Phototox Version 2.0

"How to get started"

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Risiken erkennen – Gesundheit schützen

1. Create Reader data file

Since 96 well plate photometers (reader) produce data in different formats and arrangments, the software "Phototox" only supports one of the most common reader data formats that can be imported directly from the reader (concentration, OD; concentration, OD; ....).

However, since all modern readers are supplied with PC reader software that displays the OD´s of the 96 well plate, this 12 x 8 matrix can be imported in the program.

The software "Phototox" imports data, which are saved in the plain ASCII Text format and arranged in a layout scheme corresponding the pipetting scheme of a 96 well plate (12 x 8 matrix) e.g. as given in ANNEX B of the 3T3 NRU PT SOP.

The layout scheme in the "Phototox" software can be edited and changed according to the pipetting scheme used (See Section 2.2).

To generate an ASCII (*.txt) file, copy the 12 x 8 formatted reader OD data to the Windows clipboard and paste them in a program (Text Editor), which is able to save data in ASCII Text format (*.txt).

You can use Windows Notepad.exe or any other Editor, even Windows Excel can save pasted data in the *.txt format (see Table 1).

Save your data as a text file (*.txt).

0.048	0.046	0.045	0.046	0.046	0.045	0.047	0.046	0.047	0.048	0.049	0.05
0.05	0.303	0.317	0.3	0.299	0.301	0.286	0.235	0.073	0.051	0.35	0.05
0.046	0.303	0.303	0.311	0.312	0.286	0.304	0.234	0.073	0.051	0.334	0.052
0.044	0.327	0.326	0.328	0.323	0.293	0.282	0.237	0.08	0.051	0.334	0.05
0.045	0.308	0.302	0.301	0.314	0.298	0.282	0.215	0.074	0.057	0.334	0.053
0.045	0.316	0.349	0.316	0.316	0.298	0.292	0.206	0.075	0.054	0.359	0.05
0.044	0.333	0.335	0.339	0.335	0.301	0.291	0.192	0.067	0.05	0.355	0.048
0.047	0.049	0.054	0.05	0.049	0.049	0.056	0.052	0.054	0.055	0.052	0.052

Table 1: Reader data txt File (12 x 8 matix, example Bithionol +UV). Plate layout as shown in the 3T3 NRU PT SOP ANNEX B.

2. Import Reader data file in Phototox 2.0

2.1 Create a project

Before any data can be imported, create a project (this is a series of related experiments, e.g. repeat experiments, the minimum being 1 plate +UV and 1 plate –UV).

In a project, each experiment is saved as one data set, which contains identification data, measured dose effect values and fitted dose effect curves.
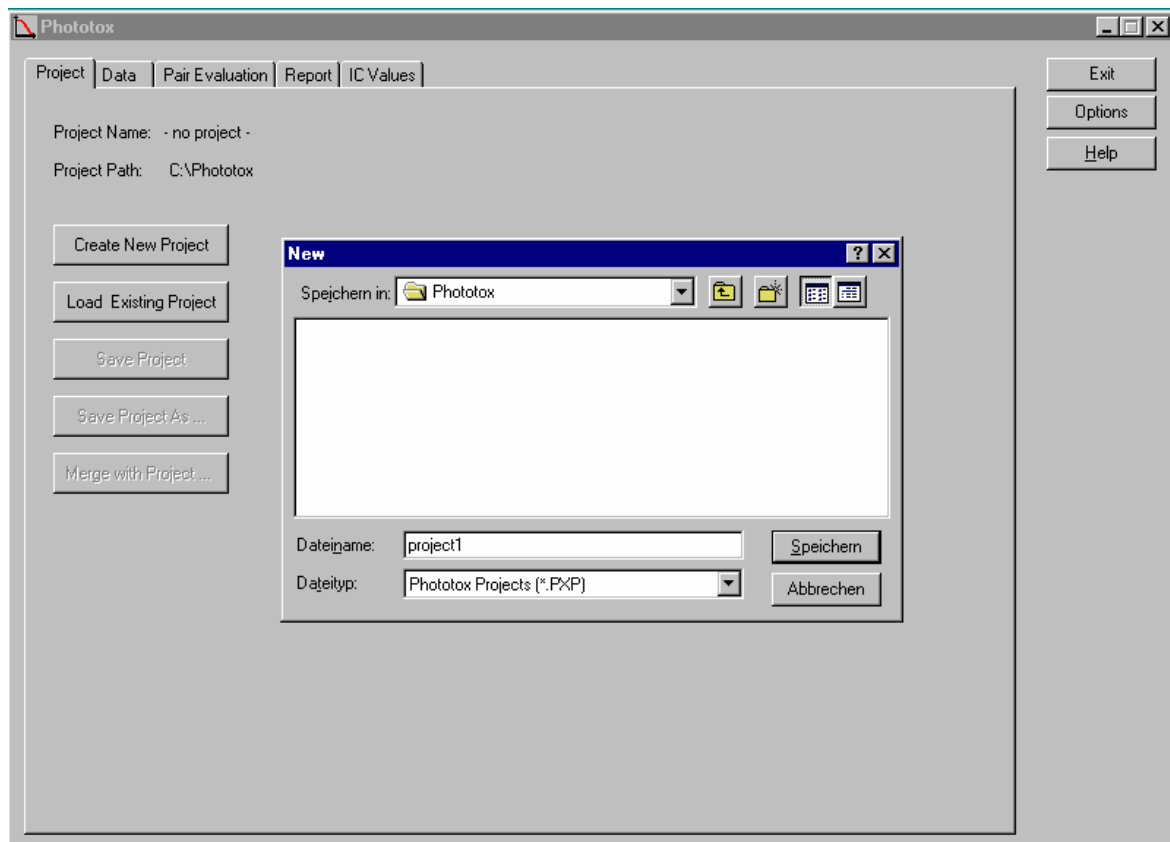

Figure 1: Creating a new project

To create a new project, open the program, select "**Project**" and click the button "**Create New Project**". A dialog box will open, for entering the project name. Save the new project.

2.2 Configuration of the layout of the 96-well plate

The layout (96-well plate configuration) in Figure 2 is a recommendation shown in the 3T3 NRU PT SOP ANNEX B. This layout can be changed and edited according your needs.
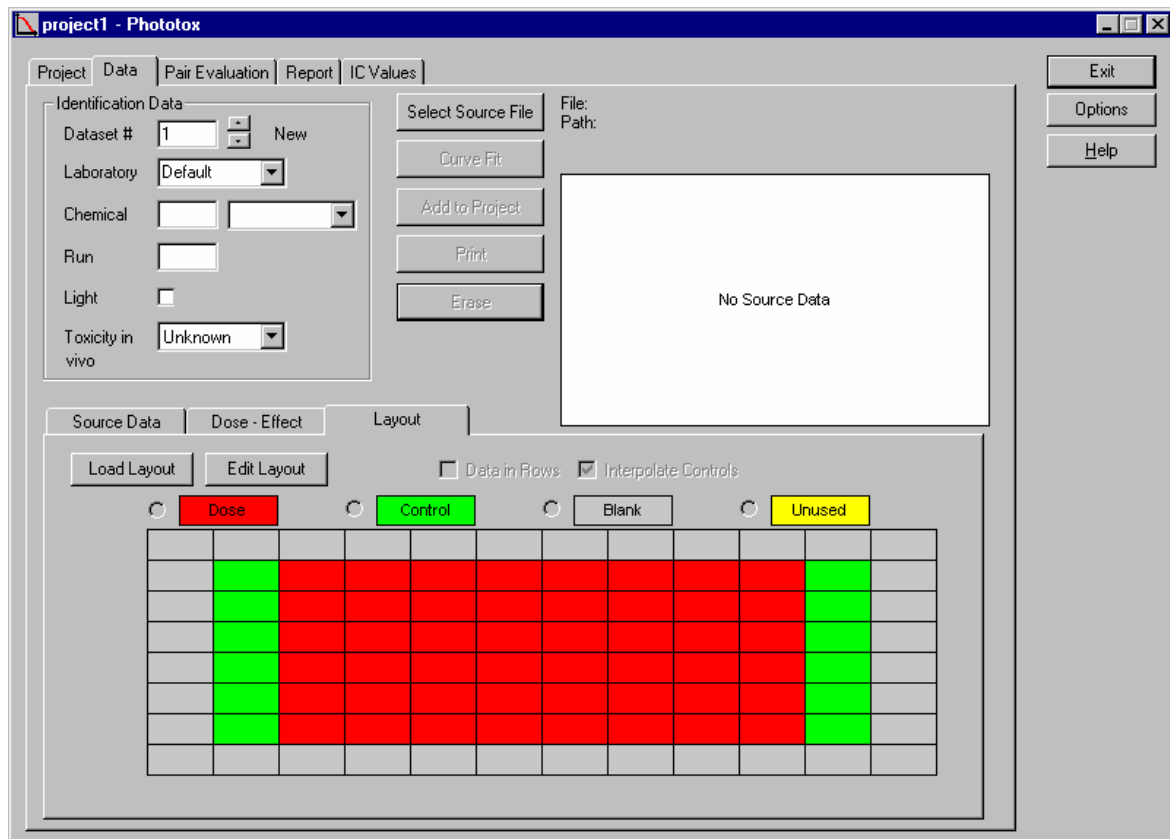
Figure 2: Configuration of the layout of the 96-well plate

☞   **IMPORTANT:** The pipetting scheme of the 96 well plate (12 x 8 matrix) and the layout scheme within the "Phototox" software must match each other.

2.3 Selecting of a source file and manual entries

To import a 96 well plate data (*.txt) file, generated according to Section 1, open "**Data**", click the button "**Select Source File**" and click the selected .txt file. The raw data will be displayed.

Next:

- For "**Chemical**" enter the chemical name and/or the chemical number (use "**Option**" to select which)

- For "**Run**" enter number of repeat experiment for this chemical

- For "**Light**" tick check box. if the actual experiment is +UVA-vis irradiation. (Figure 3 shows the raw data of Bithionol, +UV)

- Finally, since the imported 12 x 8 data file (*.txt) does not contain information about the used concentrations of the test chemical, you have to **enter** manually the test chemical **concentrations** used in the experiment.
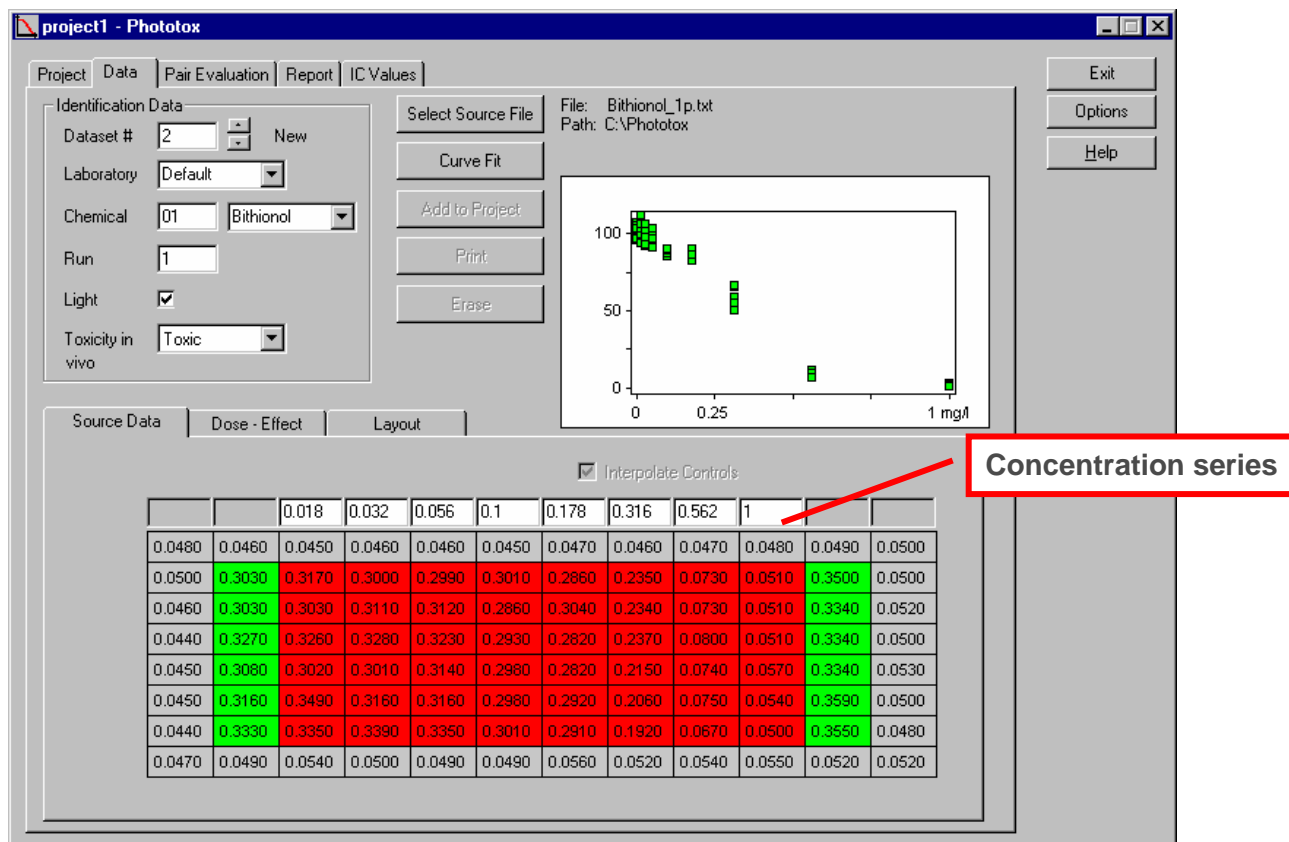
Figure 3: Selecting a Source File and manual entries

☞ **IMPORTANT:** Manual entries (Chemical, Run, Light and concentrations) must be complete to allow adding of the result to a project.

3. Curve Fit

After the concentrations have been entered, click the button "**Curve Fit**" to produce the curves, which will be displayed in the graph window a few seconds later.

After "**Curve Fit**" add the data to the project file, using the button "**Add to Project**".

Repeat the steps with the –UV experiment (example: Bithionol -UV): See Figure 4 + 5.

Figure 4: +UV experiment (Bithionol)

project1 - Phototox

Tabs: Project | Data | Pair Evaluation | Report | IC Values — Exit | Options | Help

Identification Data
- Dataset # 2 — New
- Laboratory: Default
- Chemical: 01 — Bithionol
- Run: 1
- Light: ✓
- Toxicity in vivo: Toxic

Select Source File — Curve Fit — Add to Project — Print — Erase — IC50: 0.35022

File: Bithionol_1p.txt
Path: C:\Phototox

Graph: N= 5 E0=0.2616

Source Data | Dose - Effect | Layout — ☑ Interpolate Controls

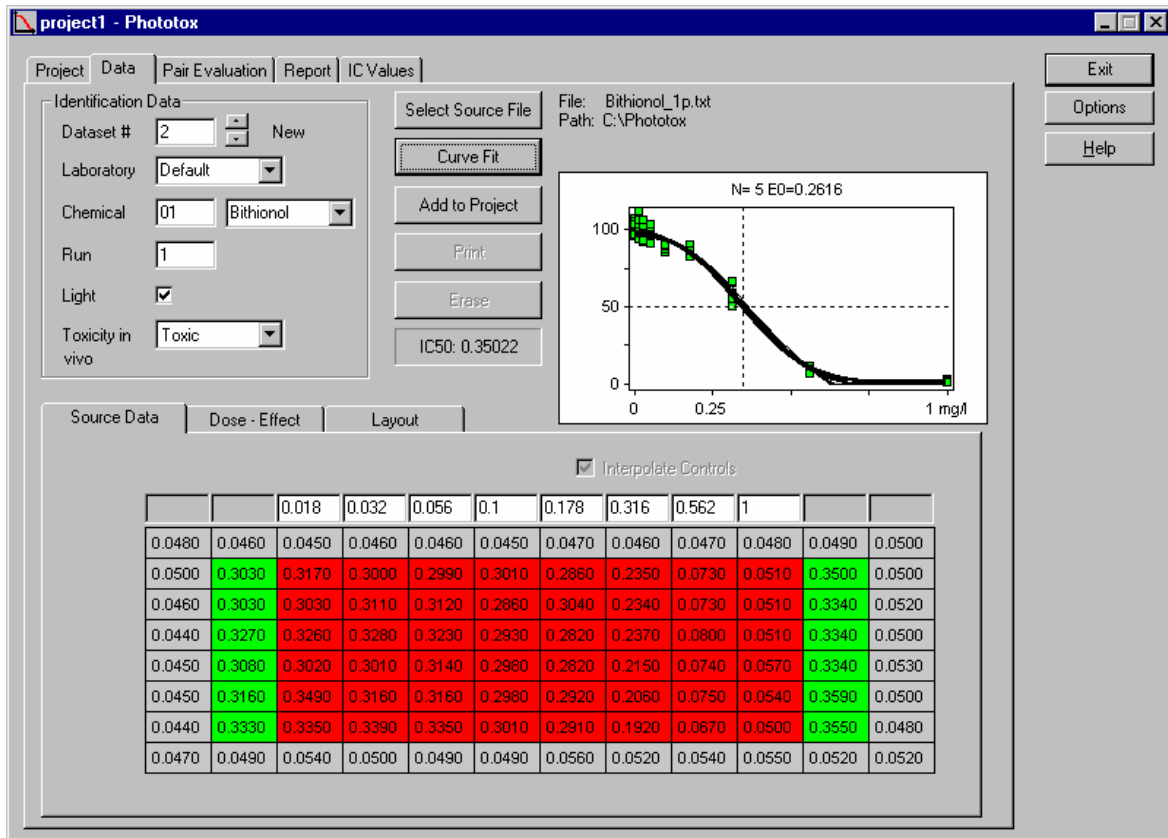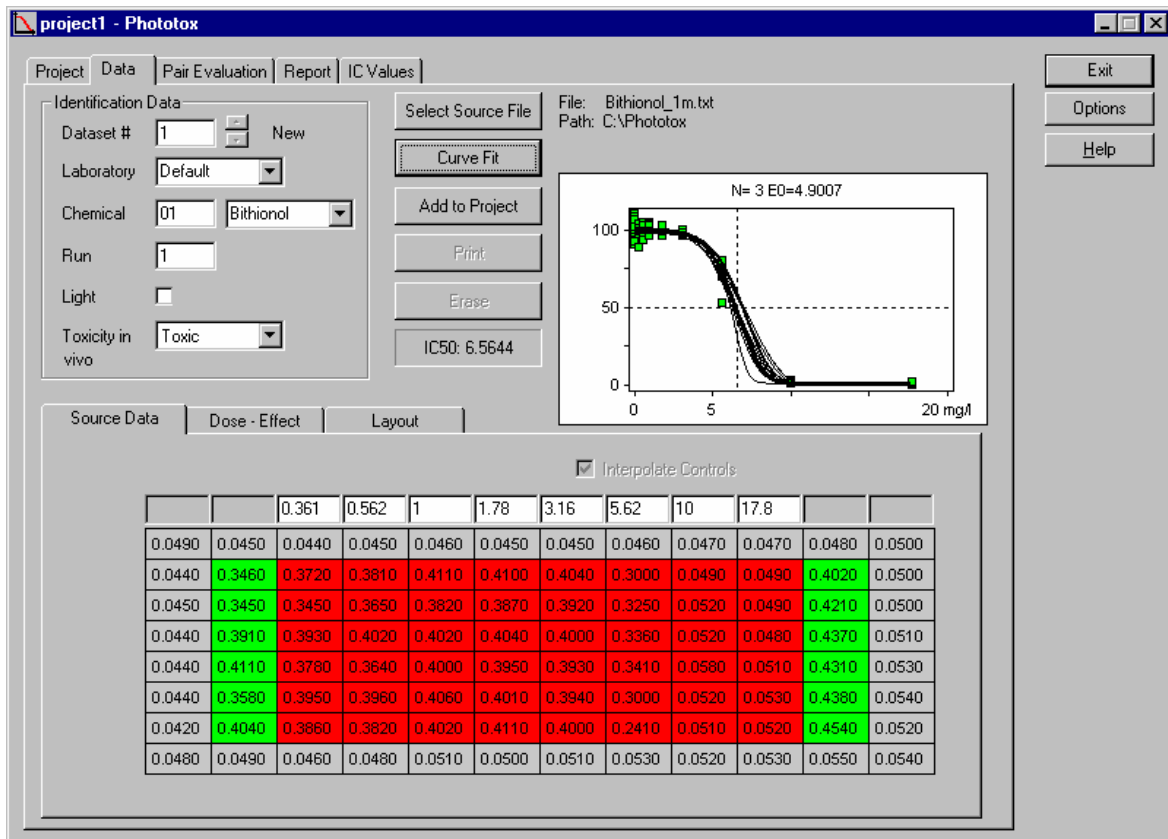|		0.018	0.032	0.056	0.1	0.178	0.316	0.562	1		
0.0480	0.0460	0.0450	0.0460	0.0460	0.0450	0.0470	0.0460	0.0470	0.0480	0.0490	0.0500
0.0500	0.3030	0.3170	0.3000	0.2990	0.3010	0.2860	0.2350	0.0730	0.0510	0.3500	0.0500
0.0460	0.3030	0.3030	0.3110	0.3120	0.2860	0.3040	0.2340	0.0730	0.0510	0.3340	0.0520
0.0440	0.3270	0.3260	0.3280	0.3230	0.2930	0.2820	0.2370	0.0800	0.0510	0.3340	0.0500
0.0450	0.3080	0.3020	0.3010	0.3140	0.2980	0.2820	0.2150	0.0740	0.0570	0.3340	0.0530
0.0450	0.3160	0.3490	0.3160	0.3160	0.2980	0.2920	0.2060	0.0750	0.0540	0.3590	0.0500
0.0440	0.3330	0.3350	0.3390	0.3350	0.3010	0.2910	0.1920	0.0670	0.0500	0.3550	0.0480
0.0470	0.0490	0.0540	0.0500	0.0490	0.0490	0.0560	0.0520	0.0540	0.0550	0.0520	0.0520

Figure 4: +UV experiment (Bithionol)

Figure 5: –UV experiment (Bithionol)

project1 - Phototox

Tabs: Project | Data | Pair Evaluation | Report | IC Values — Exit | Options | Help

Identification Data
- Dataset # 1 — New
- Laboratory: Default
- Chemical: 01 — Bithionol
- Run: 1
- Light: ☐
- Toxicity in vivo: Toxic

Select Source File — Curve Fit — Add to Project — Print — Erase — IC50: 6.5644

File: Bithionol_1m.txt
Path: C:\Phototox

Graph: N= 3 E0=4.9007

Source Data | Dose - Effect | Layout — ☑ Interpolate Controls

		0.361	0.562	1	1.78	3.16	5.62	10	17.8		
0.0490	0.0450	0.0440	0.0450	0.0460	0.0450	0.0450	0.0460	0.0470	0.0470	0.0480	0.0500
0.0440	0.3460	0.3720	0.3810	0.4110	0.4100	0.4040	0.3000	0.0490	0.0490	0.4020	0.0500
0.0450	0.3450	0.3450	0.3650	0.3820	0.3870	0.3920	0.3250	0.0520	0.0490	0.4210	0.0500
0.0440	0.3910	0.3930	0.4020	0.4020	0.4040	0.4000	0.3360	0.0520	0.0480	0.4370	0.0510
0.0440	0.4110	0.3780	0.3640	0.4000	0.3950	0.3930	0.3410	0.0580	0.0510	0.4310	0.0530
0.0440	0.3580	0.3950	0.3960	0.4060	0.4010	0.3940	0.3000	0.0520	0.0530	0.4380	0.0540
0.0420	0.4040	0.3860	0.3820	0.4020	0.4110	0.4000	0.2410	0.0510	0.0520	0.4540	0.0520
0.0480	0.0490	0.0460	0.0480	0.0510	0.0500	0.0510	0.0530	0.0520	0.0530	0.0550	0.0540

Figure 5: –UV experiment (Bithionol)

4. Evaluation

After the data of the +UV and the –UV experiments are fitted and added to the project as two data sets, you can evaluate the data.

To evaluate the data set pair, open "**Pair Evaluation**", and select "**Chemical**", "**Lab**" and "**Pair**" (Run1 or Run2)

Both concentration-response curves (+UV / -UV) will be displayed in the left single graph. The three windows on the right show the <u>dose effect</u>, the <u>response effect</u> and the <u>photo effect</u>.

Above the left graph the MPE and PIF plus the tox. probability values are displayed. PIF and MPE values are averages over all bootstrap pairs (See Figure 6).
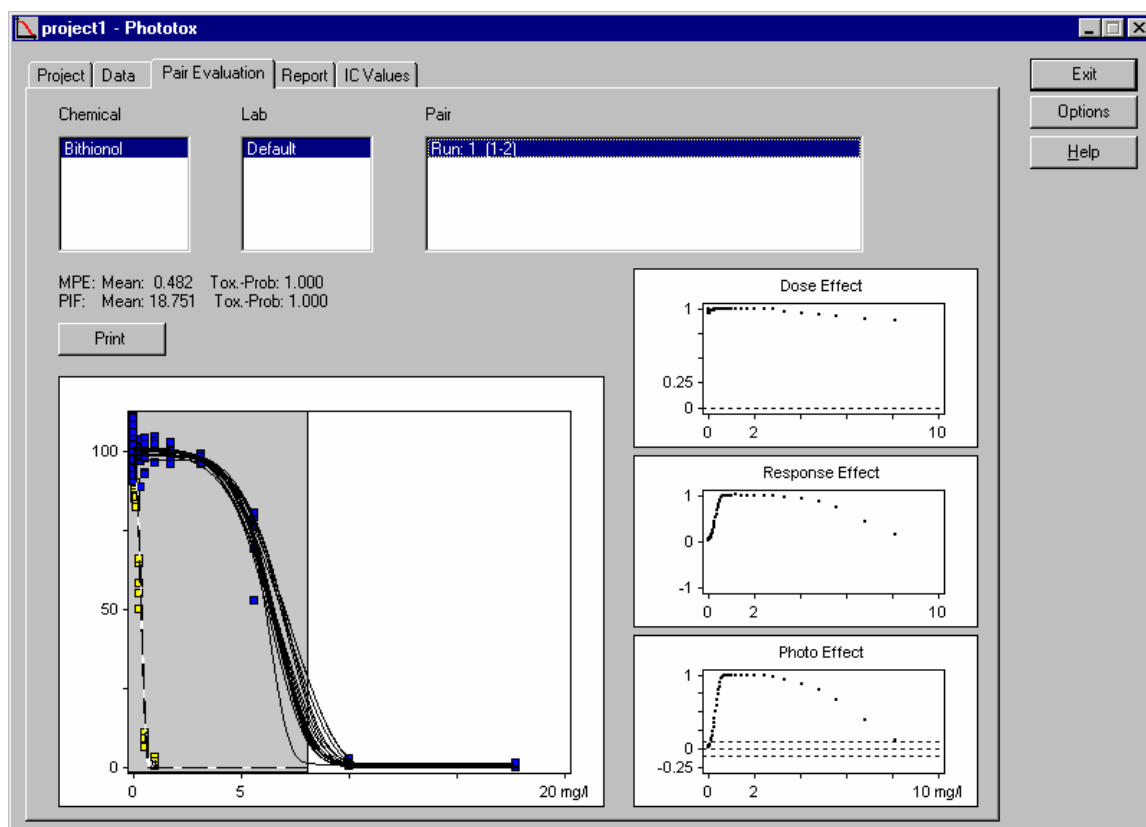

Figure 6: Pair evaluation

5. Report

Related experiments are tabulated as a project report which can be printed.
To print the report open "**Report**" and click the button "**Print**".

Chemical	Lab	Run	In Vivo	MPE Mean	Variance	Tox-Prob	PIF Mean	Variance	Tox-Prob
Bithionol	Default	1	toxic	0.482	-	1.000	18.751	-	1.000

Project | Data | Pair Evaluation | Report | IC Values

project1 - Phototox

Exit

Options

Help

Print