Project DEBtum

Realistic characterizations of the tumor-induction potential of chemicals

Research proposal prepared for STW

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&
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1. Name and address of the applicants

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2. Title

Realistic characterizations of the tumor-induction potential of chemicals.
Code name: DEBtum

3. Investigators

S.A.L.M. Kooijman	Theor.Biol.	VUA-TB
J.J.M. Bedaux	Statistician	VUA-TB
B.W. Kooi	Bioinformatics	VUA-TB
M. Luger	Programmer	STW/VUA-TB
vacant	Toxicologist, modeller	STW
W.F. ten Berge	Toxicologist	DSM
W. Slob	Statistician, modeller	RIVM
C.F. van Kreyl	Toxicologist	RIVM
H.J. van Kranen	Toxicologist	RIVM
W. de Raat	Cell Biologist	TNO-Nutrition
J. Kruse	Chemist, modeller	TNO-Nutrition

Abbreviations:
VUA-TB: Vrije Universiteit Amsterdam, Dept. Theoretical Biology
RIVM: RijksInstituut voor Volksgezondheid en Milieu (Bilthoven)
TNO-Nutr.: Toegepast Natuurwetenschappelijk Onderzoek (Zeist)

4. Estimated duration of the project

4 years.

5. Summary of the project

The proposal aims

- to evaluate existing mathematical models for tumor induction,
- to model the occurence of tumors that result from exposing experimental animals to carcinogenic compounds, in a way that is consistent with the Dynamic Energy Budget theory,
- to test model predictions against experimental and epidemiological data from the literature,
- to find characterisations for the tumor induction process that are useful for environmental risk assessment,
- to write the software package DEBtum that obtains these characterizations from standardized experiments on tumor induction,
- to compare the new method to characterize tumor induction with existing methods.

6. Estimated budget

A letter of support from DSM is attached to this proposal, in which they state to contribute financially to this project if STW will contribute too.

Letters of support from TNO-Nutrition and RIVM are also attached to this proposal, in which they state to invest in additional reseach on modelling the toxicokinetics and on testing models for tumor induction to experimental data, if this project will be executed.

6.1 Personnel (per annum)

1 grad. student (OIO; cell biology/theor. biology) 0.5 technician (comp. science)

6.2 Material support

Standard NWO (to be used for software/computer support)

6.3 Equipment

nill

6.4 Miscellaneous costs

Travel costs 10 kfl

PM: Costs for post-project care are not included

7. Names of collaborators to be employed

vacant (OIO)
Matthijs Luger (technician)

8. Description of the project

8.0. General introduction

This project concerns mathematical modelling and programming, which is the expertise of the Dept. of Theoretical biology (VUA-TB). This department has no experimental facilities and seeks supplementary expert knowledge (about tumor induction in this case) by intensive collaboration with RIVM and TNO. We believe that a strong link between theoretical and experimental research is optimal for scientific progress.

8.1. Motivation

In the systematics of the EU regulations, the potential to induce tumors and to reduce survival is crucial for restrictions of handling chemicals and emissions into the environment. The present evaluation methods can hardly be called satisfactory in a scientific sense.

Members of the team recently developed the software package DEBtox (Kooijman & Bedaux 1996), which extracts characterizations for effects of toxicants on survival, body growth, reproduction and populations growth from results of standardized aquatic toxicity experiments. Hopefully, this method will become an important tool for the assessment of Predicted NoEffects Concentrations (PNECs). The characterizations rest on the Dynamic Energy Budget (DEB) theory, which quantifies the processes of substrate (food) uptake and use by organisms as well as the change in these processes resulting from an internal concentration of toxicant. The package has been written on request by the Organisation for Economic Co-Operation and Development (OECD) and by order of the Dutch Ministry for the Environment (VROM). Discussions have been initiated within the OECD, the U.S. Environmental Protection Agency (EPA), the International Standardization Organization (ISO), the Dutch Normalization Institute (NNI) and other standardization organizations to evaluate the possibility to use this package on a routine basis. The presentation of the application of the method to the results of the OECD ring test on effects on *Daphnia* reproduction (Sheffield, March 1995) and the discussion within SETAC on the statistical

analysis of toxicity experiments (London, April 1995) indicated that we can expect a very positive attitude. These two meetings stimulated us to write DEBtox to facilitate the application of the method. The OECD workshop on the analysis of the results of toxicity tests (Braunschweig, 15-17 Oct 1996) concluded that No-Observed-Effect-Concentrations, which presently play an important role in environmental risk assessment, should be abandoned, in favour of regression methods for mechanistic process-based models (where possible). This is exactly what DEBtox offers.

STW recently granted a research proposal to develop realistic characterizations of the biodegradation potential of chemical compounds on the basis of the DEB theory. The proposal specifies the scope for software package DEBdeg, which will be set up similar to DEBtox. We expect that the packages DEBtox and DEBdeg will stimulate each other in their use, because they together provide the main input in environmental risk assessments. We also expect that the development of the package DEBtum will further enhance the implementation into environmental risk assessments schemes.

The development of the DEB theory is the core of the activity of the Dept. VUA-TB. The DEB theory represents a theoretical framework for animal energetics, which includes the processes of food uptake, growth, ageing and respiration (i.e. oxygen use or carbon dioxide production). It is tempting to extend the theory to include the processes of metabolic transformation of xenobiotics (via a coupling to the respiration rate), tumor growth (which could be treated similar to the growth of body parts, Kooijman 1993, chapter 7) and effects on the ageing process. Ageing in the DEB model results from the effects of free radicals on DNA, followed by an accumulation of 'wrong proteins'. The hazard rate is taken proportional to the concentration of these wrong proteins. Tumor inducing compounds are likely to have a similar mode of action, which translates into an acceleration of the ageing process. This explains the enhanced reduction of weight at high age by such compounds, for instance. Our previous experience with modelling toxic effects will be of help in modelling the tumor-induction potential of chemical compounds, and their effects on ageing and weight ontogeny.

The DEB theory primarily concerns energy aspects of eco-physiological behaviour. Toxicokinetic models have been developed in relation to ecotoxicological research, that involve aspects of the DEB theory in terms of the quantification of exchange rates, which depend o.a. on food uptake, respiration and changes in lipid content of the organism, and of dilution by growth. These models can be classified as a type of PBPK models. Models for toxic effects of chemicals have been developed that also involve aspects of the DEB theory in terms of identification of the physiological mode of action of chemical compounds. These effects concern the organization level of the individual, and only indirectly relate to the molecular mode of action of toxicants. These models for toxic effects can be relevant for tumor inducing compounds that are toxic, because they relate to exposure indirectly. The DEB theory and the models for toxicokinetics and general toxic effects, have to be supplemented with models for tumor induction for the purpose of this project. Several models have been proposed in the literature, which need to be evaluated with respect to their consistency with the models for ecophysiology (DEB), toxicokinetics and toxic effects. This is required, because only the combination of all these aspects can be tested against experimental data; A model for observed tumor frequencies may fail to fit experimental data, not because of lack of accuracy in the molecular aspects of tumor induction, but in the exposure aspects, for instance.

An important application of the DEB theory in this project is the translation of effects from mice to rats to humans. Body-size scaling relations for physiological variables, such as ingestion, respiration and ageing rates, are in the core of the DEB theory. They provide the rules for the required translation of tumor induction over the species, by relating tumor induction to physiological processes.

The aim of the research is to specify a range of models for toxicokinetics, (general) toxic effects and mechanisms for tumor induction and tumor development that is consistent with the DEB theory and spans a range from simple to complex to describe the various modes of actions of chemical compounds. The practical use of these models depends on the availability of data; the more elaborate the set of data, the more sophisticated the model can be to analyse the tumor-induction potential.

Frequently used quantifications of the tumor-induction potential of chemicals do not relate effects to the exposure period of the chemical, and therefore not to its concentration inside the organism. Linear extrapolation is used to predict effect; a situation that does no justice to the

importance of the problem, both for human health care and industrial interests, and for suffering of test animals. A toxicokinetic module seems to be essential for any realistic characterization for a process-based approach.

Both time and location of this proposal seem ideal in view of the following arguments:

- Matthijs Luger coded DEBtox. A great effort has been given to develop a user-friendly interface that runs under MS Windows on a PC and under Unix on workstations. We will apply a similar framework for DEBdeg, which will analyse degradation experiments. If we can appoint Matthijs, we can reduce coding efforts for DEBtum considerably.
- The project could gain a lot from the project DEBdeg, that will run during 1997–2001; and the support that we give for project DEBtox. This applies to both the techical aspects (toxicology, software development) and the implementation aspects (contact with VROM, OECD)
- A formal collaboration contract exists between the VUA-TB and RIVM, which guarantees substantial exchange with RIVM. Slob (RIVM) collaborates intensively with VUA-TB in toxicological projects.

8.2. Program

Basic to any dynamical approach to effects of chemicals is the notion that effects (here tumor induction, survival, decrease of body weight) relate to *internal* concentrations. These concentrations generally build up over some period as a result of dosing or exposure. We therefore need

- a toxicokinetic model that links exposure to internal concentrations. The simplest models assume an instantaneous partitioning among organs; more elaborate models involve more compartment models and metabolic transformation (e.g. the class of PBPK models). The DEB model ties food uptake to surface area, which is approximately proportional to weight$^{2/3}$. Effects on weight translate into effects on intake, if exposure is via food.
- an effect model that links internal concentrations to the
  - tumor induction. A linear increase might be a useful first guess (see later).
  - ageing acceleration. Since tumor induction, like ageing, is via effects on DNA, the speed up of the ageing process is a likely side effect of tumor inducing compounds. The realism of this process is indicated the fact that weight decrease in old individuals is accelerated by most tumor inducing compounds
  - mortality rate. Direct effects on survival (without the loop via DNA; non-genotoxic compounds), might be an important side effect of some compounds that should be accounted for.
- an output model that links effects to observables, i.e.
  - tumors to mortality rate. The mortality rate will probably increase with number and size of the tumors. A proportional increase might be a useful first guess. This requires a (simple) model for the growth of tumors (including their disappearence).
  - ageing acceleration to body weight. The (maximum) surface area-specific ingestion rate is probably affected by the accumulation of wrong proteins, which explains the reduction in food uptake at high age and the resulting reduction in body weight. Tumor inducing compounds enhance this process.
- Translation of tumor-induction rates from mice to rats to humans.

Many mathematical models for tumor induction exist (see e.g. Zeise et al. 1987). The RIVM has done extensive research on the Moolgavkar-Venzon-Knudson (MVK) model. A natural start of a project like this is to review existing mathematical models with respect to the above mentioned essential elements and judge aspects such as:

- biological realism of model assumptions; Goodness of fit aspects,

- balance of model details; Models that are very detailed on tumor induction, for instance, but lack any detail on physiology and general effects on physiology are less suitable for application,
- number of parameters that must be estimated versus amount (and type) of available data and other statistical aspects.

8.3. Plan of action

Year 1

- Existing mathematical models for the tumor induction compounds, and the growth of tumors will be reviewed. Some additional work is necessary to distinguish models that are related on the basis of simple vs more detailed, from models that are structurally different from each other. These differences must be made explicit and methods must be designed to choose between these models on the base of experimental data. The models must be tested with respect to their consistency with the DEB theory.
- Visits will be paid to selected institutions involved in mathematical modelling tumor induction by chemicals.
- Existing mathematical models and mechanisms of tumor induction will be compared.
- Software will be evaluated that is used to file data from experiments, in order to evaluate potential data and to make DEBtum input files compatible with such data files.
- Statistical analyses will be worked out for tumor induction data; this includes goodness of fit tests, hypotheses testing on parameter values on the basis of the likelihood ratio theory, the development of algorithms for profile likelihood functions for the parameters of most interest.
- DEBtum will be set up under Windows (PC) and X-Windows (Unix), similar to DEBtox. The basic models will be implemented. The data input and editing will be completed.

Year 2

- Pharmacokinetic models will be reviewed for application in DEBtum. This involves an optimal compromise between realism and simplicity.
- The realism of the coupling between biotransformation- and respiration rates will be studied; (the DEB theory already specifies the respiration rates and gives rules for the relative size of organs, such as the liver).
- DEB-based model formulations will be completed for tumor growth, effects of ageing on weight ontogeny and survival.
- Numerical procedures of the estimation of parameter values and testing the goodness of fit will be optimized. (The models will be formulated in terms of non-linear differential equations, which require numerical integration. We expect that these equations will be stiff, and require extra numerical care.)

Year 3

- The interaction between general toxic effects and tumor induction will be studied.
- Existing data will be used to test the model formulations on goodness of fit and effectiveness.
- The pre-release version of DEBtum will be completed and sent out for testing to selected institutions and industries.
- Various characterizations for the tumor-induction potential of compounds will be compared and the most useful ones will be identified for routine application in the analysis of bioassays.

- Support will be given to optimize the design of the experiments to test the tumor-induction potential compounds.

Year 4

- Tumor induction rates will be compared for different species, to test the predictions made on the basis of the DEB theory and to extrapolate to human species.
- Experiences and remarks by the selected institutions will be implemented in DEBtum and the final version will be prepared.
- DEBtum will be completed and applied to a set of typical data.
- Compounds will be compared with respect to their tumor-induction potential, to judge the stability of parameter estimates and the difference with existing methods.
- A PhD-thesis will be prepared by the graduate student.
- The documentation of DEBtum will be completed, which includes a series of scientific papers that describe the background.
- The work will be submitted for review by the OECD, VROM and the Gezondheidsraad.

Post-project care

- Support for DEBtum.
- Stimulate the use of DEBtum via meetings, lectures and courses.

8.4. Project organization

The tasks of project members

- Kooijman: Coordination; Supervision of modelling, especially the links with related research within the department VUA-TB; Promotor of the OIO; responsibility for the educational program.
- Bedaux: Supervision of statistical aspects, including numerical procedures for parameter estimation, profile likelihood functions and hypothesis testing. Supervision of the work by Luger. Daily support for the OIO. Copromotor of the OIO.
- Slob: Supervision of applied modelling, especially the links with the MVK model and other work within the RIVM; Modelling of alternatives for DEB-based models.
- van Kreyl, van Kranen: Supervision of cancer biology and other toxicological aspects.
- de Raat: Supervision and research of model validation, risks assessments aspects.
- Kruse: Modelling of toxicokinetics (PBPK models).
- OIO: modelling of tumor induction aspects; data acquisition from literature; testing of alternative models; testing of DEBtum; identification of useful parameters to characterize the tumor-induction potential of compounds; comparing this potential of different compounds and link them to physical and chemical properties; application of DEBtum for testing models of goodness of fit and realism.
- Luger: Coding of DEBtum; testing DEBtum on technical performance; writing of technical documentation that is presented in the helpfiles of DEBtum; organization of testing of the pre-release version by other laboratories and updating DEBtum on the basis of their remarks; preparation of future support via WWW.
- ten Berge: Advice in toxicological and practical aspects, modelling.
- Kooi: Advice on computer and modelling issues.

We plan frequent contacts between the participating groups on the basis of email-exchange.

As is usual for projects within the Dept. VUA-TB, a group of experts will be assembled to monitor and advice the progress of the research and to make sure that the research objectives are met. This group will meet about four times a year on the basis of brief progress reports that are prepared by the OIO. The actions to be taken are written out in notes, which will be checked in each meeting. The members include VUA (Dept. VUA-TB: Kooijman, Bedaux, OIO, Luger), RIVM (van Kreyl, Slob, van Kranen), DSM (ten Berge), TNO-nutrition (W.K. de Raat, J. Kruse), STW (C. Mombers). The committee will set the priorities on the basis of progress. The progress reports, that are prepared prior to each meeting, includes a detailed plan of action till the next meeting.

8.5. Project endpoints

The results of the project will be condensed in papers, and offered for publication in scientific journals. These papers include the PhD-thesis by the OIO. The results will be collected in a document entitled "The analysis of tumor induction data", with software package DEBtum; a setup similar to "The analysis of aquatic toxicity data" by Kooijman & Bedaux, 1996 with software package DEBtox and to the planned publication "The analysis of biodegradation data" for 2001. The document plus software will be available against production plus handling costs.

The floppy will contain software that runs under MS Windows and under Unix. This floppy will only contain load modules, not source code; the source code will remain at the Dept. VUA-TB, to guarantee its integrity. The Dept. VUA-TB will take care for future support, which will be provided via World Wide Web. As for DEBtox and DEBdeg, the support for DEBtum might be transfered to the OECD or to a software house in the future. As for DEBtox and DEBdeg, the Dept. VUA-TB will not seek financial profits from the sales of DEBtum. The general setup and specifications of DEBtum will be very similar to DEBtox. This includes the selection of alternative mathematical models, estimation of parameter values (including standard deviations), profile likelihood functions for the most essential parameter(s), tests of statistical hypotheses about the parameter values on the basis of the likelihood ratio theory, analyses of residuals, tests for goodness of fit, including extensive graphical support. The guiding commission will be given a strong say in adjusting the details of the specifications. Like DEBtox and DEBdeg, DEBtum will be coded in C++.

8.6. Relationship with other research

The Dept. VUA-TB aims to develop theory for energy and material fluxes through biological systems. The relationship between levels of organisation (from the molecular to the ecosystem level) is the focus of research. Applications of the theory are worked out in the fields of biotechnology, ecotoxicity and global change.
Crossfertilization is likely with other projects:

– the support of software package DEBtox.

– the development of the software package DEBdeg for the analysis of the results of bioassays for degradation of compounds.

– modelling and experimental studies of RIVM: project Genetic changes and carcinogenesis.

– Risk Assessment studies of TNO-nutrition.

8.7. Contacts

We followed the strategy to involve existing expertise in the Netherlands into this project. We will use this platform to build up international contacts during the project.

9. Utilisation

The software package DEBtum for the DEB-based analysis of the tumor-induction potential of chemicals, that has to be written in this proposal, is meant to supplement the existing software package DEBtox for the DEB-based analysis of toxic affects, and the software package DEBdeg for the DEB-based analysis of the biodegradation of compounds. These three packages will stimulate

the use of each other, because the are based on a coherent view of the interaction between biota and xenobiotics. All three packages analyse results of standardized bioassays. The care for human health, the suffering of test animals, the law on the use of animals for experiments, the substantial costs of toxicity experiments with mammals and the economic importance for the chemical industries are powerfull motivations to optimize the analysis of the results of experiments to quantify the tumor-induction potential of chemicals. See also the appending letter of support by CTB

We expect that the package DEBtum will be used by all centra that frequently deal with bioassays for genotoxicity. These centra include

- Governmental bodies that deal with environmental problems (VROM, OECD)
- Research institutions that support these bodies (RIVM, EPA)
- Standardization organizations for bioassays (ISO, NNI)
- Chemical industries that develop new chemicals and chemical products
- Research institutions that test and evaluate properties of chemicals (TNO, Notox, consultants such as BKH, DHV)

Just like DEBtox and DEBdeg, the package DEBtum will be offered for evaluation to the OECD (see section 8.1: motivation).

10. References

The purpose of the list below is to support the tekst above and to show some research results of the participants that are relevant in the context of this proposal. No attempt has been made to give an overview of the relevant literature with respect to the subject.

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11. Appendices

Letters of support by DSM, RIVM and TNO-Nutrition which specify concrete contributions to the research that is proposed here.