

Reactivity and ecotoxicity of the next-generation herbicide flufenauxirim: a density functional theory (DFT) and quantitative structure-activity relationship (QSAR) study with reference to aminocyclopyrachlor

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Flufenauxirim is a newly developed pyrimidine carboxylic acid herbicide that received provisional approval from ISO in 2025 but lacks any experimental data on its environmental effects, ecotoxicity or chemical reactivity. In this study, density functional theory (DFT) at the B3LYP/6-31G(d) level was employed to compute global and local reactivity descriptors for flufenauxirim and for the structurally related reference herbicide aminocyclopyrachlor, both in gas phase and in water using the CPCM solvation model. A series of 108 substructural variants were generated by systematic removal of functional groups to identify structural alerts. An XGBoost-based QSAR model with leave-one-out cross-validation predicted the maximum electrophilic Fukui index with excellent accuracy. A composite custom risk score was formulated to rank environmental risk. The NH_2 group was identified as a critical structural alert, and flufenauxirim was predicted to have a medium-to-high aquatic toxicity profile, warranting experimental verification.

Keywords: DFT, QSAR, flufenauxirim, aminocyclopyrachlor, *in silico* toxicology

INTRODUCTION

Carboxylic acid herbicides, particularly pyrimidine and pyridine derivatives, are widely used for the selective control of broadleaf weeds because of their auxin-mimicking mode of action. [1, 2] However, their persistence in soil and water raises concerns about adverse ecotoxicological effects on non-target organisms such as fish, daphnia, algae and soil microorganisms. [3, 4]. Aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid) has been relatively well characterized experimentally, with reported acute fish $\text{LC}_{50} > 120$ mg/L, *Daphnia magna* EC_{50} 39.7-43 mg/L and soil half-lives ranging from 3 to 112 days depending on environmental conditions [5, 6]. Flufenauxirim (6-amino-2-[2-fluoro-3-methoxy-4-(trifluoromethyl)phenyl]-5-methoxypyrimidine-4-carboxylic acid) is a next-generation herbicide that was provisionally approved by ISO in 2025 [7, 8]. It contains a trifluoromethyl group ($-\text{CF}_3$) and two methoxy substituents, which may alter its lipophilicity, electronic structure and toxicological profile compared to Aminocyclopyrachlor. To date, no experimental or computational data exist on the environmental effect or toxicity of flufenauxirim.

Density functional theory (DFT) provides a robust framework for computing molecular properties such as frontier orbital energies, chemical hardness,

electrophilicity and Fukui functions, which are directly linked to chemical reactivity and potential toxicity mechanisms [9-12]. When combined with quantitative structure-activity relationship (QSAR) modelling, these descriptors can predict acute and chronic toxicity for data-poor compounds [13, 14]. Advanced machine learning approaches such as XGBoost, together with leave-one-out cross-validation (LOOCV), have been shown to improve prediction accuracy and are increasingly accepted by regulatory agencies for *in silico* risk assessment [15, 16].

In the present study, we systematically investigated the parent herbicides flufenauxirim and aminocyclopyrachlor together with a large set of substructural variants (108 structures) generated by removing or modifying key functional groups (NH_2 , OCH_3 , F, CF_3 , Cl, COOH , OH, cyclopropyl and the pyrimidine core). The objectives were: first to compute global and local reactivity descriptors at the B3LYP/6-31G(d) level in gas phase and in water (CPCM); second to develop a high-accuracy QSAR model for predicting the maximum electrophilic Fukui index f_{max}^+ ; third to formulate a composite custom risk score for ranking environmental risk; and finally, to identify structural alerts that significantly elevate the predicted risk. The results provide the first *in silico* risk assessment framework for flufenauxirim and guide future experimental testing.

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METHODOLOGY

All calculations were carried out using the Gaussian 09W program package (Revision D.01) [17]. The molecular structures of flufenauxirim and aminocyclopyrachlor were drawn with GaussView 6.0.

In addition to the parent molecules, a series of substructural variants was generated by systematically removing or replacing functional groups while preserving the core heterocyclic scaffold. This procedure yielded 80 unique structures for flufenauxirim and 28 for aminocyclopyrachlor, covering a wide range of electronic environments. The three-dimensional starting geometries were pre-optimized with the MMFF94 force field using Avogadro 1.2.0 before the DFT calculations.

Geometry optimizations and electronic property calculations were performed at the B3LYP hybrid functional level [18, 19] with the 6-31G(d) basis set [20]. This level of theory has been shown to provide reliable geometries, vibrational frequencies and frontier orbital energies for organic molecules of herbicide size [21, 22]. Solvent effects (water) were included using the conductor-like polarizable continuum model (CPCM) [23, 24]. Each structure was optimized twice: once in the gas phase ($\epsilon = 1.0$) and once in water ($\epsilon = 78.4$). All stationary points were confirmed by vibrational frequency analysis; no imaginary frequencies were found, indicating true local minima.

From the optimized gas-phase geometries, the following global reactivity descriptors were derived using Koopmans' theorem [25]: ionization potential $I \approx -E_{\text{HOMO}}$, electron affinity $A \approx -E_{\text{LUMO}}$, chemical hardness $\eta = (I - A)/2 = (E_{\text{LUMO}} - E_{\text{HOMO}})/2$, chemical softness $S = 1/(2\eta)$, electronegativity $\chi = (I + A)/2$ and the electrophilicity index $\omega = \chi^2/(2\eta)$ [26]. The HOMO and LUMO energies (in eV) as well as the dipole moments (in Debye) were taken directly from the Gaussian output files.

Local reactivity was analyzed *via* the Fukui functions using the finite-difference approximation [27, 28]. Single-point energy calculations were performed on the gas-phase optimized geometries for the neutral (charge 0, singlet), anionic (charge -1, doublet) and cationic (charge +1, doublet) species. Mulliken population analysis provided atomic charges $q_k(N)$ for the neutral molecule, $q_k(N+1)$ for the anion and $q_k(N-1)$ for the cation. The Fukui function for electrophilic attack was computed as $f^+_k = q_k(N) - q_k(N+1)$. The maximum value over all atoms, f^+_{max} , was recorded for each structure as a measure of the most electrophilically reactive site. Nucleophilic

(f^-) and radical ($f^\bullet$) indices were also calculated but are not discussed in detail here.

The solvation free energy ΔG_{solv} (in $\text{kcal}\cdot\text{mol}^{-1}$) was obtained as the difference between the water-phase and gas-phase single-point energies at the same level of theory. All computed data including total energies (Hartree), dipole moments (Debye), HOMO, LUMO, ΔE , η , χ , ω , f^+_{max} , ΔG_{solv} and molecular descriptors such as molecular weight (MW), logP, topological polar surface area (TPSA) were extracted automatically using Python 3.14 with the CCLIB and PANDAS libraries and compiled into two Excel files which serve as the primary data repository.

A quantitative structure-activity relationship (QSAR) model was developed to predict f^+_{max} from the computed DFT descriptors and derived features. The model was built using the XGBoost algorithm [15] with leave-one-out cross-validation (LOOCV). The feature set included ΔE , η , ω , dipole moments (gas and water), ΔG_{solv} , logP, MW, TPSA, as well as interaction terms: $\log(f^+_{\text{max}})$, f^+/f^- ratio, $f^+\times\Delta E$, $f^+\times\eta$, $f^+\times\text{dipole water}$ and $f^+\times\Delta G_{\text{solv}}$. The cross-validated predictive performance was evaluated using Q^2 (the squared correlation coefficient between predicted and observed values). The model achieved an excellent Q^2 of 0.9849, confirming its high predictive power.

Finally, a composite custom risk score was formulated to integrate multiple reactivity dimensions into a single ranking metric that can be used for environmental risk prioritization. The score is defined as $\text{Risk Score} = 0.35\cdot\omega + 0.45\cdot f^+_{\text{max}} - 0.20\cdot\eta$, where ω and f^+_{max} is positively correlated with electrophilic reactivity and potential toxicity, while η (hardness) is negatively correlated (harder molecules are less reactive). The coefficients were chosen empirically based on sensitivity analysis and on the known importance of electrophilicity and local reactivity in covalent binding to biological macromolecules [29, 30]. Based on the resulting risk scores, the structures were classified into three groups: high risk (score >1.0), medium risk ($0.4 \leq \text{score} \leq 1.0$) and low risk (score <0.4).

RESULTS AND DISCUSSION

The calculated global and local reactivity descriptors for the parent molecules and selected high-risk, medium-risk, and low-risk substructural variants are summarized in Tables 1 and 2 for aminocyclopyrachlor and flufenauxirim, respectively. Additionally, visual representations of the parent molecules and selected variants are provided in Figures 1 and 2.

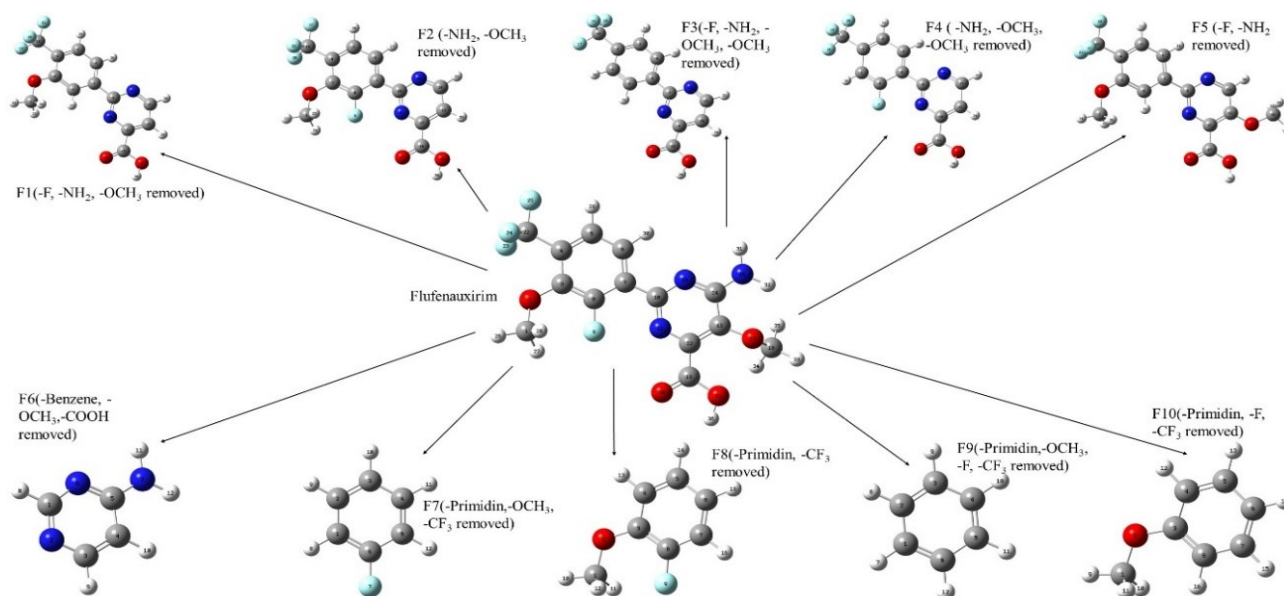


Figure 1. Chemical structures of the flufenauxirim parent molecule and selected substructural variants (A1-A10). (grey, C; white, H; red, O; blue, N; turquoise, F)

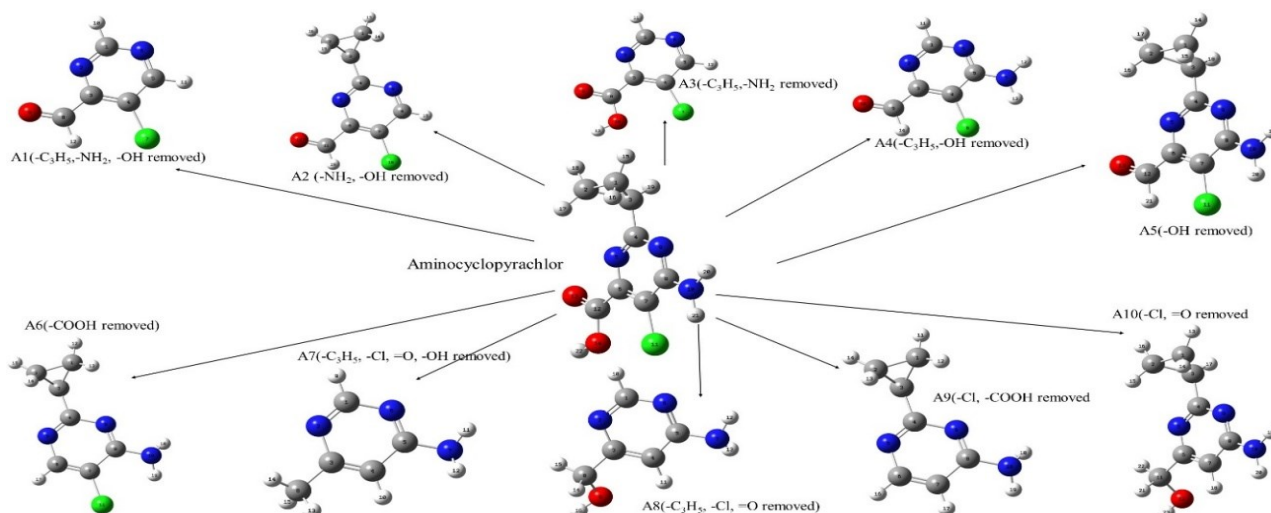


Figure 2. Chemical structures of the aminocyclopyrachlor parent molecule and selected substructural variants (F1-F10). (grey, C; white, H; red, O; blue, N; green, Cl)

Table 1. Computed reactivity descriptors and custom risk scores for flufenauxirim and its substructural variants (B3LYP/6-31G(d) CPCM water).

Molecule	ΔE Energy (eV)	η Hardness (eV)	$f_{\max}^r$	ω Electrophilicity index (eV)	Risk score	Risk group
F1	4.116	2.066	0.135	4.716	1.298	High
F2	4.457	2.229	0.143	4.712	1.268	High
F3	4.625	2.312	0.151	4.711	1.255	High
F4	4.647	2.324	0.127	4.723	1.245	High
F5	4.112	2.061	0.145	4.428	1.203	High
Flufenauxirim	4.716	2.356	0.138	3.652	0.869	Medium
F6	5.698	2.849	0.213	1.981	0.219	Low
F7	6.066	3.033	0.170	1.841	0.114	Low
F8	5.749	2.871	0.212	1.497	0.045	Low
F9	6.031	3.021	0.165	1.603	0.031	Low
F10	5.801	2.901	0.191	1.384	-0.010	Low

Table 2. Computed reactivity descriptors and custom risk scores for aminocyclopyrachlor and its substructural variants (B3LYP/6-31G(d) CPCM water).

ID	ΔE Energy (eV)	η Hardness (eV)	$f_{\max}^+$	ω Electrophilicity index (eV)	Risk score	Risk group
A1	4.651	2.323	0.168	5.827	1.650	High
A2	4.654	2.324	0.146	5.367	1.479	High
A3	5.031	2.516	0.172	4.949	1.306	High
A4	4.637	2.320	0.169	4.755	1.277	High
A5	4.644	2.321	0.171	4.458	1.173	High
Aminocyclopyrachlor	5.151	2.575	0.159	2.974	0.597	Medium
A6	5.525	2.763	0.155	2.171	0.277	Low
A7	5.752	2.872	0.166	1.890	0.162	Low
A8	5.758	2.881	0.208	1.822	0.155	Low
A9	5.754	2.878	0.181	1.817	0.142	Low
A10	5.736	2.869	0.188	1.630	0.082	Low

In both herbicide families, the removal of the amino ($-\text{NH}_2$) group consistently resulted in the highest risk scores, driven by a marked rise in electrophilicity and a decrease in chemical hardness. The parent molecule flufenauxirim exhibits a medium risk classification, yet its score is notably higher than that of aminocyclopyrachlor, indicating that flufenauxirim is intrinsically more reactive and potentially more toxic to aquatic organisms. The presence of the trifluoromethyl group and the two methoxy substituents in flufenauxirim thus appears to enhance its overall electron-accepting capacity. Conversely, variants lacking electron-withdrawing groups such as Cl, COOH or the entire pyrimidine core were assigned to the low-risk category. These structures display larger HOMO-LUMO gaps and higher chemical hardness, which are classic indicators of kinetic stability and reduced toxicity potential.

The XGBoost-based QSAR model demonstrated high predictive accuracy for the maximum electrophilic Fukui index, confirming that the selected DFT descriptors and interaction terms adequately capture the electronic factors governing local electrophilicity. This reliability reinforces the confidence in the resulting risk rankings.

A clear structural alert was identified: the amino ($-\text{NH}_2$) group functions as a critical modulator of environmental risk in pyrimidine carboxylic acid herbicides. Its removal renders the molecule more electron-deficient, as evidenced by increased electrophilicity and decreased hardness, making the derivatives more prone to covalent binding with nucleophilic sites in biological macromolecules a key mechanism of aquatic toxicity. The finding aligns with the known electron-donating nature of primary amines, and the presence or absence of this group can serve as a rapid screening criterion for toxicity.

The higher risk profile of flufenauxirim relative to aminocyclopyrachlor can be attributed to the strong electron-withdrawing effect of the $-\text{CF}_3$ group and the net electron-withdrawing contribution of the methoxy substituents in this conjugated system. The combined effect yields a molecule that is both more polarizable and more electrophilic than the reference herbicide. Experimental validation of this prediction through acute toxicity assays is strongly encouraged, as no such data currently exist for flufenauxirim.

Low-risk variants, characterized by the absence of key electron-withdrawing substituents or the pyrimidine core, exhibit high stability and low electrophilicity, consistent with the known low toxicity of simple heterocyclic cores. This suggests that the environmental risk of herbicide candidates could be substantially reduced by incorporating fewer electron-withdrawing groups or a less conjugated core, provided that herbicidal activity is maintained.

The excellent performance of the QSAR model indicates that the chosen set of DFT descriptors is sufficient for estimating local electrophilicity in related compounds, enabling application to other pyrimidine carboxylic acid derivatives within the applicability domain of the training set.

From a regulatory perspective, the proposed *in silico* framework supports early-stage screening of new herbicide candidates, aligning with the recommendations of agencies such as ECHA, EPA and OECD for the use of validated QSAR models in read-across and category approaches. For Flufenauxirim, a substance currently under regulatory review, the predicted medium-to-high risk profile highlights the need to prioritize experimental ecotoxicity testing, particularly for *Daphnia magna* and fish chronic endpoints.

Several limitations must be acknowledged. The QSAR model was trained solely on computed DFT

descriptors, and calibration against experimental toxicity data would strengthen its predictive power. Only the electrophilic Fukui function was incorporated into the risk score; future work should also consider nucleophilic and radical attack mechanisms. The empirical coefficients of the Custom Risk Score may be refined as experimental data become available. Finally, metabolic activation pathways were not considered, although they can convert a low-reactivity parent into a highly reactive intermediate *in vivo*. Despite these limitations, the integrated DFT-QSAR methodology offers a cost-effective, animal-free approach for preliminary environmental risk assessment of newly synthesized herbicides.

CONCLUSIONS

NH₂ group was identified as the strongest structural alert for high environmental risk, and Flufenauxirim is predicted to be more reactive than Aminocyclopyrachlor. Removal of electron-withdrawing groups yields low-risk variants with increased hardness and larger HOMO-LUMO gaps. The integrated DFT-QSAR approach provides a reliable framework for early-stage risk screening of new herbicide candidates, reduces the need for animal testing, and supports regulatory decision-making. For flufenauxirim, experimental acute and chronic toxicity tests are strongly recommended to validate the present predictions.

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