TerraQSAR™ - FHM

Fathead minnow 96-hr LC50 Estimation Software, vs. 2.1

User Manual

Purpose

TerraQSAR™ - FHM is a holistic, probabilistic-algorithm-based neural network software program, designed and optimized solely for the computation of acute (96-hr) median lethal concentrations (LC50) of organic (carbon-containing) substances with a defined chemical structure to the fish fathead minnow (*Pimephales promelas*).

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Introduction

Welcome to **TerraQSAR™**, a breakthrough development in toxicity estimation software, developed by TerraBase Inc.

TerraQSAR™ prediction software is based on the probabilistic neural network methodology using the molecular structure of the substances under investigation. The **TerraQSAR™ - FHM** program estimates the 96-hr lethal concentrations to 50% of a population (LC50) of the North American fish fathead minnow (*Pimephales promelas*), a widely used test species.

TerraQSAR modules use as input a chemical's SMILES code (2-D or 3-D), which is an international code for the representation of chemical structures and amenable to computer analysis.

The **TerraQSAR™ - FHM** module computes the LC50 in both mg/L and pT (log [L/mmole]) units, as well as the molecular weight (MW) of substances entered.

Theory

The field of artificial intelligence and neural network application is experiencing rapid growth in all aspects of technology. From elevator control to drug design, neural network methods have lots to contribute to product development, operating improvements, and frequently enable the customer to tackle problems, which were inaccessible hitherto.

The **TerraQSAR** products exploit the neural network methodologies developed in recent years by researchers and programmers both within and outside the company. In contrast to linear methodologies, such as simple regression methods, principal components analysis and others, neural networks make use of non-linear relationships, which makes them particularly useful for chemical/biological problems where different and/or unknown modes of action are known or likely to be present, in addition to linear relationships.

Important information on both the theory and specific aspects of this software can be found in the references given in the Literature.

Computation Process

Data Set

The **TerraQSAR™ - FHM** fathead minnow toxicity estimation program is based on a data set of measured values for 886 organic (carbon-containing) compounds. These data are widely available, both from non-commercial sources, such as the US Environmental Protection Agency's Ecotox database, or from commercial sources, such as TerraBase Inc.'s **TerraTox™: Explorer** database. In fact, these data form the basis for most fish toxicity estimation programs.

Fragments

Major fragments used in the **TerraQSAR** modules have been described in detail in several publications listed in the Literature, especially the works by Kaiser *et al.* An overview of basic fragment types considered is given in Table 1 below.

Table 1. Examples of fragments used in **TerraQSAR**.

Fragment type	Examples
Acidity fragment	C(=O)O, S(=O)(=O)O
Aliphatic ring fragment	C1CCCCC1, C1CCCC1
Aromatic ring fragment	c1ccccc1, c1ccccn1
Atom fragment	C, H, N, O
Bond fragment	C-C, C=C, C#C
Group fragment	C-O-H, C-O-C, O=C-O-C
Hydrophobicity fragment	C(C)(C)C, CCCC
Ionisation fragment	[O-], [Na+]
Polarity fragment	O=N(=O)CC(O)
Reactivity fragment	C=CC=O
Stereo fragment	Cl[C@H](C)N, Cl[C@@H](C)N
Weight fragment	molecular weight

Input Query

All **TerraQSAR** modules use the SMILES string code as input. For additional comments about the SMILES code, refer to the paragraph on SMILES Notation.

Computation

The computer evaluates the number and type of fragments present in the query string and computes the resulting estimate on the basis of the same types of fragments present in a data set of 886 compounds for which measured values have been published in the literature. Computation time varies with the complexity of the query structure and speed of the computer. Typically, for compounds without chiral centers, and molecular weight of <200, computation time on a 2 GHz machine takes <5 seconds.

Results

Figure 1 shows the measured vs. predicted values for all 886 compounds used in the development of the **TerraQSAR** fathead minnow estimation program, as obtained from the program. The data cover approximately ten orders of magnitude, ranging from pT= -3 to pT= 7, where pT is the negative logarithm of the millimolar LC50 concentration.

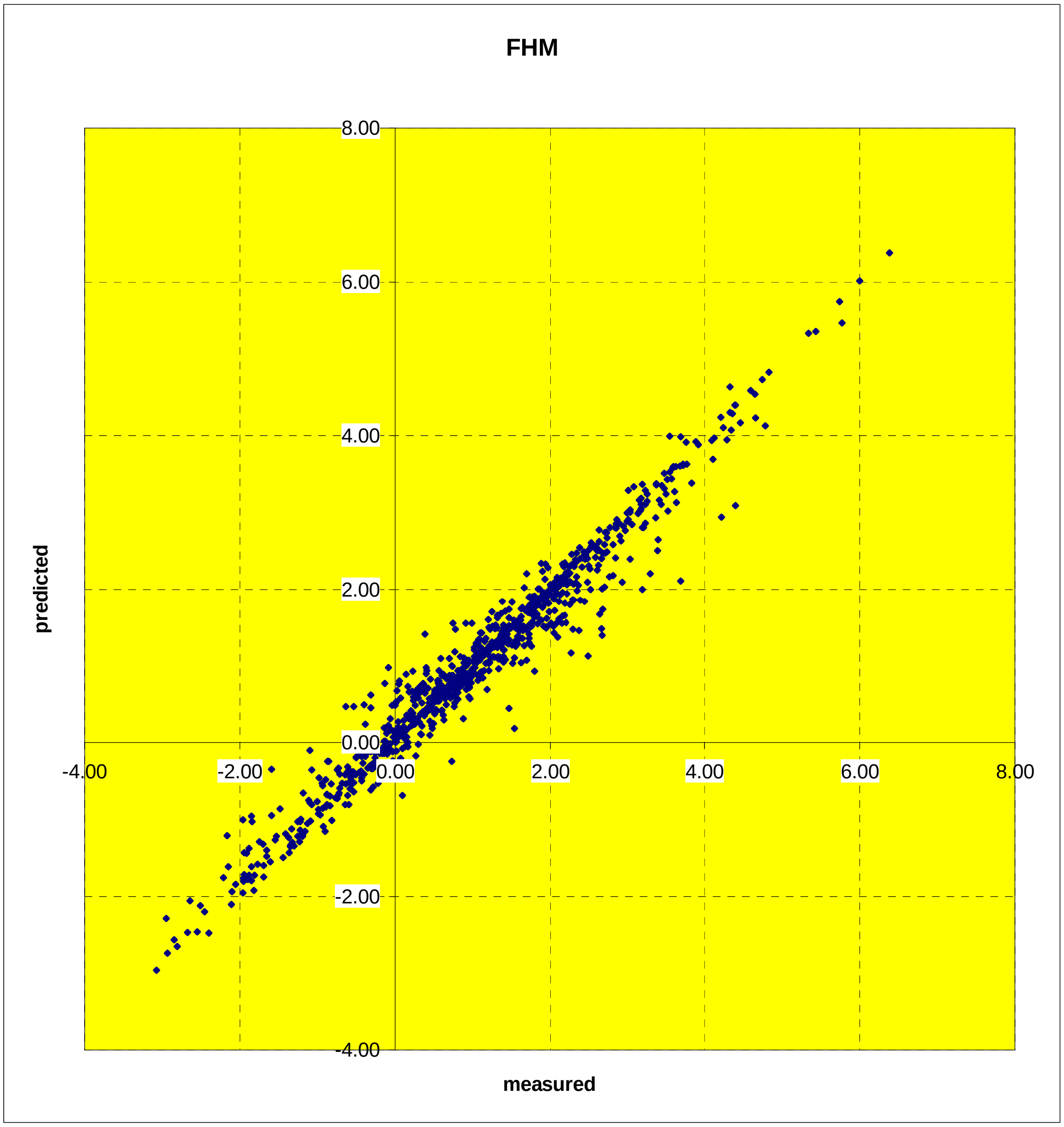

Fig. 1. Plot of the measured vs. predicted fathead minnow LC50 values for all 886 compounds in the training set; units are pT, equal to log(L/mmol).

SMILES Notation

The Simplified Molecular Input Line Entry System (SMILES) has been developed by D. Weininger at the beginning of the 1980's. It is far superior to the previously used Wiswesser Line Notation (WLN) for coding and depicting chemical structures, by being simple, intuitive, and machine readable. For an excellent tutorial on the SMILES notation, refer to the Daylight Corp. web site.

Recently, Accelrys Inc. introduced a variety of software modules allowing the visualization of SMILES codes as chemical structure drawings. In this process, Accelrys introduced changes to the common (Daylight Corp.) interpretation of SMILES codes by their software. As a result, lower case "c", formerly only interpreted as sp^2 carbon, is now interpreted as either as sp^2 or sp^3 carbon, depending on its surrounding and connections to other atoms. The determinant here is whether or not the carbon atom is part of an aromatic ring, as defined by the Hueckel rules. This has ramifications for the correct interpretation of SMILES strings by the **TerraQSAR** programs, as they are built on the backbone of the Accelrys software. Therefore, all users are cautioned to ascertain that their SMILES codes follow the rules of the Accelrys software, i.e., to ascertain that only sp^2 carbons in ring systems which satisfy Hueckel conditions for aromaticity are given in lower case "c"; all other sp^2 carbons, whether in rings or not, must be entered as capital "C". Some examples of valid and not valid SMILES strings are listed below in Table 2.

Table 2. Valid and not valid examples of Accelrys' SMILES code.

Substance	SMILES not valid	SMILES valid
cyclopentadiene [a]	c1cccC1	C1=CC=CC1
coumarin [a]	c1cc2OC(=O)ccc2cc1	c1cc2OC(=O)C=Cc2cc1

[a] The SMILES strings shown as "not valid" are valid *per se*, however, the interpretation of these codes are the hydrogen-saturated compounds cyclopentane and 3,4-dihydrocoumarin, respectively.

European (SIDS) Evaluation

The SIDS Evaluation program by the European Community undertook a comparative evaluation of six fish toxicity QSAR programs, including the **TerraQSAR - FHM** (fathead minnow) program. The following text outlines the main steps and results, for a complete copy of the report, see Pavan et al. (2005).

Method outline

1. Preliminary analysis of SIDS acute fish toxicity data.
2. Generation of molecular structure files for the SIDS chemicals (Smiles, mol files), for further calculation of both two-dimensional molecular descriptors and three-dimensional descriptors. An excel file containing chemical names, CAS numbers and SMILES for 177 chemicals was kindly provided by Eva Wedebye (DK).
3. Development of a list of literature-based models to make predictions of SIDS endpoints. The focus was on models for fish toxicity.
4. Selection of transparent and reproducible models: recovery of the training set used to develop the models and checking of the test method used to generate it; identification of the molecular descriptors used and assessment of the transparency of the algorithm.
5. Estimation of predictive ability by internal validation techniques (cross-validation, bootstrap, response randomization).
6. Evaluation of QSAR applicability domains by making predictions of SIDS test data: checking the domain of applicability with respect to descriptor ranges and any structural rules defining the group of substances for which the models are valid.
7. Application of the models to the SIDS chemicals.
8. Evaluation of predictive performance in terms of explained variance (Q^2_{ext}) and the prediction reliability (order of magnitude between estimated and experimental data). Predictive

performance was assessed for the full set of SIDS substances, and for subsets based on different hypotheses about the applicability domain.
9. Comparative analysis of the model quality.

Results of Method

Table 3, below, is an excerpt from “Comparative Assessment of QSAR Models for Aquatic Toxicity” (Pavan et al., 2005), comparing six different QSAR models for the “Danish dataset” of 177 SIDS compounds. As is evident from Table 3, the TerraQSAR™ - FHM program delivers estimates for all (177) test compounds (versus 125 compounds by the nearest competitor), and has the highest Q^2*ext* of all tested programs. A copy of the complete report can be accessed through the reference (Pavan et al., 2005).

Table 3. Model performance comparison.

Model	N. Train	SIDS Train	Q^2_{LOO}	$Q^2_{boostrap}$	SDEP	R^2	Test MOA	N.Test	Unknown SIDS predictions	Total SIDS predictions	Q^2ext
NPN	58	8	91.51	91.66	0.421	92.18	NPN	14	37	51	89.06
							Mixed	28	97	125	90.86
PN	86	5	89.59	89.64	0.336	90.07	PN	2	4	6	N.A.
							Mixed	25	98	123	86.66
N	144	13	87.06	87.11	0.461	87.55	NPN + PN	13	41	54	92.18
							Mixed	24	97	121	91.63
MIXED	114	9	75.94	75.83	0.495	77.57	Mixed	22	51	73	87.10
E-State	121	8	68.28	9.30	0.505	84.04	Mixed	17	69	86	89.43
Terra QSAR	886	N.A	N.A	N.A	N.A	94.56	Mixed	57	120	177	99.39

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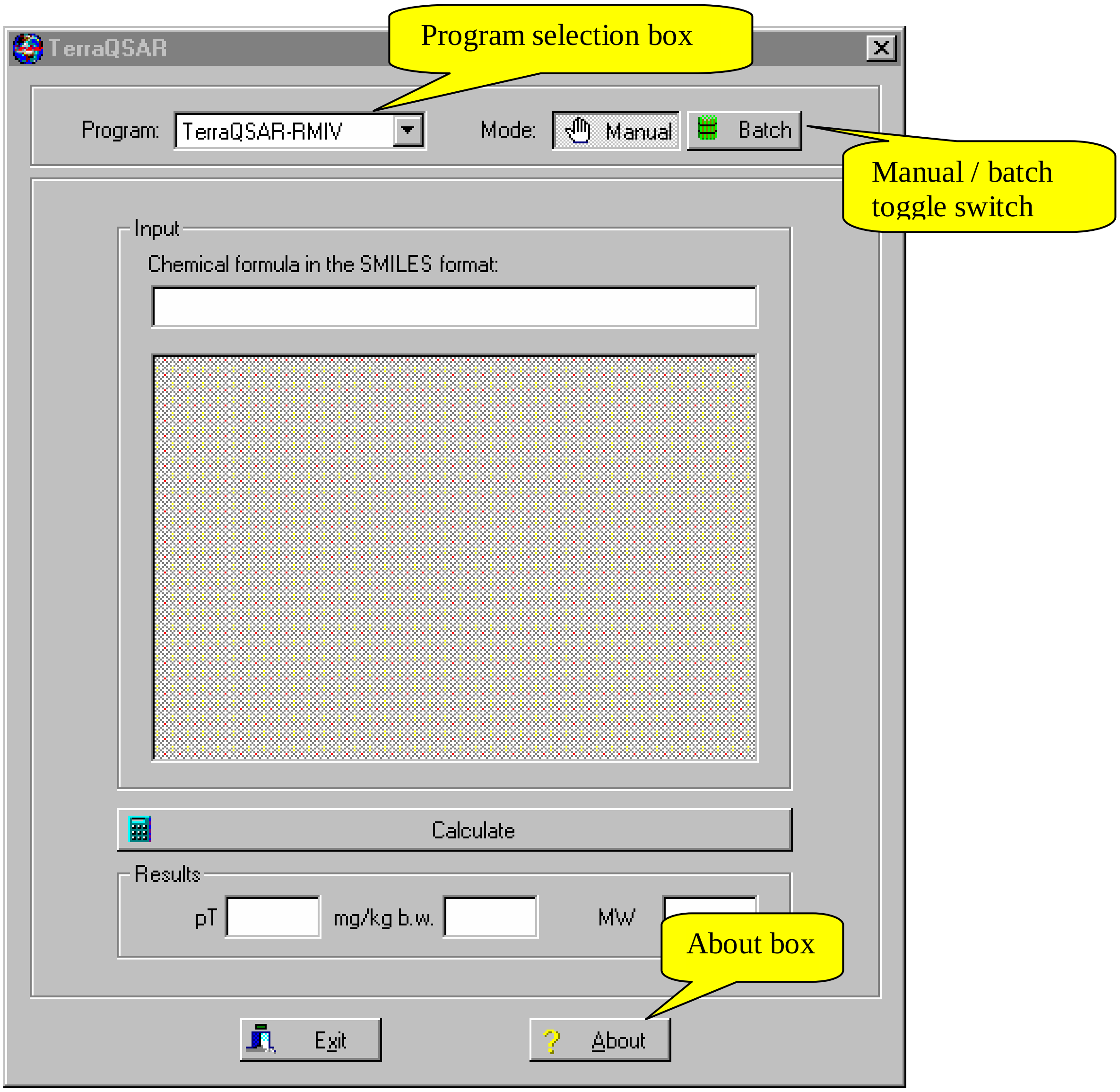
Program Interface

The program interface of the **TerraQSAR** toxicity prediction modules is shown in Figure 2. It is simple, intuitive, and highly functional.



Program: TerraQSAR-RMIV

Mode: Manual

Program selection box

Manual / batch toggle switch

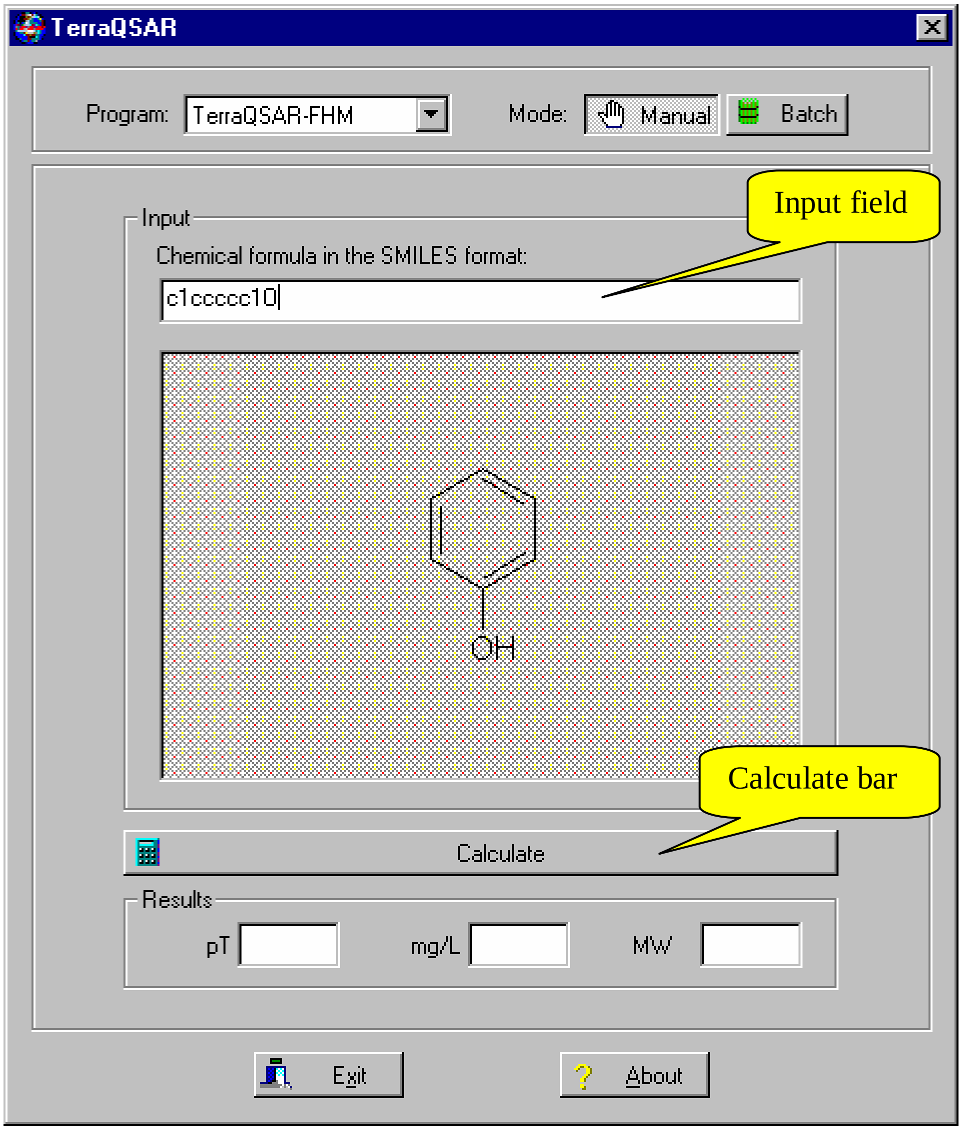
About box

Fig. 2. The Program Interface as it appears on startup.

The Program selection box allows the user to the selected program, e.g. FHM for “Fathead minnow LC50”, “RMIV for “Rat / Mouse *iv*. LD50”, etc. The second box provides a toggle switch between switch between Manual mode and Batch mode (available in the Professional versions only). The About box contains important user information.

In the Manual mode, the user enters a SMILES (Simplified Molecular Input Line Entry System) string, either by typing it into the Input field, or by pasting it from memory, when copied into memory from another source, such as a TerraBase Inc. database output. Please consult the section on SMILES Notation for important advice.

Example 1: Phenol has the SMILES string “c1ccccc1O”. Copying this string into memory, for example from this text (making sure the quotation marks are omitted), and pasting it into the Input field, will result in the appearance of the chemical structure of phenol in the shaded, rectangular field below, as shown in Figure 3.



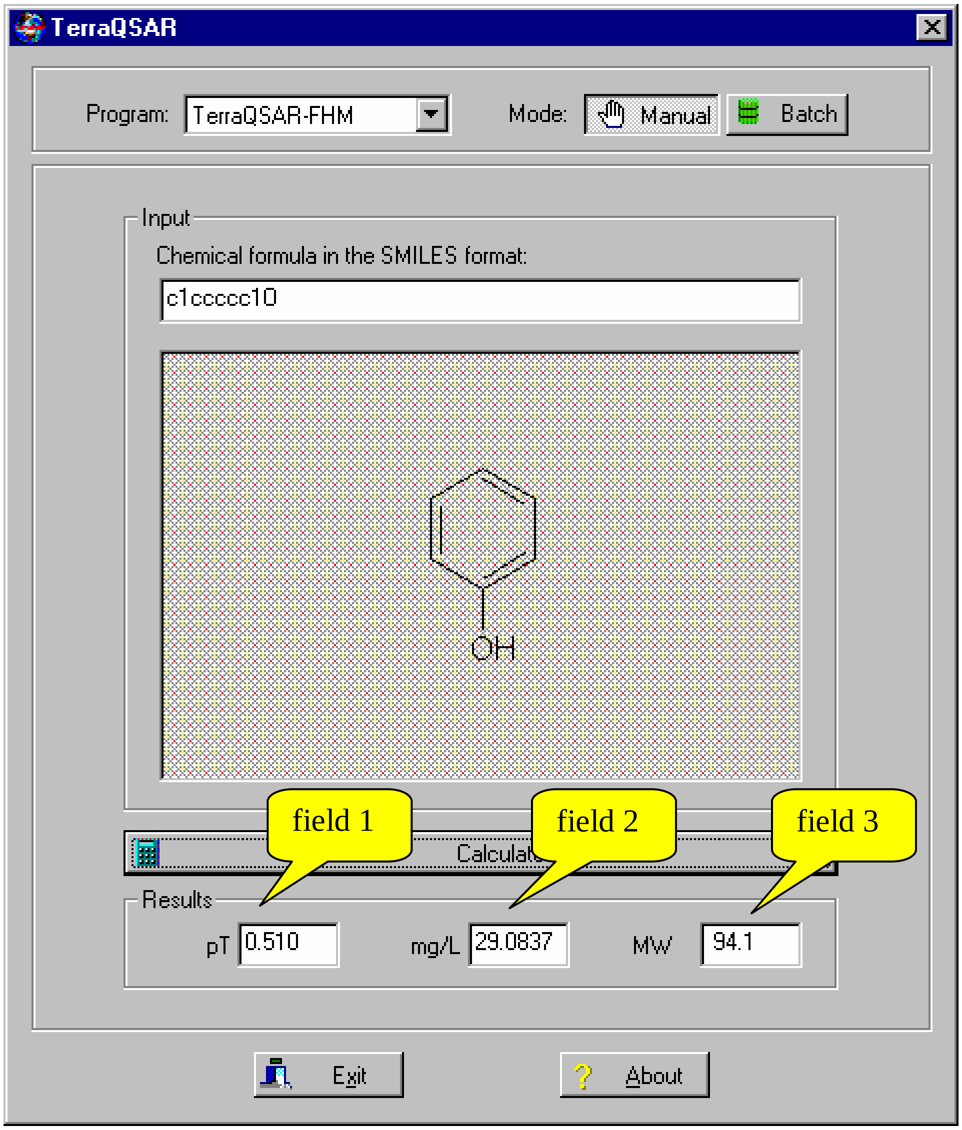
Program: TerraQSAR-FHM

Mode: Manual

Chemical formula in the SMILES format: c1ccccc1O

Fig. 3. Result of entering or pasting the SMILES string for phenol (c1ccccc1O) into the input field: the structure of phenol (excluding hydrogen atoms) will appear in the field below.

Once the user has ascertained that the structure of the compound is that of the desired chemical, a simple click of the Calculate bar below the structure field will result in the three fields below the bar to be filled with the predicted values for the compound, as shown in Figure 4. Field 1 (pT) is the negative logarithm of the LC50 concentration in mmol/L; field 2 (mg/L) is the LC50 value in mg/L; and field 3 (MW) shows the molecular weight of the compound. Computation time varies with the complexity of the structure and the computer specifics, ranging from a nearly instantaneous result (~1 sec) for small structures to minutes for large molecules with highly complex structures.



Program: TerraQSAR-FHM

Mode: Manual

Chemical formula in the SMILES format: c1ccccc1O

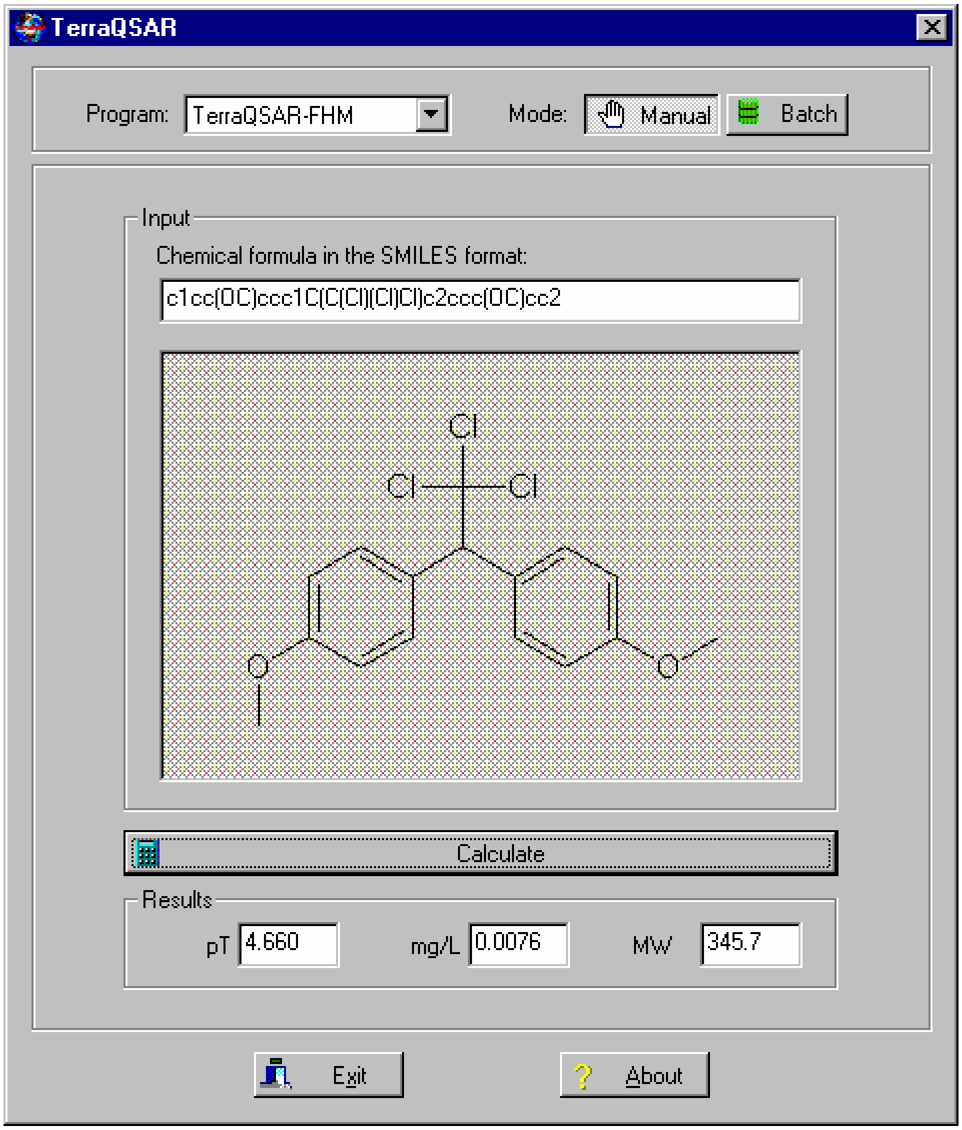
pT 0.510

mg/L 29.0837

MW 94.1

<u>Fig. 4</u>. Result of the execution of “Calculate”. The fields show the following values: Field 1 (pT) is the negative logarithm of the millimolar LC50 concentration, field 2 (mg/L) is the LC50 value in mg/L, and field 3 shows the molecular weight of the compound. Computation time ca. 2 sec at 2 GHz.

Example 2: A more complex molecule, the insecticide p,p'-Methoxychlor, with the SMILES "c1cc(OC)ccc1C(C(Cl)(Cl)Cl)c2ccc(OC)cc2" and structure shown in Figure 5, has a computed LC50 value of 0.0076 mg/L, as shown in Figure 5.



Program: TerraQSAR-FHM

Mode: Manual

Chemical formula in the SMILES format: c1cc(OC)ccc1C(C(Cl)(Cl)Cl)c2ccc(OC)cc2

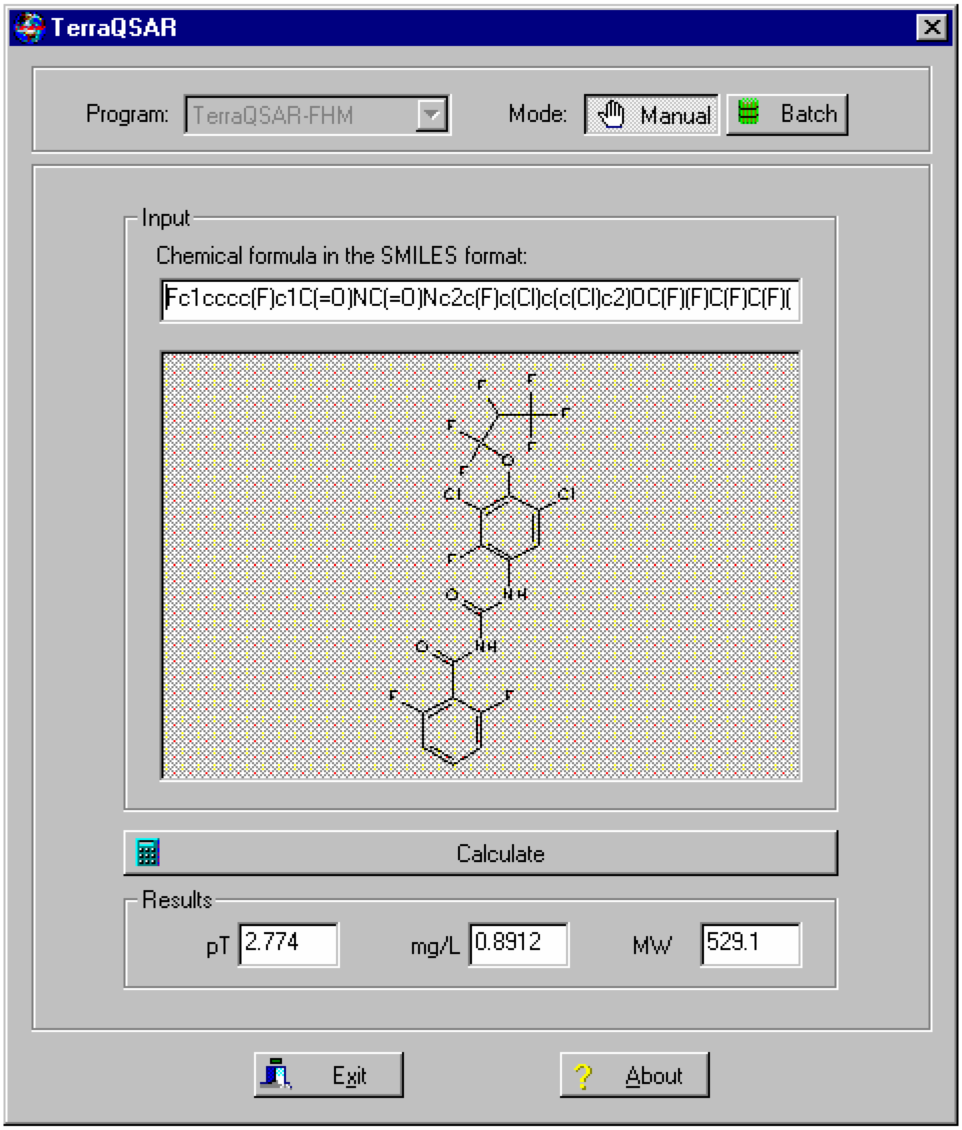
pT 4.660

mg/L 0.0076

MW 345.7

<u>Fig. 5</u>. Result of computation for the insecticide p,p'-Methoxychlor, SMILES string shown in the input field, depicting the compound p,p-Methoxychlor, with its computed 96-hr LC50 value for fathead minnow of 0.0076 mg/L (pT: 4.66).

Example 3: The insecticide Noviflumuron, CAS 121451-02-3, has a predicted fathead minnow 96-hr LC50 of 0.89 mg/L, as shown in Figure 6.



Program: TerraQSAR-FHM

Chemical formula in the SMILES format:

```
Fc1cccc(F)c1C(=O)NC(=O)Nc2c(F)c(Cl)c(c(Cl)c2)OC(F)(F)C(F)C(F)(
```

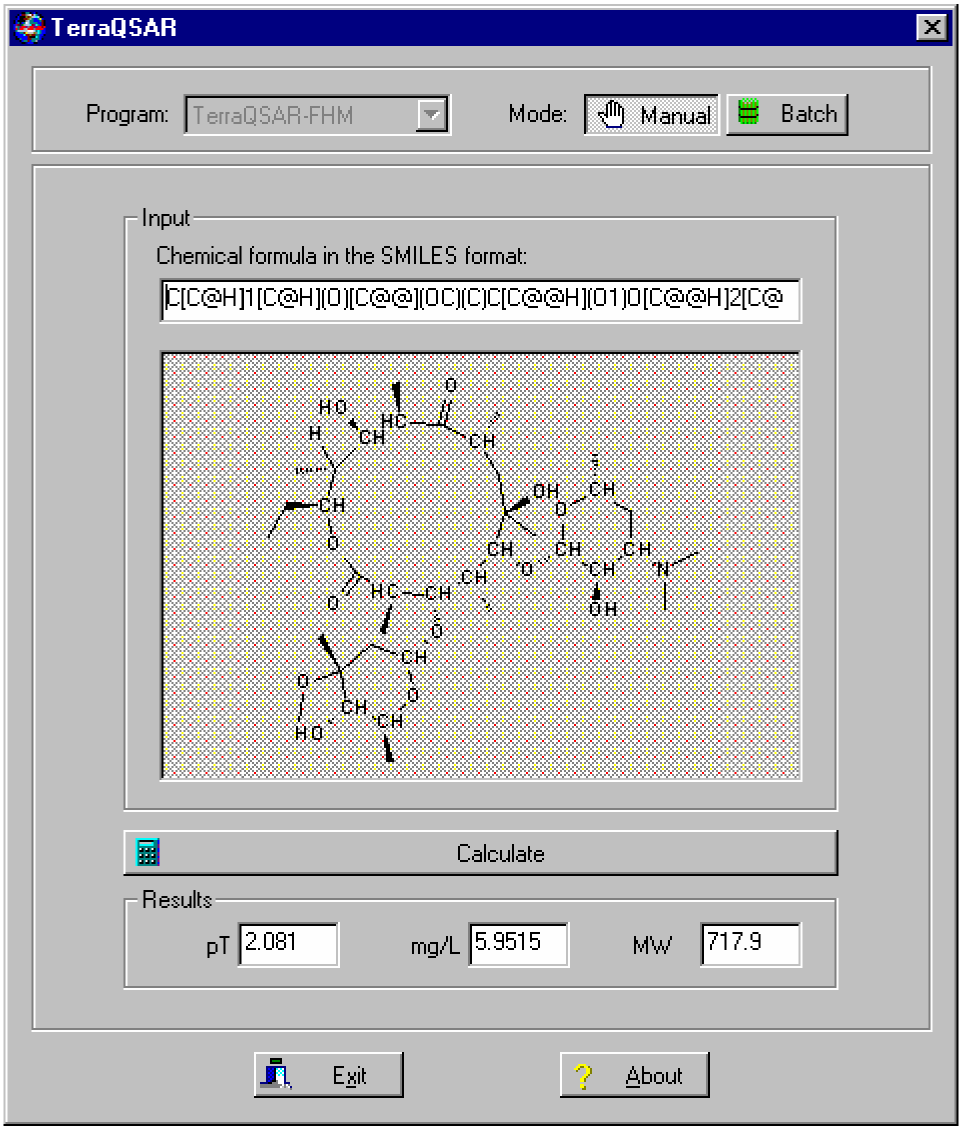
pT 2.774

mg/L 0.8912

MW 529.1

Fig. 6. Result of computation for the SMILES string shown in the input field, depicting the insecticide Noviflumuron, with its computed 96-hr LC50 value for the fathead minnow of 0.89 mg/L.

Example 4: The antibiotic compound Erythromycin B, CAS 527-75-3, with the 3D-SMILES code C[C@H]1[C@H](O)[C@@](OC)(C)C[C@@H](O1)O[C@@H]2[C@@H](C)C(=O)O[C@@H](CC)[C@@](C)([H])[C@@H](O)[C@@H](C)C(=O)[C@H](C)C[C@](O)(C)[C@@H]([C@@H]2C)O[C@@H]3O[C@H](C)C[C@H](N(C)C)[C@H]3O (only a part of the SMILES code is visible in the Input field) has a predicted fathead minnow 96-hr LC50 of 5.95 mg/L, as shown in Figure 7; computation time ca. 50 sec at 2 GHz.



Program: TerraQSAR-FHM

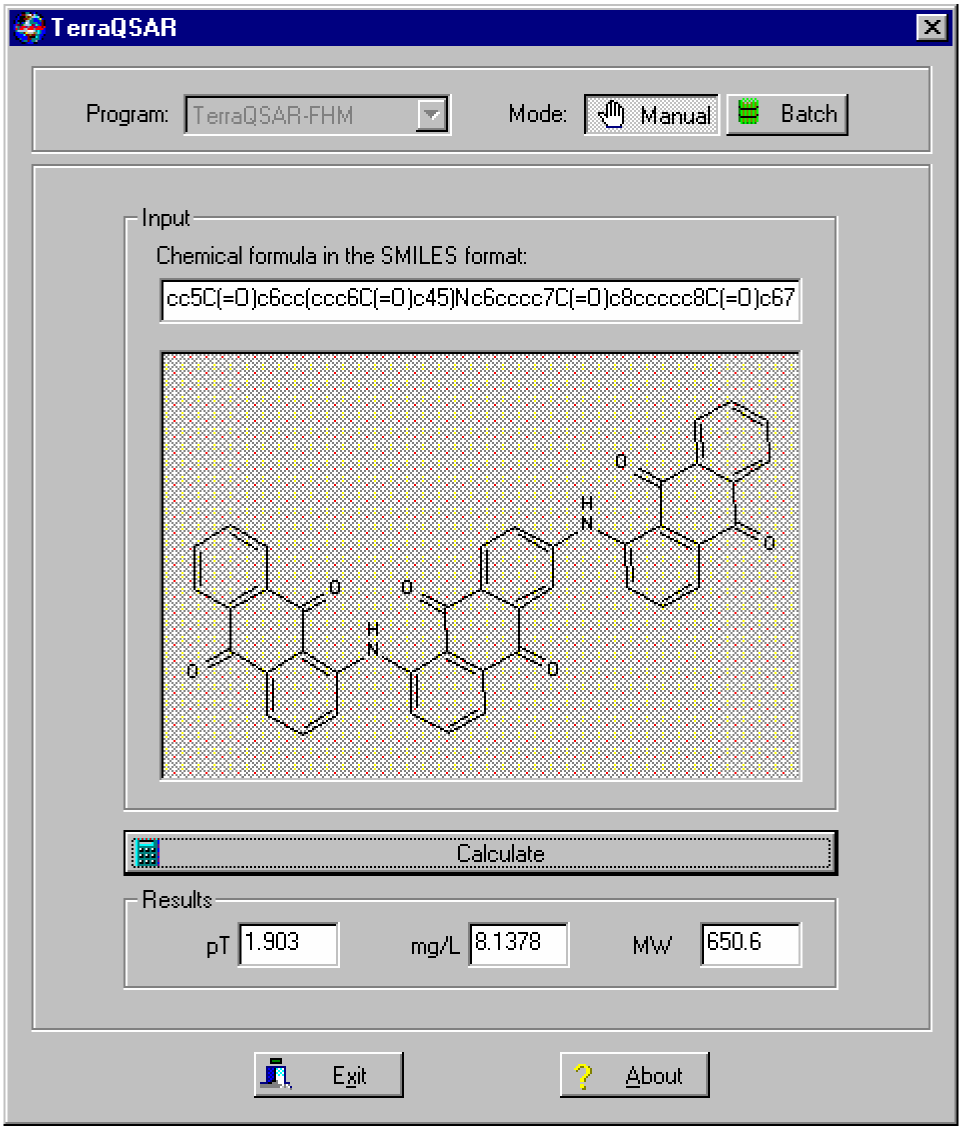
Mode: Manual

Chemical formula in the SMILES format: C[C@H]1[C@H](O)[C@@](OC)(C)C[C@@H](O1)O[C@@H]2[C@

pT 2.081 mg/L 5.9515 MW 717.9

Fig. 7. Result of computation for the SMILES string shown in the input field, depicting the antibiotic Erythromycin B, with the computed 96-hr LC50 value for the fathead minnow of 5.95 mg/L.

Example 5:
An anthraquinone dye, CAS 4478-06-2, with the molecular formula C42H22N2O6, and the SMILES code:
c1cccc2C(=O)c3c(cccc3C(=O)c12)Nc4cccc5C(=O)c6cc(ccc6C(=O)c45)Nc6cccc7C(=O)c8ccccc8C(=O)c67 (only a part of the SMILES code is visible in the Input field) and the structure shown, has the predicted fathead minnow 96-hr LC50 of 8.1 mg/L, as shown in Fig. 8.



Program: TerraQSAR-FHM

Mode: Manual Batch

Input

Chemical formula in the SMILES format:

cc5C(=O)c6cc(ccc6C(=O)c45)Nc6cccc7C(=O)c8ccccc8C(=O)c67

Results

pT 1.903 mg/L 8.1378 MW 650.6

<u>Fig. 8</u>. Result of computation for the SMILES string shown in the input field, depicting an anthraquinone dye, with the computed 96-hr LC50 value for the fathead minnow of 8.1 mg/L.

Technical Requirements

Operating system: PC with Windows 95, 98, NT, 2000, or ME, XP (SP2), or Vista, operating system.
Central processor unit (CPU): No specific requirement, duration of computations will increase with decrease in CPU speed; 2.0 GHz or higher recommended.
Mouse or other pointing device: required.
Screen setting: Variable, 800 x 640, or higher.
CD-ROM drive: required.
Other: Presence of the **TerraQSAR** CD in the CD-ROM drive is required for program execution.

Installation Instructions

The **TerraQSAR** software is a fully functional, stand-alone system, easy to install or uninstall. It consists of two installation parts; we recommend installation in the order mentioned.

Part 1. Installation of the Accelrys software

To install/un-install the Accelrys supporting software, necessary for the proper functioning of the **TerraQSAR** program, use the Windows – Control – Install/Remove Software command to run the “setup.exe” in the Accelrys folder and follow the instructions.

Part 2. Installation of TerraQSAR

To install/un-install **TerraQSAR**, use the Windows – Control – Install/Remove Software command to run the “setup.exe” in the TerraQSAR folder and follow the instructions.

Customer Support

TerraBase Inc. is committed to effective customer support. With the rapid change in PC technology, operating systems and other software and hardware changes, the occasional hiccup is bound to happen. We will try our best to help customers with problems related to our products, in most cases free of charge. Contact our help department with any question and concern about our products, either by EMAIL, FAX, or MAIL.

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