



<https://www.docs.isalos.novamechanics.com/>

The objective of this practical session is to investigate how the physicochemical properties of per- and polyfluoroalkyl substances (PFAS) influence their environmental fate and potential human exposure. We will apply a structured modelling workflow that integrates environmental fate simulations, statistical analysis, and machine-learning modelling using three computational tools: SimpleBox4Planet (<https://www.enalosccloud.novamechanics.com/proplanet/simplebox4planet/>) and Isalos Analytics Platform (<https://enalosccloud.novamechanics.com/novamechanicssystem/userregistration/>).

In addition to evaluating the influence of key physicochemical properties, the practical will aim to identify combinations of property values and levels that minimize environmental mass, thereby supporting the design of safer alternative PFAS within a Safe-and-Sustainable-by-Design (SSbD) framework. Furthermore, a machine-learning surrogate model will be developed to predict environmental mass directly, reducing the need to repeatedly run the underlying mechanistic mass-balance and differential-equation models and thus lowering the computational cost of screening.

Please, follow the steps presented in the following link to download and install Isalos Analytics on your local PCs: <https://www.docs.isalos.novamechanics.com/installation.html>.

Isalos version used: 2.1.2

Step-by-step guide for implementing in Isalos

Design of Experiment using Isalos

Step 1: Import data

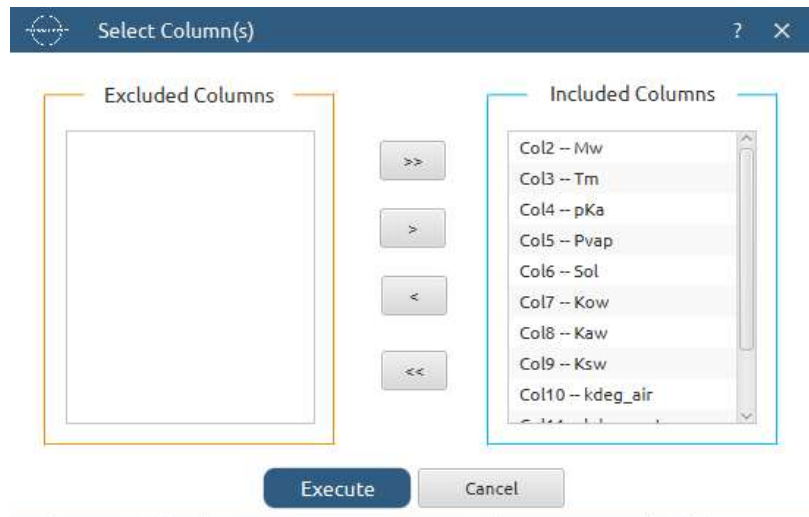
Manually insert data as shown in the table below into Isalos on the input spreadsheet (left).

Mw	Tm	pKa	Pvap	Sol	Kow	Kaw	Ksw	kdeg_air	kdeg_water	kdeg_sed	kdeg_soil
200.0	40.0	-3.0	1.0E-9	10.0	100.0	1.0E-8	0.1	1.0E-7	1.0E-8	1.0E-11	1.0E-10
800.0	350.0	4.0	0.01	10000.0	1000000.0	0.001	100.0	1.0E-6	1.0E-7	1.0E-9	1.0E-8

	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)	Col9 (D)	Col10 (D)	Col11 (D)	Col12 (D)	Col13 (D)
User Header	User Row ID	Mw	Tm	pKa	Pvap	Sol	Kow	Kaw	Ksw	kdeg_air	kdeg_water	kdeg_sed	kdeg_soil
1		200.0	40.0	-3.0	1.0E-9	10.0	100.0	1.0E-8	0.1	1.0E-7	1.0E-8	1.0E-11	1.0E-10
2		800.0	350.0	4.0	0.01	10000.0	1000000.0	0.001	100.0	1.0E-6	1.0E-7	1.0E-9	1.0E-8

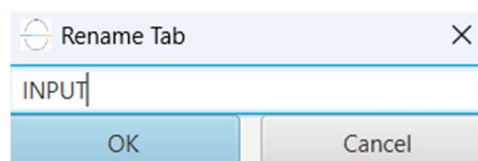
Step 2: Manipulate data

Select all columns by navigating *Data Transformation > Data Manipulation > Select Column(s)* and click on 'Execute' button.



User Header	Col1	Col2 (D)	Col3 (D)	Col4 (D)	Col5 (D)	Col6 (D)	Col7 (D)	Col8 (D)
User Row ID	Mw	Tm	pKa	Pvpap	Sol	Kow	Kaw	Ksw
1	200.0	40.0	-3.0	1.0E-9	10.0	100.0	0E-7	0E-7
2	800.0	350.0	4.0	0.01	10000.0	1000000.0	0.001	
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								

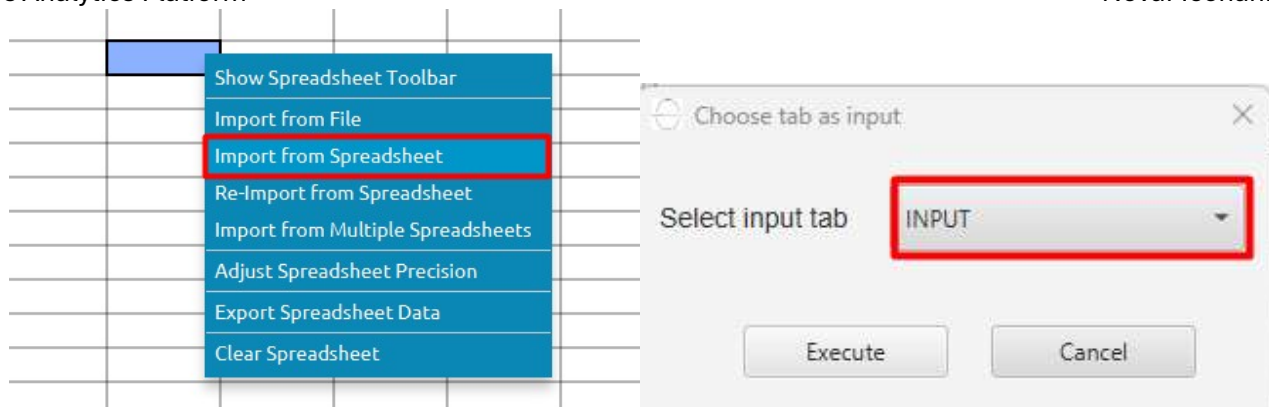
Rename the sheet to 'INPUT' by right-clicking on the node and selecting 'Rename'.



Step 3: Create new sheet for Plackett Burman

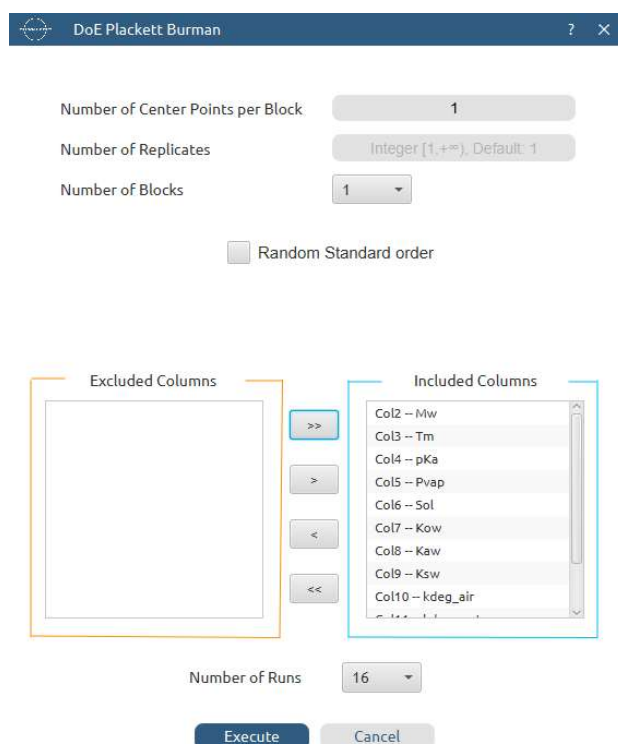
Create a new sheet and name it as 'PLACKETT BURMAN'.

Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet'. Then select 'INPUT'.



Step 4: Plackett Burman

Design Plackett Burman by selecting from the tabs *DOE > Screening > Plackett Burman*. Once the configuration window appears, make the selections as indicated below and proceed by clicking the 'Execute' button.



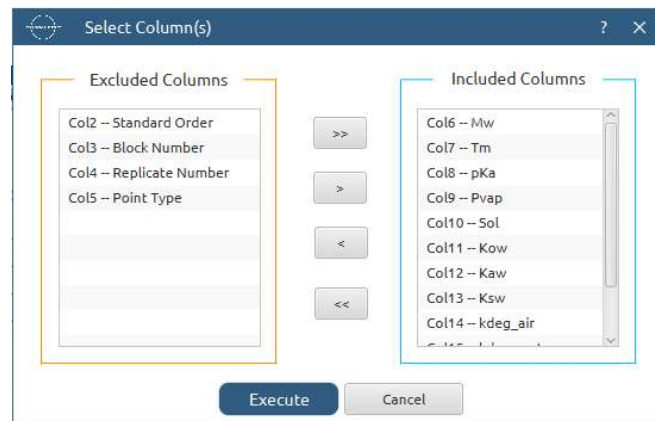
Hint:

The analysis began with a screening design, specifically a Plackett–Burman design, to identify the most influential factors affecting the environmental fate response and to provide an initial indication of whether the system could be described using a linear model. Since the Plackett–Burman design is primarily intended for screening purposes, it can only estimate main effects and does not capture interaction or quadratic terms.

Step 5: PREPROCESS: Import & Select

Create a new sheet and name it as 'PREPROCESS'. Import data from the output of the 'PLACKETT BURMAN' sheet.

Then select from tabs *Data Transformation > Data Manipulation > Select Column(s)*. Ensure to replicate the selection of columns as indicated below, and then click the 'Execute' button.



Step 6: Export the output as a CSV file

Export the output to a CSV file by right-clicking on the output spreadsheet (on the right) and selecting 'Export Spreadsheet Data.' Choose 'CSV' as the file extension and click the 'Execute' button.

Name the file 'ScreeningDesign' and save it. Subsequently, open the file, delete the first column, and save the file again.

10.0	1000000.0	0E-7	100.0	0.0000010	1E-
10000.0	100.0	0.001	100.0	0.0000010	1E-
10.0	1000			0.0000010	0E-
10000.0	1000			1E-7	0E-
10000.0	1000			1E-7	0E-
10000.0	1000			1E-7	1E-
10000.0	100.0	0E-7	0.1	0.0000010	0E-

Compute environmental mass using SimpleBox4Planet

Step 7: Access Sensitivity Exploration

Open the following URL link:

<https://AN/www.enaloscloud.novamechanics.com/proplanet/simplebox4planet/> and navigate to the bottom of the page. Select 'Sensitivity Exploration' button.

Step 8: Upload Input File

When a new window appears, click on 'Upload CSV' button and select the file 'ScreeningDesign.csv' which created in the previous step.

Step 9: Execute & Download Results

After the data is successfully loaded, click on the 'Execute' button to generate the total mass produced (in kg).

Finally, click on the 'Download Results (Excel)' button to download the results, naming the file 'ScreeningDesignResults.xlsx'.

Sensitivity Exploration

Buttons: Add Row, Remove Selected, Upload CSV, Download CSV Template, Clear, Execute, Download Results (Excel)

Input scenarios (Mw → kdeg(soil)). Add rows manually or upload CSV.

Row	Mw [g mol ⁻¹]	Tm [°C]	pKa	Pvap [Pa]	Sol25 [mg L ⁻¹]	Kaw	Kow	Ksw	kdeg(air) [s ⁻¹]	kdeg(water) [s ⁻¹]	kdeg(sed) [s ⁻¹]	kdeg(soil) [s ⁻¹]	Total (kg)
☐	200	40	-3	0.01	10	100	0.001	100	1e-7	1e-7	1e-11	1e-8	4.19432e+07
☐	200	40	4	1e-9	10	1000000	0.001	0.1	0.000001	1e-8	1e-9	1e-8	3.83110e+07
☐	200	350	-3	1e-9	10000	1000000	1e-8	100	1e-7	1e-7	1e-9	1e-8	4.52409e+07
☐	800	40	-3	0.01	10000	100	0.001	0.1	0.000001	1e-7	1e-9	1e-8	1.73039e+07
☐	200	40	4	0.01	10	1000000	1e-8	100	0.000001	1e-7	1e-9	1e-10	3.17755e+09
☐	200	350	4	1e-9	10000	100	0.001	100	0.000001	1e-7	1e-11	1e-10	2.84037e+08
☐	800	350	-3	0.01	10	1000000	0.001	100	0.000001	1e-8	1e-11	1e-10	3.19557e+09
☐	800	40	4	1e-9	10000	1000000	0.001	100	1e-7	1e-8	1e-11	1e-8	4.73348e+07

Execution completed for 17 rows.

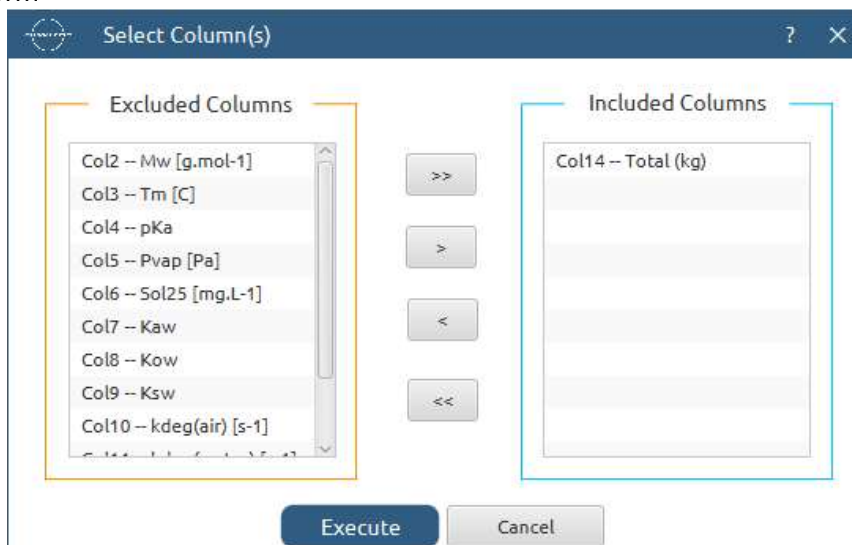
Statistical Analysis using Isalos

Step 10: Create Sheet & Import Results

Create a new sheet in Isalos Analytics and name it as 'RESULTS-SCREENING'. Right-click on the input (left) spreadsheet and select 'Import from File' to import the ScreeningDesignResults.xlsx created in the previous step.

Step 11: Select Output Column

Then navigate to *Data Transformation > Data Manipulation > Select Column(s)* and select only the column corresponding to the generated output, as shown below.



Step 12: PREPROCESS-v2: Merge Data

Create a new sheet and name it as 'PREPROCESS-v2'. Right-click on the input (left) spreadsheet and select 'Import from Multiple Spreadsheets'. Select the 'PREPROCESS' as the Left Spreadsheet and 'RESULTS-SCREENING' as the Right Spreadsheet, then click on 'Execute' button.

	Col1	Col2	Col3	Col4	Col5	Col6
User Header	User Row ID					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						

Show Spreadsheet Toolbar
 Import from File
 Import from Spreadsheet
 Import from Multiple Spreadsheets
 Adjust Spreadsheet Precision
 Export Spreadsheet Data
 Clear Spreadsheet

Multiple Spreadsheet Joiner

?

×

Join Configuration Steps

Step 1: PREPROCESS

RESULTS-SCREENING

Add

Delete

Join Type

☒ Concatenation ☐ Left Join ☐ Right Join ☐ Inner Join ☐ Full Outer Join

Left Spreadsheet

PREPROCESS

Right Spreadsheet

RESULTS-SCREENING

Join Column

☒ Common header name ☐ Different header names

Execute

Cancel

Step 13: PREPROCESS-v2: Select Columns

Navigate to *Data Transformation > Data Manipulation > Select Column(s)* and select the columns shown below.

Select Column(s)

?

×

Excluded Columns

Col14 -- User Row ID_RESULTS

>>

>

<

<<

Included Columns

Col2 -- Mw

Col3 -- Tm

Col4 -- pKa

Col5 -- Pvap

Col6 -- Sol

Col7 -- Kow

Col8 -- Kaw

Col9 -- Ksw

Col10 -- kdeg_air

Execute

Cancel

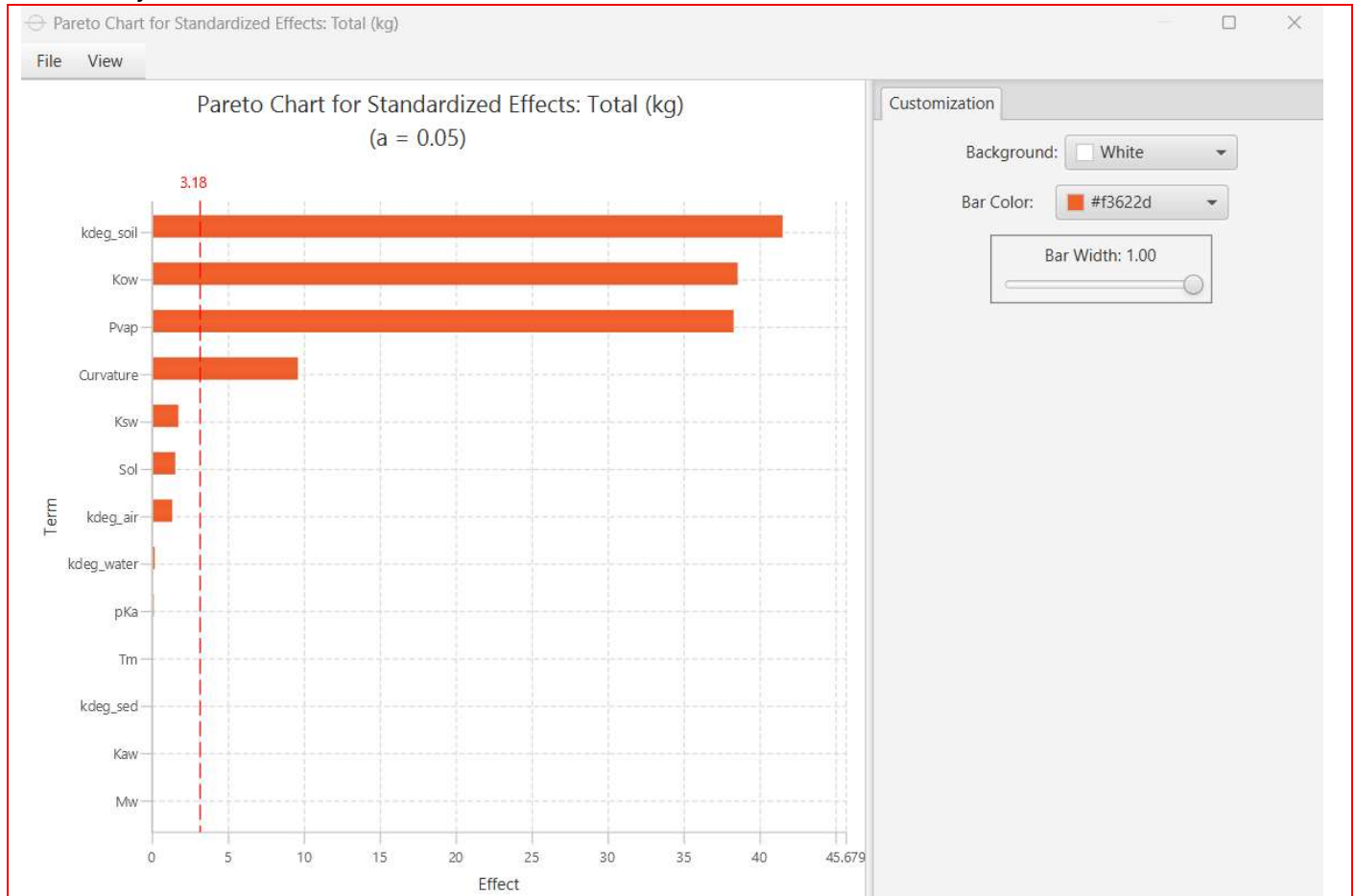
Step 14: PARETO

Create a new sheet and name it as 'PARETO'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v2'. Navigate to *DOE > Post DoE Analysis > Pareto Analysis* and select the configuration as shown below.

The screenshot shows the 'Pareto Analysis' dialog box. It has a title bar with a help icon, the text 'Pareto Analysis', and a close button. The dialog contains the following fields and controls:

- Dependent Variable:** A dropdown menu showing 'Col14 -- Total (kg)'.
- Analysis Type:** A dropdown menu showing 'Main Effects'.
- Level Of Significance:** A text input field containing '0.05'.
- Excluded Columns:** An empty list box on the left.
- Factors:** A list box on the right containing 'Col2 -- Mw', 'Col3 -- Tm', 'Col4 -- pKa', and 'Col5 -- Pvpap'. It has a scroll bar.
- Covariates:** An empty list box at the bottom right.
- DOE type:** Two radio buttons. The first is 'Factorial / Screening' (selected) and the second is 'Response Surface'.
- Include Center Points:** A checked checkbox.
- Buttons:** 'Execute' and 'Cancel' buttons at the bottom.

Output:

**Observation:**

The analysis indicates that kdeg(soil), Kow and Pvp effects exceed the statistical significance threshold and are therefore identified as the dominant factors influencing the environmental fate response. All remaining variables fall below the threshold and are considered non-significant within the explored design space and thus are excluded from further analysis.

Step 15: ANCOVA-v1

Create a new sheet and name it as 'ANCOVA-v1'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v2'.

Navigate to *Statistics > Analysis of (Co)Variance > ANCOVA* and select the configuration as shown below.

- Based on the Pareto plot, the model equation was selected for further analysis using ANCOVA.

ANCOVA

?

×

Confidence Level (%)

95

Dependent Variable

Col14 -- Total (kg)

Sum of Squares for Tests

Adjusted (Type III)

Coding for Factors

(-1, 0, +1)

Excluded Columns

>

<

Factors

Specify Reference Levels

>

<

Covariates

Col2 -- Mw
Col3 -- Tm
Col4 -- pKa
Col5 -- Pvap

Formula Type:

☐ Custom

☒ Main Effects

☐ Full Factorial

Specify Custom Model Formula

Formula:

Mw + Tm + pKa + Pvap + Sol + Kow + Kaw + Ksw + kdeg_air + kdeg_water +

Execute

Cancel

Output

A table analyzing the variance/covariance of each term in the formula is outputted in the output spreadsheet.

User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value	Significant
	Mw	1	10926826080 512	10926826080 512	0.0000736	0.9935661	No
	Tm	1	41062784400 128	41062784400 128	0.0002766	0.9875281	No
	pKa	1	91610262254 976	91610262254 976	0.0006170	0.9813727	No
	Pvap	1	9.1253460713 92502E+18	9.1253460713 92502E+18	61.4603111	0.0014298	Yes
	Sol	1	1.4800480394 7008E+16	1.4800480394 7008E+16	0.0996830	0.7679976	No
	Kow	1	9.2533811682 59553E+18	9.2533811682 59553E+18	62.3226430	0.0013924	Yes
	Kaw	1	1.5515130155 52E+13	1.5515130155 52E+13	0.0001045	0.9923334	No
	Ksw	1	1.8980345580 18035E+16	1.8980345580 18035E+16	0.1278349	0.7387548	No
	kdeg_air	1	1.1277733781 05536E+16	1.1277733781 05536E+16	0.0759569	0.7965049	No
	kdeg_water	1	22057373547 5328	22057373547 5328	0.0014856	0.9711014	No
	kdeg_sed	1	21426020880 256	21426020880 256	0.0001443	0.9909907	No
	kdeg_soil	1	1.0726843825 873908E+19	1.0726843825 873908E+19	72.2465925	0.0010507	Yes
	Error	4	5.9390171660 37034E+17	1.4847542915 092586E+17			
	Total	16	2.9744932456 64485E+19				

Observation:

Only Pvp, Kow, and kdeg_soil are statistically significant ($p < 0.05$), indicating that these variables are the main drivers of the response, while all other factors show no significant effect.

Step 16: Factorial-Plots

Create a new sheet and name it as 'FACTORIAL-PLOTS'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v2'.

Navigate to *DOE > Post DoE Analysis > Factorial Plot Analysis* and select the configuration as shown below.

Factorial Plot Analysis

Dependent Variable

Col14 -- Total (kg)

Analysis Type

Main Effects

Excluded Columns

>

<

Factors

Col2 -- Mw

Col3 -- Tm

Col4 -- pKa

Col5 -- Pvpap

Specify Factor Values

Covariates

>

<

DOE type:

Factorial / Screening

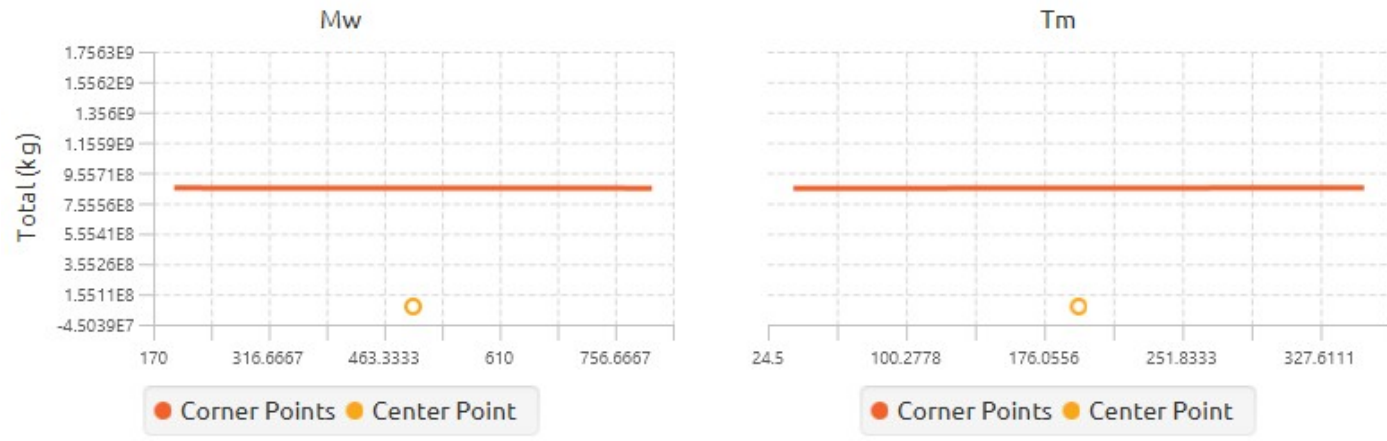
Response Surface

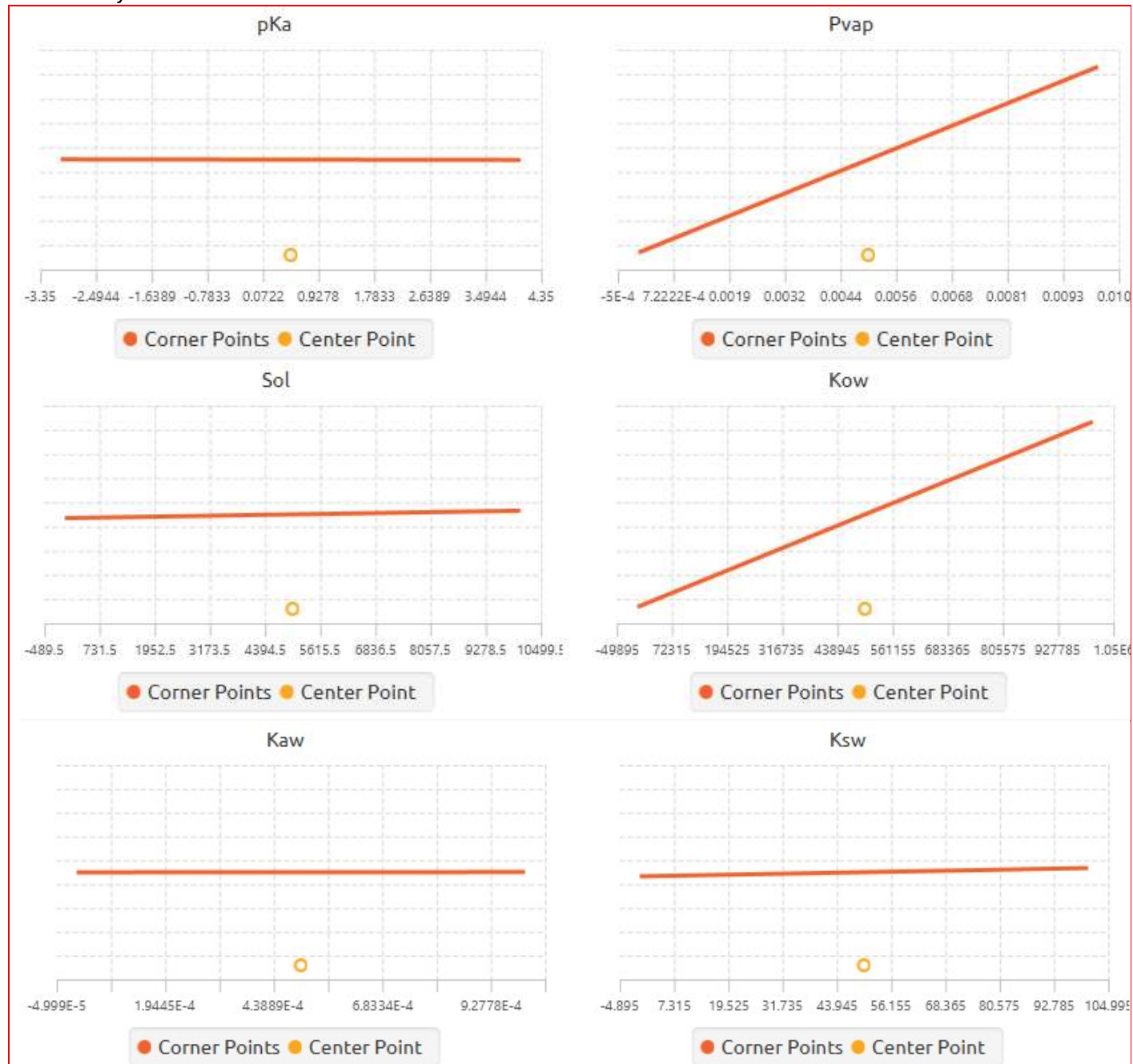
Include Center Points

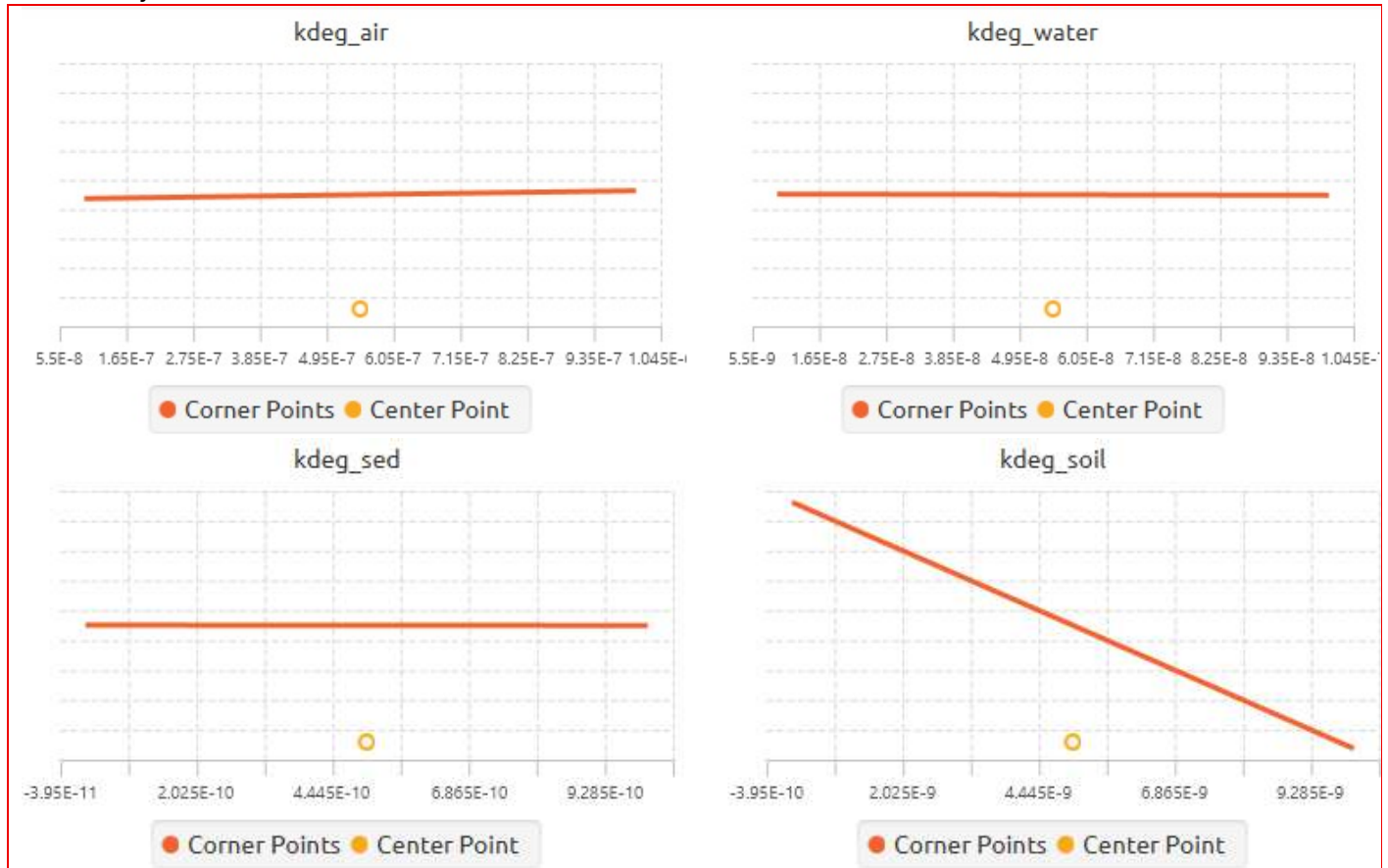
Execute

Cancel

Output:







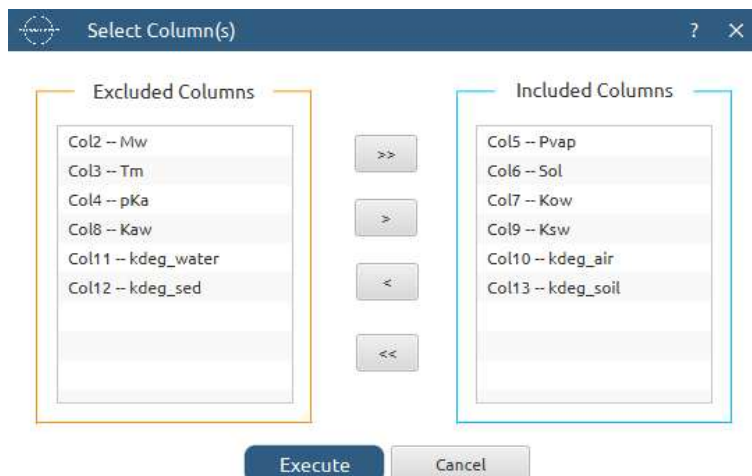
Observation:

The factorial plot analysis indicates that a linear model is not sufficient to describe the system behavior. For all examined factors, the center points do not lie on the straight line connecting the low and high factor levels, indicating the presence of curvature in the response. This deviation suggests that the relationship between the physicochemical properties and the environmental fate response is non-linear, thereby supporting the need for an RSM design to adequately capture these curvature effects.

Step 17: PREPROCESS- v3: Import & Select

Create a new sheet and name it as 'PREPROCESS-v3'. Import data from the 'INPUT' sheet.

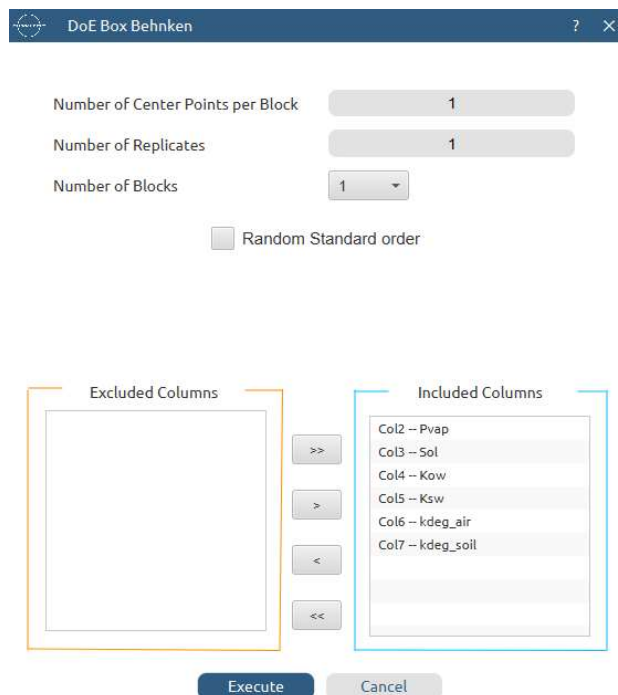
Navigate to *Data Transformation > Data Manipulation > Select Column(s)* and select columns according to the specified selections below.

**Hint:**

Since the significant factors had already been identified (step 10) during the screening stage, the least influential variables were excluded from the subsequent RSM analysis in order to reduce model complexity and focus on the most relevant physicochemical drivers. Accordingly, kdeg(soil), Kow, and Pvap were retained as clearly significant factors. In addition, Ksw, Sol, and kdeg(air) were also included in the RSM study, although they were slightly below the significance threshold, because their effects were close enough to the cutoff to suggest potential relevance in the presence of curvature or interaction effects.

Step 18: BOX BEHNKEN

Create a new sheet and name it as 'BOX BEHNKEN'. Import data from the 'PREPROCESS-v3' sheet. Navigate to *DOE > Response Surface > Box Behnken*. Follow the specified selections outlined below and click on 'Execute'.



DoE Box Behnken

Number of Center Points per Block: 1

Number of Replicates: 1

Number of Blocks: 1

☐ Random Standard order

Excluded Columns: (empty)

Included Columns:

- Col2 -- Pvap
- Col3 -- Sol
- Col4 -- Kow
- Col5 -- Ksw
- Col6 -- kdeg_air
- Col7 -- kdeg_soil

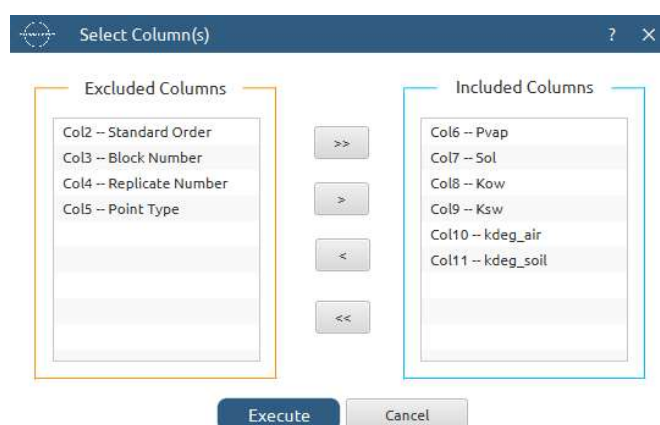
Execute Cancel

Hint:

The Box–Behnken design was selected because it efficiently estimates quadratic models while requiring fewer experimental runs compared to full factorial designs, and avoids extreme corner points, making it suitable for stable environmental modelling studies.

Step 19: PREPROCESS- v4: Import & Select

Create a new sheet and name it as 'PREPROCESS-v4'. Import data from the 'BOX BEHNKEN' sheet. Navigate to *Data Transformation > Data Manipulation > Select Column(s)* and select columns according to the specified selections below.



Select Column(s)

Excluded Columns:

- Col2 -- Standard Order
- Col3 -- Block Number
- Col4 -- Replicate Number
- Col5 -- Point Type

Included Columns:

- Col6 -- Pvap
- Col7 -- Sol
- Col8 -- Kow
- Col9 -- Ksw
- Col10 -- kdeg_air
- Col11 -- kdeg_soil

Execute Cancel

Step 20: RESULTS-RSM: Create Sheet & Paste Data

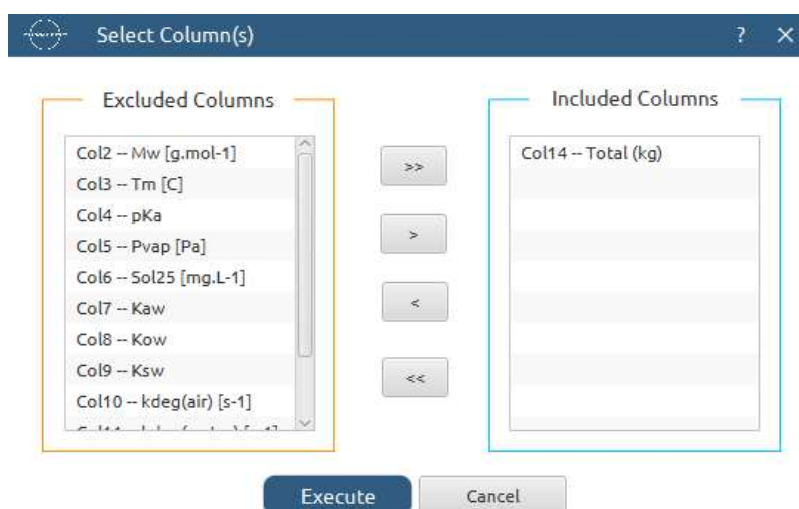
Create a new sheet and name it as 'RESULTS-RSM'. Copy and paste the table below on the input (left) spreadsheet.

Mw	Tm	pKa	Pvap	Sol	Kow	Kaw	Ksw	kdeg_air	kdeg_water	kdeg_sed	kdeg_soil	Total (kg)
500	195	0.5	1E-09	10	500050	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.37001e+07
500	195	0.5	0.01	10	500050	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.37655e+07
500	195	0.5	1E-09	10000	500050	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.37001e+07
500	195	0.5	0.01	10000	500050	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.37655e+07
500	195	0.5	1E-09	10	500050	0.0005	100	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.38901e+07
500	195	0.5	0.01	10	500050	0.0005	100	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.39547e+07
500	195	0.5	1E-09	10000	500050	0.0005	100	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.38901e+07
500	195	0.5	0.01	10000	500050	0.0005	100	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.39547e+07
500	195	0.5	0.005	10	100	0.0005	50.05	1E-07	5.5E-08	5.05E-10	5.05E-09	6.12784e+07
500	195	0.5	0.005	10000	100	0.0005	50.05	1E-07	5.5E-08	5.05E-10	5.05E-09	6.12784e+07
500	195	0.5	0.005	10	1000000	0.0005	50.05	1E-07	5.5E-08	5.05E-10	5.05E-09	8.08496e+07
500	195	0.5	0.005	10000	1000000	0.0005	50.05	1E-07	5.5E-08	5.05E-10	5.05E-09	8.08496e+07
500	195	0.5	0.005	10	100	0.0005	50.05	0.000001	5.5E-08	5.05E-10	5.05E-09	5.31330e+07
500	195	0.5	0.005	10000	100	0.0005	50.05	0.000001	5.5E-08	5.05E-10	5.05E-09	5.31330e+07
500	195	0.5	0.005	10	1000000	0.0005	50.05	0.000001	5.5E-08	5.05E-10	5.05E-09	7.31556e+07
500	195	0.5	0.005	10000	1000000	0.0005	50.05	0.000001	5.5E-08	5.05E-10	5.05E-09	7.31556e+07
500	195	0.5	0.005	5005	100	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	1E-10	3.53034e+07
500	195	0.5	0.005	5005	1000000	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	1E-10	3.18569e+09
500	195	0.5	0.005	5005	100	0.0005	100	5.5E-07	5.5E-08	5.05E-10	1E-10	3.00012e+08
500	195	0.5	0.005	5005	1000000	0.0005	100	5.5E-07	5.5E-08	5.05E-10	1E-10	3.18593e+09
500	195	0.5	0.005	5005	100	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	1E-08	1.99702e+07
500	195	0.5	0.005	5005	1000000	0.0005	0.1	5.5E-07	5.5E-08	5.05E-10	1E-08	3.84344e+07
500	195	0.5	0.005	5005	100	0.0005	100	5.5E-07	5.5E-08	5.05E-10	1E-08	3.51830e+07
500	195	0.5	0.005	5005	1000000	0.0005	100	5.5E-07	5.5E-08	5.05E-10	1E-08	3.86233e+07
500	195	0.5	1E-09	5005	500050	0.0005	0.1	1E-07	5.5E-08	5.05E-10	5.05E-09	8.06886e+07
500	195	0.5	0.01	5005	500050	0.0005	0.1	1E-07	5.5E-08	5.05E-10	5.05E-09	8.07525e+07
500	195	0.5	1E-09	5005	500050	0.0005	100	1E-07	5.5E-08	5.05E-10	5.05E-09	8.08734e+07
500	195	0.5	0.01	5005	500050	0.0005	100	1E-07	5.5E-08	5.05E-10	5.05E-09	8.09367e+07
500	195	0.5	1E-09	5005	500050	0.0005	0.1	0.000001	5.5E-08	5.05E-10	5.05E-09	7.29903e+07
500	195	0.5	0.01	5005	500050	0.0005	0.1	0.000001	5.5E-08	5.05E-10	5.05E-09	7.30557e+07
500	195	0.5	1E-09	5005	500050	0.0005	100	0.000001	5.5E-08	5.05E-10	5.05E-09	7.31807e+07
500	195	0.5	0.01	5005	500050	0.0005	100	0.000001	5.5E-08	5.05E-10	5.05E-09	7.32455e+07
500	195	0.5	0.005	10	500050	0.0005	50.05	1E-07	5.5E-08	5.05E-10	1E-10	3.16038e+09
500	195	0.5	0.005	10000	500050	0.0005	50.05	1E-07	5.5E-08	5.05E-10	1E-10	3.16038e+09
500	195	0.5	0.005	10	500050	0.0005	50.05	0.000001	5.5E-08	5.05E-10	1E-10	3.15254e+09
500	195	0.5	0.005	10000	500050	0.0005	50.05	0.000001	5.5E-08	5.05E-10	1E-10	3.15254e+09
500	195	0.5	0.005	10	500050	0.0005	50.05	1E-07	5.5E-08	5.05E-10	1E-08	4.54982e+07
500	195	0.5	0.005	10000	500050	0.0005	50.05	1E-07	5.5E-08	5.05E-10	1E-08	4.54982e+07
500	195	0.5	0.005	10	500050	0.0005	50.05	0.000001	5.5E-08	5.05E-10	1E-08	3.78057e+07
500	195	0.5	0.005	10000	500050	0.0005	50.05	0.000001	5.5E-08	5.05E-10	1E-08	3.78057e+07
500	195	0.5	1E-09	5005	100	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-10	1.55376e+08
500	195	0.5	0.01	5005	100	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-10	1.74744e+08

500	195	0.5	1E-09	5005	1000000	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-10	3.12269e+09
500	195	0.5	0.01	5005	1000000	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-10	3.18736e+09
500	195	0.5	1E-09	5005	100	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-08	3.24302e+07
500	195	0.5	0.01	5005	100	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-08	3.27370e+07
500	195	0.5	1E-09	5005	1000000	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-08	3.85112e+07
500	195	0.5	0.01	5005	1000000	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	1E-08	3.85193e+07
500	195	0.5	0.005	5005	500050	0.0005	50.05	5.5E-07	5.5E-08	5.05E-10	5.05E-09	7.38488e+07

Step 21: Select Output Column

Navigate to *Data Transformation > Data Manipulation > Select Column(s)* and select only the column corresponding to the generated output, as shown below.



Step 22: PREPROCESS-v5: Merge Data

Create a new sheet and name it as 'PREPROCESS-v5'. Right-click on the input (left) spreadsheet and select 'Import from Multiple Spreadsheets'. Select the 'PREPROCESS-v4' as the Left Spreadsheet and 'RESULTS-RSM' as the Right Spreadsheet, then click on 'Execute' button.

	Col1	Col2	Col3	Col4	Col5	Col6
User Header	User Row ID					
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						

Show Spreadsheet Toolbar
Import from File
Import from Spreadsheet
Import from Multiple Spreadsheets
Adjust Spreadsheet Precision
Export Spreadsheet Data
Clear Spreadsheet

Step 23: PREPROCESS-v5: Select Columns

Navigate to Data Transformation > Data Manipulation > Select Column(s) and select the columns shown below.

Step 24: PARETO-RSM

Create a new sheet and name it as 'PARETO-RSM'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v5'.

Navigate to *DOE > Post DoE Analysis > Pareto Analysis* and select the configuration as shown below.

Pareto Analysis

Dependent Variable

Col8 -- Total (kg)

Analysis Type

Main Effects + Two-Factor...

Level Of Significance

0.05

Excluded Columns

>

<

Factors

Col2 -- Pvap

Col3 -- Sol

Col4 -- Kow

Col5 -- Ksw

Covariates

>

<

DOE type:

Factorial / Screening

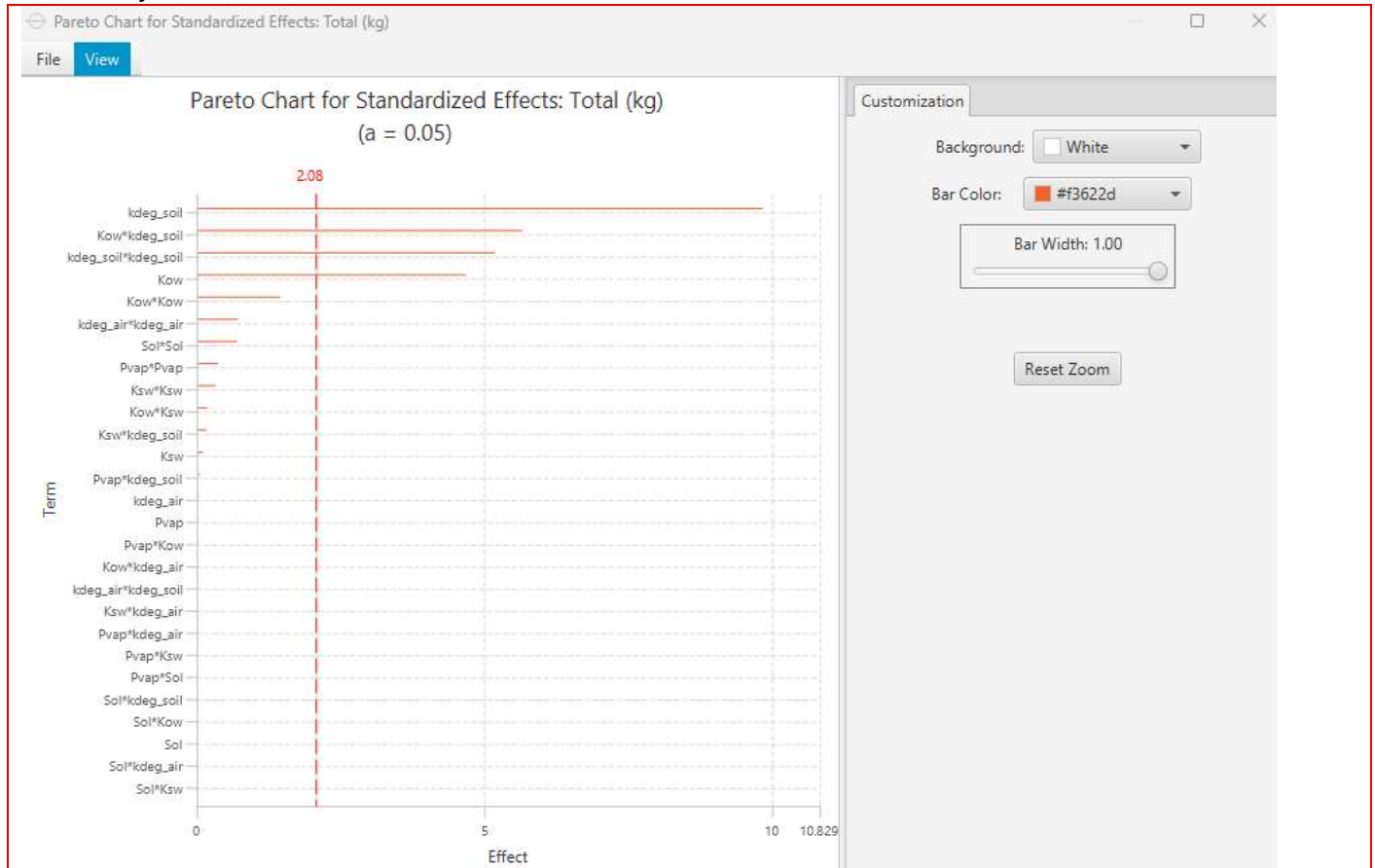
Response Surface

Include Center Points

Execute

Cancel

Output:



Observation:

The Pareto analysis of the Box Behnken results showed that kdeg(soil), the interaction term Kow × kdeg(soil), the quadratic term $kdeg(soil)^2$, and Kow were the most important statistically significant contributors to the environmental fate response.

Step 25: ANCOVA-v2

Create a new sheet and name it as 'ANCOVA-v2'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v5'.

Navigate to *Statistics > Analysis of (Co)Variance > ANCOVA* and select the configuration as shown below.

- Based on the Pareto plot, the model equation was selected for further analysis using ANCOVA.

ANCOVA

Confidence Level (%) 95

Dependent Variable Col8 -- Total (kg)

Sum of Squares for Tests Adjusted (Type III)

Coding for Factors (-1, 0, +1)

Excluded Columns

Factors

Covariates

Col2 -- Pvap
Col3 -- Sol
Col4 -- Kow
Col5 -- Ksw

Formula Type: ☒ Custom ☐ Main Effects ☐ Full Factorial

Specify Custom Model Formula

Formula: Pvap + Sol + Kow + Ksw + kdeg_air + kdeg_soil + Sol:Ksw + Sol:kdeg_air + Sol:Kow + Sol:kdeg_soil + Pvap:Sol + Pvap:Ksw + Pvap:kdeg_soil + Ksw:kdeg_air + Ksw:kdeg_soil + Kow:kdeg_air + Kow:kdeg_soil + kdeg_air:kdeg_soil + Sol:Sol + Pvap:Pvap + Kow:Kow + Ksw:Ksw + kdeg_air:kdeg_soil + kdeg_soil:kdeg_soil

Execute Cancel

Custom Formula Creation

Included Columns

Pvap
Sol
Kow
Ksw
kdeg_air
kdeg_soil

Add interactions
up to order 2

Add terms
up to order 2

Add Custom Interaction

Delete Selected

Reset Selections

Included Terms

Pvap
Sol
Kow
Ksw
kdeg_air
kdeg_soil
Sol:Ksw
Sol:kdeg_air
Sol:Kow
Sol:kdeg_soil
Pvap:Sol
Pvap:Ksw
Pvap:kdeg_soil
Ksw:kdeg_air
Ksw:kdeg_soil
Kow:kdeg_air
Kow:kdeg_soil
kdeg_air:kdeg_soil
Sol:Sol
Pvap:Pvap
Kow:Kow
Ksw:Ksw
kdeg_air:kdeg_soil
kdeg_soil:kdeg_soil

Move up Move down

Pvap + Sol + Kow + Ksw + kdeg_air + kdeg_soil + Sol:Ksw + Sol:kdeg_air + Sol:Kow + Sol:kdeg_soil + Pvap:Sol + Pvap:Ksw + Pvap:kdeg_soil + Ksw:kdeg_air + Ksw:kdeg_soil + Kow:kdeg_air + Kow:kdeg_soil + kdeg_air:kdeg_soil + Sol:Sol + Pvap:Pvap + Kow:Kow + Ksw:Ksw + kdeg_air:kdeg_soil + kdeg_soil:kdeg_soil

Save Cancel

The model formula is:

Sol + Pvap + Kow + Ksw + kdeg_air + kdeg_soil + Sol:Ksw + Sol:kdeg_air + Sol:Kow + Sol:kdeg_soil + Pvap:Sol + Pvap:Ksw + Pvap:kdeg_soil + Ksw:kdeg_air + Ksw:kdeg_soil + Kow:kdeg_air + Kow:kdeg_soil + kdeg_air:kdeg_soil + Sol:Sol + Pvap:Pvap + Kow:Kow + Ksw:Ksw + kdeg_air:kdeg_soil + kdeg_soil:kdeg_soil

A table analyzing the variance/covariance of each term in the formula is outputted in the output spreadsheet.

User Row ID	Source	DF	Adj SS	Adj MS	F-Value	P-Value	Significant
	Pvap	1	22381512293 643264	22381512293 643264	0.0798612	0.7802522	No
	Sol	1	7.6298469371 2046E+16	7.6298469371 2046E+16	0.2722465	0.6072888	No
	Kow	1	2.7202310894 12095E+18	2.7202310894 12095E+18	9.7062675	0.0052328	Yes
	Ksw	1	3.8517975091 54202E+16	3.8517975091 54202E+16	0.1374390	0.7145538	No
	kdeg_air	1	9.6751387913 54982E+16	9.6751387913 54982E+16	0.3452261	0.5630932	No
	kdeg_soil	1	5.0492630551 93214E+18	5.0492630551 93214E+18	18.0166671	0.0003620	Yes
	Sol*Ksw	1	1024.0	1024.0	0E-7	1.0000000	No
	Sol*kdeg_air	1	3072.0	3072.0	0E-7	0.9999999	No
	Sol*Kow	1	1024.0	1024.0	0E-7	1.0000000	No
	Sol*kdeg_soil	1	1024.0	1024.0	0E-7	1.0000000	No
	Pvap*Sol	1	0.0	0.0	0.0	1.0	No
	Pvap*Ksw	1	488448.0	488448.0	0E-7	0.9999990	No
	Pvap*kdeg_air	1	1123328.0	1123328.0	0E-7	0.9999984	No
	Pvap*Kow	1	2.5316212636 16E+14	2.5316212636 16E+14	0.0009033	0.9763067	No
	Pvap*kdeg_soil	1	87619468420 1984	87619468420 1984	0.0031264	0.9559386	No
	Ksw*kdeg_air	1	15678464	15678464	0E-7	0.9999941	No
	Ksw*kdeg_soil	1	77842069124 47488	77842069124 47488	0.0277754	0.8692322	No
	Kow*kdeg_air	1	10188097843 2	10188097843 2	4E-7	0.9995246	No
	Kow*Ksw	1	97645071945 32864	97645071945 32864	0.0348415	0.8537197	No
	Kow*kdeg_soil	1	8.9737194133 18893E+18	8.9737194133 18893E+18	32.0198242	0.0000129	Yes
	kdeg_air*kdeg_soil	1	10878125056	10878125056	0E-7	0.9998447	No
	Sol*Sol	1	1.4042415963 895296E+17	1.4042415963 895296E+17	0.5010583	0.4868182	No
	Pvap*Pvap	1	3.8234150912 41267E+16	3.8234150912 41267E+16	0.1364262	0.7155582	No
	Kow*Kow	1	5.9621136592 16681E+17	5.9621136592 16681E+17	2.1273880	0.1594826	No
	Ksw*Ksw	1	32128851779 216384	32128851779 216384	0.1146414	0.7382825	No
	kdeg_air*kdeg_soil	1	1.4587380348 8896E+17	1.4587380348 8896E+17	0.5205036	0.4785833	No
	kdeg_soil*kdeg_soil	1	7.5301137288 80362E+18	7.5301137288 80362E+18	26.8687828	0.0000389	Yes
	Error	21	5.8853573521 65053E+18	2.8025511200 785968E+17			
	Total	48	6.4093296737 3832E+19				

Observation:

Kow, kdeg_soil, and their interaction (Kow:kdeg_soil) are statistically significant, along with the quadratic term $kdeg_soil^2$, indicating that both linear and nonlinear effects of soil degradation and its interaction with hydrophobicity (Kow) play a key role in the response.

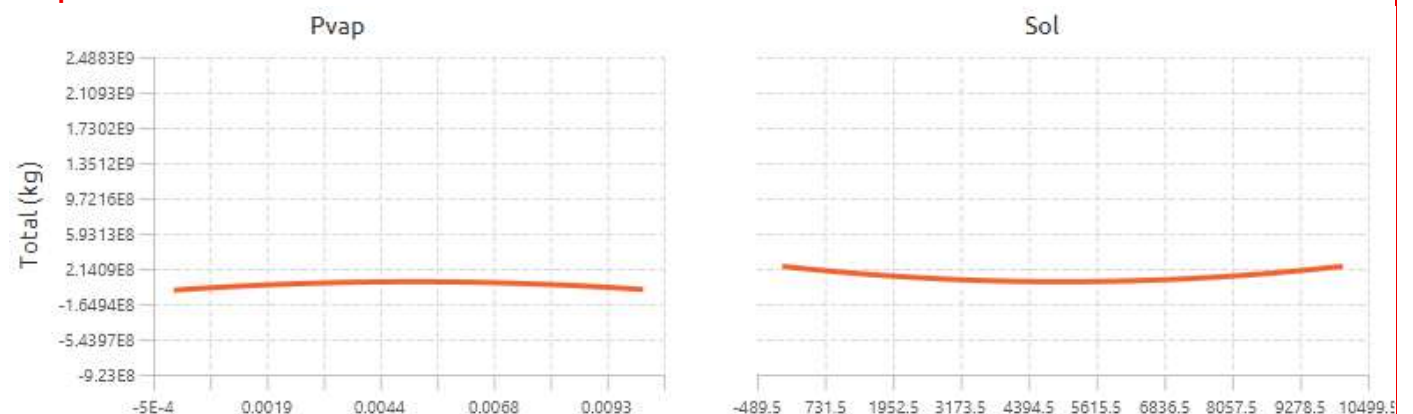
Step 26: Factorial-Plots-RSM

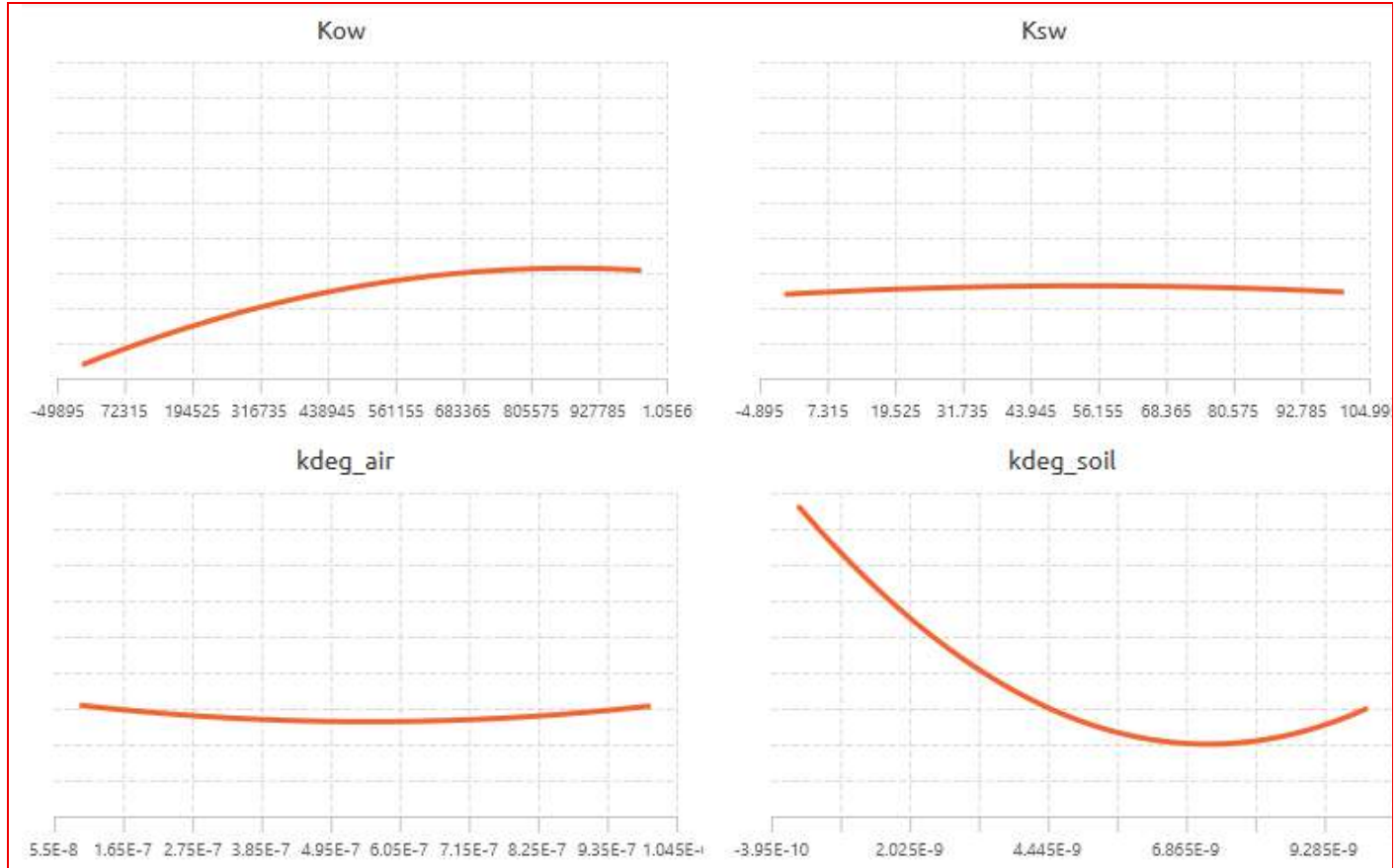
Create a new sheet and name it as 'FACTORIAL-PLOTS-RSM'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v5'.

Navigate to *DOE > Post DoE Analysis > Factorial Plot Analysis* and select the configuration as shown below.

The screenshot shows the 'Factorial Plot Analysis' dialog box. The 'Dependent Variable' is set to 'Col8 -- Total (kg)'. The 'Analysis Type' is 'Main Effects + Two-Factor...'. The 'Excluded Columns' list is empty. The 'Factors' list contains 'Col2 -- Pvp', 'Col3 -- Sol', 'Col4 -- Kow', and 'Col5 -- Ksw'. The 'Specify Factor Values' button is visible. The 'Covariates' list is empty. The 'DOE type' is set to 'Response Surface' (indicated by a selected radio button). The 'Include Center Points' checkbox is unchecked. The 'Execute' and 'Cancel' buttons are at the bottom.

Output:



**Observation:**

The factorial plots obtained from the Box-Behnken design further highlight the presence of curvature effects.

Step 27: OPTIMIZATION

Create a new sheet and name it as 'OPTIMIZATION'. Right-click on the input (left) spreadsheet and select 'Import from Spreadsheet' and select the 'PREPROCESS-v5'.

Navigate to *DOE > Post DoE Analysis > Multi-Objective Optimization* and select the configuration as shown below.

Multi Objective Optimization

Analysis Type: Main Effects + Two-Factor...

Confidence Level: 95

Excluded Columns: [Empty Box]

Factors:

- Col2 – Pvpap
- Col3 – Sol
- Col4 – Kow
- Col5 – Ksw
- Col6 – kdeg_air
- Col7 – kdeg_soil

Specify Factor Values

Dependent Variables:

Col8 – Total (kg) Target 1E+7 Scale: 1 Importance: 1

Specify Dependent Variable Ranges

☐ Optimize on the Discretized Space (Only factor values that appear in the dataset will be considered)

Execute Cancel

Output:

User Row ID							
	Optimal Settings - Solution						
	Pvap	Sol	Kow	Ksw	kdeg_air	kdeg_soil	
	0.0015009	1957.0324061	579703.6465934	59.6863679	5E-7	0E-7	
	Response Prediction						
	Response	Predicted Value	Standard Error	(95.0%) CI Lower	(95.0%) CI Upper	(95.0%) PI Lower	(95.0%) PI Upper
	Total (kg)	10000000.0000001	293067091.4053503	-599466380.7206407	619466380.7206409	-1248369907.513438	1268369907.513438
	Desirabilities						
	Total (kg)	Overall					
	1.0	1.0					

Observation:

Following the Box–Behnken design, a multi-objective optimization was performed with the target of minimizing the total environmental mass to approximately 10,000,000 kg. The optimal solution suggests that low degradation in soil ($kdeg(soil) = 1.0 \times 10^{-8} \text{ s}^{-1}$) combined with relatively high Kow and moderate Pvpap and Sol values leads to the desired outcome. The predicted total mass is close to the target, with an overall desirability of 1.0, indicating an excellent optimization result. However, the wide confidence and prediction intervals highlight a high level of uncertainty in the prediction, suggesting that while the model captures the trend, variability in the system remains significant and should be considered when interpreting the results.

These optimal conditions can be interpreted within an SSbD framework, as they suggest combinations of physicochemical properties that minimize environmental persistence and accumulation, thereby guiding the design of safer PFAS alternatives.

Final Isalos Workflow

Following the above-described steps, the final workflow on Isalos will look like this:

