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In silico approaches using CASE Ultra and QSAR Toolbox for predicting genotoxicity and carcinogenicity on diverse groups of chemicals

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ABSTRACT

Humans are daily exposed to a wide range of chemicals in their environment, many of which may exert harmful effects on health. Hence, knowledge of these chemicals for their genotoxicity and carcinogenicity potential is crucial for protecting human health. Genotoxicity, in particular, serves as an early indicator of carcinogenic risk. The assessment of both genotoxicity and carcinogenicity is vital for regulatory bodies and has led to the development of alternative non-animal testing methods. One such method is *in silico* approach, which relies on predictive software tools for faster, more cost-effective screening.

This paper examines two *in silico* tools, CASE Ultra 1.9.0.8 (MultiCASE, USA) and QSAR Toolbox 4.5 (OECD), to evaluate their ability to predict the genotoxicity and carcinogenicity of various chemicals. The *in silico* tools CASE Ultra, QSAR Toolbox, and its profilers demonstrated remarkable performance, with balanced accuracy rates of 80%, 85%, and 62%, for genotoxicity and 79%, 86% and 66% for carcinogenicity, respectively. These promising results underscore the potential of computational approaches in risk assessment, offering a valuable complement to traditional testing methods for evaluating the genotoxicity and carcinogenicity of chemicals. Such tools can play a crucial role in regulatory decision-making and public health protection.

1. Introduction

Genotoxicity and carcinogenicity are essential toxicological endpoints for all chemical substances, including pharmaceuticals, industrial chemicals, pesticides, food additives, and cosmetic ingredients for health hazard assessment.

As per the CAS registry, there are more than 279 million registered substances, and this number is continuously increasing [1]. Identifying hazardous chemicals involves toxicological evaluation in animals and other organisms. However, conducting animal studies for individual chemicals is not feasible. It comes with challenges involving time demands, ethical concerns, huge costs, and the complex nature of genotoxicity and carcinogenicity mechanisms. Additionally, as cosmetics are considered non-essential products used esp. for beautification, countries like the European Union (as per Regulation (EC) No 1223/2009), India, New Zealand, etc. have banned animal testing due to ethical concerns and limited relevance to humans [2–4].

Hence, in the last few years, efforts have been made to address these issues, one of them being NAMs (new approach methods), which involve *in silico*, *in chemico*, *in vitro*, and *ex vivo* approaches that can reduce costs, bring mechanistic insights, and address animal welfare concerns [5]. The computational approach has become a valuable tool and is widely used to support Next Generation Risk Assessment (NGRA), which is defined as an exposure-led, hypothesis driven risk assessment approach that integrates *in silico*, *in chemico* and *in vitro* approaches which can assess the toxicity of chemicals when existing chemicals do not have adequate safety data or are new molecules, especially for cosmetics since the ban on animal testing, validated alternative methods like *in vitro* human skin models, *in silico* computer predictions (QSAR), and *ex vivo* human tissue tests are recommended [6]. Over the past five years, major regulatory bodies such as the OECD, FDA, and ECHA have developed guidance supporting NAMs. OECD Test guideline 497, adopted in June 2025 for defined approaches on skin sensitization, recommends the use of a combination of *in vitro* and *in silico* models to arrive at a rule-based conclusion on potential dermal sensitization [7]. The FDA's Modernization Act 3.0, signed in Feb 2024, allows NAMs in place of animal testing for drug development, giving increasingly viable alternatives to animal testing [8]. ECHA, under Annex IX in its Read Across Assessment

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2468-1113/© 2025 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

Framework, has encouraged the use of valid QSAR models for various endpoints related to genotoxicity, skin sensitization, and reproductive/developmental toxicity [9]. Recent advancements in machine learning and artificial intelligence (AI) have also shown that scientists integrate multi-omics data, including genomics, transcriptomics, and proteomics, to explicate the molecular mechanisms that cause chemical-induced genetic damage [10].

The Qualitative Structure-Activity Relationship (QSAR) methodology is used in various stages of the development of substances by predicting properties that correlate with toxicity endpoints, structure–activity relationship models for new chemical formulations, physico-chemical properties, and building/retrieving information on chemical databases [11]. Hence, the success of the *in silico* tool depends on the range of adverse effects that the models cover, the predictive performance of the models, and the explanatory details provided in the prediction results. Two commonly used software programs for predictive toxicity are CASE Ultra and OECD QSAR toolbox. The CASE Ultra is a combination of statistical algorithms and expert-derived rules that uses a proprietary machine-learning algorithm in combination with molecular descriptors generated by fragmentation of training set structures [12,13]. While in case of OECD QSAR Toolbox offers a structured and transparent approach for genotoxicity and carcinogenicity assessment, primarily through its profiling system, category formation, and mechanistic read-across capabilities [14]. While OECD QSAR is free and has more options for customization for more accurate predictions and mechanistic understanding, however its usage is complex and less user-friendly, which requires significant expert judgment to ensure that analog selection, justification, and uncertainty analysis are robust and scientifically defensible [15]. In comparison with CASE Ultra, its user-friendly interface and speed, make it particularly useful for routine screening and early-phase risk evaluation. However, the limited transparency of its internal algorithms and the restricted customization options pose challenges for users requiring detailed mechanistic justifications [16].

Both tools are recognized by regulatory authorities, and the complementary use of Case Ultra and OECD QSAR Toolbox provides a more comprehensive and defensible prediction strategy. While Case Ultra can rapidly identify potential hazards, the OECD Toolbox can support these findings with mechanistic and structural justifications, thereby facilitating a more robust and scientifically rooted regulatory submission. Currently, ICH M7 guideline has recommended QSAR predictions for pharmaceutical impurities only for mutagenicity, which can be used for regulatory submissions [17]. However, assessment of other endpoint assessments are also required, which will support quick and non-animal evaluation. To support this, EFSA has updated its guideline on Smoke flavorings, where QSAR methodology can be implemented for the whole spectrum of genotoxicity endpoints, provided that the conditions mentioned are met [18]. Therefore, it is crucial to determine the effectiveness of computer-based systems in predicting the toxic effects of chemicals on humans.

Hence, in this study, we evaluated *in silico* tools for their capability to predict genotoxicity and carcinogenicity for diverse groups of chemicals.

2. Material and methods

A total of 200 chemicals were selected randomly to ensure broad representation across various chemical categories. The dataset includes industrial substances, pharmaceuticals, pesticides, food additives, biocides, flavoring agents, natural products, cosmetic ingredients, nitrosamines. This diverse selection allowed robust *in silico* analysis across multiple use classes and toxicological profiles. The substances were chosen based on the availability of established data from peer-reviewed literature sources [19–27]. Such a dataset supports the generation of meaningful predictions and comparative toxicological evaluations for various genotoxicity and carcinogenicity endpoints using two *in silico* tools, namely, CASE Ultra (v1.9.0.8) by MultiCASE (USA) and QSAR Toolbox (v4.5) by OECD. The list contains 53 IARC-listed carcinogens (30 group 2B, 14 Group 2A, 9 Group 1 carcinogens respectively) and 66 are prop65 classified carcinogens [28,29]. The endpoints targeted were based on *in silico* analysis of established data for various studies, viz. (i) Ames, (ii) *in vitro* mammalian cell gene mutation, (iii) *in vitro* chromosomal aberration, (iv) *in vivo* micronucleus, and (v) rodent carcinogenicity.

We compared the results compiled from the public domain and those obtained from our *in silico* analysis. CASE Ultra is a commercial application whereas QSAR toolbox is a free software application, supporting chemical hazard assessment. The QSAR Toolbox was used in the Classic User Interface. Chemicals were entered into the QSAR Toolbox using CAS RN or SMILES. For each compound, data available in the Toolbox from different databases related to genotoxicity and carcinogenicity were used, and for those that didn't have any information, data gap filling using the QSAR model was used to predict the endpoints.

Additionally, toolbox alerts using profilers were also considered. Only positive, negative, and equivocal results were considered for evaluation. For profilers, endpoints showing alerts were considered positive, and those showing no alerts were considered negative. In the case of CASE Ultra, chemicals were identified using CAS RN or SMILES, and results were considered positive when the alerts were fired as positive or known positive; likewise, negative or known negative results were considered negative. For both CASE Ultra and QSAR Toolbox, out-of-domain results were not considered for evaluation.

In addition to assessing the overall balanced accuracy across all substances, a category-specific analysis was also conducted for three major chemical groups: pharmaceuticals (41 chemicals), pesticides (50 chemicals), and industrial chemicals (62 chemicals) for meaningful statistical evaluation. Other categories (e.g., food additives, biocides, flavouring agents, natural products, cosmetic ingredients and nitrosamines) were excluded from this sub-analysis due to their limited representation ($n < 13$), which could compromise the statistical robustness and reliability of the category-level comparisons.

Databases used for different endpoints in CASE Ultra and OECD QSAR toolbox are as follows:

In the CASE Ultra following databases were used for genotoxicity and Carcinogenicity: Model for chromosomal aberrations for CHO and CHL cell lines (v1.9.0.8), Micronucleus test (v1.9.0.8), Mouse lymphoma activated and inactivated (v1.9.0.8), for mutagenicity expert and statistical based model (v1.9.2.0), female and male mouse Carcinogenicity, female and male rat Carcinogenicity model (v1.9.0.8).

In case of OECD QSAR Toolbox the following profilers were employed for genotoxicity assessment: OECD HPV Chemical Categories (v3.0), DNA binding by OASIS (v.2.8), DNA binding by OECD(v2.3), Protein binding by OASIS (v3.8), Protein binding by OECD (v2.3), DNA alerts for AMES, CA and MNT by OASIS (v3.8), Ames test alerts by ISS (v2.6), *in vivo* mutagenicity (Micronucleus) alerts by ISS (v2.6), Protein binding alerts for Chromosomal aberration by OASIS (v1.7).

For carcinogenicity, following profilers were included: OECD HPV Chemical Categories (v3.0), DNA binding by OASIS (v2.8), DNA binding by OECD (v2.3), Protein binding by OASIS (v3.8), Protein binding by OECD (v2.3), Carcinogenicity alerts by ISS (v2.6), DNA alerts for AMES, CA and MNT by OASIS (v3.8), Ames test alerts by ISS (v2.6), *in vivo* mutagenicity (Micronucleus) alerts by ISS (v2.6), Oncologic Primary Classification (v4.2), Protein binding alerts for Chromosomal aberration by OASIS (v1.7).

For carcinogenicity, the following data sources were included: Biocides and plant protection ISSBIOC (v1.1), Carcinogenicity and mutagenicity ISSCAN (v1.4), CPDB (v1.1), cell transformation assay ISSCTA (v1), ECHA REACH (v5.2021), Food TOX Hazard EFSA (v1.3), ISSSTY (v1.1), and Genotoxicity & Carcinogenicity ECVAM (v2.1).

For genotoxicity, following data sources were included: Genotoxicity & Carcinogenicity ECVAM(v2.1), Genotoxicity OASIS (v1.8), Genotoxicity pesticides EFSA (v2.0), Micronucleus ISSMIC (v1.2), Micronucleus OASIS (v1.5), Toxicity Japan MHLW (v3.0), Transgenic Rodent

Database (v1.0), Food TOX Hazard EFSA (v1.3), Bacterial mutagenicity ISSSTY (v1.1), ECHA REACH (v5.2021).

3. Results

Our study yielded significant insights into the predictive performance of CASE Ultra and the QSAR toolbox (including its profilers). A summary of our results can be found in Tables 1-5 (Supplementary file), where we have compiled the genotoxicity and carcinogenicity alerts provided by CASE Ultra and the QSAR toolbox. Based on the compiled information, the compounds were classified into True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) and calculated using below formula:

Sensitivity = [True Positive / (True Positive + False Negative)] x 100.

Specificity = [True Negative / (True Negative + False Positive)] x 100.

Positive Predictive Value (PPV) = [True Positive / (True Positive + False Positive)] x 100.

Negative Predictive Value (NPV) = [True Negative / (True Negative + False Negative)] x 100.

Accuracy = [True Positive + True Negative / (True Positive + True Negative + False Positive + False Negative)] x 100.

False Positive Rate (FPR) = [False Positive/ (False Positive + True Negative)] x 100.

False Negative Rate (FNR) = [False Negative/ (False Negative + True Positive)] x 100.

Balanced accuracy = Sensitivity + Specificity /2.

The detailed results of accuracy, sensitivity, specificity, PPV, and NPV of the *in silico* software are given below

Overall predictive performance for genotoxicity and carcinogenicity:

CASE Ultra achieved an overall accuracy of 79%, sensitivity of 81%, specificity of 78%, positive predictive value of 74%, negative predictive value of 84% and balanced accuracy of 80%.

QSAR toolbox obtained an overall accuracy of 86%, sensitivity of 81%, specificity of 90%, positive predictive value of 86%, negative predictive value of 87%, and balanced accuracy of 86%. In the case of profilers, alerts showed an overall accuracy of 65%, sensitivity of 57%, specificity of 71%, positive predictive value of 59%, negative predictive value of 70% and balanced accuracy of 64%.

Overall genotoxicity:

CASE Ultra: Accuracy 79%, Sensitivity 81%, Specificity 79%, PPV 70%, NPV 87%, FPR 21%, FNR 19% balanced accuracy 80%.

QSAR Toolbox: Accuracy 86%, Sensitivity 77%, Specificity 92%, PPV 86%, NPV 86%, FPR 8%, FNR 23%, balanced accuracy 85%.

QSAR Toolbox Profilers: Accuracy 64%, Sensitivity 52%, Specificity 71%, PPV 51%, NPV 73%, FPR 29%, FNR 48%, balanced accuracy 62%.

Ames assay:

CASE Ultra: Accuracy 94%, Sensitivity 97%, Specificity 93%, PPV 73%, NPV 99%, FPR 7%, FNR 3%.

QSAR Toolbox: Accuracy 88%, Sensitivity 55%, Specificity 96%, PPV 76%, NPV 90%, FPR 4%, FNR 45%.

QSAR Toolbox Profilers: Accuracy 79%, Sensitivity 83%, Specificity 78%, PPV 40%, NPV 96%, FPR 22%, FNR 17%.

In vitro genotoxicity (Ames, Mammalian cell gene mutation assay, Chromosomal aberration):

CASE Ultra: Accuracy 82%, Sensitivity 81%, Specificity 83%, PPV 74%, NPV 88%, FPR 17%, FNR 19%.

QSAR Toolbox: Accuracy 87%, Sensitivity 77%, Specificity 93%, PPV 87%, NPV 87%, FPR 7%, FNR 23%.

QSAR Toolbox Profilers: Accuracy 69%, Sensitivity 44%, Specificity 81%, PPV 54%, NPV 74%, FPR 19%, FNR 56%.

In vivo genotoxicity (*In vivo* micronucleus assay):

CASE Ultra: Accuracy 69%, Sensitivity 80%, Specificity 60%, PPV 60%, NPV 80%, FPR 40%, FNR 20%.

QSAR Toolbox: Accuracy 83%, Sensitivity 75%, Specificity 88%, PPV 82%, NPV 83%, FPR 12%, FNR 25%.

QSAR Toolbox Profilers: Accuracy 55%, Sensitivity 69%, Specificity 45%, PPV 48%, NPV 66%, FPR 55%, FNR 31%.

Rodent carcinogenicity:

CASE Ultra: Accuracy 79%, Sensitivity 81%, Specificity 76%, PPV 83%, NPV 73%, FPR 24%, FNR 19%, balanced accuracy 79%.

QSAR Toolbox: Accuracy 87%, Sensitivity 93%, Specificity 79%, PPV 87%, NPV 88%, FPR 21%, FNR 7%, Balanced accuracy 86%.

QSAR Toolbox Profilers: Accuracy 67%, Sensitivity 65%, Specificity 71%, PPV 76%, NPV 58%, FPR 29%, FNR 35%, balanced accuracy 66%.

Balanced accuracy was calculated for pharmaceuticals, industrial chemicals, and pesticides to assess model performance across distinct chemical domains, as shown below in Figs. 1 and 2. This stratified approach helped to identify potential strengths or limitations of the prediction tools when applied to chemically or functionally distinct groups, thereby supporting more informed application in risk assessment contexts. Overall, these results indicated the superior predictive capabilities of CASE Ultra and QSAR toolbox across a wide range of endpoints pertaining to genotoxicity and carcinogenicity.

4. Discussion

Many accepted *in vitro* and *in vivo* tests enable hazard identification with respect to DNA damage and its fixation. This damage caused by gene mutations, chromosomal damage, and numerical chromosome changes has the potential to lead to mutagenicity and carcinogenicity.

ICH guidelines recommend a battery of tests for genotoxicity testing of pharmaceuticals, which includes conducting a gene mutation study in bacteria, a DNA damage test relevant for mammalian cells, and one *in vivo* test for genetic damage [30].

Long-term studies are conducted to evaluate chemicals' carcinogenic potential, which involves huge costs and ethical concerns. Any cause of concern derived from laboratory investigations, animal toxicology studies, and human data may lead to a need for carcinogenicity studies. Testing for the chemical's carcinogenic potential is also dependent on the duration of exposure (at least 6 months) and if there is concern about the chemical's carcinogenic potential [31]. Additionally, the acquisition of certain specific key units of information, including the results of genetic toxicology, intended patient population, clinical dosage regimen, pharmacodynamics in animals and humans (selectivity, dose–response), and repeated-dose toxicology studies will also help to decide the need for carcinogenic study [32].

To overcome these tedious procedures, an *in silico* system can be helpful, which consists of a knowledge base comprised of structural alerts that are statistically or mechanistically related to toxicity [33]. To support *in silico* approaches, the OECD has built a 5-point framework to validate QSAR models, which includes:

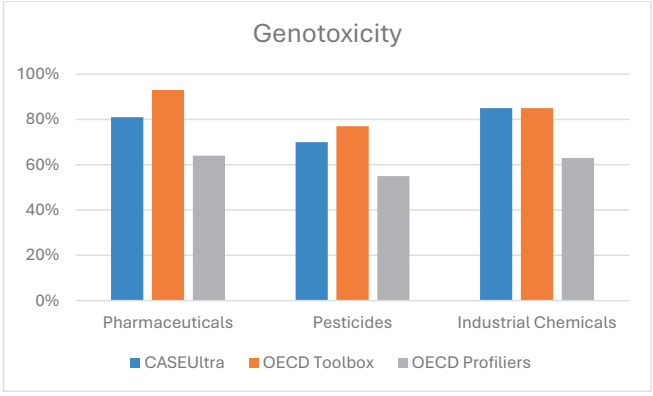


Fig. 1. Balanced accuracy of *in silico* genotoxicity predicition by chemical class.

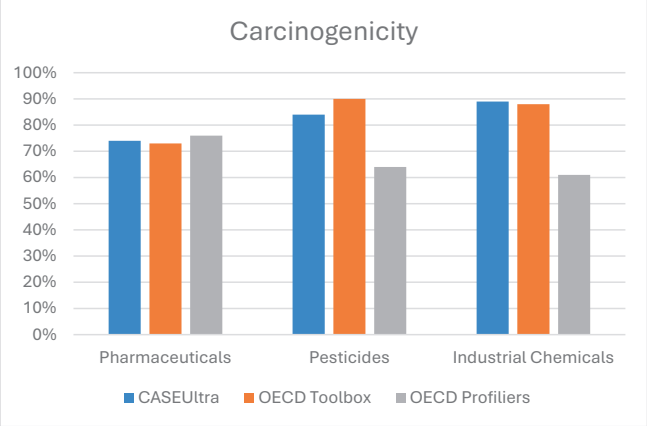


Fig. 2. Balanced accuracy of *in silico* carcinogenicity predicition by chemical class.

1. A defined endpoint
2. An unambiguous algorithm
3. A defined domain of applicability
4. Measures that are appropriate for goodness to fit, robustness, and predictivity
5. A mechanistic interpretation, if possible [34].

An earlier study conducted by Varun et al. (2024) showed that the OECD QSAR Toolbox demonstrated effective performance in predicting phototoxic potential of drugs and chemicals with a balanced accuracy of 79% demonstrating the reliability of the *in silico* tool in early safety assessment [35]. However, the predictive capability of *in silico* tools in diverse groups of chemical data was not available. Hence, we checked the reliability of 2 software tools on a diverse group of chemicals (the majority being industrial substances, pharmaceutical products, pesticides, food additives, biocides, flavoring agents, natural products, cosmetic ingredients, and nitrosamines).

In the current study, overall we obtained balanced accuracy of 80%, 86%, and 64% for CASE Ultra, QSAR Toolbox, and its profilers, respectively. For *Ames* assay, we obtained accuracy of 94%, 88% and 79% for CASE Ultra, QSAR Toolbox and its profiliers, which demonstrates a high level of concordance with laboratory findings, where repeatability between laboratories ranges from 80% to 85% [36]. For *in vitro* genotoxicity, CASE Ultra demonstrated a sensitivity of 81% and specificity of 83%, while the OECD QSAR Toolbox showed specificity of 93% and sensitivity of 77%. OECD profilers exhibited a sensitivity and specificity of 44% and 81% respectively. These results are comparable to conventional *in vitro* assays, which typically report sensitivity and specificity between 80% and 95% [37]. For *in vivo* mutagenicity, we have obtained sensitivity and specificity of 80 and 60% for CASEUltra, 75 and 88% for OECD toolbox, and 69 and 45% for its profilers. These data are also comparable to *in vivo* mutagenicity studies, which detected carcinogens at a sensitivity and specificity of 41 and 61% respectively [22]. In case of rodent carcinogenicity, the CASEUltra, OECD toolbox, and it's profiliers have shown an accuracy of 79%, 87% and 67% which are in line with findings that suggest rodent carcinogenicity studies predict human carcinogens with an accuracy of 65-73% [38]. It can also be seen that, except for a few chemicals (3 in CASEUltra, 4 in OECD Toolbox, and 20 in OECD profiliers), the rest aligned with the IARC classification. For prop65 listed chemicals, except for 5 chemicals for CASEUltra, 3 chemicals for OECD, and 22 chemicals in OECD profilers, others have shown false negative results. In addition, balanced accuracy was evaluated for genotoxicity and carcinogenicity predictions across pharmaceuticals, pesticides, and industrial chemicals domains. For genotoxicity, pharmaceuticals showed the highest balanced accuracy (64 to 93%), suggesting that well-characterized drug compounds with vast experimental data will enhance *in-silico* predictions as compared to pesticides and industrial compounds, which have structural variability and bioactivation-dependent pathways which can make models difficult to predict [39]. On the other hand, carcinogenicity assessment for pesticides (64 to 90%) and industrial chemicals (61 to 88%) was higher than compared to pharmaceuticals (73 to 76%). The reason could be partly due to the broader and longer-term environmental exposure to pesticides and industrial chemicals, leading to more epidemiological studies and research on their long-term health effects, including cancer [40,41]. While also rigorously tested, pharmaceutical compounds are typically used for shorter durations, and under controlled conditions, limiting the availability of long-term, population-level data on carcinogenicity [42]. The increase in the number of false positive and false negative cases for chemicals in OECD profilers might be due to the non-usage of metabolic alerts for prediction, which might have significantly changed the accuracy. OECD QSAR toolbox has a unique property where it recommends the data sources and profilers which are required for a particular endpoint. However, if the results are not suitable, it has options for customization, like data gap filling using read across and QSAR prediction. Additionally, for profilers to increase the accuracy, the usage of simulations for metabolism using observed and simulated S9 metabolism can be used to identify potential bioactivated mutagens [43]. All these results have shown that computational models can nearly replicate the reliability of laboratory-based assays. It is important to note that the effectiveness of these *in silico* tools depends on the availability of well-defined chemical structures. Consequently, these *in silico* tools are not suitable for substances with unknown or complex compositions, such as mixtures, formulations, and oils (such as plant extracts and cosmetics).

Our analysis revealed that all software effectively predicted genotoxicity and carcinogenicity. As diverse groups of chemicals were analyzed, this study also represents the most comprehensive attempt to date that uses computational toxicology for genotoxicity and carcinogenicity risk assessment.

5. Conclusion

In conclusion, our results showed computational models like CASE Ultra and QSAR Toolbox demonstrated predictive accuracies comparable to traditional experimental methods across various genotoxicity and carcinogenicity endpoints.

For genotoxicity, balanced accuracy values were 80% for CASE Ultra, 85% for QSAR Toolbox, and 62 % for QSAR Toolbox Profilers, with varying sensitivities and specificities reflecting differences in how each model identifies true positives and negatives. In rodent carcinogenicity prediction, the models showed balanced accuracies ranging from 66% to 86%, with sensitivities between 65% and 93%. Positive and negative predictive values were within acceptable ranges, supporting the utility of these tools in early hazard identification.

However, the scope of these software for a broader range of domains, e.g., medical devices, food, and animal feed additives, has yet to be studied. Hence, these results show that these software tools offer a valuable means for quick and reliable initial assessment of chemicals for their genotoxicity and carcinogenic properties, and integration of these software in regulatory frameworks can enhance screening efficiency, aligning with the principles of 3Rs (Replacement, Reduction, and Refinement). It also emphasizes the importance of understanding model characteristics when applying them for regulatory or risk assessment purposes.

CRediT authorship contribution statement

Gowrav Adiga Perdur: Conceptualization, Methodology, Data curation, Writing – original draft preparation. **Zabiullah AJ:** Writing – review & editing. **Mohan Krishnappa:** Writing – review & editing. **Kamil Jurowski:** Writing – review & editing. **Varun Ahuja:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.comtox.2025.100380.

Data availability

Data will be made available on request.

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