KATE2020

Ecotoxicity Prediction System—Internet Version
Operating Manual (March 25, 2024, version)

KATE2020 is a tool for the prediction of chemical ecotoxicity based on the following data:

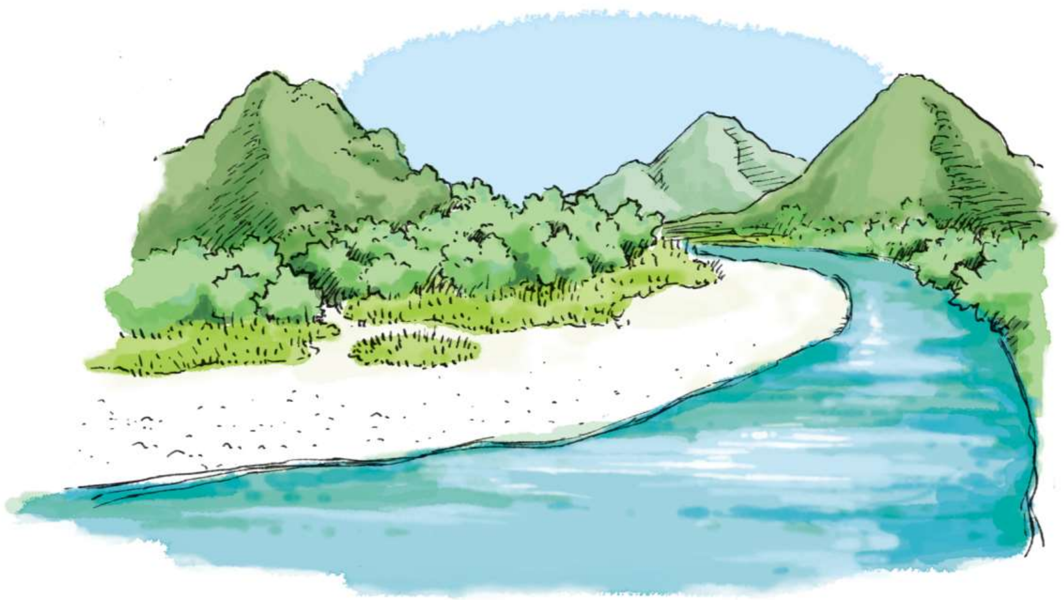
- 50% lethal concentration (LC50) in the fish acute toxicity test
- 50% effective concentration (EC50) in the *Daphnia magna* immobilization test
- 50% effective concentration (EC50) in the algal growth inhibition test
- No-observed-effect concentration (NOEC) in the fish early-life-stage toxicity test
- No-observed-effect concentration (NOEC) in the *Daphnia magna* reproduction test
- No-observed-effect concentration (NOEC) in the algal growth inhibition test

Values predicted by KATE2020 are for reference use only and cannot be used to satisfy the requirements for ecotoxicity test results necessary for notification under Japanese Chemical Substances Control Law (Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc.).

For questions, please contact the administrators at
Health and Environmental Risk Division, National Institute for Environmental Studies KATE Contact Desk
kate@nies.go.jp

Operating Manual Revision History

Version	Date of issue	Revision history
0.1	March 29, 2018	Manual for KATE2017 beta version
0.8	January 30, 2019	Provisional version
1.0	May 23, 2019	Official version
1.0.1	June 4, 2019	Minor update
1.0.2	July 30, 2019	Explanation of JSME Editor was updated
2.0	March 30, 2022	Manual for KATE2020 version 3.0
	March 30, 2022	KATE2020 version 3.0 released
3.0	March 30, 2023	Manual for KATE2020 version 4.0
	March 30, 2023	KATE2020 version 4.0 released
4.0	March 25, 2024	Manual for KATE2020 version 5.0
	March 25, 2024	KATE2020 version 5.0 released



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List of Abbreviations

EC50: 50% effective concentration

The concentration of a chemical dissolved in test water expected to produce a certain effect in 50% of test organisms.

KATE: KAshinhou Tool for Ecotoxicity

The name of the ecotoxicity QSAR system developed by the Center for Health and Environmental Risk Research of the National Institute for Environmental Studies, Japan. It is pronounced as "Kate".

KOWWIN™

A program for estimating log P values of organic compounds. The program is part of the EPI (Estimation Programs Interface) Suite™, which is a suite of estimation programs developed by the US EPA for rapid toxicity screening of chemicals.

LC50: 50% lethal concentration

The concentration of a chemical dissolved in test water that causes death in 50% of test organisms.

Log P: Logarithm of the octanol/water partition coefficient

The logarithm of the ratio of the concentration of a chemical between the solvents 1-octanol and water at equilibrium. It is considered an index of the hydrophobicity of a chemical substance. Log P values ignore ionized query chemical. (http://www.eic.or.jp/ecoterm/?act=view&serial=295; description retrieved from EIC Net on March 1, 2022)

NOEC: No observed effect concentration

The concentration of a chemical causing no statistically or biologically significant increase in the frequency or intensity of any effect in the tested group compared with the control group. Concentration division just under LOEC (Least Observed Effect Concentration). (http://www.env.go.jp/chemi/report/ierac18/1-ref2.pdf; description retrieved from the glossary of the Ministry of the Environment of Japan on March 1, 2022)

(Q)SAR: (Quantitative) structure–activity relationship

The relationship between the structural characteristics or the physicochemical constant of a chemical and its biological activities (e.g., toxicity) is called the Structure–Activity Relationship (SAR), and the quantitative relationship is called the Quantitative Structure–Activity Relationship (QSAR). When both are referred to, (Q)SAR is used. For example, SAR refers to an estimation of the toxicity level of a chemical based on the presence of a specific functional group. A model to quantitatively calculate the toxicity or other properties of a chemical based on the structure is called a QSAR model. (http://www.env.go.jp/chemi/report/ierac18/1-ref2.pdf; description retrieved from the glossary of the Ministry of the Environment of Japan on March 1, 2022)

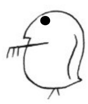
SMARTS: SMiles ARbitrary Target Specification

An extension of SMILES that is used to describe molecular substructures (see http://www.daylight.com/dayhtml_tutorials/languages/smarts/ for a tutorial on using the notation; accessed on March 1, 2022)

SMILES: Simplified Molecular-Input Line-Entry System

Line notation that uses ASCII characters to describe the molecular structure of a chemical (see http://www.daylight.com/smiles/index.html for a tutorial on using the notation; accessed on March 1, 2022)

US EPA: United States Environmental Protection Agency



1. Introduction

(1) What are KATE and KATE2020?

KATE (KAshinhou Tool for Ecotoxicity) is a quantitative structure–activity relationship (QSAR)–based tool for the prediction of chemical ecotoxicity. The tool was developed by the Center for Environmental Risk Research of the National Institute for Environmental Studies (currently Health and Environmental Risk Devision, National Institute for Environmental Studies) under contract with the Japanese Ministry of the Environment[1].

The beta version of the tool was released as KATE2017 on NET in 2018, which was then updated in early 2019. Version 1.0 of KATE2017 on NET was officially released in mid-2019. In early 2020, version 1.0 of KATE2020 was released, followed in early 2021 by version 2.0. KATE2020 (version 2.0) operates over the internet and is available at https://kate.nies.go.jp/onnet2020-e.html.

Latest version, KATE 2020 version 4.0, uses ecotoxicity test data published by the Japanese MOE[3] as well as fish acute toxicity test data from the US EPA fathead minnow database[5] as chemicals dataset. QSAR equations are updated when additional test results become available.

A query chemical is entered into KATE2020 by using simplified molecular-input line-entry system (SMILES) notation, which can be obtained from within KATE2020 by entering a CAS number or by drawing a chemical structure in the provided JavaScript Molecule Editor (JSME Editor). The SMILES string is then used to perform QSAR prediction based on log P value. Currently, KATE2020 predictions are based on the following data:[2]

- 50% lethal concentration in the fish acute toxicity test (OECD TG 203)
- No-observed-effect concentration in the fish early-life-stage toxicity test (OECD TG 210)
- 50% effective concentration in the *Daphnia magna* acute immobilization test (OECD TG 202)
- No-observed-effect concentration in the *Daphnia magna* reproduction test (OECD TG 211)
- 50% effective concentration and no-observed-effect concentration in the algal growth inhibition test (OECD TG 201)

(2) Major Updates from KATE2020 version 4.0 to version 5.0

<u>Improvements of user interfaces</u>

i) Input screen and multiple chemicals prediction results screen were improved
ii) Download functionality of multiple chemicals prediction results was added
iii) Display of structure class definition was improved

<u>Updates to QSAR models</u>

i) Reviewing toxicity test results for alga acute and chronic, recalculated toxicity of some chemicals based on the latest test guideline, some chemicals (e.g., mixture of isomers) were altered from training set to support chemicals.
ii) Two aliphatic primary amines and one epoxide, whose toxicity testing was done in FY2022, were added to training set.
iii) Recalculated statistics of regression equations of QSAR classes

(3) Major Updates from KATE2020 version 3.0 to version 4.0

<u>Improvements of user interfaces</u>

i) Log in system was revised so that users can use KATE2020 without user ID and password.
ii) Some interfaces (prediction results screen, verify QSAR screen etc.) were modified.

<u>Updates to QSAR models</u>

i) Reviewing toxicity test results for fish acute, recalculated toxicity of some chemicals based

on the latest test guideline, some chemicals were altered from training set to support chemicals.
ii) Two thiols (fish and Daphnid acute) and one imide (Daphnid acute) compounds, whose toxicity testing was done in FY2021, were added to training set.
iii) Recalculated statistics of regression equations of QSAR classes

Updates to QSAR class names
i) Scrutinizing the names of all QSAR classes, some of them were fixed.

(4) Major Updates from KATE2020 version 2.0 to version 3.0

Updates to QSAR models
i) Reviewing toxicity test results for daphnid acute, recalculated toxicity of some chemicals based on the latest test guideline, some chemicals were altered from training set to support chemicals.
ii) Recalculated statistics of regression equations of QSAR classes

Updates to QSAR class names
i) Scrutinizing the names of all QSAR classes that satisfy statistic criteria ($R^2 \geq 0.7$, $Q^2 \geq 0.5$, and $n \geq 5$), some of them were fixed.

(5) Major Updates from KATE2020 version 1.1 to version 2.0

Updates to QSAR models
i) One of the criteria for QSAR classes displayed on the prediction results screen by default was changed from $Q^2 \geq 0.6$ to $Q^2 \geq 0.5$.
ii) The algal chronic toxicity QSAR equation "CNOS_X basic aromatic n unreactive" was changed in accordance with the correction of toxicity data.

Updates to displays and user interfaces
i) An individual structure judgement result for each substructure was added.
ii) The ability to display a format for printing was added.
iii) An issue in batch mode where prediction would stop before completion when an error was encountered was fixed.
iv) Some expressions were corrected.
iv) An issue in batch mode where prediction would stop before completion when an error was encountered was fixed.

Updates to structure class names
i) Prefixes such as "CN_X" were removed or added.
ii) Typos were fixed.
iii) Unnecessary notations were removed.
iv) Prefix notations were unified.
v) The "reactive/unreactive" notations were corrected.
vi) Predicted toxicity types were corrected.

(6) Major Updates from KATE2020 version 1.0 to version 1.1

i) The predicted toxicity value was updated to be displayed in exponential notation (ex. 2.3e-7) when the value is greater than 10^{-5}, equal to 10^6, or less than 10.
ii) The predicted toxicity value was updated to be displayed to two significant figures.
iii) An issue where prediction was not always executed, even when the input information was correct, was fixed.
iv) Other slight changes in some expressions were made.

(7) Major Updates from KATE2017 on NET to KATE2020 version 1.0

Updates to QSAR models
i) The tool for estimating log P was changed from ClogP to KOWWIN™. Some QSAR models

were modified in accordance with this change.

ii) KOWWIN™ measured value was removed from the priority list for log P value to leave only two options: 1. user input value and 2. KOWWIN™ estimated value.

iii) Chemical substances with log P > 6.0 were excluded from calculation of the QSAR equation.

iv) Additional training set data and QSAR classes were added, and some were removed.

v) Chemicals that were not used for QSAR calculation (support chemicals with data with log P > 6.0, data with an inequality sign, and outliers) were updated to be displayed as information.

<u>Updates to displays and user interfaces</u>

i) Data lists of training set data and support chemicals included in the QSAR class were added.

ii) Definition lists of structure class corresponding to QSAR class were added.

iii) A sorting function was added to the chemical list on the "Verify QSAR" screen.

iv) The prediction and confidence intervals were linked to the additional regression line calculated by excluding training set data.

v) A checkbox to skip KOWWIN™ calculation was added.

(8) Development History up to the Release of KATE2017 on NET

The beta version of the KATE system was released in January 2008 and KATE2009 on the Internet was released in March 2009. In March 2011, KATE2009 was updated to KATE2011 with the addition of chemicals data, updated rules for structure classification, updated structure judgement, and addition of substructures related to skin sensitization. KATE2011 is still available at https://kate.nies.go.jp/onnet.html (Japanese only).

In March 2018, the beta version of KATE2017 on NET was released. This release was followed by the official release of version 1.0 in January 2019. The main changes between KATE2011 and KATE2017 on NET were updates to the substructure language and search program from FITS (a combined substructure language and search program) to a combination of SMARTS notation and the CDK search program, and the addition of predictions for algal and chronic toxicity, toxicity data with inequality signs, and structure classes. In addition, the QSAR models received a significant update, the log P calculation module was changed from ClogP (Daylight Chemical Information Systems, Inc.) to KOWWIN™ (US EPA), displays, user interfaces were updated, and the display language was changed to English.

(9) About Support Chemicals

In KATE2020, the following data are not used for QSAR model construction and are displayed as "Support Chemicals" for information purposes only:

i) data with estimated log P > 6.0

ii) data with an inequality sign (such as limit test)

iii) mixture of isomers

However, data with an inequality sign that fall within the applicability domain of log P are also used for the structure judgement.

(10) About log P

KATE2020 utilizes the log P prediction model KOWWIN™ (with permission from the US EPA) to obtain log P values used for the toxicity prediction[6]. Users must acknowledge that they agree to the KOWWIN™ Licensing Policy each time at login to KATE2020. The policy is provided here for reference:

KOWWIN v1.69 (April 2015)

(11) Disclaimer

KATE2020 is provided as a tool for obtaining information on the potential degree of ecotoxicological effects of chemicals. The Japanese MOE and the National Institute for Environmental Studies give no guarantee about the accuracy of ecotoxicity values provided by KATE2020 and assume no responsibility whatsoever for any damages resulting from the use of ecotoxicity values predicted by KATE2020.

In addition, values predicted by KATE2020 cannot be used to satisfy the requirements for ecotoxicity test results necessary for notification under the Japanese Chemical Substance Control Law (Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc.).

For copyright information and instructions for linking to KATE2020, please visit the policy page of the KATE website: https://kate.nies.go.jp/spolicy-e.html.

(12) Acknowledgments

KATE2020 uses data obtained from the following software applications and libraries. We express our sincere appreciation to each of the development teams.

- ☐ Open Babel (http://openbabel.org/wiki/Category:Installation)
- ☐ JSME Molecular Editor (https://jsme-editor.github.io/)
  - B Bienfait and P Ertl, JSME: A free molecule editor in JavaScript, J. Cheminform. 5:24 (2013). doi:10.1186/1758-2946-5-24.
- ☐ CDK (Chemistry Development Kit) (https://cdk.github.io/)
  - E Willighagen et al., The Chemistry Development Kit (CDK) v2.0: Atom typing, depiction, molecular formulas, and substructure searching, J. Cheminform. 9:33 (2017). doi:10.1186/s13321-017-0220-4.
  - JW May and C Steinbeck, Efficient ring perception for the Chemistry Development Kit, J. Cheminform. 6:3 (2014). doi:10.1186/1758-2946-6-3.
  - C Steinbeck et al., Recent developments of the Chemistry Development Kit (CDK) - an open-source Java library for chemo- and bioinformatics, Curr. Pharm. Des 12:2111-2120 (2006). doi:10.2174/138161206777585274.
  - C Steinbeck et al., The Chemistry Development Kit (CDK): An open-source Java library for chemo- and bioinformatics, J. Chem. Inf. Comput. Sci. 43:493-500 (2003). doi:10.1021/ci025584y.
- ☐ KOWWIN™

(https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface)
(all URLs accessed March 1, 2023)

(13) References

1) https://kate.nies.go.jp (accessed March 30, 2023)
2) http://www.env.go.jp/chemi/sesaku/01.html (accessed March 30, 2023)
3) http://www.env.go.jp/chemi/sesaku/seitai.html (accessed March 30, 2023)
4) https://archive.epa.gov/med/med_archive_03/web/html/fathead_minnow.html (accessed March 1, 2023)
5) A Furuhama, T Toida, N Nishikawa, Y Aoki, Y Yoshioka, and H Shiraishi: Development of an ecotoxicity QSAR model for the KAshinhou Tool for Ecotoxicity (KATE) system, March 2009 version, SAR QSAR Environ. Res., 21 (5), 403 (2010).
6) https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface/ (accessed March 1, 2023)

2. Overview of KATE2020

(1) QSAR prediction Procedures

Figure 2-1 shows a summary of how KATE2020 performs toxicity prediction for a query chemical.

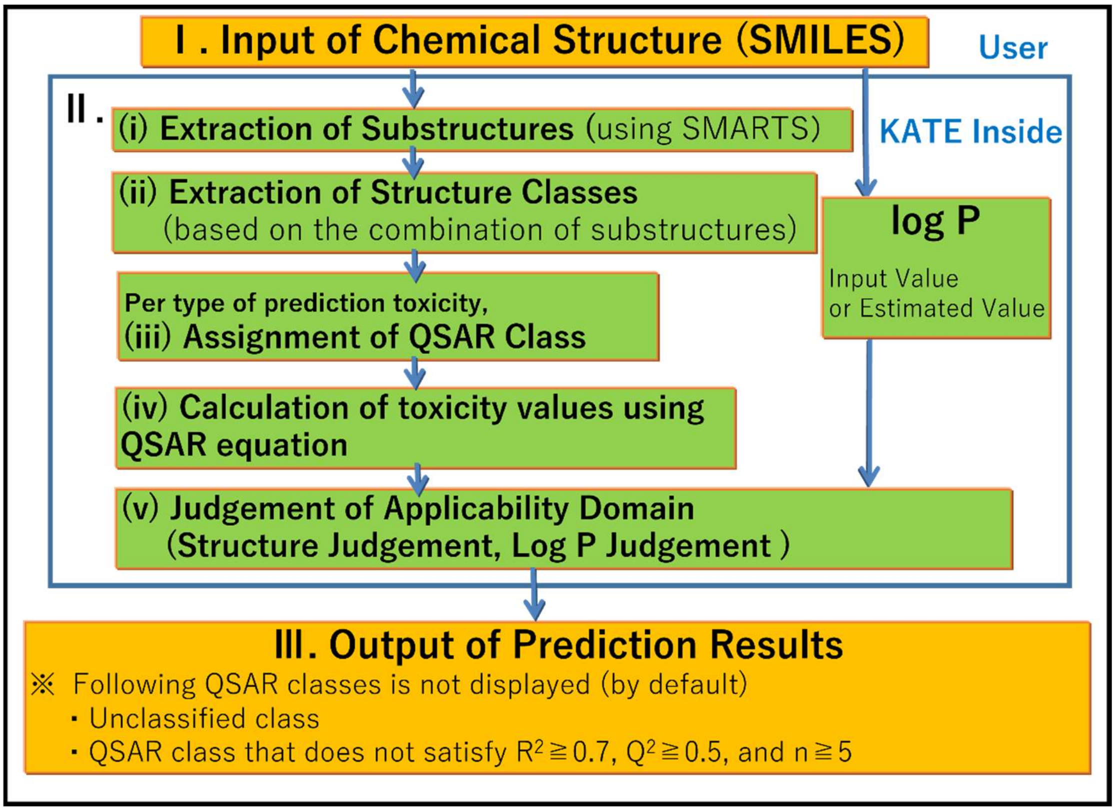


Figure 2-1. Summary of QSAR prediction in KATE2020

I. The structure of the query chemical is input using SMILES notation.

II. Based on the SMILES string input by the user, KATE2020 calculates QSAR equations, predicts toxicity values, and judges the applicability domain. This is accomplished in five steps:

(i) Extraction of the substructures of the query chemical

(ii) Extraction of structure classes*1 based on combination of multiple substructures

(iii) Assignment of QSAR class*2 corresponding to the structure class for each type of predicted toxicity type (one structure class may be assigned to more than one QSAR class for each type of predicted toxicity).

(iv) Calculation of toxicity values using the QSAR equation*3 for each assigned QSAR class

(v) Judgement of applicability domain (structure judgement and log P judgement)

*1 Classification by “AND/OR” combination of substructures

*2 Classification based on the chemical structure for each type of predicted toxicity

*3 A simple linear regression equation using log P as the descriptor that is created for the training set data included in the QSAR class.

III. Output of prediction results

(i) When a query chemical is not assigned to a QSAR class, the chemical is assigned to Unclassified class*4.

(ii) Unclassified class and QSAR classes not satisfying $R^2 \geq 0.7$, $Q^2 \geq 0.5$, and $n \geq 5$ are not displayed*5 by default.

*4 QSAR class that is not assigned to any QSAR class.

*5 R^2, Q^2, and n are the coefficient of determination, internal validation index (leave-one-out method), and the number of training set data respectively, which have been calculated for each QSAR class in advance.

Figure 2-2 shows a representative prediction flow using 1-pyridin-3-ylethanone as the query chemical.

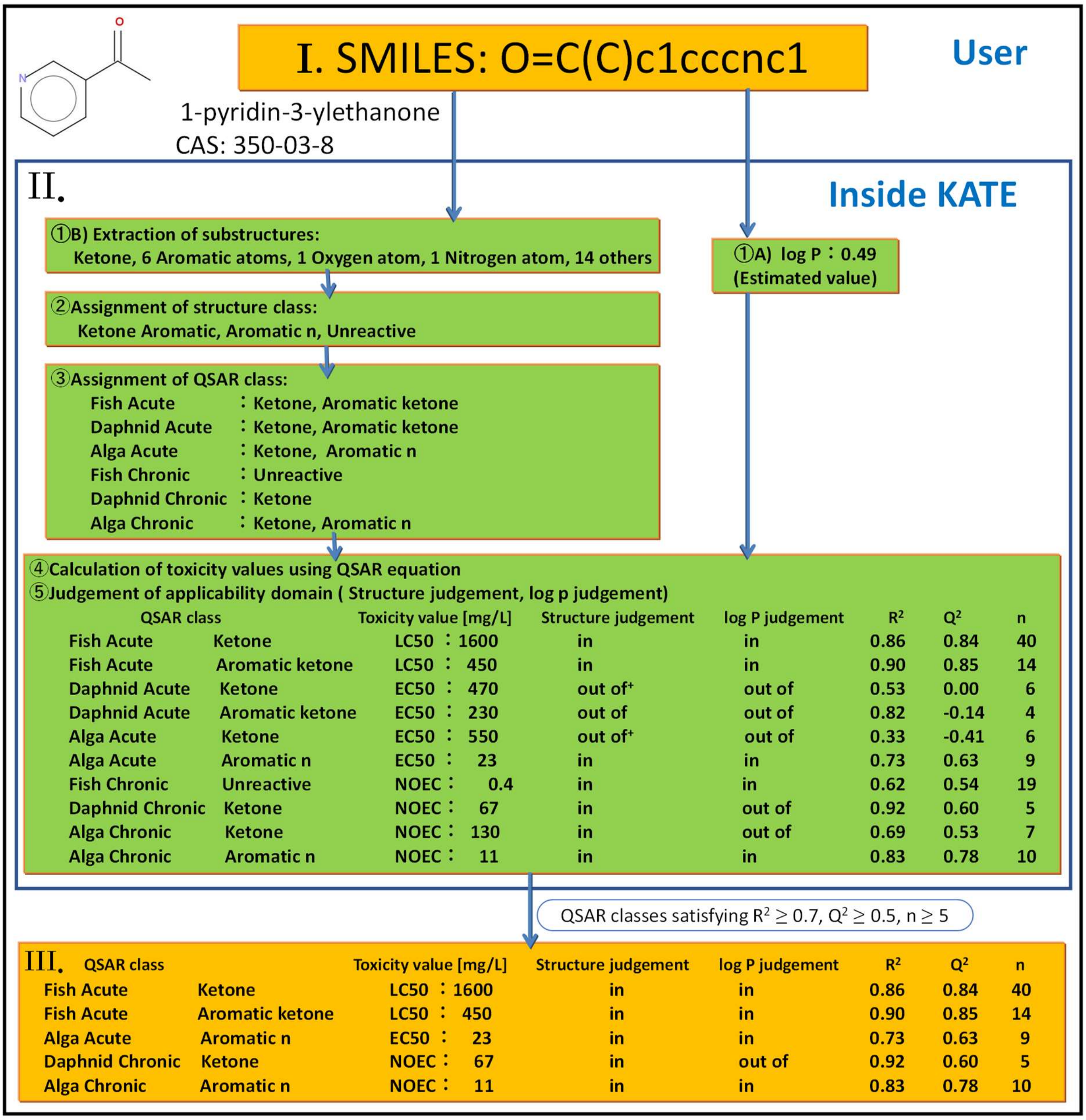


Figure 2-2. Representative prediction flow using 1-pyridin-3-ylethanone as the query chemical

*Actual names of Ketone, Aromatic Ketone, Aromatic n and Unreactive in Figure 2-2 are as follows.

Ketone	: COns_X ketone unreactive
Aromatic Ketone	: COS_X ketone unreactive aromatic
Aromatic n	: CNOS_X basic aromatic n unreactive
Unreactive	: CNO_X unreactive Fish chronic, w/ N,O

Assignment of QSAR Equations, Prediction of Toxicity Values, and Judgement of Applicability Domain

KATE2020 performs the following process to construct the QSAR equations and predict toxicity values based on the structure of the query chemical.

(i) Extraction of substructures

Based on the list of substructure definitions (SMARTS), the number of each substructure contained in the query chemical is determined. The CDK library is utilized for the calculation of the number of substructures by SMARTS.

(ii) Extraction of structure classes

Based on the list of structure class definitions ("AND/OR" combination of substructures), all the structure classes that match the structure of the query chemical are extracted.

(iii) Assignment of QSAR class

The system assigns QSAR classes that correspond to the structure class of the query chemical for each type of predicted toxicity based on the list of QSAR class definitions, where each QSAR class is defined by a type of predicted toxicity and a structure class. KATE2020 may assign more than one QSAR class for each type of predicted toxicity. If the query chemical is not classified to any QSAR class, it is assigned to "Unclassified class".

(iv) Calculation of toxicity values by using QSAR equations

There is a QSAR equation corresponding to each QSAR class, and so the log P value of the query chemical is substituted into each QSAR equation to calculate the log (1/toxicity value((in mmol/L); the system then converts that value into a toxicity value (in mg/L) using the molecular weight of the query chemical.

(v) Judgement of applicability domain

KATE2020 judges whether a predicted toxicity value of the query chemical is within the applicability domain. It performs two types of judgment: A) structure judgement, and B) log P judgement. When the results of both judgement types fall within the applicability domain, the predicted toxicity value of KATE2020 is considered appropriate.

A) Structure Judgement

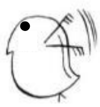
KATE2020 judges whether the structure of the query chemical falls within the applicability domain of the QSAR class classified in (ii) by comparing the "substructures for structure judgement" [*1] (Figure 2-3). There are three possible judgements: in, in (conditionally), and out of. A QSAR class judged to be "in" or "in (conditionally)" is considered to be within the applicability domain in terms of structure.

in: Within the applicability domain

All the "substructures for structure judgement" of the query chemical are found in the "substructures for structure judgement" extracted from the training set data[*2] in the QSAR class (pink and orange area in Figure 2-3), or the query chemical contains no "substructures for structure judgement".

in (conditionally): Conditionally within the applicability domain

The query chemical does not meet the condition of "in", but all the "substructu structure judgement" of the query chemical are found in "substructures for st



judgement” extracted from the training set data in the QSAR class or those from the Narcotic Group class *3 (pink, orange and yellow areas in Figure 2-3).

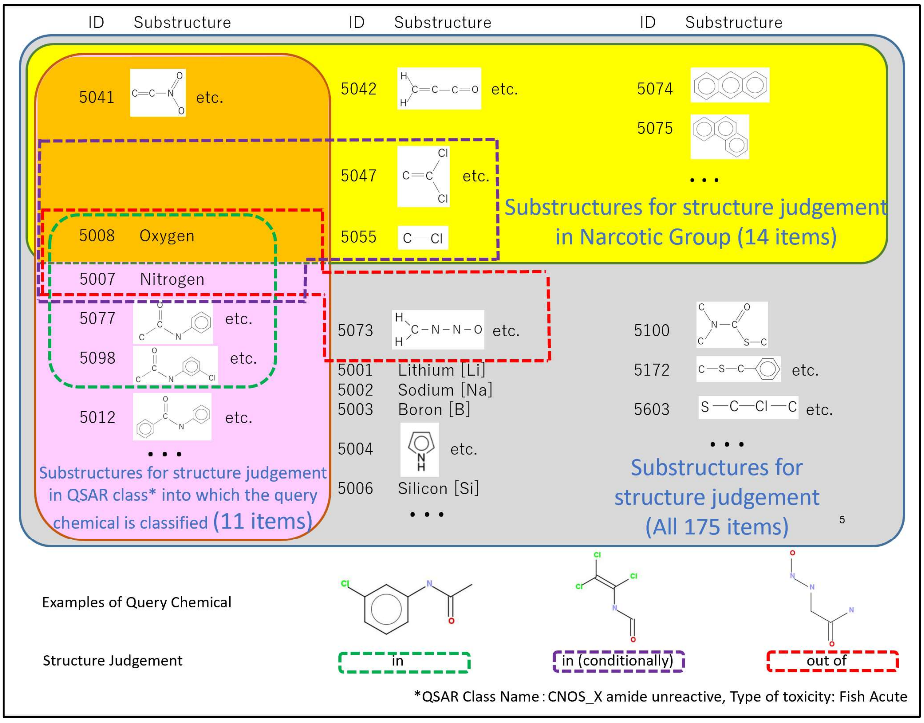
out of: Out of the applicability domain

The query chemical does not meet the conditions of “in” or “in (conditionally)”; that is, in the query chemical, there is at least one “substructures for structure judgement” that is in neither the “substructures for structure judgement” extracted from the training set data of the QSAR class nor those from the Narcotic Group class (grey area in Figure 2-3).

*1 Substructures introduced for the structure judgement in KATE, which contain toxicologically characteristic structures, are also used for structural classification (for details see the KATE2020 technical guidance document (to be published)).

*2 The “substructures for structure judgement” extracted from the training set data also includes substructures of chemicals with an inequality sign whose log P judgement is “in” (within the applicability domain).

*3 Baseline toxicity not based on specific physiological activity (narcotic effect). In KATE2020, QSAR classes whose toxicity is explained only by a simple narcotic effect are defined for each type of predicted toxicity. Examples include aliphatic hydrocarbons, sulfoxide, aliphatic ethers, aromatic ethers, aliphatic ketones, aromatic ketones, and alcohols. These QSAR classes are grouped and defined as the Narcotic Group for each type of predicted toxicity.



* The green frame encloses substructures extracted when predicting with the leftmost chemical in “Examples of Query Chemical”. The same applies to the purple and red frames.

* “etc.” indicates multiple substructures have the same ID, so only one example is shown.

Figure 2-3. Example of Structure Judgement

B) Log P Judgement

KATE2020 judges whether the log P value falls within the applicability domain, based on whether the log P value of the query chemical is between the minimum and maximum log P values of all training set data in the QSAR class concerned.

In KATE2020, all chemical substances with log P > 6, which are highly hydrophobic and have low prediction accuracy, are judged as being out of the applicability domain. This is a new feature in KATE2020.

in: Within the applicability domain (Figure 2-4).

out of: Out of the applicability domain (Figure 2-5).

out of+: Out of the applicability domain, but the log P value of the query chemical takes a value between the minimum and maximum log P values of all chemicals (both the training set data and the support chemicals) in the QSAR class concerned (Figure 2-6).

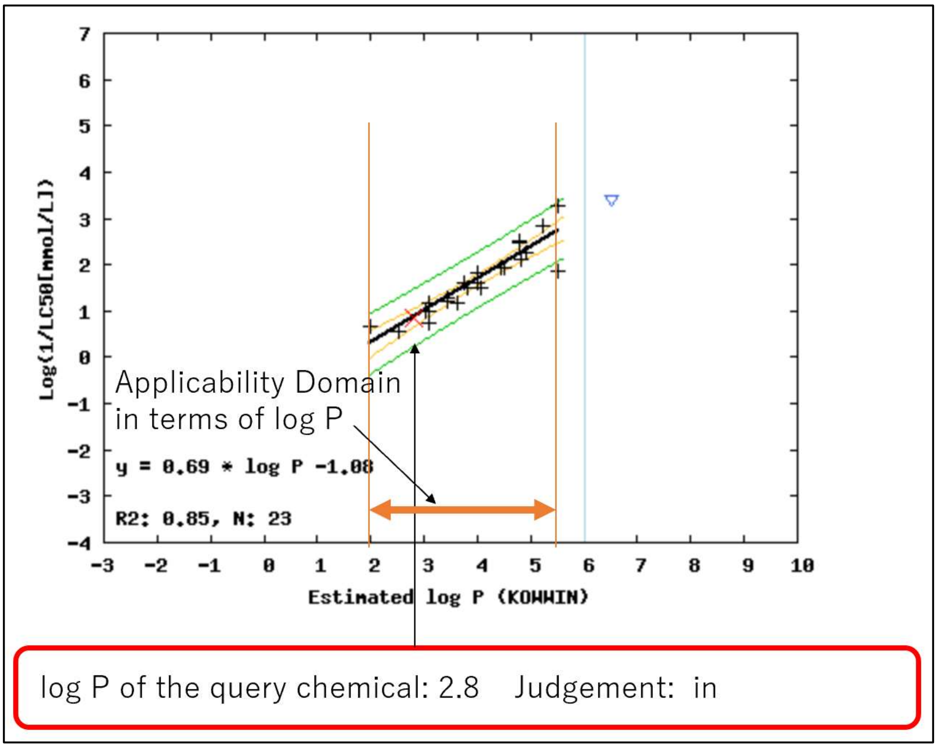


Figure 2-4. Example of log P judgement (in)

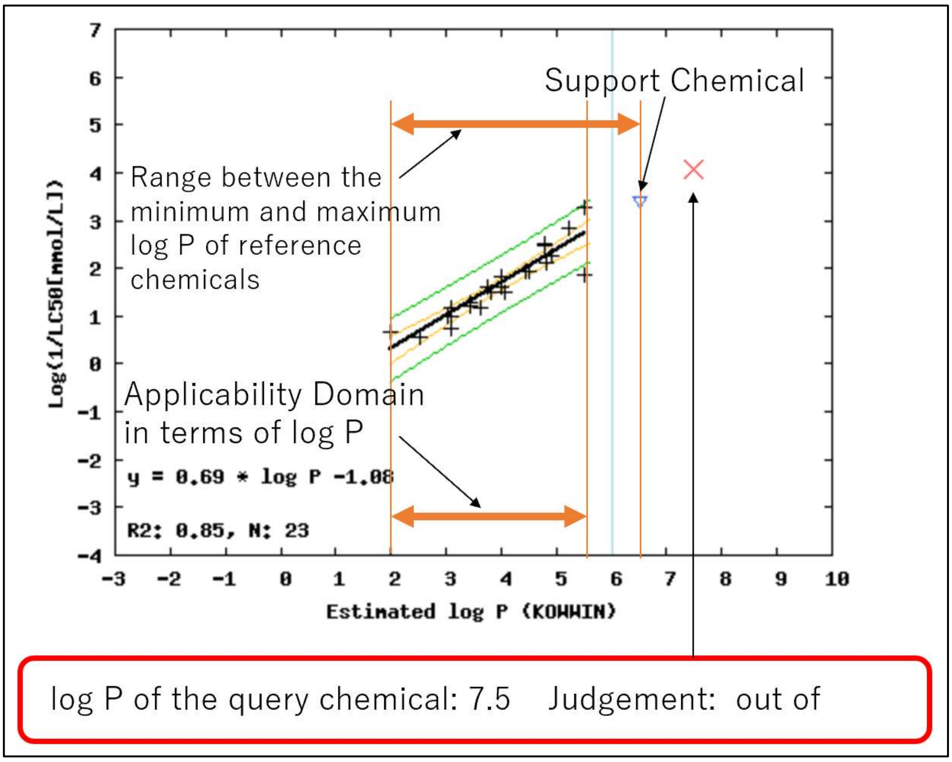


Figure 2-5. Example of log P judgement (out of)

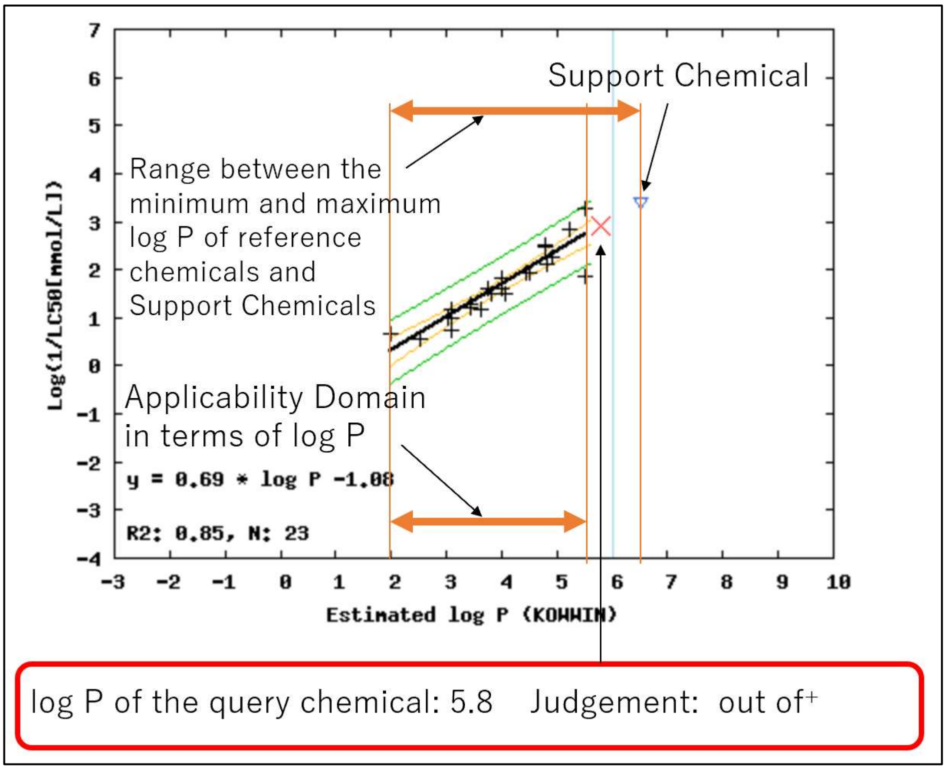


Figure 2-6. Example of log P judgement (out of+)

3. Log In

Since the release of KATE2020 ver. 4.0 in March 2023, users no longer need user ID and password to login. Accessing KATE2020 login screen (https://kate2.nies.go.jp/nies/index.php) in figure 3, users only have to ①agree with the term of agreement and then ②click “Start the session” button. Data entered by the user, such as SMILES, and output results (e.g., predicted toxicity values and QSAR classes) are stored only in the session, not on the KATE2020 server, and they are automatically deleted when the session expires. The session will expire when web browser is closed or user stopped using KATE2020 for one hour. Furthermore, the previous information is overwritten and deleted every time a prediction is made.

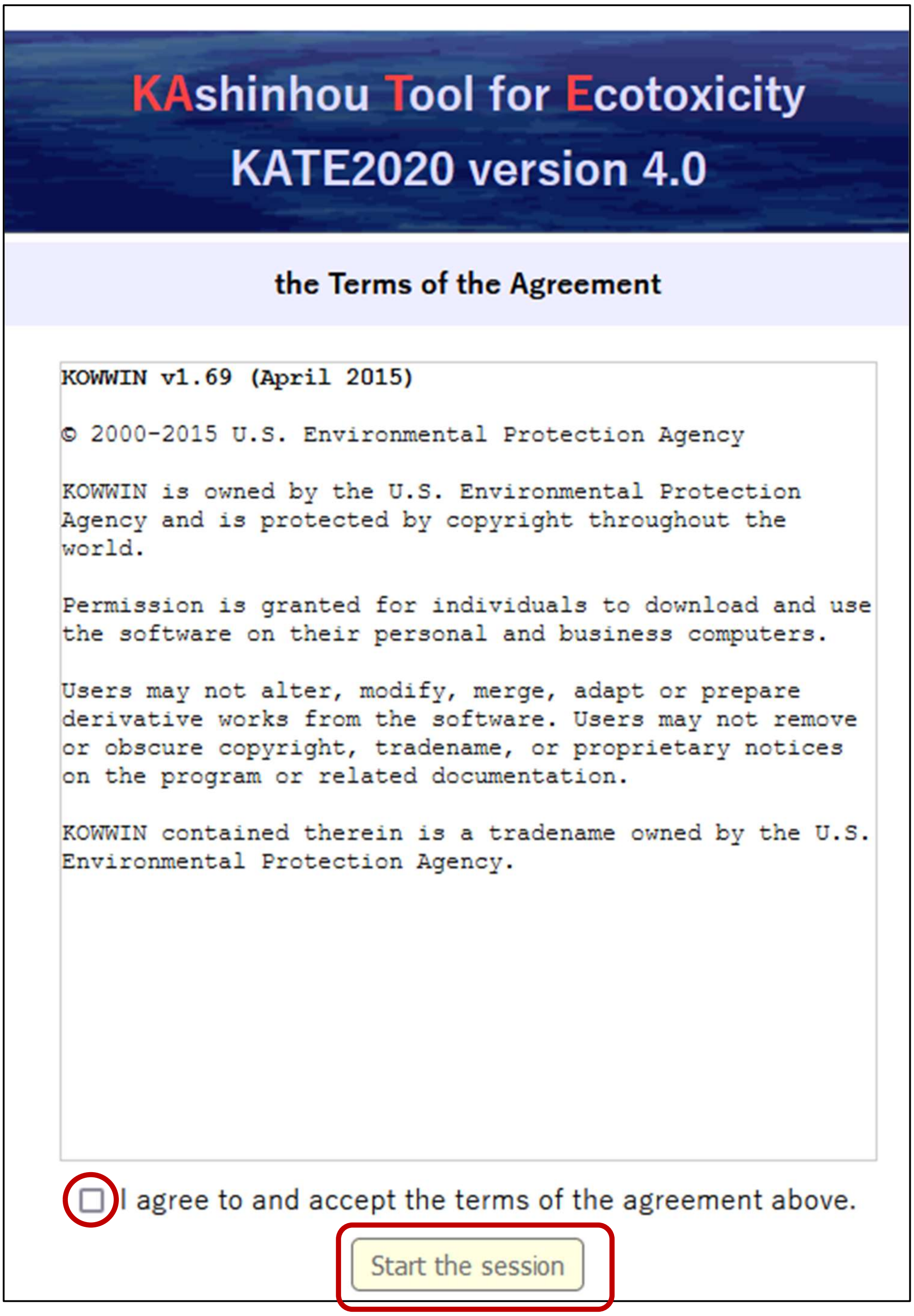


Figure 3. Login screen

4. Input of Query Chemical

After login, the input screen is displayed (Figure 4-1). KATE2020 performs predictions based on the SMILES string of a query chemical. In the center of the screen is a field for the input of a SMILES string. Above that are fields for entering a CAS number or chemical name for conversion to a SMILES string. SMILES strings can also be generated from a drawing of a chemical structure created in the JSME Editor tool within KATE2020.

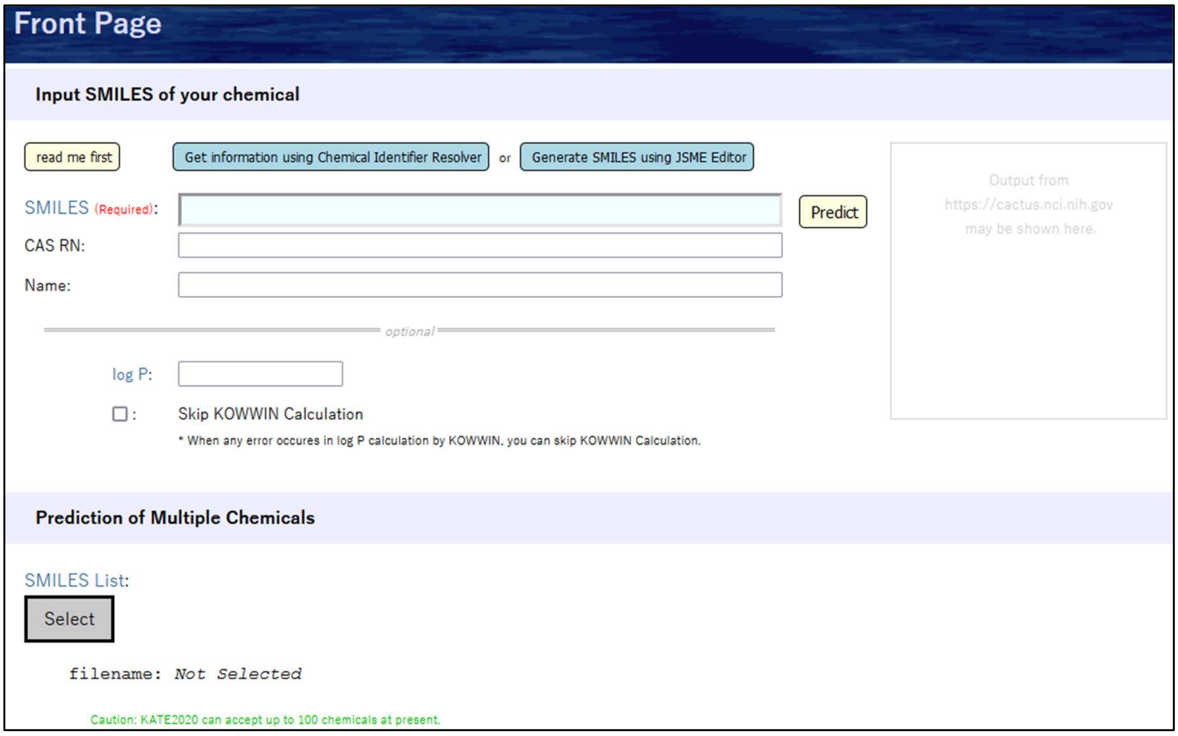
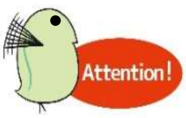


Figure 4-1. Input screen

(1) Acceptable and Unacceptable SMILES Strings

SMILES strings for almost all organic compounds, as well as some inorganic nitrogen compounds (e.g., hydrazine), can be entered into KATE2020. However, the string must



- contain C or N
- not include elements other than H, C, N, O, F, Si, P, S, Cl, As, Br, Sn, and I
- not include ions (although, ammonium [N+] and [n+] can be entered)
- not express a mixture (i.e., a SMILE string that includes ".")
- be converted to the protonated form if it includes [Na], [K], [Li], [Na+], [K+], or [Li+] (e.g., "c1ccccc1O[Na]" should be entered as "c1ccccc1O").

Unacceptable SMILES strings will prompt the system to return an error message.

(2) Direct Input of a SMILES String

Enter a SMILES string in the SMILES input field (Figure 4-1), click the "Predict" button (Figure 4-2), and the QSAR Prediction Results screen will be displayed. In this example, the SMILES string for pyridine-3-ylmethanamine has been entered.

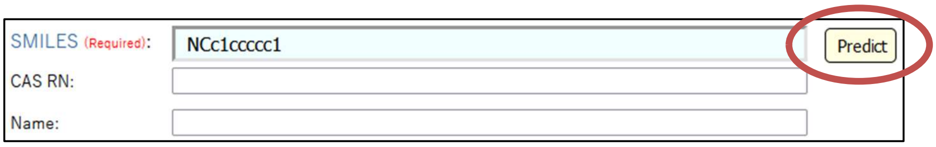


Figure 4-2. "Predict" button next to the SMILES input box

(3) Convert a Drawing of a Chemical Structure into a SMILES String

You can draw a chemical structure using the JavaScript Molecule Editor (JSME Editor) within KATE2020 and convert the drawing to a SMILES string.

Step 1: Click "JSME Editor" under the SMILES string input box (Figure 4-3) to open the JSME Editor window (Figure 4-4).

Figure 4-3. Link to the "JSME Editor"

Step 2: In the editor window, draw the structure of the query chemical and click the "Submit smiles to KATE" button. The chemical structure will be converted into the corresponding SMILES string and the string will be automatically entered in the SMILES input field (Figure 4-5). Click the "Predict" button to display the QSAR Prediction Results screen. Detailed instructions on how to use the JSME Editor can be found at the developers' webpage: https://jsme-editor.github.io/ (accessed March 1, 2024)

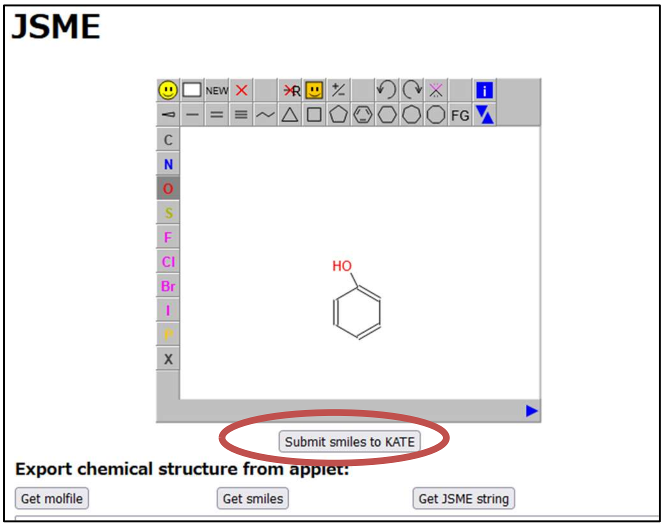


Figure 4-4. JSME Editor

Figure 4-5. Conversion of a structure drawn in JSME Editor into a SMILES string

(4) Convert a CAS Number or Chemical Name into a SMILES String

KATE2020 includes tools to convert a CAS number or chemical name into a SMILES string and vice versa.

Enter the CAS number or chemical name and click the “Get information using Chemical Identifier Resolver” button (Figure 4-6). The structure associated with the CAS number or chemical name will be converted to a SMILES string, and the string will be automatically entered in the SMILES field. In addition, the IUPAC name, SMILES string, and chemical structure of the query chemical will be presented above the CAS number entry box, and the IUPAC name will be entered into the Name field (Figure 4-7). Click the “Predict” button to display the QSAR Prediction Results screen.

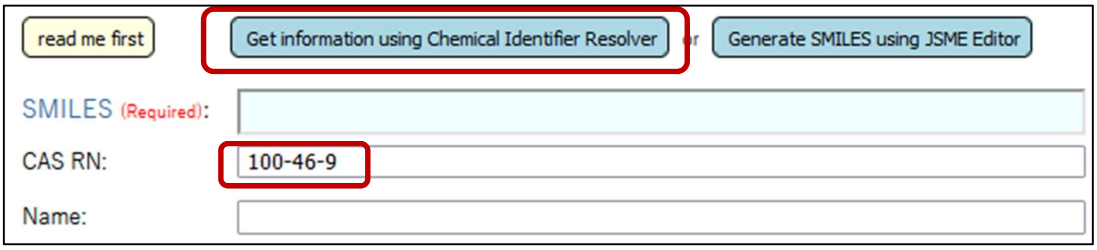


Figure 4-6. CAS input box and “CAS to SMILES, IUPAC Name” button

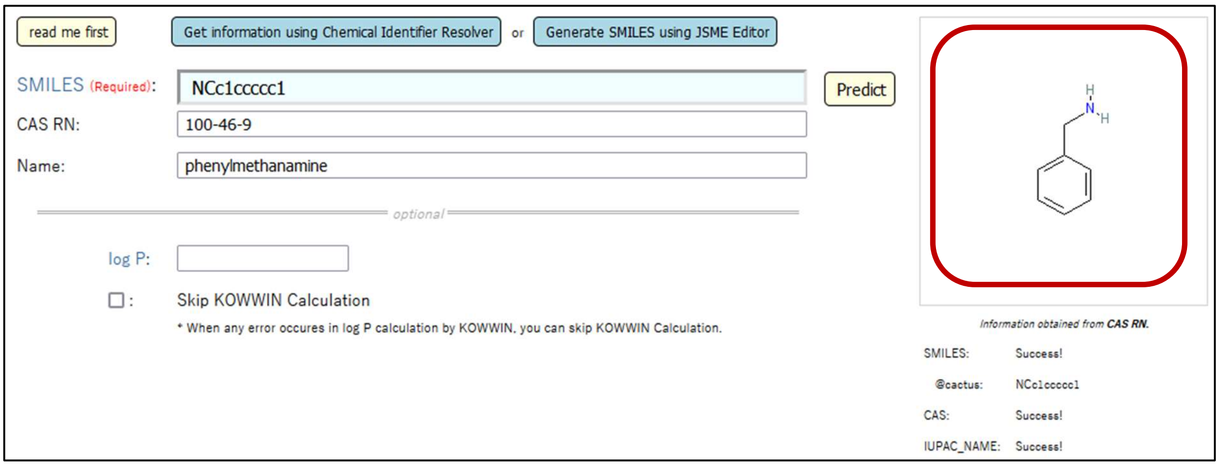


Figure 4-7. Conversion of a CAS number to a SMILES string

(5) User-input Log P Values

If the log P value of the query chemical is known, the value can be entered in the field below the SMILES string input box (Figure 4-8). The entered log P value will be preferentially used in the toxicity prediction.

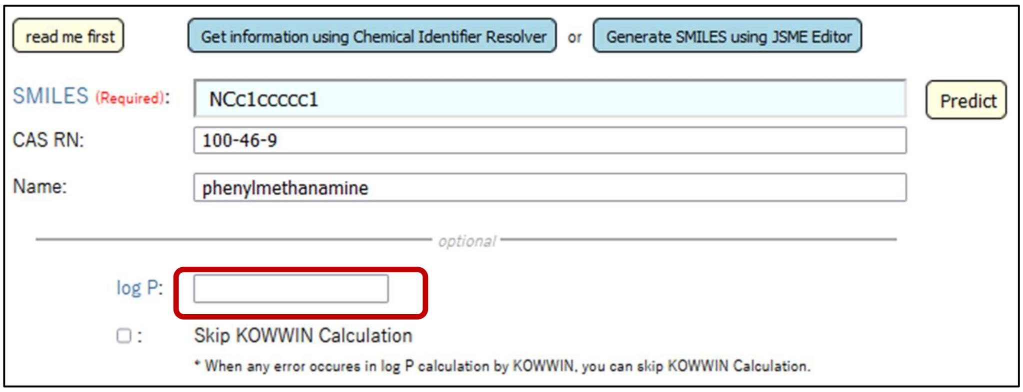


Figure 4-8. Input of a known log P value

(6) User-input CAS Number and IUPAC Name

If a CAS number or IUPAC name is entered in the CAS or Name fields, the information will displayed on the QSAR Prediction Results screen exactly as it was entered (Figure 4-9).

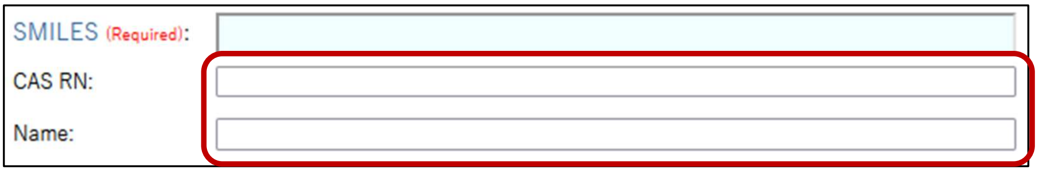


Figure 4-9. Input of CAS number and IUPAC name for inclusion at the top of the QSAR prediction results

(7) Skip KOWWIN™ Calculation

If a SMILES string that cannot be used by KOWWIN™ for the estimation of log P is entered, an error will be reported during the prediction process. To continue with the prediction, the "Skip KOWWIN Calculation" box should be clicked, and a user-define log P value should be entered (Figure 4-10). If a log P value is not entered, QSAR classes will still be assigned, but no predicted toxicity values will be calculated. A user-defined log P value can also be entered on the QSAR Prediction Results screen.

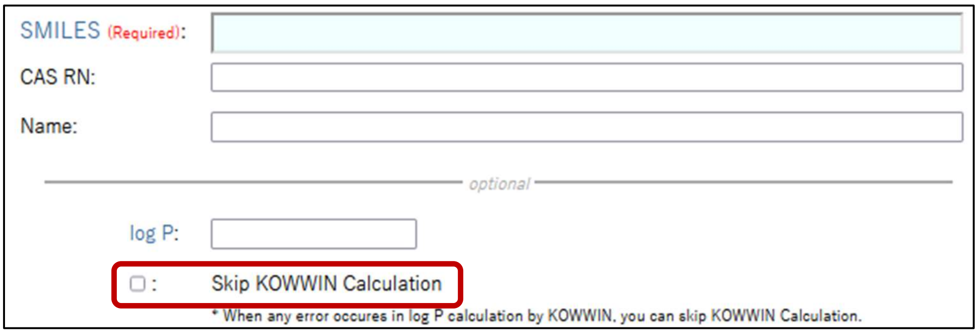
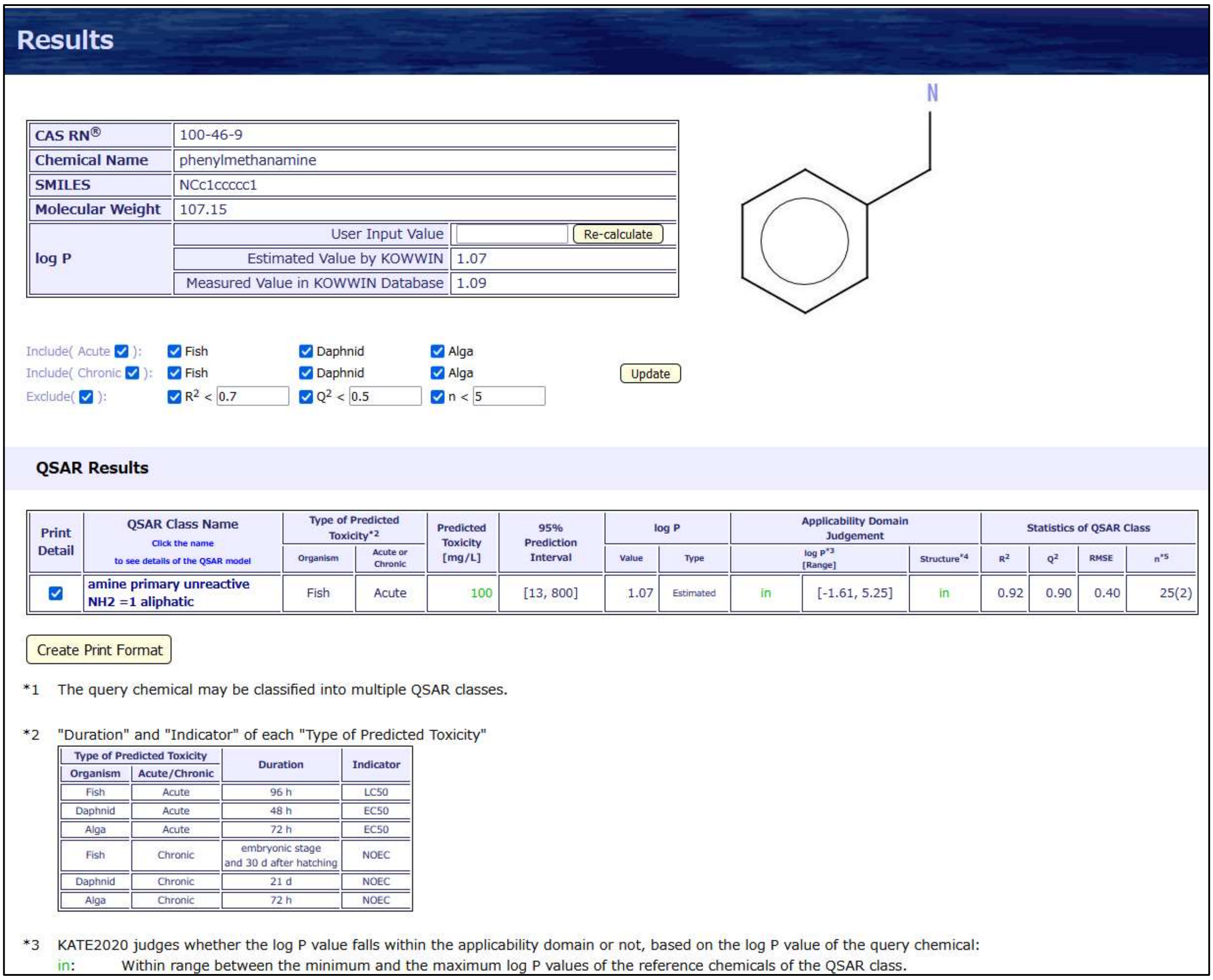


Figure 4-10. Option to skip KOWWIN™ calculation

5. QSAR Prediction Results

After entering the necessary information about the query chemical in the input page and clicking the “Predict” button, the QSAR Prediction Results screen is displayed (Figure 5-1). This screen provides a summary of the query chemical, the QSAR classes to which the query chemical was assigned for each type of predicted toxicity, the ecotoxicity prediction results for each QSAR class, and the statistics associated with each QSAR class.



Results

CAS RN®	100-46-9	
Chemical Name	phenylmethanamine	
SMILES	NCc1ccccc1	
Molecular Weight	107.15	
log P	User Input Value	[ ] Re-calculate
	Estimated Value by KOWWIN	1.07
	Measured Value in KOWWIN Database	1.09

Include(Acute ☑): ☑ Fish ☑ Daphnid ☑ Alga

Include(Chronic ☑): ☑ Fish ☑ Daphnid ☑ Alga Update

Exclude(☑): ☑ R^2 < 0.7 ☑ Q^2 < 0.5 ☑ n < 5

QSAR Results

Print Detail	QSAR Class Name Click the name to see details of the QSAR model	Type of Predicted Toxicity*2		Predicted Toxicity [mg/L]	95% Prediction Interval	log P		Applicability Domain Judgement			Statistics of QSAR Class			
		Organism	Acute or Chronic			Value	Type	log P*3 [Range]		Structure*4	R^2	Q^2	RMSE	n*5
☑	amine primary unreactive NH2 =1 aliphatic	Fish	Acute	100	[13, 800]	1.07	Estimated	in	[-1.61, 5.25]	in	0.92	0.90	0.40	25(2)

Create Print Format

*1 The query chemical may be classified into multiple QSAR classes.

*2 "Duration" and "Indicator" of each "Type of Predicted Toxicity"

Type of Predicted Toxicity		Duration	Indicator
Organism	Acute/Chronic		
Fish	Acute	96 h	LC50
Daphnid	Acute	48 h	EC50
Alga	Acute	72 h	EC50
Fish	Chronic	embryonic stage and 30 d after hatching	NOEC
Daphnid	Chronic	21 d	NOEC
Alga	Chronic	72 h	NOEC

*3 KATE2020 judges whether the log P value falls within the applicability domain or not, based on the log P value of the query chemical:
in: Within range between the minimum and the maximum log P values of the reference chemicals of the QSAR class.

Figure 5-1. QSAR Prediction Results screen

(1) Summary of the Query Chemical

The upper part of the QSAR Prediction Results screen shows a summary of the information used for the prediction (Figure 5-2).

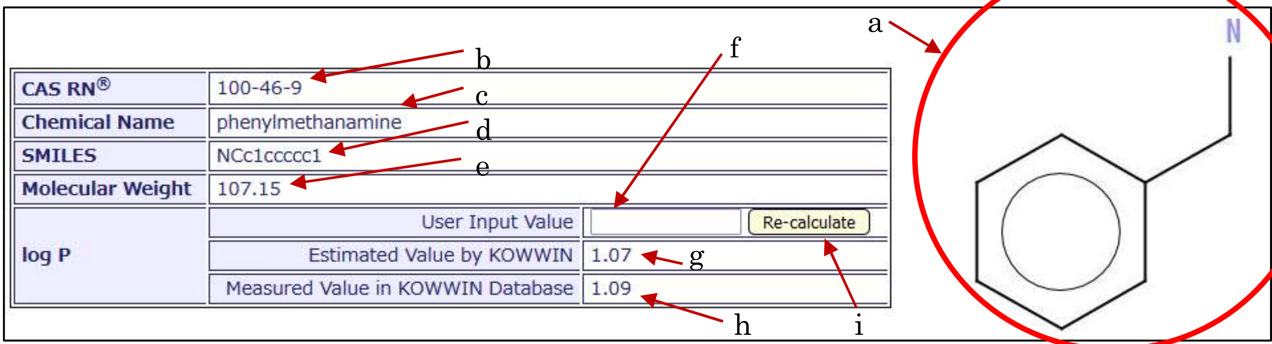


Figure 5-2. Summary of the query chemical

a Chemical structure of the query chemical.

b CAS number (displayed only when entered by the user).

Note: The CAS number is verified by means of the check digit only (the final digit in the CAS number), not the whole CAS number, and the number is tagged with "(incorrect)" if the check digit is incorrect . This may identify if an incorrect number has been input, but not whether the SMILES string matches the CAS number.

c Name of chemical substance (displayed only when entered by the user).

d SMILES string of the query chemical.

e Molecular weight of the query chemical calculated by Open Babel

f Log P value entered by the user.

g Log P value estimated by KOWWIN™.

h Measured log P value in the KOWWIN™ database.

Note: If there is more than one log P value for the query chemical in the KOWWIN™ database, all values in the database will be displayed.

i "Re-calculate" button: Clicking updates the QSAR prediction results with the log P value entered in the "User Input Value" field.

(2) QSAR Prediction Result

The middle section of the QSAR Prediction Results screen provides the QSAR class names of the query chemical, type of predicted toxicity, predicted toxicity results (in green), 95% prediction interval, log P value used for the query chemical, applicability domain judgement, and statistics related to the QSAR classes (Figure 5-3). The results are presented as a table with a series of checkboxes that can be used to filter the results.

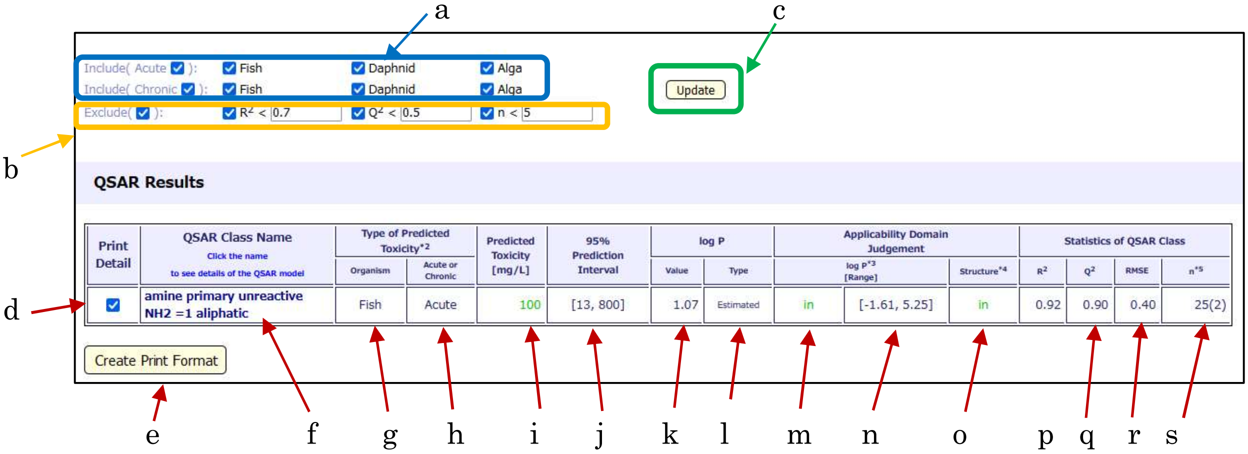


Figure 5-3. QSAR Prediction Results screen

<u>Checkboxes</u>

a “Include” checkboxes indicate the type of predicted toxicity results to show. All the boxes are checked by default.

b “Exclude” checkboxes: If a QSAR class meets any of the conditions stipulated for R^2 (coefficient of determination), Q^2 (internal validation index), or n (number of training set data), the results are not displayed. Which QSAR classes are shown can be specified by unchecking the boxes or changing the values. By default, all the boxes are checked, and the following values are entered: $R^2 < 0.7$, $Q^2 < 0.5$, or $n < 5$. QSAR classes with either value less than these limits are not displayed.

※When the leftmost checkbox is checked, all three checkboxes on the right side are checked. For example, checking the checkbox in the parentheses to the right of “Exclude” will check R2, Q2, and n checkboxes at the same time.

When no QSAR class is displayed for all types of predicted toxicity, “No applicable results” is displayed (Figure 5-4).

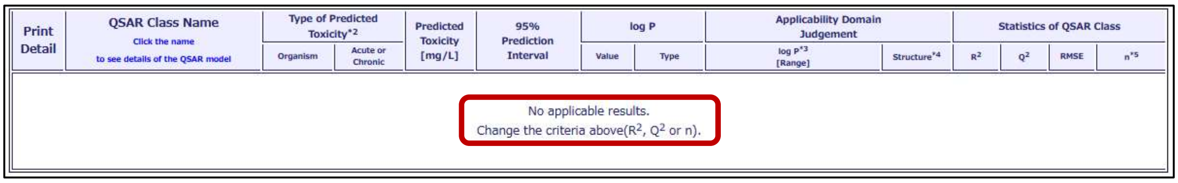


Figure 5-4. QSAR Prediction Results (No QSAR Class Shown)

c “Update” button: Clicking updates the QSAR results in line with any changes that have been made to the checkboxes.

Batch Printing

d Check boxes for inclusion in the final print format. By default, checkboxes are selected when the QSAR class meets the criteria $R^2 \geq 0.7$ and $Q^2 \geq 0.5$ and $n \geq 5$, the log P judgement is “in”, and the structure judgement is “in” or “in (conditionally)”.

e Button for displaying the final print format for review.

QSAR class names and links

f QSAR class names. Click the name to go to the Verify QSAR screen.

Type of predicted toxicity

g Organism (fish, daphnid, or alga)

h Acute or chronic

The following combinations are available for e and f:

Type of predicted toxicity		Testing method	Test duration	Indicator
Organism	Acute/ chronic			
Fish	Acute	Fish acute toxicity test (OECD TG 203)	96 h	LC50
Daphnid	Acute	Daphnia magna immobilization test (OECD TG 202)	48 h	EC50
Alga	Acute	Algal growth inhibition test (OECD TG 201)	72 h	EC50
Fish	Chronic	Fish early-life-stage toxicity test (OECD TG 210)	Embryonic stage and 30 d after hatching*	NOEC
Daphnid	Chronic	Daphnia magna reproduction test (OECD TG 211)	21 d	NOEC
Alga	Chronic	Algal growth inhibition test (OECD TG 201)	72 h	NOEC

*Although the test duration of the fish early-life-stage test differs by species and the number of days before hatching, it is set as “embryonic stage and 30 days after hatching” for *Oryzias latipes* used in the ecotoxicity tests conducted by the Japanese Ministry of the Environment.

Predicted values

i Predicted toxicity value

j 95% prediction interval of the predicted toxicity value

log P

k Type of log P used for the query chemical, which is determined in the following priority order:

1. User Input: log P value entered by the user
2. Estimated: log P value estimated by KOWWIN™

l Log P value of the query chemical

Judgement of applicability domain

m Log P judgement result.

n [Minimum value, maximum value] of the descriptor log P of the training set data in the QSAR class (applicability domain in terms of log P).

o Structure judgement result.

Statistics

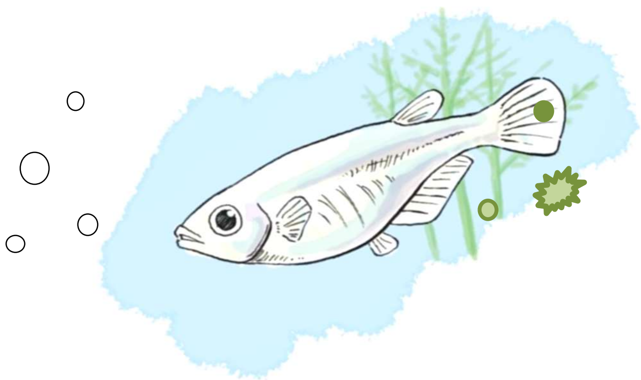
p R^2 (coefficient of determination) of the QSAR equation

q Q^2 (internal validation index by the leave-one-out method) of the QSAR equation (for details, see the KATE2020 technical document※).

r RMSE (root mean square error) of the QSAR equation

s Number of training set data used for the QSAR equation (support chemicals are not included). The value in parenthesis is the number of support chemical data points.

※https://kate2.nies.go.jp/nies/doc/KATE_TechnicalDocument-e.pdf



6. Details of QSAR Class Information

In the QSAR prediction results, clicking on a link in the “QSAR Class Name” column will open a new window showing detailed information of the QSAR class (Figures 6-1 to 6-3). On this screen, a graph of the regression equation for the QSAR class, detailed information on the training set data and support chemicals, definition of the structure class, and substructures of the query chemical are shown. The page can be considered to consist of nine sections, each of which is described below.

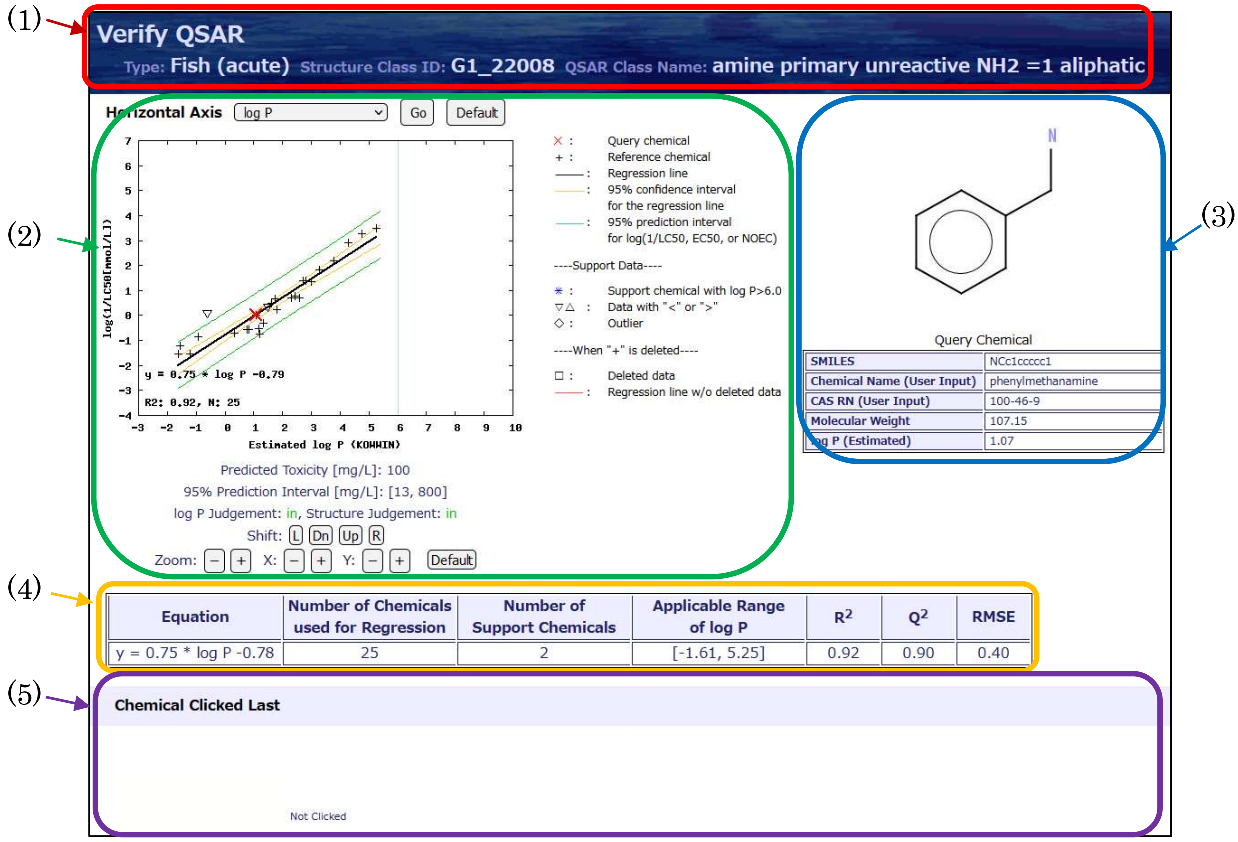


Figure 6-1. Verify QSAR screen (top)

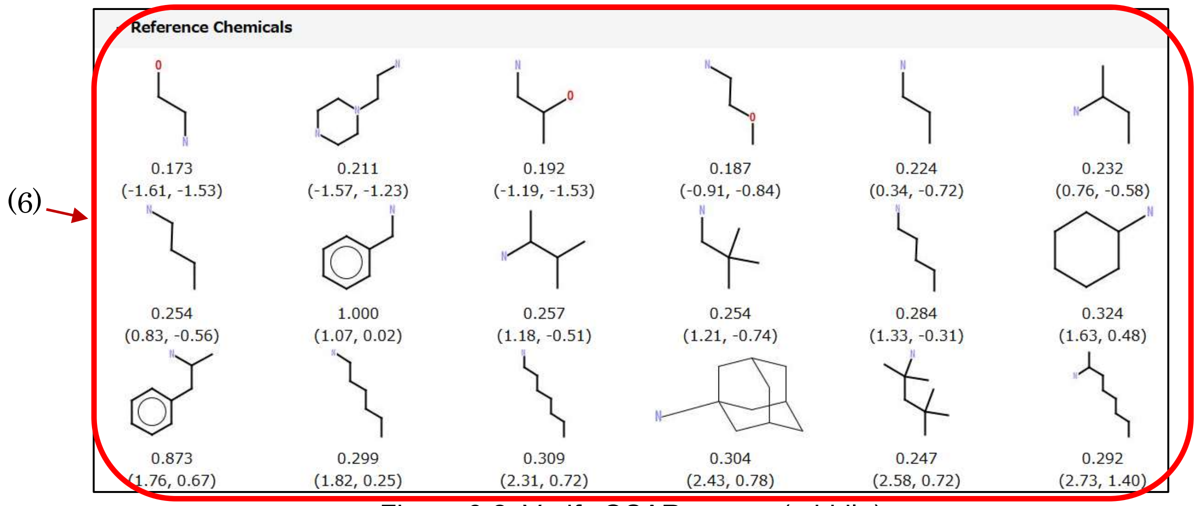
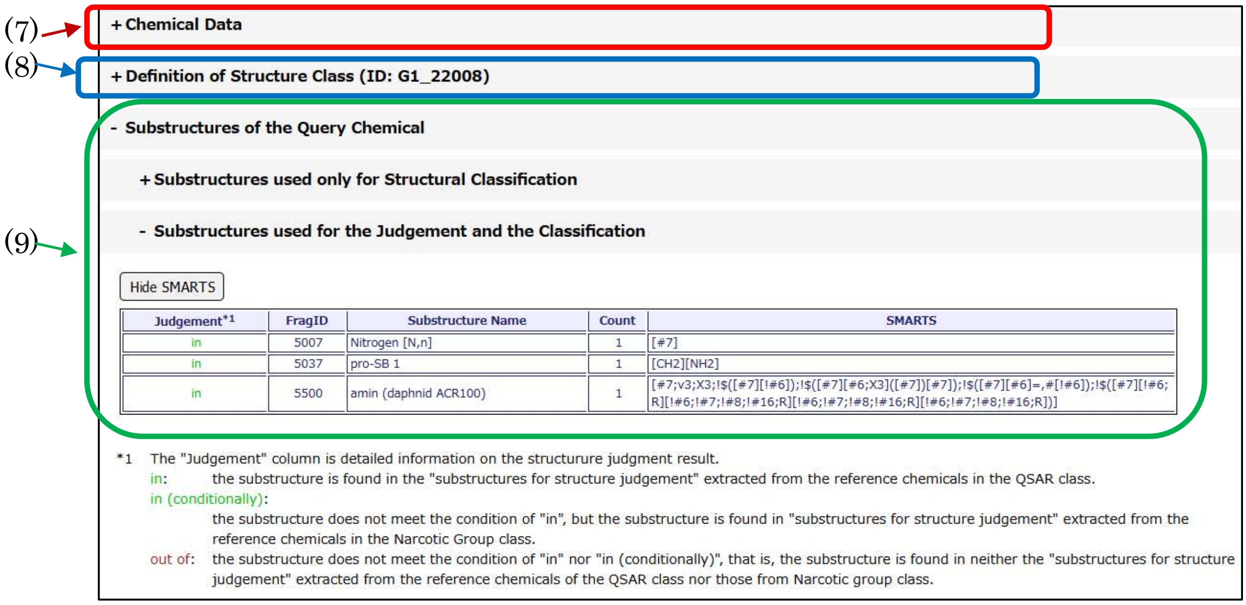


Figure 6-2. Verify QSAR screen (middle)



Judgement*1	FragID	Substructure Name	Count	SMARTS
in	5007	Nitrogen [N,n]	1	[#7]
in	5037	pro-SB 1	1	[CH2][NH2]
in	5500	amin (daphnid ACR100)	1	[#7;v3;X3;!$([#7][!#6]);!$([#7][#6;X3]([#7])[#7]);!$([#7][#6]=,#[!#6]);!$([#7][!#6;R][!#6;!#7;!#8;!#16;R][!#6;!#7;!#8;!#16;R][!#6;!#7;!#8;!#16;R])]

Figure 6-3. Verify QSAR screen (bottom)

(1) Summary of QSAR Class

The dark blue band at the top of the page provides summary information (Figure 6-4).

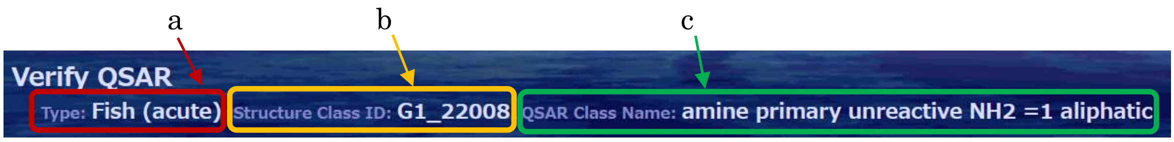


Figure 6-4. QSAR class basic information

- a Type of predicted toxicity for the QSAR class
- b Structure class ID corresponding to the QSAR class (see "(8) structure class definition")
- c Name of the QSAR class

(2) Graph

The graph of the regression equation consists of four parts (Figure 6-5).

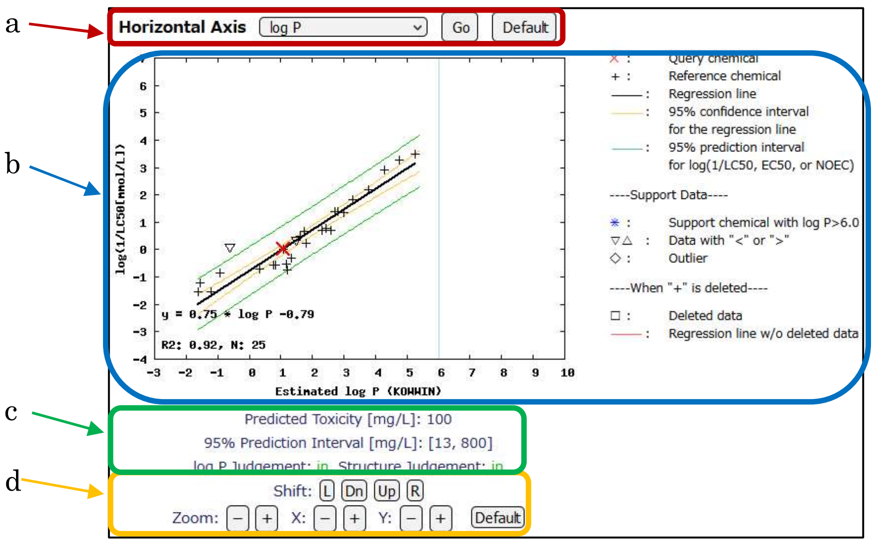


Figure 6-5. Graph of the regression equation (toxicity vs. log P)

a Field to select the horizontal axis

Change from “log P” (default) to “Predicted Variable” and click the “Update” button to draw a graph of “measured toxicity value vs. predicted toxicity value”, where toxicity value is presented as log(1/{LC50, EC50, or NOEC} in mmol/L). Click the “Default” button to return to the default display.

b Graph and legend

The graph of the regression equation is displayed on the left with the legend on the right. The following symbols are used in the legend:

×: Query chemical
+: Training set
Black line: Regression line
Orange curve: 95% confidence interval of the regression equation
Green curve: 95% prediction interval of log(1/{LC50, EC50, or NOEC})

------Support Data------
*: Plot for a chemical with log P > 6.0
▽△: Plot for a chemical with an inequality sign
◇: Plot for an outlier

------When a training set (shown as “+” in the graph) is deleted by clicking on the symbol in the graph------
□: Deleted data
Red line: Regression line calculated without the deleted data

------Bottom-left of the graph------
First line Regression equation
Second line “R^2” Coefficient of determination of the regression equation
“N” Number of training set data (support chemical data are not included)

c Information for the query chemical

1st line: Predicted toxicity value (in mg/L]
2nd line: 95% prediction interval of predicted toxicity value (in mg/L]
3rd line: Log P judgment and structure judgment results

d Graph display buttons

First line

Shift	L:	Shift view left	R:	Shift view right
	Dn:	Shift view down	Up:	Shift view up

Second line

Zoom	−: Zoom out whole graph	+: Zoom in whole graph
X	−: Zoom out the X axis	+: Zoom in the X axis
Y	−: Zoom out the Y axis	+: Zoom in the Y axis

Selection of individual chemicals

The "Chemical list" dropdown gives a list of training set data. When a point (+) on the graph (Figure 6-6) is clicked or one of the structures on the list is clicked (Figure 6-7), the chemical is excluded from the calculation, and an updated regression line is shown in red (Figure 6-6). The prediction interval (green curves) and confidence interval (orange curves) are also recalculated without the selected chemical. Multiple chemicals can removed from the calculation.

When one or more training set data are removed from the calculation, the number of chemicals removed is displayed in the upper left of the graph (Figure 6-6) and, in addition to the original QSAR equation, the modified QSAR equation is displayed after an arrow (Figure 6-6). Updated information on R^2 and N is also displayed.

When a training set is removed, the + symbol for the chemical is changed to a □ (Figure 6-6), and the structure is highlighted by a violet frame in the training set data list (Figure 6-7). Click the point again to deselect.

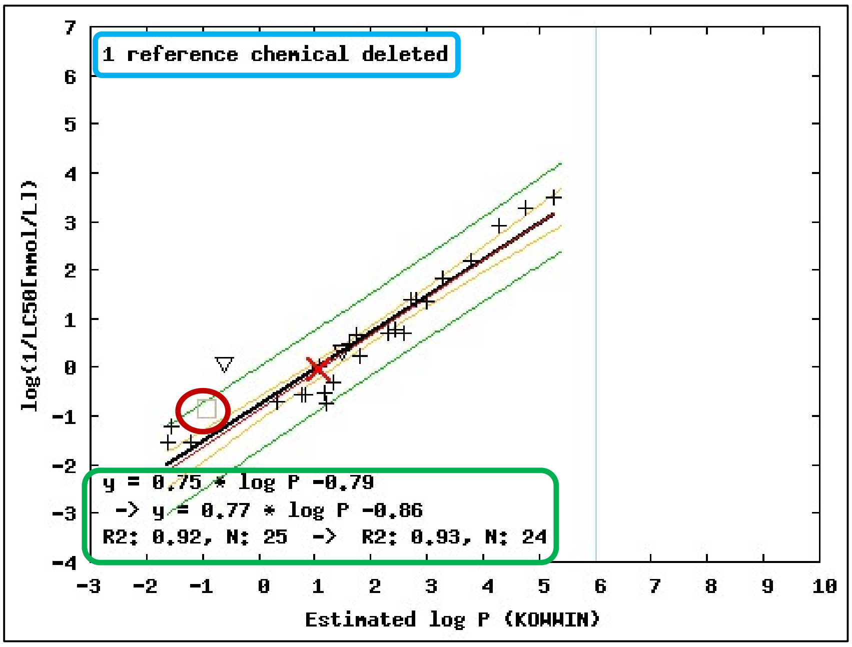


Figure 6-6. Selection of training set (graph)

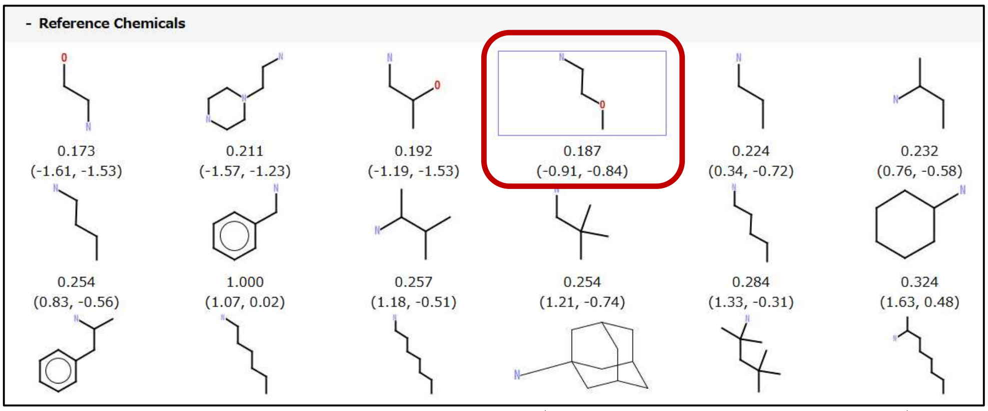
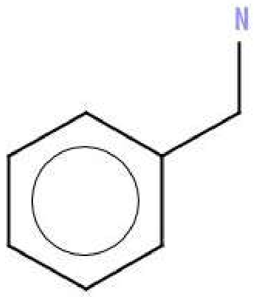


Figure 6-7. Selection of training set (Training set data dropdown)

(3) Information About the Query Chemical

The chemical structure and basic information about the query chemical are displayed to the right of the graph legend (Figure 6-8).



Query Chemical

SMILES	NCc1ccccc1
Chemical Name (User Input)	phenylmethanamine
CAS RN (User Input)	100-46-9
Molecular Weight	107.15
log P (Estimated)	1.07

Figure 6-8. Query chemical information

SMILES: SMILES string of the query chemical

Chemical Name (User Input): Chemical name entered by the user

CAS RN (User Input): CAS number entered by the user

Molecular Weight: Molecular weight of the query chemical

log P: Log P value of the query chemical. "(User Input)" is appended for a user-defined value and "(Estimated)" is appended for a value estimated by KOWWIN™.

(4) Information About the Regression Equation

Information about the regression equation (QSAR equation) is displayed below the graph (Figure 6-9).

Equation	Number of Chemicals used for Regression	Number of Support Chemicals	Applicable Range of log P	R^2	Q^2	RMSE
y = 0.75 * log P -0.78	25	2	[-1.61, 5.25]	0.92	0.90	0.40

Figure 6-9. Information about the regression equation

Equation: Regression equation (QSAR equation)

Number of Chemicals used for Regression: Number of training set data used for the regression calculation

Number of Support Chemicals: Number of support chemicals identified

Applicable Range of log P: Minimum and maximum log P values for the training set data

R^2: Coefficient of determination of the QSAR equation

Q^2: Internal validation index by the leave-one-out method of the QSAR equation (for details, see the KATE2020 technical guidance document (to be published))

RMSE: Root mean square error of the QSAR equation

(5) Chemical Clicked Last

The "Chemical Clicked Last" section provides information about the last clicked chemical within the Training set data or Support Chemicals list. In its default state (i.e., when no chemical has been clicked), the page reports "Not Clicked" (Figure 6-10).

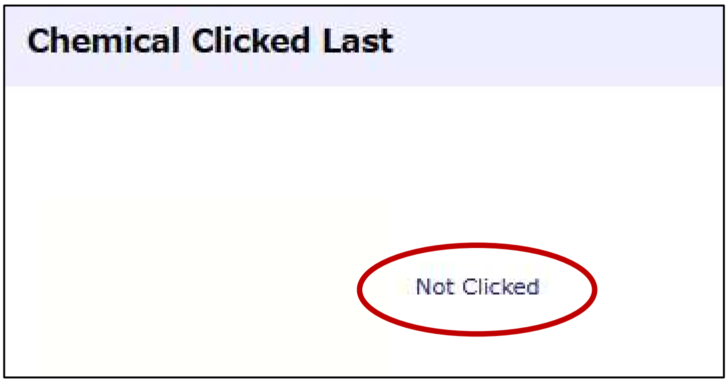


Figure 6-10. Chemical Clicked Last section: before a chemical has been clicked

When a chemical is clicked, the following information is displayed (Figure 6-11):

- Chemical structure
- SMILES string
- CAS number
- IUPAC name
- Coordinates on the regression graph
- Square of residual
- Molecular weight
- Measured toxicity value information ({LC50, EC50, or NOEC}; species; reference)
- Note (information about toxicity test etc., if applicable)

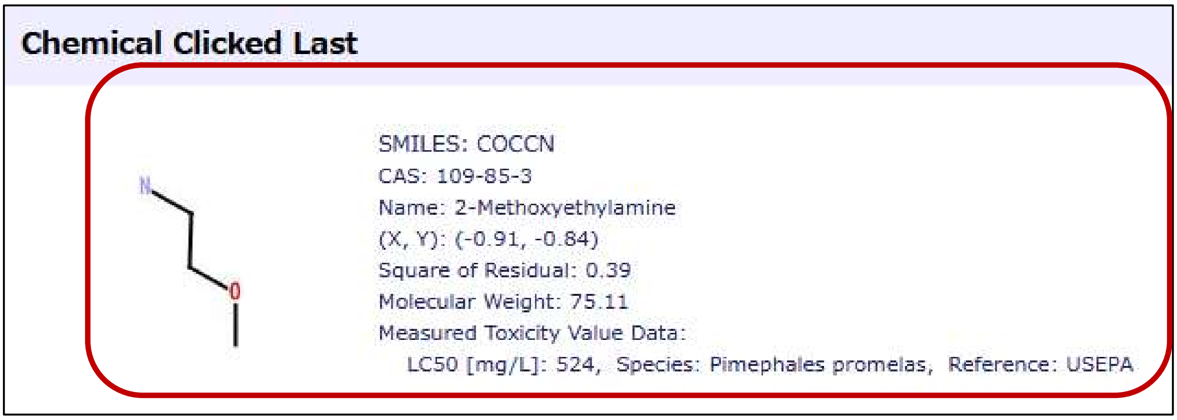


Figure 6-11. Chemical Clicked Last section: after a chemical has been clicked

(6) List of Chemical Structures

The “Chemical list” dropdown provides lists containing the structural formulas of the training set and support chemicals within the QSAR class (Figure 6-12).

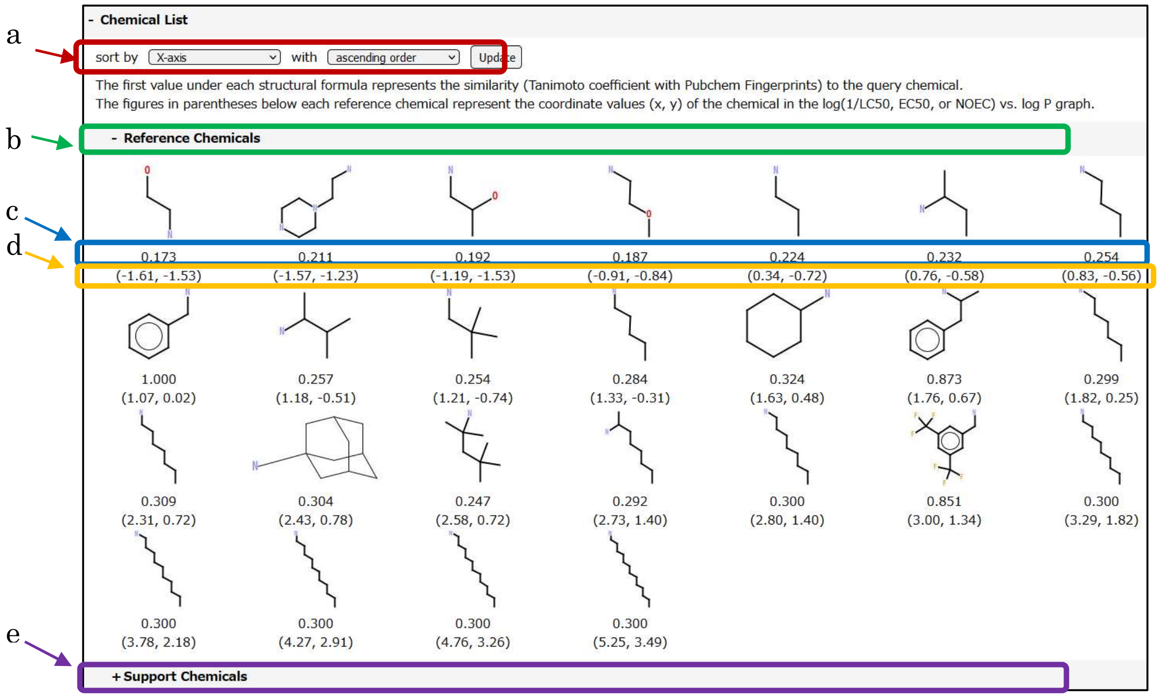


Figure 6-12. “Chemical List” dropdown

Each of these lists can be sorted using the dropdown menus and “Update” button at the top of the page (Figure 6-12a). The available options are sort by CAS number, X coordinate (log P), Y coordinate (log (1/{LC50, EC50, or NOEC})), square of residual, and similarity, and each can be arranged in ascending or descending order. By default, the chemicals are arranged in ascending order of X coordinate (log P). Here, X coordinate, not X axis, is used in case “Horizontal Axis” (see “a” in (2)) is selected as “log P”; that way, even if the chemicals are sorted by X coordinate, when the graph of “Measured toxicity value vs. Predicted toxicity value” is displayed they are still sorted by log P value.

Clicking the “Training set data” dropdown reveals a list containing the structural formulas of the training set data (Figure 6-12 b). The values displayed directly below the chemical structures (Figure 6-12 c) are their similarity with the query chemical (Tanimoto coefficient using PubChem fingerprints; for details, see the KATE2020 guidance document [to be published]); the coefficient falls between 0 and 1, and a structure with higher similarity to the query chemical has a value closer to 1. The values displayed in parentheses (Figure 6-12 d) are the X coordinate (i.e., log P value) and Y coordinate (log(1/{LC50, EC50, or NOEC})) of the chemical substance in the default graph.

Clicking the “Support Chemicals” dropdown (Figure 6-12 e) reveals three additional lists showing the structures of the support chemicals divided into those with data containing an inequality sign, outlier chemicals, and chemicals with a log P > 6 (Figure 6-13).

Figure 6-13. “Support Chemicals” dropdown

(7) Chemical Data

The “Chemical Data” dropdown provides the chemical details of the training set data (Figure 6-14) and support chemicals in the class. Both lists are formatted as shown below.

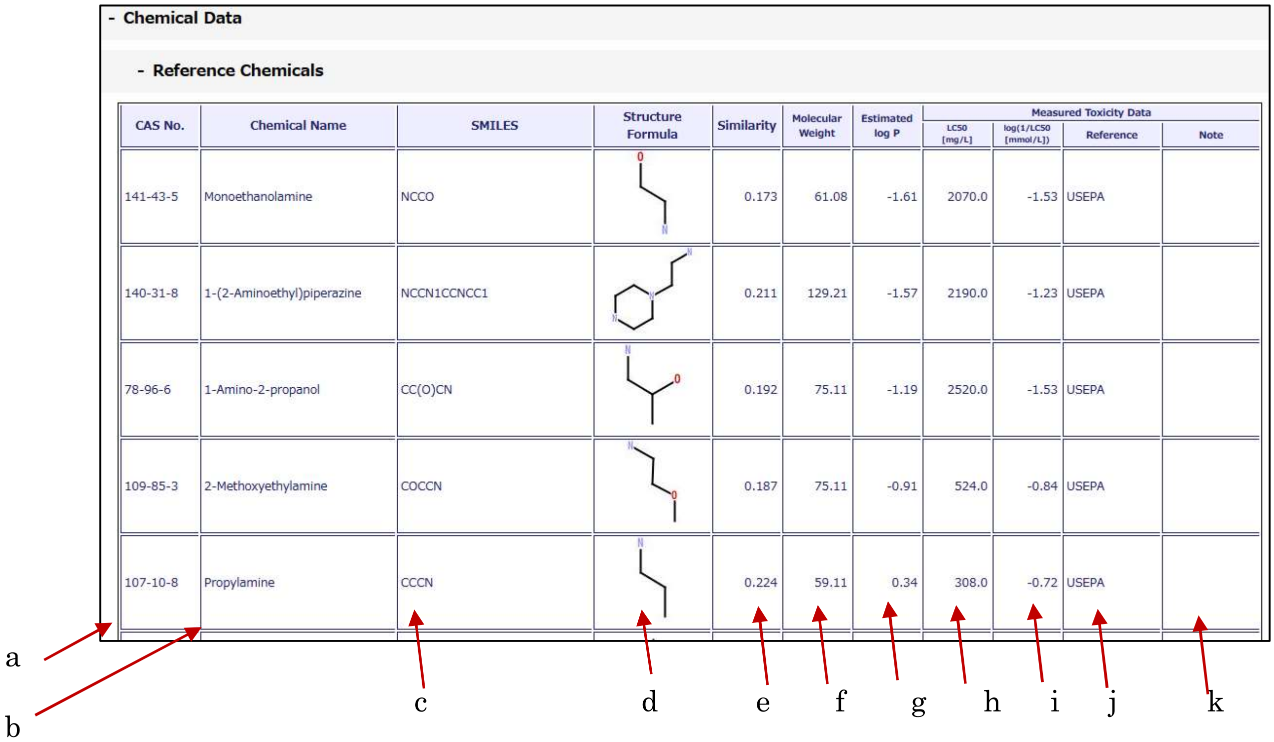


Figure 6-14. “Chemical Data” dropdown

a CAS No.: CAS number

b Chemical Name: Chemical name used in KATE2020

c SMILES: SMILES string used in KATE2020

d Structure Formula: Structural formula

e Similarity: Similarity between the chemical in the list and the query chemical

f Molecular Weight: Molecular weight

g Estimated log P: Log P value estimated using KOWWIN™

h EC50*: Toxicity value (in mg/L) based on the results of ecotoxicity tests

i log(1/EC50* [mmol/L]):

*For h and i, the LC50, EC50, or NOEC corresponding to the type of predicted toxicity of the QSAR class is automatically displayed.

j Species: Species for which the original toxicity data were determined

k Reference: Source of the original toxicity data. The year indicates the year the test was implemented. “MOE” means the data were obtained from the following webpage: http://www.env.go.jp/chemi/report/ierac18/2-1-2-1.pdf (results of ecotoxicity tests conducted by the Japanese MOE [in Japanese]; accessed March 1, 2023). “USEPA” means the data were fish acute toxicity test results obtained from the US EPA fathead minnow database as a training set data dataset.

l Note: Other information about the chemical substance

The order in which the data are displayed is determined by the sorting filter at the top of the page (see “a” in (6)).

(8) Structure Class Definition

Clicking the “Definition of Structure Class” dropdown reveals information about the structure class corresponding to the QSAR class (Figure 6-15). The assigned structure class is highlighted in yellow, with the structure class ID in the "Structure ID" column, the name of the structure class or substructure in the "Description" column The "Decision Tree" column contains the definition of the structure class or substructure.

Definition of Structure Class (ID: G1_22008)

Group: Amine primary — Show all structures

Structure ID	Description	Decision tree
-	amine CNH2	ID:3100 > 0
-	amine primary unreactive	└ R_00033 = false
-	NH2 amine unreactive	└ G1_00010 = false
-	amine primary unreactive NH2=1	└ ID:3100 = 1
G1_22008	amine primary unreactive NH2=1 aliphatic	└ ID:4510 = 0
GA_22008	amine primary unreactive NH2=1 aliphatic alga	└ RA_00033 = false

Figure 6-15. “Structure Class Definition” information

(9) Substructures of the Query Chemical

The “Substructures of the Query Chemical” section contains two dropdowns. Clicking the lower “Substructures used for the Judgement and the Classification” dropdown reveals a list of the substructures used in both the structural classification and the structural judgement (Figure 6-16).

Substructures used for the Judgement and the Classification

f → Hide SMARTS

Judgement[*1]	FragID	Substructure Name	Count	SMARTS
in	5007	Nitrogen [N.n]	1	[#7]
in	5037	pro-SB 1	1	[CH2][NH2]
in	5500	amin (daphnid ACR100)	1	[#7;v3;X3;!$([#7][!#6]);!$([#7][#6;X3]([#7])[#7]);!$([#7][#6]=,#[!#6]);!$([#7][!#6;R][!#6;!#7;!#8;!#16;R][!#6;!#7;!#8;!#16;R][!#6;!#7;!#8;!#16;R])]

a b c d e

Figure 6-16. “Substructures used for the Judgement and the Classification” dropdown

a Judgement: This is a new function in KATE2020 version 2.0. Structure judgment result for the substructure. This provides detailed information about the structure judgment of the QSAR class, and when the structure judgment of the QSAR class is “out of” or “in (conditionally)”, you can see which of the substructures contained in the query chemical are the cause of the judgment result.

Three judgments are possible:

in: The substructure is found in the “substructures for structure judgement” extracted from the training set data in the QSAR class.

in (conditionally): The substructure does not meet the condition of “in”, but the substructure is found in “substructures for structure judgement” extracted from the training set data in the Narcotic Group class.

<u>out of</u>: The substructure does not meet the conditions of "in" or "in (conditionally)"; that is, the substructure is found in neither the "substructures for structure judgement" extracted from the training set data of the QSAR class nor those from the Narcotic group class.

b FragID: ID of the substructure. This is a four-digit number and was arbitrarily set for convenience during the development of KATE. At present, the FragIDs used in this table all start with the number 5 (for details, see the KATE2020 technical guidance document (to be published)).

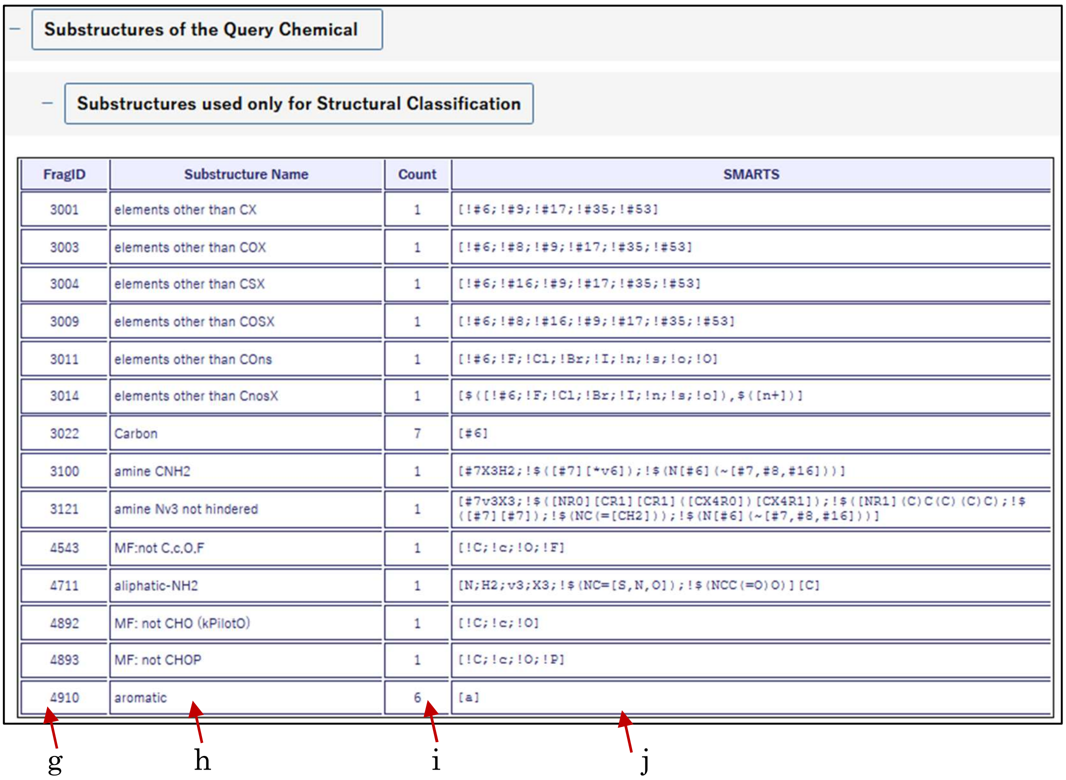
c Substructure Name: Name of each substructure (please note that the names may change in the future).

d Count: The number of substructures in the query chemical corresponding to SMARTS

e SMARTS: Definition of the substructure in SMARTS notation

f Show/Hide SMARTS Button: Click to hide or display the "SMARTS" column.

Clicking the "+Substructures used only for Structural Classification" dropdown reveals a list of the substructures used only for the structural classification (Figure 6-17).



− Substructures of the Query Chemical

− Substructures used only for Structural Classification

FragID	Substructure Name	Count	SMARTS
3001	elements other than CX	1	[!#6;!#9;!#17;!#35;!#53]
3003	elements other than COX	1	[!#6;!#8;!#9;!#17;!#35;!#53]
3004	elements other than CSX	1	[!#6;!#16;!#9;!#17;!#35;!#53]
3009	elements other than COSX	1	[!#6;!#8;!#16;!#9;!#17;!#35;!#53]
3011	elements other than COns	1	[!#6;!F;!Cl;!Br;!I;!n;!s;!o;!O]
3014	elements other than CnosX	1	[$([!#6;!F;!Cl;!Br;!I;!n;!s;!o]),$([n+])]
3022	Carbon	7	[#6]
3100	amine CNH2	1	[#7X3H2;!$([#7][*v6]);!$(N[#6](~[#7,#8,#16]))]
3121	amine Nv3 not hindered	1	[#7v3X3;!$([NR0][CR1][CR1]([CX4R0])[CX4R1]);!$([NR1](C)C(C)(C)C);!$([#7][#7]);!$(NC(=[CH2]));!$(N[#6](~[#7,#8,#16]))]
4543	MF:not C,c,O,F	1	[!C;!c;!O;!F]
4711	aliphatic-NH2	1	[N;H2;v3;X3;!$(NC=[S,N,O]);!$(NCC(=O)O)][C]
4892	MF: not CHO (kPilotO)	1	[!C;!c;!O]
4893	MF: not CHOP	1	[!C;!c;!O;!P]
4910	aromatic	6	[a]

Figure 6-17. "Substructures of a Query Chemical" dropdown (used only for structural classification)

g FragID: ID of the substructure. This is a four-digit number and was arbitrarily set for convenience during the development of KATE. At present, the FragIDs used in the table all start with a number 3, 4, 6, or 7 (for details, see the KATE2020 technical guidance document (to be published)).

h Substructure Name: Name of each substructure (please note that the names may change in the future).

i Count: The number of substructures in the query chemical.

j SMARTS: Definition of the substructure in SMARTS notation.

7. Prediction of Multiple Chemical Substances

Files containing multiple SMILES strings can be input to perform sequential prediction for multiple chemical substances.

(1) Input File: “SMILES List”

KATE utilizes a “SMILES list”, an input file containing a series of SMILES strings, to sequentially predict the ecotoxicity of multiple query chemicals. In KATE2020, the specifications of the “SMILES list” are different from those in KATE2017.

In the SMILE list file, enter one or more headers in the first line (case-insensitive) and the information on each query chemical in the subsequent lines in text form.

As headers, you can enter “SMILES” (required) as well as “ID” (ID defined by user), “LogP” (log P value entered by user), “NAME” (name of chemical substance), and “CAS” (CAS number). Separate columns with tabs.

Below are two examples of SMILES lists:

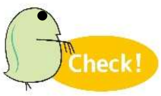
Example 1

```
SMILES
CCCCOC(=O)CS
CC(=C)CS
CC1(CC2(C)CC3(Br)C1)CC(Br)(C2)C3
CCCCCCCCCBr
CCCCCCCCC(Br)CBr
CCCCCCCCCCBr
```

*In the example above, the first line specifies the column name “SMILES”, and the subsequent lines specify “SMILES” strings of query chemicals.

Example 2

NAME	LogP	SMILES	CAS	id
name1	0.8	CCCCOC(=O)		A10
name2		CC(=C)CS		A50
name3	1.3	NCc1cccnc1	3731-52-0	F20



*The order of columns is arbitrary.
*Columns should be separated by tabs (⌴ denotes a tab character). The number of tabs should be the same in each line.
*Any of the columns can be omitted except the “SMILES” column.

In KATE2020, to see how to format the SMILES list, click “SMILES list” above the “Select” button (Figure 7-1).

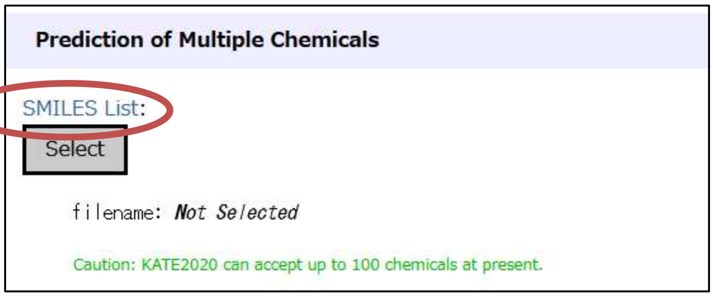


Figure 7-1. Link to details about formatting a SMILES list

(2) Prediction Procedures

Step 1. Click the “Select” button on the Input screen (Figure 7-2).

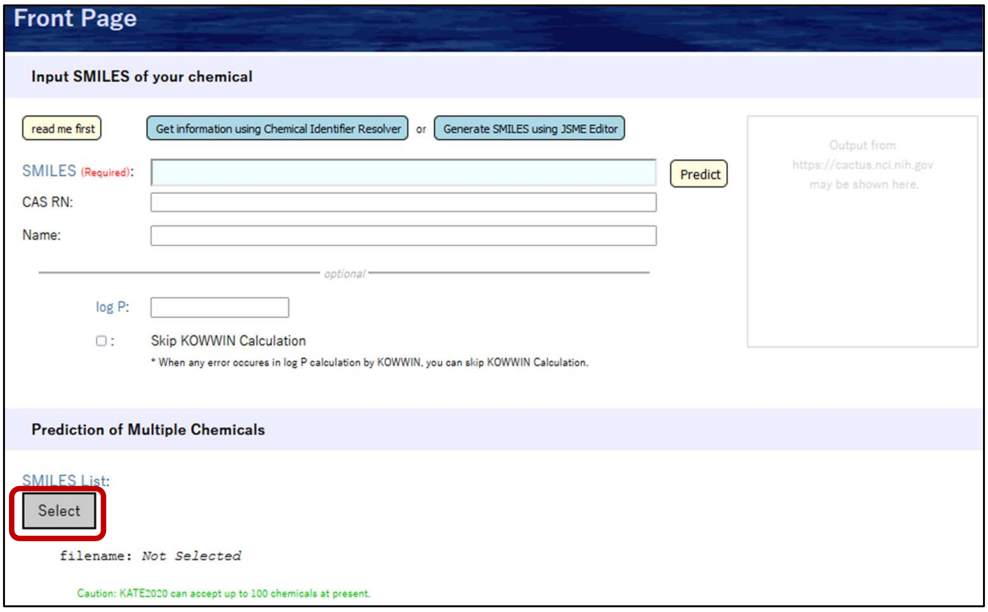


Figure 7-2. Select Button for “Prediction of Multiple Chemicals”

Step 2. Select the input SMILES list and click the “Open” button (Figure 7-3).

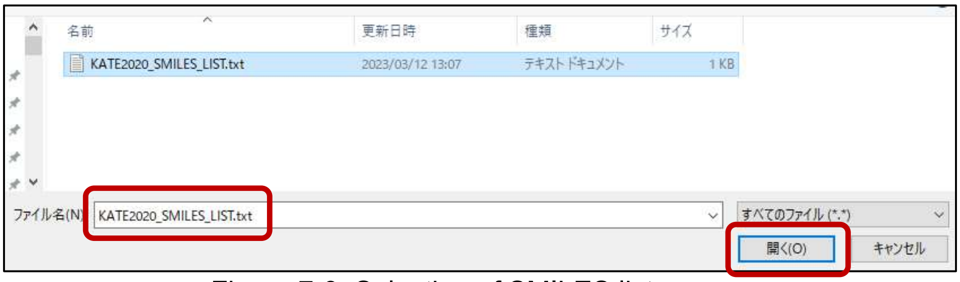


Figure 7-3. Selection of SMILES list

Step 3. Click the “Predict” button to start the prediction (Figure 7-4).

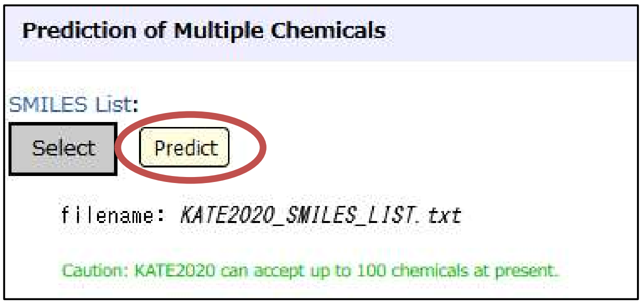
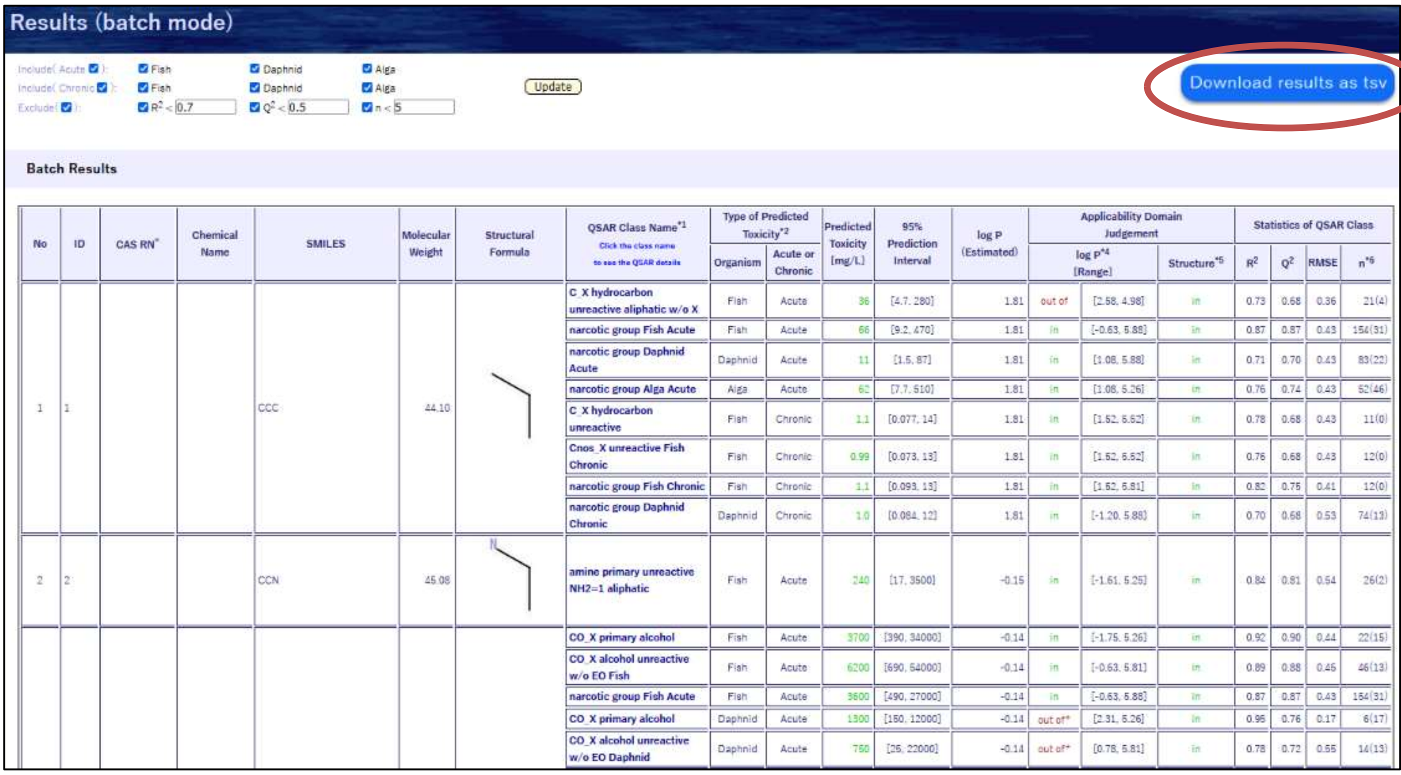


Figure 7-4. “Predict” Button for using a SMILES list

After the calculation, the prediction results are displayed (Figure 7-5).



No	ID	CAS RN*	Chemical Name	SMILES	Molecular Weight	Structural Formula	QSAR Class Name*1 Click the class name to see the QSAR details	Type of Predicted Toxicity*2 Organism	Acute or Chronic	Predicted Toxicity [mg/L]	95% Prediction Interval	log P (Estimated)	Applicability Domain Judgement log P*4	[Range]	Structure*5	R^2	Q^2	RMSE	n*6
1	1			CCC	44.10		C_X hydrocarbon unreactive aliphatic w/o X	Fish	Acute	36	[4.7, 280]	1.81	out of	[2.58, 4.98]	in	0.73	0.68	0.36	21(4)
							narcotic group Fish Acute	Fish	Acute	66	[9.2, 470]	1.81	in	[-0.63, 5.88]	in	0.87	0.87	0.43	154(31)
							narcotic group Daphnid Acute	Daphnid	Acute	11	[1.5, 87]	1.81	in	[1.08, 5.88]	in	0.71	0.70	0.43	83(22)
							narcotic group Alga Acute	Alga	Acute	62	[7.7, 510]	1.81	in	[1.08, 5.26]	in	0.76	0.74	0.43	52(46)
							C_X hydrocarbon unreactive	Fish	Chronic	1.1	[0.077, 14]	1.81	in	[1.52, 5.52]	in	0.78	0.68	0.43	11(0)
							Cnos_X unreactive Fish Chronic	Fish	Chronic	0.99	[0.073, 13]	1.81	in	[1.52, 5.52]	in	0.76	0.68	0.43	12(0)
							narcotic group Fish Chronic	Fish	Chronic	1.1	[0.093, 13]	1.81	in	[1.52, 5.81]	in	0.82	0.75	0.41	12(0)
							narcotic group Daphnid Chronic	Daphnid	Chronic	1.0	[0.084, 12]	1.81	in	[-1.20, 5.88]	in	0.70	0.68	0.53	74(13)
2	2			CCN	45.08	N	amine primary unreactive NH2=1 aliphatic	Fish	Acute	240	[17, 3500]	-0.15	in	[-1.61, 5.25]	in	0.84	0.81	0.54	26(2)
							CO_X primary alcohol	Fish	Acute	3700	[390, 34000]	-0.14	in	[-1.75, 5.26]	in	0.92	0.90	0.44	22(15)
							CO_X alcohol unreactive w/o EO Fish	Fish	Acute	6200	[690, 54000]	-0.14	in	[-0.63, 5.81]	in	0.89	0.88	0.45	46(13)
							narcotic group Fish Acute	Fish	Acute	3600	[490, 27000]	-0.14	in	[-0.63, 5.88]	in	0.87	0.87	0.43	154(31)
							CO_X primary alcohol	Daphnid	Acute	1300	[150, 12000]	-0.14	out of*	[2.31, 5.26]	in	0.95	0.76	0.17	6(17)
							CO_X alcohol unreactive w/o EO Daphnid	Daphnid	Acute	750	[25, 22000]	-0.14	out of*	[0.78, 5.81]	in	0.78	0.72	0.55	14(13)

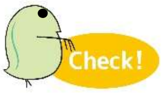
Figure 7-5. Results obtained from using a SMILES list

Click on the QSAR class name in the QSAR Class Name column to go to the Verify QSAR screen. You can also download the prediction results as a tab-delimited file by clicking on 'Download results as tsv' in the top right-hand corner of the screen. The contents of the file are shown in Figure 7-6.

ID	CAS RN	Chemical Name	SMILES	Molecular Weight	QSAR ID	QSAR Class Name	Organism	Acute or Chronic	Predicted Toxicity	95% Prediction Interval	log P Value	log P Type	log P Judgement	log P Range	Structure Judgement	R2	Q2	RMSE	n
1			CCC	44.1	12120341	C_X hydrocarbon unreactive aliphatic w/o X	Fish	Acute	36	[4.7, 280]	1.81	Estimated	out of	[2.58, 4.98]	in	0.73	0.68	0.36	21(4)
1			CCC	44.1	12899941	narcotic group Fish Acute	Fish	Acute	66	[9.2, 470]	1.81	Estimated	in	[-0.63, 5.88]	in	0.87	0.87	0.43	154(31)
1			CCC	44.1	22899941	narcotic group Daphnid Acute	Daphnid	Acute	11	[1.5, 87]	1.81	Estimated	in	[1.08, 5.88]	in	0.71	0.7	0.43	83(22)
1			CCC	44.1	32899941	narcotic group Alga Acute	Alga	Acute	62	[7.7, 510]	1.81	Estimated	in	[1.08, 5.26]	in	0.76	0.74	0.43	52(46)
1			CCC	44.1	12100151	C_X hydrocarbon unreactive	Fish	Chronic	1.1	[0.077, 14]	1.81	Estimated	in	[1.52, 5.52]	in	0.78	0.68	0.43	11(0)
1			CCC	44.1	12500151	Cnos_X unreactive Fish Chronic	Fish	Chronic	0.99	[0.073, 13]	1.81	Estimated	in	[1.52, 5.52]	in	0.76	0.68	0.43	12(0)
1			CCC	44.1	12899851	narcotic group Fish Chronic	Fish	Chronic	1.1	[0.093, 13]	1.81	Estimated	in	[1.52, 5.81]	in	0.82	0.75	0.41	12(0)
1			CCC	44.1	22899851	narcotic group Daphnid Chronic	Daphnid	Chronic	1	[0.084, 12]	1.81	Estimated	in	[-1.20, 5.88]	in	0.7	0.68	0.53	74(13)
2			CCN	45.08	12200841	amine primary unreactive NH2=1 aliphatic	Fish	Acute	240	[17, 3500]	-0.15	Estimated	in	[-1.61, 5.25]	in	0.84	0.81	0.54	26(2)
3			CCO	46.07	10600041	CO_X primary alcohol	Fish	Acute	3700	[390, 34000]	-0.14	Estimated	in	[-1.75, 5.26]	in	0.92	0.9	0.44	22(15)
3			CCO	46.07	12102041	CO_X alcohol unreactive w/o EO Fish	Fish	Acute	6200	[690, 54000]	-0.14	Estimated	in	[-0.63, 5.81]	in	0.89	0.88	0.45	46(13)
3			CCO	46.07	12899941	narcotic group Fish Acute	Fish	Acute	3600	[490, 27000]	-0.14	Estimated	in	[-0.63, 5.88]	in	0.87	0.87	0.43	154(31)
3			CCO	46.07	20600041	CO_X primary alcohol	Daphnid	Acute	1300	[150, 12000]	-0.14	Estimated	out of+	[2.31, 5.26]	in	0.95	0.76	0.17	6(17)
3			CCO	46.07	22102241	CO_X alcohol unreactive w/o EO Daphnid	Daphnid	Acute	750	[25, 22000]	-0.14	Estimated	out of+	[0.78, 5.81]	in	0.78	0.72	0.55	14(13)
3			CCO	46.07	22899941	narcotic group Daphnid Acute	Daphnid	Acute	260	[30, 2200]	-0.14	Estimated	out of+	[1.08, 5.88]	in	0.71	0.7	0.43	83(22)
3			CCO	46.07	30600041	CO_X primary alcohol	Alga	Acute	28000	[260, 2.9e+6]	-0.14	Estimated	out of+	[2.31, 5.26]	in	0.91	0.79	0.36	6(16)
3			CCO	46.07	32102041	CO_X alcohol unreactive w/o halogen, acid, EO	Alga	Acute	12000	[380, 360000]	-0.14	Estimated	out of+	[1.08, 5.26]	in	0.95	0.9	0.35	6(14)
3			CCO	46.07	32899941	narcotic group Alga Acute	Alga	Acute	5100	[480, 55000]	-0.14	Estimated	out of+	[1.08, 5.26]	in	0.76	0.74	0.43	52(46)
3			CCO	46.07	12899851	narcotic group Fish Chronic	Fish	Chronic	30	[1.4, 630]	-0.14	Estimated	out of	[1.52, 5.81]	in	0.82	0.75	0.41	12(0)
3			CCO	46.07	22102251	CO_X alcohol unreactive w/o EO Daphnid	Daphnid	Chronic	24	[1.4, 410]	-0.14	Estimated	in	[-1.20, 5.81]	in	0.82	0.75	0.52	14(8)
3			CCO	46.07	22899851	narcotic group Daphnid Chronic	Daphnid	Chronic	16	[1.2, 210]	-0.14	Estimated	in	[-1.20, 5.88]	in	0.7	0.68	0.53	74(13)
3			CCO	46.07	32102051	CO_X alcohol unreactive w/o halogen, acid, EO	Alga	Chronic	1100	[24, 54000]	-0.14	Estimated	out of+	[0.69, 5.26]	in	0.87	0.81	0.59	10(9)

Figure 7-6.tsv file of prediction results of multiple chemicals

(3) Additional information



- If there is a line in the SMILES list that contains an error, that line will be skipped, and an error message will be displayed in the corresponding row of the prediction results.
- The maximum number of chemical substances the system can predict at once is 100.
- The time needed for prediction depends on the structures of the chemical substances contained in the SMILES list. It usually takes no longer than 30 min to finish the calculation. If it is taking longer, there may be a problem with the calculation. To address the issue, try to reduce the number of chemical substances in the SMILES list or enter a single chemical substance in order to check if the system is operating normally. If you find an issue with a particular SMILE, please contact the KATE Contact Desk (KATE@nies.go.jp).

8. Printing the Prediction Results

In the prediction results screen, click the button **Create Print Format** to format the prediction results for printing. The resulting screen will contain the prediction results for all the QSAR classes that were selected on the main results screen (Figure 8).

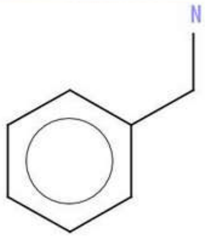
(1) → Ecotoxicity Prediction by KATE2020 version 4.0
March 12, 2023 at 13:28 (JST)

[http://kate.nies.go.jp]

国立研究開発法人 国立環境研究所 National Institute for Environmental Studies

(2) → **Results**

CAS RN®		
Chemical Name		
SMILES	NCc1ccccc1	
Molecular Weight	107.15	
log P	User Input Value	
	Estimated Value by KOWWIN	1.07
	Measured Value in KOWWIN Database	1.09

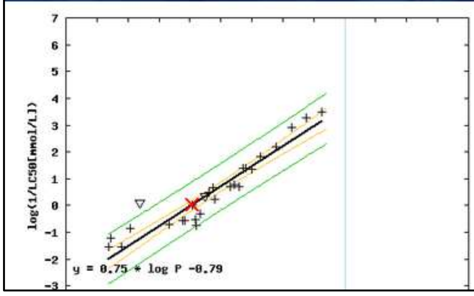


Include(Acute ☑): ☑ Fish ☑ Daphnid ☑ Alga
Include(Chronic ☑): ☑ Fish ☑ Daphnid ☑ Alga
Exclude(☐): ☐ R^2 < 0.7 ☐ Q^2 < 0.5 ☐ n < 5

QSAR Results

Print Detail	QSAR Class Name*1	Type of Predicted Toxicity*2		Predicted Toxicity [mg/L]	95% Prediction Interval	log P		Applicability Domain Judgement			Statistics of QSAR Class			
		Organism	Acute or Chronic			Value	Type	log P*3 [Range]		Structure*4	R^2	Q^2	RMSE	n*5
☑	amine primary unreactive NH2 =1 aliphatic	Fish	Acute	100	[13, 800]	1.07	Estimated	in	[-1.61, 5.25]	in	0.92	0.90	0.40	25(2)
☑	amine unreactive NH2 =1 aliphatic	Daphnid	Acute	30	[0.21, 4300]	1.07	Estimated	in	[-1.61, 3.00]	in	0.78	-2.11	0.32	4(1)
☑	amine unreactive NH2 =1 aliphatic (alga)	Alga	Acute	9.3	[0.00087, 100000]	1.07	Estimated	in	[-1.61, 3.00]	in	0.09	-3.92	0.59	4(0)
☐	CNO_X unreactive (Fish chronic), excl. (CnosX w/o n+)	Fish	Chronic	0.22	[0.0097, 5.0]	1.07	Estimated	in	[-1.61, 5.99]	in	0.62	0.54	0.57	19(2)
☐	N_X amine aliphatic NH2=1	Daphnid	Chronic	0.14	[0.0026, 7.8]	1.07	Estimated	in	[-1.61, 3.19]	in	0.45	0.29	0.76	19(0)
☐	amine unreactive NH2 =1 aliphatic	Daphnid	Chronic	1.2	[0.020, 77]	1.07	Estimated	in	[-1.61, 1.63]	in	0.23	-2.19	0.25	4(0)
☐	amine unreactive NH2 =1 aliphatic (alga)	Alga	Chronic	1.8	[0.00010, 31000]	1.07	Estimated	in	[-1.61, 3.00]	in	0.32	-2.65	0.62	4(0)

(3) → Type: **Fish (acute)** Structure Class ID: **G1_22008** QSAR Class Name: **amine primary unreactive NH2 =1 aliphatic**



× : Query chemical
\+ : Reference chemical
—— : Regression line
—— : 95% confidence interval for the regression line
—— : 95% prediction interval for log(1/LC50, EC50, or NOEC)

----Support Data----

* : Support chemical with log P>6.0
▽△ : Data with "<" or ">"
◇ : Outlier

----When "+" is deleted----

□ : Deleted data
—— : Regression line w/o deleted data

Figure 8. Print Format screen

(1) Title, Date and time when the prediction results were output (Japan Standard Time)
(2) QSAR results (Checks are unchangeable)
(3) Results for individual QSAR classes