Manual for PEARLNEQ v6

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WAGENINGEN
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1 Wageningen Environmental Research

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Abstract

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Aged sorption is a process by which a plant protection product can become more strongly adsorbed to soil over time, resulting in, among other things, reduced leaching to groundwater. The inclusion of aged sorption is part of a higher tier in the groundwater leaching assessment in the EU registration procedure for plant protection products. Data of experimental studies that demonstrate an increase in sorption over time can be used to derive model-input parameters for assessments using the FOCUS Groundwater scenarios. This report describes the use of the new version of PEARLNEQ, version 6, that can be used to estimate aged sorption parameters for the active ingredient of the plant protection product on the basis of a soil incubation experiment with this substance. The new version can calculate (optionally) the 95% confidence intervals of the fitted parameters by bootstrapping which are probably more accurate than the intervals provided by PEST.

Keywords: aged sorption, pesticide, inverse modelling, PEARLNEQ, non-equilibrium sorption, PEST, soil, leaching, plant protection product, PEARL

Referaat

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Niet-evenwichtssorptie is een proces waardoor naar verloop van tijd een gewasbeschermingsmiddel sterker gebonden wordt aan bodemdeeltjes, hetgeen onder andere kan leiden tot minder uitspoeling naar het grondwater. Het meenemen van niet-evenwichtssorptie is een onderdeel van een hogere tier van de beoordeling van de uitspoeling naar het grondwater in de EU toelatingsprocedure. Gegevens van experimentele studies die de toename van de sorptie in de tijd aantonen kunnen gebruikt worden om de invoergegevens van modellen af te leiden voor berekeningen met de FOCUS Grondwaterscenario's. Dit rapport beschrijft het gebruik van de nieuwe versie van PEARLNEQ, versie 6, die gebruikt kan worden om een schatting te maken van de niet-evenwichtssorptie parameters van de actieve stof van het gewasbeschermingsmiddel op basis van een incubatie-experiment met grond waaraan deze stof is toegevoegd, De nieuwe versie kan ook desgewenst de 95% betrouwbaarheidsintervallen berekenen van de gefitte parameters door middel van bootstrapping, die waarschijnlijk nauwkeuriger zijn dan de intervallen berekend door PEST.

Trefwoorden: lange-termijn sorptie, bestrijdingsmiddel, inverse modellering, PEARLNEQ, niet-evenwichtssorptie, PEST, bodem, uitspoeling, gewasbeschermingsmiddel, PEARL

Preface

EFSA has developed guidance on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. The Standing Committee on Plants, Animals, Food and Feed agreed that this guidance will be applicable as of 1 April 2021 (date of dossier submission) to dossiers submitted under Regulation (EC) No 1107/2009.

PEARLNEQ is one of the tools that can be used to analyse such studies. This manual has been prepared to support the user of version 6 of PEARLNEQ.

In this new version of PEARLNEQ, a methodology has been implemented to estimate more accurately the 95% confidence interval of the fitted parameters. In addition, several modifications have been implemented to increase the precision of the input for the PEST model or to improve the applicability of the model.

Contents

Summary

This manual describes the PEARLNEQ v6 software package. This package can estimate long-term sorption parameters using results of aged sorption studies with soil using a sub-model for sorption and transformation that is identical to the sub-model used for that purpose in FOCUS_PEARL.

The sub-model assumes two types of sorption sites: equilibrium sites and non-equilibrium sites. The sorption isotherms for both sites are described with Freundlich equations. The content sorbed at the equilibrium site is assumed to be continuously at equilibrium, and the content sorbed at the non-equilibrium site is described with a pseudo first-order sorption rate equation. The software package offers two options for describing the transformation rate in soil. The first option is that the transformation rate in soil is proportional to the amount in the liquid phase plus the amount sorbed at the equilibrium site. The second option is that the transformation rate in soil is proportional to the amount in the liquid phase. So, for both options, the content sorbed at the non-equilibrium site is not subject to transformation.

The mathematical equations describing the sub-model are solved via a Fortran program. An additional Fortran program generates the necessary input files for the PEST optimisation package. Instructions are given for how to obtain optimised parameters using an example dataset and, subsequently, instructions are given how to obtain parameters using your own data.

The PEST optimisation package also provides estimates of confidence intervals of the parameters. However, these may be inaccurate. Therefore, a procedure is provided to estimate more accurately the 95% confidence intervals by bootstrapping. A disadvantage is that this requires much more computation time.

Samenvatting

Deze handleiding beschrijft het PEARLNEQ v6 softwarepakket. Dit pakket kan de niet-evenwichtssorptie parameters schatten door gebruik te maken van niet-evenwicht sorptie ('aged sorption') studies met een sub-model voor sorptie en omzetting dat identiek is aan het sub-model voor dat doel in FOCUSPEARL.

Het sub-model gaat uit van twee soorten sorptieplekken, evenwichtssorptieplekken en niet-evenwichtssorptieplekken. De sorptie-isothermen voor beide plekken worden beschreven met de Freundlich vergelijkingen. Het gehalte gesorbeerd op de evenwichtssorptieplek is continu in evenwicht en het gehalte gesorbeerd op de niet-evenwichtssorptieplek wordt beschreven met een pseudo eerste-orde sorptie snelheidsvergelijking. Het softwarepakket biedt twee opties voor het beschrijven van de omzettingssnelheid in de grond. De eerste optie is dat de omzettingssnelheid recht evenredig is met de hoeveelheid van de stof in de vloeibare fase van de bodem vermeerderd met de hoeveelheid die gesorbeerd is op de evenwichtssorptieplek. De tweede optie is dat de omzettingssnelheid recht evenredig is met de hoeveelheid in de vloeibare fase. In beide opties is de hoeveelheid die gesorbeerd is op de niet-evenwichtssorptieplek niet beschikbaar voor omzetting.

De wiskundige vergelijkingen van het sub-model worden opgelost via een Fortran programma. Een aanvullend programma genereert de benodigde invoerbestanden van het PEST optimalisatiepakket. Instructies worden gegeven op welke manier geoptimaliseerde parameters verkregen kunnen worden met behulp van een voorbeeld dataset en hierop aansluitend worden instructies gegeven hoe de parameters verkregen kunnen worden met eigen gegevens.

Het PEST optimalisatie pakket levert ook schattingen van het betrouwbaarheidsinterval van de parameters. Echter, deze kunnen onnauwkeurig zijn. Daarom is een procedure uitgewerkt om het 95% betrouwbaarheidsinterval meer nauwkeuriger te kunnen schatten door middel van bootstrapping. Een nadeel van deze methode is wel dat er veel meer rekentijd voor nodig is.

1   Introduction

This document describes a PEARLNEQ-PEST combination which can be used to estimate the parameters for long-term sorption kinetics in the PEARL model on the basis of an incubation experiment for a certain soil and a certain pesticide. The combination also provides the transformation half-life at reference temperature (when long-term sorption kinetics are included in PEARL, the definition of this half-life changes so it has to be recalculated; see Boesten and van der Linden, 2001). If the incubation experiment has been carried out at multiple temperatures, the molar enthalpy of transformation for the transformation rate in soil can be optimised simultaneously.

The differences with the previous release (PEARLNEQ v5) are as follows:
- When the option 'equal' is used for weighing residuals, PEARLNEQ calculates weighing factors to ensure that the two types of measurements (i.e. the remaining mass in the system and the concentration in the liquid phase) obtain an approximately equal weight in the fitting procedure.
- The weight factors are written with more decimals to the PEST input file; in previous versions, only three decimals were written (e.g. 0.020 instead of 0.0204) which may result in the option 'inverse' rounding errors in the order of percents.
- The lower limit of the initial mass in the system has been set to 0.001 mg (previously 0.1 mg).
- Additionally, a procedure is provided to estimate more accurately the confidence intervals of the parameters using bootstrapping.
- A small bug was repaired in the calculation procedure of the system variables at the last sampling time (in the previous version the values of the system variables at this last sampling time were those from one timestep before the last sampling time; this happened only if the calculation stopped at the last sampling time, so no error occurred if the calculation proceeded somewhat longer; as the timestep is typically in the order of 0.01 d, the error made was small).

Recently, the European Commission provided guidance on aged sorption (EC, 2021) indicating that PEARLNEQ is one of the recommended tools that may be used to obtain aged sorption parameters. However, note that PEARLNEQ contains additional features that may not be in line with this guidance (e.g. the option to estimate the Arrhenius activation energy described at p. 23 of the guidance, or the option to obtain confidence intervals by bootstrapping, as described in Chapter 9).

2 Precautionary remark

This PEARLNEQ-PEST software tool should be seen as an introduction to fitting results of experiments on long-term sorption kinetics to the sorption sub-model used in the PEARL model. The tool shows you how PEST can be coupled to a Fortran program that contains this PEARL sorption sub-model (i.e. PEARLNEQ.EXE) but it should not be seen as a ready-to-use tool. The tool provides you with example input files for the PEST optimisation package and shows you how to organise this optimisation. We had to make a number of assumptions for generating these PEST input files (e.g. upper and lower bounds of parameters, weighing factors for each measurement, etc.). We do not claim that these assumptions are defensible for your problem; they are our best guesses, but they may not be appropriate for your problem. It is your responsibility to check the appropriateness of the result obtained. We do not accept any responsibility for use of PEARLNEQ.

3 Description of the incubation experiment

The PEARLNEQ-PEST tool can be used to fit the results of the following experiment. A number of jars is filled with soil. Each jar contains the same mass of moist soil. At the start of the experiment, the same initial mass of pesticide is added to the moist soil in all jars. The jars are incubated at a fixed temperature (or at a few temperatures). At certain points in time, the remaining total amount of pesticide is measured via an extraction with organic solvent. At the same time, the concentration in the liquid phase of the moist soil is measured. The liquid phase can be collected by centrifuging the moist soil over a filter. As an alternative for centrifuging, a desorption experiment can be carried out by adding a certain volume of aqueous solution (*e.g.* $CaCl_2$ solution) and subsequent shaking for about 24 h.

It may also be useful to have additional results of measurements of an adsorption isotherm with an equilibration time of about 24 h using the same soil and pesticide.

4   Theoretical background

PEARLNEQ assumes a Freundlich two-site sorption sub-model: one site for equilibrium sorption and a second site for long-term sorption kinetics. The operational definition for the equilibrium sorption sites is that they have reached equilibrium after about 24 h shaking of a well-stirred suspension of the soil in water. The long-term sorption sites do not reach equilibrium within 24 h. PEARLNEQ assumes first-order degradation kinetics; it offers two options for describing the degradation kinetics. The first option is that the degradation rate is proportional to the number of molecules present in liquid phase and those sorbed to the equilibrium site. The second option is that the degradation rate is proportional to only the number of molecules present in liquid phase (see Beltman et al., 2008). However, molecules sorbed on the non-equilibrium site are assumed not to degrade in both options. This conceptual model is presented in Figure 1. The sub-model for sorption and degradation kinetics used in PEARLNEQ can be described as follows (Leistra et al., 2001):

$$M_p = V c_L + M_s (X_{EQ} + X_{NE}) \tag{1}$$

$$X_{EQ} = K_{F,EQ}\, c_{L,R} \left(\frac{c_L}{c_{L,R}} \right)^N \tag{2}$$

$$\frac{dX_{NE}}{dt} = k_d \left(K_{F,NE}\, c_{L,R} \left(\frac{c_L}{c_{L,R}} \right)^N - X_{NE} \right) \tag{3}$$

$$K_{F,NE} = f_{NE}\, K_{F,EQ} \tag{4}$$

$$\frac{dM_p}{dt} = -k_t (V c_L + M_s\, X_{EQ}) \tag{5A}$$

$$\frac{dM_p}{dt} = -k_t V c_L \tag{5B}$$

$$K_{F,EQ} = m_{OM}\, K_{OM,EQ} \tag{6}$$

where:
M_p = total mass of pesticide in each jar (μg), acronym Mas
V = the volume of liquid in the soil incubated in each jar (mL), acronym VolLiq
M_s = the mass of dry soil incubated in each jar (g), acronym MasSol
c_L = concentration in the liquid phase (μg/mL), acronym ConLiq
$c_{L,R}$ = reference concentration in the liquid phase (μg/mL), acronym ConLiqRef
X_{EQ} = content sorbed at equilibrium sites (μg/g)
X_{NE} = content sorbed at non-equilibrium sites (μg/g)
$K_{F,EQ}$ = equilibrium Freundlich sorption coefficient (mL/g), acronym CofFreEql
$K_{F,NE}$ = non-equilibrium Freundlich sorption coefficient (mL/g), acronym CofFreNeq
N = Freundlich exponent (-), acronym ExpFre
k_d = desorption rate coefficient (d^{-1}), acronym CofRatDes
f_{NE} = a factor for describing the ratio between the equilibrium and non-equilibrium Freundlich coefficients (-), acronym FacSorNeqEql

k_t = degradation rate coefficient (d^{-1})

m_{OM} = mass fraction of organic matter in the soil (kg/kg), acronym CntOm

$K_{OM,EQ}$ = coefficient of equilibrium sorption on organic matter (mL/g), acronym KomEql

PEARLNEQ does not use the transformation rate coefficient (k_t) as input parameter, but the half-life at reference temperature (acronym DT50Ref, *DegT50*). They are related as follows (assuming first-order kinetics):

DegT50 = ln (2) / k_t (7)

The effect of soil temperature on the transformation rate coefficient in soil is described by the Arrhenius equation:

$$f_T = \exp\left(\frac{-E}{R}\left[\frac{1}{T} - \frac{1}{T_{REF}}\right]\right)$$ (8)

where

f_T = the multiplication factor for the rate coefficient (-)

E = molar enthalpy of transformation (kJ/mol), acronym MolEntTra

T = temperature of the soil during the incubation (K)

T_{REF} = the reference temperature for the specified *DegT50* (K)

R = the gas constant (kJ mol^{-1} K^{-1}).
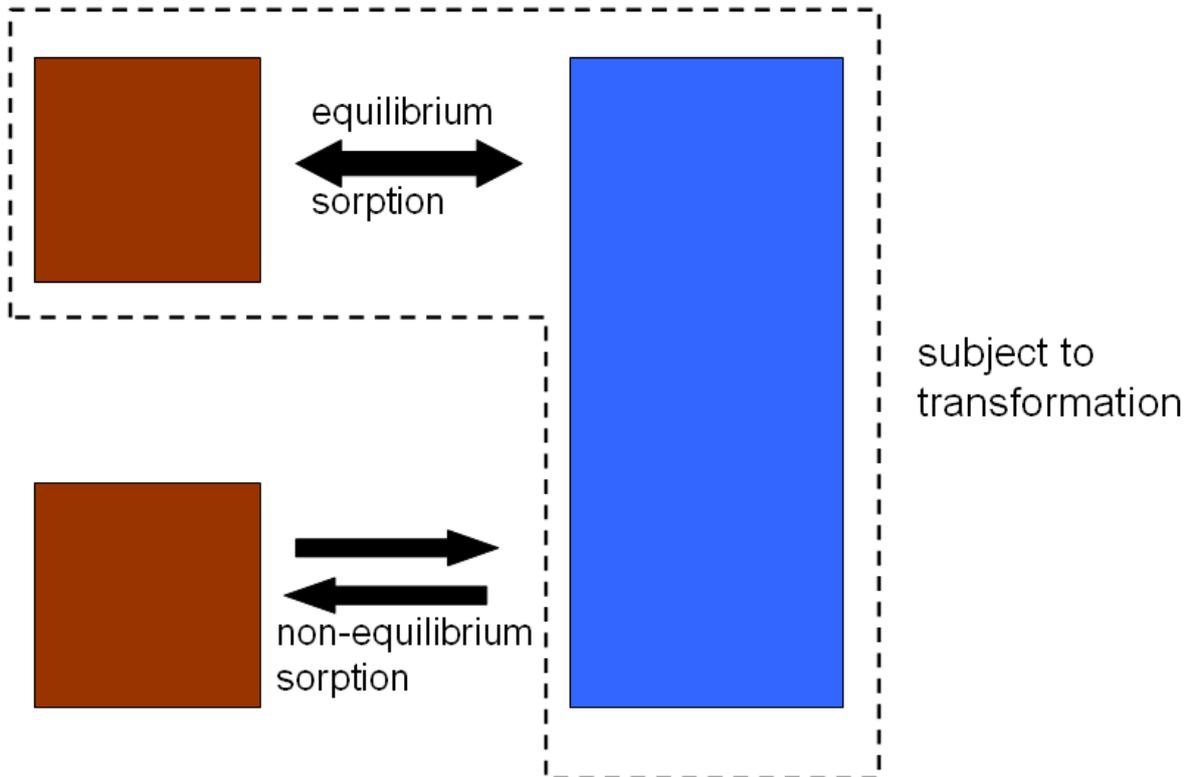
Figure 1   *Conceptual representation of the PEARLNEQ model. Note that there are two options for the transformation process as described by Eqns 5-A and 5-B. Only the option described by Eqn 5-A is shown here.*

Often, no concentration measurements in the soil pore water are available, but instead, at each sampling point in time, a certain volume of water (usually a CaCl$_2$ solution) is added to soil and the suspension is shaken for about 24 h, after which the concentration in the supernatant is measured. In

such a case, the fit has to be based on these concentration measurements in the supernatant of the soil-water suspension.

This is simulated in PEARLNEQ as follows:
A)   it is assumed that full equilibrium is reached for the equilibrium sorption site during the desorption experiment (i.e., shaking for 24 h).
B)   it is assumed that desorption from the non–equilibrium sorption site can be ignored during the desorption experiment.

Assumption A is justifiable because this is precisely the operational definition of the equilibrium sorption site. Assumption B is justifiable because desorption coefficients for long-term kinetics are usually in the order of 0.01 d^{-1}, which implies that amounts desorbed within 1 day are negligibly small.

Using these assumptions, the concentration in the liquid phase of the supernatant after desorption can be estimated by stating that (i) the total content of substance in the moist soil and the soil-water suspension have to be equal, and (ii) the content sorbed at the non-equilibrium sites in the moist soil and in the soil-water suspension are equal. Using Equation 1 then results in the following equation:

$$V\,c_{L,MS} + M_s\left(X_{EQ,MS} + X_{NE}\right) = \left(V + V_{ADD}\right)c_{L,SUS} + M_s\left(X_{EQ,SUS} + X_{NE}\right) \qquad (9)$$

where:
the subscript MS indicates the moist-soil system
the subscript SUS indicates the soil-water suspension system and
V_{ADD} = volume of liquid (usually CaCl$_2$ solution) added to the soil at each sampling point just before starting the 24 h desorption experiment (mL), acronym VolLiqAdd.

At each sampling point in time, Equation 9 can be rewritten (using Eqn 2) into an equation that contains only one unknown variable, i.e. the concentration in the liquid phase of the soil suspension ($c_{L,SUS}$). PEARLNEQ always provides as output the concentration in the soil-water suspension as a function of time. If $V_{ADD} = 0$, then this implies that the concentration in the moist soil is given.

PEARLNEQ provides as output also the so-called apparent sorption coefficient ($K_{D,APP}$). This is used for studies in which a certain volume of water (usually a CaCl$_2$ solution) is added to soil and the suspension is shaken for about 24 h. It is then defined as the total content sorbed at the end of the shaking period divided by $c_{L,SUS}$. This is calculated by PEARLNEQ as:

$$K_{D,APP} = \frac{X_{EQ,SUS} + X_{NE}}{c_{L,SUS}} \qquad (10)$$

PEARLNEQ solves the set of Eqn 1 to Eqn 9 numerically using Euler's method for integration of the state variables M_p and X_{NE}. The time step for integration, Δt, is calculated as:

$$\Delta t = \frac{A}{\max(k_t f_T, k_d)} \qquad (11)$$

where A is an accuracy parameter (-) which was set to 0.003. Van den Berg et al. (2016) have shown that such a value of A should give accurate results.

The concentration in the liquid phase is calculated via an iteration procedure as described in Appendix 4 of FOCUS (2006). Appendix 3 shows a test of the PEARLNEQ results against an analytical solution for the case of a linear isotherm ($N=1$), indicating good correspondence between numerical and analytical results.

5 Fitting procedure for parameters with PEST

The provided package assumes that the following variables need to be optimised:
- The initial mass of the pesticide (MasIni)
- The ratio between the equilibrium and non-equilibrium Freundlich coefficients (FacSorNeqEql)
- The desorption rate coefficient (CofRatDes)
- The half-life at reference temperature (DT50Ref) for the selected transformation option (Eqn 5-A or Eqn 5-B)
- The coefficient of equilibrium sorption on organic matter (KomEql)
- The molar enthalpy of transformation (MolEntTra); this can only be optimised if the experiment has been carried out at multiple temperatures.

It is assumed that all other variables are known.

The provided package assumes that the measurements that are fitted and consist for each point in time of:
- A mass of pesticide in μg
- A concentration of pesticide in liquid phase in μg/mL.

PEST needs a number of input parameters for the fitting procedure (e.g. initial guesses of the fitting parameters, upper and lower bounds of parameters, criteria for the convergence of the iteration procedure, weighing factor for each measurement, etc.). The package provides values for all these input parameters, and we do not claim that these are the best choice for your dataset. Our experience is that the weighing factor for each measurement is the most important input parameter. Therefore, we offer two options for weighing:
1. 'equal', which gives an equal weight factor to all masses and an equal weight factor to all concentrations in the liquid phase
2. 'inverse', which gives a weight factor that is proportional to the inverse of the observed value (if the observed value is zero, the weight factor is set to 1.0)

The PEST optimisation is based on the sum of squares of differences between measured and fitted values with the understanding that each difference is multiplied with its weight factor. So, the weight of a measurement in the fitting procedure is determined by its contribution to this sum of squares. The option 'equal' implies that differences for high observed values get more weight (i.e. contribute more to the sum of squares) than differences for low observed values. As described above, the fitting procedure considers two quantities: mass of pesticide and the concentration in the liquid phase. Giving them the same weight factor may lead to the situation that the estimation of the parameters is dominated by one of the two. For example, if the mass is initially 50 μg and the concentration in the liquid phase is in the order of 1 μg/mL, then the fitting procedure would be dominated by the decline of the mass of pesticide. This would be inefficient use of the data. Therefore, in case of the option 'equal', all masses are given a weight factor of 1.0, and all concentrations in the liquid phase are given a weight factor equal to the average of all measured masses divided by the average of all measured concentrations. So, e.g., if the average of all masses would be 25 and the average of all concentrations 0.5, then the concentrations will be given a weight factor of 50, thus ensuring approximately equal weight of the two quantities in the optimisation procedure.

The option 'inverse' implies that each measurement gets more or less equal weight for the parameter estimation. This option ensures that, for example, an 5% difference between an observed and a fitted value obtains the same weight (i.e. contributes equally to the sum of squares) irrespective whether the value is high or low. This then is consistent with the concept of relative errors. The alternative option 'equal' gives all differences between observed and fitted values the same weight factor, which is consistent with the concept of absolute errors. This 'inverse' option gave the best results in a few tests and is considered the normal procedure for aged sorption studies, according to EFSA (2018, p.16). Measured masses and concentrations will usually be many orders of magnitude higher than

their detection limits, so there is a priori no reason why the relative error of low values should be higher than that of high values. In principle, the user can check whether the differences between fitted and measured values are more consistent with the concept of relative errors than with the concept of absolute errors and use this check to decide between 'inverse' or 'equal' options.

Other important input are the initial guesses of the fitting parameters. While using PEARLNEQ, we noticed that the results regularly depend on these initial guesses. We therefore advise you to always perform a number of runs with different initial guesses.

The provided package can handle multiple observations for each point in time. The user can specify up to nine replicate sets. A replicate set can contain measurements at different time points and different temperatures. There is no restriction in the number of measurements in a replicate set. The different replicate sets do not necessarily need to contain the same number of measurements or the same time points of measuring. However, there is a restriction with respect to the temperatures. The user needs to specify in a separate table at which temperature the measurements are performed, and each replicate set should contain at least one measurement performed at each of the temperatures specified in the list.

We advise you to analyse the results carefully, especially the 95% confidence intervals of your parameters (Chapter 7 describes where you can find these). If the interval is wide for a certain parameter, this indicates that the estimated variable is very uncertain. As a consequence, it may be not meaningful to use it any further in the risk assessment; an alternative option would be to give it less weight in the subsequent averaging of parameter values.

6 Installation of PEARLNEQ

PEARLNEQ is distributed in a zip file. Unzip the file and specify a path (e.g. c:\pearlneq). Be sure there is no space within the specified path, because this will cause failure. We suggest installing and running PEARLNEQ on your hard disk and a 64-bit system (running on a network disc or a 32-bit system may result in failure).

The package contains five directories: Neq_Bin, PEST, Neq_Fortran_source_files, Neq_Example and Bootstrapping.
- The Neq_Bin directory contains the PEARLNEQ executables, PEARLNEQ.EXE and PEARLMK.EXE. The PEST optimisation software is available in the PEST directory. As PEST is available freeware (http://www.pesthomepage.org), we included relevant executables of the latest version (version 17.1). Separate installation of PEST is not necessary.
- The Neq_Fortran_source_files directory contains the Fortran source files used to generate the PEST input files and the program that calculates the sorption kinetics.
- The Neq_Example directory contains results from an example study: bentazone in a Dutch sandy soil: the Vredepeel dataset (Boesten and Van der Pas, 2000).
- The Bootstrapping directory contains executables and batch files to assess the confidence interval of the parameters for long-term sorption kinetics in a more sophisticated way.

7   Running the example

The following steps must be followed.

1. Run the example to check if everything works and get experience with the system. Go to the Neq_Example directory, and run the example, example.bat.
   - The batch file will first call PEARLMK. This pre-processing program needs the example.mkn file as input and generates the input files for PEST, i.e. example.pst, example.tpl and example.ins (see Figure 2, RunId = 'example'). The 'pst' file is the PEST control file. The 'tpl'-file provides the template for the input file for PEARLNEQ and the 'ins'-file describes the location of the simulated values in the 'out'-file. Please note that in case of replicate datasets (in the example there are two replicates), there is an 'ins'-file for every replicate (example1.ins, example2.ins, etc).
   - Next PEST programs PESTCHEK, TEMPCHEK and INCHEK are executed to check respectively the 'pst' file, the 'tpl'-file and the 'ins'-file(s). Press 'enter' after each check to proceed.
   - Then, optimisation starts. PEST calls PEARLNEQ several times (see Figure 2; in the example 39 times).
   - If you get an error message after the first step (PEARLMK), type 'control-break' to stop the process and check the error messages available in the example.err file.
2. After successful optimisation, read the results from the file example.rec. The file can be opened with a text editor, e.g. Notepad. The relevant results, including parameter values, 95% confidence intervals and correlation matrices, can be found at the end of this file (Section OPTIMISATION RESULTS, see Appendix 2). The meaning of the short acronyms in this rec-file is as follows:
   fsne = FacSorNeqEql
   crd = CofRatDes
   dt50 = DT50Ref
   masini = MasIni
   komeql = KomEql
   met = MolEntTra.
   PEST also generates parameter sensitivity files etc. Details can be found in the PEST manual (Doherty, 2005).
3. PEARLNEQ will create an output file (example.out) and a log file (example.log). The output files are self-explanatory. The output file contains the result of the last run which is in PEST by definition the run with the optimised parameters.
4. The results from the output file (example.out) are the source for the best fit and can be used to create graphs.
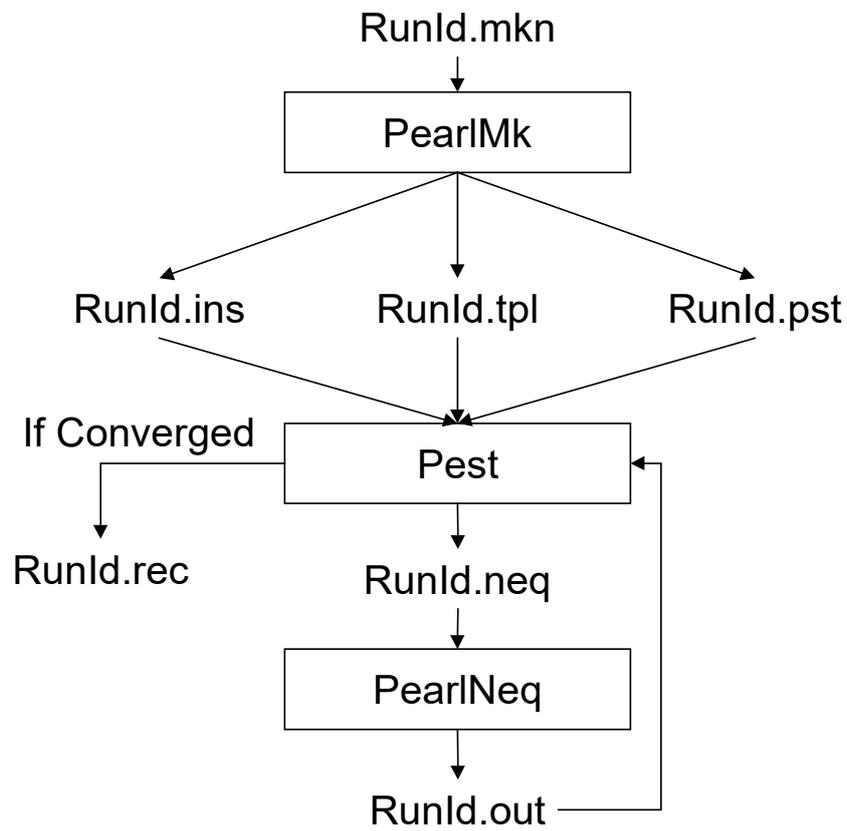
Figure 2 *Dataflow diagram for the* PEARLNEQ-PEST *combination. The acronym RunId is 'example' for the example provided. Note that in this example, there are two ins-files (example1 and example2) because the example contains two replicates.*

8   Run PEARLNEQ with your own data

1. We assume that you have carried out an appropriate incubation experiment as described before. The first step of optimising your own data consists of **editing the file <u>example.mkn</u>**, which can be found in the example subdirectory of the **PEARLNEQ directory**. Open the file with a text editor e.g. Notepad. *Please make a copy of this file and the.bat file before editing.* Make sure there is no space in the new name. This will give an error. An example of this input file is listed in Appendix 1. The following parameters must be provided:

    - <u>TimEnd</u> (d): The duration of the incubation experiment. This determines the length of the simulation period.
    - <u>MasIni</u> (µg): The initial total mass of pesticide in each jar. *In contrast to initial versions of PEARLNEQ, this parameter will be optimised.* There is no default value for this parameter because it depends on the setup of the experiment.
    - <u>MasSol</u> (g): The mass of dry soil incubated in each jar.
    - <u>VolLiqSol</u> (mL): Volume of liquid in the moist soil in each jar *during* incubation.
    - <u>VolLiqAdd</u> (mL): Volume of liquid added to the soil *after* incubation (i.e. the amount of liquid added to perform a conventional desorption equilibrium experiment).
    - <u>CntOm</u> (kg.kg^{-1}): Mass fraction of organic matter in the soil.
    - <u>ConLiqRef</u> (µg mL^{-1}): Reference concentration in the liquid phase.
    - <u>ExpFre</u> (-): Freundlich exponent; use value taken from adsorption isotherm measured for this pesticide-soil combination.
    - <u>KomEql</u>mL g^{-1}): coefficient of equilibrium sorption on organic matter. *This parameter will be optimised.* We suggest to use as initial guess a value taken from adsorption isotherm measured for this pesticide-soil combination; in case you have no organic matter content of the soil, set the organic matter to 1.0 and specify the measured Freundlich equilibrium coefficient (see Eqn 6).
    - <u>FacSorNeqEql</u> (-): factor describing the ratio $f_{NE} = K_{F,NE}/K_{F,EQ}$ as defined by Eqn 4. *This parameter will be optimised*, but you have to specify an initial guess here. We suggest a value of 0.5. As results may depend on initial guesses, we advise you to always perform a number of runs with different initial guesses.
    - <u>CofRatDes</u> (d^{-1}): the desorption rate coefficient. *This parameter will be optimised*, but you have to specify an initial guess here. We suggest a value of 0.02 d^{-1}. As results may depend on initial guesses we advise you to always perform a number of runs with different initial guesses.
    - <u>OptSor option:</u> sorption option to be used: 'Neql' for fits including long-term sorption kinetics (standard option) and 'Eql' for fits considering only equilibrium sorption.
    - <u>DT50Ref</u> (d): the transformation half-life under reference conditions, applying to the equilibrium domain for the option of Eqn 5-A and to the liquid phase for the option of Eqn 5-B. *This parameter will be optimised*, but you have to specify an initial guess here. This initial guess could be the 'classical' half-life, which applies to the total soil system (i.e. the equilibrium domain + the non-equilibrium domain).
    - <u>TemRefTra</u> (C): The reference temperature, for which the half-life will be provided (set to incubation temperature if data for only one temperature are available and set to 20° C if you have data for multiple temperatures).
    - <u>MolEntTra</u> (kJ mol^{-1}): the molar enthalpy of transformation. *This parameter will be optimised if you have carried out the experiment at multiple temperatures; otherwise it is a model-input*. In any case, you have to specify a value (e.g. 60 kJ mol^{-1}) which will be used as an initial guess in case of data for more than one temperature.
    - <u>Tem</u> (C) table: List of temperatures at which the incubation experiment has been carried out. One temperature is OK if only data for one temperature are available.
    - <u>NumRepSet</u> (-): Number of replicate sets.
    - <u>Opt_weights option</u>: options for weights. Two options for weighing are offered: 'equal' which gives an equal weight to all pesticide masses and an equal (but different) weight to all concentrations (see Chapter 5 for details) and 'inverse' which gives a weight that is proportional

to the inverse of the observed value; if the observed value is zero, the weight is set equal to 1.0 in any case; you can inspect the weights in the 'pst' file.

- Opt_transformation option: option for the concept of the degradation rate. The two options are 'EqlDom' (transformation in the equilibrium domain as described by Eqn 5-A) and 'LiqPhs' (transformation in the liquid phase only as described by Eqn 5-B).
- Observations table: List of observations. Column 1 contains the time (d), the Column 2 the temperature, Column 3 contains the total mass of pesticide in the system (µg) and Column 4 contains the concentration of pesticide (µg mL^{-1}) measured in the pore water of moist soil (then VolLiqAdd = 0) or in the water phase after a desorption experiment (in which case VolLiqAdd is not zero). Column 5 contains the number (integer) of the replicate set, and Column 6 contains the characters 'OBS'. You can specify up to nine replicate sets. A replicate set can contain measurements at different time points and different temperatures. There is no restriction in the number of measurements in a replicate set. The different replicate sets do not necessarily need to contain the same number of measurements or the same time points of measuring. However, each replicate set should contain at least one measurement performed at each of the temperatures specified in the Temperature table. Measurements in the Observations table should be sorted: firstly by column 5 (number of replicate set; integer); secondly by Column 2 (temperature); and thirdly by Column 1 (time). Specify missing values or values you do not want to include in the optimisation procedure (e.g. outliers) as -99.999.

2. Modify the contents of the example.bat file (with right mouse button, select edit): **replace 'example' everywhere by the name of the copied input file.** Repeat step 1-5 of Chapter 7. Note that in the example two replicate sets are used. Each replicate set is linked to an PEST instruction file (*.ins). The PEST program INCHEK is executed to check the 'ins'-file(s). In case you remove or add replicate sets, you need to modify example.bat such that 'ins'-file(s) are checked by the INCHEK program.

3. If the optimisation is not successful, you can try re-running PEARLNEQ with different initial guesses of MasIni, DT50Ref, FacSorNeqEql, KomEql and CofRatDes.

The optimisation with PEST varies the values of the parameters within a range specified in the PEST control.pst file. This file contains default ranges as specified by the PEARLMK program. If you would like to modify the range, please open the.pst file during the first pause in the execution with the.bat file (see an example pst file in Figure 3).

```
pcf
* control data
restart estimation
  6  60    6 0  4
1 2 single point 1 0 0
5.0 2.0 0.1 0.01 15
3.0 4.0 1.0e-3
0.1
50 0.001 5 10 0.001 4
1 1 1
* parameter groups
FSNE    relative 0.01  0.00001  always_3  2.0 best_fit
CRD     relative 0.01  0.00001  always_3  2.0 best_fit
DT50    relative 0.01  0.00001  always_3  2.0 best_fit
MASINI  relative 0.01  0.00001  always_3  2.0 best_fit
KOMEQL  relative 0.01  0.00001  always_3  2.0 best_fit
MET     relative 0.01  0.00001  always_3  2.0 best_fit
* parameter data
FSNE    none factor      0.5000  0.01     10.0  FSNE    1.00  0.00  1
CRD     none factor      0.0100  1.e-5     0.5  CRD     1.00  0.00  1
DT50    none factor     14.0000  1.0     500.0  DT50    1.00  0.00  1
MASINI  none factor     54.6400  0.001  1000.0  MASINI  1.00  0.00  1
KOMEQL  none factor      2.1000  0.1    40000.0  KOMEQL  1.00  0.00  1
MET     none factor    110.0000 10.0     200.0  MET     1.00  0.00  1
* observation groups
group_1
group_2
group_3
group_4
```

Figure 3   *First part of an example pst file showing the ranges of the fitting parameters in the section 'parameter data'. The minima and maxima are given in the fifth and sixth column (e.g. 0.01 and 10.0 for FSNE).*

9   Assessment of confidence intervals of parameters by bootstrapping

Introduction

As described above, PEARLNEQ uses PEST for estimating the long-term sorption parameters (i.e. the desorption rate coefficient k_d and the factor for describing the ratio between the equilibrium and non-equilibrium Freundlich coefficients f_{NE}). PEST is based on the Levenberg-Marquardt algorithm; it provides best-fit values and 95% confidence intervals of the fitted parameters. Intervals of 95% confidence can be used to calculate the relative standard error (RSE) which is recommended as a criterion for parameter acceptance by EFSA (2018). However, in the output file containing these intervals, PEST gives a warning that these intervals may be inaccurate because they are calculated on the basis of a linearity assumption which may be invalid (Doherty, 2005). So, it is no surprise that the 95% confidence limits from PEST sometimes are indeed inaccurate (ter Horst et al., 2013). Uncertainty in model parameters may be important for regulatory decision-making. For example, groundwater scenario calculations are commonly based on averages of sorption parameters derived from different soils. So, in case of large differences between parameter uncertainties derived from different studies, it may be advisable to give values with a large uncertainty less weight than parameters with a small uncertainty (or to discard values with a large uncertainty). However, a prerequisite is then that the uncertainties are assessed adequately. Thus, we decided to work on improving the uncertainty assessment of k_d and f_{NE}.

Ter Horst et al. (2013) analysed the effect of system parameters on the expected error in the long-term sorption parameters and found that the error in these parameters is mostly less than 25% if the CV of the parameter derived from PEST is lower than about 20%. This was based on a fixed experimental design and on simulated errors derived from one source (a set of adsorption experiments with one pesticide by co-authors). However, assuming an acceptable error of 25% is an arbitrary choice, and it seems useful to develop a more generic procedure for estimating the confidence interval more accurately. So, we take a different approach: we start from the dataset under consideration and attempt to improve the estimation procedure for the confidence interval of the parameters.

We started with assessing the uncertainty by MCMC (Monte-Carlo Markov Chain) using the Metropolis-Hastings algorithm for stepping through the parameter space. However, this was not successful because the autocorrelation in the chain of draws of k_d or f_{NE} remained too large; this is possibly caused by the fact that PEARLNEQ fits 5-6 parameters simultaneously. An alternative is to use parametric bootstrapping (Davison & Hinkley, 1997). This bootstrapping has the disadvantage that the computation time is about one order of magnitude longer. However, it is more robust, as it does not have such an autocorrelation problem. For these reasons, we decided to develop a parametric bootstrapping procedure.

Note that the bootstrapping method was not considered in the 2021 version of the Aged Sorption guidance (EC, 2021) and should therefore not be used for refining the RSE value in the context of the regulatory guidance for aged sorption.

Procedure of parametric bootstrapping procedure

The bootstrapping procedure works as follows:
- The model is fitted to the dataset by using PEST
- The error in the measurements is estimated from the residuals of the fit
- A large number of datasets (e.g. 1000) is generated based on the best-fit values of the parameters and based on the estimated errors assuming that these are normally distributed
- These new datasets are fitted with PEST and confidence intervals are estimated from the distribution of the fitted parameter values

So the first step (after fitting the data to the model) is to estimate the error in the measurements. We developed this bootstrap procedure with the limitation of assuming that each measurement is equally important in the fitting procedure, i.e. using the option 'inverse'. As described before, the option 'inverse' gave best results in a few tests and is considered the standard for studies on long-term sorption kinetics (EFSA, 2018, p.16).

As described before in Chapter 5, the option 'inverse' is consistent with the concept of relative errors. So, we assume that M_p and c_L have normally distributed relative errors. The use of relative errors means that the errors can be expressed in terms of the coefficient of variation (CV). So, we need to estimate CVs of c_L and M_p from the fit by PEST to the dataset. The CV of M_p, CV_M (-), was estimated by

$$CV_M = \sqrt{\frac{\Sigma\left(\frac{M_{p,m} - M_{p,f}}{M_{p,f}}\right)^2}{n-p}}$$
(12)

where $M_{p,m}$ is the measured mass (µg), $M_{p,f}$ is the fitted mass (µg), n is the number of measurements and p is the number of fitting parameters. The same approach was used for the CV of c_L. There are five or six fitting parameters (six if data at different temperatures are available). However, we have two types of measurements (c_L and M_p) that are fitted simultaneously. It therefore seems appropriate to divide the numbers of parameters between these two types, so, using $p = 2.5$ or $p = 3$ for each.

We checked the use of $p = 2.5$ by generating 1000 datasets for a pesticide-soil incubation system with $k_d = 0.026$ d^{-1}, $f_{NE} = 0.64$, $DegT50_{EQ} = 24$ d, $N = 0.9$, and $K_F = 2.8$ L/kg. Each dataset consisted of duplicate values of M_p and c_L for nine times (0, 15, 30, …, 105 and 120 days). The values of M_p and c_L were generated assuming normally distributed errors of 6% and 3%, respectively. The 1000 datasets were fitted with PEARLNEQ/PEST, and CVs of M_p and c_L were derived from the differences between the generated and fitted values using the above procedure (using $p = 2.5$ as only data at one temperature were used). The average CVs of M_p and c_L were found to be 6.06% and 2.98%, respectively, so, very close to the true values. Thus the estimation procedure for the CVs of M_p and c_L generated on average correct CVs (using $p = 5$ instead of $p = 2.5$ gave average CVs of 6.61% and 3.25%, so, using $p = 5$ is clearly not correct).

However, the standard deviations of the 1000 generated CVs of M_p and c_L using $p = 2.5$ were found to be 1.10% and 0.54%, respectively. This indicates that there is some uncertainty when estimating a CV from a single dataset (i.e. the situation in practice) that consists of duplicate values for nine times. We tested this further by generating 1000 datasets consisting each of 18 values randomly drawn from a normal distribution with a mean of 1.0 and a CV of 6% (i.e. the same CV as M_p). Calculating back the CVs of the sample population of 18 values gave a mean CV of 5.93% with a standard deviation of 1.03%. This 1.03% is close to the 1.10% obtained for M_p, so, the uncertainty in the estimated CVs is probably just a consequence of having only 18 datapoints.

The next step is to generate a large number of datasets using these estimated CVs and to fit these with PEST. The last step is then to estimate the 95th confidence interval from the statistical populations of the fitted k_d and f_{NE} values. This was done using

$$P_i = \frac{i - \frac{1}{2}}{B}$$
(13)

where i is the rank number of a sorted array of the parameter values, P_i is the percentile corresponding with the parameter value of rank number i and B is the total number of values of the statistical population (i.e. the number of datasets generated). This generates a large number of pairs of percentiles and parameter values from which the 95th confidence interval was estimated by linear interpolation. Note that the bootstrapping procedure described here aims at improving the uncertainty assessment of k_d and $f_{NE.}$ The confidence interval of all other parameters optimised should be taken from the PEST output.

We tested this procedure in a series of example calculations (see Appendix 4). A series of calculations for datasets artificially generated by assuming normally distributed relative errors showed that bootstrapping confidence intervals were usually very close to those of PEST for k_d whereas the widths from bootstrapping were mostly smaller for f_{NE}. If the widths were small, also the widths for f_{NE} from bootstrapping and PEST were close to each other (as was to be expected and thus can be seen as a check of the bootstrapping procedure). Although based on a limited number of cases, Appendix 4 seems to indicate that PEST confidence intervals for the aged sorption fitting procedure are reasonably accurate, especially for k_d. So the user should not expect a spectacular lowering of widths of confidence intervals by applying bootstrapping. Given also the comparatively long computation time, the use of bootstrapping seems only advisable if the widths of the confidence intervals play a critical role.

Instructions for the bootstrapping procedure
The necessary files for the bootstrap procedure are in the subdirectory 'bootstrapping'. The procedure requires that you have obtained a satisfactory fit of the data by PEARLNEQ/PEST based on an input file called `source.mkn` and that the results of the fit are available in the corresponding PEST output file `source.rec`. The subdirectory contains as an example `source.mkn` and `source.rec` files used for run nr 9 of the example calculations described in Appendix 4. Note, that if you have used a different name during the optimisation setup (e.g. example.mkn), you have to rename the *.mkn and *.rec files to source.mkn and source.rec.

For the bootstrapping procedure to work, the file `source.mkn` must fulfil the following requirements:
- The Observations table should be the last part of the file
- There should be no empty lines in the file below the last line (i.e. the line with the 'end_table' statement which closes the Observations table).

So, copy your `source.mkn` and `source.rec` files to the 'bootstrapping subdirectory', go to this subdirectory and proceed as follows:
1. Specify the number of bootstrap datasets (*B*) to be generated in the file `nr_of_datasets.txt`.
2. Generate these *B* datasets by running `generate_data.bat` resulting in *B* 'mkn'-files. The initial guesses for the parameters are automatically set to the optimised values from the provided source.rec file.
3. Running `generate_data.bat` also gives a file `source_output.txt` with a listing of all differences between measured and fitted values together with the calculated standard deviations of the relative differences of M_p and c_L that were used to generate the datasets.
4. Furthermore this generates a file `run_pearlneq.bat` which should be run to fit these datasets with PEST; this results in *B* 'rec'-files.
   After the fitting of the datasets has been completed, this 'bat'-file calls the file `cleanup.bat` which removes PEST output files other than the 'rec'-file (besides this file, PEST generates some 20 other output files for each fit of a dataset).
5. Postprocess these *B* 'rec'-files by running `postprocess.bat` which will generate an output file `bootstrap_output.txt` containing the bootstrapping results.

The output file contains (1) a list of all *B* fitted parameter values of k_d and f_{NE}, (2) the cumulative frequency distribution of k_d and f_{NE} and (3) the derived 95% confidence intervals of these parameters and their median using increasing numbers of datasets. An example of the last item is shown below.

Factor for describing the ratio between the equilibrium and non-equilibrium Freundlich coefficients (-)

```
------------------------

2.5th, 50th and 97.5th percentiles based on first    100 values  :      0.248    0.455    0.706

2.5th, 50th and 97.5th percentiles based on first    200 values  :      0.267    0.443    0.762

2.5th, 50th and 97.5th percentiles based on first    300 values  :      0.258    0.444    0.790

2.5th, 50th and 97.5th percentiles based on first    400 values  :      0.267    0.444    0.795

2.5th, 50th and 97.5th percentiles based on first    500 values  :      0.258    0.445    0.790

2.5th, 50th and 97.5th percentiles based on first    600 values  :      0.263    0.444    0.789

2.5th, 50th and 97.5th percentiles based on first    700 values  :      0.265    0.445    0.788

2.5th, 50th and 97.5th percentiles based on first    800 values  :      0.267    0.442    0.786
```

```
2.5th, 50th and 97.5th percentiles based on first   900 values  :       0.270    0.443    0.785
2.5th, 50th and 97.5th percentiles based on all     999 values  :       0.271    0.444    0.784
```

Thus the user can check whether the number of datasets chosen was sufficiently large for obtaining an accurate 95% confidence interval.

The generation of the bootstrapping datasets is a random process which requires a seed value. This is automatically generated based on the clock time of the computer (with echo to the file `seed.txt`) and thus will differ between different generation procedures. In case you want for some reason the same seed, then provide a file `prescribed_seed.txt` which contains as the only information this seed value; the value must be a negative integer lower than −10000, so e.g. −12345.

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Justification

Akkoord Extern contactpersoon

functie: Senior beleidsmedewerker gewasbescherming

naam: Eelco Riemens

datum: 25 mei 2021

Akkoord Intern contactpersoon

naam: Erwin van Boekel

datum: 28 mei 2021

Annex 1   Example input file

```
*----------------------------------------------------------------------------------------
* STANDARD FILE for pearlmk version 6
* Program to fit the half-life, activation energy and parameters for long-term sorption
* kinetics of pesticides in soil
*
* This file is intended for use with the PEST program
* Please refer to the manual of PEARLNEQ
*
*----------------------------------------------------------------------------------------

* Model control
Yes               ScreenOutput
0.0               TimStart        (d)                Start time of experiment
500.0             TimEnd          (d)                End time of experiment
0.01              DelTim          (d)                Time step of Euler's integration procedure

* System characterization
54.64             MasIni          (ug)               Initial guess of initial mass
45.36             MasSol          (g)                Mass of soil in incubation jar
6.64              VolLiqSol       (mL)               Volume of liquid in the moist soil
0.0               VolLiqAdd       (mL)               Volume of liquid ADDED
0.047             CntOm           (kg.kg-1)          Organic matter content

* Sorption parameter
1.0               ConLiqRef       (mg.L-1)           Reference liquid concentration
0.87              ExpFre          (-)                Freundlich exponent
2.1               KomEql          (L.kg-1)           Initial guess of Coefficient for equilibrium sorption
0.5               FacSorNeqEql    (-)                Initial guess of ratio KfNeq/KfEql
0.02              CofRatDes       (d-1)              Initial guess of desorption rate constant
Neql              OptSor          (-)                Option for type of sorption process to be simulated:
'Neql' or 'Eql'

* Transformation parameters
14.00             DT50Ref         (d)                Initial guess of half-life at ref. temperature
20.0              TemRefTra       (C)                Reference temperature
110.0             MolEntTra       (kJ.mol-1)         Initial guess of molar activation energy

* Temperature at which the incubation experiments have been carried out
table Tem (C)
1  5.0
2  15.0
end_table

* Number of replicate sets (range 1 - 9)
* A set of replicates can contain observation at different time points and temperatures
* Each replicate set should contain at least one measurement performed at each of the temperatures specified
in table Tem
* 1st sort by Rep. (column 5), 2nd sort by Tem (column 2), 3rd sort by Tim (column 1)
* specify missing values or values you do not want to include in the optimisation procedure (e.g. outliers)
as -99.999
* PEARLMK will give these observations a weight of zero, meaning that the observation takes to part in the
optimisation
2                 NumRepSet       (-)

* Option for weights of Observations:
*'equal' gives an equal weight to all pesticide masses and an equal (but different) weight to all
concentrations
*'inverse' gives weight equal to inverse value of each measurement (if measurement is zero then weight is
1.0)
inverse    Opt_weights

* Option for description of transformation rate
* 'EqlDom' uses rate based on amount of substance in equilibrium domain
* 'LiqPhs' uses rate based on amount of substance in liquid phase
EqlDom     Opt_transformation

* Provide the results of the measurements
* Tim  Tem  Mas       ConLiq   Rep.  observation ID
* (d)  (C)  (ug)      (ug/mL)
table Observations
2      5    52.2400   5.9340    1      OBS
10     5    50.7800   4.4670    1      OBS
42     5    46.0200   3.9340    1      OBS
87     5    37.8200   2.8560    1      OBS
157    5    33.1800   1.9390    1      OBS
244    5    25.5300   1.4640    1      OBS
358    5    18.1900   0.8660    1      OBS
451    5    10.4300   0.6360    1      OBS
2      15   51.5600   5.8530    1      OBS
6      15   48.3100   4.3540    1      OBS
10     15   44.6900   3.5730    1      OBS
42     15   23.9400   1.5650    1      OBS
87     15   10.9600   0.6560    1      OBS
157    15    3.2800   0.1500    1      OBS
```

```
244    15     1.4600    0.0310    1    OBS
2       5    51.0200    5.5230    2    OBS
10      5    50.4000    5.6450    2    OBS
42      5   -99.9999    3.3930    2    OBS
87      5    39.4000    3.0080    2    OBS
157     5    32.4500    1.9170    2    OBS
244     5    26.2100    1.4660    2    OBS
358     5    22.4400    0.8980    2    OBS
451     5     8.4200    0.5670    2    OBS
2      15    51.1000    5.9380    2    OBS
6      15    46.4800    4.5310    2    OBS
10     15    54.4400    4.3290    2    OBS
42     15    22.3400    1.7290    2    OBS
87     15    10.8300    0.6860    2    OBS
157    15     2.9900    0.1550    2    OBS
244    15     1.4200    0.0300    2    OBS
end_table
```

Annex 2   Results of the default example

Results (taken from last section of REC-file)

These are the results of the default example, provided with the package.

```
                    OPTIMISATION RESULTS



Parameters ----->

Parameter         Estimated          95% percent confidence limits
                  value            lower limit       upper limit
 fsne             0.396764         0.260668          0.532859
 crd              5.660101E-03     2.669861E-03      8.650341E-03
 dt50             15.2563          14.1249           16.3877
 masini           56.6135          53.5068           59.7203
 komeql           2.79245          2.34462           3.24027
 met              105.646          101.796           109.496


Note: confidence limits provide only an indication of parameter uncertainty.
      They rely on a linearity assumption which  may not extend as far in
      parameter space as the confidence limits themselves - see PEST manual.


See file example.sen for parameter sensitivities.



Observations ----->

Observation        Measured       Calculated     Residual      Weight     Group
                   value          value
o1                 52.2400        56.1193        -3.87932       1.9142E-02  group_1
o2                 5.93400        4.87707         1.05693       0.1685      group_1
o3                 50.7800        54.1942        -3.41422       1.9693E-02  group_1
o4                 4.46700        4.66290        -0.195900      0.2239      group_1
o5                 46.0200        47.2533        -1.23326       2.1730E-02  group_1
o6                 3.93400        3.90901         2.498741E-02  0.2542      group_1
o7                 37.8200        39.2265        -1.40653       2.6441E-02  group_1
o8                 2.85600        3.07750        -0.221498      0.3501      group_1
o9                 33.1800        29.7653         3.41472       3.0139E-02  group_1
o10                1.93900        2.16413        -0.225132      0.5157      group_1
o11                25.5300        21.5310         3.99896       3.9170E-02  group_1
o12                1.46400        1.44149         2.250611E-02  0.6831      group_1
o13                18.1900        14.4324         3.75758       5.4975E-02  group_1
o14                0.866000       0.884725       -1.872510E-02  1.155      group_1
o15                10.4300        10.5670        -0.137019      9.5877E-02  group_1
o16                0.636000       0.611510        2.448954E-02  1.572      group_1
o17                51.5600        54.2417        -2.68173       1.9395E-02  group_2
o18                5.85300        4.70441         1.14859       0.1709      group_2
o19                48.3100        49.8048        -1.49476       2.0700E-02  group_2
o20                4.35400        4.27968         7.432244E-02  0.2297      group_2
o21                44.6900        45.7471        -1.05710       2.2376E-02  group_2
```

o22	3.57300	3.89342	-0.320422	0.2799	group_2
o23	23.9400	23.5524	0.387621	4.1771E-02	group_2
o24	1.56500	1.83091	-0.265914	0.6390	group_2
o25	10.9600	9.93362	1.02638	9.1241E-02	group_2
o26	0.656000	0.643754	1.224635E-02	1.524	group_2
o27	3.28000	3.36264	-8.264049E-02	0.3049	group_2
o28	0.150000	0.140282	9.718440E-03	6.667	group_2
o29	1.46000	1.41841	4.159341E-02	0.6849	group_2
o30	3.100000E-02	3.173109E-02	-7.310900E-04	32.26	group_2
o31	51.0200	56.1193	-5.09932	1.9600E-02	group_3
o32	5.52300	4.87707	0.645931	0.1811	group_3
o33	50.4000	54.1942	-3.79422	1.9841E-02	group_3
o34	5.64500	4.66290	0.982100	0.1771	group_3
o35	-99.9999	47.2533	-147.253	0.000	group_3
o36	3.39300	3.90901	-0.516013	0.2947	group_3
o37	39.4000	39.2265	0.173472	2.5381E-02	group_3
o38	3.00800	3.07750	-6.949845E-02	0.3324	group_3
o39	32.4500	29.7653	2.68472	3.0817E-02	group_3
o40	1.91700	2.16413	-0.247132	0.5216	group_3
o41	26.2100	21.5310	4.67896	3.8153E-02	group_3
o42	1.46600	1.44149	2.450611E-02	0.6821	group_3
o43	22.4400	14.4324	8.00758	4.4563E-02	group_3
o44	0.898000	0.884725	1.327490E-02	1.114	group_3
o45	8.42000	10.5670	-2.14702	0.1188	group_3
o46	0.567000	0.611510	-4.451046E-02	1.764	group_3
o47	51.1000	54.2417	-3.14173	1.9569E-02	group_4
o48	5.93800	4.70441	1.23359	0.1684	group_4
o49	46.4800	49.8048	-3.32476	2.1515E-02	group_4
o50	4.53100	4.27968	0.251322	0.2207	group_4
o51	54.4400	45.7471	8.69290	1.8369E-02	group_4
o52	4.32900	3.89342	0.435578	0.2310	group_4
o53	22.3400	23.5524	-1.21238	4.4763E-02	group_4
o54	1.72900	1.83091	-0.101914	0.5784	group_4
o55	10.8300	9.93362	0.896377	9.2336E-02	group_4
o56	0.686000	0.643754	4.224635E-02	1.458	group_4
o57	2.99000	3.36264	-0.372640	0.3344	group_4
o58	0.155000	0.140282	1.471844E-02	6.452	group_4
o59	1.42000	1.41841	1.593410E-03	0.7042	group_4
o60	3.000000E-02	3.173109E-02	-1.731090E-03	33.33	group_4

Explanation of the observations and groups in the 'Observations' table in the PEST *.rec output file

Group:
- group_1 – contains all observations of Mas[1] and ConLiq[2] belonging to replicate set 1 and the first temperature at which the incubation experiments have been carried out (5 C in the example)
- group_2 – contains all observations of Mas[1] and ConLiq[2] belonging to replicate set 1 and the second temperature at which the incubation experiments have been carried out (15 C in the example)
- group_3 – contains all observations of Mas[1] and ConLiq[2] belonging to replicate set 2 and the first temperature at which the incubation experiments have been carried out (5 C in the example)
- group_4 – contains all observations of Mas[1] and ConLiq[2] belonging to replicate set 2 and the second temperature at which the incubation experiments have been carried out (15 C in the example)

Observations:
The observations with the odd numbers (o1, o3 etc.) are masses and those with the even numbers (o2, o4 etc.) are concentrations in the liquid phase.

[1]Mas: the total mass of pesticide in the system (µg)

[2]ConLiq: the concentration of pesticide (µg mL^{-1}) measured in the pore water of moist soil (then VolLiqAdd = 0) or in the water. phase after a desorption experiment (in which case VolLiqAdd is not zero).

# Annex 3 Comparison between an analytical solution and PEARLNEQ

In this appendix an analytical solution for the remaining mass of pesticide is compared with the PEARLNEQ solution. The system properties were:

- Mass of dry soil (MasSol) (g)   1.0000
- Volume of water in moist soil (VolLiqsol) (mL)   0.2000
- Volume of water added (VolLiqAdd (mL)   0.0000
- Initial mass of pesticide (MasIni) (ug)   10.0000
- Reference concentration (ConLiqRef) (ug.mL-1)   1.0000
- Equilibrium sorption coefficient (CofFreEql) (mL.g-1)   1.0000
- Non-equili. sorption coefficient (CofFreNeq) (mL.g-1)   0.5000
- Freundlich exponent (ExFre) (-)   1.0000
- Desorption rate coefficient (CofRatDes) (d-1)   0.0100
- Half-life transformation (DT50Ref) (d)   1.0000
- Reference temperature (TemRefTra) (K)   293.1500

The transformation rate concept of Eqn 5-A was used.
The analytical solution was taken from Appendix 4 of FOCUS (2006).

The figure shows that the PEARLNEQ solution coincides very well with the analytical solution.

Mass of substance in jar ($\square$g)
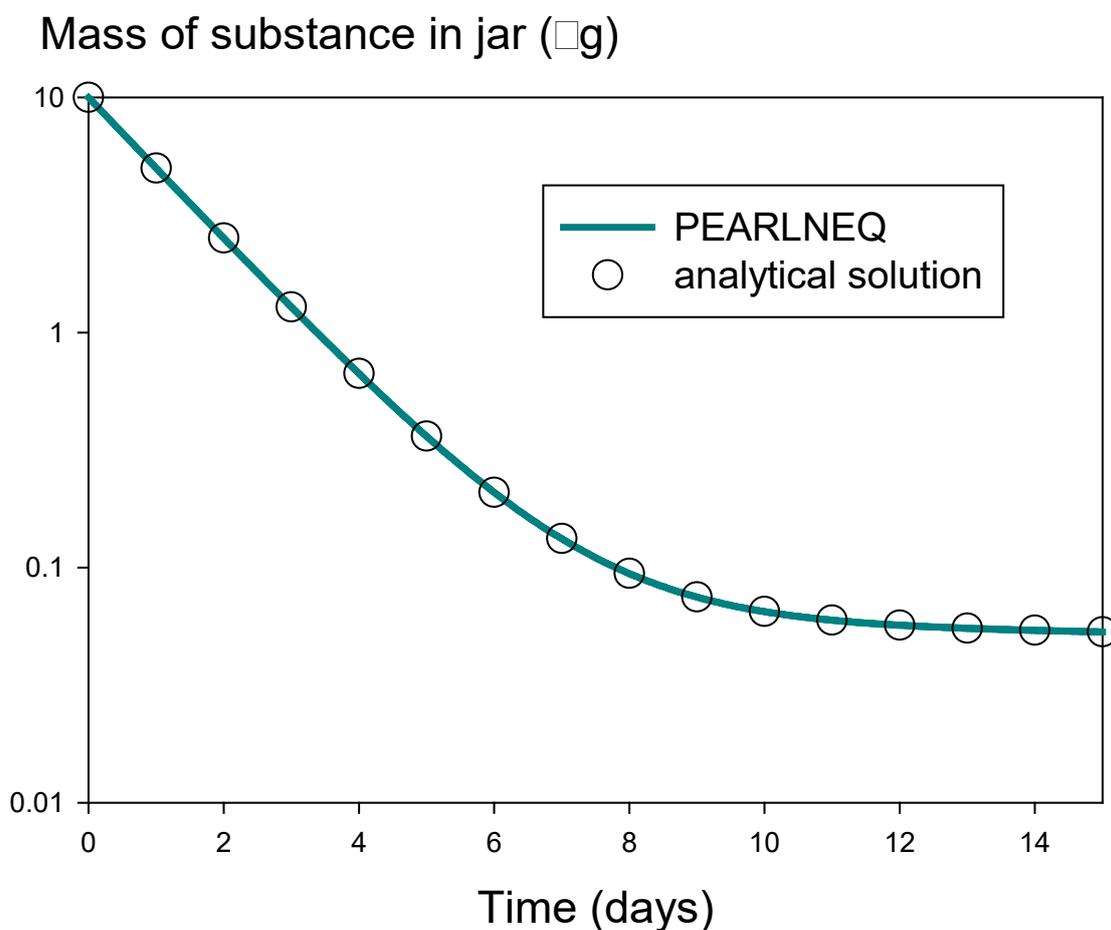
Comparison between the analytical solution and the PEARLNEQ solution

Annex 4 Example calculations of bootstrapping confidence intervals

A series of example calculations was carried out to illustrate the bootstrapping application. The procedure was as follows:

- a single dataset was generated assuming normally distributed errors in remaining mass (M_p) and concentration in liquid phase (c_L) with coefficients of variation (CVs) of 6% and 3%, respectively; this dataset is called the 'source dataset'; we expect these 6% and 3% to be realistic (e.g. ter Horst et al., 2013, found 7% and 4% for experiments with four soils and one pesticide)
- this dataset was fitted with PEST and from the differences between fitted and measured values the CVs of M_p and c_L were estimated using Eqn 12 as described in Chapter 9
- based on these fitted M_p and c_L values and their estimated CVs, a large number (999) of datasets (further called 'bootstrap data sets') were generated assuming normally distributed errors in M_p and c_L and these were all fitted with PEST
- 2.5 and 97.5 percentiles of k_d and f_{NE} estimated by PEST for the source dataset were compared to the 2.5 and 97.5 percentiles of k_d and f_{NE} estimated from the best-fit values derived from the bootstrap datasets)

The source and bootstrap datasets consisted of duplicate values of M_p and c_L for nine times (0, 15, 30, 45, 60, 75, 90, 105 and 120 days). The Freundlich exponent was fixed to 0.9. Calculations were made for three combinations of k_d and f_{NE} based on an earlier analysis of k_d and f_{NE} data (Boesten, 2017), representing small, medium and large effects of aged sorption:

Effect of aged sorption	k_d (d^{-1})	f_{NE} (-)
Small	0.032	0.33
Medium	0.026	0.64
Large	0.014	2.99

Calculations were made for three combinations of K_F and the half-life for the equilibrium part of the aged sorption system, $DegT50_{EQ}$. These combinations were selected in such a way that they would result in a FOCUS leaching concentration of about 1 µg/L for the FOCUS Kremsmuenster scenario based on calculations assuming no aged sorption (Boesten, 2017). This procedure was followed to ensure that the corresponding substances would require a higher-tier approach such as including aged sorption. These Kremsmuenster calculations showed that this leaching concentration was approximately found if the K_{om} is equal to 1.8 times the $DegT50_{SFO}$ (i.e. the $DegT50$ assuming single first-order kinetics). The organic matter content of the top layer in Kremsmuenster is 0.036, which gives then $K_F = 0.07 \, DegT50_{SFO}$. The $DegT50_{EQ}$ can be approximated by $DegT50_{SFO} / (1 + f_{NE})$ (Boesten, 2017). Calculations were made for $DegT50_{SFO}$ values of 20, 40 and 60 d from which values of K_F and $DegT50_{EQ}$ were derived using these approximations. This resulted in the combinations of input parameters for the nine cases as shown in Table 1.

Table 1   *Pesticide properties used for example calculations based on the Kremsmuenster scenario.*

Nr	DegT50$_{SFO}$ (d)	K_F (L/kg)	k_d (d^{-1})	f_{NE} (-)	DegT50$_{EQ}$ (d)
1			0.032	0.33	15
2	20	1.4	0.026	0.64	12
3			0.014	2.99	5
4			0.032	0.33	30
5	40	2.8	0.026	0.64	24
6			0.014	2.99	10
7			0.032	0.33	45
8	60	4.2	0.026	0.64	37
9			0.014	2.99	15

The results in Table 2 and Table 3 indicate that the confidence intervals based on bootstrapping were either approximately equal to those obtained from a single PEST fit or smaller. This is further illustrated by the widths of the confidence interval divided by the median parameter value (called 'relative widths') as shown in Figure 1: the relative widths of the intervals from PEST and bootstrapping were usually very close to each other for k_d whereas the widths from bootstrapping were mostly smaller for f_{NE}. One would expect that the confidence intervals from PEST and bootstrapping are the same if they are small: Figure 1 shows that this is indeed the case. Table 4 gives the numbers of the relative widths of the confidence intervals for the different datasets. Combination with Table 1 shows that the relative widths decrease with increasing value of f_{NE}.

Table 2   *Upper and lower limits of the 95% confidence interval of the desorption rate coefficient k_d and its median value (derived from PEST or bootstrapping) for the example calculations with pesticide properties as shown in Table 1.*

	k_d (d^{-1}) × 10 000					
	Lower limit		Median		Upper limit	
Set	PEST	Bootstrap	PEST	Bootstrap	PEST	Bootstrap
1	276	279	318	317	360	358
2	227	229	247	248	268	268
3	128	128	134	135	141	141
4	226	240	304	301	381	378
5	219	225	262	262	306	307
6	132	132	140	140	149	149
7	178	210	294	294	410	429
8	198	211	264	263	330	329
9	129	130	139	139	147	147

Table 3   *Upper and lower limits of the 95% confidence interval of the desorption rate coefficient f_{NE} and its median value (derived from PEST or bootstrapping) for the example calculations with pesticide properties as shown in Table 1.*

Set	f_{NE} (-) × 1 000					
	Lower limit		Median		Upper limit	
	PEST	Bootstrap	PEST	Bootstrap	PEST	Bootstrap
1	301	319	348	349	395	380
2	619	636	671	671	723	711
3	2971	2988	3157	3156	3343	3359
4	276	296	334	336	392	382
5	568	586	650	653	732	727
6	2867	2873	3069	3069	3271	3303
7	271	290	358	360	445	442
8	508	527	612	619	717	711
9	2774	2773	2973	2968	3172	3187

## Width confidence interval (%) from bootstrapping
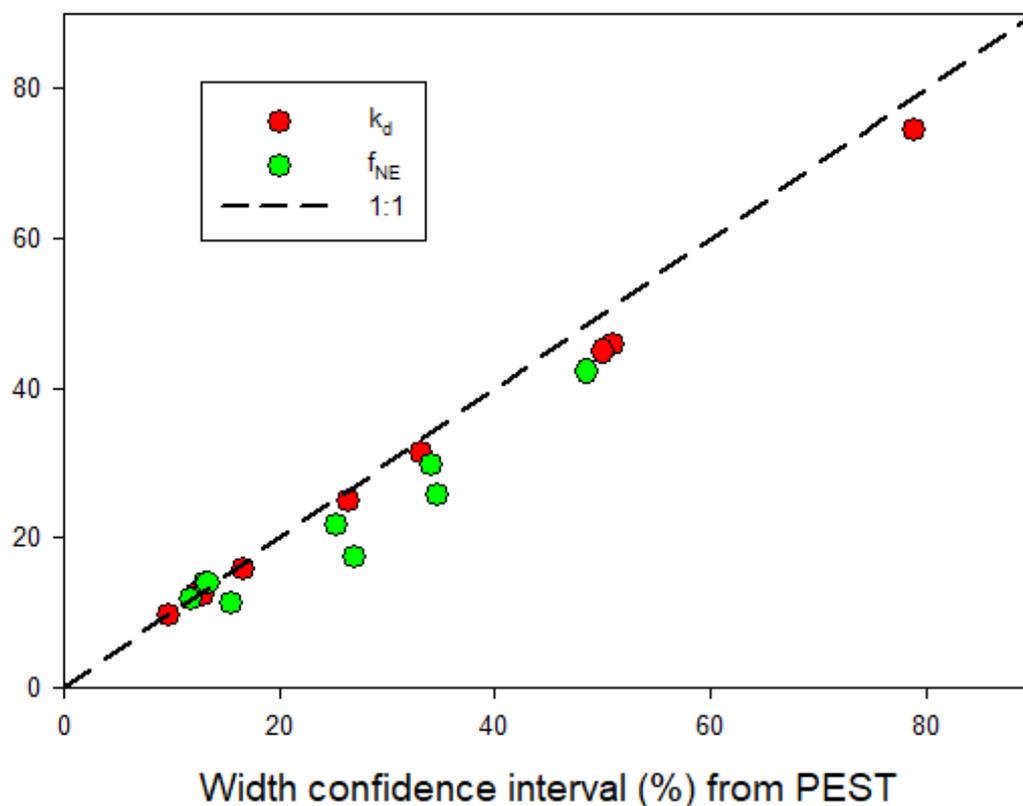
Figure 1 *Relative width of the 95% confidence interval (defined as the width of the interval expressed as a percentage of the median) as estimated with the bootstrapping procedure as a function of the corresponding width estimated with PEST from the source data set.*

Table 4 *Relative widths of the 95% confidence interval of k_d and f_{NE} in percents (derived from PEST or bootstrapping) for the example calculations with pesticide properties as shown in Table 1.*

Set	% for k_d		% for f_{NE}	
	PEST	Bootstrap	PEST	Bootstrap
1	26.4	24.9	27.0	17.5
2	16.6	15.8	15.5	11.2
3	9.7	9.7	11.8	11.8
4	51.0	46.2	34.7	25.6
5	33.2	31.3	25.2	21.7
6	12.1	12.9	13.2	14.1
7	78.9	74.8	48.6	42.2
8	50.0	44.9	34.2	29.2
9	12.9	12.2	13.4	13.9

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