

# A Workflow for *In Silico* Assessment of Genetic Toxicity and Application to Pharmaceutical Genotoxic Impurities under ICH M7



**TOX NAVIGATION**  
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## BACKGROUND and OBJECTIVES

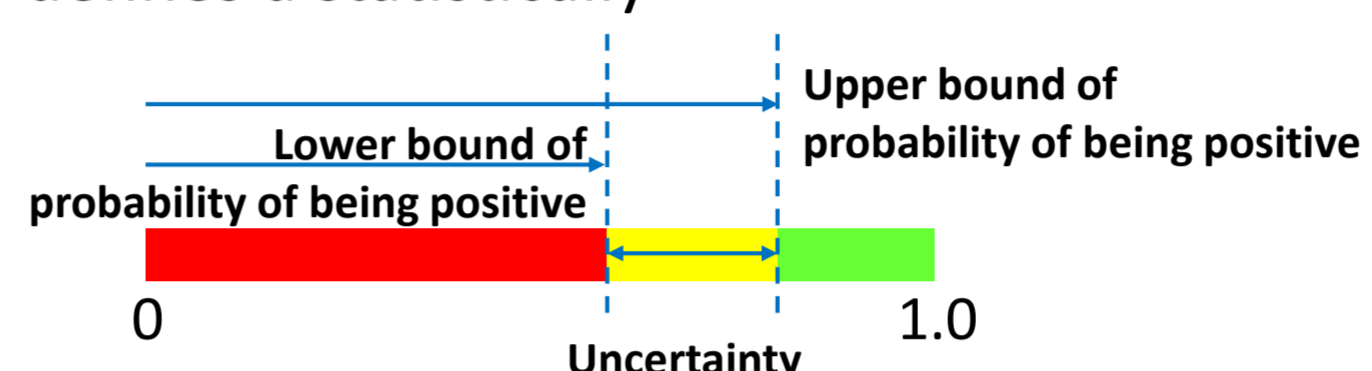
The ICH M7 guideline provides a framework for assessing and controlling DNA reactive impurities in a pharmaceutical product. When no adequate experimental mutagenicity and/or carcinogenicity results are available, an assessment of Structure-Activity Relationships (SAR) that focuses on bacterial mutagenicity predictions should be performed. Currently genotoxic impurities are identified periodically using an expert panel. This creates a time delay between impurity elucidation and assessment. **If potential genotoxicity can be identified during or shortly after elucidation, projects can be run more efficiently** and the number of compounds needing expert assessment can be significantly reduced. **This poster describes an early screening workflow for genotoxicity that can be applied to alert process and analytical chemists much earlier than is currently possible.**

## MATERIAL and METHODS

**Dataset:** 53 structures, *in silico* prediction of their potential DNA reactivity with a variety of mainly commercial tools, expert judgment and, when available, AMES data<sup>1</sup>.

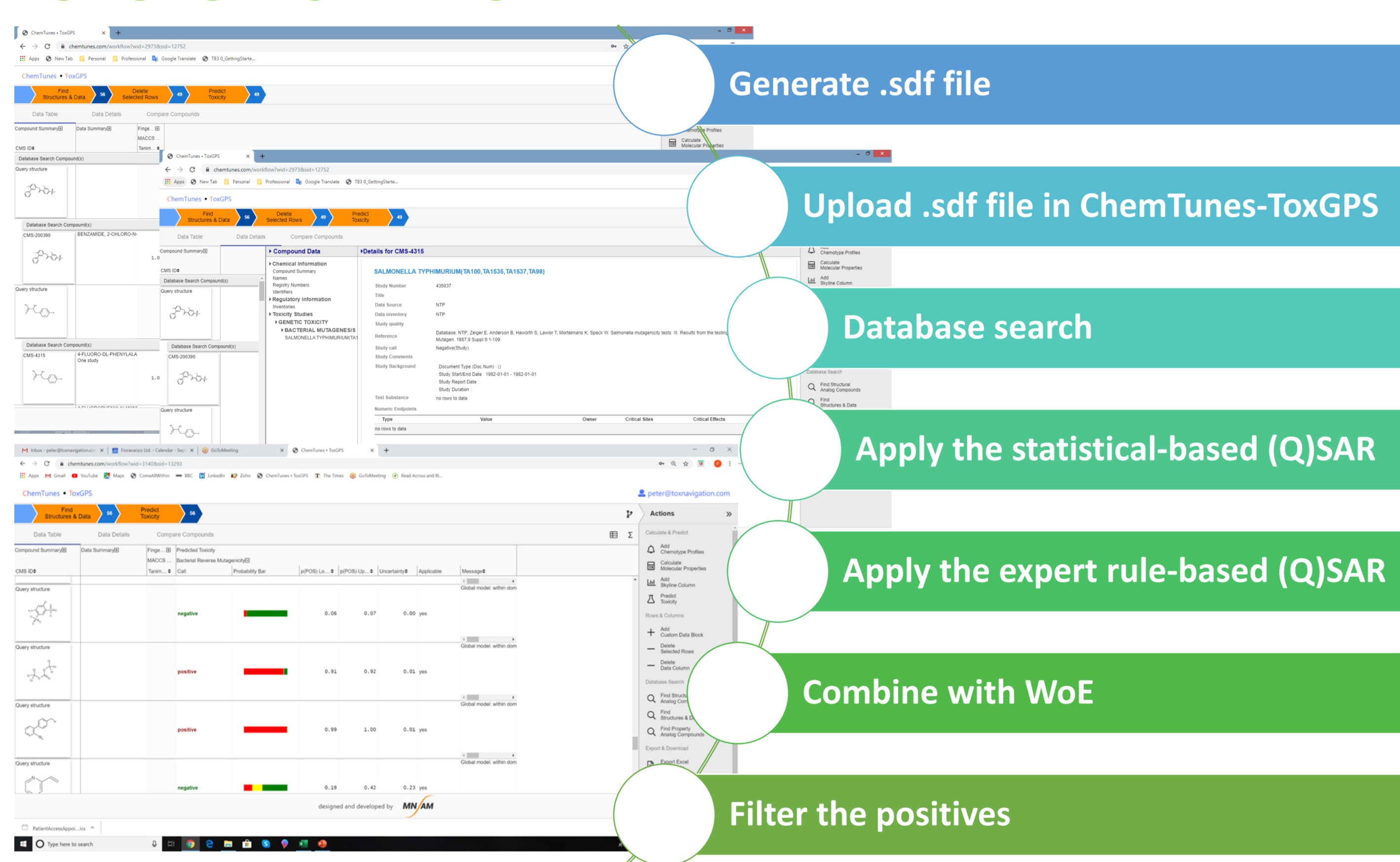
**(Q)SAR models<sup>2</sup>:** the ToxGPS Bacterial Reverse Mutagenesis model utilizes three model types: (1) global QSAR models; (2) local mode of action (MoA) QSAR models that take mechanistic knowledge into account; (3) chemotype alerts (structural alerts).

**Weight of Evidence (WoE) algorithm:** The WoE method<sup>3</sup> is based on Dempster-Shafer decision theory (DST). After applying all relevant QSAR models and chemotype alerts to a given query molecule, a set of predictions is obtained, each with an associated uncertainty estimate. DST defines a statistically



rigorous way to combine all predictions to arrive that the final WoE result, again with uncertainty quantitatively taken into account. For a binary classification scenario, WoE predictions are "positive", "uncertain", or "negative".

## TOXGPS WORKFLOW



The original expert assessment was reviewed. 4 compounds were reassessed as new AMES data was available. The final dataset contains 24 negative compounds (45%) and 29 positive ones (55%), most of them are problematic compounds requiring expert opinion for (Q)SAR results interpretation. Four classification scheme based on ToxGPS predictions or on published (Q)SAR predictions were compared:

Schema 1 (ToxGPS workflow) – WoE combination of statistical and expert rule-based (Q)SAR

Schema 2 – Classified if the literature statistical and expert rule-based predictions agree

Schema 3 – Classified according to literature with statistical-based (Q)SAR predictions

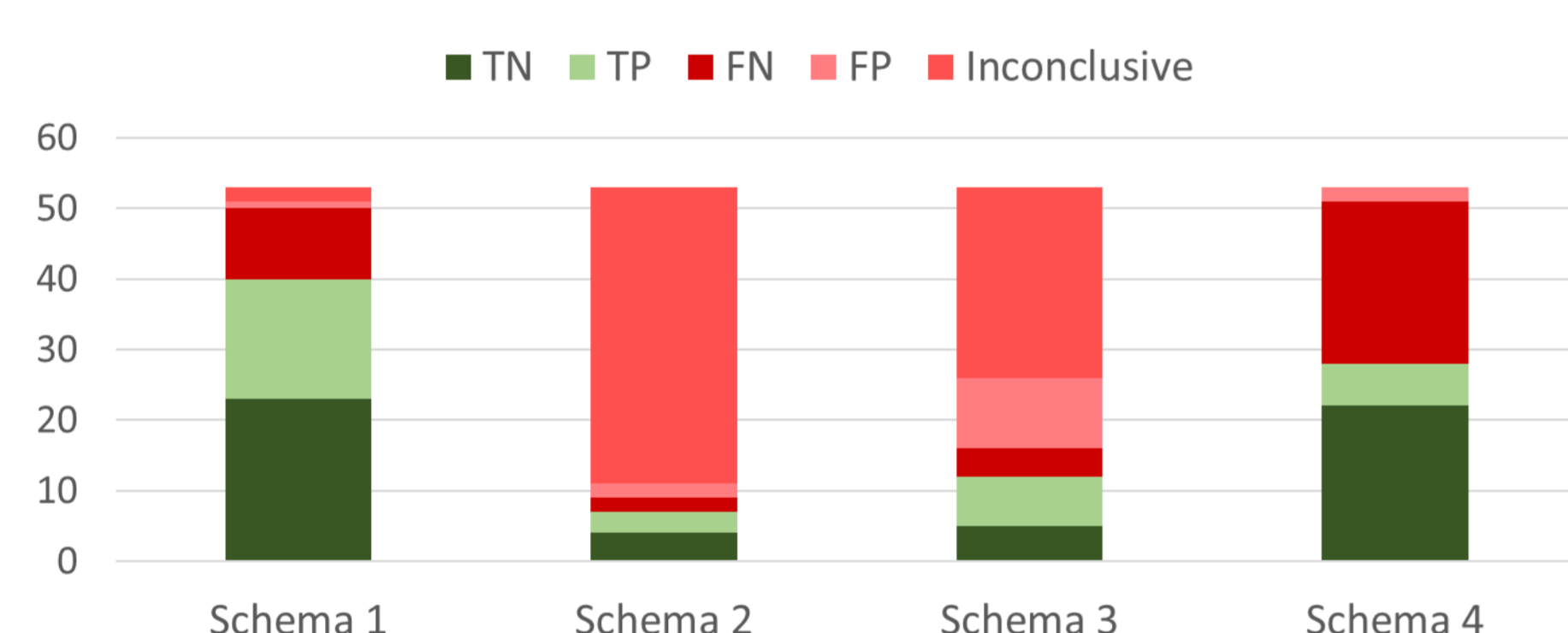
Schema 4 – Classified according to literature with expert rule-based (Q)SAR predictions

Out of domain and uncertain predictions are defined as inconclusive and would require either an expert judgment or an AMES test to conclude on the genotoxic potential.

## Results

Schema 1 and 4 are the only ones able to classify more than 50% of the dataset and Schema 1 shows the best quality of prediction in terms of Matthews Correlation Coefficient (MCC). Furthermore Schema 1 **minimises the number of False Positives** addressing one of the main concern of an early genotoxicity screening and **maximises the number of True Positives** (17 vs 6 in Schema 4) allowing an easy and early identification of a larger number of impurities with a genotoxic potential.

## RESULTS



	Schema 1	Schema 2	Schema 3	Schema 4
True Neg	23 43%	4 8%	5 9%	22 42%
True Pos	17 32%	3 6%	7 13%	6 11%
False Neg	10 19%	2 4%	4 8%	23 43%
False Pos	1 2%	2 4%	10 19%	2 4%
Inconclusive	2 4%	42 79%	27 51%	0 0%

Total number of compounds: 53, 24 negative, 29 positive

The **Matthews Correlation Coefficient** is a measure of the quality of binary classifications, it returns a value between -1 and +1. A coefficient of +1 represents a perfect prediction, 0 no better than random prediction and -1 indicates disagreement between prediction and observation.

**MMC Schema 1 = 0.61**  
**MMC Schema 4 = 0.17**

## False positive

The False Positive retrieved with the screening workflow is assigned to ICH M7 class 4 (non mutagenic) by an expert review by using ChemTunes-ToxGPS<sup>2</sup> for read-across.

**Chemotype alert: Alkylating agent**

This alert is shared with a known negative and the benzonitrile is negative as well in the AMES test. Experimental data from ChemTunes<sup>2</sup>

**Predictions for endpoint: Bacterial Reverse Mutagenicity**  
UNCERTAIN Probability (POSITIVE) = [0.377, 0.625]

## False negative

ID	Schema 1	Schema 4	Probability Bar	ToxGPS expert rule-based	Comments
24	neg	neg	Green	neg	Experimental data available in ChemTunes
30	neg	neg	Green	neg	False negative
34	neg	neg	Green	neg	Experimental data available in ChemTunes
37	neg	neg	Green	pos	Positive analogues available in ChemTunes
40	neg	pos	Red	pos	False negative
44	neg	neg	Green	pos	False negative
48	neg	neg	Green	pos	False negative
50	neg	neg	Green	pos	Borderline positive (Q)SAR
52	neg	neg	Green	pos	Experimental data available in ChemTunes
53	neg	neg	Green	pos	Positive analogues available in ChemTunes

9 of the 10 FN of Schema 1 are also FN in Schema 4, 6 FN are correctly reviewed as positives by viewing the experimental data automatically retrieved by ChemTunes<sup>2</sup>.

## Inconclusive

**Chemotype alert: azide – this alert suggests genotoxic potential. In the literature<sup>1</sup> it is considered positive.**

**Predictions for endpoint: Bacterial Reverse Mutagenicity**  
UNCERTAIN Probability (POSITIVE) = [0.377, 0.625]

**Model name: Global**  
Probability (POSITIVE) [0.377, 0.625]

A conservative approach is suggesting potential genotoxicity. In the literature<sup>2</sup> it is considered positive.

## REFERENCES

- Amberg et al. Regul. Toxicol. Pharmacol. (2016) 77, 13-24; Amberg et al. Regul. Toxicol. Pharmacol. (2019) 102, 53-64; Mishima et al. Genes and Environment (2018) 40:19; Sutter et al. Regul. Toxicol. Pharmacol. (2013) 67, 39-52; 2019 Pharmaceutical Industry and Regulators Symposium, Anax and McDonald.
- ChemTunes-ToxGPS<sup>®</sup> at MN-AM, <https://www.mn-am.com/products/toxgps>
- Rathman et al. Computational Toxicology 6 (2018) 16-31
- Honma et al. Mutagenesis, 2019 vol: 34 (1) pp: 41-48

## SUMMARY AND FUTURE

- ToxGPS workflow has shown to be a viable tool for identifying genotoxicity during or shortly after elucidation of drug impurities as it is able to:
  - automatically process an .sdf file
  - minimise the number of false positive and false negative for **problematic** compounds (21%)
  - maximise the number of true positive and true negative for **problematic** compounds (75%)
  - reduce the number of inconclusive predictions for **problematic** compounds (4%)
- ChemTunes-ToxGPS has shown to be a viable tool for ICH M7 classification as **the final number of misclassified compounds is 4 (8%)**, in the same range scored by other well established tools<sup>4</sup>
- QSAR modeling based on biologically meaningful grouping using mechanistically selected chemotypes and molecular descriptors
- Robust risk assessment system providing rigorous method for quantitative weight-of-evidence
- Final outcome combines the evidences of QSAR models and chemotype rule-based predictions to provide good prediction performance
- Include in the workflow the assessment for class 4