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**Degradation rates in the environment:
extrapolation of standardized tests**

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SAMENVATTING

In de risicobeoordeling van chemische stoffen in het milieu is de schatting van afbraaksnelheden essentieel voor een realistische evaluatie van blootstelling van organismen in de verschillende compartimenten. Aangezien QSAR's (quantitative structure activity relationships) voor afbraak nog verre van algemeen toepasbaar zijn, werd een onderzoek uitgevoerd naar de mogelijkheden om resultaten van gestandaardiseerde degradatietoetsen te extrapoleren naar een aantal relevante milieucompartimenten. Het ontwerp van de OECD-laboratoriumtoetsen is zodanig dat, althans op het screeningsniveau, algemene toepasbaarheid dicht benaderd wordt. Daar staat dan tegenover dat deze toetsen geenszins een afspiegeling vormen van afbraak in het veld. Het blijkt echter dat bij de meeste stoffen waarvan biodegradatiegegevens beschikbaar zijn, verwezen wordt naar een van OECD standaardtoetsen.

Het ontbreekt het Beoordelingssysteem Nieuwe Stoffen nog aan een systematische procedure om de resultaten van een (combinatie van) OECD-toets(en) te vertalen naar omzettingssnelheden onder realistische omstandigheden. In dit rapport wordt een methode gepresenteerd waarmee op basis van toetsresultaten een snelheidsconstante kan worden afgeleid voor afbraak in een biologische zuiveringsinstallatie (rwzi), oppervlaktewater en aerobe bodem. Het afleiden van eerste orde biodegradatie-snelheidskonstanten in de verschillende compartimenten voor stoffen die positief zijn in een OECD-toets voor Ready Biodegradability berust o.a. op de aanname dat de halfwaardetijd omgekeerd evenredig is met de totale concentratie van micro-organismen. Een eerste aanzet tot validatie wordt gegeven door de resultaten van een literatuurstudie naar de afbraak van 20 stoffen in het veld te vergelijken met het positieve resultaat in een OECD Ready Biodegradability test.

SUMMARY

Assessing the risk of chemicals for the environment requires an estimation of degradation rates in order to evaluate a realistic exposure concentration for organisms. As QSAR's (quantitative structure activity relationships) for degradation are far from generally applicable, methods are proposed to extrapolate results obtained from standardized degradation tests to the most relevant environmental compartments. The design of the OECD laboratory test methods is such that, at least at the screening level, almost general applicability is achieved. Inevitably, tests conditions do not reflect biodegradation in the real world. However, if data are available on biodegradation it appears that for most chemicals references are made to one of those standardized OECD methods.

The Evaluation System New Chemicals is still lacking a systematical procedure to translate the result of an OECD test (or a combination of test results) to transformation rates under realistic circumstances. In this report a methodology is presented to evaluate standardized test results to rate constants for degradation in a biological wastewater treatment plant, surface water and aerobic soil. The extrapolation of a positive result in OECD screening tests relies a.o. on the assumption that the half-life is inversely proportional to the total concentration of micro-organisms. A first attempt was made to validate this extrapolation by comparing results of reported field studies on biodegradation of 20 chemicals with positive results in OECD Ready Biodegradability tests.

1 INTRODUCTION

The environmental fate of chemicals and their partitioning over the distinguished compartments is most often evaluated by means of mathematical models. The resulting exposure concentrations as a part of the model output may be regarded a prerequisite of the environmental hazard assessment of a chemical. However, the correct input of rate constants for exchange and degradation processes is essential for a meaningful application of model calculations.

For most chemicals exchange rates can be evaluated within acceptable accuracy margins from elementary properties of both the environment and the compound. More difficult is the estimation of rate constants of degradation processes, particularly for biodegradation.

Degradation processes can be both abiotic (e.g. photochemical reactions and hydrolysis) and biotic. In principle there are two approaches obtaining parameters that characterize the degradation rate of a chemical in a particular compartment: a theoretical making use of QSARs (Quantitative Structure Activity Relationships) and an experimental on the basis of standardized test results. Although considerable progression has been made, the general applicability of QSARs is limited to predictions which are *quantitative* only for the reaction between organic chemicals and hydroxyl radicals in air (1) and *qualitative* for hydrolysis (2). In the framework of the project Evaluation System New Chemicals, QSAR's developed by Atkinson (1) are summarized in a RIVM report (3). TNO has incorporated these QSARs in an expert system for the estimation of the life-time of organic compounds in ambient air (4).

Despite large efforts it has not been possible until now to formulate generally applicable QSARs for the major elimination process of chemicals, i.e. aerobic biodegradation in the compartments surface water, soil and the aeration tank of a communal wastewater treatment plant. The only possibility is then to interpret data of experimental studies which in most cases pertain to results of standardized tests.

In this report methods are presented for extrapolating results of a standardized test, or a combination of different tests, to several environmental compartments. The primary objective of this study is to provide substance regulating offices with data they so urgently need: biodegradation rates in communal wastewater treatment plants, surface water and sludge amended agricultural soil. The extrapolation of a positive result of a so-called Ready Biodegradability Test to the aerator of a communal wastewater treatment plant has already been reported (5). However extrapolation to surface water and aerobic soil is, from a microbiologist's point of view, rather controversial and may provoke a technical-scientific discussion. The reason is that the conditions in standardized tests are artificial and do not reflect the real world.

Information extracted from reported experimental studies on biodegradation kinetics in environments that resemble as close as possible natural systems, such as mesocosms and simulation tests, are summarized as a first attempt to validate the here proposed extrapolation methods. In Ch 5 all extrapolation methods are summarized in a table in order to enhance the usefulness of this report.

2 BIODEGRADABILITY TESTS ACCORDING TO THE OECD GUIDELINES

2.1 Detergents: "screening" and "confirmatory"

The large-scale introduction of synthetic surfactants in consumer products during the early sixties was accompanied by environmental problems which were considered as unacceptable. This was not because these chemicals did not degrade in nature but because biodegradation in communal wastewater treatment plants was simply too slow to achieve a high level of elimination. As a response legislation became in force stipulating that *elimination of surface active properties* of these chemicals in systems treating domestic wastewater be at least 80%. Test procedures were designed relying on specific analytical parameters for *primary degradation* which, as a consequence, means for surfactants that a parent compound is converted into a chemical that has lost its foaming properties. OECD experts developed appropriate test methods and related analytical techniques. In 1971 for anionic (6) and in 1976 for nonionic surfactants (7), the following test system was published by the OECD:

- The OECD Screening Test, a static flask test which is relatively simple and considered as an "acceptance test". This 19 d test is very stringent and sorts out "soft" surfactants that do not have to be tested further as a high biodegradability is expected in sewage works. The test is not a "rejection test" as a negative result does not necessarily mean that the chemical is not eliminated in an activated sludge reactor.
- The OECD Confirmatory Test, based on a simulation of the conditions existing in an activated sludge reactor. This test should be used for any surfactant which may not have passed the OECD Screening Test, in order to confirm or disprove the first results obtained. The results obtained at this stage are the only ones to be taken into consideration in the refusal or acceptance of surfactants that did not pass the OECD Screening Test.

The OECD Confirmatory Test is one of the few test systems in which a real world situation is simulated without the need for radio labelled chemicals. The semi-specific analytical method is sufficiently sensitive for monitoring primary degradation at concentration levels that are representative for domestic wastewater being in the range of 0.1 - 3 mg/L.

2.2 Industrial chemicals: the three-tiered OECD test system

Taking benefit from experience gained during the design of test systems for surfactants, the OECD experts in 1981 published a test system that was meant to evaluate the biodegradability of industrial chemicals as a property that form part of their ecotoxicity (8). This test system is in principle suitable for a wide variety of chemicals and was adopted by the EC in 1984 (9). This system is also known under the name "OECD-hierarchy" as three different levels are distinguished:

- "Ready Biodegradability" (RBT), analogous to the OECD Screening Test, *for quickly selecting "soft" chemicals* in order to avoid time and money consuming further research. The main difference with the detergent OECD Screening Test is that RBT methods cover a wide range of physico-chemical and biological properties. This is the reason why at this stage, unlike the test system for detergents, different methods are required (see Table 1) which, however, all rely on the principle that biodegradation is monitored as the degree of mineralisation, by means of summary parameters such as oxygen uptake, carbon dioxide production or elimination of

dissolved organic carbon (DOC). Without employing ^{14}C -techniques this is only possible if the test compound is the sole carbon and energy source for microorganisms. The test duration is 28 d, allowing some adaptation of the microorganisms to the compound but mineralisation as a test criterion adds some extra stringency as it prevents chemicals to pass the test which are only converted into persistent products. In 1990 a harmonisation was finalized which reduced the differences in stringency between the different types of RBTs (10).

- "Inherent Biodegradability" (IBT), *to demonstrate the potential degradability of a compound*. Unlike in RBTs, the conditions for biodegradation are optimal as is indicated by the relatively high population densities given in Table 2. Also these methods have a screening function: to select "hard" chemicals as a negative result indicates that a chemical is too persistent so that, tentatively, no further research on biodegradation has to be done.
- "Simulation" (ST), designed to measure *the rate of biodegradation in a specified environmental compartment*. At this stage the activated sludge test is the only method for which an OECD-protocol exists. It describes a method which is almost similar to the OECD Confirmatory Test, the difference, however, is that mineralisation (without ^{14}C techniques) is analyzed in stead of primary degradation.

Table 1. Ready Biodegradability Tests, revised in 1990 (10). The concentration of microorganisms in the third column corresponds to the maximum amount effluent or activated sludge that is allowed to use as inoculum in the test. These values are derived from literature (11).

RBT	summary parameter	population density (CFU/ml)
Modified OECD Screening Test	DOC	$(0,5 - 2,5) \cdot 10^2$
CO ₂ Evolution	CO ₂	$(2 - 10) \cdot 10^5$
Manometric Respirometry	O ₂	$(2 - 10) \cdot 10^5$
DOC Die-Away	DOC	$(2 - 10) \cdot 10^5$
Closed Bottle	O ₂	$(0,5 - 2,5) \cdot 10^3$
MITI(I)	O ₂	$(2 - 10) \cdot 10^5$

Table 2. Inherent Biodegradability Tests, OECD (1981), ref. (8). The concentration of microorganisms is derived from literature (11) and corresponds to the maximum amount of activated sludge applied in the test system.

IBT	summary parameter	population density (CFU/ml)
Zahn-Wellens Test	DOC	$(0,7 - 3) \cdot 10^7$
SCAS	DOC	$(2 - 10) \cdot 10^7$
MITI(II)	O ₂	$(0,7 - 3) \cdot 10^6$

2.3 Tests that are equivalent to RBTs and IBTs

A test method is equivalent to an OECD Ready Biodegradability Test if the test conditions determining the stringent character of the test are comparable: test duration (28 d), pass level at day 28 (> 60 of 70% of the theoretical) and the quality and quantity of the inoculum. In Table 3 tests are listed that satisfy such criteria and therefore all extrapolation methods that are proposed to RBTs should be considered also applicable to these test methods.

Table 3. Tests that are equivalent to the 1990 revised Ready Biodegradability Tests (10).

test	summary parameter	population density (CFU/ml)
ISO 7827	DOC	$(2 - 10) \cdot 10^5$
NEN 6513	DOC	$(2 - 10) \cdot 10^5$
NEN 6515	O ₂	$(2 - 10) \cdot 10^5$

If the test systems in Tables 1 and 3 are modified in such a way that the conditions become less stringent then the RBT or equivalent test changes into an IBT. Some examples are: prolonging the test duration, the use of higher amounts of inoculum or the use of inoculum that is adapted to the compound.

3 EXTRAPOLATION OF "READY BIODEGRADABILITY"

3.1 Premises and assumptions

An organic compound is referred to as "readily biodegradable" specified by the OECD Guidelines for Testing of Chemicals if the results of a RBT meet the following criteria:

- 1.) not later than at day 28 mineralisation should be at least 60% (O₂ en CO₂ tests) or 70% (DOC tests) of the theoretical, pertaining to complete oxidation. In most cases biodegradation starts after the elapse of some time, t_L (lag phase), after which a plateau of at least 60 or 70% (depending on the type of RBT) should be reached within a specified period of time, t_w. Whether a lag period precedes biodegradation (t_L > 0) or not (t_L = 0), unconditionally should hold that:

$$t_L + t_w \leq 28 \text{ d} \quad (1)$$

- 2.) the time elapsed from the start of the mineralisation process, defined as 10% of the theoretical, till the required plateau is reached should be no longer than 10 d. In OECD terminology this condition is known as the "10 d time window":

$$t_w \leq 10 \text{ d} \quad (2)$$

Rarely condition 1.) is fulfilled but not condition 2.). In most cases the length of the lag period is comparable to t_w and apparently the process is not described by a rate law which exclusively is first order in the concentration of the test compound. Although the concentration-time plot usually has a sigmoid shape, for compounds that have passed a RBT the following assumption is made:

Assumption 1: a half-life not longer than 5 d, in terms of first order degradation kinetics, is derived from the "10 d time window". The first order rate constant of the mineralisation process in a RBT, k_{RBT}, has the following lower limit:

$$k_{RBT} \approx \frac{\ln 2}{(1/2) \cdot t_w} \geq \frac{0,693}{5} \geq 0,14 \text{ d}^{-1} \quad (3)$$

Essential for the extrapolation of a positive result from a RBT to other aerobic compartments are the following three assumptions:

Assumption 2: the first order rate constant, k_i, is proportional to the population density in aerobic water, regardless whether the water in which the microorganisms are present is synthetic mineral medium of a RBT, surface water or pore water in soil:

$$k_i = \text{constant} \cdot [\text{microorganisms}]_i \quad (4)$$

From Table 1 it can be shown that the range 3·10⁴ - 30·10⁴ CFU/ml is representative for the population density in a RBT. As a consequence of Assumption 2, the constant in Eqn 4 is approximately equal to 0.14·10⁻⁵ ml CFU⁻¹d⁻¹.

Assumption 3: degradation of a chemical exclusively takes place in the water phase. Chemicals that are associated with sediment or soil particles are not available for degradation.

Assumption 4: the partitioning of a compound between the particle and the aqueous phase is governed by a thermodynamic equilibrium occurring at a rate which is fast with respect to degradation processes.

3.2 Extrapolation

3.2.1 Surface water

Temporal and spatial variation of population density in surface water may exceed several orders of magnitude, however $4 \cdot 10^4$ CFU/ml is regarded as a representative value. This value is obtained by extrapolating the cell density of fresh water sediment ($1,8 \cdot 10^9$ cells/cm³, ref. 12), presumably having a dry weight content equal to 1500 g/L, to the water column where the dry matter content is 30 mg/L. As this population density is in the range of bacteria concentrations in RBTs, it is assumed that the first order rate constant for microbial degradation in surface water, k_{water} , equals k_{RBT} :

$$k_{\text{water}} = k_{\text{RBT}} \frac{[\text{microorganisms}]_{\text{water}}}{[\text{microorganisms}]_{\text{RBT}}} = k_{\text{RBT}} \geq 0,14 \text{ d}^{-1} \quad (5)$$

3.2.2 Soil

The evaluation of the first order biodegradation rate constant in aerobic soil is based on Assumptions 3 and 4 and on the supposition that all microorganisms counted in soil, exclusively reside in soil moisture or pore water and may contribute to biodegradation. The rate constant for degradation in aerobic soil, k_{soil} , is related to the rate constant in pore water, k_{pw} , and the (dimensionless) distribution coefficient, K_d , which is the mass ratio of the chemical in the particle phase and the pore water phase:

$$k_{\text{soil}} = \frac{k_{\text{pw}}}{1 + K_d} \quad (6)$$

where K_d is obtained from the relationship $\rho_d \cdot K_p$, with ρ_d (kg/L) being the dry weight of solid phase per volume moistened soil and K_p (L/kg) the partition coefficient of the compound in a solids/pore water system. K_p for hydrophobic compounds can be estimated in a simple fashion, making use of the empiric relationship $K_p = 0,5 \cdot f_{\text{oc}} \cdot K_{\text{ow}}$, where f_{oc} is the fraction organic carbon of the particle phase and K_{ow} is the octanol-water partition coefficient of the chemical. The next step is to find the relation between k_{pw} and k_{RBT} . To this end the concentration of microorganisms in pore water must be known if the assumption is used that the rate constant for biodegradation is proportional to the population density in the aqueous phase. In Table 4 the number of microorganisms per mass moist soil is converted into number (CFU) per volume pore water. The samples which were at field capacity, were collected from different localities at two different times. All CFU values, referenced to pore water, are in the range between 2 and $200 \cdot 10^6$ CFU/ml with the range $3 \cdot 10^6$ - $30 \cdot 10^6$ CFU/ml being representative. It is striking that in this series there is no positive correlation between the organic carbon content and the biomass. Based on the difference in population density with respect to a RBT it is assumed that:

$$k_{pw} = 100 \cdot k_{RBT} = 14 \text{ d}^{-1} \quad (7)$$

Table 4. Number of microorganisms per gram soil at field capacity converted to population density in pore water.

sample location (ref.)	% water	% organic carbon	population density (CFU/g moist soil)	population density (CFU/ml pore water)
Maasdijk (13)	13,4	0,6	(13 - 31)·10 ⁶	(97 - 230)·10 ⁶
Maasdijk (14)	5,3	1,8	1,6·10 ⁶	30·10 ⁶
Rolde (13)	13	2,8	(5 - 7)·10 ⁶	(38 - 54)·10 ⁶
Rolde (14)	18,6	3,3	2,1·10 ⁶	11·10 ⁶
Kootwijk (13)	9,4	0,6	(0,8 - 1)·10 ⁶	(9 - 11)·10 ⁶
Kootwijk (14)	6,1	2,2	0,11·10 ⁶	2·10 ⁶
Katwijk (13)	4,2	< 0,1	(0,6 - 0,7)·10 ⁶	(14 - 17)·10 ⁶
Katwijk (14)	3,7	0,3	1,2·10 ⁶	32·10 ⁶

3.2.3 Communal wastewater treatment plants (wwtp's)

De biodegradation rate in a wwtp is empirically derived from a positive RBT result. There is wide consensus that a compound is completely eliminated from the effluent if it is "readily biodegradable". It has been shown that this is also true if the compound does neither adsorb onto sludge nor volatilize so that biodegradation is the only removal mechanism in the aeration tank. The extent of elimination is supposed to be dependent on the mode of operation of the plant, analogously to the BOD removal from domestic wastewater. The lower the sludge loading rate of the installation - and the longer the hydraulic retention time, (T_a) - the higher the removal percentage of both BOD and the chemical is. From mass balance considerations it follows for a non adsorbing and non-volatile chemical that the concentration ratio of the aqueous phase flowing into (C_i) and out of (C_e) the aeration tank depends on the first order rate constant for degradation from the water phase and the hydraulic retention time:

$$\frac{C_i}{C_e} = 1 + T_a \cdot k_{wwtp, \text{ aqueous phase}} \quad (8)$$

It is assumed that if the sludge loading rate is relatively high, corresponding to $T_a = 3$ hr, elimination due to biodegradation (even if sorption and volatilization are negligible) is still at least 90% and at a low sludge loading rate ($T_a = 10$ hr) at least 97%. Correlating these "empirical" removal percentages to the hydraulic retention time gives the first order biodegradation constant *in the aqueous phase*:

$$k_{wwtp, \text{ aqueous phase}} = 3 \text{ hr}^{-1} \quad (9)$$

It should be emphasized that in a wwtp other removal mechanisms may play a significant role. Thus the above mentioned removal figures for chemicals that are "readily biodegradable" should be considered as conservative and may be even higher if partition coefficients for sludge-water and air-water exceed a certain level (5). In the calculation procedure for predicting the emission of chemicals by a wwtp (SimpleTreat, ref. 5) the rate constant in the water phase of the activated sludge is assumed to be 3 hr^{-1} for all chemicals that are positive in a RBT.

3.3 Extrapolations compared to biodegradation rates measured in the field

A vast body of literature is available on biodegradation of chemicals in surface water and, though to a lesser extent, in communal wastewater treatment plants. With the exception of agricultural pesticides, which in view of their application are usually not easily biodegradable, less is known of biodegradation of chemicals in the aerobic soil compartment. In order to validate the here proposed extrapolation models, a selection was made from results of field studies reported in the open and "grey" literature that exclusively deal with fairly biodegradable chemicals. Field study are in this context defined as investigations on biodegradation rates both in natural systems and in artificial systems that simulate as close as possible the real world. An example of the latter is the activated sludge test, which has proved to be predictive to a communal wastewater treatment plant. The below listed RBT results are partly derived from interlaboratory exercises and tests conducted in the RIVM.

3.3.1 Surface water

Compounds tested according the OECD system can be ranked in categories of increasing persistence. On the most persistent side of the biodegradability scale are those chemicals that failed to pass an IBT. On the other end of the scale we find the very "soft" chemicals that not only passed a RBT but reached the pass level already within 14 d. Table 5 contains 5 chemicals that degraded consistently fast both in ring tests and in natural aquatic systems.

Table 5. Chemicals giving a positive result in RBT ringtest programmes within 14 days compared to fast biodegradation observed in surface water ($t_{1/2} < 5$ d).

compound	ringtest programme	$t_{1/2}$ RBT (d)	real world study, lit. ref.	$t_{1/2}$ field (d)
aniline	EC (1981-1982)	<14	Subba-Rao et al. (1982), 15	<5
benzoic acid	EC (1981-1982)	<14	Simkins & Alexander (1984), 16	<5
1-naphtol	EC (1983-1984)	<14	Rogers et al. (1984), 17	<5
2-phenylphenol	EG (1983-1984)	<14	Gonsior et al. (1984), 18	<5
4-nitrophenol	NNI (1980)	<14	Spain et al. (1980, 1983), 19 & 20	<5

A literature search was made for papers reporting half-lives shorter than 14 d in natural waters in order to obtain a validation set of chemicals to be tested in a RBT. All chemicals proved "readily biodegradable" (see Table 6), tentatively corroborating that a positive result in a RBT can be extrapolated to the aquatic ecosystem.

Table 6. Readily biodegradable compounds compared to half-lives derived from reported field studies

test compound	$t_{1/2}$ RBT (d)	$t_{1/2}$ field (d)	real world study, lit. ref.
3-cresol	<28	< 1 d	Pfeander & Bartholomev (1982), 21
4-cresol	<28	< 3 d	Paris et al. (1983), 22; Spain et al.(1983), 20
NTA	<28	< 7 d	Larson (1983), 23
diethanolamine	<28	< 7 d	Boethling & Alexander (1979a), 24
phenol	<28	< 2 d	Paris et al. (1983), 22; Rubin et al.(1985), 25
quinoline	<28	< 4 d	Rogers et al. (1984), 17
monochloro-acetic acid	<28	< 8 d	Boethling & Alexander (1979b), 26
4-acetylphenol	<28	< 2 d	Paris et al. (1983), 22
4-methoxyphenol	<28	< 14 d	Paris et al. (1983), 22

3.3.2 Soil

The number of compounds of which information is available that can be used to validate Eqs (5) and (6) is rather limited. Two synthetic detergents, linear alkylbenzene sulphonate (LAS) and linear alkylethoxylate (LAE), have been investigated thoroughly - also biodegradation in agricultural soil - as their production forms the major share of all consumer surfactants. Reported partition coefficients for LAS in activated sludge are in the range 1000 - 3000 L/kg and for LAE approximately two times higher. Usually soil-water partition coefficients - necessary for the prediction of biodegradation rates - are approximately a factor 10 lower (see Table 7). Predicted biodegradation half-lives for LAS are in good agreement with field studies and although a little pessimistic also for LAE. However these results are acceptable taking into account for the uncertainty of the soil-pore water partition coefficients.

A study on the biodegradation of several chlorophenol congeners in different soil types was conducted in the RIVM in 1988 (30). The reported degradation rates vary sometimes two orders of magnitude and are less consistent the more persistent the compound is. Although 4-chlorophenol is the least persistent and proved positive in a RBT, degradation half-lives still range over more than one order of magnitude. Nevertheless extrapolation seems to overestimate biodegradation in the real world. Moreover the half-life in the field study of 4-chlorophenol pertains to primary degradation in stead of mineralisation. Generally, however, primary degradation of this type of compounds is immediately followed by ultimate degradation.

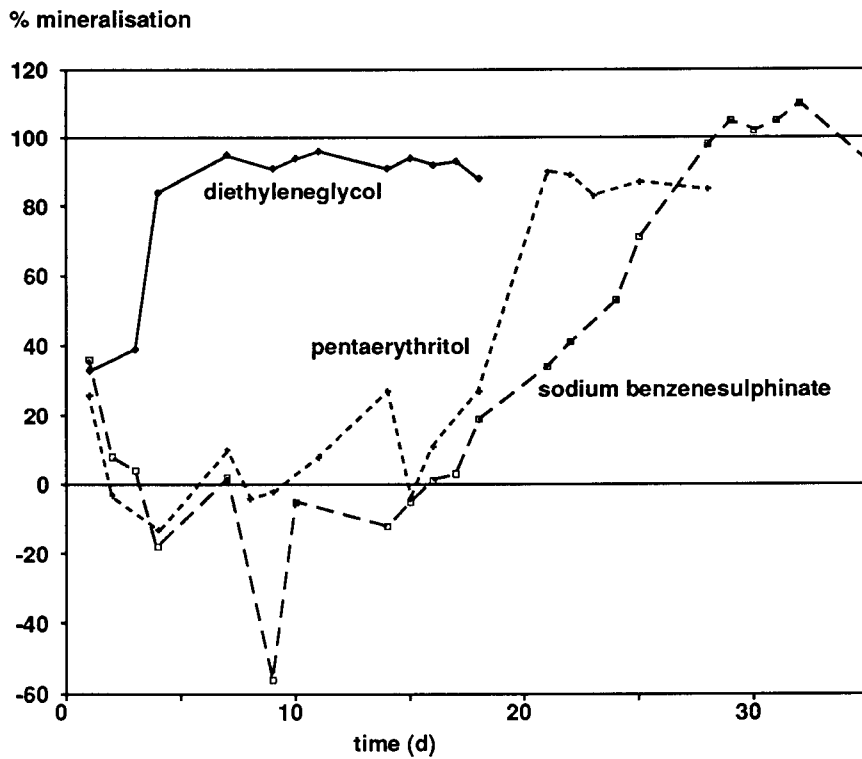
Table 7. Estimated and measured half-lives of three compounds in aerobic soil.

compound	assumed value of K_p (L/kg)	half-life calculated from $\ln 2/k_{soil}$	half-life from field studies (ref.)
LAS	100 - 300	5 - 15 d	1 - 5 d (27) 18 - 26 d (28) <1 - 3 m (29)
LAE	200 - 600	10 - 30 d	1 - 10 d (27)
4-chlorophenol	10 - 30	0,8 - 1,5 d	1 - 30 d (30)

3.3.3 Communal wastewater treatment plants

In OECD and EC interlaboratory exercises it appeared that pentaerythritol, diethyleneglycol en sodium benzenesulphinate are typically borderline cases of "ready biodegradability". To avoid false-positives it should be confirmed that these compounds are consistently and completely eliminated in a communal wastewater treatment plant. Degradation is the only elimination route for these chemicals in a wwtp as air-water and sludge-water partition coefficients exclude any other elimination pathway.

As is shown by Fig 1, these compounds which should be considered as the more persistent among chemicals that are positive in a RBT, are completely mineralized in an activated sludge test. This means that also in communal wastewater treatment plants complete mineralization occurs because the test is a good simulation of the real world. Therefore the conclusion can be drawn that Eqn (9) is rather a pessimistic extrapolation. Unlike the extrapolation to surface and pore water, which relies on the proportionality between the biodegradation rate constant and the population density, the high value of 3 per hr is the consequence of the postulate that a positive RBT-chemical always be completely eliminated in a wwtp, regardless its physico-chemical properties.



Figuur 1 Complete mineralisation of three compounds, considered as borderline cases of "ready biodegradability", in a laboratory system simulating a wwtp very closely.

It would be interesting to see if this value for the rate constant would also be obtained if use was made of the proportionality between population density and rate constant. Table 8 shows that indeed the rate constant for biodegradation in the aqueous phase and the number of microorganisms in activated sludge are consistent with other aerobic water systems. Differences between "population density referenced" first order rate constants, in the last column of Table 8, are less than one order of magnitude.

Table 8. First order rate constants for microbial degradation in the aqueous phase in different aerobic systems referenced to population density.

system	population density (CFU·ml ⁻¹)	k (d ⁻¹)	k/population density (d ⁻¹ ml CFU ⁻¹)
RBT	(3 - 30)·10 ⁴	0,14	(0,5 - 4,7)·10 ⁻⁶
surface water	3,6·10 ⁴	0,14	4·10 ⁻⁶
pore water	(3 - 30)·10 ⁶	14	(0,5 - 4,7)·10 ⁻⁶
aeration tank wwtp	(1 - 10)·10 ⁷	72	(0,7 - 7)·10 ⁻⁶

4 EXTRAPOLATION OF OTHER BIODEGRADATION TEST METHODS

4.1 "Inherent Biodegradability" and "simulation"

No more than a *negative* result of a Ready Biodegradability Test can be extrapolated to the real world as these test are so stringent that no selection can be made on persistence in natural systems, a *positive* result of an Inherent Biodegradability Test has not any predictive value. The circumstances in an IBT are so favourable for microbial degradation that rather a negative result should be evaluated to the environmental compartments. In other words: if a compound failed to pass both a RBT and an IBT then such results should be interpreted as follows:

$$k_{water} = k_{soil} = k_{wwtp, aqueous phase} = 0 \quad (10)$$

A positive result in two out of three IBT's, listed in Table 2, are sometimes assigned a predictive value if complete biodegradation is measured. The Zahn-Wellens Test may be considered as an industrial wastewater treatment plant and the SCAS Test (semi-continuous activated sludge test) as communal wastewater treatment plant, though operating at a very low sludge loading rate which facilitate biodegradation. In this report, according to the OECD test philosophy, this extrapolation is rejected. A positive result in a SCAS-test, however, is a fair indication for biodegradation in a wwtp, but for a compound that is not "readily biodegradable" complete elimination in a wwtp can be exclusively derived from a positive result in an activated sludge test at the "Simulation" level. A compound that is negative in a RBT but positive in a activated sludge simulation test behaves like a "readily biodegradable" compound, *however only in a wwtp*, or in other words: Eqs (8-9) are applicable but not Eqs (5-7).

4.2 Standardized tests for primary degradation

4.2.1 Detergents

"OECD Screening Test" for anionic and nonionic detergents is even more stringent than RBT's and therefore a positive result is evaluated to the real world in a similar fashion as for "Ready Biodegradability", however with the following restriction: rate constants for degradation in water, soil and the aerator of a wwtp pertain only to *primary* degradation.

Extrapolation of the "OECD Confirmatory Test" - in case a compound is negative in the OECD Screening Test but positive in the OECD Confirmatory Test - is quite analogous to the activated sludge test (ST): *primary* degradation rate constant is given only by Eqn (9) but not by Eqs (5-7).

4.2.2 Hydrolysis

The pseudo-first order degradation rate constant for hydrolysis can be determined with method 111 of the OECD Guidelines for Testing of Chemicals (31). Provided that temperature and pH are comparable to test conditions, the obtained rate constant k_{hyd} can be applied directly if no data are available on other degradation processes, such as biodegradation:

$$k_{water} = k_{pw} = k_{wwtp, aqueous phase} = k_{hyd} \quad (11)$$

Hydrolysis is an abiotic transformation process but can be microbially catalyzed, therefore the rate constants given by Eqn (11) should be considered as a lower limit, to be used if no other information is available.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Extrapolation scheme

Table 9 summarizes how to extrapolate test results or a combination of test results, obtained from the OECD system for biodegradability testing (8), to the compartments surface water, soil and *the aqueous phase of activated sludge* in a wwtp. This table is also applicable to detergents if "RBT: +" is replaced with "positive in the OECD Screening Test" and "RBT: -/AST*: +" is replaced with "negative in the OECD Screening Test but positive in the OECD Confirmatory Test". However, the rate constants then only pertain to *primary* degradation.

Table 9. First order rate constants (d^{-1}) for aerobic mineralisation in surface water, soil (for different solids-pore water distributions) and a communal wastewater treatment plant (wwtp). AST* is the activated sludge test; + and - mean "passed the biodegradation test" and "failed to pass the biodegradation test", respectively.

test result -->	RBT: +	RBT: - IBT: +	RBT: - AST*: +	RBT: - IBT: -
k_{water}	0,14			0
$k_{\text{soil}}, K_d = 1$	7			0
$k_{\text{soil}}, K_d = 10$	1,3			0
$k_{\text{soil}}, K_d = 100$	0,14			0
$k_{\text{soil}}, K_d = 1000$	0,014			0
$k_{\text{soil}}, K_d = 10000$	0,0014			0
$k_{\text{wwtp, aqueous phase}}$	72		72	0

5.2 Future research

Most extrapolation methods, presented here, are based upon empirical rather than mechanistic considerations. The "10 days window" in the Ready Biodegradability test is largely determined by the growth rate of specific degrading organisms utilizing the compound as the only carbon and energy source. These conditions are different from natural water systems where the concentration of the chemical is low with respect to other carbon sources that are available for the microorganisms. Nevertheless, we believe that through future research the extrapolation methods will be supported by a kinetic approach.

In this report it is assumed that also in activated sludge biodegradation exclusively takes place in the aqueous phase. A kinetic approach would also provide more insight in the ongoing scientific debate whether or a chemical is only available for biodegradation in the water phase.

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