Project DEBdeg

Realistic characterizations of the biodegradation of compounds

Research proposal prepared for STW

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Date: 29 april 96

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1. Name and address of the applicants

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2. Title

Realistic characterizations of the biodegradation of compounds.
Code name: DEBdeg

3. Investigators

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W.M.de Vos	Microbiol.	LUW
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B. Brandt	Modeller	STW/VUA
M. Luger	Programmer	STW/VUA
A.O. Hanstveit	Microbiol.	PG/TNO-Nutr.
P.I. Muttzall	Technician	PG/TNO-Nutr.
H. Oldersma	Technician	EC/TNO-Nutr.
J. Blok	Microbiol.	STW/BKH

Collaboration:

J.de Bruijn	Toxicologist	VROM-Den Haag
J. Struys	Toxicologist	RIVM-Bilthoven
W. Slob	Biomathematician	RIVM-Bilthoven
N. Nyholm	Envir.Engineer	Tech.Univ.Denmark-Lyngby
D.M. Jones	Newcastle Res. Grp.	Fossil Fuels,U.K.

Abbreviations:
STW: Stichting voor Technische Wetenschappen Utrecht
VUA-TB: Vrije Universiteit Amsterdam, Dept. Theoretical Biology
VUA-MB: Vrije Universiteit Amsterdam, Dept. Microbial Physiology
LUW: Landbouwuniversiteit Wageningen
TNO-Nutr.: TNO-Nutrition & food research, Dept. Environmental Toxicology
BKH: Bongaerts, Kuyper and Huiswaard Consulting Engineers Delft
PG: Procter & Gamble
EC: European Commission, DG XI

4. Estimated duration of the project

4 years

5. Summary of the project

The proposal aims

- to model the microbiological degradation of organic pollutants in fresh and seawater realistically on the basis of the DEB theory
- to test model predictions against experimental data and data from the literature
- to find characterisations for the degradation process that are useful for environmental risk assessment
- to write the software package DEBdeg that obtains these characterizations from standardized experiments on biodegradation of compounds
- to compare the new method to characterize biodegradation with existing methods

6. Estimated budget

6.1 Personnel (per annum)

VUA: 0.75 post-doc (microbiol./theor.biol)
1 grad.student (oio; microbiol./mol.biol.)
0.5 technician (comp. science)
TNO: 0.1 senior scientist (30 kfl)
BKH: 0.05 senior scientist (15 kfl)

6.2 Material support

Standard NWO (to be used for software/computer support)
20 kfl chemicals+chemical analysis LUW. These are total costs, not per year. (Degradation studies involve expensive measurements, including work with radioactive labelled compounds.)

6.3 Equipment

nill

6.4 Miscellaneous cost

20 kfl travel costs (total over 4 year) for frequent visits of the oio and postdoc to TNO and LUW and incidental visits to Lyngby and New Castle.

Two workshops will be organized during the project. STW will possibly receive additional applications for these costs.

6.5 Support

Letters of support by Procter & Gamble, RIVM and Nyholm are appended to this proposal. (Prof.Dr.N. Nyholm, at the TU-Denmark, is the coordinator of the ISO commission for Biodegradation.)

As stated in their letter of support, Procter & Gamble is willing to contribute financially to this project. The details have to be worked out after approval of this proposal by STW. This contribution might cover the costs for TNO and BKH.

The EU,TNO-Nutrition and Procter & Gamble will cover the costs for experimental support (both personell and material); See under 8.1: 'motivation'; This essential support is excluded in this estimated budget because it is complementary. This experimental research will be done, even if this proposal is declined. Both TNO and Procter & Gamble believe, however, that this experimental research will benefit a lot from the theoretical support that is provided by this proposal. (This present proposal only concerns modelling and programming, but gets is basic inputs from supplementary experimentally oriented research.) The research proposals are flexible enough to tune the experimental design and the variables to be measured to the needs of this theoretical study. This is possible because the purpose of these two projects is closely related to

this theoretical one: to develop more realistic bioassays for biodegradation. In practice this means that, within certain constraints, the present proposal is supported by 6 man-year of experimental research with very little extra money for interfacing. The experimental research will be executed at TNO-Nutr. in Delft; the VUA and LUW will contribute in guiding the research theoretically and experimentally, respectively. Additional expertise is brought in by TNO-Nutrition, BKH, Procter & Gamble and RIVM. See 8.4 project organization.

7. Names of collaborators to be employed

VUA: Paul Hanegraaf (post doc)
Bernd Brandt (oio)
Matthijs Luger (techn.)
TNO: Arnbjorn Hanstveit (senior scientist)
BKH: Han Blok (senior scientist)

Since the mentioned persons at the VUA are not under permanent contract, changes are possible.

8. Description of the project

8.0. General introduction

This project concerns modelling and programming, which is the expertise of the Dept. of Theoretical biology (VUA-TB). This department has no experimental facilities and seeks experimental support and supplementary expert knowledge (of biodegradation in this case) by intensive collaboration with experimentally oriented groups. The collaborating partners for this project are
- TNO, a non-profit organization which has a long experience in practical ecotoxicological research with intensive contacts with government and industry
- BKH, a consultancy bureau which recently prepared an extensive review of bioassays for biogradation for the Commission of the European Communities. J. Blok (BKH) and J. Struys (RIVM) collaborated to study biodegradation; See further under 8.4: project organization and 10: References.
- LUW, which has knowledge about fundamental issues of microbial mediated degradation
We believe that a very strong link between theoretical and experimental research for is optimal for scientific progress.

8.1. Motivation

The European Union regulates the use of chemicals through a system of environmental hazard identification (based on standard ecotoxicity and biodegradability tests) and environmental risk assessment. Environmental risk assessment for chemical pollutants requires two main inputs: predicted environmental concentrations (PECs) and translations of these concentrations into biological effects (PNECs: Predicted No-Effect Concentrations).

In the systematics of the EU regulations, the degradation constants of substrates in different environmental compartments are crucial for determining the PEC values. But neither relevant methods nor adequate calculation methods are available for this purpose.

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 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 will be initiated this year 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) indicates 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 asked Kooijman to join the steering committee to guide the discussion on statistical analysis of toxicity experiments, which is planned for Oct 1996. SETAC asked Bedaux to give a course on DEBtox. An invitation for STW and/or reviewers of this proposal is appended for a siminar that will be given on DEBtox.

The development of the DEB theory is the core of the activity of the Dept. VUA-TB and represents a theoretical framework for animal and microbial energetics. Since this includes the processes of both substrate uptake and population growth, it is tempting to extend the methods to include the processes of biodegradation, because lack of reliable knowledge about this process is a weak element in the prediction of environmental concentrations (as functions of site and time). The processes of chemical transformation, transport and photo degradation are elements of the prediction of environmental concentrations that are much better understood and quantified. If the application of the DEB theory to biodegradation would be successful, the combination with the above mentioned characterizations of effects would provide a consistent and scientifically sound basis for risk assessment. Our previous experience with modelling toxic effects will be of help in modelling the biodegradation of toxic compounds that induce an extra lag-phase (apart from the processes of adaptation and selection).

Existing quantifications of biodegradation mainly aim at the fraction of the compound that is left after a standardized period (28 days) and not at degradation processes directly. They also aim at discharges in sewage water treatment plants (Jager and Visser 1994), rather that in surface water and in the sea, which would be much more realistic in practice. Not being process-oriented, the present models do not consider the effects of co-metabolism and diauxic effects. When kinetic models are applied, they usually are inspired from chemical kinetics, such as n-th order kinetics, rather than from microbial dynamics.

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

- An experimentally oriented study started at TNO in January 1996 in collaboration with the Newcastle Research Group on Fossil Fuels and Environmental Geochemistry, to develop realistic experiments to quantify the biodegradation of oil sludges. This study would greatly benefit from this theoretically oriented proposal, while providing essential data to validate the methods that are to be developed within this proposal. (Funding from the EU).

- An experimentally oriented study starts at TNO in medio 1996 in collaboration with Procter & Gamble (Strombeek-Bever), to develop realistic bioassays to quantify the biodegradation of organic pollutants. The above mentioned remark on the highly beneficial exchange between experimental and theoretical studies also applies here.

- Matthijs Luger coded DEBtox. His contract will end in June 1996. 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 plan to apply a similar framework for DEBdeg, which will analyse degradation experiments. If we can appoint Matthijs, we can reduce coding efforts considerably.

- Paul Hanegraaf will receive his PhDegree medio 1996. His thesis deals with the application of the DEB theory to microbial growth and he developed extensions for uptake of different substrates simultaneously. If we can appoint Paul, we have a 'flying start'.

- Bernd Brandt received his Masters Degree (Ir) with honour. During his study, he gained experimental experience with biodegradation studies under supervision by prof.de Vos; After his study, he extended this experience on biodegradation in Australia (Macquarie University, Sydney) for some months.

- The project leader Bas Kooijman worked at TNO in the period 1977-1985 and was TNO consultant in the period 1985-1995. He worked earlier with Hanstveit a.o. on biodegradation problems (Kooijman and Hanstveit 1981, 1979, Kooijman et al 1983). This guarantees that the collaboration between VUA and TNO will be effective. A formal collaboration contract exists between the VUA-TB and RIVM, which guarantees substantial exchange with RIVM. Slob (RIVM) collaborates intensively with the VUA in toxicological projects.

- At this moment the ISO commission on biodegradation is reconsidering the design and evaluation of biodegradation tests, to make them more realistic and more suitable for risk assessment. Chairman of this group, prof Nyholm, collaborated earlier to develop applications of the DEB theory to characterize toxic effects on algal growth (Kooijman et al 1996). Drs.Ir. Blok collaborates with prof. Nyholm on biodegradation issues.

- DEB-based biodegradation analysis will help the international acceptance of DEB-based toxicity analysis, and vice versa, on the basis of consistency arguments.

8.2. Program

Basic to population and ecosystem dynamics are the rules that individual organisms apply for the uptake and use of substrates (food) and nutrients. The Dynamic Energy Budget (DEB) theory, that has been developed by the Dept. VUA-TB, quantifies the mechanisms behind these rules, which specify the processes of feeding, digestion, storage, development, maintenance (including heating), growth, propagation (i.e. reproduction or division) and aging (Kooijman 1993). The term 'dynamic' relates to the changes of these rules during development through the embryo, juvenile and adult stages of animals, or through the cell cycle of unicellular organisms. The rules are strongly interrelated by regulation processes, which explains why they must be studied simultaneously. The theory has been developed originally for heterotrophs (animals, fungi, some bacteria) and recently extended to include autothrophs (plants, algae, other bacteria). The mathematics of the dynamics has been worked out for populations of individuals that apply these rules, using simple assumptions for the interaction of the individuals (such as feeding from the same resource). The Dept. VUA-TB has been involved in a variety of projects that test and evaluate fundamental biological issues, but also in practical applications of the theory such as the purification of sewage water while avoiding the production of sludge.

Ecologically relevant effects of toxicants on individuals can be modelled successfully by relating the parameters of the DEB-model to the concentration of the toxicant inside the organism. Physiologically realistic models have been worked out for the toxicokinetics, which account for changes in size and storage (lipid content). A important practical side result of the application of the DEB theory to effects of toxicants is a method to obtain a no-effect concentration (Kooijman 1995). This is implemented in the software package DEBtox (Kooijman and Bedaux 1996).

We aim to model the processes of degradation of compounds by bacteria. The first study concerns the degradation of compounds that are used as energy source, excluding other energy sources. We first focus on one-species populations of bacteria and account for the following elements

- the bacteria apply the DEB rules for the uptake of the compound

- the bacteria apply the DEB rules for propagation

- the toxic effects of the compound on the processes of assimilation maintenance and/or growth are taken to be consistent with the rules of DEBtox.

- the compound follows simple rules that affect bioavailability

In our studies of toxic effects, we focussed on low exposure levels, where only the most sensitive physiological process is affected. In this project we have to deal with high exposure levels too, which requires the study of simultaneous effects.

The aim is to quantify biodegradation in a way that is applicable to a large class of organic compounds. We will test the applicability, using a limited set of model compounds, which include crude oil and some chlorinated aromats and detergent chemicals, which is selected in collaboration with Procter & Gamble. The availability of labelled compounds will restrict the choice considerably.

Then we study mixed-species populations, where the parameter values for the processes op substrate uptake and use and for the effects differ among species, which leads to a selection process during exposure. We also include changes in bioavailability in this step.

Then we study the situation where the bacteria have several energy substrates available in the bioassay to which the compound of interest is added. Generally the bacteria will first use the most energy-rich substrate and then turn to less energy-rich ones when the concentration of

the energy-rich substrates becomes low. This switching of substrates will be modelled and the processes of co-metabolism quantified.

The models will then be simplified to reduce the number of parameters to be estimated, to arrive at formulations that can be applied on a routine basis for the analysis of standardized bioassays. This is done in such a way that allows the interpretation of the resulting few parameters in terms of the original ones.

Together with these modelling efforts, a bioassay and a measurement program will be designed such that the required parameters can be estimated in an optimal way, given the constraints on the total costs of the bioassay.

8.3. Plan of action

Year 1

- Existing models for the microbiological degradation of compounds will be reviewed (most of this work has already been done; the report by Blok and Balk 1994 provides a good and recent review). 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 tests must be formulated to choose between these models experimentally. The models must be tested with respect to their consistency with the DEB theory.

- Selected institutions will be visited that are involved in modelling biodegradation processes of xenobiotic compounds (see contacts 8.5 and 8.6, P. Chapman Zeneca, U.K.).

- Different methods to quantify microbial degradation will be compared experimentally.

- Support for the development of bioassays will be given from a model perspective, where the concentration of compound is monitored in combination with microbial activity (including the measurement of biomass).

- DEB-based model formulations will be completed for the degradation of pure compounds that have no toxic effects, using mixed species cultures. (Formulations for single species cultures already exist.)

- Statistical analyses will be worked out for biodegradation models; 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; the statistics of stochastic differential equations will be studied as an alternative to generalized non-linear regression methods.

- DEBdeg 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

- Toxic effects of compounds that are to be degraded will be identified and modelled (such effects will depend on the concentration of the compound).

- Bioassays will be set up to monitor the effect of the compound on microbial activity, including a protocol for the measurement of activity. This includes the processes of adaptation and selection. Experiments will be set up to characterize these processes in a practical way.

- The sequential use of different substrates will be studied and modelled with experimental support from outside this project.

- Model formulations for toxic effects and use of more substrates simultaneously will be implemented in DEBdeg.

- The phenomenon of bioavailability will be studied in detail, including the dynamics of chemical species.

- 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 equation will be stiff, and require extra numerical care.)

Year 3

- Modelling sequential use of substrates will be completed.
- The experimental results on adaptation and selection will be analyzed and modelled.
- The pre-release version of DEBdeg will be completed and send out for testing to selected institutions and industries.
- Various characterizations for the biodegradability 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 bioassay(s) to test the degradation of compounds.

Year 4

- Experiences and remarks by the selected institutions will be implemented in DEBdeg and the final version will be prepared.
- DEBdeg will be completed and applied to a set of typical data from the newly developed bioassays and the results will be compared (and reported) with standard bioassays.
- Schemes will be worked out that integrate information about biodegradability (DEBdeg) and about toxic effects (DEBtox) in a way that makes optimal use of the DEB theory that is behind both inputs.
- A PhD-thesis will be prepared by the graduate student.
- The documentation of DEBdeg will be completed, which includes a series of scientific papers that describe the background.
- The book "The analysis of biodegradation data" will be completed and submitted for review by the OECD.

Post-project care

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

8.4. Project organization

The team that will be brought together in this project has very good propects to be most efficient and effective. This is because it has the best knowhow on all aspects of biogradation that is available (both academic and practical, covering microbiological, chemical, environmental, modelling, statistical, computational, industrial and legislation aspects) and many of the team members know each other from earlier collaborations, so they know the expertise of each of the members. (Some of these contacts are mentioned at other places in this proposal.)

The tasks of project members

- Kooijman: Coordination; Supervision of modelling; First promotor of Brandt; Editor of book "The analysis of biodegradation data", which provides the scientific background for DEBdeg (see project endpoints 8.5.).
- de Vos: Supervision of experimental part of the work of Brandt; Second promotor of Brandt.

- Hanegraaf: Study director of Brandt; modelling microbiological aspects of biodegradation, including toxic effects on microbial physiology, uptake of mixtures of substrates, selection in mixtures of species, adaptation; consultant for experimentalists; organization of progress meetings and workshops: preparation of progress reports; organization and integration of inputs from collaborating groups; writing scientific papers (see project endpoints 8.5.).

- Brandt: modelling of degradation aspects; data acquisition from literature; executing a limited number of pilot experiments to support the experimentalists Oldersma and Muttzall scientifically; provides material for progress reports to Hanegraaf; testing of DEBdeg; identification of useful parameters to characterize the biodegradability of compounds; evaluation of concentration levels of compounds that are likely to remain in the environment when the process of biodegradation is ceased; comparing the degradation of different compounds and link them to physical and chemical properties; application of DEBdeg for testing models of goodness of fit and realism.

- Hanstveit: Supervisor of experimental work at TNO; Advice on biodegradation issues; writing papers on the results of the experimental work by Oldersma and Muttzall in a form that can be used by Hanegraaf and Brandt, including material for the progress reports. Communication with the ISO commission on biodegradation.

- Luger: Coding of DEBdeg; testing DEBdeg on technical performance; writing of technical documentation that is presented in the helpfiles of DEBdeg; organization of testing of the pre-release version by other laboratories and updating DEBdeg on the basis of their remarks; preparation of future support via WWW; exercise this support on DEBtox.

- Blok: Data acquisition from literature; Comparison of methods that are developed within this project with standard methods; writing at least one scientific paper on this issue; providing material for the progress reports. Evaluation of methods for characterizing biodegradation in a report for the OECD.

- Oldersma, Muttzall: execution of experiments according to the instructions by Hanstveit, in interaction with Hanegraaf and Brandt; technical support for Brandt.

- Bedaux: Supervision of statistical aspects, including numerical procedures for parameter estimation, profile likelihood functions and hypothesis testing. Supervision of the work by Luger.

- Stouthamer, Schraa: Advice on biodegradation and microbiological issues; Reviewers for the PhD-thesis of Brandt. (Prof. Stouthamer is editor of a journal on Biodegradation, which is published by Kluwer; He collaborated earlier with the Dept. VUA-TB in a project on the reduction of sludge production in sewage treatment plants; Hanegraaf will get his PhDegree from Stouthamer and Kooijman.)

- 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 persons that are employed to excute the project (Hanegraaf, Brandt, Hanstveit, Blok). 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, Hanegraaf, Brandt; and Dept. Microbial Physiology: Stouthamer), TNO-Nutr (Hanstveit), LUW (de Vos, Schraa), RIVM (Struys, Slob), PG (Masscheleyn), BKH (Blok), VROM (de Bruijn) and STW. 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.

Two workshops will be organized during the project, which will include the groups in Newcastle and Lyngby.

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 Bernd Brandt. The results will be collected in a book entitled "The analysis of biodegradation data", with software package DEBdeg; a setup similar to "The analysis of aquatic toxicity data" by Kooijman & Bedaux, 1996 with software package DEBtox. The latter publication is available against production costs (45 Hfl for book, 160 pages, plus 1.5 Mb floppy, published by the VU University Press). The VU University Press is willing to publish DEBdeg, if the sales of DEBtox turns out not to be disappointing. The publication "The analysis of biodegradation data" will be a multi-author book, edited by Kooijman (et al?), with a full description of the scientific background for DEBdeg; It basically comprises the papers that result from this project, plus a chapter on the methodological aspects of DEBdeg; the technical documentation of DEBdeg will be available in DEBdeg itself as helpfiles.

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, the support for DEBdeg might be transfered to the OECD or to a software house in the future. As for DEBtox, the Dept. VUA-TB will not seek financial profits from the sales of DEBdeg. The general setup and specifications of DEBdeg will be very similar to DEBtox. This includes the selection of several types of bioassays, the selection of alternative models for each bioassays (i.e. degradation routes), estimation of parameter values (including standard deviations), profile likelihood functions for the most essential parameters, 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 specifiactions. Like DEBtox, DEBdeg 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.

Two current research projects will especially benefit from this project:

- modelling the eco-physiology of microbial mats; In this project the Dept. VUA-TB collaborates with the Depts of Microbiology of the UvA (Stal) and of the RUG (van Gemerden) in the NWO priority program "Verstoring aardsystemen". The project aims to evaluate effects of disturbance on DMS productions.

- modelling the eco-physiology of the alga *Emiliania huxleyi*; In this project the Dept. VUA-TB collaborates with the Dept. of Biochemistry of the RUL (Westbroek), the Dept. of Microbiology of the RUG (Gieskes), and NIOZ (Riegman) in the NOP program Global Change. The project aims to quantify the effect of primary production on the global carbon cycle.

Both projects involve ecosystem modelling where the degradation of organic compounds and the recycling of nutrients is essential for systems dynamics.

The proposed project will find support from two other projects in the Dept. VUA-TB

- the dynamics of structured populations (i.e. populations of individuals that differ in one of more respect, such as age, cell size, nutrient reserves). This study is part of the NWO priority program on Non-Linear Dynamics (Kooijman is national coordinator of the theme population dynamics); the study will be intensified in 1996 as part of the NWO priority program Massaal Parallel Rekenen (Kooi is coordinator of this project, which involves UU, RUL, UvA, CWI); microbial dynamics and microbial food chains are important topics in this study.

- modelling (subcellular) metabolic control systems, in collaboration with prof. Westerhoff (mathematical biochemistry VUA/UvA). This effort aims to brigde the molecular and cellular levels of organization.

The Dept. Environmental Toxicity of TNO-Nutrition Delft aims at testing the toxicity and biodegradation of compounds for industry and government. This includes the development of

new testing methods and the application of these methods on a routine basis for industry and government. (This department was recently part of the Institute of Environmental Sciences, TNO-IMW.)

The proposal fits very well in the research program of both departments. The Dept. VUA-TB collaborates with the RIVM in a formalized way, on environmental and ecotoxicological issues.

8.7. Contacts in the Netherlands

J.de Bruijn: Ministry for the Environment VROM Den Haag
J. Struys and W. Slob: RIVM Bilthoven
D. van Wijk: AKZO Arnhem

8.8. International Contacts

D.M. Jones: NRG Fossil Fuels, Newcastle (U.K.)
P. Masscheleyn: Procter & Gamble, Strombeek-Bever (Belgium)
N. Nyholm: Tech. Univ. Denmark, Lyngby (Denmark)

9. Utilisation

The main biological input in environmental risk assessment for chemical compounds concerns degradation and toxic effects. The software package DEBdeg for the DEB-based analysis of the biodegradation, that has to be written in this proposal, is ment to supplement the existing software package DEBtox for the DEB-based analysis of toxic affects. Both packages analyse results of standardized bioassays. The bioassays for biodegradation are used very frequently by many laboratoria. One reason is that the law in all countries of the European Union require that biodegradation rates are established for all chemicals and chemical products that are to be traded. Similar requirements exist for all countries of the OECD. For this reason, the bioassays for biodegradation are among the best standardized by the OECD (see references). As explained, there is a great need to improve the realism of the bioassays and the predictions of actual degradation rates in the environment. We expect that the package DEBdeg will be used by all centra that frequently deal with bioassays for biodegradation. These centra include

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

Just like DEBtox, the package DEBdeg will be offered for evaluation to the OECD (see section 8.1: motivation). For this purpose, the OECD will probably seek advice by the ISO. Nyholm, who is chairman of the ISO commission for Biodegradation, wrote a letter of support to this proposal (this letter is appended to this proposal). We expect that the OECD will encourage the use of the new method during a testing period, parallel to the existing methods, and will organize a workshop to evaluate the results. If the evaluation works out positively, we can be sure that the package will be used intensively all over the world. (The OECD includes the European Union, USA, Canada, Australia, Japan and many other countries; The US-EPA generally follows the OECD in this type of environmental issues.)

Since the development of clean technologies will increase in importance in the future, we can expect an increased emphasis on a fast and complete degradation of compounds that are released in the environment. This not only concerns the production of new products, but also recycling

technologies. The availability of reliable methods to measure degradation rates is essential in for this purpose.

The first print of the book "The analysis of aquatic toxicity data", which accompanies DEBtox, comprises 1000 copies. If the sales turn out not to be disappointing, it is reasonable to assume that the first print of "The analysis of biodegradation data", which will accompany DEBdeg, will also comprise 1000 copies, since bioassays on biodegradation are even more frequently applied than bioassays on toxicity. See further under 8.5: Project endpoints.

Please note that the development of risk assessment schemes themselves is outside the scope of this proposal. The focus is on the optimization of biological information as a substrate for such schemes.

10. References

This list of references does not include the huge literature on biodegradation, because any small selection seems to be arbitrary. Many references are given in Blok and Balk 1994. This list just aims to present some expertise of the people participating in the proposal.

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Seminar

Kwantificeren van toxische effecten: Theorie en praktijk

Woensdag 5 juni 1996
de Boelelaan 1085, zaal D107
Amsterdam

Programma

13:30	Opening (Jack de Bruijn, VROM)
13:40	Het concept NEC (Bas Kooijman)
14:30	Discussie
14:45	Thee
15:00	De statistiek van NECs (Jacques Bedaux)
15:45	Discussie
16:00	Demonstratie DEBtox (Matthijs Luger)
16:30	Discussie
16:55	Aanbieding van DEBtox aan VROM en sluiting
17:00	Receptie