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ETX 2.0

A Program to Calculate Hazardous Concentrations and Fraction Affected, Based on Normally Distributed Toxicity Data

P.L.A. van Vlaardingen*, T.P. Traas,
A.M. Wintersen and T. Aldenberg

This investigation has been performed for the account of the Directorate-General for Environmental Protection, Directorate for Chemicals, Waste and Radiation, in the context of the projects 'Setting (Inter)national Environmental Quality Standards'.

*Corresponding author. RIVM, Expert Centre for Substances, P.O. Box 1, 3720 BA Bilthoven, the Netherlands. E-mail: etx.info@rivm.nl

ETX 2.0 was programmed by Arjen Wintersen, working for Augustijn Onderzoek, WG-plein 406, 1054 SH Amsterdam.

National Institute for Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, the Netherlands.
Telephone: 31-30-2749111; fax: 31-30-2742971

Rapport in het kort

E_TX 2.0. Een programma om 'hazardous concentrations' en 'fraction affected' te berekenen, gebaseerd op normaal verdeelde toxiciteitsgegevens.

Dit rapport is geschreven als handleiding bij het software programma *E_TX* 2.0. De rekentech-
nieken die met dit programma worden aangeboden worden onder andere gebruikt binnen het
RIVM project '(Inter)nationale normstelling stoffen' (INS) maar ook bij de Europese risico-
beoordeling van bestaande stoffen. In deze projecten worden milieurisicogrenzen afgeleid
voor chemische stoffen. Zowel het INS- als het EU-raamwerk staat het gebruik van statisti-
sche extrapolatie toe wanneer voldoende toxiciteitsgegevens beschikbaar zijn. De resultaten
van deze extrapolatie dienen als basis voor een milieurisicogrens (INS) of een geen-effect
niveau (EU bestaande stoffen) van een chemische stof. In de wetenschappelijke literatuur is
recent een methode beschreven om deze statistische berekening uit te voeren. Het programma
E_TX 2.0 maakt deze methode toegankelijk voor hen die werkzaam zijn in de vakgebieden van
de risicobeoordeling en/of normstelling. Het programma wordt samen met dit rapport ver-
spreid op CD-ROM.

Trefwoorden: computerprogramma; soortsgoedigheidsverdeling; statistische extrapolatie;
risicobeoordeling.

Abstract

E_TX 2.0. A Program to Calculate Hazardous Concentrations and Fraction Affected, Based on Normally Distributed Toxicity Data.

This report was written as a manual for the software program, *E_TX* 2.0. The calculation techniques offered here are currently in use in the RIVM project, 'Setting (inter)national environmental quality criteria' (INS), and the EU risk assessment for existing substances. Environmental risk limits for chemical substances are derived here. Both the INS and EU frameworks allow for statistical extrapolation in the presence of sufficient toxicity data, which serves as a basis for an environmental risk limit (INS) or a predicted no-effect concentration (EU-existing substances) of a chemical substance. A statistical technique for achieving this result, recently described in the scientific literature, is made accessible to those working on risk assessment and/or standard-setting through the *E_TX* 2.0 program. A CD-ROM is delivered along with the report.

Keywords: software; species sensitivity distribution; statistical extrapolation; risk assessment; hazardous concentration.

Contents

SAMENVATTING	9
SUMMARY.....	11
1. INTRODUCTION.....	13
1.1 REFERRING TO <i>E_TX</i> 2.0.....	13
2. WHAT CAN <i>E_TX</i> 2.0 DO FOR ME?	15
3. USER'S GUIDE.....	17
3.1 PROGRAM TYPE AND SYSTEM REQUIREMENTS	17
3.2 INSTALLING <i>E_TX</i> 2.0.....	17
3.3 REMOVING <i>E_TX</i>	21
3.4 STARTING <i>E_TX</i>	21
3.5 SAVING DATA IN <i>E_TX</i>	21
3.6 STARTING A NEW PROJECT IN <i>E_TX</i>	22
3.7 LEAVING <i>E_TX</i>	22
3.8 CHANGING THE SETTINGS FOR THE DECIMAL CHARACTER	23
3.8.1 <i>Windows 98, NT, 2000 and ME</i>	23
3.8.2 <i>Windows XP</i>	23
3.9 COMMENTS ON <i>E_TX</i>	23
4. REFERENCE MANUAL	25
4.1 STRUCTURE OF THE PROGRAM	25
4.1.1 <i>Data input</i>	25
4.1.2 <i>Types of calculations</i>	26
4.1.3 <i>Output</i>	26
4.2 GLOSSARY OF KEYWORDS.....	29
4.2.1 <i>Exposure concentration (EC)</i>	29
4.2.2 <i>Expected Ecological Risk (EER)</i>	29
4.2.3 <i>Extrapolation factor</i>	29
4.2.4 <i>Fraction affected (FA)</i>	29
4.2.5 <i>SSD Histogram and PDF</i>	30
4.2.6 <i>Goodness-of-fit</i>	30
4.2.7 <i>HC₅ and HC₅₀</i>	31
4.2.8 <i>JPC</i>	31
4.2.9 <i>PEC</i>	31
4.2.10 <i>Small Sample method</i>	31
4.2.11 <i>Statistics SSD</i>	32
4.2.12 <i>SSD</i>	33
5. WORKING WITH <i>E_TX</i>.....	35
5.1 MENU BAR AND BUTTONS	35
5.1.1 <i>File</i>	35
5.1.2 <i>Edit</i>	35
5.1.3 <i>Calculate</i>	35
5.1.4 <i>Export</i>	35
5.1.5 <i>Tools</i>	35
5.1.6 <i>Help</i>	36
5.1.7 <i>Buttons</i>	36
5.2 NAVIGATING THROUGH <i>E_TX</i>	36
5.3 ENTERING DATA.....	37
5.3.1 <i>Toxicity data</i>	37
5.3.2 <i>Assigning units to your data</i>	38
5.3.3 <i>Small sample data</i>	38
5.3.4 <i>Environmental concentrations</i>	39

5.4	CLEARING THE CONTENTS OF THE INPUT CELLS	40
5.4.1	<i>Clearing a single cell</i>	40
5.4.2	<i>Clearing all cells</i>	41
5.5	IMPORTING DATA	41
5.6	ADDING LABELS TO TOXICITY DATA	41
5.6.1	<i>Creating a label collection and using labels</i>	41
5.6.2	<i>Editing an existing label collection</i>	43
5.6.3	<i>Deselecting a label collection</i>	43
5.7	PERFORMING CALCULATIONS.....	43
5.7.1	<i>Calculating an SSD</i>	43
5.7.2	<i>Calculating a small sample HD₅</i>	43
5.7.3	<i>Calculating an FA</i>	44
5.7.4	<i>Calculating an EER and JPC</i>	44
5.8	EXPORTING RESULTS.....	44
5.9	PREFERENCES	45
5.9.1	<i>Changing graph appearance in the current project</i>	45
5.9.2	<i>Changing the axis settings</i>	46
5.9.3	<i>Changing the default graph appearance</i>	47
5.9.4	<i>Changing the default file storage directory</i>	47
5.10	COPYING GRAPHS	48
5.11	USE OF THE DECIMAL SYMBOL	48
6.	LIMITATIONS	49
6.1	SSD CALCULATIONS	49
6.2	CALCULATION OF THE FRACTION AFFECTED	49
7.	EXAMPLES	51
7.1	CALCULATING AN SSD	51
7.2	CALCULATING A FRACTION OF AFFECTED SPECIES	53
7.3	CALCULATING AN EXPECTED ECOLOGICAL RISK	54
7.4	CALCULATING AN HD ₅ FOR BIRDS OR MAMMALS FROM A SMALL DATA SET	56
	ACKNOWLEDGEMENTS	59
	REFERENCES	61
	LIST OF ABBREVIATIONS	63
	APPENDIX 1 INSTALLATION PROBLEMS.....	65
	APPENDIX 2 ERROR MESSAGES.....	67

Samenvatting

Dit rapport is de handleiding van het softwareprogramma *E_TX 2.0*. De rekentechnieken die dit programma biedt, zijn in gebruik binnen het RIVM project 'Internationale normstelling stoffen' (INS). Binnen dit project worden milieurisicogrenzen ('milieunormen') afgeleid in opdracht van het ministerie van VROM. Het richtsnoer voor de afleiding van deze risicogrenzen staat het gebruik van een statistische extrapolatietechniek toe, bij voldoende toxiciteitsgegevens. Het resultaat hiervan dient als basis voor een milieunorm. Een statistische techniek die voor dit doel geschikt is, is recentelijk beschreven in de wetenschappelijke literatuur. Het programma *E_TX 2.0* maakt de beschreven methode meer toegankelijk voor hen die werkzaam zijn op het gebied van normstelling. Het programma is ook bruikbaar bij risicoschattingen zoals die bijvoorbeeld worden uitgevoerd in Europese risicobeoordelingen van bestaande stoffen. Het programma wordt verspreid op CD-ROM, samen met dit rapport.

E_TX 2.0 rekent op basis van de beschikbare toxiciteitsgegevens een zogenaamde soortsgoedigheidsverdeling (SSD) uit. Vervolgens toetst het programma of deze verdeling voldoet aan de criteria van een normale verdeling. Van de SSD worden het 5^e percentiel en de mediaan berekend, beide met hun 90% betrouwbaarheidsinterval. Met de berekende SSD kan ook een fractie aangetaste soorten worden geschat bij een gegeven milieuconcentratie, of een verwacht ecologisch risico (EER) bij een serie van milieuconcentraties. Het programma biedt ook de mogelijkheid om van zeer kleine datasets het 5^e percentiel te schatten met behulp van de zogenaamde *small sample* methode.

Summary

This report is the manual of the software program *E_{TX} 2.0*. The calculation techniques that are offered with this program are used within the RIVM project 'Setting international environmental quality criteria' (INS). Within this project, environmental risk limits ('environmental standards') are derived, by order of the Dutch Ministry of Housing, Spatial Planning and the Environment (VROM). The guidance followed for derivation of environmental risk limits allows for statistical extrapolation when sufficient toxicity data are available. The result of this extrapolation serves as a basis for an environmental standard. A statistical technique to achieve this result has recently been described in the scientific literature. The program *E_{TX}* makes this method more accessible to those that work in the field of risk assessment of chemicals. *E_{TX}* can also be used in effect assessments as carried out in e.g. the European risk assessments for existing substances. The program is distributed on CD-ROM together with this report.

E_{TX} 2.0 calculates a normal distribution through the toxicity data entered by the user. This gives a so-called species sensitivity distribution (SSD). This distribution is subsequently tested on normality using statistical criteria. Of the calculated distribution, the estimated 5th percentile and median are presented, each with their respective two-sided 90% confidence interval. With the calculated SSD also the fraction of affected species at a given environmental concentration can be estimated, or an expected ecological risk (EER) at a series of environmental concentrations. The program also offers the opportunity to estimate the 5th percentile of very small data sets using the so-called 'small sample' method.

1. Introduction

This report is a user's manual to *E_TX* 2.0. This program implements the theory of calculating hazardous concentrations and fraction affected from species sensitivity distributions as described in Aldenberg and Jaworska (2000). The program was developed in a stepwise manner, triggered by (i) the publication of the paper by Aldenberg and Jaworska (2000), (ii) the publication of the book 'Species sensitivity distributions in ecotoxicology' (Posthuma *et al.*, 2002) and (iii) the possibility to incorporate the 'small sample' method based on the papers of Luttkik and Aldenberg (1997) and Aldenberg and Luttkik (2002).

The first version of *E_TX* was *E_TX* 1.3a (Aldenberg, 1993) which runs under MS-DOS. *E_TX* 1.3a enabled estimation of hazardous concentrations using logistic, normal and triangular distributions.

The sole purpose of *E_TX* 2.0 is to offer the user the calculation methods as described in the supporting literature. The selection of data, applicability of the supporting theory to the data entered and interpretation of results is left entirely to the responsibility of the user.

E_TX 2.0 is a software program written in Microsoft® Visual Basic.NET (Edition 2003). Data can be entered simply as a column of values in the data input section. After performing the calculation, the output section shows statistics and graphical representations in different sheets.

Earlier versions of this program (in Microsoft® Excel, called '*E_TX* 2000') are now obsolete. All features and possibilities in earlier Excel versions are still present in *E_TX* 2.0.

1.1 Referring to *E_TX* 2.0

If you use the program in an official publication, book or report, it can be referred to it in the following way:

Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T. 2004. *E_TX* 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68 pp.

2. What can *E_TX 2.0* do for me?

As stated in the introduction, *E_TX 2.0* offers you the opportunity to apply statistical theory common to species sensitivity distributions (SSDs). This theory is most commonly applied in the field of ecotoxicology. In this scientific discipline, datasets will usually consist of end-points derived from toxicity studies with a given substance and a particular species or process representing a pre-defined environmental compartment or ecosystem. To speak in a more practical sense, your data may (e.g.) be chronic NOECs for freshwater organisms.

You can use *E_TX 2.0* to calculate the following items:

- The program calculates a normal distribution through your data set.
- The program will show the results of three goodness-of-fit tests that you can use to decide whether your data follow a normal distribution. The three tests are known as Kolmogorov-Smirnov, Anderson-Darling and Cramér-von Mises.
- Next, the program calculates the median HC₅ (hazardous concentration) plus its two-sided 90% confidence limit and the median HC₅₀ plus its two-sided 90% confidence limit. At the HC₅ and HC₅₀, the corresponding median FA (fraction affected) is given (i.e. 5% and 50%, respectively) together with its two-sided 90% confidence limit.
- Results are graphically presented in a histogram and in a cumulative density function (the latter is commonly referred to as SSD).

If you have calculated a species sensitivity distribution, you have the possibility to calculate the FA at a given exposure concentration. The program gives you the median FA and its lower and upper estimates (5 and 95% confidence). If you have a series of exposure concentrations, you can also enter this series. This series is tested for normality of distribution with the Anderson-Darling goodness-of-fit test. Next, the expected ecological risk (EER) is calculated, making use of both the SSD and environmental concentrations. The result is graphically presented as a joint probability curve (JPC).

If you want to estimate the hazardous dose for 5% of mammals or birds (HD₅), but you only have a very small data set, you may want to use (what we call) the 'small sample' method. A standard deviation from a known, external data set of toxicity data is used to estimate the 5th percentile of your data set. The lower and upper limit of the two-sided 90% confidence interval are also calculated.

Note that the 'small sample' method is dependent on knowledge of both the toxicity of your compound to a few species, as well as an expectation of the variation in toxicity data of that compound, derived from a high number of data (in the form of an external standard deviation). The method of deriving HC₅ and HC₅₀ values or FA values can be applied generically to any set of toxicity data ($n > 1$).

3. User's guide

3.1 Program type and system requirements

E_TX 2.0 is a Microsoft© Windows application that runs using the Microsoft©.NET (dotnet) framework. The program should therefore be run on Personal Computers (PC) equipped with an operating system that supports Microsoft applications. The Visual Basic.NET compact framework (version 1.1) is included on the CD and will be installed if necessary. Proper functioning of *E_TX* was tested on several combinations of Windows operating systems and Microsoft Office versions. We cannot guarantee proper functioning of *E_TX* under Windows 98. In most occasions, we encountered no problems, while in some cases installation under Windows 98 was not successful for unknown reasons. For later versions of Windows, installation was successful in most cases. In those cases where installation was unsuccessful, installation of a recent version of Microsoft's Data Access Components (which can also be found on your *E_TX*-CD) solved the problem. In Appendix 1, a list of combinations tested and possible solutions when installation problems are encountered, is given.

An operating system (like Windows NT or XP) is necessary. For proper functioning, *E_TX* does not need MS Office. However, to use the export option, MS Excel is needed, because export reports are generated in an MS Excel spreadsheet. However, the program works equally well without making use of this export option.

3.2 Installing *E_TX* 2.0

- Insert the *E_TX*-CD in the CD drive of your PC.
- Start the Windows Explorer (Start button, Programs, Accessories, Windows Explorer).
- In the left side of your screen, click on the drive that is your CD drive.
- In the right half of your screen, double click on the file **Setup.Exe**.
- The installation procedure will now start. The following screen may appear:

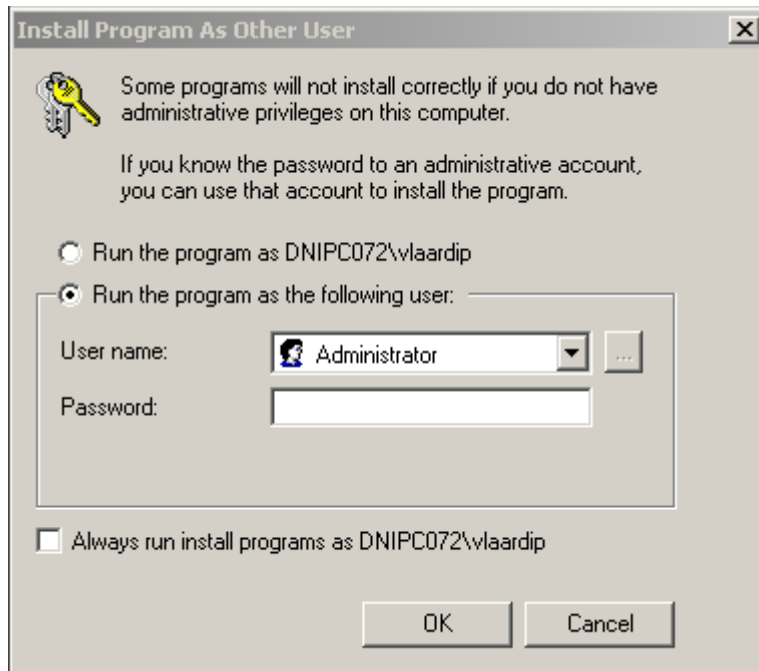


Figure 1. Installation procedure – User screen.

You are asked to decide whether a person with administrator rights should install the program for you via the displayed dialog box. The choice for one of the options depends on your company's protocols. It is advised to install the program using a user account with administrator rights. Click **OK** after having selected the correct user.

– In the following screen, click **Next**.

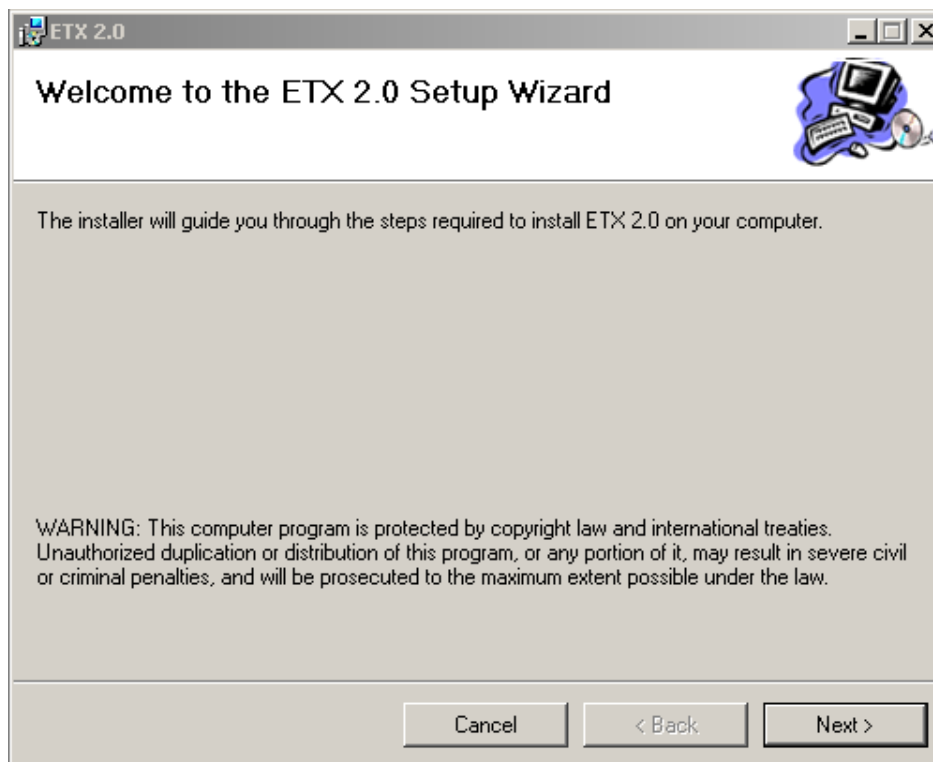


Figure 2. Installation procedure - Welcome screen.

- Carefully read the license agreement that is displayed in the following dialog box:

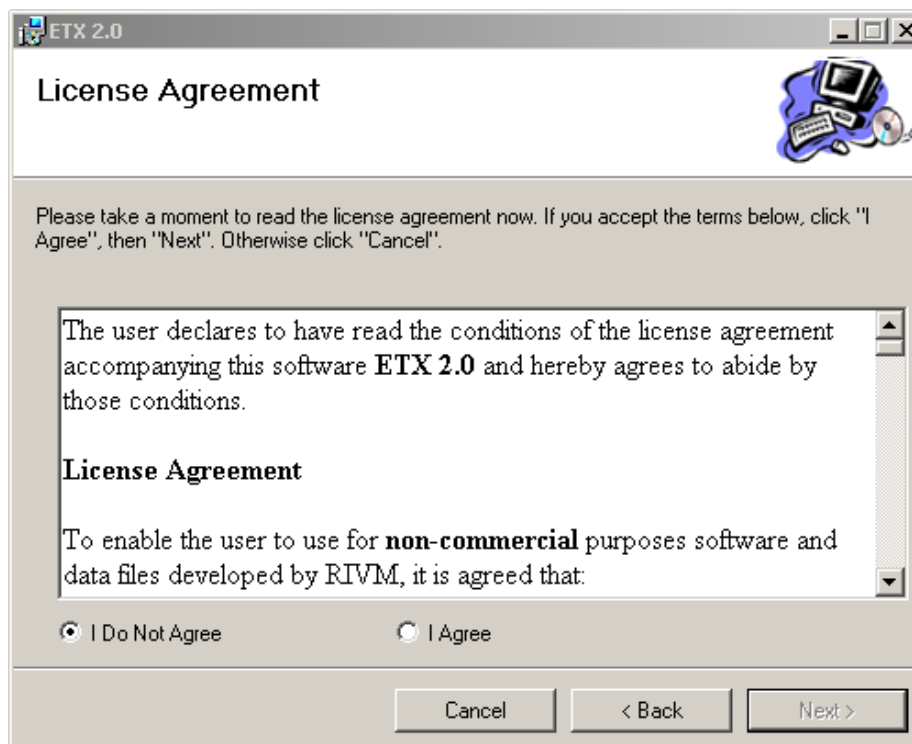


Figure 3. Installation procedure – License Agreement screen.

- If you agree with the content of this agreement, select the **I agree** option, and then click **Next**.

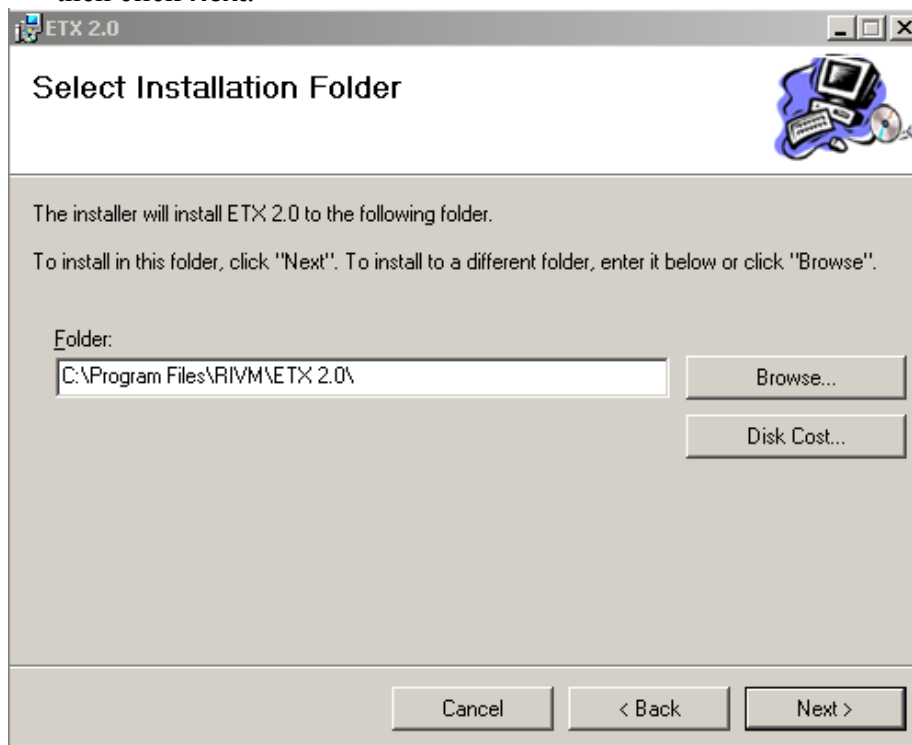


Figure 4. Installation procedure – Select Folder screen.

- In the dialog box displayed above, select the Folder in which you want ETX to be installed.

The default directory is C:\Program Files\RIVM\ETX 2.0. If you want to install $E_T X$ in an other directory, click **Browse** and select the directory of your choice.

- Click on **Next** to continue.

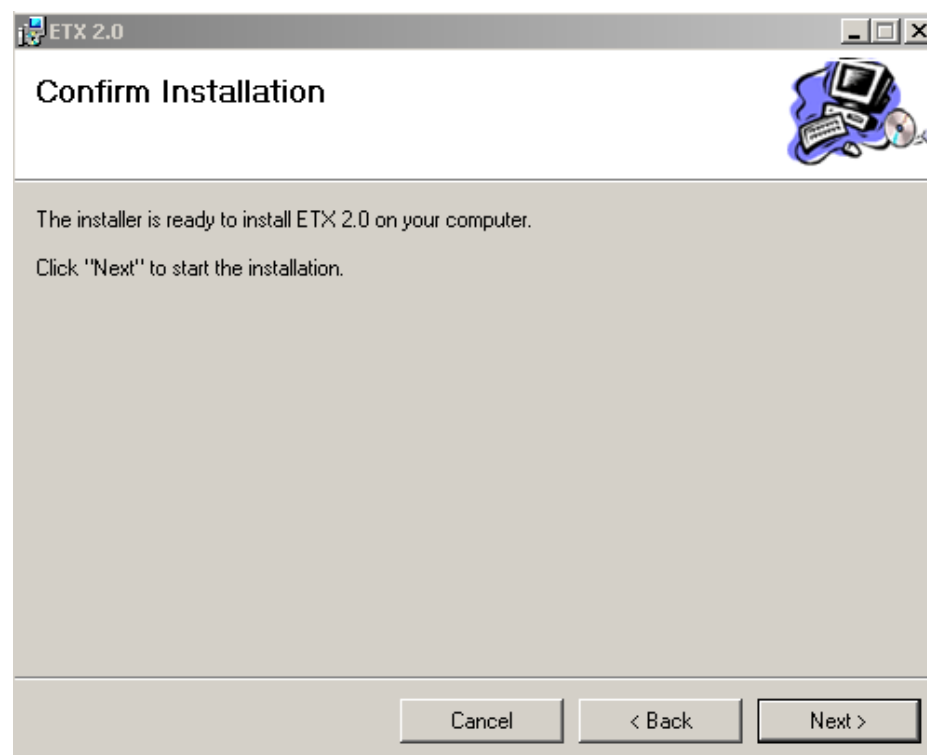


Figure 5. Installation procedure – Confirm Installation screen.

- In the dialog box displayed above, click **Next** if you want the installation to be carried out.
- Installation will now be performed. If the .NET framework is not yet installed on your computer, it will be installed. Installation of this framework takes several minutes.
- When the Installation is complete, the following screen appears. Click on **Close** to leave this screen and to finish the installation procedure.

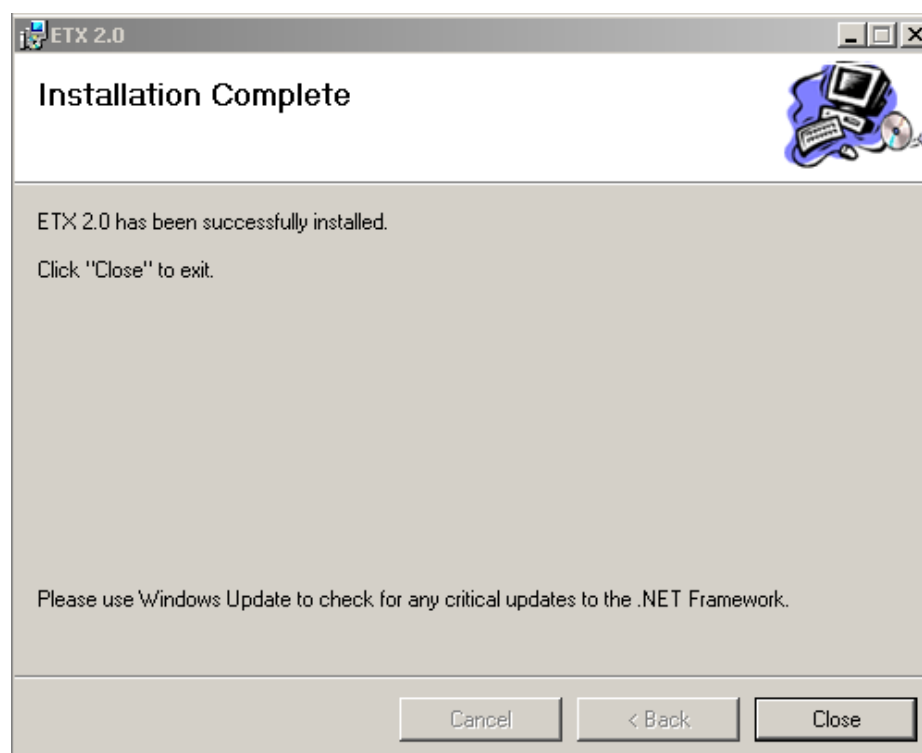


Figure 6. Installation procedure –Installation Complete screen.

3.3 Removing E_{TX}

If you want to de-install (or remove) E_{TX} , do the following.

- Under the Start button, click on Settings, Control Panel.
- In the Control Panel, double click the Add/Remove Programs icon.
- Select E_{TX} from the list of currently installed programs. After clicking on E_{TX} in this list, you can select Remove.
- E_{TX} will now be removed from you computer.

3.4 Starting E_{TX}

When E_{TX} is installed successfully, the program can be found under the Start button in the lower left corner of your screen. It will be placed under: Programs, RIVM ETX, E_{TX} 2.0. Click on the E_{TX} 2.0 icon to start the program.

3.5 Saving data in E_{TX}

If you save data in E_{TX} , your data will be stored in a Project. This is a file with the extension: etx. To save a Project, Click on **F**ile on the Menu bar, then click **S**ave.

In order to set or change the default directory where your E_{TX} projects will be stored, see sections 5.1.5 and 5.9.4.

N.B. Your calculations and calculated results will *not* be stored together with your data. However, if you open an etx-Project that you had saved earlier, and press calculate again, all

results and output will be generated, together with the settings that were active at the time your Project was saved.

3.6 Starting a new project in E_TX

The E_TX opening screen is always an empty project. You can start directly with entering data here. Starting a new project can be performed by clicking on **File, New** in the Menu bar at the top of your E_TX screen. Opening an existing project can be performed by clicking **File, Open input file**. The following dialog box appears:

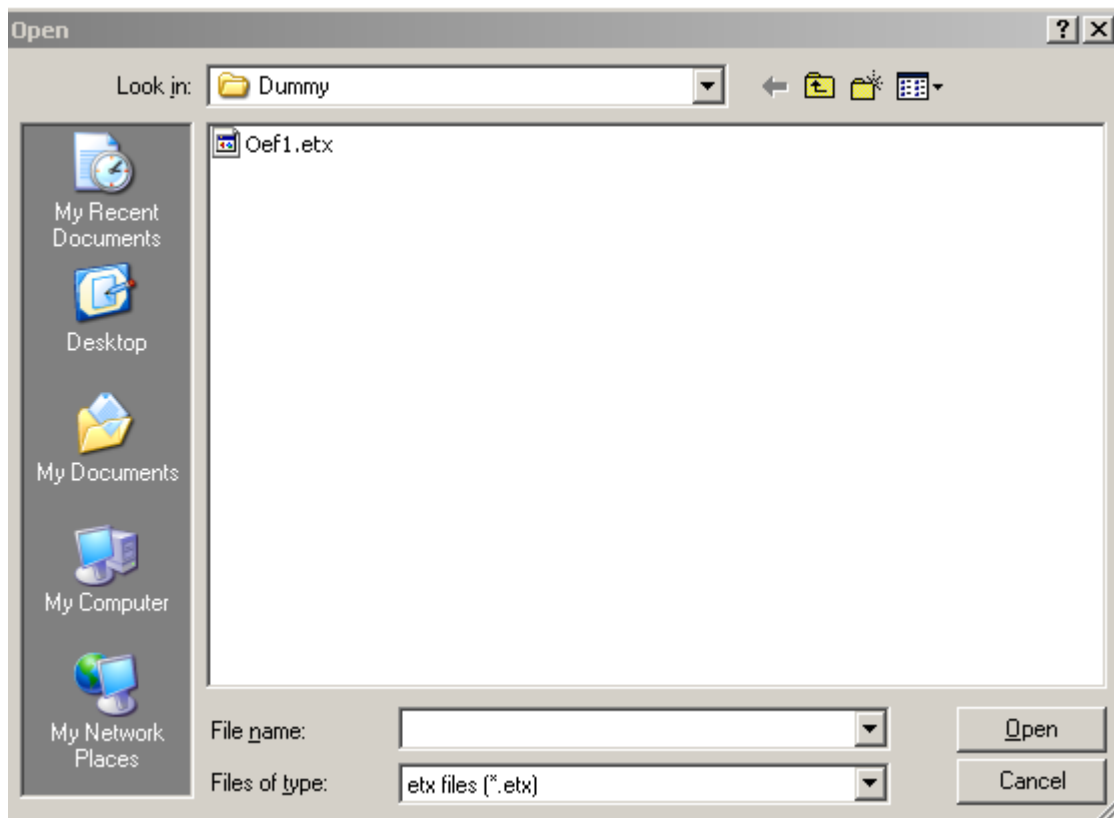


Figure 7. The dialog box shown after clicking **Open input file**.

Select the E_TX project of your choice and click on **Open**. Your Project will now be retrieved and opened.

3.7 Leaving E_TX

You can leave E_TX 2.0 by choosing **File, Exit** from the Menu bar.

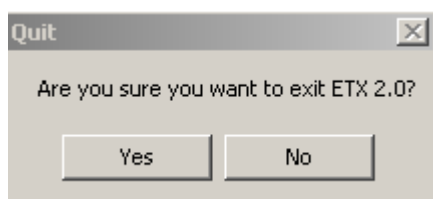


Figure 8. Dialog box shown upon leaving E_TX .

Answer the question appearing in the dialog box (Figure 8) by clicking **Yes** or **No**.

3.8 Changing the settings for the decimal character

The choice of the character used as decimal symbol or digit grouping symbol in numerical values within $E_T X$ is determined by the regional settings of your PC. In English or American settings, the comma ',' is mostly used as digit grouping symbol, while the period '.' is used as decimal sign. In the Dutch language and settings, this is opposite, here the period '.' is used as digit grouping symbol and the comma ',' is the decimal sign. Please take notice that the regional settings will also apply to values printed along the axes of figures.

You may want to change these settings. Below, you find the route to the Control Box in which you can change your settings for several Microsoft operating systems.

3.8.1 Windows 98, NT, 2000 and ME

Click on the Start button in the lower left corner of your screen, then select Settings, Control Panel and Regional Settings (double click). In the Regional Settings properties box that has appeared, select the Number tab. In this screen you can either select a Region and obtain its accompanying settings or Customise your settings. After having made your selection, click on Apply and on OK. Changing of settings will have no effect unless you restart $E_T X$.

3.8.2 Windows XP

Click on the Start button in the lower left corner of your screen, then select Settings, Control Panel, Regional and Language Options. Select the Regional Options tab. In this screen you can either select a Region and obtain its accompanying settings or Customise your settings. After having made your selection, click on Apply and on OK. Changing of settings will have no effect unless you restart $E_T X$.

3.9 Comments on $E_T X$

If you have comments on the program, its functioning or other ideas, you can send these by e-mail to etx.info@rivm.nl. Please note that this is *not* a helpdesk address. We will not respond to questions, but we will collect comments, possible errors and ideas for future development. Unfortunately, we are unable to help all users with problems or questions.

4. Reference manual

In section 4.1 we outline the different sections in which the program can be divided. We also name all sheets and briefly explain their contents. Section 4.2 explains the meaning of each of the items you may encounter in *ETX*. A list of abbreviations is included at page 63 of this report. How to use the information given in this chapter is explained to you in chapter 5 while chapter 7 tells you how to perform some calculations in practice.

4.1 Structure of the program

The program is divided in two major sections: an input and an output section. The input section has two subsections: **Input toxicity data** and **Input exposure data**. Each will be briefly discussed in the section 4.1.1. To get results, you have to invoke a calculation (section 5.7), which gives results in the **Output** section. The output section is divided in three sections, which will be introduced in section 4.1.3.

4.1.1 Data input

We discern two types of data: toxicity data and environmental concentrations.

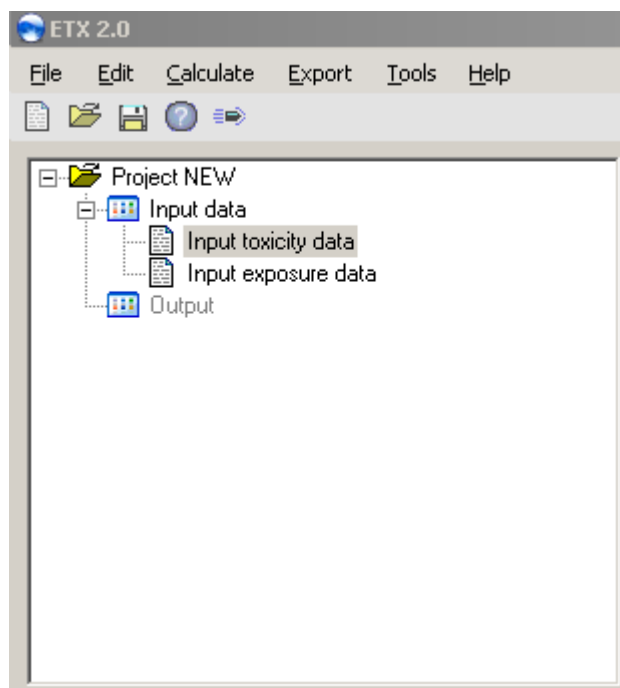


Figure 9. Subdivision of the input section.

4.1.1.1 Input toxicity data

Figure 9 shows the two sections of **Input data**.

- If you want to calculate an SSD and the accompanying HC_5 and HC_{50} , you should enter your data in the **Input toxicity data** sheet. If you want to calculate an FA, you need an SSD first. The output section will remain inaccessible until you have calculated an SSD.
- For calculation of the HD_5 for birds and mammals, there is room to enter a (small) data set of no more than 10 values. We call this the 'small sample' method. To use this method you have to select the check box labelled with **Use small sample method** in the lower right corner of the input screen (see Figure 10). You also have to enter a standard deviation

from an external data set. Entering data for the 'small sample' method is also done in the **Input toxicity data** sheet.

The image shows a software dialog box titled "Small sample". Inside the box, there is a checkbox labeled "Use small sample method". Below this, there is a label "Pre-defined standard deviations:" followed by a dropdown menu. At the bottom, there is a label "Standard deviation:" followed by a text input field.

Figure 10. Small sample box.


4.1.1.2 Input exposure data

After having entered data in the **Input toxicity data** sheet and after you have calculated the accompanying SSD, you can use the **Input exposure data** sheet to calculate either an FA or an EER. There is the possibility for input of a single exposure concentration and a separate input section in case you have a series of exposure concentrations (like a measurement series).

4.1.2 Types of calculations

You can perform four types of calculations.

1. Calculate an SSD only.
2. Calculate an SSD and an FA. An FA can only be calculated when you have entered data in **Input toxicity data** and generated an SSD.
3. Calculate an SSD and an EER. An EER can only be calculated when you have entered data in **Input toxicity data** and generated an SSD.
4. Calculate a (small sample) HD₅. A maximum of 10 data can be entered. Calculation of an SSD is not needed in this case.

All calculations are performed by clicking **Calculate, Go** on the Menu bar in each of the two Input sections, or by clicking the **Calculate** button: . Please note that pressing the **enter** key on your keyboard does *not* invoke a calculation.

After selecting the **Calculate** button (or using the menu), the calculation is performed and the results will appear in one or more of the output worksheets or graphs (see section 4.1.3).

NB. The program will also generate results and graphics if the outcome of the goodness-of-fit tests imply that it is less probable that your data derive from a normal distribution. The program only calculates, it does not make decisions. It is up to you to decide whether you accept or reject the presented outcome of the calculations.

4.1.3 Output

4.1.3.1 SSD related output

The **Output** section contains three subsections and each of the subsections contains one or more sheets. Figure 11 shows the available output sheets in the program tree below **Output**. The contents of each sheet are outlined below.

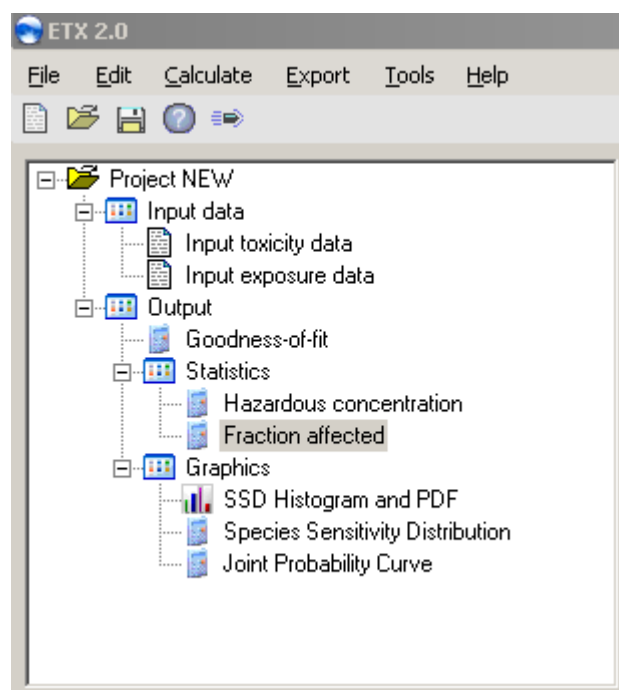


Figure 11. Subdivision of the output section for SSD calculations.

4.1.3.1.1 Goodness-of-fit

This sheet shows you the results of three goodness-of-fit (GOF) tests performed on your toxicity data. A brief explanation on the interpretation of these tests is also given. If you have entered a series of environmental concentrations in the **Input exposure data** sheet and have invoked a calculation, you will find the GOF test on these data here.

4.1.3.1.2 Statistics

In this section, two sheets can be found, the content of each is described below.

Hazardous concentration

In this sheet the mean and standard deviation (s.d.) of the normal distribution through your data are reported as well as the sample size. Furthermore, the HC₅, FA at the HC₅, the HC₅₀ and the FA at the HC₅₀ are reported. For each of these parameters, the lower and upper limit of the 90% confidence interval around the median estimate are reported.

Fraction affected

The estimated FA (plus lower and upper estimate of the two-sided 90% confidence interval) at the exposure concentration (EC) that you have entered in the **Input exposure data** sheet in the **single PEC** input cell is reported in this sheet. If you have entered a series of exposure concentrations in the **Input exposure data** sheet, the EER is reported.

4.1.3.1.3 Graphics

There are 3 sheets that show graphical output.

SSD Histogram and PDF

A histogram (or frequency distribution) of your toxicity distribution is presented when you have calculated an SSD. The bin width (classwidth) is calculated according to the method of Scott (1992) as:

$$\text{binWidth} = 3.5 \cdot \frac{\text{standard deviation}}{n^{1/3}}$$

The placement of bins on the x -axis is described in more detail in section 4.2.5. The height of the bars is expressed as the number of data they represent, which is plotted on the right hand y -axis. The normal distribution plotted in this graph is the probability density function (PDF) that is associated with your toxicity data sample. The left hand y -axis shows the density of the toxicity data. The left hand y -axis can also be used to read the densities of the bars in the histogram. For both the PDF and the histogram, the integral of all data, i.e. the sum of class-width \times density, equals 1.

Species sensitivity distribution

If you have calculated an SSD, this is the cumulative density function (CDF) that is associated with your toxicity data sample. The dots in the graph are placed at the so-called Hazen plotting positions: $p_i = (i - 0.5)/n$ (see Aldenberg *et al.*, 2002).

Joint probability curve

This graph shows the joint probability curve (JPC). To obtain a JPC you have to enter toxicity data in the **Input toxicity data** sheet and a series ($n > 1$) of exposure concentrations in the **Input exposure data** sheet. After having calculated an SSD, the JPC will be generated.

4.1.3.2 Small sample output

When the 'small sample' method is used, no SSD is required. The **Output** section only shows the **Small sample results** sheet (Figure 12). The content of the output sheet is outlined below.

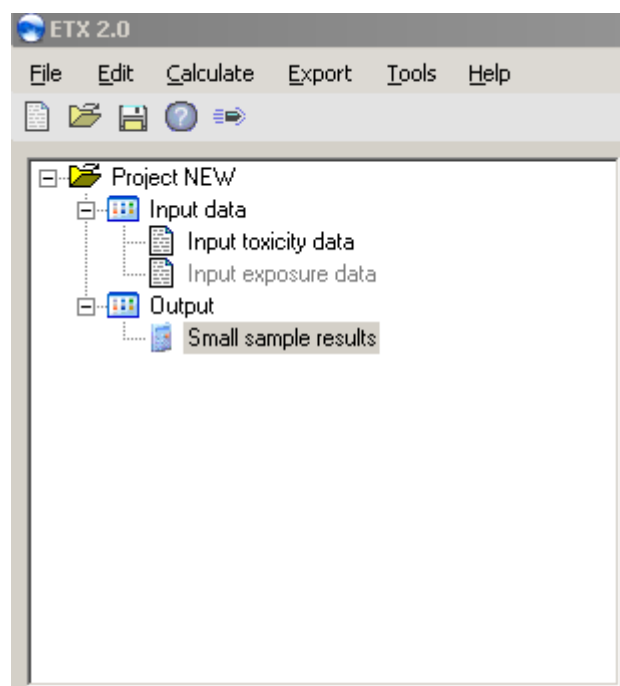


Figure 12. Subdivision of the output section for small sample calculations.

4.1.3.2.1 Small sample results

The median estimate of the HD_5 of your small data set is calculated and presented together with its 90% confidence interval limits. Also presented are the extrapolation factors that can be used to directly calculate the lower limit (LL), median or upper limit (UL) estimate of the HD_5 from the sample mean.

4.2 Glossary of keywords

In this section, a description of keywords is given, listed in alphabetical order. Keywords are those items you may encounter on the input or output screens of *E_TX*. This section gives a brief explanation of each keyword, but we do not go deeply into the theoretical background of each item in this manual. For more background information, we refer to the underlying literature, which is listed in the Reference section of this manual.

4.2.1 Exposure concentration (EC)

In this manual and in *E_TX* 2.0, the parameter 'exposure concentration' is synonym with 'environmental concentration'. Both can be abbreviated as EC and both are interchangeable in manual and program.

4.2.2 Expected Ecological Risk (EER)

An EER can be calculated if a set of predicted exposure concentrations (ECs) is entered in the input section of the program. Mean and s.d. are scaled relative to the mean and s.d. of the species sensitivity distribution. The EER literally is the probability that a randomly drawn species for a random draw of exposure is affected.

The following statistical parameters will appear in the **Fraction affected** output screen (in order of appearance):

SEC_{mean}, SEC_{sd} and EER

SEC stands for scaled environmental concentration.

- The SEC_{mean} is the scaled mean of the environmental concentration distribution. It is reported here as the log₁₀ transformed value.
- SEC_{sd} is the scaled s.d. of the environmental concentration distribution. It is reported here as the log₁₀ transformed value.
- EER is the expected (mean) ecological risk, given the SSD and the distribution of environmental concentrations. The EER is the area below the curve in the **joint probability curve (Output, Graphics)**.

4.2.3 Extrapolation factor

In the **Small Sample Output** sheet, extrapolation factors are displayed. These can be found in the **HD₅ (median) results** box on your screen.

Extrapolation factors are factors to be applied multiplicatively to the geometric mean of the original (not log-transformed) toxicity data. Extrapolation factors depend on the FA, sample size, confidence level and on the standard deviation of the SSD (Aldenberg and Luttik, 2002). In *E_TX* 2.0, extrapolation factors are given for an FA of 5% (HD₅) for the upper and lower limit of the 90% confidence interval of the HD₅.

4.2.4 Fraction affected (FA)

An FA can be calculated when one single predicted exposure concentration (EC, synonym with PEC) value is available. This EC value can be entered in the input section of the program. The following statistical parameters will appear in the **Output** screen. In order of appearance:

LL FA, Median FA and UL FA

Reported is the median estimate of the FA at the PEC you have entered. Also reported is the two-sided 90% confidence interval of the FA: LL FA represents the 5% confidence limit and

UL FA represents the 95% confidence limit. These calculations are based on repeated linear interpolation and are approximate values. The procedure is explained in section 8.2.3 of Aldenberg and Jaworska (2000). A precise answer can be obtained using the functions described in section 8.2.1 of that paper.

This routine does not yield results when your standardised mean logarithmic effect concentration is ≤ -5 or ≥ 5 . As a check, the value of the standardised logarithmic concentration calculated from your PEC value is shown in the **Fraction affected** output sheet. If this value is outside the range mentioned above, no results will be given. The message 'The method does not yield results because your standardised PEC exceeds the limits of ≤ -5 or ≥ 5 ' will appear in red. The sheet will show 'Out of bounds!' as error values.

4.2.5 SSD Histogram and PDF

This graph is a histogram or frequency distribution in which the toxicity data are represented by bars. On the x -axis the \log_{10} of toxicity values (NOEC, LC_{50} s etc.) is plotted. On the right hand y -axis, the frequency of the toxicity values is plotted. Toxicity values are distributed over frequency classes (often referred to as bins) that have a width calculated according to Scott (1992):

$$\text{binWidth} = 3.5 \cdot \frac{\text{standard deviation}}{n^{1/3}}$$

The placing of bins on the x -axis is worked out as follows:

1. The number of bins is calculated using the equation mentioned above;
2. Bins are divided over the range of toxicity data by centering around the mean (=sample mean, or mean of the toxicity data);
3. Sample values coinciding with a bin limit are dropped in the next lower bin.
4. In case the lowest sample value coincides with the lower limit of the lowest bin, this value is dropped in this lower bin.

In case of an even number of bins, we identify *two* middle bins. The mean of the toxicity data now coincides with the separation between the two middle bins. More precise, the mean value itself is chosen to coincide with the highest value of the left bin (due to point 3 above). In case of an uneven number of bins, there is *one* middle bin. In these cases, the middle of the middle bin is chosen to coincide with the mean of the toxicity data.

4.2.6 Goodness-of-fit

Whether the sample of toxicity data derives from a normal distribution can be assessed with goodness-of-fit tests. Two different types of tests are implemented, based on quadratic (vertical) distance and on the largest vertical distance. Well-known quadratic tests are the Anderson Darling test and the Cramér-von Mises test (cf. D'Agostino and Stephens, 1986). The Kolmogorov-Smirnov test is a well-known vertical distance test (D'Agostino and Stephens, 1986).

1. The Anderson-Darling goodness-of-fit test highlights differences between the tail of the distribution and the input data and is generally regarded as a very powerful general test (Aldenberg *et al.*, 2002).
2. The Kolmogorov-Smirnov test focuses on differences in the middle of the distribution and is not very sensitive to discrepancies of fit in the tail of the distribution.

Interpreting Critical Values

If a test statistic is above the 5% critical value, normality is rejected at the 5% critical value, indicating doubts about normality.

If a test statistic is below the 5% critical value, normality is accepted (not rejected) at the 5% critical value.

If a higher critical value is accepted (e.g. at 2.5% significance level), then the probability that these data derive from a normal distribution is smaller than at 5%, but it is not impossible that the sample derives from a normal distribution.

Some people find it confusing that a higher significance level, say 10%, has a lower critical value. 'Rejected' occurs more often with a higher significance level. In conclusion: a GOF test does NOT say that a sample cannot derive from a normal distribution, just that it becomes less probable with decreasing significance levels.

4.2.7 HC₅ and HC₅₀

Your toxicity dataset refers to a selection of species, which is treated as a sample drawn from a population (in a statistical sense). By definition, the calculated normal distribution encompasses all species inhabiting the environmental compartment of interest. It is up to you to determine whether your sample of species is representative for the population of species (in a biological sense) you want to derive HC values for. The distribution is the –estimated– function that relates the relative sensitivity of the species thought present in a given environmental compartment to (the logarithm of) the toxicant concentration. This distribution is a normal distribution (by definition) when you use *E_TX* 2.0 for your calculations. HC is short for 'hazardous concentration'. HC₅ and HC₅₀ are the 5th and 50th percentile (median is synonymous for the latter) of the normal distribution that is fitted through the toxicity data you have entered. HC₅ and HC₅₀ are expressed as a toxicant concentration (in the same units as the toxicity data you have entered in the input section). Hence, they represent the toxicant concentration that is hazardous to 5 or 50 percent of 'all' species. See section 4.2.11 for explanation of the HC parameters calculated by *E_TX* 2.0.

4.2.8 JPC

The abbreviation stands for joint probability curve. This is a graphical representation of the risk of a substance to the species, that may be used in risk characterisation. The type of graph *E_TX* shows is a cumulative profile plot. It is constructed by plotting FA values from the SSD (in CDF form) on the *y*-axis against exposure concentration distribution (ECD) values (also in CDF form) on the *x*-axis at corresponding log-exposure concentrations. The area under the curve (AUC) is equal to the expected ecological risk. For more detail, see Aldenberg *et al.* (2002). The numerical value of the AUC, or EER, is shown in the **Fraction affected** sheet (**Output, Statistics**).

4.2.9 PEC

If an environmental concentration of the substance of interest is the outcome of some model calculation rather than a measured value –or series of values– in the field, it is called a *predicted* environmental concentration. If you have a single PEC value and an SSD, you can calculate the fraction of species described by that SSD that is (potentially) affected by the substance.

4.2.10 Small Sample method

The idea to estimate a percentile of an SSD and its uncertainty for a very small sample of toxicity data has emerged from the field of pesticide registration. Due to the limited size of the sample, there is no reliable information on the standard deviation of the SSD. A standard deviation from a *different* sample, to which one attaches more confidence because it has higher reliability, is used to estimate the HD₅. Luttik and Aldenberg (1997) published this method for *logistically* distributed toxicity data.

The method that is currently implemented in *E_TX* 2.0 offers the possibility to calculate HD₅ values for birds or mammals based on *normally* distributed toxicity data (Aldenberg and Lutik, 2002). From a dataset of 55 pesticide LD₅₀ values for birds a pooled variance estimate of the standard deviations of all LD₅₀ values was calculated. The same was done for a dataset of 69 pesticide LD₅₀ values for mammals. From both datasets the pooled variance estimates for carbamates and organophosphorous compounds were also calculated. All pooled variance estimates are listed in the **Input toxicity data** sheet in the **Small sample** box (under **Pre-defined standard deviations**). These estimates can be used as an ('external') standard deviation that is assumed to describe the spread of the population from which your (small) sample of bird/mammal data has been drawn.

Recommendations to the 'small sample' method:

- (i) When there are indications that the (small) sample standard deviation does not reflect the population standard deviation, even when $n \geq 4$, consider using the pooled standard deviation (i.e. use the 'small sample' technique). A maximum of 10 entries is allowed for in the **Input toxicity data** sheet.
- (ii) Use the LL HD₅ values or corresponding assessment factors when a 5% probability for overestimation of the HD₅ is desired. When the median HD₅ or its corresponding assessment factor is used, 50% probability for HD₅ overestimation is allowed.
- (iii) When there are indications that the available data are derived from a test with a sensitive species, one could consider using the median estimate of the HD₅ or the corresponding extrapolation factor.

4.2.11 Statistics SSD

If an SSD has been calculated by *E_TX*, i.e. if a normal distribution has been fitted through your toxicity data, the following statistical parameters will appear in the **Hazardous concentration** output screen (in order of appearance).

Mean, s.d. and *n*

Mean and s.d. are the sample mean and the sample standard deviation ($n-1$) of the normal distribution. These parameters are shown in log₁₀ units and are the parameters that describe the normal distribution fitted through your toxicity data set. *n* is the sample size, i.e. the number of toxicity data. It is shown because the size of *n* directly determines the size of the uncertainty in the HC₅ and HC₅₀ (Aldenberg and Jaworska, 2000). These sample statistics are used to estimate the SSD parameters.

HC₅ results: **LL HC₅**, **HC₅**, **UL HC₅** and **sprHC₅**

These parameters are shown in the same concentration units as you entered your toxicity data in. Presented are the estimated 5th percentile of the normal distribution (HC₅) and its two-sided 90% confidence limits, called the lower limit (LL) and upper limit (UL), respectively. The HC₅ itself is a normally distributed statistic, and sprHC₅ is a measure of the width of the HC₅ distribution. It is calculated as the ratio of UL and LL.

FA at HC₅ results: **FA_{lower}**, **FA_{median}** and **FA_{upper}**

The median estimate of the FA at the toxicant concentration HC₅, is 5%, by definition, since the HC₅ is the 5th percentile of the SSD. The two-sided 90% confidence interval for the FA is also reported: FA_{lower} represents the 5% confidence limit of the FA and FA_{upper} represents the 95% confidence limit.

HC₅₀ results: **LL HC₅₀**, **HC₅₀**, **UL HC₅₀** and **sprHC₅₀**

These parameters are shown in the same concentration units as you entered your toxicity data in. Presented are the estimated median or 50th percentile of the normal distribution (HC_{50}) and its two-sided 90% confidence limits, called the lower limit (LL) and upper limit (UL), respectively. The HC_{50} itself is a normally distributed statistic, and $sprHC_{50}$ is a measure of the width of the HC_{50} distribution. It is calculated as the ratio of UL and LL.

FA at HC_{50} results: **FA_{lower}**, **FA_{median}** and **FA_{upper}**

The median estimate of the FA at the toxicant concentration HC_{50} , is 50%, by definition, since the HC_{50} is the 50th percentile of the SSD. The two-sided 90% confidence interval for the FA is also reported: FA_{lower} represents the 5% confidence limit of the FA and FA_{upper} represents the 95% confidence limit.

4.2.12 SSD

E₇X calculates an SSD from your sample of toxicity data assuming that the (continuous) distribution describing the sensitivity of the population underlying your sample is normally distributed over log concentration. This Gaussian distribution is graphically presented in the **SSD Histogram and PDF** sheet (to be found under **Output, Graphics**). The data and the fitted distribution are also presented as a CDF plot in the **SSD** sheet (**Output, Graphics**). The statistical parameters derived from this distribution are presented in the **Hazardous concentration** sheet (**Output, Statistics**).

5. Working with *E_TX*

5.1 Menu bar and buttons

The menu bar and buttons that are available in *E_TX* are shown in Figure 13. Throughout the program, the same menu bar and buttons will appear. All functions that may be accessed via the menu bar will not be described here individually, we will give a general description of each menu item. Most items you will encounter when you work through the manual or by just trying them!

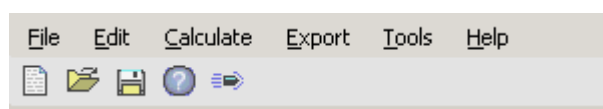


Figure 13. Menu bar and buttons in *E_TX*.

5.1.1 File

Options provided here allow you to:

- start a new *E_TX* project (**N**ew).
- open an existing *E_TX* project (**O**pen input file). When you have saved your work in an *E_TX* project, *E_TX* does *not* save the calculated data and graphs. If you want to see the results again, simply press Calculate after opening an existing *E_TX* project and your results will reappear. If you want to save all data, choose **E**xport and all your results will be placed in an Excel spreadsheet.
- save your work (**S**ave).
- save your project under a different name (**S**ave as).
- leave *E_TX* (**E**xit).

5.1.2 Edit

The options provided here allow you to handle individual cells or ranges of cells, that you can either delete, cut, copy and paste.

5.1.3 Calculate

The only option provided here is to invoke a calculation. The function of this menu is equal to that of the calculate button (section 5.1.7). For more information on the types of calculations you can perform, see section 5.7.

5.1.4 Export

The only option provided here is to export the data you have entered and calculated, to a separate file. The data will be exported in Microsoft Excel spreadsheet format. See also section 5.8. To change the default directory where projects and exported files are stored, see section 5.9.

5.1.5 Tools

There are four options here: **L**abels, **S**ort toxicity data, **S**ort labels and **P**references.

- Use **L**abels to attach label to the entries in the toxicity data sheet. For more information please see section 5.6.
- By clicking on **S**ort toxicity data, the toxicity data in the **Input toxicity sheet** will be sorted in increasing order. NB: this operation can not be undone.

- By clicking on **Sort labels**, the labels in the **Input toxicity sheet** will be sorted in alphabetical order. NB: this operation can not be undone.
- Use **Preferences** to define the default settings for the looks of your graphs and to set the default directory where projects and exported files are stored. For more information please see section 5.9.






5.1.6 Help

In the **Help** menu, there are two options to choose: **E_TX Help topics** and **About E_TX 2.0**. When you select the help topics, you can use the E_TX 2.0 manual online. The content of this menu is equal to the content of this manual.

About E_TX 2.0 gives you information on the version of this program. Information on earlier versions of E_TX is also provided.

5.1.7 Buttons

The buttons have the following names and function:

-  New. This button opens a New E_TX project.
-  Open existing project.
-  Save. Allows you to save the current project.
-  Help. Allows you to access the Help file.
-  Calculate. Invokes a calculation.

5.2 Navigating through E_TX

After starting up E_TX, the following screen will appear:

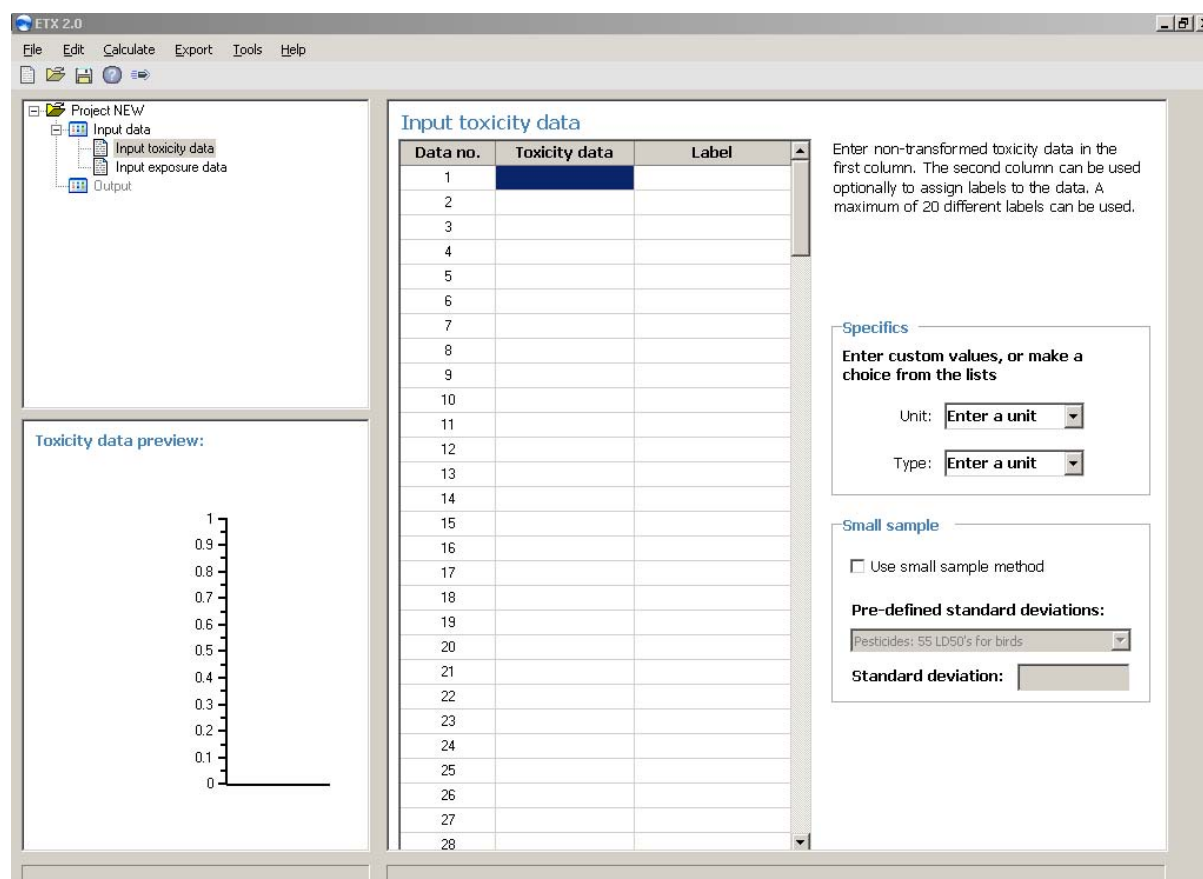


Figure 14. *E_{7X}* appearance after starting the program.

This screen is divided in three major parts: in the upper left corner: the navigating section, in the lower left corner: the **Toxicity data preview** section and the largest part is the right half of the screen: the **Input toxicity data** section. In the navigating screen, you find + or – signs. By clicking on these you can *unfold* or *fold* a specific part of the program and *view* or *hide* its contents. Use these buttons to find out that each *E_{7X}* project contains an **Input data** section and an **Output** section. If you select a specific section or sheet by clicking on it, it will be displayed in the right half of your screen. In the following, you will be guided through each of the different parts of the program.

5.3 Entering data

5.3.1 Toxicity data

- Go to the **Input toxicity data** sheet in the **Input data** section of *E_{7X}*.
- Go to the **Input toxicity data** box (this is the right part of your screen).
- Next, in the cells under **Toxicity data**, enter your toxicity values as a column, with one value per input cell. Enter the toxicity data in the original (non-transformed) values.
- For information on entering the unit of your data or the type of endpoint, see section 5.3.2.
- For information on labelling of your toxicity data, see section 5.6.
- Make sure *all* entries have the same unit: mg/l, µg/kg, ppm etc.
- You cannot enter the value 0 (zero). Your data will be log₁₀-transformed for calculation and log(0) does not exist. If you enter a zero value, the error message **Please enter only**

positive values will be displayed at the bottom of your screen. Delete the zero value or enter a positive non-zero value to continue.

- You need not sort the data.
- You may leave blank cells, empty cells will not be included in the graph or in calculations.
- For HC_5 and HC_{50} calculation a maximum number of 200 data can be entered.
- For FA and FA-confidence limits the maximum number of data (n) = 75 for FA at HC_{50} and $n = 200$ for FA at HC_5 .

When you have finished entering data, see section 5.7.1 on how to calculate an SSD.

5.3.2 Assigning units to your data

You may enter the unit of toxicity data and/or exposure data. It is optional to do this, but there are two reasons to do so. First, if you are about to enter both toxicity and exposure data e.g. to calculate a fraction affected, the data should be entered in E_{TX} in the same units! Second, if you enter the unit of your data, this unit will be printed in the output of your data that is exported to Excel.

To assign a unit to your data go to the **Input toxicity data** section. In the lower right half of your screen, you find the **Specifics** box (Figure 15):

Figure 15. The specifics box.

- In the scrollbar to the right of **Unit**, you may type a unit of your choice or select the unit from the pulldown menu.
- In the scrollbar to the right of **Type**, you may type the endpoint of the toxicity data you are about to enter, or you can select an endpoint from the pulldown menu.
- The **Specifics** box functions as a piece of scrap paper. Both options in the box remind you that all toxicity data should be of the same unit and type. Unit and type are not linked to any calculation. They reappear in the **Input exposure data** section, to remind you that the units of data entered in that section should be identical to the units of the data entered in the **Input toxicity data** section.
- Use of the Specifics box is optional. E_{TX} works equally well when you clear the contents of the **Unit** and **Type** boxes.

5.3.3 Small sample data

- Go to the **Input toxicity data** sheet in the **Input data** section of E_{TX} .
- In the **Input toxicity data** box (this is the right part of your screen), click on the **Use small sample approach** check box in the **Small sample** box. The following dialog box will appear:

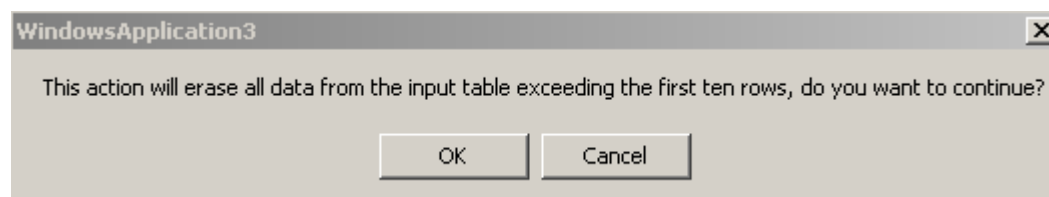


Figure 16. Small sample verification box.

If you had already typed data in the input section, these data will be lost when you switch to the Small sample input mode. Remember that this is a different method that does not require calculation of an SSD. So, if you want to keep the data you had entered before, click on **Cancel** and Save your work in an E_{TX} project. Then start a new project for your 'small sample' calculation. Otherwise, click on **OK**.

- The number of toxicity data that you can enter is now limited to 10.
- Enter your toxicity values as a column, with one value per input cell. Enter the toxicity data in the original (non-log transformed) format.
- Make sure *all* entries have the unit of mg/kg body weight.
- You cannot enter the value 0 (zero). Your data will be \log_{10} -transformed for calculation and $\log(0)$ does not exist.
- You need not sort the data.
- You may leave blank cells, empty cells will not be included in calculations.
- Enter a standard deviation. In the **Small sample** box at the lower right corner of your input screen, you can choose one of several pre-defined pooled standard deviations. The accompanying value that will be used for calculations appears in the **Standard deviation** box.

When you have finished entering data, see section 5.7.2 on how to calculate an HD_5 .

5.3.4 Environmental concentrations

5.3.4.1 One environmental concentration

This calculation applies when you have only one environmental concentration, e.g. a PEC resulting from the exposure part of a risk assessment rather than a series of measurements.

- Go to the **Input exposure data** sheet in the **Input data** part of E_{TX} .
- You need to have an SSD before you can enter exposure data. Refer to section 5.3.1 for information on this subject. If you enter exposure data without having an SSD, the following message will pop up:

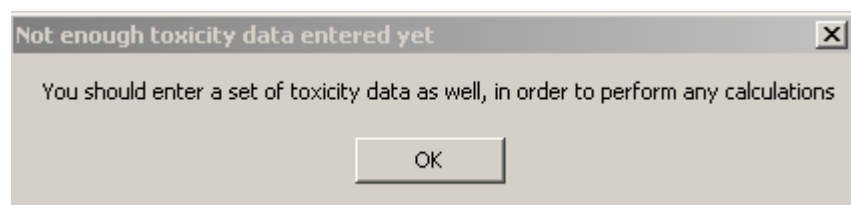


Figure 17. Message displayed upon FA calculation when no toxicity data have been entered.

- Enter your PEC value in the **Single PEC** box at the right part of your screen.
- Make sure that the unit of your PEC value is identical to the unit of the toxicity values that you have entered to generate your SSD! The unit of your toxicity data will be displayed in the **Specifics** box when you have made use of it while entering of your toxicity data.

- Enter a non-log transformed PEC value!
- This routine does not yield results when your standardised logarithmic PEC is ≤ -5 or ≥ 5 . The value of the standardised logarithmic concentration PEC will be shown in the **Fraction affected** sheet (**Output, Statistics**).
- You cannot enter the value 0 (zero). Your data will be \log_{10} -transformed for calculation and $\log(0)$ does not exist.

When you have finished entering data, see section 5.7.3 on how to calculate an FA.

5.3.4.2 *A series of environmental concentrations*

This calculation applies when you have a series of environmental concentrations, e.g. a measurement series of the compound of interest in an environmental compartment.

- Go to the **Input exposure data** sheet in the **Input data** part of E_{TX} .
- Go to the **Input environmental concentrations** box (this is the right part of your screen).
- In the cells under **Exposure data**, enter your toxicity values as a column, with one value per input cell.
- Enter the toxicity data in the original (non-transformed) values.
- Make sure that the unit of your environmental concentrations is identical to the unit of the toxicity values that you have entered to generate your SSD! The unit of your toxicity data will be displayed in the **Specifics** box when you have made use of it during entering of your toxicity data.
- Make sure *all* entries have the same unit: mg/l, $\mu\text{g}/\text{kg}$, ppm etc.
- You cannot enter the value 0 (zero). Your data will be \log_{10} -transformed for calculation and $\log(0)$ does not exist.
- You need not sort the data.
- You may leave blank cells, empty cells will not be included in the graph or in calculations.

When you have finished entering data, see section 5.7.4 on how to calculate an EER and JPC.

5.4 Clearing the contents of the input cells

5.4.1 Clearing a single cell

You can clear (or delete) data in a single input cell as follows.

- Select the cell you want to by clicking in the cell.
- Click on **E**dit, **D**elete in the Standard menu bar or press the Delete button on your keyboard to clear the content of the selected cell. The following message will appear:

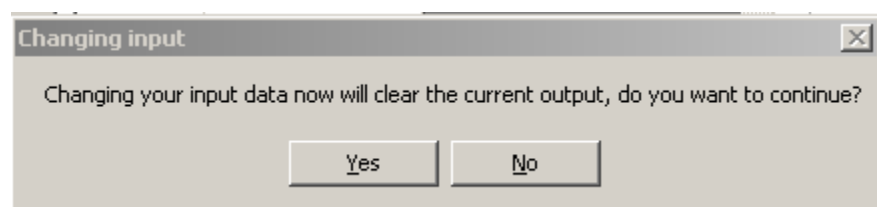


Figure 18. Message displayed when cell contents are about to be deleted.

- Click on **Yes** if to continue. The content of the cell will be deleted.

5.4.2 Clearing all cells

In order to clear a range of cells, do the following:

- Select the uppermost cell of the range of cells you want to clear, by clicking in the cell.
- Press the Shift button together with the Arrow Down (↓) button (both on your keyboard). While holding the shift button pressed down, you can select multiple cells by using the ↓ button repeatedly.
- When you have reached the bottom cell of the range you want to clear, stop holding down keys. Your selection of cells is now highlighted.
- Click on **Edit**, **Delete** in the Standard menu bar or press the Delete button on your keyboard to clear the content of the selected cells.

In order to clear all cells, do the following:

- Select the upper cell by clicking in it.
- Press the Shift button together with the Ctrl button. While holding these buttons pressed down, click on the Arrow Down (↓) button (all buttons are on your keyboard).
- All cells will now be selected (highlighted).
- Click on **Edit**, **Delete** in the Standard menu bar or press the Delete button on your keyboard to clear the content of the selected cells.

5.5 Importing data

You can also enter data (both toxicity data and environmental concentrations) from e.g. MS Excel. The procedure is as follows:

- The data you want to import should be placed in a column.
- Select the column containing your data and copy it
- Switch to *E7X* and click once in the upper cell of the data column of the sheet in which you want to import your data.
- Paste the data.

Please refer to section 5.11 on how to use the decimal symbol in these type of operations.

5.6 Adding labels to toxicity data

NB. This function is optional. All other program functions can be executed equally well when the toxicity data are not labelled.

When entering toxicity data, you have the possibility to label your entries. This means that you can add a text label to toxicity data. You might, for example, want to discriminate between the different taxonomic levels in your SSD and add labels like *algae*, *crustacea*, *insecta* (or abbreviations). Apart from adding text labels, you can customise the appearance of the symbols that are linked to your labels. These symbols will be used to construct your SSD.

5.6.1 Creating a label collection and using labels

Before you can enter labels that will also appear in your SSD graph, you have to create a label collection. To create a label collection, do as follows:

- Click on **T**ools, **L**abels in the Menu bar and the **Input toxicity data labels** dialog box shown in Figure 19 will appear:

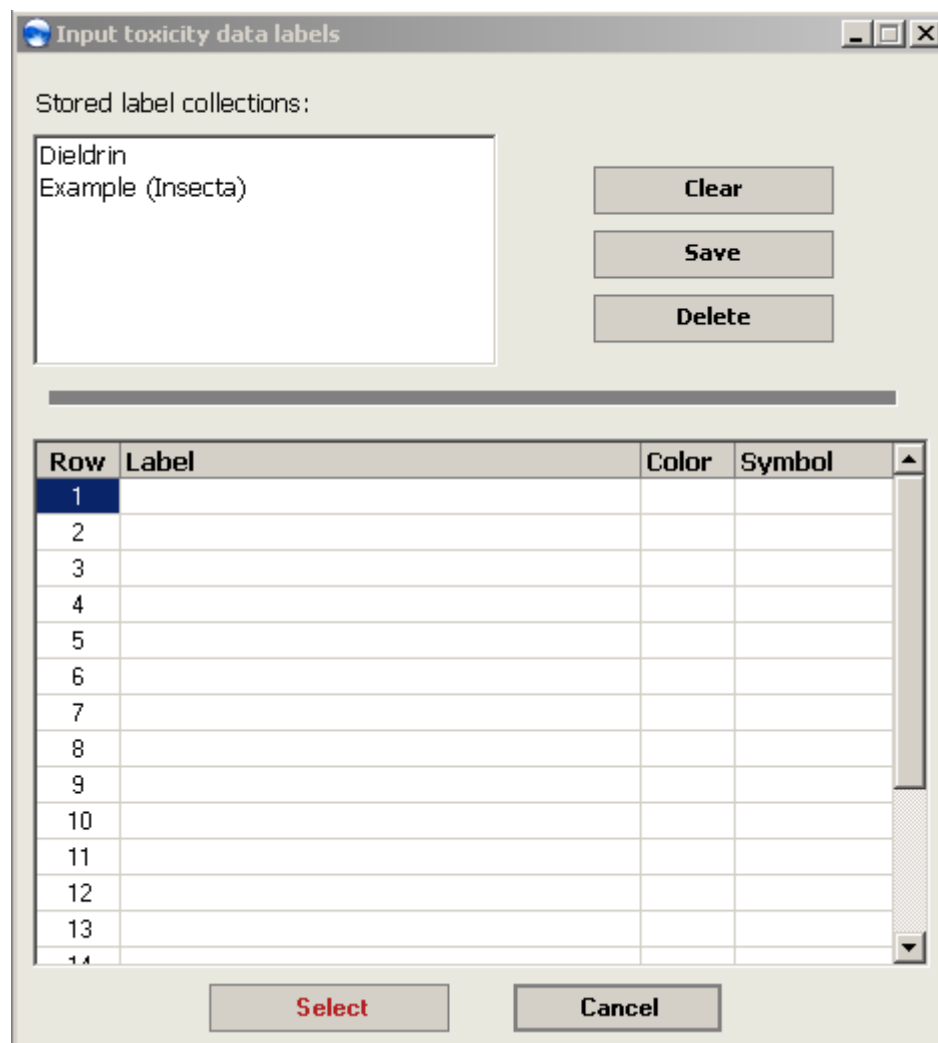


Figure 19. The **Input toxicity data labels** box.

- Next, type the name of the label in the column **Label** and assign a **Color** and a **Symbol** to the label in the following columns. Complete your label collection in this way.
- After having completed your label collection, click the **Save** button in the top part of the **Input toxicity data labels** box and type the name of your label collection. The name will now appear in the **Stored label collections** box visible at the top of the dialog box.
- You can select a label collection by clicking on its name in the **Stored label collections** box and subsequently press the **Select** button at the bottom of the label box. You then automatically return to the **Input toxicity data** sheet.
- Back in the **Input toxicity data** screen you see the selected label collection displayed in the lower right corner of your screen:



Figure 20. The active label collection display.

- Back in the **Input toxicity data** screen, behind each of the toxicity data that you enter, there is a **Label** cell. Using the pull down menu in each cell, you can now choose one of the

taxonomic groups (or 'labels') that are present in the label collection you have just selected.

5.6.2 Editing an existing label collection

Editing an existing label collection can also be performed via the **Input toxicity data labels** dialog box:

- Click on **T**ools, **L**abels in the menu bar and the **Input toxicity data labels** dialog box shown in Figure 19 will appear.
- Select the label collection in the **Stored label collections** box by clicking on it.
- Edit the label collection.
- Press the **Save** button to save any changes.
- Press the **Select** button if you wish to select the current label collection. You will automatically leave this dialog box after clicking **Select**.

5.6.3 Deselecting a label collection



In case you want to clear the connection between the toxicity data you have entered and the selected label collection, do the following:

- In the **Input toxicity data** screen you see the **Label collection display** in the lower right corner of your screen (Figure 20).
- Click on the **Deactivate** button to clear all labels entries behind your toxicity data.
- Note that this action will not erase your label collection or its settings, only its connection with the current dataset.

5.7 Performing calculations


5.7.1 Calculating an SSD


When you have finished entering toxicity data in the **Input toxicity data** sheet, you are ready to calculate an SSD.

- Select **Calculate, Go** from the Standard menu bar or press the **Calculate** button:  to invoke the calculation of the SSD and its statistics.
- After performing the calculation, *E7X* will automatically switch to the **Hazardous concentration sheet** in the **Output, Statistics** section.
- Switch manually to the **Goodness-of-fit** section in order to check the result of the normality tests performed on your toxicity data.
- Two graphs will be generated as well: the histogram and an SSD. Both can be found under **Output, Graphics**.
- If you have deleted or added data in the input section, choose **Calculate, Go** from the Standard menu bar (or press ) again for an update of statistics and graphics.

5.7.2 Calculating a small sample HD₅



When you have finished entering toxicity data in the **Input toxicity data** sheet using the 'small sample' method, you are ready to calculate an HD₅.

- Select **Calculate, Go** from the Standard menu bar or press the **Calculate** button:  to invoke the calculation of the HD₅ and its statistics.
- After performing the calculation, *E7X* will automatically switch to the **Small sample results sheet** in the **Output, Statistics** section.

- If you have deleted or added data in the input section, choose **Calculate, Go** from the Standard menu bar (or press ) again for an update of statistics.



5.7.3 Calculating an FA

When you have calculated an SSD and finished entering exposure data in the **Input exposure data** sheet, you are ready to calculate an FA.

- Select **Calculate, Go** from the Standard menu bar or press the **Calculate** button:  to invoke the calculation of the FA and its statistics.
- After performing the calculation, E_{TX} will automatically switch to the **Fraction affected sheet** in the **Output, Statistics** section.
- If you have deleted or changed your PEC in the input section, choose **Calculate, Go** from the Standard menu bar (or press ) again for an update of statistics.

5.7.4 Calculating an EER and JPC

When you have finished entering a series of environmental concentrations in the **Input exposure data** sheet, you are ready to calculate an EER and JPC.

- Select **Calculate, Go** from the Standard menu bar or press the **Calculate** button:  to invoke the calculation of the EER and its JPC.
- After performing the calculation, E_{TX} will automatically switch to the **Fraction affected sheet** in the **Output, Statistics** section. This sheet shows the EER in the lower one of the two boxes.
- Switch manually to the **Goodness-of-fit** section in order to check the result of the normality test performed on your exposure data.
- A joint probability curve will be generated as well. It can be found under **Output, Graphics**.
- If you have deleted or added data in the input section, choose **Calculate, Go** from the Standard menu bar (or press ) again for an update of statistics and the JPC.

5.8 Exporting results

Once you have generated results and statistics, you might want to save these results or store them in some other format. This can be done by using the **Export** function. You can find the **Export** function in the menu bar. Clicking on Export shows you one menu option: **Export current output**. After selecting this option, your data, statistical output and graphs will be exported to an MS Excel spreadsheet (extension .xls). The default filename is identical to the name of your E_{TX} project. If you have not yet stored your data in an E_{TX} project, the default filename will be NEW.xls. The following dialog box appears:

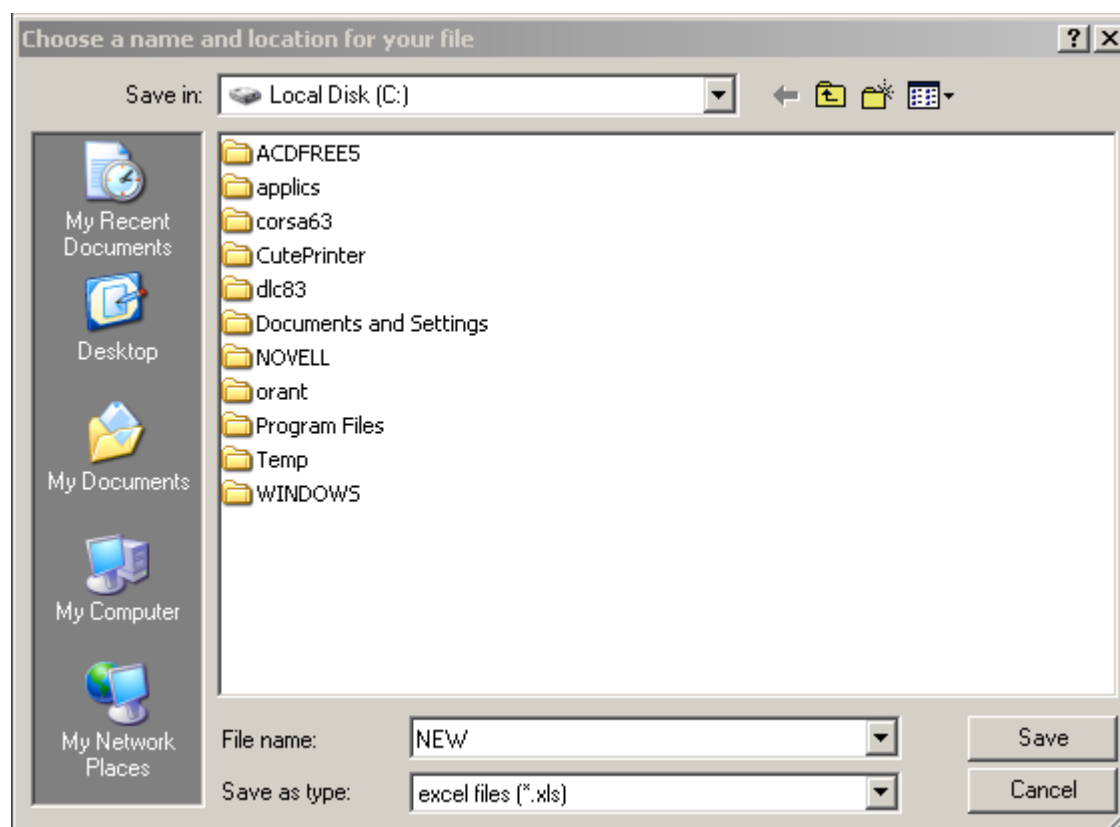


Figure 21. Save export file dialog box.

After having selected the location of your choice, click on **Save**. You will be asked if you want to open the export file directly or not:

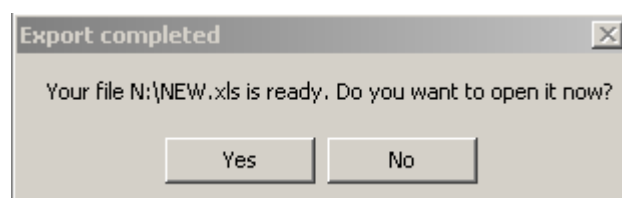


Figure 22. Export completed dialog box.

In order to have the exported (Excel)filenames corresponding with your E_{TX} -project names, save your E_{TX} data in an E_{TX} -project before exporting data to an Excel sheet. The name of your E_{TX} -project will then be chosen as default filename for your exported data file.

5.9 Preferences

5.9.1 Changing graph appearance in the *current* project

The three graphs that may be generated with E_{TX} 2.0 can be edited to a limited extent. This section explains how.

- Go to one of the graphs that you have generated: **SSD Histogram and PDF**, **Species sensitivity distribution** or **Joint probability curve** under **Graphics**.
- In the graph of your choice, click with the right mouse button (left button for left handed mousers).
- A pop up-window appears in which you can select various parts of the graph that you might want to edit (e.g. Title, Title font, labels, line colour etc.).

- All changes that you have made using this option will be saved along with your project. When you re-open a project and press calculate, the settings you last made will appear in your graphs.

5.9.2 Changing the axis settings

There are three possible graphs in E_{TX} : an **SSD Histogram and PDF**, a **Species sensitivity distribution** and a **Joint probability curve**. The axis settings of the **SSD Histogram and PDF** can not be changed. Axis settings for the other two graphs can be changed as follows:

- Go to one of the graphs that you have generated: **SSD Histogram and PDF**, **Species sensitivity distribution** or **Joint probability curve** under **Graphics**.
- In the graph of your choice, click with the right mouse button (left button for left handed mousers).
- A pop up-window appears in which you can select various parts of the graph that you might want to edit (e.g. Title, Title font, labels, line colour etc.). Select **Axes settings** from the appearing menu. The following dialog box appears:

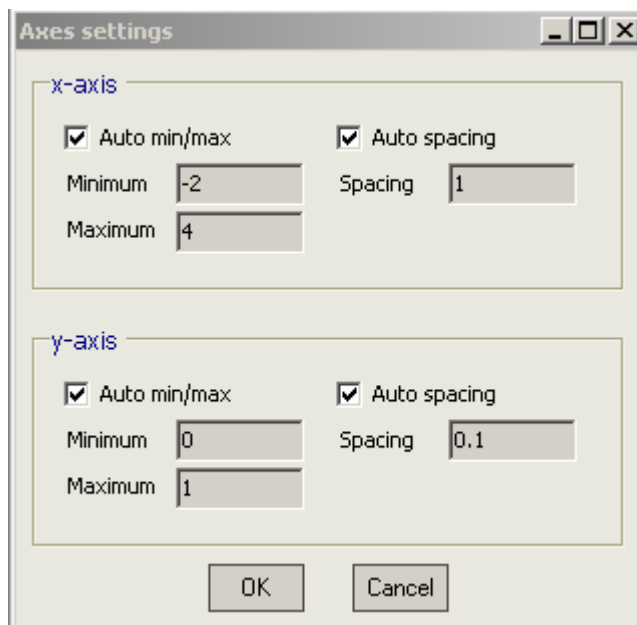


Figure 23. Axes settings dialog box.

Change the settings to your wishes and confirm by clicking **OK**.

5.9.3 Changing the default graph appearance

You may enter default values for the appearance of each of the three graphs via the **Tools, Preferences** menu that you can access via the menu bar. Figure 24 shows the dialog box that appears when you select this option.

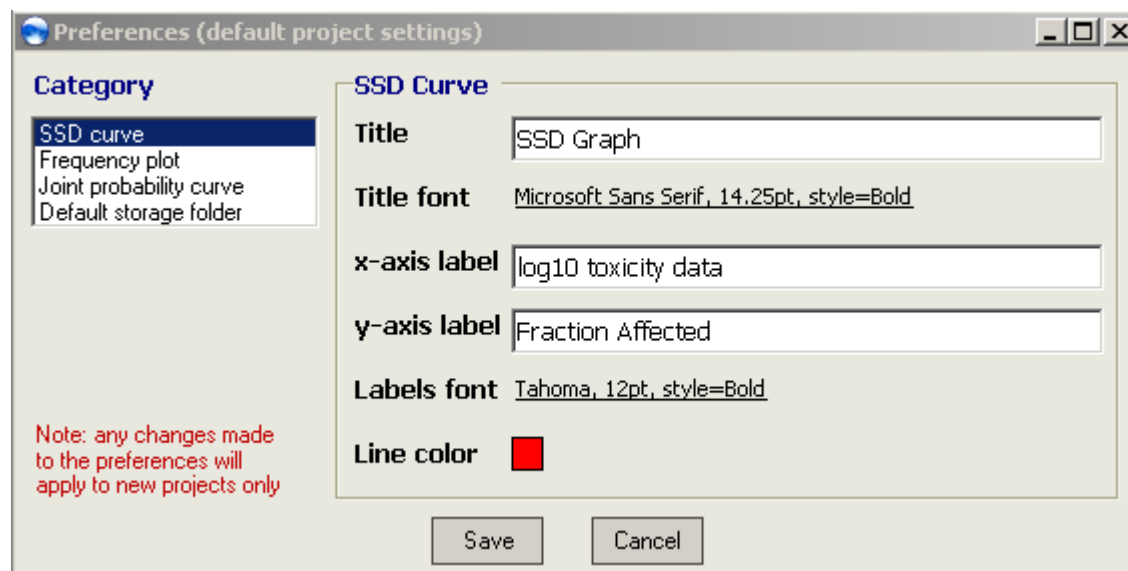


Figure 24. The default preferences dialog box.

- Select the graph of which you want to change the default settings in the box under **Category**.
- Edit the defaults in right half of the dialog box.
- After having made your changes, click on Save to save changes or on Cancel if you do not wish to keep your changes.
- Note that any changes you make will only apply to new projects that you will start after the current project. If you want to change the appearance of graphs in the current project, use the method described in the section above (5.9.1).

5.9.4 Changing the default file storage directory

You can select or change one directory where both your ETX -project files as well as exported data files will be saved. To change this directory:

- Click on **Tools, Preferences**. The box shown in Figure 24 appears.
- Click on **Default storage folder** in the **Category** box. The box shown in Figure 25 appears.

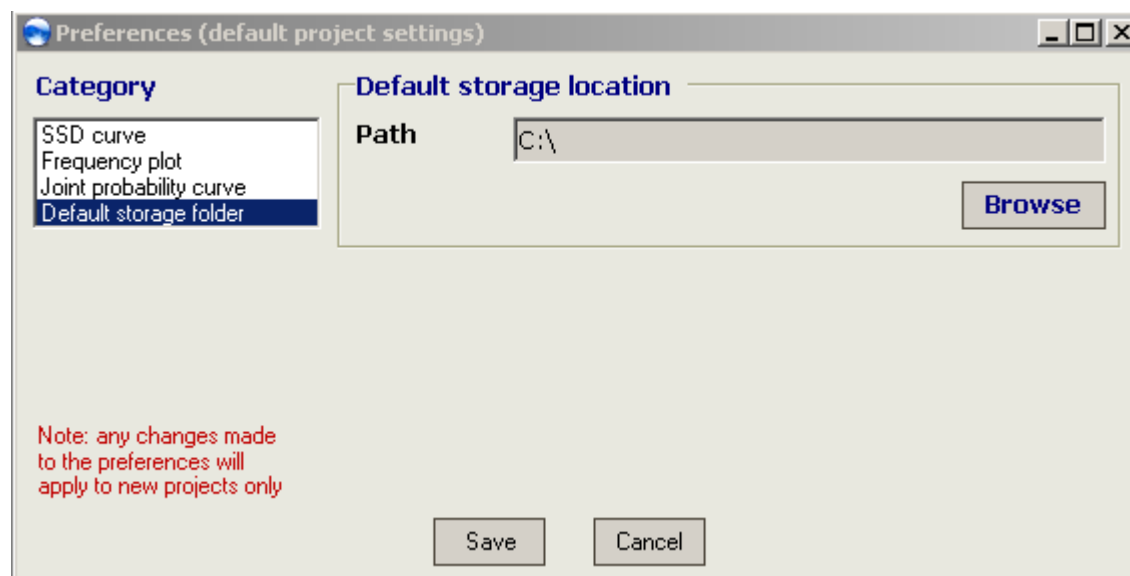


Figure 25. The default file storage location - dialog box.

- Type the directory of your choice behind **Path** or select a directory by browsing for it.
- Confirm your selection by clicking on **Save**. You will now leave the **Preferences** dialog.

5.10 Copying graphs

The three graphs that may be generated with E_{TX} can be copied to other programs. This section explains how.

- Go to one of the graphs that you have generated: **SSD Histogram and PDF**, **Species sensitivity distribution** or **Joint probability curve** under **Graphics**.
- In the graph of your choice, click with the right mouse button (left button for left handed mousers).
- A pop up-window appears; select **C**opy.
- You can now paste your graph in another program.

5.11 Use of the decimal symbol

The default setting of E_{TX} is that thousand separators will be ignored. In E_{TX} , the regional settings of your computer are applied to the figures you enter in input cells and to the graphs that are displayed under **Graphics**. For help on changing of these regional settings, please see section 3.8.

The default setting of E_{TX} has the following consequences. Example: if the period is your decimal symbol, the comma is usually the thousand separator. As long as you enter data with only a period as a decimal symbol, all is ok. You should enter figures higher than 999 *without* thousand separator: e.g. type 10000 rather than 10,000. As soon as E_{TX} recognises a thousand separator, a warning will be displayed in the status bar at the bottom of your E_{TX} screen: **Your data contains illegal decimal symbols**. This warning is displayed because otherwise, entering a figure like 13,1 would be entered as 131 and there is a fair chance that you might not notice this type of unexpected changes!

Therefore, two golden rules are:

1. Do *not* use thousand separators when entering values.
2. The user determines the decimal symbol (settings) and is responsible for correct use.

6. Limitations

6.1 SSD calculations

- For HC₅ and HC₅₀ calculation a maximum number of 200 data can be entered.
- For calculation of the FA at the HC₅₀ of your SSD (which is 50, by definition) and its accompanying lower and upper limit, the maximum number of toxicity data (*n*) is 75.

6.2 Calculation of the fraction affected

The calculation of the fraction affected that is calculated at a given environmental concentration (PEC) is limited by the height of the PEC value. The standardised logarithmic value of this PEC should be within the range -5 to 5. As a check, the value of the standardised logarithmic concentration calculated from your PEC value is shown in the **Fraction affected** output sheet. If this value is outside the range mentioned above, no results will be given. The message 'The method does not yield results because your standardised PEC exceeds the limits of <-5 or >5' will appear in red. The sheet will show 'Out of bounds!' as error values.

7. Examples

This section is a practical guide, which helps you through the calculation of an SSD or FA or other options in a stepwise manner.

7.1 Calculating an SSD

- ▶ Open *E_TX* 2.0 or open a new project.
- ▶ In **Input data**, go to the **Input toxicity data** sheet, that is in the right part of your screen.
- ▶ Type the following data in the input cells in the column **Toxicity data**:

0.97

3.33

3.63

13.5

13.8

18.7

154

- ▶ In this example we will not add labels to the data. Leave the cells in the column **Label** empty.

These data are NOEC values for toxicity of cadmium to seven soil organisms, and are expressed in ($\mu\text{g Cd/g soil}$). The data are taken from Van Straalen and Denneman (1989) and are also used as an example in Aldenberg and Jaworska (2000).

- ▶ Go to the **Specifics** box in the **Input toxicity data** sheet. Click in the box to the right of **Unit**, and type: $\mu\text{g/g}$.
- ▶ In the **Specifics** box, click the pull down menu (downpointing arrow) and select NOEC.
- ▶ After having selected 'NOEC' in the **Specifics** box, press **Calculate, Go** in the Standard Menu bar. After performing the calculation, *E_TX* automatically switches to the **Hazardous concentration sheet** in the **Statistics section** (Figure 26, shown at the next page).
- ▶ Go to the **Goodness-of-fit** sheet and check if your data are normally distributed (Figure 27, shown at the next page). In this example it is probable that the data derive from a normal distribution.

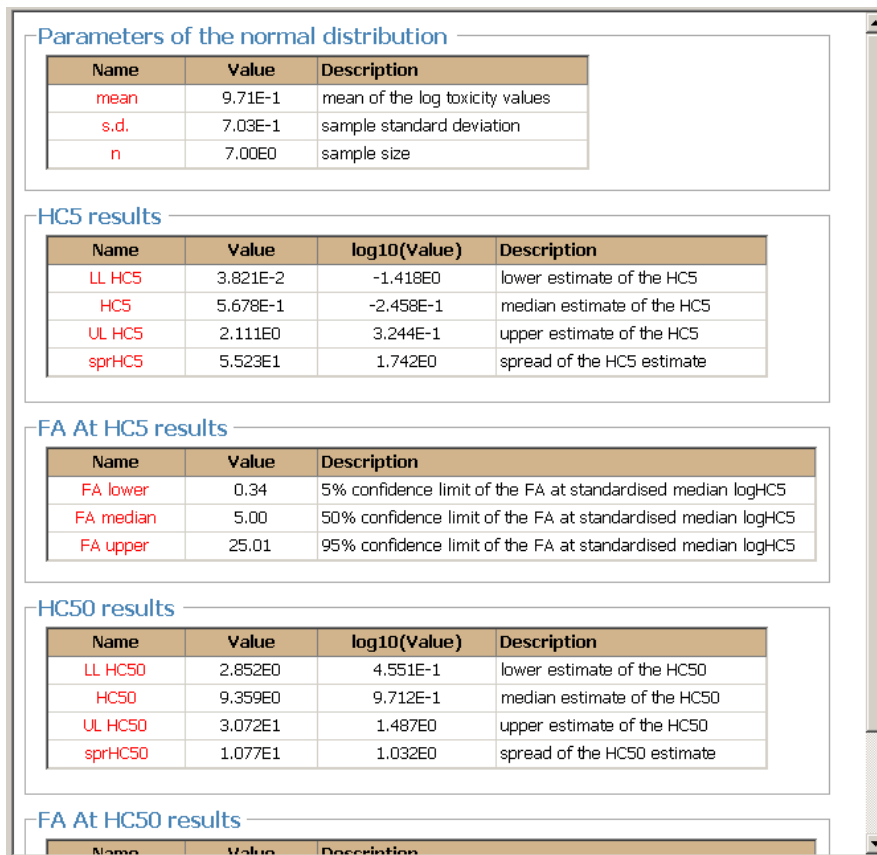


Figure 26. Output of statistical parameters for HC5, HC₅₀ and FA calculation.

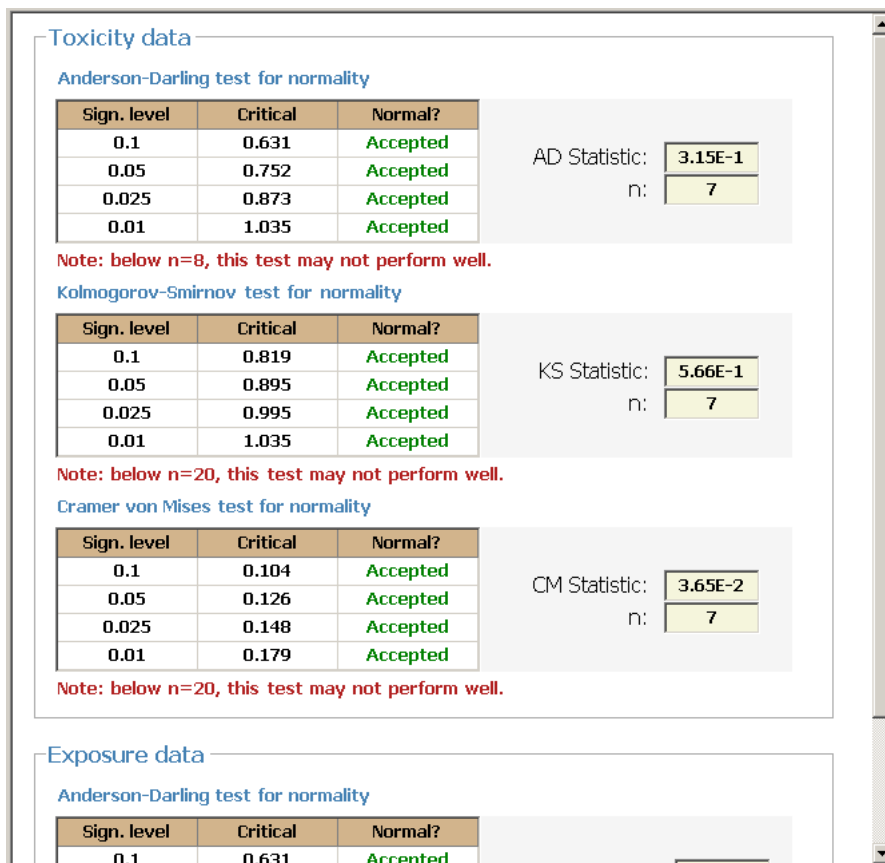


Figure 27. Output goodness-of-fit tests.

► Go to **Output, Graphics** to find the **SSD Histogram and PDF** sheet and the **SSD graph** sheet to view the graphical output (Figure 28).

SSD Histogram and PDF

SSD graph

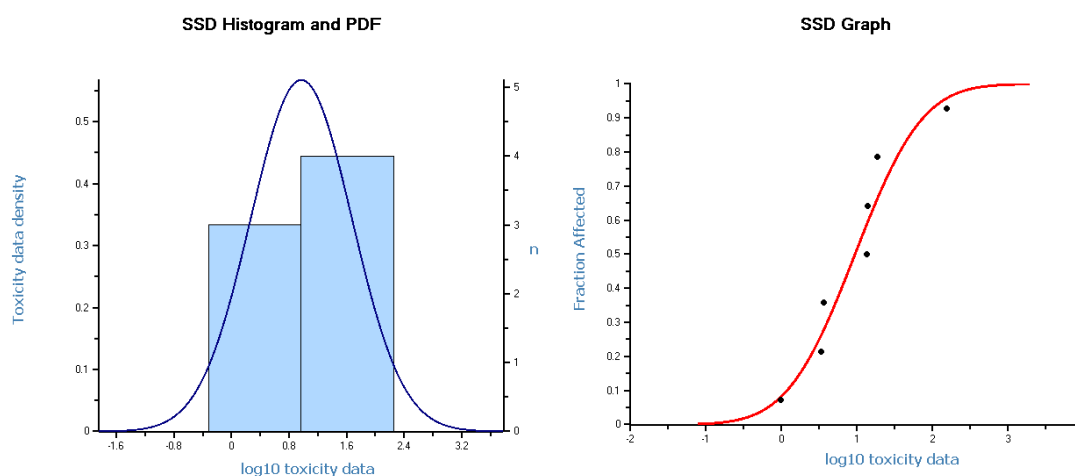


Figure 28. Histogram and PDF (left panel) and species sensitivity distribution (CDF, right panel).

7.2 Calculating a fraction of affected species

In order to calculate the fraction of species that is (potentially) affected at a given exposure concentration, we need the SSD of these species. So, you have to calculate an SSD first. In this example, we will use the SSD that was generated in section 7.1. Next, suppose we have one environmental concentration of 12 mg Cd/kg soil. The toxicity data were entered in the units of $\mu\text{g Cd/g soil}$. This is identical to mg Cd/kg soil. We have verified that units of toxicity data and PEC value are identical. Proceed as follows.

- Calculate an SSD (see section 7.1).
- Go to the **Input exposure data** sheet. In the right part of your screen, you see the box **Single PEC**.
- In the **Single PEC** box, click in the empty cell next to 'Enter a single PEC'; type 12 and press [enter].
- After having entered the PEC value, press **Calculate, Go** in the Standard Menu bar.
- E_{TX} will now switch automatically to the **Fraction affected** sheet in the **Output** section.
- Note that the value of the standardised logarithmic concentration calculated from this PEC is 0.5136. Since this value is >-5 and <5 , an FA can be calculated by E_{TX} (see section 6 for limitations).
- The upper half of this sheet (the **FA results** box) shows your result (Figure 29): the median percentage of species in the soil that will be affected by this Cd concentration is 55.9%. The variation in the data tells you that there is 90% confidence that the percentage of affected species is between 31.6% and 78%.

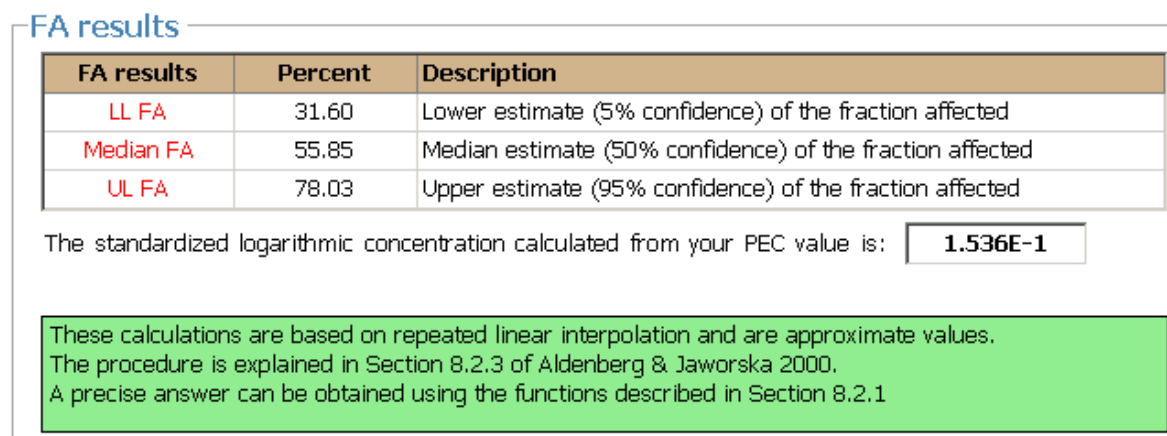


Figure 29. The fraction of species affected by 12 mg/kg Cd.

7.3 Calculating an expected ecological risk

Suppose you have a series of Cd concentrations, measured in a field soil. The measured concentrations are 1.5, 7, 3, 12, 1, 11 and 6 (mg Cd per kg soil). You can now calculate an EER and generate its graphical representation as a joint probability curve. In order to calculate the EER for the organisms in the field soil, we need the SSD of these species for Cd. In this example, we will use the SSD that was generated in section 7.1.

- ▶ Calculate an SSD (see section 7.1).
- ▶ Go to the **Input exposure data** sheet in the **Input data** section.
- ▶ In the right part of your screen, called **Input environmental concentrations**, click in the first cell of the column under **Exposure data**. Enter the following values, each next value in a new input cell:

1.5
7
3
12
1
11
6

- ▶ After entering the last value, press **Calculate, Go** in the Standard Menu bar.
- ▶ In order to check if your data follow a normal distribution, go to the **Goodness-of-fit** sheet in the **Output** section. In the lower half of this sheet, in the **Exposure data** box, the results of the test for normality of your exposure data are shown (Figure 30). In this example it is probable that the data derive from a normal distribution.

Expected Ecological Risk

FA results	Value	Description
SEC Mean	-4.882E-1	Scaled mean of (log10) environmental concentration distribution
SEC sd	5.996E-1	Scaled S.D. of (log10) environmental concentration distribution
Exp. Ecol. Risk	3.377E1	Expected Mean Ecological Risk, given the SSD and the EC distribution

The mean and standard deviation are scaled relative to the mean and SD of the Species Sensitivity Distribution.
The expected (mean) ecological risk is the area below the Joint Probability Curve.

Figure 30. Results of GOF test for exposure data.

► Go to the **Fraction affected** sheet (**Output, Statistics** section). In the lower half of this sheet (the **Expected ecological risk** box) you see two statistical parameters that are used to calculate the EER. The EER itself amounts to 33.8%, which is the percentage of species likely to be affected, given the SSD and EC distribution based on the data you have entered.

Expected Ecological Risk

FA results	Value	Description
SEC Mean	-4.882E-1	Scaled mean of (log10) environmental concentration distribution
SEC sd	5.996E-1	Scaled S.D. of (log10) environmental concentration distribution
Exp. Ecol. Risk	33.77	Expected Mean Ecological Risk, given the SSD and the EC distribution

The mean and standard deviation are scaled relative to the mean and SD of the Species Sensitivity Distribution.
The expected (mean) ecological risk is the area below the Joint Probability Curve.

Figure 31. Results of the expected ecological risk calculation.

► Go to the **Joint probability curve** sheet (**Output, Graphics** section). The graph in Figure 32 should be displayed.

JP Curve

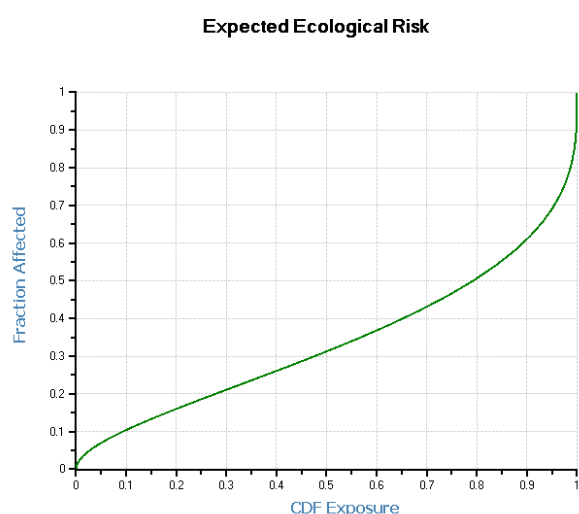


Figure 32. The joint probability curve belonging to the SSD from example 7.1 and the exposure data from example 7.3.

7.4 Calculating an HD_5 for birds or mammals from a small data set

In contrast to the previous examples, you do not have to generate an SSD before you can calculate an HD_5 for a small sample of toxicity data.

- ▶ Go to the **Input exposure data** sheet in the **Input data** section.
- ▶ In the **Input toxicity data** box (this is the right part of your screen), click on the **Use small sample approach** check box in the **Small sample** box. The following dialog box will appear:



Figure 33. Small sample verification box.

If you had already typed data in the input section, these data will be lost when you switch to the Small sample input mode. Remember that this is a different method that does not require calculation of an SSD. You will have to start a separate project for each type of calculation that you perform. So, if you want to keep the data you had entered before, click on **Cancel** and save your work in an E_{TX} project. Then start a new project for your 'small sample' calculation. Since we want to use the 'small sample' method, click on **OK**.

- ▶ In the part of your screen called **Input toxicity data**, the number of input cells is now reduced to 10.

▶ In the **Input toxicity data** sheet, click in the first cell of the column under **Toxicity data**. If there are any values present, delete these data. Type the following –fictitious– data in the input cells, each next value in a new input cell:

120
550
630

Let us say that these values are three LD_{50} values for birds, for a pesticide that is not a carbamate nor an organophosphorous compound.

- ▶ From the list of **Pre-defined standard deviations** that is also shown in the **Small sample** box, in the lower right corner of your screen, select the value for 'LD₅₀ data of 55 pesticides for birds'. The figure 0.465 will appear in the **Standard deviation** cell.

▶ Click on **Calculate, Go** in the **Standard** menu bar.

▶ After performing the calculation, E_{TX} automatically switches to the **Small sample output** sheet in the **Output, Statistics** section (Figure 34).

Parameters

Name	Value	Description
mean	2.540E0	mean of the log toxicity values
n	3	sample size
s.d.	0.465	preselected standard deviation

HD5 (median) results

Name	Value (mg/kg bwt)	Extrapolation factor (EF)
LL HD5	2.154E1	1.609E1
HD5 (median)	5.954E1	5.819E0
UL HD5	1.646E2	2.105E0

Figure 34. Output of the small sample calculation with *E7X 2.0*.

► The estimated 5th percentile of HD₅ values is 60 mg/kg_{bwt}. There is 90% confidence that the HD₅ will lie between 22 and 165 mg/kg_{bwt}. The extrapolation factors shown in the second column can be used to calculate each of the three reported parameters directly from the sample mean. For further details we refer to Aldenberg and Luttik (2002).

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List of abbreviations

AUC	area under the curve
CDF	cumulative distribution function
DGM/SAS	Directorate General for Environmental Protection, Directorate for Chemicals, Waste and Radiation
EC	exposure concentration, in the context used here it is synonym to environmental concentration
ECD	exposure concentration distribution
EER	expected ecological risk
EU	European Union
<i>E_TX</i>	<i>EcoToX</i>
FA	fraction affected
GOF	goodness-of-fit
HC ₅	hazardous concentration, 5 th percentile of normally distributed toxicity data
HC ₅₀	hazardous concentration, median or 50 th percentile of normally distributed toxicity data
HD ₅	hazardous dose, 5 th percentile of normally distributed toxicity data
INS	Setting (Inter)national Environmental Quality Standards
JPC	joint probability curve
LC ₅₀	toxicant concentration causing 50% mortality in test population
LL	lower limit (of a confidence interval)
MS	Microsoft ©
<i>n</i>	sample size
NOEC	no observed effect concentration
PAF	potentially affected fraction; identical to FA, but PAF is no longer used in <i>E_TX</i> 2.0
PDF	probability density function
PEC	predicted environmental concentration
RIVM	National Institute for Public Health and the Environment
RIKZ	National Institute for Coastal and Marine management
RIZA	Institute Inland Water Management and Waste Water Treatment
s.d.	standard deviation
SEC	scaled environmental concentration
SSD	species sensitivity distribution
UL	upper limit (of a confidence interval)
VROM	Dutch Ministry of Housing, Spatial Planning and the Environment

Appendix 1 Installation problems

This appendix gives you an overview of combinations of Microsoft operating systems and Microsoft Office combinations we have tested plus the results of these tests. For some problems you may have benefit of a solution that is offered here.

Table 1. List of results: installation of E_TX 2.0 on MS operating systems and MS Office versions plus reference to possible solutions where appropriate.

MS Windows version	MS Office version	Test result	Solution
Windows 98 SE	not installed	Installation successful	
Windows 98	Office 97	Installation unsuccessful	
Windows 2000	Office 97	Installation unsuccessful	try solution 1
Windows 2000	2000	Installation successful	
Windows NT	not installed	Installation successful	
Windows NT	Office 97	Installation successful	
Windows XP	not installed	Installation successful	solution 2
Windows XP	Office 97	Installation successful	solution 2
Windows XP	Office 2000	Installation successful	solution 2
Windows XP	Office XP	Installation successful	solution 2
Windows XP	Office 2003	Installation successful	solution 2

Solution 1

With older versions of Windows, you might need to install Microsoft's Data Access Components (MDAC) or a recent update of MDAC. One version of MDAC is placed as a compressed file on the E_TX -CD. It is called **MDAC_TYP.exe** (version 2.80). You may install the file provided to you on the E_TX -CD, however, since updates of MDAC frequently appear we advise you to visit the Microsoft website to check for possible more recent updates. Visit: <http://www.microsoft.com> and search for MDAC.

If there are no MDAC installed on your PC or there are no recent updates available, follow the installation procedure described below.

Installation of MDAC from the E_TX -CD.

- Insert the E_TX -CD in the CD drive of your computer.
- Start the Windows Explorer (Start button, Programs, Accessories, Windows Explorer).
- In the left side of your screen, click on the drive that is your CD drive.
- In the right half of your screen, double click on the file **MDAC_TYP.exe**.
- The installation procedure will now start. Complete the installation procedure.

Now try to install E_TX . We refer to section 3.2 for the installation procedure.

Solution 2

If you have a machine with a recent Windows version, but installation is still unsuccessful, try manual installation of E_TX on your hard disk. Do as follows:

Manually Installing *E_TX*

- Insert the *E_TX*-CD in the CD drive of your computer.
- Start the Windows Explorer (Start button, Programs, Accessories, Windows Explorer).
- Create a new directory on your hard disk (e.g. C:\My Documents\ETX TEMP) in which the installation files can be placed.
- Files\RIVM\E_TX 2.0).
- Double click the file *E_TX* 2.0.msi that is on the hard disk of your computer to start MS Installer.
- Normally, *E_TX* should now be installed without problems.

Manually installing .NET framework

If the compact .NET framework is not installed on your computer, try to run the setup program manually:

- Insert the *E_TX*-CD in the CD drive of your computer.
- In the dialog box enter E:\dotnetfx.exe, where E is the letter of your CD drive.
- Normally, the .NET framework should now be installed without problems.
- Next carry out the procedure described above, under 'Manually installing *E_TX*'.

Appendix 2 Error messages

This appendix gives an overview of the error messages that can be displayed when using *E_TX* 2.0. A short description or solution to the problem is given where appropriate.

Error messages displayed during starting

Message

Could not initialize application, make sure a recent version of Microsoft's Data Access Components (MDAC) are installed on your PC

Solution

See solution 1 in Appendix 1.

Error messages displayed during calculations or entering data

Message

You should enter a set of toxicity data as well, in order to perform any calculations

Solution

E_TX can not perform calculation of an SSD and accompanying statistics (FA, HC etc.) unless you enter a toxicity data set in the **Input toxicity data** sheet. The minimum number of data is three.

The 'small sample' method can be used without calculating an SSD first.

Message

You haven't entered sufficient data!

Solution

E_TX will not perform calculation of an SSD and accompanying statistics (FA, HC etc.) unless you enter a toxicity data set of at least three values in the **Input toxicity data** sheet.

E_TX will not perform calculation of an HD₅ using the *small sample method* unless you enter at least one toxicity value in the **Input toxicity data** sheet.

Message

You haven't entered sufficient exposure data to calculate the Expected Ecological Risk and JPC curve

Solution

E_TX will not perform calculation of an EER and JPC unless you enter at least three exposure concentrations in the **Input exposure data** sheet.

Message

A label collection is active, this means you have to assign labels to all entries in the toxicity input screen before you can calculate results

Solution

Go to the **Input toxicity data** sheet and check if all toxicity entries have a label attached to it. If you do not want your toxicity data to be labelled, go to the **Input toxicity data** sheet and click on the **De-activate** button in the lower right corner of your screen.

Message

You have to enter a prefixed standard deviation in order to perform small sample calculations

Solution

In the **Small Sample** box in the **Input toxicity data** sheet, select a predefined standard deviation or enter a custom value.

Error messages displayed when saving an E_{TX} file*Message*

An error occurred while saving the file.

Solution

A single solution for this problem can not be given. Try copying the dataset or datasets you have entered to an other application. Then quit E_{TX} , and restart E_{TX} in order to retry your work.

Error messages displayed when opening an E_{TX} file*Message*

An error occurred while opening the file.

Solution

Your file may be damaged or in the wrong format. Try to open your data in another application and copy them to E_{TX} .

Error messages displayed when exporting results*Message*

Please (re)calculate your results before exporting

Solution

You have made changes to the data but you have not yet pressed the calculate button. Consequently, the results you are about to export do not belong to the dataset, which is currently entered in E_{TX} . In order to continue, you have to update your results first by performing a calculation.

Message

An error occurred while exporting, please make sure that a version of Microsoft Excel 97 or higher is installed on your PC.

Solution

A solution is given in the message; an Excel version as recent as Excel 97 should be used.