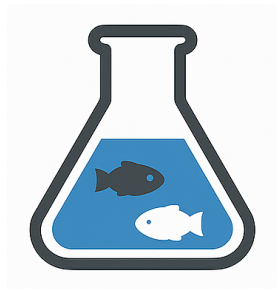


LC₅₀/EC₅₀ Calculator

User Manual

Version 1.0.0



Dr. Branimir K. Hackenberger

Department of Biology
Josip Juraj Strossmayer University of Osijek

BioQuant-SCIOM d.o.o.

October 2, 2025



Contents

1	Introduction	1
1.1	About the Program	1
1.1.1	Key Features	1
1.2	System Requirements	1
2	Installation	3
2.1	Windows Installation	3
2.1.1	Download	3
2.1.2	Installation Procedure	3
2.2	Ubuntu/Linux Installation	3
2.2.1	AppImage Method (Recommended)	3
2.2.2	DEB Package for Debian/Ubuntu	4
2.2.3	Snap Package	4
2.3	macOS Installation	4
2.3.1	DMG Installation	4
2.3.2	Homebrew Installation	5
3	Theoretical Foundations	7
3.1	LC ₅₀ and EC ₅₀ Concept	7
3.2	Mathematical Foundations	7
3.2.1	Probit Analysis	7
3.2.2	Logit Analysis	8
3.2.3	Weibull Analysis	8
3.2.4	Spearman-Kärber Method	8
3.3	Additional Parameters	8
3.3.1	EC ₁₀ and EC ₉₀	8
3.3.2	NOEC and LOEC	9
3.3.3	MATC	9
3.3.4	Benchmark Doses (BMD)	9
3.3.5	PNEC Values	9
4	Using the Program	11
4.1	Launching the Program	11
4.1.1	Windows	11
4.1.2	Linux	11
4.1.3	macOS	11
4.2	Program Interface	12
4.2.1	Interface Areas	12
4.3	Data Entry	12
4.3.1	Basic Data	12



4.3.2	Experimental Data	13
4.3.3	Calculation Results	13
5	Usage Examples	15
5.1	Example 1: Acute Toxicity to <i>Daphnia magna</i>	15
5.1.1	Scenario	15
5.1.2	Experimental Design	15
5.1.3	Input Procedure	15
5.1.4	Expected Results	16
5.1.5	Interpretation	16
5.2	Example 2: Algal Growth Inhibition	17
5.2.1	Scenario	17
5.2.2	Procedure	17
5.2.3	Results	17
5.3	Example 3: Spearman-Kärber for Terrestrial Organisms	17
5.3.1	Scenario	17
5.3.2	Spearman-Kärber Method Specifics	17
5.3.3	Data	18
5.3.4	Results	18
6	Advanced Features	19
6.1	Automatic Settings Memory	19
6.1.1	Structure of settings.json	19
6.2	CSV Log File	19
6.2.1	Data Import/Export	20
6.3	PDF Reports	20
7	Troubleshooting	21
7.1	Common Errors	21
7.2	Recommended Practices	21
7.2.1	Data Preparation	21
7.2.2	Method Selection	22
7.2.3	Results Validation	22
7.3	FAQ	22
8	Technical Specifications	23
8.1	Program Architecture	23
8.2	API Documentation	23
8.2.1	Rust Functions	23
8.2.2	JavaScript Functions	24
8.3	Data Structures	24
9	References and Standards	27
9.1	International Standards	27
9.2	Literature	27
9.3	Web Resources	28
A	List of Organisms	29
A.1	Aquatic Organisms	29
A.2	Terrestrial Organisms	29



B	Formulas and Constants	31
B.1	Transformation Functions	31
B.2	Statistical Constants	31
B.3	Assessment Factors	31
C	Contact and Support	33
C.1	Technical Support	33
C.2	Bug Reporting	33
C.3	Licensing	33
	About the Author	35



1. Introduction

1.1 About the Program

The LC₅₀/EC₅₀ Calculator is an advanced professional software designed for precise calculation of lethal concentration (LC₅₀) and effective concentration (EC₅₀) of chemical substances on test organisms. The program is developed in the Rust programming language with the Tauri framework, ensuring high performance and security.

1.1.1 Key Features

- **Four statistical methods:** Probit, Logit, Weibull, and Spearman-Kärber
- **Automatic calculation of additional parameters:** EC₁₀, EC₉₀, LOEC, NOEC, MATC
- **Benchmark doses (BMD):** BMC₁₀ and BMC₂₀ with 95% confidence intervals
- **Risk assessment:** PNEC calculations for different ecosystems
- **Data visualization:** Interactive graphs with regression curves
- **PDF reports:** Automatic generation of professional reports
- **CSV log:** Permanent storage of all analyses
- **Settings memory:** Automatic saving of user preferences

1.2 System Requirements

Table 1.1: Minimum system requirements

Component	Requirement
Operating System	Windows 10/11, macOS 10.15+, Ubuntu 20.04+
RAM	Minimum 4 GB (recommended 8 GB)
Disk Space	200 MB for installation
Screen Resolution	Minimum 1280×720 (recommended 1920×1080)
.NET Framework	Version 4.7.2 or newer (Windows only)



2. Installation

2.1 Windows Installation

2.1.1 Download

1. Go to <https://sciom.hr/lc50-calculator/download>
2. Download `LC50Calculator-Setup.exe`
3. Verify SHA-256 checksum for security

2.1.2 Installation Procedure

1. Run `LC50Calculator-Setup.exe` as administrator
2. Accept license terms
3. Select installation directory (recommended: `C:\Program Files\LC50Calculator`)
4. Select components:
 - ✓ Main program
 - ✓ Desktop shortcut
 - ✓ Start menu shortcuts
 - ✓ Context menu for CSV files
5. Click "Install"
6. Wait for installation to complete
7. Click "Finish" to launch the program

Windows Defender Warning

During first installation, Windows Defender may display a warning. Click "More info" then "Run anyway" as the software is safe and digitally signed.

2.2 Ubuntu/Linux Installation

2.2.1 AppImage Method (Recommended)



```
1      # Download AppImage
2      wget https://sciom.hr/downloads/LC50Calculator
        -1.0.0.AppImage
3
4      # Make it executable
5      chmod +x LC50Calculator-1.0.0.AppImage
6
7      # Run
8      ./LC50Calculator-1.0.0.AppImage
```

Listing 2.1: AppImage installation

2.2.2 DEB Package for Debian/Ubuntu

```
1      # Download DEB package
2      wget https://sciom.hr/downloads/lc50calculator_1
        .0.0_amd64.deb
3
4      # Install dependencies
5      sudo apt update
6      sudo apt install libgtk-3-0 libwebkit2gtk-4.0-37
7
8      # Install package
9      sudo dpkg -i lc50calculator_1.0.0_amd64.deb
10
11     # Fix any dependency issues
12     sudo apt --fix-broken install
```

Listing 2.2: DEB installation

2.2.3 Snap Package

```
1      sudo snap install lc50calculator
```

Listing 2.3: Snap installation

2.3 macOS Installation

2.3.1 DMG Installation

1. Download LC50Calculator-1.0.0.dmg
2. Double-click the DMG file
3. Drag the application icon to the Applications folder
4. Eject DMG
5. Launch from Applications



2.3.2 Homebrew Installation

```
1      # Add SCIOM tap
2      brew tap sciom/tools
3
4      # Install
5      brew install --cask lc50calculator
```

Listing 2.4: Homebrew installation

macOS Gatekeeper

On first launch, macOS may block the application. Go to System Preferences → Security & Privacy and click "Open Anyway".



3. Theoretical Foundations

3.1 LC₅₀ and EC₅₀ Concept

LC₅₀ (Lethal Concentration 50) represents the concentration of a test substance that causes death in 50% of exposed organisms during a specified time period.

EC₅₀ (Effective Concentration 50) denotes the concentration that causes a specific effect (e.g., growth inhibition, immobilization) in 50% of test organisms.

3.2 Mathematical Foundations

3.2.1 Probit Analysis

Probit transformation is based on the assumption of normal distribution of tolerance:

$$\text{Probit}(p) = \Phi^{-1}(p) + 5 \quad (3.1)$$

where Φ^{-1} is the inverse function of the standard normal distribution, and p is the mortality proportion.

Abbott's Correction

For boundary values, Abbott's correction is applied:

$$p_{\text{corr}} = \frac{0.5}{n} \quad \text{for } p = 0 \quad (3.2)$$

$$p_{\text{corr}} = \frac{n - 0.5}{n} \quad \text{for } p = 1 \quad (3.3)$$

Linear Regression

After transformation, linear regression is applied:

$$Y = a + b \cdot \ln(X) \quad (3.4)$$

where:

- Y - probit value
- X - concentration
- a - intercept
- b - slope

LC₅₀ is calculated as:

$$\ln(\text{LC}_{50}) = \frac{5 - a}{b} \quad (3.5)$$



3.2.2 Logit Analysis

Logit transformation uses the logistic function:

$$\text{Logit}(p) = \ln \left(\frac{p}{1-p} \right) \quad (3.6)$$

For LC_{50} :

$$\ln(\text{LC}_{50}) = \frac{0-a}{b} \quad (3.7)$$

3.2.3 Weibull Analysis

Weibull transformation:

$$W(p) = \ln(-\ln(1-p)) \quad (3.8)$$

LC_{50} is calculated as:

$$\ln(\text{LC}_{50}) = -\frac{a}{b} \quad (3.9)$$

3.2.4 Spearman-Kärber Method

A non-parametric method that requires no distribution assumptions:

$$\log \text{LC}_{50} = \log x_0 + h \left(\sum_{i=1}^n p_i - 0.5 \right) \quad (3.10)$$

where:

- x_0 - lowest dose
- h - logarithmic interval between doses
- p_i - mortality proportions

Standard error:

$$SE = h \sqrt{\sum_{i=1}^n \frac{p_i(1-p_i)}{n_i}} \quad (3.11)$$

95% confidence interval:

$$\text{CI}_{95\%} = \text{LC}_{50} \times e^{\pm 1.96 \cdot SE} \quad (3.12)$$

3.3 Additional Parameters

3.3.1 EC_{10} and EC_{90}

For the Probit method:

$$\text{EC}_{10} = \exp \left(\frac{\Phi^{-1}(0.1) + 5 - a}{b} \right) \quad (3.13)$$

$$\text{EC}_{90} = \exp \left(\frac{\Phi^{-1}(0.9) + 5 - a}{b} \right) \quad (3.14)$$



3.3.2 NOEC and LOEC

NOEC (No Observed Effect Concentration) - the highest concentration with no observed effect.

LOEC (Lowest Observed Effect Concentration) - the lowest concentration with an observed effect.

The program automatically determines NOEC and LOEC based on statistical analysis (threshold: 10% increase in mortality).

3.3.3 MATC

Maximum Acceptable Toxicant Concentration:

$$\text{MATC} = \sqrt{\text{NOEC} \times \text{LOEC}} \quad (3.15)$$

3.3.4 Benchmark Doses (BMD)

BMC_{10} and BMC_{20} are calculated using the bootstrap method (1000 iterations):

$$\text{BMC}_x = \exp\left(\frac{\text{target}_x - a}{b}\right) \quad (3.16)$$

where target_x is the transformed value for $x\%$ response.

3.3.5 PNEC Values

Predicted No Effect Concentration according to REACH guidelines:

Table 3.1: PNEC assessment factors

Ecosystem	AF	Formula
Aquatic	1000	POD/1000
Terrestrial	1000	POD/1000
Sediment	10	POD/10

where POD (Point of Departure) is determined hierarchically:

1. NOEC (if available)
2. BMC_{10} lower bound
3. $\text{LOEC}/3$



4. Using the Program

4.1 Launching the Program

4.1.1 Windows

- Double-click the desktop icon
- Or: Start menu → LC50 Calculator
- Or: Right-click on CSV file → Open with → LC50 Calculator

4.1.2 Linux

```
1      # From terminal  
2      lc50calculator  
3  
4      # Or from application menu  
5      # Applications -> Science -> LC50 Calculator
```

4.1.3 macOS

- Launchpad → LC50 Calculator
- Or: Applications → LC50 Calculator
- Or: Spotlight search (Cmd+Space) → "LC50"



4.2 Program Interface

LC₅₀/EC₅₀ Calculator
Kalkulator za izračun letalne ili efektne koncentracije (LC₅₀/EC₅₀) pomoću statističkih metoda

Podaci o eksperimentu

Testirana tvar: Jedinica koncentracije: Metoda izračuna:

Datum početka testiranja: Trajanje testiranja (sati): Organizam:

Jedinica učinka:

Koncentracije

Unesite svoje podatke u donja polja. Svaki podatak mora biti u posebnom redu. Sva tri polja moraju sadržavati jednak broj podataka.

Koncentracije	X0	Xt
0.01	20	0
0.03	20	1
0.1	20	4
0.3	20	12
1.0	20	19
3.0	20	20

Figure 4.1: Main program interface

4.2.1 Interface Areas

1. **Experiment data** - basic test information
2. **Data input** - concentrations and results
3. **Control buttons** - calculate, save, PDF
4. **Results** - displayed after calculation
5. **Graph** - visualization of regression analysis

4.3 Data Entry

4.3.1 Basic Data

Table 4.1: Basic data fields

Field	Description
Test Substance	Name or chemical formula (e.g., "CdCl ₂ ")
Concentration Unit	mg/L, µg/L, g/L or custom
Calculation Method	Probit, Logit, Weibull or Spearman-Kärber
Start Date	Date when experiment started
Test Duration	24h, 48h, 72h, 96h or custom
Organism	Select from list or enter custom
Effect Unit	/1, /mm ² , /FU or custom



4.3.2 Experimental Data

Data is entered in three columns, each data point on a new row:

Input Format

Concentrations	X_0	X_t
0.1	20	0
0.5	20	2
1.0	20	5
5.0	20	15
10.0	20	20

4.3.3 Calculation Results



Figure 4.2: Calculation results



5. Usage Examples

5.1 Example 1: Acute Toxicity to *Daphnia magna*

5.1.1 Scenario

Testing the toxicity of copper sulfate (CuSO_4) on water fleas according to OECD 202 protocol.

5.1.2 Experimental Design

- **Test organism:** *Daphnia magna* (< 24h old)
- **Duration:** 48 hours
- **Temperature:** $20 \pm 1^\circ\text{C}$
- **pH:** 7.8 ± 0.2
- **Dissolved oxygen:** $> 6 \text{ mg/L}$
- **Photoperiod:** 16h light : 8h dark

5.1.3 Input Procedure

1. **Basic data:**

- Test substance: CuSO4
- Unit: mg/L
- Method: Probit
- Date: 2025-01-15
- Duration: 48 h
- Organism: *Daphnia magna*
- Effect unit: /1

2. **Experimental data:**

Table 5.1: Data for *Daphnia magna* test

Concentration (mg/L)	X_0	X_t
0.01	20	0
0.03	20	1
0.1	20	4
0.3	20	12
1.0	20	19
3.0	20	20



3. Click **Calculate LC₅₀/EC₅₀**

5.1.4 Expected Results

Table 5.2: Analysis results for CuSO₄

Parameter	Value
LC ₅₀	0.217 ± 0.034 mg/L
EC ₁₀	0.089 mg/L
EC ₉₀	0.531 mg/L
NOEC	0.03 mg/L
LOEC	0.1 mg/L
R ²	0.982
Slope	2.847

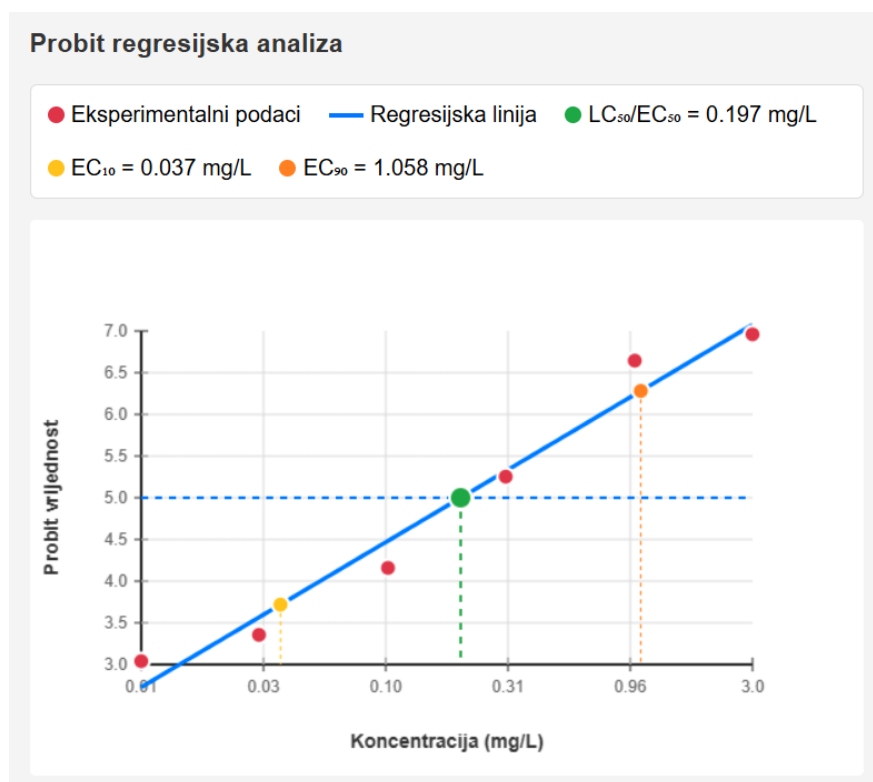


Figure 5.1: Probit analysis for CuSO₄ on *Daphnia magna*

5.1.5 Interpretation

- LC₅₀ = 0.217 mg/L classifies CuSO₄ as **very toxic** to aquatic organisms
- R² = 0.982 shows excellent model fit
- EC₉₀/EC₁₀ ratio = 5.96 indicates a moderately steep dose-response curve
- PNEC_{aquatic} = 0.000217 mg/L (applying factor of 1000)



5.2 Example 2: Algal Growth Inhibition

5.2.1 Scenario

Growth inhibition test on *Pseudokirchneriella subcapitata* with the herbicide atrazine according to OECD 201.

5.2.2 Procedure

1. Basic data:

- Test substance: **Atrazine**
- Unit: $\mu\text{g/L}$
- Method: **Weibull** (better for growth curves)
- Duration: 72 h
- Organism: *Pseudokirchneriella subcapitata*
- Effect unit: /FU (fluorescence units)

2. Data:

Table 5.3: Data for algal growth inhibition test

Concentration ($\mu\text{g/L}$)	Control FU	Inhibited FU
0	1000	0
10	1000	50
30	1000	180
100	1000	420
300	1000	750
1000	1000	950

5.2.3 Results

- $\text{EC}_{50} = 112.4 \mu\text{g/L}$
- $\text{EC}_{10} = 28.7 \mu\text{g/L}$
- $\text{BMC}_{10} = 31.2 \mu\text{g/L}$ (95% CI: 24.8-38.9)
- $\text{PNEC}_{\text{aquatic}} = 0.112 \mu\text{g/L}$

5.3 Example 3: Spearman-Kärber for Terrestrial Organisms

5.3.1 Scenario

Contact toxicity test of pesticide on earthworms *Eisenia fetida*.

5.3.2 Spearman-Kärber Method Specifics

- Requires geometric progression of concentrations
- Does not require normal distribution
- Ideal for data with 0% and 100% mortality



5.3.3 Data

Table 5.4: Geometric progression for Spearman-Kärber

Concentration (mg/kg)	N	Mortality
1	10	0
3	10	1
9	10	3
27	10	6
81	10	9
243	10	10

Note: Factor between concentrations = 3 (geometric progression)

5.3.4 Results

- $LC_{50} = 34.7$ mg/kg
- 95% CI: 22.1 - 54.5 mg/kg
- Method does not provide R^2 value (non-parametric)

6. Advanced Features

6.1 Automatic Settings Memory

The program automatically saves last used settings in:

Table 6.1: Settings locations

OS	Path
Windows	C:\Users\[USERNAME]\AppData\Roaming\ LC50Calculator\settings.json
macOS	~/Library/Application Support/LC50Calculator/settings.json
Linux	~/.config/LC50Calculator/settings.json

6.1.1 Structure of settings.json

```
1      {
2          "test_substance": "CuSO4",
3          "unit": "mg/L",
4          "method": "Probit",
5          "organism": "Daphnia magna",
6          "test_duration": "48 h",
7          "concentrations": "0.01|n0.03|n0.1|n0.3|
8                          n1.0",
9          "total_organisms": "20|n20|n20|n20|n20",
10         "dead_organisms": "0|n1|n4|n12|n19"
11     }
```

Listing 6.1: Example settings.json file

6.2 CSV Log File

Each save adds a record to log.csv:

```
1      Timestamp,Test Substance,Organism,Test Date,Test
2      Duration,
3      Method,Concentration Unit,Effect Unit,LC50/EC50,
4      EC10,EC90,
5      LOEC,NOEC,MATC,Slope,Intercept,R-squared,BMC10,
6      BMCL10,
7      BMCU10,BMC20,BMCL20,BMCU20,POD,PNEC Aquatic,
8      PNEC Terrestrial,PNEC Sediment
```

Listing 6.2: Structure of log.csv file



6.2.1 Data Import/Export

Importing CSV Data

The program can load existing CSV files with data. The format must be:

- First column: concentrations
- Second column: total number of organisms
- Third column: number of dead/affected organisms
- Separator: comma (,) or semicolon (;)

6.3 PDF Reports

Automatically generated PDFs contain:

1. Title page

- Substance and organism name
- Test date and duration
- Institutional data

2. Analysis results

- All calculated values
- Statistical parameters
- Confidence intervals

3. Graphical display

- Regression curve
- Experimental data
- LC/EC values

4. Data table

- Concentrations
- Number of organisms
- Mortality percentage

5. Interpretation

- Model quality assessment
- Regulatory suitability
- Recommendations

7. Troubleshooting

7.1 Common Errors

Table 7.1: Common errors and solutions

Error	Solution
"All arrays must have the same length"	Check that all columns have the same number of rows
"All concentrations must be positive"	Remove zero or negative values
"Insufficient data for regression"	Enter at least 2 rows of data
"Spearman-Kärber requires geometric progression"	Use concentrations in constant ratio (e.g., 1, 2, 4, 8...)
" $R^2 < 0.7$ "	Model fits poorly, try another method
"PDF generation failed"	Check write permissions and disk space

7.2 Recommended Practices

7.2.1 Data Preparation

1. **Number of concentrations:** Minimum 5 + control
2. **Range:** Cover 0-100% mortality
3. **Spacing:** Geometric progression (factor 2-3)
4. **Replicates:** At least 3 per concentration
5. **Control:** Mortality $< 10\%$



7.2.2 Method Selection

Table 7.2: Method selection guide

Method	When to Use
Probit	First choice, normal distribution, standard in ecotoxicology
Logit	Alternative to Probit, often gives similar results
Weibull	Asymmetric data, growth curves, hormesis effects
Spearman-Kärber	Non-parametric alternative, geometric progression

7.2.3 Results Validation

Acceptability Criteria

- $R^2 > 0.9$ for regulatory purposes
- $R^2 > 0.8$ for research purposes
- Control mortality $< 10\%$
- CV between replicates $< 30\%$
- LC_{50} within range of tested concentrations

7.3 FAQ

Q: Can I use the program for plants?

A: Yes, use EC_{50} for growth or germination inhibition.

Q: What if I don't have 100% mortality?

A: The program automatically applies Abbott's correction.

Q: How do I export results for publication?

A: Use the PDF report or copy from the CSV log file.

Q: Does the program support IC_{50} ?

A: Yes, IC_{50} is equivalent to EC_{50} for inhibition.

8. Technical Specifications

8.1 Program Architecture

The program is built with modern technologies:

Table 8.1: Technology stack

Component	Technology
Backend	Rust 1.75+
Frontend	HTML5, CSS3, JavaScript (ES6+)
Framework	Tauri 1.6
PDF generation	pdf-writer 0.10
Statistics	statrs 0.16
UI rendering	WebView2 (Windows), WebKit (macOS/Linux)

8.2 API Documentation

8.2.1 Rust Functions

```
1      #[tauri::command]
2      fn calculate_lc50(data: ExperimentData)
3      -> Result<ProbitResult, String>
4
5      #[tauri::command]
6      fn calculate_logit(data: ExperimentData)
7      -> Result<LogitResult, String>
8
9      #[tauri::command]
10     fn calculate_weibull(data: ExperimentData)
11     -> Result<WeibullResult, String>
12
13     #[tauri::command]
14     fn calculate_spearman_karber(data: ExperimentData
15     )
16     -> Result<SpearmanKarberResult, String>
17
18     #[tauri::command]
19     fn generate_pdf(pdf_data: PdfData)
20     -> Result<String, String>
21
22     #[tauri::command]
23     fn save_log_csv(csv_content: String)
24     -> Result<String, String>
```



Listing 8.1: Main Rust functions

8.2.2 JavaScript Functions

```
1      async function calculateLC50() {
2          const data = collectData();
3          const result = await invoke('
4              calculate_lc50', { data });
5              displayResults(result);
6      }
7
8      function collectData() {
9          return {
10              concentrations: [...],
11              total_organisms: [...],
12              dead_organisms: [...]
13          };
14      }
15
16      function drawChart(results) {
17          // Canvas rendering logic
18      }
19
20      async function generatePDF() {
21          const chartPng = getChartAsBase64();
22          await invoke('generate_pdf', { ...data,
23              chartPng });
24      }
```

Listing 8.2: Frontend functions

8.3 Data Structures

```
1      #[derive(Debug, Serialize, Deserialize)]
2      pub struct ExperimentData {
3          pub concentrations: Vec<f64>,
4          pub total_organisms: Vec<usize>,
5          pub dead_organisms: Vec<usize>,
6      }
7
8      #[derive(Debug, Serialize)]
9      pub struct ProbitResult {
10          pub lc50: f64,
11          pub slope: f64,
12          pub intercept: f64,
13          pub r_squared: f64,
14          pub probit_data: Vec<ProbitPoint>,
15          pub regression_line: Vec<(f64, f64)>,
16          pub additional_values: AdditionalValues,
17      }
18
19      #[derive(Debug, Serialize)]
```



```
20      pub struct AdditionalValues {  
21          pub ec10: f64,  
22          pub ec90: f64,  
23          pub loec: Option<f64>,  
24          pub noec: Option<f64>,  
25          pub benchmark_doses: BenchmarkDose,  
26          pub risk_assessment: RiskAssessment,  
27          pub matc: Option<f64>,  
28      }
```

Listing 8.3: Basic structures



9. References and Standards

9.1 International Standards

- **OECD Guidelines:**
 - OECD 201: Alga Growth Inhibition Test
 - OECD 202: Daphnia sp. Acute Immobilisation Test
 - OECD 203: Fish, Acute Toxicity Test
 - OECD 207: Earthworm, Acute Toxicity Tests
 - OECD 211: Daphnia magna Reproduction Test
- **ISO Standards:**
 - ISO 14442:2006 - Water quality guidelines
 - ISO 11348 - Vibrio fischeri bioluminescence
 - ISO 10706 - Chronic toxicity to Daphnia magna
- **EU Regulations:**
 - REACH Regulation (EC) No 1907/2006
 - CLP Regulation (EC) No 1272/2008
 - Water Framework Directive 2000/60/EC
- **US EPA:**
 - ECOTOX Database Standard Operating Procedures
 - Series 850 - Ecological Effects Test Guidelines

9.2 Literature

1. Finney, D.J. (1971). *Probit Analysis* (3rd ed.). Cambridge University Press.
2. Hamilton, M.A., Russo, R.C., Thurston, R.V. (1977). Trimmed Spearman-Kärber method for estimating median lethal concentrations. *Environmental Science & Technology*, 11(7), 714-719.
3. OECD (2006). Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. Series on Testing and Assessment No. 54.
4. Newman, M.C. (2013). *Quantitative Ecotoxicology* (2nd ed.). CRC Press.



5. Wheeler, J.R., Grist, E.P.M., Leung, K.M.Y., Morritt, D., Crane, M. (2002). Species sensitivity distributions: data and model choice. *Marine Pollution Bulletin*, 45, 192-202.
6. Ritz, C., Baty, F., Streibig, J.C., Gerhard, D. (2015). Dose-Response Analysis Using R. *PLOS ONE*, 10(12), e0146021.
7. EFSA (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal*, 11(7), 3290.

9.3 Web Resources

- SCION website: <https://scion.hr>
- Program download: <https://lcec50calculator.scion.hr>
- GitHub repository: <https://github.com/Branimir-K-Hackenberger/lcec50-calculator>
- OECD Test Guidelines: <https://www.oecd.org/chemicalsafety/testing/>
- US EPA ECOTOX: <https://cfpub.epa.gov/ecotox/>
- REACH helpdesk: <https://echa.europa.eu/support>

A. List of Organisms

A.1 Aquatic Organisms

Table A.1: Standard aquatic test organisms

Species	Group	Application
<i>Daphnia magna</i>	Crustacea	Acute and chronic toxicity, standard
<i>Ceriodaphnia dubia</i>	Crustacea	Chronic toxicity, more sensitive
<i>Danio rerio</i>	Pisces	Acute toxicity, embryotoxicity
<i>Oryzias latipes</i>	Pisces	Endocrine disruptors
<i>Pimephales promelas</i>	Pisces	US EPA standard
<i>Pseudokirchneriella subcapitata</i>	Algae	Growth inhibition, primary producers
<i>Desmodesmus subspicatus</i>	Algae	EU alternative
<i>Chlorella vulgaris</i>	Algae	Freshwater alga
<i>Lemna minor</i>	Macrophyta	Aquatic plants
<i>Myriophyllum spicatum</i>	Macrophyta	Submerged macrophyte
<i>Vibrio fischeri</i>	Bacteria	Bioluminescence, rapid test
<i>Tetrahymena pyriformis</i>	Protozoa	Single-celled eukaryotes
<i>Chironomus riparius</i>	Insecta	Sediment toxicity
<i>Gammarus pulex</i>	Crustacea	Running waters
<i>Hyalella azteca</i>	Crustacea	Sediment, North America

A.2 Terrestrial Organisms

Table A.2: Standard terrestrial test organisms

Species	Group	Application
<i>Eisenia fetida</i>	Oligochaeta	Acute and chronic, OECD standard
<i>Eisenia andrei</i>	Oligochaeta	Alternative to <i>E. fetida</i>
<i>Enchytraeus crypticus</i>	Enchytraeidae	Reproduction, more sensitive
<i>Folsomia candida</i>	Collembola	Soil arthropods
<i>Lactuca sativa</i>	Dicotyledonae	Germination, root growth
<i>Avena sativa</i>	Monocotyledonae	Monocotyledons
<i>Brassica napus</i>	Dicotyledonae	Oilseed rape
<i>Apis mellifera</i>	Hymenoptera	Bees, pollinators
<i>Bombus terrestris</i>	Hymenoptera	Bumblebees



Species	Group	Application
<i>Aleochara bilineata</i>	Coleoptera	Beneficial insects
<i>Pardosa sp.</i>	Araneae	Spiders, predators
<i>Porcellio scaber</i>	Isopoda	Decomposers
<i>Lumbricus terrestris</i>	Oligochaeta	Deep-burrowing earthworms

B. Formulas and Constants

B.1 Transformation Functions

Table B.1: Transformation functions for different methods

Method	Transformation
Probit	$Y = \Phi^{-1}(p) + 5$
Logit	$Y = \ln\left(\frac{p}{1-p}\right)$
Weibull	$Y = \ln(-\ln(1-p))$
Gompertz	$Y = -\ln(-\ln(p))$
Angular	$Y = \arcsin(\sqrt{p})$

B.2 Statistical Constants

Table B.2: Critical values for different significance levels

Parameter	90% CI	95% CI	99% CI
z-value	1.645	1.960	2.576
t (df=10)	1.812	2.228	3.169
t (df=20)	1.725	2.086	2.845
t (df=30)	1.697	2.042	2.750

B.3 Assessment Factors

Table B.3: REACH assessment factors (AF)

Available Data	AF
Acute L(E)C ₅₀ for 3 trophic levels	1000
Lowest chronic NOEC for 1 species	100
Chronic NOEC for 2 trophic levels	50
Chronic NOEC for 3 trophic levels	10
SSD (5% HC ₅)	1-5
Field data	1-5



C. Contact and Support

C.1 Technical Support

Contact	Details
Email	support@sciom.hr
Phone	+385 31 123 456
Web	https://sciom.hr/support
Working hours	Mon-Fri, 8:00-16:00 CET

C.2 Bug Reporting

To report issues, please include:

1. Program version (Help → About)
2. Operating system
3. Problem description
4. Steps to reproduce
5. Screenshot of error
6. CSV file with data

Email: bugs@sciom.hr

C.3 Licensing

License Terms

- LICENSE GRANT - By this license SCIOM d.o.o. grants you the following rights: - Installation and use of the SOFTWARE - Making one backup copy of the SOFTWARE. - Use of the SOFTWARE and results produced by its use for commercial and non-commercial purposes without any obligations to the owner or author of the SOFTWARE
- RESTRICTIONS - Distribution of copies of the SOFTWARE to third parties with or without compensation is not permitted. - Modification, adaptation, translation or creation of new derivative works based on the SOFTWARE is not permitted. - Decompilation, disassembly or reverse engineering of the SOFTWARE is not permitted.



- COPYRIGHT - All rights, including but not limited to copyright, in and to the SOFTWARE and all copies thereof are owned by SCIOM d.o.o.
- WARRANTIES AND DISCLAIMERS - The SOFTWARE is provided "AS IS" without warranties of any kind. SCIOM d.o.o. disclaims all warranties, whether express or implied.
- LIMITATION OF LIABILITY - SCIOM d.o.o. shall in no event be liable for any damages (including, without limitation, damages for loss of business profits, business interruption, loss of business information, or any other pecuniary loss) arising from the use or inability to use this SOFTWARE.
- ACADEMIC LICENSE - Special licensing options are available for academic institutions. Please contact info@sciom.hr for more information.
- TERMINATION - This Agreement is effective until terminated or the SOFTWARE is uninstalled. Your rights under this Agreement will terminate automatically without notice from SCIOM d.o.o. if you fail to comply with any term of this Agreement.
- GOVERNING LAW - This Agreement shall be governed by the laws of the Republic of Croatia and international agreements.
- Copyright © 2025 SCIOM d.o.o. All rights reserved.

For different licensing terms, contact: info@sciom.hr

About the Author

Dr. Branimir K. Hackenberger is a tenured full professor at the Department of Biology, Josip Juraj Strossmayer University of Osijek. He is an expert in ecotoxicology with over 30 years of experience in developing methods for assessing the toxicity of chemical substances and their impact on the environment.



SCIOM d.o.o.
Fruškogorska 5, 31000 Osijek, Croatia
<https://sciom.hr>