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# MODELING LIFETIME AND DEGRADABILITY OF ORGANIC COMPOUNDS IN AIR, SOIL, AND WATER SYSTEMS

## (IUPAC Technical Report)

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# Modeling lifetime and degradability of organic compounds in air, soil, and water systems

## (IUPAC Technical Report)

*Abstract*: Degradability of organic compounds in air, soil, and water is the most important factor for evaluating their environment fate as well as possible adverse effects to humans and the environment. The primary degradation process in the troposphere is the reaction with the hydroxyl radical. For water and soil compartments, the primary degradation process is biodegradation. The objectives of this report are: (i) to review published models and their evaluation studies, (ii) to perform an in-house evaluation of general models for estimating tropospheric degradation and biodegradation of organic compounds, and (iii) to recommend reliable procedures for estimating degradability of organic compounds in the environment. The extensive evaluation procedure has shown that the most accurate method for estimating tropospheric degradation is Atkinson's group contribution method. Although this method has some limitations, it seems to be a method of choice. A viable alternative to Atkinson's method is a direct calculation, performed today almost routinely, of the reaction rate constants with hydroxyl radicals. Recently, a methodology based on reliable semiempirical potential energy surfaces was developed that enables the calculation of reaction rate constants within a factor of 2 of their measured values. A partial least squares (PLS) model and a set of seven biodegradation rules have been found to be the most reliable in estimating complete biodegradation of organic compounds. However, it is recommended to use all four evaluated methods to estimate biodegradation in the environment. If their results agree, such estimates are very reliable.

## 1. INTRODUCTION

The persistence of commercial chemicals in the environment [1,2] is one of the most important factors in evaluating their fate in the environment as well as their possible adverse effects [3]. Thus, our ability to measure or reliably estimate possible degradation processes or pathways is of critical importance to environmental risk assessment of commercial chemicals. The primary degradation process in the troposphere is the reaction of chemicals with the hydroxyl (OH) radical, while for the water and soil compartments the primary degradation process is biodegradation. Consequently, the main objective of this project was the collection and evaluation of qualitative and quantitative models for estimating the lifetime and degradability of organic compounds in air, soil, and water systems. An earlier evaluation study has been performed within the EU project QSAR for Predicting the Fate and Effects of Chemicals in the Environment [4–6]. This evaluation study has shown that 200 models were published for various degradation processes in air, soil, and water systems by the first quarter of 1994. The large majority of degradation models were developed for a small set of chemicals, usually functional analogs, and their applicability is quite limited. Furthermore, the evaluation of biodegradation models has shown that they are of limited assistance since their predictive level is low, generally below 70%, and their chemical or application domains are either uncertain or undefined.

The major obstacle that precluded the development of better and reliable biodegradation models in the past was the absence of standardized and uniform biodegradation databases. Several years ago, two databases of “high-quality” biodegradation data became generally available, i.e., the BIODEG database of evaluated and standardized biodegradation data and the so-called MITI database containing the results of a single screening test for “ready” biodegradability in aerobic aqueous medium. Consequently, the last five years has been a period of a very intensive development of new and better qualitative and quantitative biodegradability models by the application of new and advanced computational and statistical methods. The major advances were made in: (i) developing a new generation of qualitative biodegradability rules by the application of artificial intelligence techniques [7–9], (ii) developing a new generation of quantitative biodegradability models by the application of partial least squares (PLS) discriminant analysis [10], and (iii) the application of MultiCASE program to select structural fragments that are critical for biodegradability of organic pollutants and to create automated predictions of aerobic biodegradation of chemicals [11,12].

In this report, an extensive evaluation of general models for estimating tropospheric degradation and complete biodegradation (mineralization) will be given (i.e., the most important environmental endpoints for predicting the persistence of a chemical). First, we will describe the structure and characteristics of large data collections used to develop and evaluate, and, consequently, the main characteristics of those models will be described. The body of this report concerns the results and discussion of extensive evaluation of general models for estimating tropospheric degradation and biodegradation. Models will be evaluated in terms of their accuracy and range of applicability. A particular emphasis will be given to the results of external validation (i.e., estimation of degradation values that are not used in the development of a particular model). All limitations of individual models will be clearly described. Finally, recommendations will be given on the reliable application of predictive models for estimating lifetime and degradability of organic compounds in the environment.

## 2. DATABASES USED TO DEVELOP AND EVALUATE DEGRADATION MODELS

### 2.1 Gas-phase reaction rates with OH radicals

The reaction with the OH radical is the major chemical loss process for the majority of organic compounds emitted into the troposphere. This removal process is critical for the effects of present and future emission of anthropogenic halocarbons on the ozone formation in urban as well as rural areas, stratospheric ozone depletion, long-range transport of chemicals, acid deposition, and global climate change. A large amount of experimental data concerning the tropospheric transformation of emitted organic compounds with the OH radical has been obtained from laboratory and ambient air studies over the past three decades [13–16]. However, to allow that the available data are efficiently used by other scientists (e.g., chemical modelers), they must be continuously critically reviewed and evaluated [15,17].

The database of evaluated kinetic data for the reactions of OH radical with various organic compounds has now about 500 recommended reaction rate constants [13,14,16]. The information on reaction rate constants covers the entire temperature range for which kinetic and mechanistic data are available. Data from relative rate constant studies are re-evaluated on the basis of the recommended rate constants for the reference reactions. The overall error limits for the absolute rate constants are expected to be 10–15%, except for the most recent studies for which the overall error limits have been reduced to 6–10%. For relative rate constant studies the two least-squares standard deviations seems to be more realistic due to the additional systematic uncertainties associated with the rate constant for the reference reaction. In addition to providing a recommended set of kinetic data, those reviews and evaluations provide an up-to-date status of the kinetic and mechanistic information available. Also, these reviews are the most reliable source of data for the development of techniques for rate constant and mechanism estimations.

## 2.2 Biodegradation data

Two databases of “high-quality” biodegradation data are generally available. The first is the BIODEG database from Syracuse Research Corporation [18,19] with evaluated biodegradation data for about 300 diverse commercial chemicals. The second database is a collection of so-called MITI-I data [10,20], results of a single uniform biodegradation test for nearly 900 commercial chemicals.

The MITI-I test is a screening test for “ready” biodegradability in an aerobic aqueous medium and has been described by OECD [21] and EU [22] test guidelines (OECD 301 C and EU C.4-F). The MITI-I test was developed in Japan, and it now constitutes one of the six standardized “ready” biodegradability tests described by EU and OECD regulations. For the MITI-I test, 100 mg/L of test substance is inoculated and incubated with 30 mg/L sludge. Biological oxygen demand (BOD) is measured continuously during the 28-day test period. The pass level for “ready” biodegradability is reached, if the BOD amounts to $\geq 60\%$ of theoretical oxygen demand (ThOD). Biodegradation data determined according to the MITI-I test protocol are now available for 894 substances of widely varying chemical structures [20,23,24]. The majority of data has been published [23], and a smaller fraction has been obtained through the Japanese Existing Chemicals Law program directed by the MITI [24].

The biodegradation data from both databases are discrete values (i.e., chemicals are classified as “ready” or “not ready” biodegradable). This type of biodegradation data is perfectly suited for a direct evaluation and development of qualitative models or classification rules. On the other hand, this type of data is not well suited for evaluation and development of quantitative models since their biodegradation estimates and/or predictions are continuous values, which must be reinterpreted or reclassified to make them compatible and comparable with the binary nature of BIODEG and MITI data.

## 2.3 High production volume chemicals (HPVCs)

In 1993, the European Union (EU) adopted the Regulation (EEC) 793/93 on existing chemical substances [3] as a first step toward managing the potential risks posed by chemicals to man and the environment. This regulation is divided into three main parts: data collection, priority setting and risk assessment. The data collection part focused first on the high production volume chemicals (HPVCs), i.e., those chemicals that have been imported or produced in the EU in quantities exceeding 1000 metric tonnes per year. The list of HPVCs consists of 2492 substances, of which 1073 are single compounds to which a structural formula can be attributed.

## 3. TROPOSPHERIC DEGRADATION

## 3.1 Description of models

### *3.1.1 Atkinson’s method*

The Atkinson’s group contribution method [13,25–27], developed to estimate OH radical reaction rate constants, is based on: (a) four possible reaction pathways and (b) an additive fragment contribution scheme. The four possible reactions by which the OH radicals can react with organic compounds are: (i) hydrogen atom abstraction, (ii) addition to double and triple bonds, (iii) addition to aromatic rings, and (iv) reaction with nitrogen-, sulfur-, and phosphorus-containing groups. It is assumed that the reaction rate constants can be estimated separately and that the estimation of the overall reaction rate constant of a chemical can be made by the additive group contribution approach. The overall reaction rate is the sum of all possible individual reaction rates. The use of well-established reaction pathways has a sound physical and mechanistic background. The assumption that their reaction rate constants can be estimated separately has never been proven. The group contribution scheme is based on statistical arguments. The most recent version of this method [27] has 89 empirical parameters, i.e., 26 group rate constants and 63 substituent factors, to estimate the rate constants for three of four possible reaction pathways. The reactions rate constants for the addition to aromatic rings are estimated from the electrophilic substituent constants ($\sigma^+$).

### 3.1.2 MOOH method

Recently, Klamt [28,29] has developed the system of nonlinear quantitative structure–activity relationship (QSAR) models for the estimation of OH-radical reactivity from molecular orbital (MO) calculations. To make predictions, the ground state of an organic molecule has to be optimized using the AM1 parameterization in the MOPAC program [30]. This system of models covers the three most important reactions of OH radicals with organic chemicals, i.e., hydrogen abstraction from aliphatic carbons, addition to carbon double bonds, and addition to aromatic rings. Reactions with oxygen-containing compounds have been modeled in a later version of the system [29], but reactions with nitrogen-, sulfur-, and phosphorus-containing groups are not taken into account. The models developed are based on calculated descriptors such as charge-limited effective HOMO-energy or energy-limited effective HOMO-charge. The six newly calculated MO descriptors have been devised and all are combinations of MO energies and atomic charges on appropriate reaction centers. A nonlinear optimization procedure was performed to obtain regression coefficients. The calculated MO energies, as well as the atomic and MO coefficients used to derive those descriptors, have a clear physical meaning. However, the physical meaning of the MO descriptors derived has never been clarified. Unfortunately, this promising approach cannot be applied to nitroaromatic compounds and halomethanes.

## 3.2 Evaluation and validation of models

### 3.2.1 Atkinson's method

The Atkinson's group contribution method has been validated by the authors [26,27], as well as by an independent study [31]. The validation of the most recent version of Atkinson's group contribution method [27] has shown that for about 90% of 485 organic compounds the calculated reaction rate constants are within a factor of 2 of the experimental reaction rate constants. Disagreements most commonly occur between experimental and calculated rate constants for halogen-containing compounds, and in particular for haloalkanes, haloalkenes, and halogenated ethers [32]. Disagreements also arise for ethers, especially for polyethers and cycloethers [25,33–35], certain haloalkenes [25], and haloalkanes-containing $–CX_3$ (X = F, Cl, Br) groups [13]. Also, the group rate constants and the substituent group factors of nitrogen containing organic compounds are only valid for alkyl-substituted amines [27]. Finally, this method is not working for perhalogenated compounds. It seems that the present estimation technique [27] is reasonably reliable when applied for chemical classes used in its derivation. However, its use for compounds that belong to classes other than those used in its development is discouraged.

The results of an independent validation study with 369 chemicals [31] show that 90% of reaction rate constants were estimated within a factor of 2. Only for 14 compounds (~4%), the estimated reaction rate constants deviate more than a factor of 3 from the experimental values. Furthermore, this study showed that larger deviations are apparent for several chemical classes: compounds with 3 halogens on the same carbon atom, phosphates, small heterocyclic rings (epoxides and aziridines), nitroalkanes, and aromatics that are not benzene derivatives. Unfortunately, this validation study has been performed with an older version of Atkinson's method [26]. However, Kwok and Atkinson [27] have confirmed that some of those problems still apply to the latest version of their estimation method. We have recently estimated reaction rate constants for those 14 compounds by the updated version of Atkinson's method [36], and deviations were larger than a factor of 3 for only 5 compounds. Significant deviations were found for trifluoromethane (factor of 5.2), 1,1,1-trifluoroethane (factor of 8.8), and *o*-nitrophenol (factor of 4.8). Atkinson's method was also evaluated within an EU project on modeling the fate of environmental pollutants [37], and it was found that this method is very accurate. For a dataset of 460 organic chemicals, the estimated reaction rate constants have a mean squared error of only 0.051 log units (factor of 1.12).

It is fair to conclude that the evaluation results have shown that Atkinson's method is very accurate and can be applied to a large and diverse range of chemical classes. Thus, we would recommend it as a method of choice, particularly for novices and laymen in this area. However, Atkinson's method should not be used to estimate tropospheric degradation of haloalkenes and haloalkanes with $–CX_3$ (X = F, Cl, Br) groups, perhalogenated compounds, and ethers (especially polyethers, cycloethers, and halogenated ethers). Also, the estimates obtained for compounds from chemical classes not used in the development of Atkinson's method should be used with caution, and those results will require a careful analysis and confirmation by other estimation methods.

#### *3.2.2 MOOH method*

The MOOH method has also been evaluated and validated by the original author [28,29] as well as by an independent study [37]. The system of models for hydrogen abstraction, addition to aromatic ring, and addition to double bonds has been developed using a training set of about 170 organic compounds. The average error of the estimated rate constants for the training set was 0.19 log units (factor of 1.54), once a small number of outliers were excluded. A test set of 38 chemicals was used to validate the predictive potential of the models. The average residual within the test set was 0.28 log units (factor of 1.9), and all estimated reaction rate constants were within a range of 0.48 log units of measured values. The result of this external validation test is very satisfactory, but it is not clear why only a part of available data has been used as the test set. It must also be pointed out that this method cannot be applied to nitroaromatic compounds and halomethanes. An extension of this method for oxygenated compounds (alcohols, aldehydes, ketones, organic acids, and their esters) was achieved, using about 100 organic compounds. The average error of the estimated rate constants for the training set of 93 chemicals was 0.21 log units (factor of 1.6). Again, a small number of outliers were excluded from the training set.

The MOOH method and its extension were also evaluated within the EU project on modeling the fate of environmental pollutants [37]. For the large dataset of 460 organic chemicals, it was found that the estimated reaction rate constants have much larger mean squared error than reported in the original studies [28,29], i.e., 0.609 log units (factor of 4.06). It was also revealed that the MOOH method systematically underestimates the reaction rate constants with hydroxyl radical.

The evaluation results show that the MOOH method is less accurate than Atkinson's method. However, there are still two areas where the MOOH method can be useful: (i) for chemicals for which Atkinson's method gives unreliable estimates and (ii) for compounds from chemical classes not used in the development of Atkinson's method.

### 3.3 Tropospheric degradation of high production volume chemicals

The Atkinson's method (AOP 1.83) was applied to estimate the reaction rate constants with the OH radical in the troposphere for 917 single compounds from the EU list of HPVCs. The MOOH method and its extension were applicable for 864 discrete HPVCs. To illustrate the degradability of these compounds in the troposphere, the estimation results are graphically presented in Fig. 1 [37]. It is clear that both methods estimate that the large majority of HPVCs will be easily degraded in the troposphere and that their half-lives will be below 10 days. Estimated tropospheric half-lives are based solely on the reaction with OH radicals. Thus, if other transformation pathways are feasible for HPVCs, such as reaction with ozone and the nigh-time reaction with nitrate radicals, their half-lives will be even shorter.

To compare the estimates obtained by those two methods, the MOOH predictions of the tropospheric half-lives have been plotted against the AOP predictions for the HPVCs list. The result is illustrated by Fig. 2. Most MOOH predictions are located above the diagonal line Y = X, indicating that the MOOH method systematically yields a longer tropospheric half-life. Such behavior of the MOOH method has already been noted in the previous section. Those results are in line with our conclusion that the estimates obtained by Atkinson's method are more accurate and that it should be used as a method of choice.

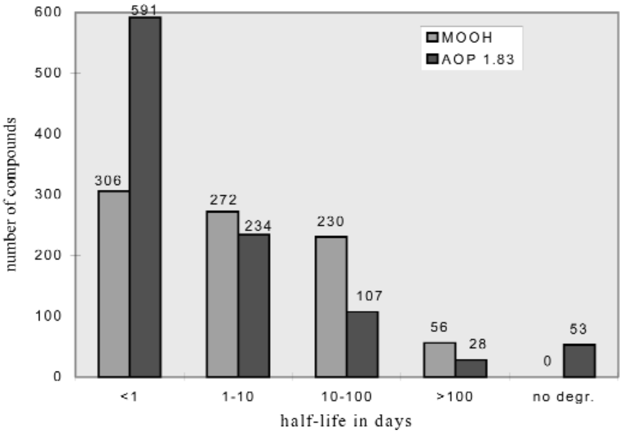

**Fig. 1** Number of compounds in the EU HPVCs list as a function of the estimated tropospheric half-life.

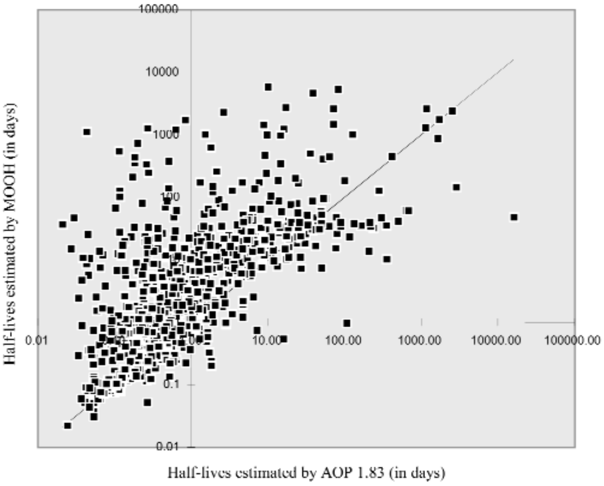

**Fig. 2** MOOH estimates of atmospheric half-lives for reaction with OH radicals vs. AOP estimates for the HPVCs.

### 3.4 Calculation of reaction rate constants

A viable alternative to Atkinson's and MOOH methods for estimating tropospheric degradation and tropospheric half-lives of organic chemicals is a direct calculation of their reaction rate constants with hydroxyl radical. The tremendously fast developments in computing technology, hardware, and software have enabled the calculation of the energy profiles of gas-phase reactions with OH radicals. The abstraction of hydrogen atoms by hydroxyl radicals, as well as its addition to the double bonds, have been a subject of theoretical investigations with semiempirical [38–40] and accurate *ab initio* [41–52] MO calculations during the last decade. The early efforts have focused on studying structural and energetic properties of reactants, products, and transition-state structures of hydroxyl radical reactions with small hydrocarbons and their halogenated derivatives [41–43,48–51,53].

More recently, a detailed analysis of the minimum energy path has been performed [39,45,47,54] by the variational transition-state theory augmented with the multidimensional semiclassical tunneling approximations of hydrogen abstraction from ethane, fluoroethane, and chloroethane by hydroxyl radical. This approach has been used to calculate the reaction rate constants for temperatures from 200 to 1000 K. The contribution from the tunneling effect was evaluated using the semiclassical zero-curvature and small-curvature tunneling approximations. The calculated thermal reaction rate constants agree well with the experimental results [54].

For molecular systems that are much larger than those described above, a detailed *ab initio* analysis of reaction-path dynamics is computationally too demanding to be generally useful. Thus, the standard PM3 Hamiltonian is reparametrized to obtain reliable semiempirical potential energy surfaces for the reaction of ethane with the OH radical and to derive an affordable methodology for calculating reaction rate constants at low computational cost [39]. The specific reaction parameters so obtained are used to calculate the reaction rate constants of several alkanes with up to eight carbon atoms, and all calculated reaction rate constants were within a factor of 1.5 of experimental values. Furthermore, the same PM3 reaction parameters were used to calculate the reaction rate constants for the fluoroethane and chloroethane abstraction reactions. The computed rate constants differ from experiment by, at most, a factor of 2.5. Recently, the same approach was applied to polyhalogenated alkanes, and it was found to be successful too [40]. Thus, it was demonstrated that the specific reaction parameters can be used for analogous reactions of the same mechanism, and it seems that the reaction rates for larger hydrocarbons and their halogenated derivatives can be calculated reliably at a low computational cost.

## 4. BIODEGRADATION

### 4.1 Description of models

#### *4.1.1 BIODEG models*

The Biodegradation Probability Program (BIODEG) by Howard *et al.* [18,55] estimates the probability for the rapid aerobic biodegradation of an organic chemical in the presence of mixed populations of environmental microorganisms. Thus, it classifies organic chemicals as either being easily or slowly biodegradable. Estimates are based upon fragment constants that are developed using multiple linear and nonlinear regression analyses. The evaluated dataset (BIODEG database) [19] was used to select 36 chemical fragments plus a molecular weight fragment that have a potential effect on biodegradability. 295 chemicals were used to derive the fragment probability values that are applied in the BIODEG models. The dataset consists of 186 easily biodegradable chemicals and 109 slowly biodegradable chemicals. The linear and nonlinear models perform equally well. In the evaluation of biodegradation models, we will only consider the linear regression model.

#### *4.1.2 PLS biodegradation model*

This model predicts whether a substance is readily biodegradable or not-readily biodegradable under MITI-I test conditions. The MITI-I test is a screening test for “ready” biodegradability in an aerobic aqueous medium, and it has been described as an Organization for Economic Cooperation and Development (OECD) and EU test protocol [21,22]. The PLS model was developed in several steps. The biodegradation data measured according to the MITI-I test protocol were collected and evaluated for a large dataset of 894 substances of widely varying chemical structures. A set of 127 predefined structural fragments was selected, and a descriptor matrix was developed indicating the absence or presence of each fragment in the 894 chemicals. A model was developed in which the MITI data were correlated with the structural fragments using PLS discriminant analysis. PLS is a projection method that relates the information in the biodegradation dataset to the systematic variation in the structural fragments using latent vectors. The PLS model for biodegradation generates predictions on a continuous scale. Thus, a transformation is needed to compare those predictions with the original binary data for biodegradability. The continuous scale is divided into two areas, >0.55 and <0.45, corresponding to readily biodegradable and not-readily biodegradable chemicals, respectively. Estimated values between 0.45 and 0.55 should be considered as borderline cases and preferably should not be used [24].

The examination of the full dataset of 127 fragments indicated that 44 fragments have positively signed PLS regression coefficients, and thus have an enhancing effect on the biodegradability of a chemical. The two most important positively signed fragments are long nonbranched alkyl chains. These structures are generally known to be susceptible to oxidation, resulting in the formation of carboxylic acids, via primary n-alkyl alcohols and aldehydes. Other fragments associated with a significant positive effect on the structure's biodegradability are the presence of one or more hydroxyl group(s) attached to a chain structure, and one or more carbonyl, ester, or acid groups attached to either a chain or ring structure. Chain structures with these fragments are susceptible to common oxidation processes that involve the formation of carboxylic acids through the intermediate formation of aldehydes as described before. The aromatic ring structures with these fragments degrade through the formation of catechol followed by ring opening.

Of the 127 structural fragments, 83 fragments were associated with negatively signed regression coefficients, indicating that they had a retarding effect on the biodegradability. The most important fragments with a retarding effect on biodegradability are fragments indicating the presence of one or more aromatic rings, and fragments related to the presence of one or more halogen substituents on either a chain or ring structure. These findings are consistent with general experience. The degradability of halogenated chemicals has been the subject of many experimental studies, and it has been reported generally that aerobic biodegradation decreases with increasing degree of halogenation. Several explanations have been postulated for the retarding effect of halogens on biodegradability, i.e., to the possible inhibitory effects of the substance to the microbial community and/or to an electron-withdrawing effect of halogen substituents, making oxidation of the ring or chain structure more difficult. In any case, such structures are not expected to be degradable under aerobic conditions in a wastewater treatment plant or in the environment.

The PLS biodegradation model has shown very good classification ability, about 85% for both degradable and nondegradable chemicals. However, no predictions were made for about 10% of all chemicals because their estimated values were between 0.45 and 0.55. As described earlier, this is the borderline area between readily biodegradable or not-readily biodegradable substances, and those estimates are not reliable and should not be used.

The influence of interactions between fragments within the same molecule was also investigated [24]. A two-step variable selection was performed in the development of the model with fragment–fragment interactions to keep the model size manageable. For the variable selection, only 97 fragments were included that were present in at least five substances. The most important fragment–fragment interactions were then selected on the basis of their PLS regression coefficients, i.e., the additional 706 descriptor variables. With these additional variables, the model classification ability increased to 89% overall. The improved classification ability with the addition of fragment–fragment interaction variables is almost entirely related to the "not ready" biodegradable substances since their predictions increased from 86% to 92%.

### 4.1.3 MultiCASE model

The MultiCASE program [57,58] is a fully automated system that analyzes the activity of a given set of compounds and automatically identifies structural and physicochemical descriptors that seem to be responsible for the observed activity. The MultiCASE modeling program has been applied to a dataset of MITI-I values for 894 compounds used in the PLS study [24] in order to investigate whether the implicit variable selection as performed by the MultiCASE program could improve the classification of chemicals as "ready" or "not ready" biodegradable. The dataset was divided into a training set (643 chemicals) and a test set (251 chemicals). Eleven metalloorganic compounds and 2 ambiguous structures were removed from the training set, which leaves 630 chemicals for analysis. This training set was again separated into two files, one with all biodegradable compounds ($n = 269$) and one with all nonbiodegradable compounds ($n = 361$). MultiCASE generated all possible structural fragments that are present in both files, and the first file was searched for substructure fragments explaining biodegradability in the MITI-I test (biophores), while the second file was searched for fragments explaining nonbiodegradability in the test, respectively (biophobes). The program located 48 biophores that could explain all 269 biodegradable compounds, as well as 10 biophobes. Finally, a multiple linear regression (MLR) relationship was built between the 58 selected fragments and the biodegradation data measured according to the MITI-I test protocol [12]. The model was capable of correctly classifying 92.5% of the data in the training set.

#### *4.1.4 Biodegradation rules developed by the inductive machine learning method*

The artificial intelligence technique (i.e., the inductive machine learning method [7–9]) and biodegradation data measured according to the MITI-I test protocol were used to develop structural rules for fast ultimate biodegradation [59]. The following seven rules have been derived for fast biodegradation (i.e., the chemical will biodegrade fast if any of the following seven rules applies):

- esters, amides, or anhydrides with a larger number of ester groups than rings
- all chemicals with at least one acyclic C–O bond and molecular weight below 129
- chemicals built of C, H, N, and O atoms and with larger number of esters groups than rings but without nitro group
- organic acids with molecular weight below 173 and with more acid groups than halogen atoms
- chemicals built of C, H, N, and O atoms with weight below 129 having equal number of aromatic amino groups and acid groups but without nitro group
- esters, amides, or anhydrides with molecular weight below 173 and at least one acyclic C–O bond
- chemicals built of C, H, N, and O atoms with molecular weight below 173 and at least one acyclic C–O bond, equal number of aromatic amino groups and acid groups, but without a nitro group

This set of 7 rules is based only on 11 structural descriptors, selected from a pool of 17 [8]. It was possible to correctly classify 84.3% of chemicals from the MITI database with a well-balanced classification of easily and poorly biodegradable chemicals.

An analysis of structural descriptors present in biodegradation rules was performed to extract information on requirements for either fast or slow biodegradation. The low molecular weight (below 173); presence of only C, H, N, and O atoms in a chemical; presence of C–O bonds; acyclic structures; as well as acid, ester, amide, and anhydride functional groups all seem to be stimulating features for biodegradation. On the other hand, the presence of rings, aromatic amines, halogen atoms, or a nitro group seems to retard the biodegradation process. These findings are also consistent with the general experience.

### 4.2 Validation of biodegradation models

#### *4.2.1 BIODEG model*

To assess the real value of the predictive power of biodegradation models, it is most useful to focus on the external validation of models. The linear BIODEG model based on evaluated biodegradation data [18,55] has been evaluated on a large set of consistent biodegradation data (i.e., 733 compounds tested with the MITI-I test). We expect those evaluation results to be realistic, as well as a solid indicator of the model's future performance in predicting biodegradability of new compounds, since they are based on a large dataset of structurally diverse chemicals. The results of this validation [12] are given in Table 1.

This test showed that the overall performance of the BIODEG model is only 61.1% of correct predictions. This is a relatively poor result. However, in the case of "ready" biodegradable chemicals its prediction score is high, namely, 91.1% of the predictions are correct. However, the BIODEG model was not modeled to predict the outcome of the MITI-I test, which was found to be a more strict measure of biodegradability than the evaluated biodegradation data from the BIODEG dataset. Thus, it was logical to find out that the BIODEG model predicts a significant number of chemicals to be "ready" biodegradable although they were evaluated by MITI-I test as "not ready" biodegradable chemicals. It

**Table 1** Number of chemicals predicted as "ready" or "not ready" biodegradable compared to the results of the MITI-I test. The validation set consists of a subset of 733 compounds from the MITI-I set. Compounds used in model development and metalloorganic compounds are excluded from the validations. Results are presented for the original threshold value (0.500) and optimized threshold value (0.803) of the BIODEG linear model.

| **Model** | BIODEG (0.500) | | BIODEG (0.803) | | No. of Chemicals |
|---|---|---|---|---|---|
| **predicted** | "ready" | "not ready" | "ready" | "not ready" | |
| **-correct** | 266 | 182 | 179 | 357 | 292 "ready" |
| **-error** | 26 | 259 | 113 | 84 | 441 "not ready" |
| **% correct** | 91.1 | 41.3 | 61.3 | 81.0 | |

is possible to correct this defect of the BIODEG model by tuning the threshold value used to distinguish between "ready" and "not ready" biodegradable chemicals to the MITI-I data. The optimized threshold value for the BIODEG linear model was found to be 0.803 [12]. By using the optimized threshold value, the overall performance of the BIODEG linear model is improved to 73.1%, and the number of correct predictions for "not ready" biodegradable chemicals has increased dramatically, i.e., from 41.3 to 81.0% (Table 1). However, at the same time the number of correct predictions for the "ready" biodegradable chemicals has decreased from 91.1 to 61.3%

### 4.2.2 PLS biodegradation model

The PLS model is based on 127 predefined structural fragments and the set of MITI-I values for 894 substances [24]. Eighty-five percent of the model predictions were in agreement with the observed biodegradability. The predictions were slightly better for "not ready" biodegradable substances, with 86% correct predictions vs. 84% correct predictions for "ready" biodegradable substances. No predictions were made for about 10% of the substances because the calculated scores were in the borderline area between "ready" and "not ready" biodegradation. Including fragment–fragment interactions improved the model predictions for "not ready" biodegradable substances to 92%, but had no effect on predictions for "ready" biodegradable substances. The PLS model could not be externally validated by the simple use of the MITI-I test protocol data since all data were used to develop the PLS model. Thus, the training set of 894 MITI-I test data was divided into four subsets consisting of 25% of substances from the database. Consequently, the four submodels without fragment–fragment interaction terms were developed each time using three different subsets of chemicals. For each submodel the remaining subset was used for external validation. The external validation results of these four submodels are presented in Table 2. The predictions for "not ready" biodegradation were 83 to 87% correct, and the pre-

**Table 2** Summary results of internal validation and repeated external validation.

| Model | No. of Substances in Validation | "Ready" Biodegradation % Correct Predictions | "Not Ready" Biodegradation % Correct Predictions | Total % Correct Predictions |
|---|---|---|---|---|
| Internal validation | 894 | 84% | 86% | 85% |
| Internal valid. + interaction terms | 894 | 84% | 92% | 89% |
| External valid. 1 | 223[1] | 79 | 83 | 81 |
| External valid. 2 | 223[1] | 83 | 84 | 84 |
| External valid. 3 | 224[1] | 81 | 85 | 83 |
| External valid. 4 | 224[1] | 77 | 87 | 83 |

[1] These substances were not used in the model generation step.

dictions for “ready” biodegradation were 77 to 83% correct [24]. These results show that prediction scores of internal and external validation are very similar and confirm a solid predictive capability of the PLS model.

The domain of application of any fragment model is restricted by the presence of the fragments in such substances. The PLS model can be applied to all substances having at least one of the 127 fragments in their molecular structure. The broad range of structural fragments used in developing the PLS model allows its application to a wide variety of chemical structures.

### *4.2.3. MultiCASE model*

The MultiCASE model has been evaluated by MITI-I test protocol data for 759 compounds [12,23]. Six hundred and thirty of these chemicals were used in model development. The results of the evaluation are presented in Table 3.

These results indicate that the MultiCASE model will not give a reliable estimation of biodegradability since it was able to correctly classify only the small fraction of compounds not used in model development. The fact that the selection of structural descriptors as performed by the MultiCASE model does not lead to a better performance (compared to the PLS model) is probably due to the MLR implementation of the selected fragments. MLR combined with the high number of structural descriptors gives a fair chance of overfitting the data, leading to a highly degraded performance in external validation.

The test set of 251 compounds not used in the MultiCASE model development was used to evaluate the ability of selected fragments (biophores and biophobes) to correctly classify “ready” biodegradable and “not ready” biodegradable chemicals. Seven metalloorganic compounds had to be excluded from this test set, leaving 244 compounds for evaluation. First, all compounds were searched for the presence of biophores (sites of potential microbial attack). This search resulted in 41 warnings, indicating compounds with structural fragments that may be potential biophores, whereas none of those fragments were present in the training set compounds. In all such cases, it is not possible to make predictions since the program cannot evaluate the effect of “unknown” fragments on biodegradability of those chemicals. Furthermore, one compound was too small to contain any biophore. This reduced the test set to 202 compounds. Due to the absence of a biophore, 106 compounds were predicted to be “not ready” biodegradable. This was a correct prediction for 95 compounds (89.6%) according to their MITI-I values. However, to assume that the presence of a biophore will result in a “ready” biodegradable compound is far from true. Namely, 96 compounds were predicted to be biodegradable due to the presence of a biophore, but this was correct for only 43 of those chemicals (44.8%).

The test set of 244 compounds was then searched for biophobes (biodegradation-retarding fragments). The second search resulted in 37 warnings on potential biophobes. Thus, the test set was reduced to 207 compounds. Due to the presence of a biophobe, 111 compounds were predicted to be “not ready” biodegradable, while 96 were predicted as “ready” biodegradable because of the absence of any known biophobe. According to MITI-I test data, these predictions were correct for 82 (73.9%) and 65 (67.7%) compounds, respectively.

**Table 3** Number of predictions “ready” or “not ready” biodegradable compared to the actual evaluation in the MITI-I test of 759 compounds.

| **Model** | MultiCASE (759) | | | MultiCASE (630) |
|---|---|---|---|---|
| **predicted** | “ready” | “not ready” | all | all |
| **-correct** | 231 | 354 | 585 | 583 |
| **-error** | 84 | 90 | 174 | 48 |
| **% correct** | 73.3 | 79.7 | 77.1 | 92.5 |

From the results of the external validation of the qualitative MultiCASE model on 244 compounds, it is clear that the model performs very well for the prediction of "not ready" biodegradability based on the absence of known biophores. In that case, the success rate is 89.6%, which is a very good performance for an external validation. The absence of a known biophore indicates a lack of an active site for microbial attack, and it seems to be a good indicator for a slow biodegradability of a compound.

Other assumptions based on the presence or absence of biophores or biophobes result in low prediction scores (44.8–73.9%) and cannot be used in evaluation of a compound's biodegradability. The poor performance of the model based on the presence of a biophore may be rationalized either by: (i) the presence of a biophore that will not lead to a complete biodegradation (mineralization) or (ii) the simultaneous presence of a biophobe in a molecule that will make its biodegradation difficult. Thus, it seems that additional work is necessary on this promising approach in order to derive a useful tool for estimating biodegradability.

### *4.2.4 Biodegradation rules for "ready" biodegradable chemicals*

The inductive machine learning method [7–9] and biodegradation data measured according to the MITI-I test protocol were used to develop 7 structural rules for "ready" biodegradable chemicals [59]. This set of rules, based on 11 structural descriptors classified correctly 84.3% chemicals from the training set of 762 compounds with a balanced classification of "ready" (84.9%) and "not ready" (83.7%) biodegradable chemicals.

The set of developed rules was externally validated on 293 compounds from the BIODEG database [18,55]. The evaluation test showed that the overall performance of the seven biodegradation rules is very good since 85% of the predictions were in agreement with the observed biodegradability. The predictions were slightly better for "ready" biodegradable substances, with 86.3% correct predictions vs. 83.6% correct predictions for "not ready" biodegradable substances [59]. The evaluation test shows that the prediction scores on the training set and test sets are very similar, and it confirms a solid predictive potential of the seven developed biodegradation rules for "ready" biodegradable chemicals.

## 4.3 Application of biodegradation models to the list of HPVCs

The BIODEG, PLS, and MultiCASE models have been applied to estimate the biodegradability of the HPVCs in the EU [37]. The purpose of this exercise was to compare the consistency of those models when predicting biodegradability to the set of HPVCs, as well as to provide necessary data for environmental fate and risk assessment. The results of such a comparative study may be used as additional supporting evidence on the quality of evaluated models.

The BIODEG model could generate predictions for 918 of the compounds on the list of HPVCs and gave 332 times a prediction "ready" and 586 times "not ready" biodegradable, using the threshold value of 0.806. The PLS model could give an estimate for 924 compounds: 418 times "ready" and 506 times "not ready" The MultiCASE model gave estimates for 885 compounds: 339 times "ready" and 546 times "not ready".

The combined results for all three models show that it was possible to generate predictions for the 930 compounds from the HPVC list; that is, an estimate of the biodegradation probability could be produced using one or more of the three models. More than 50% of the chemicals (530 compounds) are predicted to be readily biodegradable. Two hundred and eighteen compounds are predicted to be not readily biodegradable, and for 182 compounds results are conflicting. The conflicting results (~20%) most probably reflect the uncertainty in the biodegradability predictions, as well as the uncertainty in the measured data. The combined results are shown in Fig. 3.

In order to evaluate conflicting results, the frequency of substructure fragments used as descriptors in those models has been examined [12]. Evaluation results have shown that the presence of a single negative fragment (i.e., chloro, nitro, or amino substituent on aromatic ring, the quaternary carbon atom) may be sufficient for a "not ready" biodegradability. An exception is the aliphatic ether group that

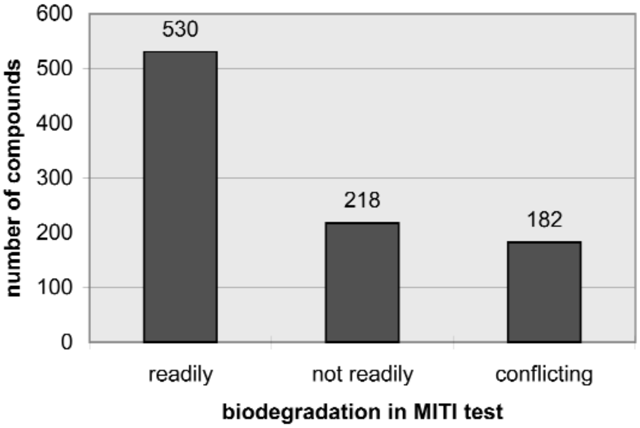

**Fig. 3** Number of compounds on the EU HPVCs list as a function of the estimated biodegradability in the ready biodegradation test.

was relatively important for BIODEG model predictions and almost unimportant for the PLS and MultiCASE models. The positive fragments are much more abundant in the HPVCs, but their presence does not correspond highly with the predictions of "ready" biodegradability. Thus, it seems that the presence of a positive fragment in a molecule is not a good indicator of its "ready" biodegradability. The same was found earlier while evaluating the qualitative MultiCASE model.

The qualitative MultiCASE model based on the absence of biophores is used to make a prediction of the nonbiodegradable compounds in the HPVCs list. Estimates were possible for 955 compounds. However, 65 biodegradable and 45 nonbiodegradable compounds were already present in the training set used to develop this model, and, consequently, all were deleted from the estimation effort. Furthermore, the evaluation process generated warnings for 226 compounds due to the presence of potential, previously unidentified biophores or biophobes, while 13 compounds were identified as too small to contain any of the known biophores. From the remaining 606 compounds, 280 are predicted to be "not ready" biodegradable owing to the lack of a known biophore. It is very likely that these compounds will be "not ready" biodegradable in the MITI-I test.

The evaluation results show that the optimal procedure for estimating biodegradation of organic compounds in the environment would be to apply four methods, i.e., the PLS model, a set of seven biodegradation rules, the qualitative MultiCASE model, as well as the BIODEG model. If their results agree, such estimates should be considered as very reliable.

## 5. SUMMARY AND RECOMMENDATIONS

The major obstacle that precluded the development of better and reliable biodegradation models in the past was the absence of standardized and uniform biodegradation data for various chemical classes [61,62]. Several years ago, two databases of "high-quality" biodegradation data became generally available. The BIODEG database contains evaluated and standardized biodegradation data for about 300 diverse commercial chemicals. The second database, the so-called MITI database, contains results of the MITI-I screening test for "ready" biodegradability in an aerobic aqueous medium for nearly 900 commercial chemicals. The MITI-I test protocol is one of the six standardized "ready" biodegradability tests recommended by EU and OECD regulations.

The last five years have been a period of very intensive development of new and better qualitative and quantitative biodegradability models by the application of new and advanced computational and statistical methods. The objectives of this report are to review published models and their evaluation studies, to perform some in-house evaluation of the general models for estimating tropospheric degradation or complete biodegradation of organic compounds, and to give recommendations for a reliable procedure to estimate lifetime and/or degradability of organic compounds in the environment. Here are the major findings and recommendations:

## Tropospheric degradation

1. There are two general models for estimating tropospheric degradation of organic compounds—the Atkinson's group contribution method and the MOOH method. An extensive evaluation has shown that Atkinson's method is far more accurate and consequently should be used as a method of choice. However, Atkinson's method should not be used to estimate tropospheric degradation of haloalkenes and haloalkanes with $–CX_3$ (X = F, Cl, Br) groups, perhalogenated compounds, and ethers (especially polyethers, cycloethers, and halogenated ethers). Its application is also discouraged for chemical classes not used in its development.
2. The recommended procedure for estimating tropospheric degradation of organic compounds would be to use both methods. If their results agree, those estimates should be considered as reliable. If their results disagree and Atkinson's method estimates faster degradation rate, its result should be considered as being more reliable since the MOOH method was found to systematically underestimate tropospheric degradation rates. Finally, if their results disagree and Atkinson's method estimates slower degradation rate, such results should be used with caution, but, again, more confidence may be placed in Atkinson's method if the compound belongs to chemical classes used in its development.
3. A viable alternative to the Atkinson's and MOOH methods for estimating tropospheric degradation and tropospheric half-lives of organic chemicals is the direct calculation of their reaction rate constants with hydroxyl radical. The dramatic developments in computing technology enabled the calculations of the energy profiles of gas-phase reactions to be performed almost routinely. Recently, an affordable methodology was developed for calculating reaction rate constants at low computational cost. It is based on reliable semiempirical potential energy surfaces, and the computed rate constants differ from experiment at most by a factor of 2. This approach is particularly suitable for estimating tropospheric degradation of haloalkenes and haloalkanes with $–CX_3$ (X = F, Cl, Br) groups.

## Biodegradation

4. There are four general approaches for estimating biodegradation of organic compounds; BIODEG models, PLS model, MultiCASE models, and a set of seven biodegradation rules. Extensive internal and external evaluation has shown that the PLS model and the set of seven biodegradation rules are the most reliable in estimating biodegradation of organic compounds. Their predictive scores range from 81 to 86%, and those two methods should be the first choice for estimating biodegradation of organic compounds. BIODEG linear models and the MultiCASE approach based on the absence of a biophore may be used as reliable evaluation methods for "not ready" biodegradable substances.
5. The recommended procedure for estimating biodegradation of organic compounds in the environment would be to use all four methods. If their results agree, such estimates should be considered as very reliable. If results of three methods agree, those estimates should be considered as reliable. If the PLS model and biodegradation rules estimate a substance to be "not ready" biodegradable and the BIODEG linear models and the MultiCASE approach evaluate the same substance to be "ready" biodegradable, most confidence should be put into the former prediction. If the PLS model and biodegradation rules estimate a substance to be "ready" biodegradable and

the BIODEG linear models and the MultiCASE approach evaluate the same substance to be "not ready" biodegradable, most confidence should be put into the latter prediction. All other combinations of estimation results should be used with caution, as they will require a careful analysis of results before a more reliable prediction is obtained.

## 6. ABBREVIATIONS AND ACRONYMS USED

| | |
|---|---|
| BOD | biological oxygen demand |
| EU | European Union |
| HOMO | highest occupied molecular orbital |
| HPVC | high production volume chemical |
| MITI | Japanese Ministry of International Trade and Industry |
| MLR | multiple linear regression |
| MO | molecular orbital |
| OECD | Organization for Economic Cooperation and Development |
| PLS | partial least squares |
| QSAR | quantitative structure–activity relationship |
| ThOD | theoretical oxygen demand |

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