case, allow a better use of a more extended amount of biological parameters.

The sources of data of possible interest for "Biologically-Based Modelling" are very extended. They may include all the in vitro tests relevant to carcinogenesis, as test for DNA deffects, tests for gene mutations, for chromosomal aberrations, as well as various tests and data providing information on cell proliferation. Dose-response relationships are an obvious and "classical" source of information, used from the very beginning for risk assessment. Toxicokinetic data represent another important set of information, essential for toxicokinetic modelling and for a proper interpretation, use and interspecies extrapolation of dose-response relationships and of parameters derived from them. Moreover, variability in cancer risk and individual susceptibility are today recognized as a point of main importance in cancer risk assessment, and involve scientific, regulatory and ethical aspects.

Biomarkers relevant to cancer risk are today recognized to represent a fundamental source of data, which may integrate and extend the power of epidemiological surveys, may provide basic information for the evaluation of the risk to which human populations, subgroups and individuals are submitted and guide prevention activity. Basically, biomarkers for cancer risk are subdivided in three categories: biomarkers of exposure, biomarkers of susceptibility and biomarkers of effects. Biomarkers of exposure include, for example, DNA adducts,

protein adducts, urinary DNA adducts and DNA repair products; these parameters allow the identification of interactions with relevant macromolecules. The cytogenetic biomarkers of early events (e.g., sister chromatid exchanges, chromosome aberrations, micronuclei and different categories of mutations) may consent the identification of early effects relevant to cancer risk in exposed individuals and may be correlated with the exposure pattern. As it is known, the complex problem of individual susceptibility to carcinogenic agents has been identified through the study of specific biomarkers. Together with biomarkers of early events (as DNA adducts), specific enzymes, specific metabolic parameters and characteristics, as well as individual variability of DNA repair efficiency, and other relevant parameters, have pointed out significant differences (up to one order of magnitude and more) among human individuals and subgroups that may be correlated with different susceptibilities. A number of epidemiological studies have confirmed this finding.

One of the most important features of "Biologically-Based Modelling" is its capability of including these data in risk mathematical description and evaluation. Some of the above mentioned parameters may be inserted in models, or, in other cases, they may be still taken into account by making reference to a distribution of values, instead of single values, for some model parameters (e.g., the transition rate of normal susceptible cell to an initiated cell may be assumed to depend not only on spontaneous rate and on its dose-related increase, but may also be related, for example, to the statistical variability and distributions of adduct formation rates and of other parameters assessed for different human subgroups or individuals, at the same exposure conditions).

One of the mathematical model mostly used for risk assessment is the well known "Multistage Model", originally proposed by Armitage and Doll in the 1950s. The fundamental principle of this model is the assumption that a single malignant cell may become fully malignant only after it has sustained a sequence of irreversible heritable changes. These changes are assumed to occur spontaneously or to be induced by the exposure to carcinogenic agents. A specific form of the Armitage-Doll model, the "Linearized Multistage Model", has been used during last decades for estimating low-dose cancer risk. This model form is characterized by a no-threshold low-dose behavior which is always linear (i.e., the low-dose risk is assumed to be a linear function of the dose), with a low-dose slope that is represented by the upper confidence limit of the model linear coefficient. The "Multistage Model" has encountered much favour in the past because it is easily mathematically tractable and predicts age-specific incidence curves that appear consistent with the ones observed in many epidemiological studies. The "Linearized Multistage Model" form may be also regarded as an empirical fitting of the experimental data, that consents to identify a low-dose linear trend which is compatible with the experimental data.

The theory of multistage model has been extended in order to consider the effects of time dependent and non-constant exposure patterns, the influence of pharmacokinetic processes ruling the distribution of carcinogenic chemicals in target tissues as well as the possible formation of active metabolites and other processes relevant for dose-response analysis and interspecies extrapolation, and the problem of multiple exposure.

However, a critical review of this model has pointed out some pitfalls. First of all, this model only considers the irreversible events (mutations) causing the transition of the involved cell through successive stages, without taking into account the clonal expansion of intermediate and malignant cell population. In other words, it cannot account for processes considered of main importance in cancer promotion. Moreover, some mathematical approximations, even if generally acceptable, may not be considered appropriate in all cases (e.g., in the case of relatively high tumour responses). These and other reasons have stimulated the identification of models more suitable for a detailed description of most relevant biological events.

The "Two-Mutation Clonal Expansion Model", and its developments, were aimed at providing a solution to these problems. This class of models allow the use of parameters which represent biological observables that can be measured or tested, and that have a precise biological meaning. The two-mutation clonal expansion models are essentially based on the assumption that target stem cells are transformed into intermediate or initiated cells as consequence of a mutation process ("initiation"); intermediate or initiated cells can divide in two cells of the same type or can die (clonal expansion of initiated cells is referred as "promotion"); lastly, during the division of initiated cells, one may sustain a second mutation ("conversion") and become malignant. The rates of the above processes may depend on the carcinogen dose at the target. This model category allows for a classification of carcinogenic agents into initiators (increasing the first mutation rate), promoters (increasing the cell proliferation rate of initiated cells), and completers (increasing the transition rate from initiated to malignant cells), with specific reference to model parameters (first mutation rate, initiated cell division and death rates, and second mutation rate, respectively). Anti-promoter and inhibitor agents may also be considered in the same framework.

Moreover, these models may incorporate and use information relative to specific intermediate lesions, detected in some carcinogenic experiments [such as enzyme altered foci (EAF) in rodent carcinogenesis and papillomas in mouse skin painting], considered to possibly represent clones of intermediate cells.

A quantitative formulation of the models has been developed, and is presented in detail in the pilot study together with the mathematical details necessary for practically treating and implementing the model(s) in a normal computer.

As it is well known, carcinogenic risk assessment is in large part effected by using animal experimental data. This is in particular necessary in the absence of epidemiological data. Physiologically based toxicokinetic (PBTK) models are of main importance for estimating target tissue doses and providing a sound basis for interspecies extrapolation. The dose effectively active at the target may represent a fraction of the exposure dose, or of its metabolite(s). PBTK models may appropriately describe both these processes. The consideration of toxicokinetics in animal data-based carcinogenic risk assessment typically imply the use of PBTK models for both estimating the effective dose in the experimental animals and the corresponding human exposure and target tissue dose. Many physiological and metabolic parameters, relevant to carcinogenic risk assessment, have been shown to substantially vary in the different species in agreement with specific relationships with body weight. Many studies have indicated that lifetime, breath duration and rate, heartbeat duration, pulse time, bloodflow rate, as well as the rates of other parameters, are approximately constant across the different species, if expressed in "physiological time", or internal time units. The "internal clock" has been shown to be related to the body weight, according to a specific relationship, that is important for interspecies extrapolation.

A detailed and quantitative presentation and discussion of PBTK models and of interspecies scaling of relevant physiological parameters is included in the pilot study final report.

Carcinogenic risk assessment is typically based on the selection and use of sets of experimental data or observations, on their interpretation in the light of theoretical models, and on data fitting of mathematical models, that are theoretically justified. This process also needs to be regarded in the light of fundamental mathematical and statistical considerations. The evaluation of uncertainty of estimated parameters and of robustness of risk estimations, the definition of the statistical distribution of relevant parameters, and sensitivity analysis of estimates are typical examples of what may be useful for a careful and critical mathematical-statistical evaluation of modelling procedures and results, in particular when applied to specific practical cases. The comparison of estimates obtained from different models in the low-dose range (i.e., the dose range where experimental data are absent and extrapolation is necessary) is particularly important, since this is the dose range of main interest for the large majority of risk assessment practical applications. Simulation exercises may be of help in these evaluations, and provide a valuable tool for hypothesis testing, studying the uncertainty of estimates, as well as for comparing results obtained using different models. A section of the pilot study has been dedicated to these topics.

Biologically-based risk assessment not only represents a theoretical exercise, but, rather, it also represents a methodology that may be used and has been profitably used to analyze specific cases, solve problems and provide risk estimates of practical interest. The examination and critical evaluation of case studies may be very useful for clarifying the characteristics of this approach, highlighting its possibilities and indicating the difficulties encountered. For example, biologically-based modelling has been used for reconsidering epidemiological studies (e.g., radon, cigarette smoking and lung cancer in the case of Colorado Plateau uranium miners; cigarette smoking and the British Doctors cohort; colon cancer in the general population of Birmingham, England), for analyzing animal experiments (e.g., effects of PCBs on initiation and promotion of enzyme altered foci (EAF) in rodents, effects of N-nitrosomorpholine on liver EAF and hepatocellular carcinoma in rodents, as well as the role of cell proliferation in urinary bladder carcinogenesis in rodents, etc.), and for providing suitable extrapolation criteria of risk from animals to humans (e.g., use of PBTK models, in various animal experiments). These examples confirm the usefulness and the advantages of biologically-based modelling procedures, also indicating that risk assessment is not only the result of the application of mathematical formulas, but, rather, it represents a multidisciplinary scientific exercise, that includes the joint evaluation of all available data, hypothesis formulation and testing, and the production of estimates whose uncertainty is carefully presented and discussed. For this purpose, the Pilot Study has examined many case studies and informative examples.

The conclusions and recommendations of the Pilot Study highlight the importance of attentively examining and discussing the mechanisms involved, or possibly involved, in the process under study, with a strict reference to available data, including in vitro and short term tests, animal bioassays, epidemiological data possibly available, cell proliferation data, target tissues, intermediate lesions, biomarkers, relevant metabolic pathways and interactions of parent compound and/or of its metabolites with DNA, and other relevant processes and parameters. The use of biologically-based risk assessment methods is recommended whenever sufficient information is available for this purpose. The evaluation of individual susceptibilities of human individuals is recognized as a major objective for future research. In any case, the use of relevant biomarkers may provide information useful for this purpose, that may be incorporated in biologically-based modelling. Interspecies extrapolation of toxicological parameters should consider both the toxicokinetics and the toxicodynamics of the process of interest. Each risk estimate should include an appropriate uncertainty evaluation. A main conclusion of the Pilot Study is that a model should properly reflect the real physical, chemical and biological processes, capturing the essential biological processes that can be observed, even if this may be so complex to be described in every detail. Within this framework, the questions about low-dose behaviour modifications due to the possible presence of threshold or saturation phenomena are more easily addressed in models that contain parameters which have a biological interpretation, and, therefore, may be measured in principle.