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Analysis of the properties of Picloram and proposal of a compound as its replacement

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Abstract: Pesticides such as Picloram, are considered toxic to humans and the environment, which is necessary search new substancesless toxics. With the analysis of the structure and some molecular descriptors, it is possible to propose new compounds, in which it can reduce the toxicity in humans without affect its power as herbicide. This paper presents the results of the molecular structure of the new compounds, starting with the Picloram. The characteristics of absorption, metabolism and toxicity of each possible replacement were evaluated. From which, a less toxic compound containing specific characteristics of an organochlorine herbicide was obtained. From the 10 compounds tested, 2 were selected, in order to carry out the proposals for replacement the pesticide. The compound 4-amino-6-chloropyridine-2-carboxylic acid (substance I) got less simulated toxicity. In the toxicity for some species, an increase of 8.36 % on average in the lethal dose was founded.
Keywords -Organochlorine Herbicide, Picloram, ADMET Properties, Toxicity.

Introduction

Organochlorine compounds, attack the central nervous system when were ingested, these substances are inhibitors of ATPase, thereby increasing the concentration of adenine triphosphate (ATP) produced. This compound is a fundamental nucleotide, used in generating of celular energy, necessary for biological work, such as breathing, circulation, nerve transmission, among others[1,2] . Blockade of oxidative phosphorylation due to inhibition of ATPase, leads to a reduction in cellular respiration and causes damage to organs in which exist direct contact[2]. Nowadays, the use of chemical pesticides for pest control is predominant, therefore the number of people poisoned by pesticides is high in agricultural areas[4,5].

4-Amino-3,5,6-trichloropyridin-2-carboxylic acid commonly known as Picloram. It is part of the family of organochlorine herbicides and it is is classified by the EPA (1995) as a compound that does not show carcinogenicity in humans. However, to high doses is very toxic. When exposed to plants, it causes an uncontrolled growth, which ends with death. In laboratory tests was determined the compound is highly hydrophilic, so it is normal to find them in bodies of water as a contaminant, however, this substance is rapidly degraded by some bacteria present in the rivers. [6]

Toxicological tests, require a lot of time and materials, so it is necessary anticipate system properties and reduce the resources used, this process is known as in-silico tests[7]. This process is widely used in the pharmaceutical industry for predicting new compounds[8,9].

The in-silico method was based on the analysis of molecular descriptors. These descriptors quantify the behaviors of each compound relative to similar molecules. With the analysis of the new substance, descriptors were obtained and make a relation to properties of molecules already studied[10,11]. For pesticides, the main method of prediction are quantitative structure-activity relationships (QSAR) [12].

For drugs, the analysis was complemented by a more detailed study, which is based on the properties of absorption, distribution, metabolism, excretion and toxicity (ADMET), this method is based on relationships QSAR detailed[8]. All these properties allow a more complete estimate of the behavior of the molecule based on its analysis.

Based on the above, in this work was made the study of Picloram and some substances derivate from this, which reduce the toxicological effects for humans and the environment.

I. Materials and Methods

The evaluation of ADMET properties, was conducted through 3 steps: assessment of the models used by the program, development of new molecules based on reactivity of Picloram and the evaluation of the properties of new substances. The **Error! Reference source not found.** shows the general methodology of research, also the main steps for the validation of the method and the properties of the new compounds are listed.

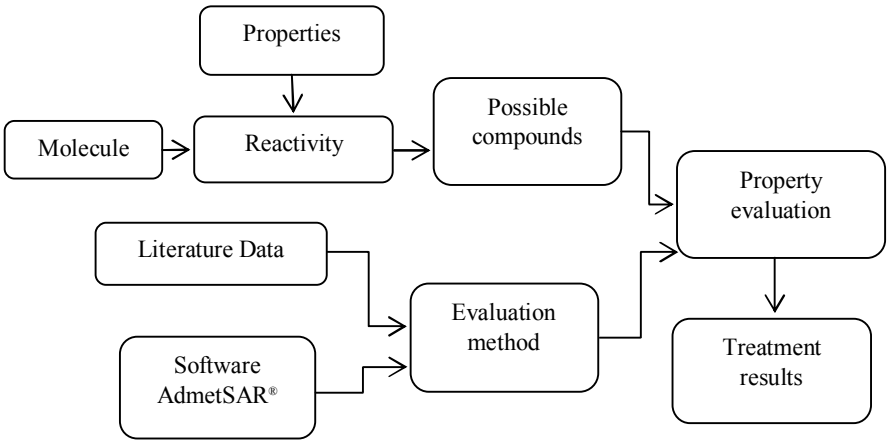

Figure1: General methodology for obtaining properties of new compounds.

a) Evaluation model:

Obtaining the ADMET properties was performed using the AdmetSAR® software[8]. To use the software, the degree of precision that had the model was verified. The model results were compared with data from the literature and government sources for the control of agricultural pesticides, such as the Agropecuarian colombian institute (ICA), the Ministry of Agriculture and Rural Development and the Environmental Protection Agency of the United States.

The analysis took into account the properties of absorption, metabolism and toxicity, because the organochlorines compounds are rapidly metabolized in humans, whereby the distribution and excretion properties, are not good indicators of the behavior of molecule inside the body[1]. The analysis was supplemented with the probability of success in predicting, in order to discard the less accurate values.

b) Development of new molecules:

Alternative substances were acquired through the possible reactions that can have the Picloram. In **Error! Reference source not found.** was showed the main functional group of the molecule and the chlorine

atoms, on which the reactions were performed, for obtaining new molecules. These parts of the molecule are the more unstable radicals, reason which the new substances are easily obtained[13].

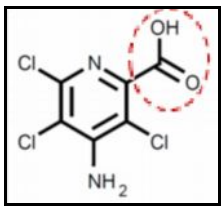

Figure2: Acid group of Picloram.

c) Evaluation of properties:

The scale of toxicological classification of the World Health Organization (WHO) for pesticides was used, for changing the qualitative values of toxicity level to quantitative values, to make a numerical analysis (run, 2009). The conversion is shown in **Error! Reference source not found.**, for harmful features a negative value was given, to symbolize the damage of the compound can produce.

Table1: Conversions for qualitative data.

Assigned value	Qualitative property	Toxicity Level
1.00	Less harmful	V
0.50		IV
0.00		III
-0.50		II
-1.00	More harmful	I

In order to facilitate analysis of the properties, the weighing of the properties was carried out with the equation 1, where the result (R) is the value according to conversion (V) and the probability (P) for each property. Thus it was found that the evaluation of toxicity is inversely related to the value obtained from R.

$$R=\sum V^{*}P \qquad (1)$$

The properties were quantitatively evaluated as shown in

Table8, for the 5 molecules that obtained the higher value in the evaluation of qualitative properties, , in order to determine the ideal replacement. These properties had some error in the correlations used by the program, this error was due to linear prediction models multi-qualifiers used[8].

Table 2: Evaluation of quantitative properties for Picloram.

Property	**Value**
Solubility (LogS)	-2.8128
Rat acute toxicity (LD50, mol/kg)	1.2693
Fish toxicity (pLC50, mg/L)	1.5001
Tetrahymena Pyriformis toxicity (pIGC50, ug/L)	1.8758

III. Results

Picloram is an herbicide, mainly due to their chlorine radicals, besides the acid group own of molecule (**Error! Reference source not found.**). This molecule is metabolized and produces chlorine anions, which are attracted to the sodium and potassium cations containing in plants. The use of these cations for forming the salt,

causes the sodium-potassium pump fail, with that, begin to deteriorate the cell biological processes, which lead to their premature death and decreased the life plant. If this compound is applied improperly on crops, it can cause severe damage to species that inhabit the sector. The toxicity (LD50) in rats for this compound in 5000 mg / kg, with which this compound is classified as moderately toxic.

To use the software, it is required to insert the compounds with the format simplified molecular input line entry system (SMILES), for which they were drawn and optimized structures with MOPAC 2012 program v 15.156W using the method FP7, that version was used due it is the version which has less error in the prediction of the structure to compounds organochlorines[14,15].

The optimized molecule was converted to SMILES format following the rules proposed by Weininger in 1988 for a single notation for each molecule, this process is performed in the program OpenBabel V.2.3.1 [16,17].

OC(=O)c1nc(Cl)c(c(c1Cl)N)Cl Smile Picloram

The validation of the model used by the AdmetSAR ® software, the most important characteristics for toxicity were evaluated in various animal species as rats, bees and fish[11,13,18]. The properties evaluated were absorption, metabolism and toxicity. Inside these properties stand the cytochrome P450 metabolism, the solubility and cancer risk by exposure to the molecule. 7 characteristics were obtained, which are specified in**Error! Reference source not found.**.The results obtained from the model of prediction of properties were compared with values from the literature. In addition, the relative error was calculated, which shows a measure of the accuracy of the system. That evaluation is the point of reference for the data obtained from the software and how is the behavior for the organochlorine compounds.

Table3: Comparison of the prediction model AdmetSAR®..

Parameter	Prediction	Probability	Real value (Akoto et al., 2015; C. Li et al., 2015; Wu et al., 2015)	Relative error
S (H2O)	-2.8128	0.81	400	(7.11)
			430	(13.59)
			562	(33.92)
Cancer	No Carcinogenic	0.7854	No Carcinogenic	No
Human Cancer	No	0.7237	No	No
			No	No
			No	No
			Negative	No
Rat toxicity DL 50	1.9801	0.613	5000	(4.38)
			5000	(4.38)
			4500	6.25
Bee toxicity	Baja	0.9051	8163.265306	1 Category
Biodegradability	No	0.8429	No	No
Metabolism CYP450 1A2	No inhibitor	0.8442	No inhibitor, substrate	No

To obtain the new molecules derived from the Picloram, It was made a search for structural transformations from the active sites and the radicals of Picloram, as they were the acid group and the chlorine anions. Three criteria were defined, to focus the information collection, first, the molecules necessary for the synthesis of Picloram, molecules whose preparation comes Picloram degradation and the main reactions of the acid group, as the addition of a base whereby the corresponding salt is obtained. Through this methodology, 10 possible compounds shown in **Error! Reference source not found.** were found. To have a means of comparison of the new molecules, the properties for Picloram was also analyzed.

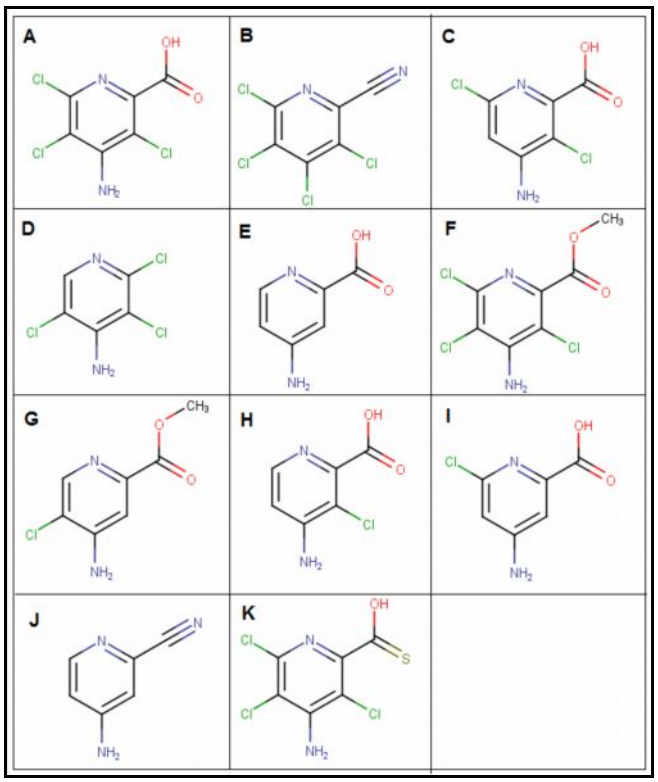

Figure3: Bi dimensional structure of the molecules studied.

The shape of the molecule was draw and optimized through the Mopac 2012 program, using the method FP7 for analysis the organochlorine substances, finally, the molecules were converted to the SMILE format proposed by Weininger, for this, the molecule was saved in format .mol, it is a file format for holding information about the atoms, bonds, connectivity and coordinates of a molecule. In **Error! Reference source not found.** the final results for each molecule are observed, before being subjected to the prediction model of properties. With the software AdmetSAR the properties were evaluated, in **Error! Reference source not found.** is shown the results obtained for Picloram. The analysis of the results is performed by applying equation 1, for which, it has that the higher the value obtained, the compound will be less toxic, this was due that was assigned a greater value to the beneficial behaviors of each characteristic evaluated.

Table4: SMILE format for the studied molecules.

Molecule	SMILE
A	OC(=O)c1nc(Cl)c(c(c1Cl)N)Cl
B	C1(=C(C(=C([C](=N1)#N)Cl)Cl)Cl)Cl
C	c1(cc(c(c(n1)C(=O)O)Cl)N)Cl
D	c1c(c(c(c(n1)Cl)Cl)N)Cl
E	c1cc(cc(n1)C(=O)O)N
F	c1(c(c(c(c(n1)C(=O)OC)Cl)N)Cl)Cl
G	c1c(c(cc(n1)C(=O)OC)N)Cl
H	c1cc(c(c(n1)C(=O)O)Cl)N
I	c1(cc(cc(n1)C(=O)O)N)Cl
J	C1=CC=C[C](=N1)#N
K	c1(c(c(c(c(n1)C(=S)OC)Cl)N)Cl)Cl

Table 5: Absorption evaluation for Picloram.

Property	Value	Probability
Blood-Brain Barrier	1.0000	0.8157
Human Intestinal Absorption	-1.0000	0.9179
Caco-2 Permeability	1.0000	0.6613
P-glycoprotein Substrate	-1.0000	0.8324
P-glycoprotein Inhibitor	1.0000	0.9880
	1.0000	0.9827
Renal Organic Cation Transporter	1.0000	0.9293
Total Absorption	**2.6267**	

The absorption was showed in **Error! Reference source not found.**, which was worked with features, that aim to indicate the level of absorption of the substance, in different parts of the human body, the main characteristics assessed are: the blood-brain barrier, which indicate the substance causes the formation of a barrier of cells in the cerebral cortex, this barrier has as its main objective the prevention of the passage of toxic substances to the brain. The absorption in the intestine, the permeability Caco-2 and the P-glycoprotein and the transporter renal, they evaluated the uptake and ease of entry into the bloodstream, through the various digestive organs such as stomach, small intestine, among others[4,8].

The metabolism was showed in **Error! Reference source not found.**, it were considered the cytochromes P450, these are part of the family of the hemeproteins, which form part of the electron transfer chain in which the substrates are metabolized, in order to be finally utilized by the body. They were evaluated from two points of view, whether they were inhibitors or if they had functionality as substrates. Finally the toxicity was evaluated in **Error! Reference source not found.**, through different species such as bees, rats and the Tetrahymena pyriformis. These species were chosen due its similarity in the metabolism to the humans, reason why they were widely used in laboratory studies[19,20]. The global results for the other molecules are shown in **Error! Reference source not found.**.

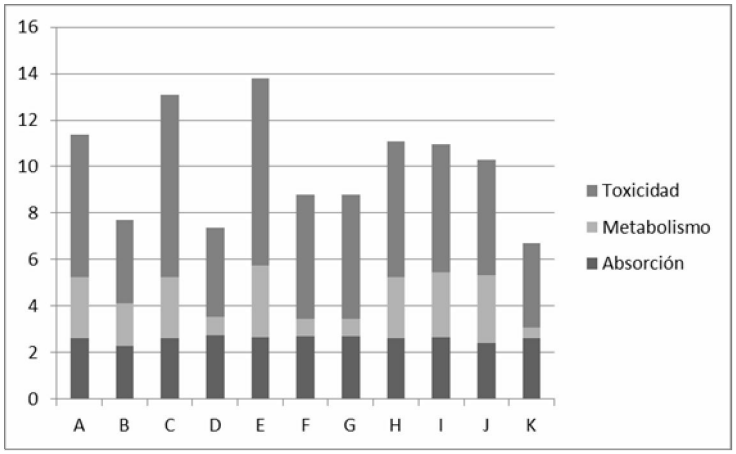

Figure4: Qualitative assessment of molecules.

Table6: Metabolism evaluation for Picloram.

Property	Value	Probability
CYP450 2C9 Substrate	-1.0000	0.8512
CYP450 2D6 Substrate	-1.0000	0.9018
CYP450 3A4 Substrate	-1.0000	0.7613
CYP450 1A2 Inhibitor	1.0000	0.6256
CYP450 2C9 Inhibitor	1.0000	0.8916
CYP450 2D6 Inhibitor	1.0000	0.9196
CYP450 2C19 Inhibitor	1.0000	0.9515
CYP450 3A4 Inhibitor	1.0000	0.8329
CYP Inhibitory Promiscuity	1.0000	0.9173

Total Metabolism	**2.6242**

Tabla 7: Toxicity evaluation for Picloram.

Property	**Value**	**Probability**
Human Ether-a-go-go-Related Gene Inhibition	1.0000	0.9885
	1.0000	0.9582
AMES Toxicity	1.0000	0.9133
Carcinogens	1.0000	0.7854
Fish Toxicity	1.0000	0.6873
Tetrahymena Pyriformis Toxicity	1.0000	0.6205
Honey Bee Toxicity	1.0000	0.9051
Biodegradation	-1.0000	0.9932
Acute Oral Toxicity	0.5000	0.6154
Carcinogenicity (Three-class)	1.0000	0.9432
Total Toxicity	**6.1160**	
Total	**11.3669**	

Table8: Evaluation of quantitative properties for Picloram.

Property	**Value**
Solubility (LogS)	-2.8128
Rat acute toxicity (LD50, mol/kg)	1.2693
Fish toxicity (pLC50, mg/L)	1.5001
Tetrahymena Pyriformis toxicity (pIGC50, ug/L)	1.8758

Table 9: Quantitative evaluation.

	Aqueous solubility (LogS)	**Rat Acute Toxicity (LD50. mol/kg)**	**Fish Toxicity (pLC50. mg/L)**	**Tetrahymena Pyriformis Toxicity (pIGC50. ug/L)**
A	-2.8128	1.2693	1.5001	1.8758
C	-2.8128	1.2693	1.5001	1.8758
E	-1.2774	1.1421	1.7317	2.9008
H	-2.8128	1.2693	1.5001	1.8758
I	-2.5285	1.1905	1.5676	2.1684

The molecules A, C, E, H and I have the highest values in the evaluation of their qualitative properties, so these compounds pass to the second phase, where the results of quantitative prediction models (**Error! Reference source not found.**). In these models were found the solubility, which is an indicator of the hydrophobicity of the molecule and the toxicity in some species, such the rats, the fish toxicity and the Tetrahymena pyriformis.

IV. Discussion

The validation of the prediction system was performed with data from the literature, with the comparison, as evidenced by the results of relative error of **Error! Reference source not found.**, it was determined that the software has a good degree of predictability, however it is observed an offset for the solubility of the compound from 7 to 33% and the evaluated oral toxicity in rats from 4 to 7%. The error in the system was correlated with the probability. For high values of error, it had a probability of success of 0.8100 and 0.613 for solubility and toxicity in rats respectively, for which, the probability was a clear indication of the reliability of data, in the analysis of new molecules. Also, it should be noted, that many of the properties evaluated are qualitative characteristics and the error is given by the difference of categories these are indicators of the toxicity of a compound. The data from the literature and from government sources, were based in specific classifications of each laboratory, reason why they had some differences.

It should be noted that in the molecule K, the halogen atoms in this case the chlorine atoms were combined with the thio group (atoms of sulfur), so that degradation will lead to highly toxic compounds, such as hydrogen sulfide and chlorine hydride. In the evaluation of this molecule was obtained the lowest result, thus the reliability of software at the time of making the prediction of new compounds is confirmed.

For Picloram was observed the properties of absorption (**Error! Reference source not found.**), metabolism (**Error! Reference source not found.**) and toxicity (**Error! Reference source not found.**).For absorption and metabolism, the Picloram is easily absorbed by the intestine and it behaves as an inhibitor of P-glycoprotein, this indicates that the entry into the bloodstream is relatively quick after being ingested, inside the body, this substance has inhibitory properties that affect the cytochrome P-450. The inhibition of these substances may affect the electron transfer chain of catalyzed reactions, these reactions in humans, degrade some toxic compounds, whereby the inhibition of these components causes an increase in the concentration of toxic metabolites, reason why, this compound can cause the dead. However, in toxicity, that substance was slightly toxic to many species, besides being a non-carcinogenic compound. This is because this substance is rapidly removed through the renal system, with which time retention in the body is low and therefore, the toxicity decreases.

From the molecules studied, it was obtained a similar result in their absorption properties, as shown in **Error! Reference source not found.**, this is because the organochlorine compounds were easily absorbed in the digestive system, and thereby these compounds are quickly discarded. In metabolism, there were significant differences for molecules B, D, F, G and K, these molecules have major changes in structural conformation, due to the loss of chlorine atoms. The other molecules not exhibit large variation in this property, this is due these compounds were similar in their structure, reason why do not significantly affect the cytochromes P450 and thus these molecules have similar behavior. The substances A, C and E were more easily metabolized by the human body, according to the prediction obtained. The E molecule will have a shorter metabolism because it lacks the halogen radicals, to difference of others, reason why does not require specific enzymes to degrade this compound.

In the property of toxicity, the molecules that had chlorine inside their structure have lower values, which makes them more toxic. That was due the derived compounds that can produce the substance. The differences in values obtained for each compound, were because the different properties that had each radical, however, it should be noted that the molecules B, D and K are the highly toxic, because each molecule has three atoms of chlorine, which is likely in the metabolism of the species tested, this compound were degraded to more toxic compounds.

The molecules with the best results are the A, C and E. The relation between these compounds is the continuous dehalogenation starting on Picloram until their form dechlorinated. Through these molecules was shown the change in toxicity while was done the degradation of the pesticide, which happens to have score 11.3, 13.7 and 13.0 in its fully dehalogenated form. These changes were note principaly in the toxicity property. The molecule E, was the unic substance with the value of one in the characteristic of biodegradability, so this molecule cause less damage to the environment in case of being in contact with it. Also the probability obtained for these values is higher.

In order to determine the ideal replacement, the 5 molecules that obtained the higher value in the evaluation of qualitative properties were the molecules A, C, E, H and I, the quantitative properties were evaluated as shown in **Error! Reference source not found.**. It should be noted that these molecules had an alteration of the acid group, while in the discarded molecules the disruption of this group causes more harmful molecules to humans, in special the substances B, D, F, G and K. Of these substances was found to have the worst values in metabolism, except the molecule B. Thus, altering the acid group can trigger more toxic substances, due to they require specific enzymes, that were capable of breaking the new links, however, it remains for future analysis, assess retention time within the body**.**

Of the five substances with better results, it was found that Compound E did not contain chlorine atoms, reason why this molecule loses the proper characteristic of herbicide organochlorines. However is left for future trials checking their level of toxicity in- live. It was found that the substance I, compared to the other three and with the reference (substance A Picloram) had the less toxic global values, making it a potential candidate for replacement the Picloram. The toxicity values on different living beings show similarity for all molecules.

Otherwise for the E substance had the greater values, this indicates that this compound requires a higher dose to intoxicate these species.

Substances E, C and H evaluated in the system, were candidates for replacement the Picloram pesticide, however, as discussed above, the compound E is discarded because it did not contain chlorine, which is an essential characteristic of pesticides organochlorines. Of the other substances is preferred to use the H molecule, because it has a lower content of chlorine, but the molecule C, according to the prediction model used, reduces the toxicity level.

Varying toxicity in species were determined, making a comparison with the Picloram, thus, it was found that for the molecule I, is improved by 4.49% in rat toxicity and Tetrahymena pyriformis and 15.59% for fish.

V. Conclusions

The validation of system prediction was performed, in order to characterize the organochlorine herbicides through the ADMET methodology, which is widely used in medicine. A tool for the prediction of new pesticides was found, in which, the main objective was reduce the toxic effect in humans, that type of model was based on QSAR methodology and was complemented by the ADMET analysis. With that modifications, the usable range and accuracy in predicting extends.

Four molecules that have the highest values were found (E, C, H and I). The E molecule meets the initial objectives of reducing the toxic effect on humans, though their herbicidal properties were altered due the compound was not halogenated. The C molecule, showed a decrease in toxicity, also had the chlorine characteristic in the organochlorine molecules, however this compound was present in degradation of Picloram, reason why the risk of high concentrations of Picloram in the preparation of this substance. The H and I molecules also decrease the toxic effect and they had a halogen radical, which it meets the requirements for a possible replacement of Picloram.

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