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Potential Health Risks of Chloroacetanilide Herbicides: An In Silico Analysis

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Abstract

The extensive use of herbicidal products in agriculture and forestry has raised concerns over potential adverse effects on human health and the environment. Chloroacetanilide herbicides are a group of synthetic chemicals used to control weeds in agriculture and forestry. However, so[me of their members have been characterized as possible carcinogens. The genotoxicity and carcinogenicity of two chloroacetanilide herbicides, delachlor and xylachlor, are discussed. This article proposes to use tools to predict their potential toxicities based on their chemical structure. Four software tools, Vega Hub, Toxtree, Lazar, and TEST, are used to predict the potential genotoxic and carcinogenic effects of the herbicides. Vega Hub uses QSAR models, Toxtree uses a decision tree approach, Lazar uses data mining algorithms, and TEST uses QSAR methods to estimate toxicity. The canonical Simplified Molecular Input Line Entry Specification (SMILES) systems of delachlor and xylachlor are entered into each software tool to create a prediction. The study found that delachlor and xylachlor is a class 3 highly toxic compounds with potential mutagenic and carcinogenic effects based on Toxtree and Vega Hub. Meanwhile, Lazar and TEST predicted that delachlor and xylachlor are unlikely to be mutagenic. This study to determine the toxicity of the herbicides delachlor and xylachlor has shown that the possible effects of these herbicides on health and the environment need to be further investigated. The results provide valuable insights into chloroacetanilide herbicide toxicity and help develop safer, more environmentally friendly alternatives.

Keywords: Genotoxicity, carcinogenicity, in silico, chloroacetanilide herbicides

1. INTRODUCTION

The agricultural sector is responsible for producing the food people need to sustain their lives. However, plant diseases, insects, and weeds reduce productivity and quality. Therefore, agrochemicals such as herbicides help farmers to control these adverse effects

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and increase crop yields [1]. However, concerns about potential adverse effects on human health and the environment have arisen from the widespread use of these chemicals. One such concern, which can pose a significant threat to public health and the environment, is the potential genotoxic and carcinogenic effects of herbicides [2, 3].

Chloroacetanilide herbicides are a group of synthetic chemicals that are widely used for the control of weeds in agriculture and forestry. These herbicides are widely used to grow cereals, corn, soybeans, cotton, and many other crops. Due to their effectiveness in controlling weeds that can cause significant yield losses, the use of these herbicides has increased significantly in recent years [4].

Chloroacetanilide herbicides share the 2-chloroacetanilide molecular core, differing only in type and arrangement of substitutions [5]. In spite of the importance of the production of herbicides, there is one aspect that is still dramatic: the carcinogenic potential of the chloroacetanilide herbicides. Some of the members of the chloroacetanilide herbicide family have been characterized by the US Environmental Protection Agency as possible carcinogenic compounds [6]. However, the carcinogenic mechanism of chloroacetanilide compounds remains unclear, although some experiments suggest that the carcinogenic properties are related to the herbicides' ability to nucleophilically react with DNA [7]. Studies have shown that exposure to some of the chloroacetanilide herbicides can cause a range of adverse effects on non-target organisms, including humans [8-11]. These effects include developmental abnormalities, reproductive toxicity, and carcinogenicity. Therefore, it is essential to evaluate the potential toxicity of these herbicides and develop safer alternatives to minimize their environmental and health impacts.

Delachlor and xylachlor are two of the herbicides of the chloroacetanilide group (Figure 1). Delachlor is an herbicide widely used to control weeds in crops such as sugarbeet and cereals. It is also used in combination with other herbicides to control weeds in rice fields. Its chemical formula is $C_{15}H_{22}ClNO_2$. Xylachlor is an herbicide that has been used for pre-emergence or pre-plant control of annual rgrasses in cereals and other crops. Its chemical formula is $C_{13}H_{18}ClNO$ [12].

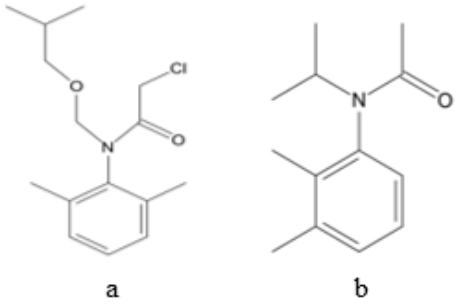

Figure 1 Chemical structures of a) delachlor and b) xylachlor

In silico tools are computer-based methods for predicting chemicals' potential toxicity from their chemical structures. Without the need for animal testing or expensive laboratory equipment, *in silico* toxicity prediction tools may provide an accurate assessment of a xenobiotic's toxicity [13]. This is beneficial because it allows researchers to assess the potential toxicity of a compound or drug quickly and easily without having to incur the cost and time associated with more traditional methods. In addition, by analysing the structure of the molecule and its potential interactions with the system, *in silico* tools can provide a more comprehensive assessment of a compound's toxicity than traditional methods [14].

The study aimed to use four software tools, Vega Hub, Toxtree, Lazar, and TEST, to predict the potential toxicity of these herbicides based on their chemical structures. The findings of this study will provide valuable insights into the toxicity of chloroacetanilide herbicides and aid in the development of safer and more sustainable alternatives.

2. METHODOLOGY

The study used four *in silico* tools to predict the potential genotoxic and carcinogenic effects of delachlor and xylachlor: Vega Hub, Toxtree (Estimation of Toxic Hazard- A Decision Tree Approach), Lazar and TEST (Toxicity Estimation Software Tool). The software tools were selected for their ability to predict the toxicity of chemicals based on their chemical structure. Each of them is open source and free public software.

Vega Hub is a software tool that uses quantitative structure-activity relationship (QSAR) models to predict the toxicity of chemicals based on their physical, chemical and biological properties [15]. QSARs use mathematical models to predict levels of toxicity based on the physical properties of the molecular structure of a chemical, known as molecular descriptors. The tool predicts the toxicity of chemicals based on their similarity to known toxic compounds. It assigns a consensus score to each prediction. This software has an open structure for data sharing and modelling and can be easily used by any user [16].

Toxtree was developed in accordance with the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation to assess the toxic effects of chemical substances. It is a software tool that uses a decision tree approach to predict the toxicity of chemicals based on their structural alerts. The tool predicts the toxicity of chemicals by identifying structural features associated with toxicity [17].

Lazar is an open-source web application that uses data mining algorithms to derive predictions for untested compounds from experimental training data, which can be any dataset containing chemical structures and biological activities. Lazar offers researchers a versatile solution by providing both a user-friendly interface and a large database [18].

The Toxicity Estimation Software Tool (TEST) has been developed to facilitate the prediction of chemical toxicity using Quantitative Structure Activity Relationship (QSAR) techniques. TEST analyses the molecular structure of an organic chemical entered by the user to determine toxicity values and physical properties. TEST has no external software requirements and users can enter a chemical to be evaluated via a provided chemical sketch window, a text file of the structure or a database of structures. Various advanced QSAR methods are used within TEST to calculate the necessary molecular descriptors [19].

The canonical Simplified Molecular Input Line Entry Specification (SMILES) systems of delachlor (CC1=C(C(=CC=C1)C)N(COCC(C)C)C(=O)CCl) and xylachlor (CC1=C(C(=CC=C1)N(C(C)C)C(=O)CCl)C) were entered into each software tool to predict their potential toxicity and carcinogenicity [20, 21]. The software tools use a variety of algorithms and models to predict the toxicity and carcinogenicity of chemicals based on their chemical structures.

3. RESULTS

Toxtree predicted that delachlor is a class 3 highly toxic compound with potential mutagenic and carcinogenic properties based on structural alerts. Vega Hub predicted that delachlor is a mutagenic and potentially carcinogenic compound with a consensus score of 0.45, indicating moderate confidence in the prediction. Therefore, Vega Hub predicted that delachlor is not genotoxic, but the model predictions were not in agreement. On the other hand, TEST predicted that delachlor is non-mutagenic with a consensus score of 0.28, while LAZAR predicted that delachlor is non-mutagenic with a probability of 0.419 but cannot predict carcinogenicity.

For xylachlor, toxtree predicted that it is a class 3 highly toxic compound with potential mutagenic and carcinogenic properties based

on structural alerts. Vega hub predicted that xylachlor is a mutagenic and potentially carcinogenic compound with a consensus score of 0.525, indicating a moderate level of confidence in the prediction. However, vega hub also predicted that xylachlor is not genotoxic, indicating that its carcinogenic properties may be due to mechanisms other than dna damage. Test predicted that xylachlor is non-mutagenic with a consensus score of 0.26, while lazar predicted that xylachlor is non-carcinogenic and non-mutagenic with probability scores of 0.217 and 0.213 respectively.

4. DISCUSSION

In silico toxicology prediction tools, also known as computational toxicology, offer several advantages in the field of toxicology. Firstly, they are cost-effective and reduce the need for animal testing, which can be expensive and time-consuming. Secondly, they provide quick and efficient identification of potentially toxic substances, which can be useful in the early stages of drug development. Additionally, they can be used to predict the toxicity of chemicals that have not yet been tested, allowing for more informed decision-making in terms of public health and environmental safety [14]. However, *in silico* toxicology prediction tools do have their limitations. One of the biggest limitations is the lack of complete accuracy, as the predictive models are based on assumptions and extrapolations from existing data. Furthermore, these tools are not yet able to fully replace animal testing, as there are still certain aspects of toxicity that cannot be accurately predicted *in silico* [22].

There have been many studies in the literature on the potential health risks of the chloroacetanilide family of herbicides. The most common chloroacetanilide herbicides are acetochlor, alachlor, butachlor, metolachlor, s-metolachlor, pretilachlor, propachlor and propisochlor. These herbicides may have adverse effects on human health, according to some studies. These herbicides are classified by the USEPA as Class B2, L2, and C carcinogens and are reported to have moderate to high chronic toxicity [23, 24]. For example, one study has shown that chloroacetanilide herbicides such as alachlor increase the likelihood of developing Parkinson's disease [25]. Ecotoxicological studies suggest that these herbicides are the causal agents for DNA damage and tumor induction in rats, fish, and human lymphocyte cells found in *in vitro* studies [26-29].

This study was conducted using *in silico* analyses to determine the potential toxicity of delachlor and xylachlor herbicides. Analyses through four different software such as Toxtree, Vega Hub, TEST and LAZAR showed that both herbicides exhibited mutagenic and carcinogenic properties in Toxtree and VEGA software. Nonetheless, the results of TEST and LAZAR predicted that delachlor and xylachlor were non-mutagenic, while xylachlor was non-carcinogenic.

Our results suggest that two commonly used herbicides, delachlor and xylachlor, may have potentially harmful effects on human health and the environment. These results emphasize the importance of using *in silico* tools to determine the toxicity of herbicides as well as other chemicals.

5. CONCLUSION

Our study highlights the need for continued research and monitoring of herbicides such as delachlor and xylachlor and underscores the importance of using advanced computational tools to identify and mitigate potential health and environmental risks associated with chemical use in agriculture and forestry. The discrepancies in the predictions among the software tools may be due to the differences in the algorithms and models used, as well as the limitations of the *in silico* approach. *In silico* predictions are based on the chemical structure of the compound and do not take into account the complex interactions and

metabolic processes that occur *in vivo*. Therefore, the *in silico* predictions should be interpreted with caution and confirmed by *in vitro* and *in vivo* experiments. Despite the limitations of the *in silico* approach, it can provide valuable insights into the potential toxicity of chemicals and aid in the development of safer and more sustainable alternatives. By taking a proactive approach to chemical safety, we can ensure that we are protecting human health and the environment for generations to come.

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The authors contributed equally to the study.

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The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

REFERENCES

[1] A. De, R. Bose, A. Kumar, S. Mozumdar, "Worldwide Pesticide Use". In: Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles. SpringerBriefs in Molecular Science. Springer, New Delhi, 5-6, 2014.

[2] B. Alewu, C. Nosiri, "Pesticides and human health," Pesticides in the modern world–effects of pesticides exposure. InTech, pp. 231–50, 2011.

[3] World Health Organization (WHO), "Public health impact of pesticides used in agriculture". World Health Organization, 1990. [Online]. Available: https://apps.who.int/iris/handle/10665/39772

[4] W. Yang, B. A. Holmén, "Relative effects of surfactants and humidity on soil/air desorption of chloroacetanilide and dinitroaniline herbicides," Environmental science & technology, vol. 42, no. 18, pp. 6843–6848, 2008.

[5] W. Liu, J. Gan, S. K. Papiernik, S. R. Yates, "Structural Influences in Relative Sorptivity of Chloroacetanilide Herbicides on Soil," Journal of Agricultural and Food Chemistry, vol. 48, no. 9, pp. 4320–4325, 2000.

[6] K. L. Dearfield, N. E. McCarroll, A. Protzel, H. F. Stack, M. A. Jackson, M. D. Waters, "A survey of EPA/OPP and open literature on selected pesticide chemicals: II. Mutagenicity and carcinogenicity of selected chloroacetanilides and related compounds," Mutation Research/Genetic Toxicology and Environmental Mutagenesis, vol. 443, no. 1–2, pp. 183–221, 1999.

[7] J. D. Coates, R. A. Bruce, J. D. Haddock, "Anoxic bioremediation of hydrocarbons," Nature, vol. 396, no. 6713, pp. 730–730, 1998.

[8] J. Ashby, L. Kier, A. Wilson, T. Green, P. Lefevre, H. Tinwell, G. Willis, W. Heydens, M. Clapp. "Evaluation of the Potential Carcinogenicity and Genetic Toxicity to Humans of the Herbicide Acetochlor." Human & Experimental Toxicology, vol. 15, no. 9: pp. 702–35, 1996.

[9] H. Bian, J. Chen, X. Cai, P. Liu, Y. Wang, L. Huang, X. Qiao, and C. Hao, "Dechlorination of chloroacetanilide herbicides by plant growth regulator sodium bisulfite," Water Research, vol. 43, no. 14, pp. 3566–3574, Aug. 2009.

[10] P. J. Dierickx, "Glutathione- dependent cytotoxicity of the chloroacetanilide herbicides alachlor, metolachlor, and propachlor in rat and human hepatoma-derived cultured cells," Cell biology and toxicology, vol. 15, pp. 325–332, 1999.

[11] S. Furukawa, T. Harada, D. Thake, M. J. Iatropoulos, J. H. Sherman, "Consensus diagnoses and mode of action for the formation of gastric tumors in rats treated with the chloroacetanilide herbicides alachlor and butachlor," Toxicologic Pathology, vol. 42, no. 2, pp. 386–402, 2014.

[12] K. A. Lewis, J. Tzilivakis, D. J. Warner, A. Green, "An international database for pesticide risk assessments and management," Human and Ecological Risk Assessment: An International Journal, vol. 22, no. 4, pp. 1050–1064, 2016.

[13] OECD, "Fundamental and guiding principles for (Q) SAR analysis of chemical carcinogens with mechanistic considerations," Series on testing and assessment. 2015. [Online]. Available: https://one.oecd.org/document/env/jm/mono(2015)46/en/pdf

[14] G. J. Myatt, E. Ahlberg, Y. Akahori, D. Allen, A. Amberg, L. T. Anger, A. Aptula, S. Auerbach, L. Beilke, P. Bellion, R. Benigni, J. Bercu, E. D. Booth, D. Bower, A. Brigo, N. Burden, Z. Cammerer, M. T. D. Cronin, K. P. Cross, L. Custer, M. Dettwiler, K. Dobo, K. A. Ford, M. C. Fortin, S. E. Gad-McDonald, N. Gellatly, V. Gervais, K. P. Glover, S. Glowienke, J. Van Gompel, S. Gutsell, B. Hardy, J. S. Harvey, J. Hillegass, M. Honma, J.-H. Hsieh, C.-W. Hsu, K. Hughes, C. Johnson, R. Jolly, D. Jones, R. Kemper, M. O. Kenyon, M. T. Kim, N. L. Kruhlak, S. A. Kulkarni, K. Kümmerer, P. Leavitt, B. Majer, S. Masten, S. Miller, J. Moser, M. Mumtaz, W. Muster, L. Neilson, T. I. Oprea, G. Patlewicz, A. Paulino, E. Lo Piparo, M. Powley, D. P. Quigley, M. V. Reddy, A.-N. Richarz, P. Ruiz, B. Schilter, R. Serafimova, W. Simpson, L. Stavitskaya, R. Stidl, D. Suarez-Rodriguez, D. T. Szabo, A. Teasdale, A. Trejo-Martin, J.-P. Valentin, A. Vuorinen, B. A. Wall, P. Watts, A. T. White, J. Wichard, K. L. Witt, A. Woolley, D. Woolley, C. Zwickl, and C. Hasselgren, "In silico toxicology protocols," Regulatory Toxicology and Pharmacology, vol. 96, pp. 1–17, 2018.

[15] H.-J. Klimisch, M. Andreae, U. Tillmann, "A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data,"Regulatory Toxicology and Pharmacology, vol. 25, no. 1, pp. 1–5, 1997.

[16] E. Benfenati, A. Manganaro, G. C. Gini, "VEGA-QSAR: AI inside a platform for predictive toxicology.," PAI@ AI* IA, vol. 1107, pp. 21–28, 2013.

[17] G. Patlewicz, N. Jeliazkova, R. J. Safford, A. P. Worth, B. Aleksiev, "An evaluation of the implementation of the Cramer classification scheme in the

Toxtree software," SAR and QSAR in Environmental Research, vol. 19, no. 5–6, pp. 495–524, 2008.

[18] A. Maunz, M. Gütlein, M. Rautenberg, D. Vorgrimmler, D. Gebele, C. Helma, "Lazar: a modular predictive toxicology framework". Frontiers in pharmacology, 4, 38, 2013.

[19] T. M. Martin, "User's Guide for T.E.S.T. (Toxicity Estimation Software Tool)", 2020.

[20] PubChem, "Delachlor." https://pubchem.ncbi.nlm.nih.gov/compound/32321 (accessed Apr. 02, 2023).

[21] PubChem, "Xylachlor." https://pubchem.ncbi.nlm.nih.gov/compound/162992 (accessed Apr. 02, 2023).

[22] T. Tralau, M. Oelgeschläger, R. Gürtler, G. Heinemeyer, M. Herzler, T. Höfer, H. Itter, T. Kuhl, N. Lange, N. Lorenz, C. Müller-Graf, U. Pabel, R. Pirow, V. Ritz, H. Schafft, H. Schneider, T. Schulz, D. Schumacher, S. Zellmer, G. Fleur-Böl, M. Greiner, M. Lahrssen-Wiederholt, A. Lampen, A. Luch, G. Schönfelder, R. Solecki, R. Wittkowski, A. Hensel, "Regulatory toxicology in the twenty-first century: challenges, perspectives and possible solutions". Archives of Toxicology, vol. 89, 823–850, 2015.

[23] USEPA, "Inventory of US greenhouse gas emissions and sinks: 1990–2011. EPA-430-R-13-001," 2013.

[24] M. Cheng, Q. Meng, Y. Yang, C. Chu, Q. Chen, Y. Li, D. Cheng, Q. Hong, X. Yan, J. He, "The Two-Component Monooxygenase MeaXY Initiates the Downstream Pathway of Chloroacetanilide Herbicide Catabolism in Sphingomonads". Applied and Environmental Microbiology, 83, 2017.

[25] N. Wan, G. Lin, "Parkinson's disease and pesticides exposure: new findings from a comprehensive study in Nebraska, USA," The Journal of Rural Health, vol. 32, no. 3, pp. 303–313, 2016.

[26] B. Hill, P. R. Jefferies, G. B. Quistad, J. E. Casida, "Dialkylquinoneimine metabolites of chloroacetanilide herbicides induce sister chromatid exchanges in cultured human lymphocytes," Mutation Research/Genetic Toxicology and Environmental Mutagenesis, vol. 395, no. 2–3, pp. 159–171, 1997.

[27] T. Green, R. Lee, R. B. Moore, J. Ashby, G. A. Willis, V. J. Lund, M. J. L. Clapp, "Acetochlor-induced rat nasal tumors: further studies on the mode of action and relevance to humans". Regulatory Toxicology and Pharmacology, vol. 32, no. 1, 127-133, 2000.

[28] B. Ateeq, M. A. Farah, W. Ahmad, "Detection of DNA damage by alkaline single cell gel electrophoresis in 2, 4-dichlorophenoxyacetic-acid-and butachlor-exposed erythrocytes of Clarias batrachus," Ecotoxicology and environmental safety, vol. 62, no. 3, pp. 348–354, 2005.

[29] C. D. Nwani, U. I. Ama, F. Okoh, U. O. Oji, R. C. Ogbonyealu, A. A. Ibiam, O. Udu-Ibiam, "Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish Tilapia zillii". African journal of biotechnology, vol. 12, no. 5, 2013.