Iraqi Journal of Industrial Research, Vol. 10, No. 1 (2023)

Open Access

Iraqi Journal of Industrial Research (IJOIR)

Journal homepage: http://ijoir.gov.iq

Activity Prediction of Various Herbicides against Honey Bee, Avian, and Multiple Human Leukemia, CNS, Ovarian, Prostate Cancer Cell Lines

Kafa Khalaf Hammud*

Iraqi Atomic Energy Commission, Baghdad – Iraq

Article information

Article history:
Received: December, 14, 2022
Accepted: March, 23, 2023
Available online: June, 14, 2023

Keywords:
ADMET,
QSAR,
Leukemia,
CNS,
Prostate

Corresponding Author:
Kafa Khalaf Hammud
kafaakhalaf@gmail.com

DOI:
https://doi.org/10.53523/ijoirVol10I1ID286

Abstract

Herbicides classify as chemicals targeting specific biochemical pathways in plants and may influence human or animal health according to their chemistry, concentration, environment, biological target and others. With safety concern, International Agency for Research on Cancer (IARC) classified herbicides and their metabolites as fetal developments may be a consequence of enzymatic inhibition or other mechanisms. Thirty phytotoxins were subjected to online pkCSM website, as a Quantitative Structure Activity Relationship (QSAR) prediction activity against honey bee, avian, and multiple human Leukemia, CNS, Ovarian, Prostate Cancer cell lines. Prediction outcomes were varied and influenced by chemical structure of each tested herbicide. Sulfentrazone having evidence of human non-carcinogenic character (Group E) had hepatotoxicity prediction and cancer cell lines activity less than 5 of Leukemia, CNS, Ovarian, and Prostate. Also, it had CYP1A2 inhibition, negative response of p-glycoprotein, Ames, skin sensitization, renal OCT2, and hERG. All above characters beside low intestinal absorption and Blood- Brain Barrier (BBB) presented encouraging online funding as more structurally safe having active – multiple toxicological and cellular interactions. Simetryn and Simazine that have the same core structure except (-SCH3) group replaced with chloro group gave semi identical results of many calculated characters and inactive materials to cancer cell lines and herbicide activity, honey bee and avian toxicities but not BBB, total clearance, and oral rat chronic (LOAEL) confirming structure influences upon prediction.

1. Introduction

In general, herbicides are chemicals that target plant biochemical pathways by their toxicological mechanisms. These high toxicities on plants may be with lower or higher effects on animal or human. Chemical structure, concentration, environmental conditions (time, temperature), plant (or animal or human) classification and chemistry, and others are mainly impact factors on herbicidal destroying effects upon living creatures [1]. These phytotoxins are working with variable degrees in growth inhibition or destroying weed/crop to control plantation instead of hand weeding or using nonspecific chemicals [2].

Their mainly actions are focusing on enzymes such as carboxylase, oxidase, dioxgenase, synthase, and others together with photosynthesis processes inhibitions. It is known that agricultural activities need herbicides but these chemicals have serious impacts on environment (soil, plant, food, animal, atmosphere, seawater, freshwater, rain, other water sources), human resources and public human health. In human life section, these phytotoxins are actively and generally employed on cell cycle- growth with particular effect(s) on liver, intestine, skin, lung, and / or kidney encouraging cell-organ damage. Banning of these toxics in a great degree is due to metabolism and permanent damage or disorder effect that is more seriously with chronic exposure [3].

Many microorganisms in environment are capable to metabolize herbicide(s) then access, accumulate in human through oral, dermal, and / or pulmonary path then elimination out of body may be occurred with easily detection by advanced analytical instruments. With safety concern by international agencies like the International Agency for Research on cancer (IARC), herbicides and their metabolites are classified according to benignity – injury categories. For example glyphosate is probably carcinogenic to human [4]. Even with minimum residual possibilities of these agricultural-vegetation materials in foods and living organisms, fetal developments may be a consequence of enzymatic inhibition or minimizing of essential bio- synthetic materials such as amino acids [5].

To have a deep ranking about herbicide toxicity and carcinogenicity in both severity and risk issues, many international organizations like World Health Organization (WHO), The European Food Safety Authority (EFSA), US Environmental Protection Agency (EPA), and /or IARC classified, studied and reported these chemicals even at final level of non-corresponding statements. Finding a link between herbicide trace in food and cancer is critical questionnaires with scientific evidences. Presence of incomparable conclusions based upon collected outcomes (Tables), test regulation, and exposure scenarios motivated researchers and these authorized organizations to category chemicals (including herbicides and their metabolites) according to cancer classification, Globally Harmonized System (GHS), Occupational Safety and Health Administration (OSHA), and other safety and hazard tabulations [6-11].

According to our knowledge, mathematical prediction in herbicidal toxicity and safety subjects is not observed yet by researchers especially in Adsorption- Distribution, Metabolism, Excretion, Toxicity (ADMET) and various cancers. Here, a new Iraqi study was designed to investigate the influence of thirty herbicides having various chemical-physical- biological properties on Pharmacokinetics, Crop, and Leukemia – CNS – Ovarian - Prostate Cancers by employing online websites belong to pkCSM - http://biosig.unimelb.edu.au/biosig/ - University of Melbourne – Australia as a Quantitative Structure Activity Relationship (QSAR) prediction.

2. Experimental Procedure

Herbicides: thirty herbicidal materials were chosen for this study (Table 1., Figure 1). Chemical notations with isomeric Simplified Molecular Input Line Entry System (SMILES) coding were used for direct chemical structure input of Vernolate (V), Trifluralin (T1), Trietazine (T2), Tridiphane (T3), Triclopyr (T4), Triasulfuron (T5), Triallate (T6), Tralkoxydim (T7), Terbacil (T8), Tebuthiuron (T9), Sulfentrazone (S1), Sulfallate (S2), Sulcotrione(S3), Solan (S4), Simetryn (S5), Simazine (S6), Siduron (S7), Sethoxydim (S8), Rimsulfuron (S9), Pyridate (P1), Prosulfuron (P2), Propyzamide (P3), Propazine (P4), Propachlor (P5), Prometryn (P6), Prometon (P7), Profluralin (P8), Pretilachlor (P9), Perfluidone (P10), and Pendimethalin (P11).

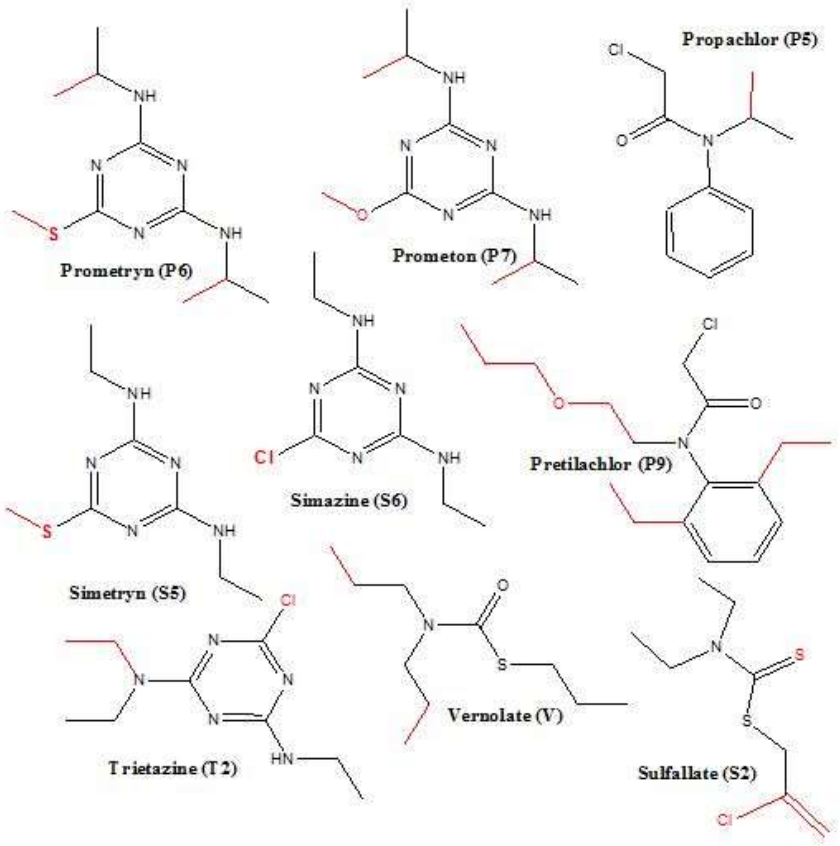

Figure (1). Chemical structures of some tested herbicides.

Online Websites and Their Predicted Characters

ADMET characters were Adsorption: Caco-2 permeability (log Papp in 10-6 cm/s), human, Intestinal absorption ((% Absorbed), Skin Permeability (log Kp), P-glycoprotein substrate and inhibitor. Distribution: BBB permeability ((log BB) and CNS permeability (log PS). Metabolism: CYP2D6 and CYP3A4 substrate, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibition. Excretion: Total Clearance (log ml/min/kg) and Renal OCT2 substrate. Toxicity: Ames, Human Maximum Tolerated Dose (log mg/kg/day), hERG inhibition, Oral Rat Acute Toxicity (LD50, mol./kg), Oral Rat Chronic Toxicity (LOAEL, log mg/kg body weight/day), Hepatotoxicity, and Skin Sensitization (Tables 2-3, Figure 2) where all these ADMET characters were predicted by [12] website.

Herbicide activity with honey bee and avian toxicities were predicted by [13] website by Yes/ No response that converted to 1.0/0.0 respectively (Table 4) and (Figure 3). CNS, Leukemia, Ovarian, and Prostate cancers were predicted according to website [14], (Tables 5-7, Figure 4.).

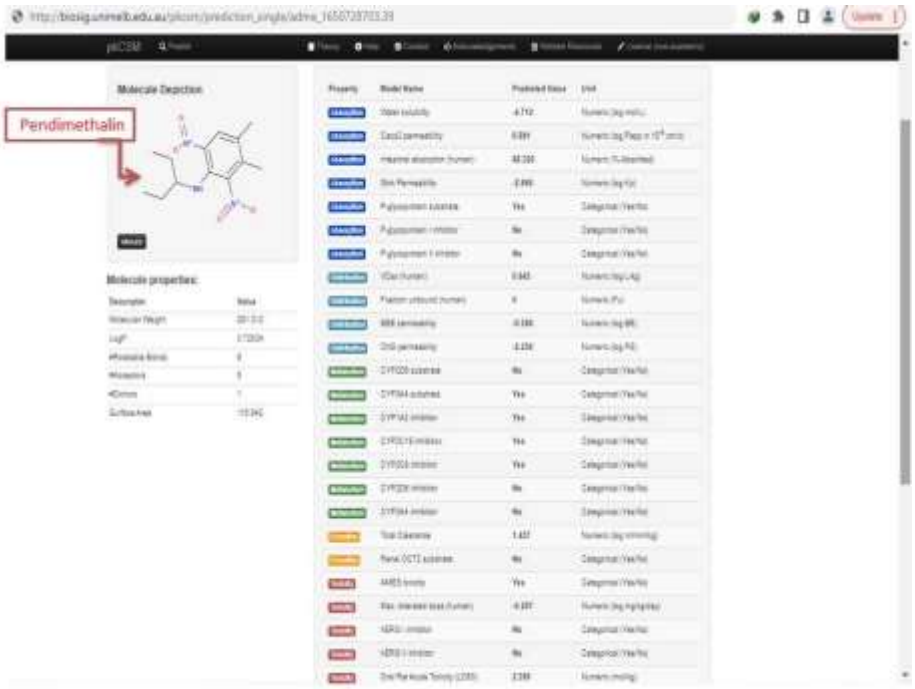

Figure (2). pkCSM prediction of Pendimethalin according to website [12].

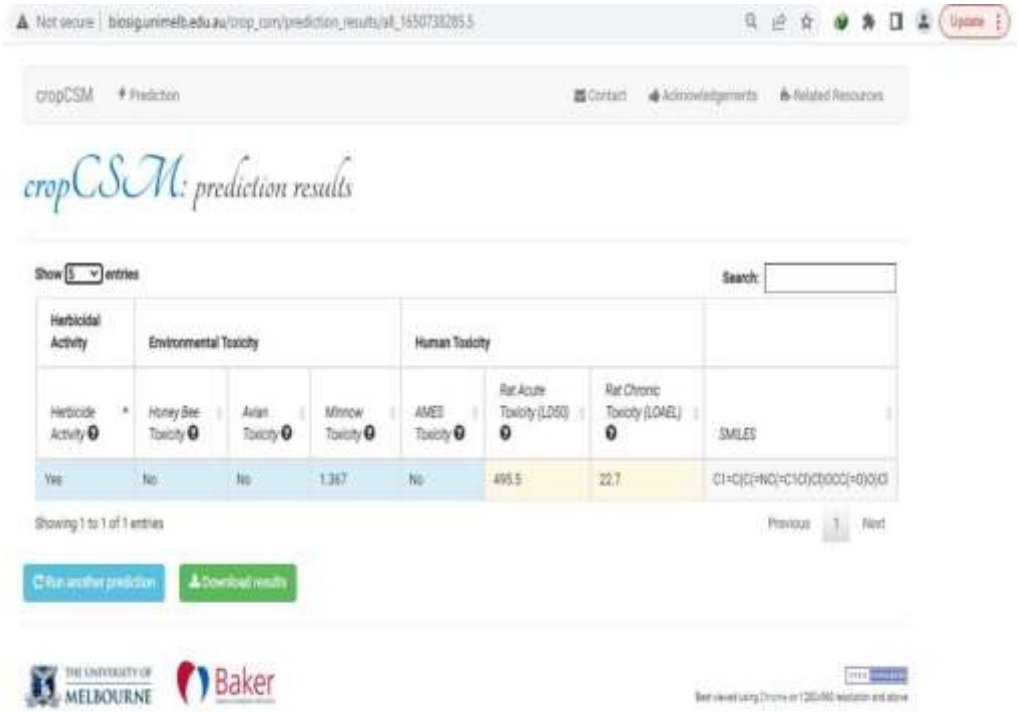

Figure (3). CropCSM prediction according to website [13].

Figure (4). pdCSM-cancer Activity and GI50% prediction according to website [14].

Table (1). SMILES and Codes of the predicted herbicides.

Code	V	T1	T2	T3	T4	T5	T6	T7	T8	T9
Name	Vernolate	Trifluralin	Trietazine	Tridiphane	Triclopyr	Triasulfuron	Triallate	Tralkoxydim	Terbacil	Tebuthiuron
Isomeric SMILES	CCCN(CCC)C(=O)SCCC	CCCN(CCC)C1=C(C=C(C=C1[N+](=O)[O-])C(F)(F)F)[N+](=O)[O-]	CCNC1=NC(=NC(=N1)Cl)N(CC)CC	C1C(O1)(CC(Cl)(Cl)Cl)C2=CC(=CC(=C2)Cl)Cl	C1=C(C(=NC(=C1Cl)Cl)OCC(=O)O)Cl	CC1=NC(=NC(=N1)OC)NC(=O)NS(=O)(=O)C2=CC=CC=C2OCCCl	CC(C)N(CC)C(=O)SCC(=C(Cl)Cl)Cl	CC/C(=N\OCC)/C1=C(CC(CC1=O)C2=C(C=C(C=C2C)C)C)O	CC1=C(C(=O)N(C(=O)N1)C(C)(C)C)Cl	CC(C)(C)C1=NN=C(S1)N(C)C(=O)NC
Code	S1	S2	S3	S4	S5	S6	S7	S8	S9	P1
Name	Sulfentrazone	Sulfallate	Sulcotrione	Solan	Simetryn*	Simazine*	Siduron	Sethoxydim	Rimsulfuron	Pyridate
Isomeric SMILES	CC1=NN(C(=O)N1C(F)F)C2=C(C=C(C(=C2)NS(=O)(=O)C)Cl)Cl	CCN(CC)C(=S)SCC(=C)Cl	CS(=O)(=O)C1=CC(=C(C=C1)C(=O)C2C(=O)CCCC2=O)Cl	CCCC(C)C(=O)NC1=CC(=C(C=C1)C)Cl	CCNC1=NC(=NC(=N1)SC)NCC	CCNC1=NC(=NC(=N1)Cl)NCC	CC1CCCCC1NC(=O)NC2=CC=CC=C2	CCC/C(=N\OCC)/C1=C(CC(CC1=O)CC(C)SCC)O	CCS(=O)(=O)C1=C(N=CC=C1)S(=O)(=O)NC(=O)NC2=NC(=CC(=N2)OC)OC	CCCCCCCCS C(=O)OC1=CC(=NN=C1C2=CC=CC=C2)Cl
Code	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Name	Prosulfuron	Propyzamide	Propazine	Propachlor	Prometryn*	Prometon*	Profluralin	Pretilachlor	Perfluidone	Pendimethalin
Isomeric SMILES	CC1=NC(=NC(=N1)OC)NC(=O)NS(=O)(=O)C2=CC=CC=C2CCC(F)(F)F	CC(C)(C#C)NC(=O)C1=CC(=CC(=C1)Cl)Cl	CC(C)NC1=NC(=NC(=N1)Cl)NC(C)C	CC(C)N(C1=CC=CC=C1)C(=O)CCl	CC(C)NC1=NC(=NC(=N1)SC)NC(C)C	CC(C)NC1=NC(=NC(=N1)OC)NC(C)C	CCCN(CC1CC1)C2=C(C=C(C=C2[N+](=O)[O-])C(F)(F)F)[N+](=O)[O-]	CCCOCCN(C1=C(C=CC=C1CC)CC)C(=O)CCl	CC1=C(C=CC(=C1)S(=O)(=O)C2=CC=CC=C2)NS(=O)(=O)C(F)(F)F	CCC(CC)NC1=C(C=C(C(=C1[N+](=O)[O-])C)C)[N+](=O)[O-]

Table (2). Pharmacokinetic characters of the predicted herbicides according to website [12].

Character	V	T1	T2	T3	T4	T5	T6	T7	T8	T9	S1	S2	S3	S4	S5
Caco-2	1.306	0.499	1.358	1.107	1.316	0.856	1.268	1.425	1.141	1.32	1.485	1.403	1.343	1.521	0.852
Intestinal absorption	91.867	90.171	93.453	90.415	89.643	56.467	87.899	92.37	91.728	93.285	89.659	90.928	97.057	90.294	92.486
Skin Permeability	-1.823	-2.647	-2.69	-2.818	-2.735	-2.735	-2.233	-2.805	-3.205	-3.164	-2.847	-1.908	-2.907	-2.361	-3.138
P-glycoprotein subs.	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
P-glycoprotein I	No	Yes	No	No	No	No	Yes	No	No	No	No	No	No	No	No
P-glycoprotein II	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
BBB	0.592	-1.097	0.465	0.379	0.087	-0.939	0.539	-0.005	-0.226	-0.016	-1.409	0.596	-0.774	0.186	-0.293
CNS	-2.513	-2.323	-3.004	-2.046	-2.948	-3.148	-1.508	-2.779	-2.844	-2.9	-3.09	-2.691	-2.516	-1.61	-3.072
CYP2D6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4	No	Yes	No	Yes	No	No	Yes	Yes	No	No	No	No	Yes	No	No
CYP1A2	No	Yes	Yes	Yes	No	No	No	Yes	Yes	No	Yes	No	No	Yes	No
CYP2C19	No	Yes	No	Yes	No	No	No	Yes	No	No	No	No	No	Yes	No
CYP2C9	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2D6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Total Clearance	0.595	0.237	0.153	0.101	0.281	0.207	0.447	1.255	-0.177	0.264	0.292	0.497	0.122	0.033	0.256
Renal OCT2	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No
Ames	No	Yes	No	Yes	No	No	Yes	No	No	No	No	Yes	No	No	No
Max. tolerated dose	0.69	0.237	1.046	0.608	1.677	0.776	0.068	0.333	0.823	0.903	0.436	0.592	0.775	0.695	1.008
hERG I	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Oral Rat Acute (LD_{50})	2.592	2.615	2.941	3.301	2.492	2.109	2.448	2.225	1.752	2.726	2.391	2.538	2.53	2.39	2.69
Oral Rat Chronic (LOAEL)	1.419	1.367	0.674	1.543	1.571	2.398	0.141	1.265	1.623	0.807	1.679	1.077	2.019	1.49	0.23
Hepatotoxicity	No	No	Yes	No	No	Yes	No	Yes	No	No	Yes	No	No	No	Yes
Skin Sensitization	Yes	No	No	Yes	No	No	Yes	No	No	No	No	Yes	No	Yes	No

Table (3a). Pharmacokinetic characters of the predicted herbicides according to website [12].

Character	S6	S7	S8	S9	P1	P2	P3
Caco-2	080	1.48	1.55	0.71	1.14	1.49	1.22
Intestinal absorption	92.14	90.31	91.14	51.06	90.14	64.20	92.26
Skin Permeability	-3.16	-2.89	-2.76	-2.74	-2.72	-2.734	-2.48
P-glycoprotein subs.	No	Yes	No	Yes	No	Yes	No
P-glycoprotein I	No	No	No	No	Yes	Yes	No
P-glycoprotein II	No	No	No	No	Yes	No	No
BBB	-0.32	0.28	-0.20	-1.34	-0.03	-0.878	0.11
CNS	-3.07	-1.72	-2.90	-3.50	-2.18	-3.44	-1.84
CYP2D6	No	No	No	No	No	No	No
CYP3A4	No	Yes	No	No	Yes	No	No
CYP1A2	No	No	No	No	Yes	No	Yes
CYP2C19	No	No	Yes	No	Yes	No	No
CYP2C9	No	No	Yes	No	Yes	No	No
CYP2D6	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	No	No	No
Total Clearance	0.10	0.41	0.56	0.23	0.32	0.09	0.001
Renal OCT2	No	No	No	No	No	No	No
Ames	No	No	No	No	No	No	No
Max. tolerated dose	1.02	0.38	0.41	0.79	1.15	0.49	0.76
hERG I	No	No	No	No	No	No	No
hERG II	No	No	No	No	Yes	No	No
Oral Rat Acute (LD_{50})	2.52	2.25	2.42	1.58	2.90	1.98	2.66
Oral Rat Chronic (LOAEL)	0.74	1.58	0.26	3.01	1.35	2.28	0.92
Hepatotoxicity	Yes	No	No	Yes	No	Yes	Yes
Skin Sensitization	No	No	No	No	No	No	Yes

Table (3b). Pharmacokinetic characters of the predicted herbicides according to website [12].

Character	P4	P5	P6	P7	P8	P9	P10	P11
Caco-2	0.97	1.58	0.94	0.32	0.35	1.77	1.34	0.89
Intestinal absorption	89.73	96.12	88.56	89.73	90.63	94.88	91.19	88.33
Skin Permeability	-3.16	-2.04	-3.06	-2.97	-2.69	-2.48	-2.83	-2.67
P-glycoprotein subs.	No	No	No	No	No	No	Yes	Yes
P-glycoprotein I	No	No	No	No	Yes	No	No	No
P-glycoprotein II	No	No	No	No	No	No	No	No
BBB	0.42	0.34	0.38	-0.38	-1.08	0.49	-1.03	-0.40
CNS	-2.92	-1.58	-2.93	-2.93	-2.38	-2.32	-2.40	-2.26
CYP2D6	No	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	Yes	Yes	Yes	Yes
CYP1A2	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
CYP2C19	No	No	No	No	Yes	Yes	Yes	Yes
CYP2C9	No	No	No	No	Yes	No	No	Yes
CYP2D6	No	No	No	No	No	No	No	No

Character	P4	P5	P6	P7	P8	P9	P10	P11
CYP3A4	No	No	No	No	No	No	No	No
Total Clearance	-0.04	0.18	0.11	0.51	0.06	0.51	0.26	1.44
Renal OCT2	No	No	No	No	No	No	No	No
Ames	No	Yes	No	No	Yes	Yes	No	Yes
Max. tolerated dose	1.22	0.72	1.17	1.19	-0.25	0.78	0.36	-0.21
hERG I	No	No	No	No	No	No	No	No
hERG II	No	No	No	No	No	No	No	No
Oral Rat Acute (LD_{50})	2.28	2.01	2.50	2.30	2.57	2.61	2.46	2.36
Oral Rat Chronic (LOAEL)	1.08	1.16	0.66	1.96	1.31	1.61	2.31	1.72
Hepatotoxicity	No	No	No	Yes	No	No	Yes	No
Skin Sensitization	No	Yes	No	No	No	Yes	No	No

Table (4). Herbicidal activity with honey bee and avian toxicity prediction According to website [13].

Code	Herbicidial activity	Honey bee toxicity	Avian toxicity	Code	Herbicide activity	Honey bee toxicity	Avian toxicity	Code	Herbicidal activity	Honey bee toxicity	Avian toxicity
V	1.0	1.0	0.0	S1	1.0	0.0	0.0	P2	1.0	0.0	0.0
T1	1.0	0.0	0.0	S2	1.0	1.0	0.0	P3	1.0	0.0	0.0
T2	1.0	0.0	1.0	S3	1.0	0.0	0.0	P4	1.0	0.0	0.0
T3	0.0	0.0	0.0	S4	1.0	0.0	0.0	P5	1.0	0.0	0.0
T4	1.0	0.0	0.0	S5	1.0	0.0	0.0	P6	1.0	0.0	0.0
T5	1.0	0.0	0.0	S6	1.0	0.0	0.0	P7	1.0	0.0	0.0
T6	1.0	0.0	0.0	S7	1.0	0.0	0.0	P8	1.0	0.0	0.0
T7	0.0	0.0	0.0	S8	1.0	0.0	0.0	P9	1.0	0.0	0.0
T8	1.0	0.0	0.0	S9	1.0	0.0	0.0	P10	0.0	0.0	0.0
T9	1.0	0.0	0.0	P1	1.0	0.0	0.0	P11	1.0	0.0	0.0

Table (5). Prediction of herbicides with CNS cancer [14].

Code	SF-268	SF-295	SF-539	SNB-19	SNB-75	SNB-78	U251	XF-498
V	4.208	4.06	4.596	4.066	4.480	4.147	4.433	4.340
T1	4.53	4.909	4.503	4.615	4.777	4.279	4.841	4.606
T2	4.127	3.829	4.282	4.284	4.200	4.056	4.151	4.166
T3	4.980	4.714	4.710	4.744	4.931	4.520	4.961	4.766
T4	4.152	4.093	4.270	4.250	4.103	4.367	4.206	4.349
T5	4.644	4.532	4.264	4.521	4.730	4.335	4.451	4.466
T6	4.625	4.521	4.857	4.212	4.679	4.395	4.553	4.542
T7	4.269	4.403	4.491	4.374	4.404	4.423	4.480	4.400
T8	4.025	4.181	4.173	4.172	4.762	4.305	4.186	4.444
T9	4.094	4.121	4.262	4.121	3.997	4.088	4.098	4.168
S1	4.691	4.454	4.457	4.312	4.772	4.473	4.595	4.574
S2	4.301	4.182	4.384	4.264	4.723	4.182	4.237	4.505
S3	4.305	3.927	4.363	4.277	4.353	4.510	4.254	4.266
S4	4.253	4.142	4.207	4.058	4.261	4.232	4.329	4.214
S5	3.871	3.939	4.152	4.056	4.227	3.990	4.066	4.041
S6	3.921	3.705	4.058	4.065	4.135	4.143	4.078	4.236
S7	4.124	4.320	4.282	3.981	4.171	4.148	4.178	4.318
S8	4.422	4.322	4.429	4.479	4.332	4.758	4.453	4.346
S9	4.342	4.386	4.258	4.386	4.791	4.485	4.289	4.478
P1	4.765	4.553	4.753	4.462	4.825	4.362	4.886	4.687
P2	4.416	4.355	4.306	4.237	4.671	4.365	4.543	4.548
P3	4.293	4.175	4.466	4.134	4.359	4.432	4.334	4.430

Code	SF-268	SF-295	SF-539	SNB-19	SNB-75	SNB-78	U251	XF-498
P4	4.269	4.171	4.185	4.100	4.217	4.257	4.105	4.288
P5	4.719	4.461	4.384	4.405	4.798	4.170	4.614	4.417
P6	4.282	4.299	4.215	4.099	4.304	4.309	4.145	4.138
P7	4.168	4.258	3.943	4.130	4.159	4.246	4.109	4.056
P8	4.627	4.743	4.434	4.634	4.646	4.312	4.649	4.549
P9	4.481	4.449	4.379	4.590	4.721	4.161	4.586	4.459
P10	4.442	4.482	4.561	4.283	4.74	4.434	4.693	4.668
P11	4.446	4.704	4.447	4.401	4.755	4.337	4.706	4.507

Table (6). Prediction of herbicides with Leukemia cancer [14].

Code	General anticancer activity	CCRF-CEM	HL-60TB	K-562	MOLT-4	P388-ADR	P388	RPMI_8226	SR
V	Inactive	4.565	4.669	4.368	4.406	4.163	4.676	4.346	4.674
T1	Active	5.404	5.439	5.063	5.31	4.658	4.804	4.861	5.123
T2	Inactive	3.98	4.202	4.143	4.186	4.766	4.539	4.422	4.864
T3	Active	5.481	5.196	5.069	5.441	5.062	4.671	5.141	5.282
T4	Inactive	4.384	4.626	4.236	4.413	4.438	4.602	4.383	4.643
T5	Inactive	4.614	4.532	4.439	4.581	5.026	4.841	4.757	4.98
T6	Inactive	4.805	5.019	4.791	4.701	4.379	4.909	4.557	5.068
T7	Inactive	4.437	4.632	4.55	4.528	5.058	5.256	4.555	4.653
T8	Inactive	4.331	4.259	4.283	4.335	4.775	3.746	4.379	4.284
T9	Inactive	4.161	4.208	4.131	4.200	4.450	4.103	4.150	4.375
S1	Inactive	4.851	4.807	4.643	4.720	4.811	4.770	4.859	4.736
S2	Inactive	4.495	4.475	5.07 0	4.746	4.860	5.1480	4.768	5.481
S3	Inactive	4.593	4.487	4.424	4.389	4.597	4.681	4.746	4.913
S4	Inactive	4.252	4.309	4.228	4.257	4.708	4.404	4.156	4.423
S5	Inactive	4.249	4.081	3.925	4.190	4.303	4.375	4.251	4.508
S6	Inactive	3.829	4.106	4.009	4.184	4.549	4.157	4.207	4.543
S7	Inactive	4.085	4.328	4.158	4.255	3.992	4.007	4.161	4.497
S8	Inactive	4.689	4.952	4.683	4.655	4.628	5.238	4.320	4.668
S9	Inactive	4.445	4.432	4.362	4.464	4.77	6.184	4.594	4.781
P1	Active	4.661	4.814	4.933	4.606	5.305	4.810	4.796	5.000
P2	Inactive	4.560	5.163	4.402	4.498	4.629	4.947	4.622	4.471
P3	Inactive	4.427	4.499	4.441	4.268	5.075	4.039	4.300	4.684
P4	Inactive	4.148	4.274	4.289	4.408	4.520	4.013	4.468	4.642
P5	Inactive	4.987	4.978	4.957	4.548	5.162	4.441	4.608	4.743
P6	Inactive	4.475	4.194	4.300	4.445	4.27	4.346	4.517	4.606
P7	Inactive	3.943	4.302	4.259	4.336	4.048	3.981	4.413	4.385
P8	Active	5.191	5.341	5.064	4.956	4.765	4.661	4.861	5.156
P9	Inactive	4.785	4.863	4.717	4.552	5.071	4.891	4.562	4.996
P10	Active	4.773	4.744	4.64	4.745	4.581	5.021	4.738	4.775
P11	Inactive	4.779	4.921	4.941	4.964	4.695	4.817	4.688	5.041

Table (7). Prediction of herbicides with Ovarian and Prostate cancers [14].

Code	Ovarian cell-line							Prostate cell-line	
	IGROV1	NCI-ADR-RES	OVCAR-3	OVCAR-4	OVCAR-5	OVCAR-8	SK-OV-3	DU-145	PC-3
V	4.197	4.180	4.403	4.344	4.349	4.311	4.155	4.398	4.445
T1	4.678	4.833	4.870	4.909	4.616	4.949	4.424	4.965	4.667
T2	4.278	4.169	4.560	3.982	4.163	4.200	4.108	4.309	4.160
T3	4.794	5.073	4.965	5.050	4.978	4.966	4.331	5.109	5.032
T4	4.215	4.168	4.268	4.263	4.248	4.233	4.071	4.298	4.287
T5	4.140	4.506	4.308	4.463	4.357	4.355	4.716	4.615	5.053
T6	4.836	4.770	5.055	4.540	4.700	4.592	4.261	4.665	4.630
T7	4.381	4.836	4.670	4.422	4.465	4.340	4.303	4.797	4.806
T8	4.186	4.192	4.198	4.229	4.352	4.213	4.117	4.197	4.143
T9	4.020	4.210	3.963	4.101	4.105	4.107	4.079	4.235	4.339
S1	4.369	4.604	4.668	4.606	4.456	4.625	4.356	4.767	4.756
S2	4.506	4.429	4.622	4.312	4.172	4.318	4.189	4.231	4.484
S3	4.430	4.849	4.351	4.465	4.291	4.333	4.536	4.929	4.818
S4	4.487	4.658	4.197	4.235	4.137	4.081	4.105	4.422	4.451
S5	3.840	4.059	4.347	3.966	4.076	4.236	4.073	4.444	4.039
S6	4.048	4.006	4.345	3.883	4.155	4.122	3.954	4.206	4.058
S7	4.137	4.283	4.312	4.170	4.154	4.029	4.188	4.504	4.286
S8	4.476	4.656	4.619	4.660	4.567	4.467	4.244	4.804	4.813
S9	4.203	4.534	4.168	4.373	4.209	4.375	4.478	4.528	4.931
P1	4.566	5.048	4.585	4.492	4.594	4.623	4.963	4.687	5.034
P2	4.134	4.48	4.419	4.426	4.418	4.258	4.538	4.673	4.732
P3	4.854	4.371	4.419	4.164	4.249	4.252	4.076	4.407	4.324
P4	4.140	4.406	4.438	4.079	4.244	4.217	4.148	4.332	4.229
P5	4.565	4.699	4.884	4.700	4.749	4.471	4.624	5.101	4.557
P6	4.051	4.417	4.387	4.088	4.216	4.342	4.307	4.479	4.341
P7	3.745	4.309	4.441	3.970	4.155	3.804	4.141	4.334	4.254
P8	4.629	4.808	4.857	4.685	4.613	4.785	4.445	4.789	4.667
P9	4.456	4.652	4.559	4.696	4.646	4.678	4.594	4.790	4.689
P10	4.303	4.452	4.619	4.697	4.435	4.479	4.369	4.587	4.475
P11	4.792	4.965	4.812	4.750	4.419	4.739	4.279	4.815	4.809

3. Results and Discussion

Thirty herbicides were selected to test their QSAR through various predictors with the help of isomeric SMILES notations and [12] as free online website (Table 1.). The easy steps of all [12, 13, 14] predictions were very helpful in proceeding of this research article. More than thirty predicted characters (Tables (2-7)) are summarized (Tables 8, 9, & 10)

Table (8). Summary of pharmacokinetic predictions.

Pharmacokinetic prediction								
Character	Min.	Max.	Character	Min.	Max.	Character	Min.	Max.
Caco-2	0.324 (P7)	1.764 (P9)	Intestinal absorption	51.056 (S9)	97.057 (S3)	Skin Permeability	-3.205 (T8)	-1.823 (V)
Character	Min.	Max.	Character	Min.	Max.	Character	Min.	Max.
BBB	-1.409 (S1)	0.596 (S2)	CNS	-3.503 (S9)	-1.508 (T6)	Total Clearance	-0.177 (T8)	1.437 (P11)
Character	Min.	Max.	Character	Min.	Max.	Character	Min.	Max.
Max. tolerated dose	-0.25 (P8)	1.677 (T4)	Oral Rat Acute (LD_{50})	1.581 (S9)	3.301 (T3)	Oral Rat Chronic (LOAEL)	0.141 (T6)	3.005 (S9)

Table (9). Summary of CNS cancer prediction.

CNS cancer prediction								
Cell line	SF-268	SF-295	SF-539	SNB-19	SNB-75	SNB-78	U251	XF-498
Min.	**3.871 (S5)**	3.705 (S6)	3.943 (P7)	3.981 (S7)	3.997 (T9)	**3.99 (S5)**	**4.066 (S5)**	**4.041 (S5)**
Max.	**4.98 (T3)**	4.909 (T1)	4.857 (T6)	**4.744 (T3)**	**4.931 (T3)**	4.758 (S8)	**4.961 (T3)**	**4.766 (T3)**

Table (9). Summary of Leukaemia cancer prediction.

Leukemia cancer prediction									
General anticancer activity	Cell line	**CCRF-CEM**	**HL-60TB**	**K-562**	**MOLT-4**	**P388-ADR**	**P388**	**RPMI-8226**	**SR**
T1, T3, P1, P8, P10	Min.	3.829 (S6)	4.081 (S5)	3.925 (S5)	4.184 (S6)	3.992 (S7)	3.746 (T8)	4.15 (T9)	4.284 (T8)
	Max.	5.481 (T3)	5.341 (P8)	5.07 (S2)	4.964 (P11)	5.305 (P1)	6.184 (S9)	4.861 (T1), (P8)	5.481 (S2)

Table (10). Summary of Ovarian and Prostate cell lines prediction.

Cell line	Ovarian cell-line							Prostate cell-line	
	IGROV1	NCI-ADR-RES	OVCAR-3	OVCAR-4	OVCAR-5	OVCAR-8	SK-OV-3	DU-145	PC-3
Min.	3.745 (P7)	4.006 (S6)	3.963 (T9)	3.883 (S6)	4.076 (S5)	3.804 (P7)	3.954 (S6)	4.197 (T8)	4.039 (S5)
Max.	4.854 (P3)	5.073 (T3)	5.055 (T6)	5.05 (T3)	4.978 (T3)	4.966 (T3)	4.963 (P1)	5.109 (T3)	5.053 (T5)

Caco-2 (cancer coli-2) is a human colon cancer cell line, intensively studied for the expression of enzymes and transportation proteins, bioactive passive diffusion via intestinal epithelial pathways, cytotherapy and food bioactive (drugs or formulation) effect on cancer due to the reproducibility and simplicity of this system [15, 16].

In general, Caco-2 permeability may be influenced by paracellular permeability – hydropholicity relationship [17]. So, herbicide with low hydrophilic character had high Caco-2 in vitro monolayer model (Tables 2 & 3) and (Figures 1 & 5). For example, S6, P6, S5, T2 have the same structural core but the presence of sulfur atom compared to oxygen atom, alkyl chain, chloroalkenyl, and alkyl ether varied Caco-2 value as (P6 and P7) or (P7 and S5), (T2 and S6), (V and S2), (P9 and P5) respectively.

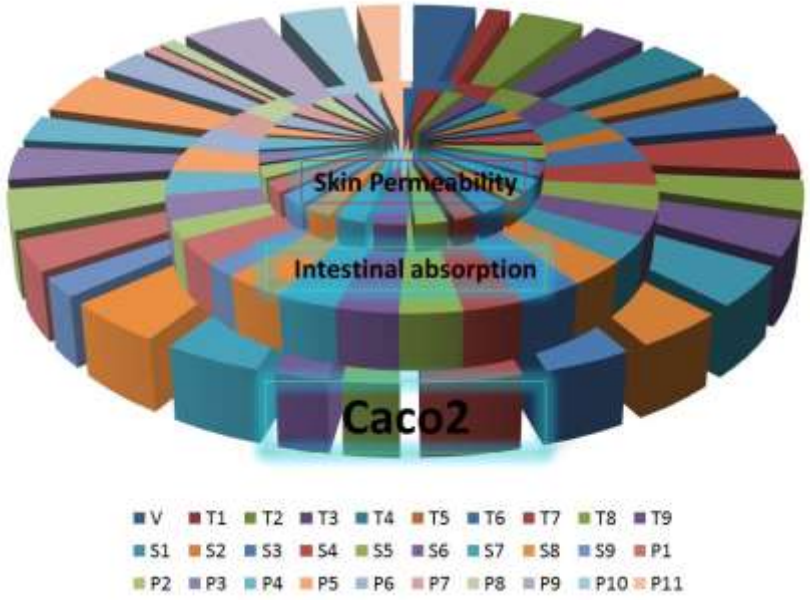

Figure (5). Skin permeability, intestinal absorption, and Caco-2 monolayer model of the tested herbicides.

Many *in vitro* and /or *in vivo* toxicological studies were following managed guidelines to evaluate chemical hazard to human and environment especially in oral absorption issues and related consequences [18-21]. Outputs of these studies permit conventional safety assessment with high transparency basing on science to protect human – animal health and leading the way to new methods companied by consuming less time- cost- resource findings [22].

Always, natural and synthesized chemicals need safety evaluation that approve using by human according to scientific approaches related to Animal/ Human/ Environment Reduction-Refinement and Replacement (AHE-3Rs) [23]. From these essential points, this in Silico study targeted many toxicological points related to herbicides effects.

One of these studied toxicological points was intestinal adsorption that had the same sequence of variation as in Caco-2 from (V) to (P11). In particular observations, replacement of chloro group in (S6) with (SCH3) group gave a little raise in this character but this replacement beside the addition of two methyl groups decreased this character as in (P7 and P6).

Oxygen presence (P7) gave more intestinal absorption compared to sulfur presence (P6), and absence of two methyl groups (S5) increased this important character compared to (P6) that may be related to polar surface area, hydrogen bonding, organic- aqueous phase solubility, atom negativity, and other related reasons(Figure 1., Tables 2-3).

Also, twenty seven of these tested herbicides exhibited high intestinal absorption (with more than 87%) presenting 90% of them that associated with their molecular characterizations.

The lowest intestinal absorption values were in S9 (51.056%), T5 (56.457%) and P2 (64.197%) that have the same core structure where replacement of (O-CH2-CH2-Cl) with (CH2-CH2-CF3) pending group gave a raise with more than 7% while structural changing between S9 and T5 gave a (5.411%) as difference between S9 and T5 (Figures 5 & 6., Tables 2-3). These structural reasons can be summarized as permeability – hydropholicity relationship that has been mentioned in Caco-2 explanation.

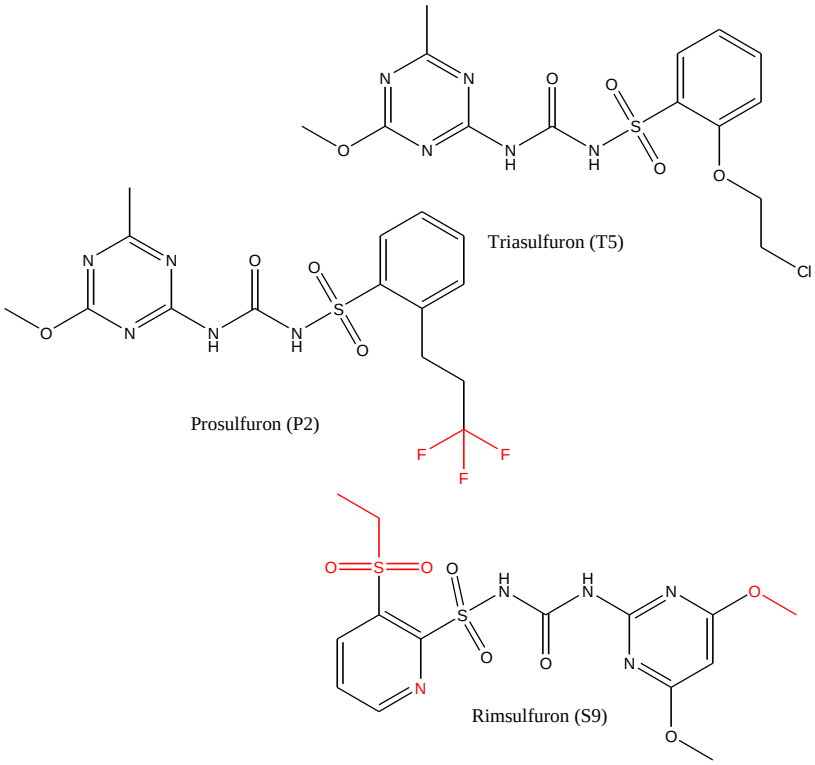

Figure (6). Chemical structures of S9, T5, and P2.

Skin is a major human barrier consisting of multi-layers with various morphological functions. Its permeation is a rate-limited nature controlling chemical (or drug) administration, discovery, and development processes. So, skin as a primary protection shield of human, an controller of losing water, and a homeostatic barrier can be influenced by chemical(s) through penetration (in the body) or remaining out of the body (non-penetration). These dermal ways are examples of infection and medication that limit chemical diffusion in bloodstream or its stability in gastrointestinal tract. As it is known, *in Silico* modeling provides non-ethical economical methodology of natural, synthesized, and hypothetical compound(s) with limited time- consuming steps. Permeation of any chemical can be occurred by diffusion, trans- or inter- cellular route according to its molecular polarity character and other physicochemical properties [24, 25].

Here, skin permeability was influenced by functional group presence or absence such as replacement of chlorine atom with the (SCH3, OCH3), hydrogen atom with Cl-CH=CH2 or CH3, or carbonyl (C=O) group with thiocarbonyl (C=S) group (Figure 1) and (Tables 2 & 3).

In another comparison of P2, S9, and T5 that have the same core structure, skin sensation did not affected by replacement of chloro group in (O-CH_2-CH_2-Cl) with (CH_2-CH_2-CF_3) pending group or addition of CH_3CH_2-SO_2, CH_3-O- , nitrogen in phenyl ring (pyridyl group) (Figures 5. & 6) and (Tables 2 & 3). In contract to the above note, presence of (Cl-CH=CH_2), carbonyl or thiocarbonyl, methyl group in V and S2 had a remarkable note related to their high skin sensation values (Figure 1) and (Tables 2 & 3).

P-Glycoprotein (P-gp) [26, 27] is a special carrier belongs to Adenosine TriPhosphate (ATP) binding cassette transporter having various interaction nature with chemical(s) classified as substrates / inducers / inhibitors. Some chemicals are bi- or tri- mixed type of classification beside one type of acting. For example, the famous antimalarial alkaloid (Quinidine) is a tri- mixed p-gp acting as substrate, inhibitor, and inducer. In another site, Artemisin is acting as non-transported inducer, non-competitive inhibitor, and non-substrate to this carrier.

There are many natural and pharmaceutics achieving P-gp inhibition like herbs (garlic, green tea, rosemary), fruits (grape, orange), natural constituents (glycoside, flavonoid, terpenoid), anticancer drug (doxorubicin), and antibiotic (erythromycin) [27, 28]. To achieve inhibitory mechanism, there are various strategies including:

- Inhibition of ATPase that affects protein pump sensitization.
- Interacting with polar heads in the lipid bilayer and changing bonding forces.
- Inhibition of transport function by reacting with cysteine located in P-gp transmembrane through thiol group.
- Decreasing of P-gp phosphorylation leading to inhibition of protein kinase.

So in general outlook, P- glycoprotein acts as a multidrug resistance protein or cell protector against toxic material found in brain, intestine, kidney, pancreas, liver, and/or testis and a secretion – elimination controller that may failure drug treatment. P- Glycoprotein may prohibit this protein system (inhibitor class) or merging drug to protein (Substrate class) [29].

p-gp substrate may have electron donor atom or group ((>C=O, -O-, -S-, R-F, -NR3, -N=) with spatial separation or three hydrogen bond acceptor with more spatial separation. In another site, hydroxyl, primary or secondary amine may decrease passive diffusion. Substrate that revealed non- effective transport (net efflux) has hydrogen bond acceptor with lower rate of transport (flipping) than active (flopping) [27, 30].

In this study, p-Glycoprotein prediction with (Yes/ No) response showed No in most of the predicted herbicides (Tables 2-3) as below:

"Yes" response for p-Glycoprotein Substrate was only with T5, S7, S9, P2, P10, and P11. Here, T5, S9, P2 had carbonyl, ether, and tertiary amine groups beside absence of hydroxyl, NH_2, or -NHR group. Also, P2 has R-F group (Figure 6.).

Interfering with the transporter leads to inhibit P-gp giving Yes response for p-Glycoprotein inhibition type I which was presenting only with T1, T6, P1, P2, and P8 beside Yes response for p-Glycoprotein inhibition type II was only with P1.

Central Nerve System (CNS) is bio- controlling environment in mammals and well developed organisms that effectively works with biological barriers. One of the most important protectors is Blood- Brain Barrier (BBB) that penetrate spinal cord and brain from bome agent(s) and exogenous material(s) and circulating back to the blood (for potentially harmful compound(s)) as well as delivering useful material(s) to brain from the blood. These dynamic regulating functions are highly responding for CNS induction and maintenance at various pathological and physiological conditions [31].

pH, calcium, magnesium, potassium, and water soluble nutrients beside their metabolites are essentials to human where they regulated by BBB via low passive permeability while fat soluble substances are transported by specific selective systems such as proteins or polypeptides to achieve their activities. Blockage mechanism of endogenous and xenobiotics minimizes CNS exposure to these substances. Excogenous compounds like herbicides and their metabolites may access / blocked CNS through the primary dynamic gate BBB [27, 31]. Changing in BBB values are mainly related to selective semipermeable BBB nature beside large surface area, negative surface charge, diffusion distance (or uniform thickness) from BBB to CNS through intracellular and intercellular paths, and structural –physicochemical properties of target molecule(s). Lipophilic, ionization, and polarity of atom(s) or group(s) regulate intra- passage of cell(s) while diffusion controls inter- movement. These regulators in both BBB and CNS membranes manage stability – specificity base of chemical(s) in human body toward drug design – development research gates [32]. In various cases, changing in attached functional group(s) varied BBB values (Figures 1, 6, & 7) and (Tables 2 & 3) as below:

- replacement of chlorine atom with the (SCH_3, OCH_3) in the same core structure increased BBB values,
- replacement of hydrogen atom with Cl-$CH{=}CH_2$ or CH_3 had a little changing in BBB,
- replacement of carbonyl (C=O) group with thiocarbonyl (C=S) group decreased BBB,
- replacement of chloro group in (O-CH_2-CH_2-Cl) (T5) with (CH_2-CH_2-CF_3) pending group in (P2), BBB values did not affect in dramatic way,
- addition of CH_3CH_2-SO_2, CH_3-O-, nitrogen in phenyl ring (pyridyl group) as in S9 compared to T5,
- and presence of (Cl-CH=CH2), carbonyl or thiocarbonyl, methyl group in V and S2 had a remarkable BBB values.

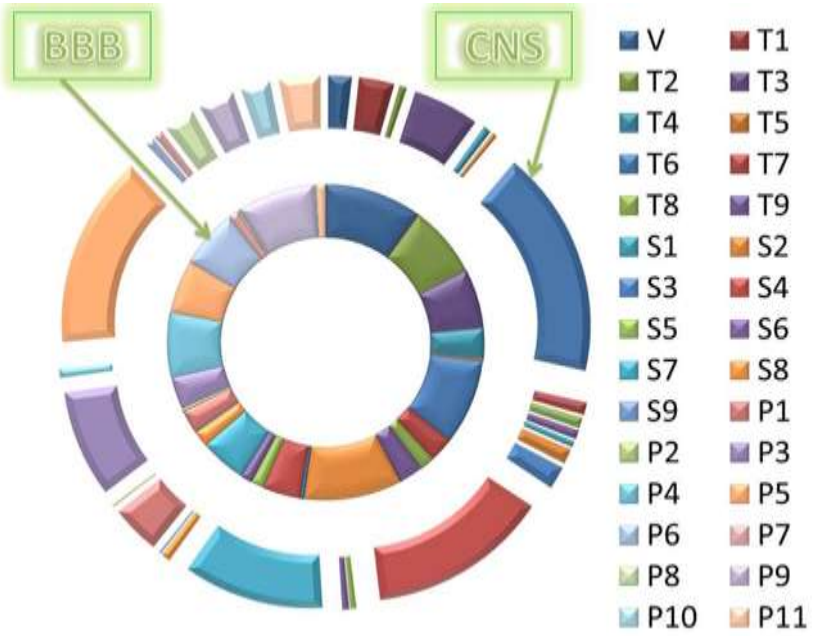

Figure (7). BBB and CNS prediction of the tested herbicids.

CNS values were ranged according to structural parameters of both brain tissue and the target molecule. CNS prediction was founded to be in high numbers in T6, P5, S4, S7, P3, T3, and P1. There was noticeable variation in CNS data compared to BBB in replacement of chlorine atom with the (SCH_3, OCH_3) in the same core, hydrogen atom with Cl-$CH{=}CH_2$ or CH_3, carbonyl (C=O) group with thiocarbonyl (C=S) group, chloro group in (O-CH_2-CH_2-Cl) (T5) with (CH_2-CH_2-CF_3) pending group in (P2), and addition of CH_3CH_2-SO_2, CH_3-O- , nitrogen in phenyl ring (pyridyl group) as in S9 compared to T5. CNS values were changed to high number by changing group (addition or replacement) (Figure 7, Tables 2 & 3.).

Cytochromes P450 (CYPs) are enzymes binding to cell membrane having heme structure. They had been found in human and other mammals with advanced identification of fifty-seven isoforms. In clinical studies, CYP3A4, CYP2D6, CYP2C19, and CYP2C9 perform drug or xenobiotic metabolism, drug –drug competing inhibition, and drug with high dose causing accumulated drug -plasma toxicity. So with these and other functions, CYPs are primer drug design and safety risk alerts. Relationship between food components, drug(s), and various CYP450 were studied and reviewed showing that some dietary components had possible effect on CYPs such as garlic, pepper, peppermint, grapefruit, orange, and others [33].

Also, several drug- metabolizing CYPs enzymes (CYP1B1, CYP2U1, and CYP3AF) found in brain endothelial cells play critical roles in human metabolism of fatty acids and specific drugs in the CNS [31]. Flexibility and presence of aromatic ring attached to hydrophobic moieties in herbicide or other xenobiotic molecules are potential interacting factors with enzymatic active site leading to CYP inhibition [34]. Tables (2. & 3.) showed high No CYPs response as below:

- CYP2D6 inhibition and substrate beside CYP3A4 substrate with **No possible response** towards all tested herbicides.
- Other CYPs presented several repeated "**Yes response**" with some tested herbicides:
  - CYP3A4 inhibition: **P11, P10, P9, P8**, P1, S3, T7, T6, T3, **T1** where this CYP has an ability to oxidize drugs among other xenobiotics [28].
  - CYP1A2 inhibition: **P11, P10, P9, P8**, P6, P5, P4, P3, P1, S4, S1, T8, T7, T3, T2, **T1**.
  - CYP2C19 inhibition: **P11, P10, P9, P8**, P1, S8, S4, S4, T7, T3, **T1**.
  - CYP2C9 inhibition: **P11, P8, P1**, S8, **T1**.

Also, CYPs shape, size (surface area), chemical composition, hydrogen bond acceptor / donor, and lipophilic nature pose a high binding affinity to polar molecule(s). Flexibility and presence of aromatic ring attached to the hydrophobic moieties in herbicides or other xenobiotic molecules are potential interacting factors with enzymatic active site leading to CYP inhibition. A noticeable safety sign of possible accumulations occurred when tested herbicide worked as inhibitor of cytochrome(s).

Major tested herbicides exhibited high intestinal absorption (with more than 87%) presenting 90% of them as mention above. By oral intake of plant parts and products having herbicidal molecules or their metabolites, these absorbed xenobiotic molecules may be transported to the liver leading to more number of enzymatically converted water- soluble compound(s) from fat- soluble compound(s) [31-34].

From Tables (1, 2, & 3), Figures (1 & 6), and above (**Yes/ No** response) observations, it can be noticed that presence of heteroatoms in chemical structures of the tested herbicides had a great influence upon CYP activity especially oxygen atom to nitrogen atom ratio. Increasing this ratio in P11, P10, P9, P8, and T1 gave repeated **Yes** inhibition predictions towards CYP1A2, CYP2C19, CYP2C9, and CYP3A4 enzymes.

Other tested factor in this *in Silico* study was total clearance which is referring to" the body capacity to eliminate the target by all needed mechanisms according to volume of blood per time (mL/minute)". So, total clearance of a molecule is pharmacokinetic key related in sum of liver or kidney elimination steps which valuable issue in drug discovery and development and xenobiotic (or its metabolic product(s)) filtration –secretion – reabsorption routes in mammals.

Physicochemical properties of target molecule and its interactions with tissue and/or other molecule(s) provide a preclinical comprehensive view of molecular deposition or biochemical pathways especially in renal section. In Silico, in vitro, and in vivo studies presents potential knowledge in understanding transportation process via both renal and non-renal systems related to molecular physicochemical properties (i.e., hydrophilic and hydrogen bonding).

This excretion character identified that these herbicides (or their metabolics) by entering mammal body including human may be removed through biliary, hepatic, and renal mechanism [35-37]. Most of the herbicides under online prediction process exhibited total clearance values below 0.6 ml/min/kg. P11 and T7 gave the highest values while P4 and T8 presented the lowest number (negative values) (Tables (2 and 3) and (Figure 8).

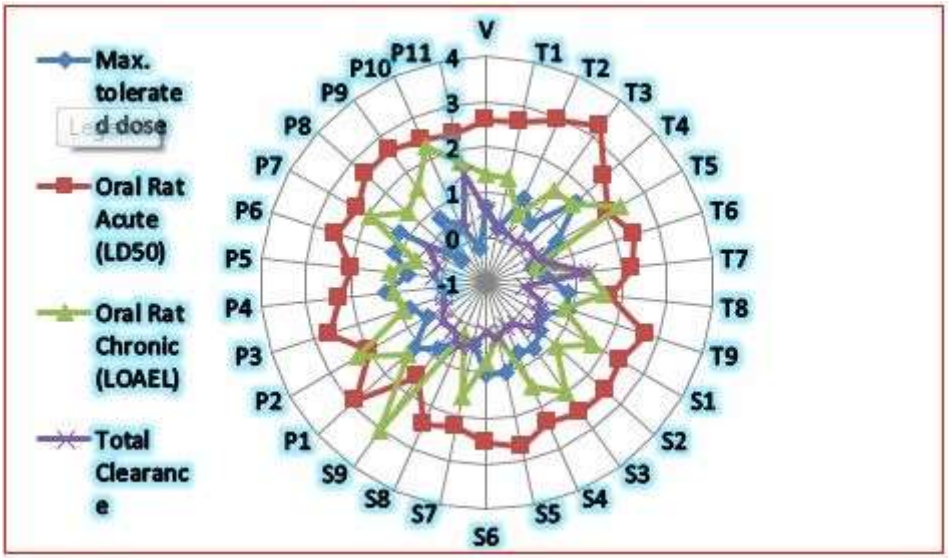

Figure (8). Total Clearance, Human Maximum Tolerated Dose, hERG inhibition, Oral Rat Acute Toxicity, and Oral Rat Chronic Toxicity (LOAEL) of the tested herbicides.

OCT2 (Organic Cation Transporter 2, electrogenic transporter) is an active sodium independent transport belongs to one of three specified gene families and targets molecule in cationic form into the filtrate by selective overlapping in renal secretion process to the renal lumen driven by the negative charge of the internal site of the cell membrane of kidney cortex in human and rat [38, 39, 40, 41]. Hydrophilic character of ionic compound(s) at physiological pH encourages net secretion by kidney after transportation process from blood and from the cell [42]. Its initial controlling steps of cationic molecule secretion is important in determining kinetics and dynamic of this molecule especially in drug- drug interaction, ligand complexity of toxin or other organic molecule through binding site and affinity character between transporter, substrate, and target molecule (endogenous or xenobiotic) [43, 44]. In this study, only T6 gave Yes response to renal organic cation transporter 2 (Figure (9).

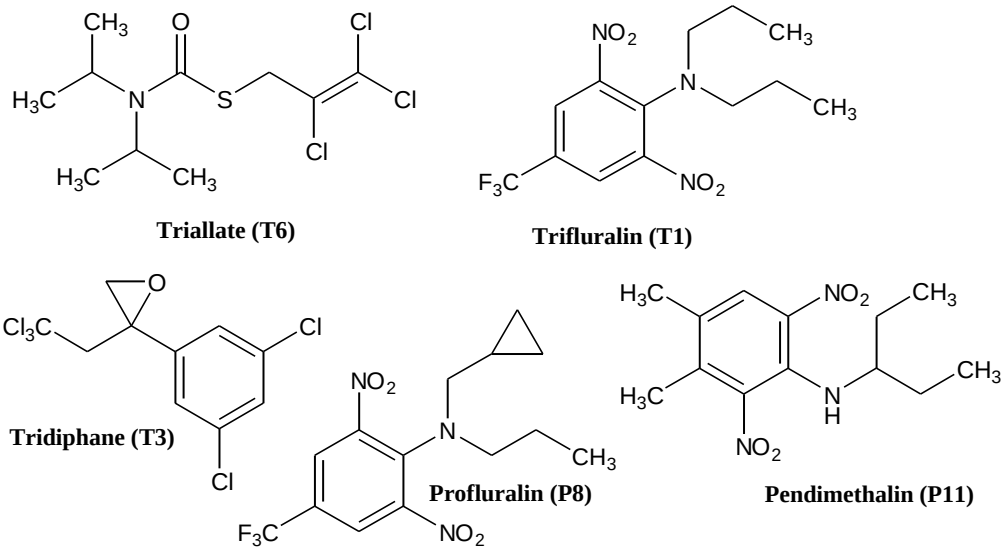

Figure (9). Chemical structures of T1, T3, T6, P8, and P11.

According to Chemical Abstract Service (CAS) [45] and American Chemical Society beside other global authorities, more than 200 million substances belonged to more than 100, 000 types were registered with a critical note that every two minutes, a new substance was introduced to the registration system.

This note needs a huge work of toxicity –mutagenicity tests beside other characterization systems. Mutagenicity as an irreversible permanent oncogenic process may led to cancer generation even with lower exposure level (lower than threshold), permitted daily exposure (PDE), and acceptance daily intake (ADI) [46 - 49].

Salmonella or Ames mutagenicity test defines as a short – term bacterial assay depending upon reverse gene mutation (damage) via various mechanisms that lead to change growth requirements in single base (or few bases) modification, insertion, deleting, or rearrangement, beside chromosome loss, rearrangement, or breakage [46].

Qualitative Structure Activity Relationship related to Ames (QSAR- Ames) prediction is an advanced study required for safety depending electrophilic and other chemical- physical characters. Also, QSAR –Ames prediction may be compared with other *in vitro*, *in vivo*, and / or other *in Silico* toxicological studies to get broad qualified – quantified point of view [47, 50].

Here, this computerized study characterized T1, T3, T6, S2, P5, P8, P9, and P11 as **mutagenic substances** with "**Yes response**" to Ames test (Tables 2 & 3).

In QSAR related to toxicity subject, "the highest dose of the test agent given during the chronic study that can be predicted not to alter the animal" represent the maximum tolerated dose. This character is important in diagnosis and treatment of numerous diseases used stimulants, antipsychotics, antidepressant or others in early or late stages of chronic health problems like carcinogenicity or long – term treatment of liver, heart, or kidney [51].

Maximum tolerated dose of the tested herbicides was ranged from (-0.25 log mg/kg/day or 0.599 mg/kg/day **belong to P8**) to (1.677 log mg/kg/day or 47.6 mg/kg/day **belong to T4**) (Figure 8) and (Tables 2 & 3).

hERG (ether-a-go-go related gene, KCNH2, Kv11.1.2) is a human gene considered as a highly selective voltage – gated channel for potassium, expresses in brain, thymus, adrenal gland, retina, heart, and smooth muscle. ERG 2 and ERG3 appear in nervous system while ERG1 channel in the human heart controlling potential repolarization (cardiac rhythm regulation) in both activation and inactivation kinetic state [52].

Chemical(s) in early drug development phase or candidate drug related to cardio-toxicity issue can be predicated via inhibition of affinity to hERG by animal, *ex vivo*, or *in Silico* studies. *In Silico* method is a favorite method because it is low costly, high throughput, computerized output, with absence of experimental lab requirements providing faster cardio-toxicity estimation [53]. Here, online estimation machine model by http://biosig.unimelb.edu.au/pkcsm/ website specified unexpected results where all 30 herbicides under testing showed **NO hERG I inhibition** while only P1 had **hERG II inhibition** (Tables 2 & 3) and (Figure 8).

EPA (U.S Environmental Protection Agency) and GHS (Globally Harmonized System of Classification and Labelling of Chemicals) classified toxicity in term of medium lethal dose (LD_{50}) and toxicity class where LD_{50} gives the required quantity of substance that kills half or the target animal (rat). This toxicity character expresses material from toxic (less than 50 mg/Kg) to safe (more than 2000 mg/Kg) [51]. Toxicity characterization may involve Oral Rat Acute Toxicity (LD_{50}, mol/kg) and Oral Rat Chronic Toxicity (LOAEL, log mg/kg body weight/day) that tested in this paper by http://biosig.unimelb.edu.au/pkcsm/ (Tables 2 & 3) and (Figure 2).

Lowest Observed Adverse Effect Levels (LOAEL) represents toxicity and chronic exposure with duration (52-104) weeks as long - term or (13 weeks) as short – term. In herbicide studies, there is a crucial need for *in Silico* foundation that may be found in food, water, soil, plant, animal and others. It is known that in Silico studies avoid legal consequences of using laboratory animals that defined by 3R (**R**eplace, **R**educe, and **R**efine) [54, 55]. Here, online computational toxicological risk assessment was done and showed that LD_{50} range was (1.581(**S9**)-3.301(**T3**)) mol./Kg while LOAEL was (0.141 (**T6**) – 3.005 (**S9**)) log mg/kg body weight/day (Tables 2-3, Figure 8) that highly influenced by difference in structures (Table 1) and related physical-chemical properties.

Hepatotoxicity is another important predictor of drug (or chemical) safety related to critical organ in human that is liver. This largest organ in human body support many functions such as digestion, metabolism, immunity, detoxification, storage and other functions by high efficient transporter that is dual blood supplement from both portal vein and hepatic artery. Bio-transformation (or detoxification) of any compound is depending upon formation of active site by addition of oxygen (Phase I) and conjugation with a functional group that initiated solubility in water (Phase II) by sulfonation, glucoronidation, and methylation. By combination of Phases (I & II), fat – soluble compound (lipophilic) is converted to corresponding water – soluble (lipophobic) compound so easier to be excreted by body through urine after transportation steps (Phase III) from hepatocyte, kidney, intestine, and any other cell. Liver may be exposed to chemical (drug or any toxic material) and this dysfunction may be depending on dose or independent. With depending mode, drug – induced liver injury is predictable causing by direct toxicity at short time with reproducibility influence, inhibition of biliary efflux, production of reactive toxic metabolics, protein modification, T & B cell cytotoxicity, and mitochondrial impairment [56].

Exogenous or endogenous, hormone, or any molecule considered as foreign compound reabsorbed by intestinal or renal tubular forward excretion after counted metabolism steps that converted target molecule to its metabolic(s) with lower toxicological activity. With these processes: metabolism, detoxification, and excretion, excess accumulation of any toxin is controlled to prevent multistep induced liver injury (acute or chronic liver failure) or liver cancer in advanced stages.

Human susceptibility to hepatotoxic material is depending upon: gender, age, nutrition, chemical composition, concentration, obesity, life style, genetic base, duration, and disease(s) especially immunodeficiency, hepatitis C, and others. Selectivity – toxicity relationship of chemical is mainly caused by presence of special group(s) that may interact with nuclear receptor(s) by enzymatic step. For example, acetaminophen (known as Panadol,

acetamidic phenol) and diclofenac (known as Voltaren, sodium carboxylate salt of secondary aromatic amine) have acute hepatocellular toxicity [57, 58].

In this prediction study, T2, T5, T7, S1, S5, S6, S9, P2, P3, P7, and P10 showed **Yes** response to hepatotoxicity where these compounds varied in their nitrogen to oxygen ratio, presence of other heteroatoms (halogen or/and sulfur), and water solubility (Tables (1-3), Figures (1, 6, 9)).

Skin as an integumentary organ can be defined as "protective barrier or guard against external environmental effect(s) to protect internal bones, muscles, and organs composed of multi-layers (epidermis and dermis)". These external environmental effects such as temperature, moister, natural or synthesized chemicals varied in their influence on skin which is skin sensations [59].

Chemicals under research, development, maintaining subjects target healthy skin and whole human body and avoiding anyone of all four types of allergy response (or hypersensitivity). Hypersensitivity type classified as type I: cutaneous test reaction, type II: antibody and related cytotoxicity, type III: antigen-antibody complex, and type IV: delayed response. So, skin sensitization is an allergic contact dermatitis of type IV occurred after more than 48 hours [60].

Sensitization symptoms may be appeared as rash, blister, and / or swollen that may be in few hours to lifetime or threat human life. Chemical reactions between any foreign material attached skin or air exposure activation, bacterial degradation, photo-activation are covalent formation with skin protein, forming adduct antigen, or / and free radical with / without electrophile – nucleophile interaction. Covalent bond formation with skin protein can be happened by nucleophilic thiol- or amine- amino acid in protein structure [61].

Changing in cell surface is a direct method in testing chemicals especially commercial (natural or synthesized) products such as pharmaceuticals and cosmeceuticals. These changing may be also tested through animals and non-animal (*in Silico*) models towards final steps: human testing.

In Silico skin sensitization has a great attention after Europe banning of animal testing since 2013 beside carcinogenicity, reproductive toxicity, and related kinetic toxicological studies.

These prediction models are online data source and commercial packed models subjected to computer automated structure evaluation (CASE) that depend on structural subunits having activity termed as biophore(s). These models are not limited to chemicals with direct sensitization, they also may have evaluated products of direct and enzymatic metabolic transformation including reduction, oxidation, hydrolysis… etc [62]. In this online *in Silico* testing model, V, T3, T6, S2, S4, P3, P5, and P9 showed skin sensitization with **Yes** response where most of them have amide (or thioamide) and chloro- groups (Figures (1, 6, & 9) and (Tables (1, 2, & 3).

Toxicity of chemicals whether used as herbicides or not used is an essential issue in safety classification and monitoring their effects on Food series (killing, inhibition, management, security, production, designing, availability, and development).

So, safety – efficacy balance is a main concern in herbicide science, health, technology, and environment. Time, cost, environmental limiting, and biological –agricultural testing of this balance as well as selectivity, action, and classification of any chemical (or herbicide) were shorten by computational models (QSAR) [63].

Recent scientific articles evaluated safety – efficacy of huge number of chemicals including crops, honey, and herbicidal activity incorporation with chemical – physical properties in high accurate identification. Online machine learning represents fast, easy, accurate prediction methods and provides scientists and drug – developers ADMET information in time of several minutes or less [64-68].

In general, **Yes/ No** response of herbicides towards Herbicidal activity with honey bee and avian toxicity that predicted by [13] website were summarized as below (Table 4):

- ☒ Herbicidal activity - **No** response: T3, T7, and P10.
- ☒ Honey bee toxicity - **Yes** response: V and S2.
- ☒ Avian toxicity – **Yes** response: only T2.

So, most of the tested herbicides have herbicidal activity with minimum toxic effects on bees and avian. For example, Vernolate (V) is safe to avian and can be used as an herbicide but toxic to honey bees.

Cancer is considered as a big challenge to scientific and medicinal regimes around the world. It needs effective and safe chemicals for treatment and diagnosis that required multiple steps of synthesis (or extraction), characterization, and practical *in vitro, in vivo, exo vivo* methods as well as time – cost consuming. QSAR strategy is a primary gate of exploring and development candidate drug and may short these multiple steps besides giving a primary image of toxicity, resistance to chemotherapy, or/ and side effects with reducing time and cost [69, 70]. Many published articles presented machine learning throughput screen studies of anticancer materials at high level of accuracy. One of these QSAR computational approaches is online screen websites targeting pharmacokinetic or pharmacodynamics properties of cancer cell lines based upon structural features [71, 72, 73, 74]. In this study, cancer cell line activity of CNS, Leukaemia, ovarian, and prostate were evaluated by [14] (Tables 5-7, Figure 4, Figures (10-12)) where summary of these cell lines were as below:

- **Active** response of all tested cell lines: T1, T3, P1, P8, and P10.
- CNS: **all were less than 5**.
- Leukaemia **(≥ 5)**: T1, T3, T5, T6, T7, S2, S8, S9, P1, P5, P8, P9, P10, and P11.
- Ovarian (**> 5**): T3, T6, and P1.
- Prostate (**> 5**): T3, T5, and P5.

These herbicides under prediction testing were characterized as (Table 8), [75]:

- Harmful if swallowed: **V, T2, T3, T4, T6, T7, T8, T9, S5, P1, and P1.**
- Causes damage to organs through prolonged or repeated exposure: **T4, T6, and S3.**
- May cause an allergic skin reaction: **T4, S9, P8, and P10.**
- Toxic to aquatic life with long lasting effects: **V.**
- Moderately toxic, classified by WHO as unlikely to be hazard as in Class U toxic, causing irritation to eye, skin, and respiratory tract: **S4.**
- Very toxic to aquatic life with long lasting effects: **T3, T4, S3, S5, and P1.**
- Causes serious eye irritation: **T4, S9, P8, and P10.**
- May irritate eyes, nose, throat, and skin: **S7.**
- May cause an allergic skin reaction: **T1, T4, T6, and S3.**
- May cause skin and eye irritation: **S6.**
- May also cause liver and kidney damage, coma or convulsions: **S6.**
- Did not induce unscheduled DNA synthesis in human cells: **T6.**
- Epidemiological study did not find significant changes in neurological function after high exposure: **T6.**
- Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential: **T7.**
- Likely to be Carcinogenic to Humans: **T7 and P5.**
- Not likely to be Carcinogenic to Humans: **P4.**
- Evidence in humans: No adequate data. Evidence in animals: Sufficient evidence. Overall summary evaluation of carcinogenic risk to humans is Group 2B: The agent is possibly carcinogenic to humans: **S2.**
- Cancer Classification: Group C Possible Human Carcinogen: **P11.**
- Group D Not Classifiable as to Human Carcinogenicity: T9 and P7.
- Group E Evidence of Non-Carcinogenicity for Humans: **T8, S1, and P6.**
- Not classifiable as to its carcinogenicity to humans (Group 3): **T1.**

Here, online prediction of Tridiphane (T3) showed extraordinary activity response to Leukaemia, Ovarian, and Prostate cell lines with **more than 5** while its activity towards CNS lines was **less than 5** (Figures (10 -12), Tables (5 -7)). Scientific literature foundations confirmed that human swallowing of T3 gives a harmful effect and it is very toxic to aquatic life lasting for long time (Table 8) [75].

Table (8). Hazard and cancer classifications of the thirty tested herbicides [75].

Name (code)	Vernolate (V)	Trifluralin (T1)	Trietazine (T2)	Tridiphane (T3)	Triclopyr (T4)
Cancer Classification	Harmful if swallowed, Toxic to aquatic life with long lasting effects.	May cause an allergic skin reaction, Suspected of causing cancer, Very toxic to aquatic life with long lasting effects , not classifiable as to its carcinogenicity to humans (Group 3).	Harmful if swallowed, Very toxic to aquatic life	Harmful if swallowed, Very toxic to aquatic life with long lasting effects.	Harmful if swallowed, May cause an allergic skin reaction, Causes serious eye irritation, Causes damage to organs through prolonged or repeated exposure, Very toxic to aquatic life with long lasting effects.
Name (code)	Sulfentrazone (S1)	Sulfallate (S2)	Sulcotrione (S3)	Solan (S4)	Simetryn (S5)
Cancer Classification:	Group E Evidence of Non-carcinogenicity for Humans	1) evidence in humans: No adequate data. 2) evidence in animals: Sufficient evidence. Overall summary evaluation of carcinogenic risk to humans is Group 2B: The agent is possibly carcinogenic to humans.	May cause an allergic skin reaction, Suspected of damaging the unborn child, Causes damage to organs through prolonged or repeated exposure, Very toxic to aquatic life with long lasting effects.	Moderately toxic, classified by WHO as unlikely to be hazard as in Class U toxic, causing irritation to eye, skin, and respiratory tract.	Harmful if swallowed, Very toxic to aquatic life with long lasting effects.
Name (code)	Prosulfuron (P2)	Propyzamide (P3)	Propazine (P4)	Propachlor (P5)	Prometryn (P6)
Cancer Classification:	no human health risks of concern at this time	Group B2: Probable Human Carcinogen	Not Likely to be Carcinogenic to Humans	Likely to be Carcinogenic to Humans	Group E Evidence of Non-carcinogenicity for Humans
Name (code)	Triasulfuron (T5)	Triallate (T6)	Tralkoxydim (T7)	Terbacil (T8)	Tebuthiuron (T9)

Cancer Classification:	Very toxic to aquatic life with long lasting effects	Harmful if swallowed, May cause an allergic skin reaction, Causes damage to organs through prolonged or repeated exposure, Very toxic to aquatic life with long lasting effects, did not induce unscheduled DNA synthesis in human cells. Epidemiological study did not find significant changes in neurological function after high exposure.	Harmful if swallowed, Suspected of causing cancer, Toxic to aquatic life with long lasting effects, Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential, Likely to be Carcinogenic to Humans	Harmful if swallowed, Very toxic to aquatic life with long lasting effects, Group E Evidence of Non-Carcinogenicity for Humans	Harmful if swallowed, Very toxic to aquatic life with long lasting effects, Group D Not Classifiable as to Human Carcinogenicity
Name (code)	Simazine (S6)	Siduron (S7)	Sethoxydim (S8)	Rimsulfuron (S9)	Pyridate (P1)
Cancer Classification:	It may cause skin and eye irritation. It may also cause liver and kidney damage, coma or convulsions.	May irritate eyes, nose, throat, and skin.	Not Likely to be Carcinogenic in Humans	Causes serious eye irritation, Very toxic to aquatic life with long lasting effects	Harmful if swallowed, Causes skin irritation. Causes an allergic skin reaction, Very toxic to aquatic life with long lasting effects.
Name (code)	Prometon (P7)	Profluralin (P8)	Pretilachlor (P9)	Perfluidone (P10)	Pendimethalin (P11)
Cancer Classification:	Group D Not Classifiable as to Human Carcinogenicity	**Causes serious eye irritation, Very toxic to aquatic life with long lasting effects**	Causes skin irritation, toxic if inhaled , Very toxic to aquatic life with long lasting effects	Harmful if swallowed, Causes serious eye irritation	Cancer Classification: Group C Possible Human Carcinogen

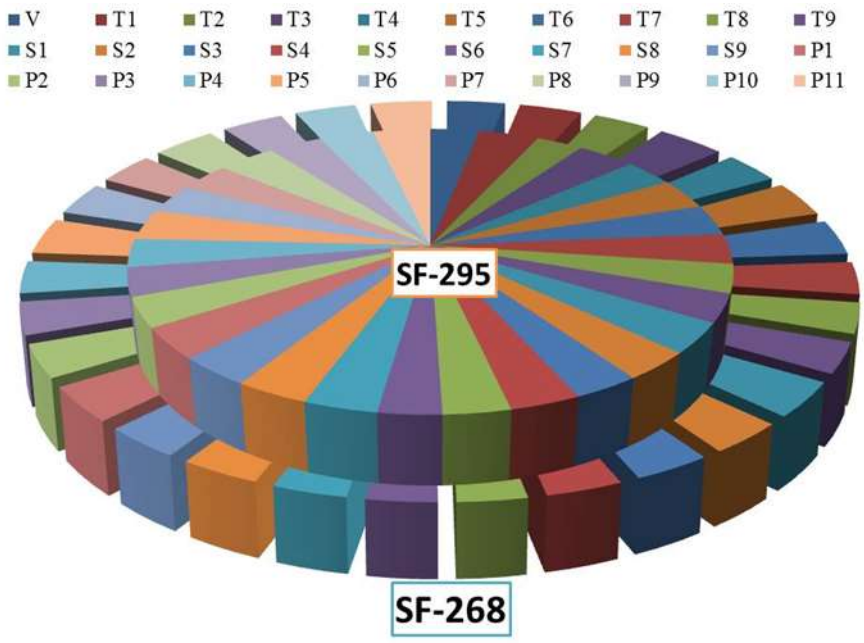

Figure (10). CNS activity prediction of two lines (SF-268 and SF-295) by http://biosig.unimelb.edu.au/pdcsm-cancer/ website.

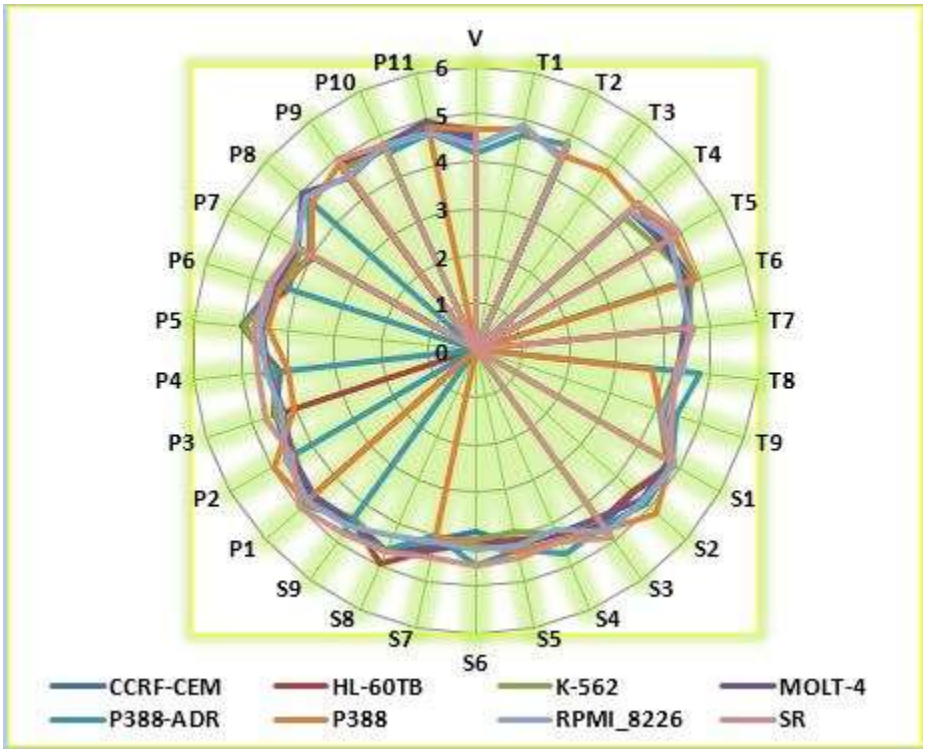

Figure (11). Leukaemia activity prediction by http://biosig.unimelb.edu.au/pdcsm-cancer/ website.

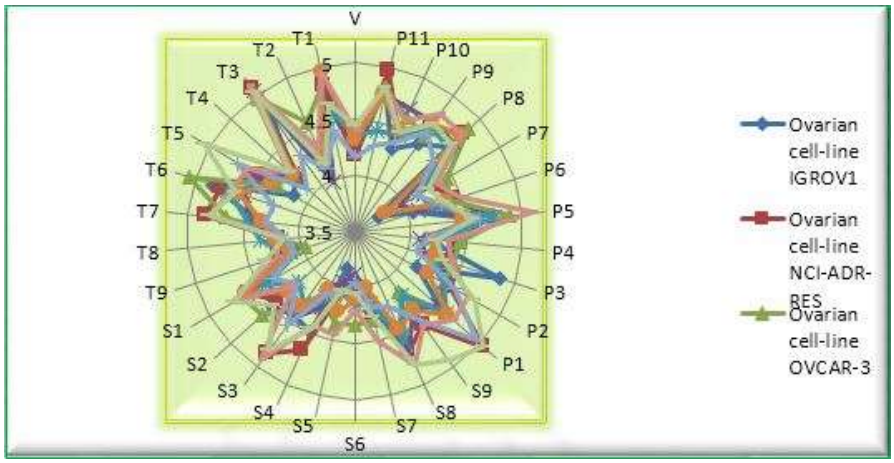

Figure (12). Ovarian and prostate activities prediction by http://biosig.unimelb.edu.au/pdcsm-cancer/ website.

4. Conclusion

Thirty herbicides were chosen and subjected to online website activity prediction belong to pkCSM - University of Melbourne – Australia as a QSAR prediction against honey bee, avian, and multiple human Leukemia, CNS, Ovarian, Prostate Cancer cell lines. Another calculated predictor of ADMET were Caco-2 permeability, human Intestinal absorption, Skin Permeability, P-glycoprotein substrate and inhibitor, BBB permeability, CNS permeability, Cytochromes P450 inhibition, Total Clearance, Renal OCT2 substrate, Ames, Human Maximum Tolerated Dose, hERG inhibition, Oral Rat Acute Toxicity, Oral Rat Chronic Toxicity, Hepatotoxicity, and Skin Sensitisation. These phytotoxins varied in their response to each calculated predictor. Sulfentrazone (S1) is a known herbicide with evidence of human non-carcinogenic character (Group E). Its prediction showed hepatotoxicity with Leukemia, CNS, Ovarian, Prostate Cancer cell lines activities less than 5. Also, it works as CYP1A2 inhibitor with negative inhibition response to p-glycoprotein, Ames test, skin sensitization, renal OCT2, and hERG. Also, S1 had the low intestinal absorption (89.659%) and lowest BBB compared to the other herbicides under testing. So, Sulfentrazone (S1) is more structurally safe, however, its active –multiple toxicological - cellular interactions must be under considerations. In comparison, Simetryn (S5) and Simazine (S6) have the same core structure except (-SCH_3) group replaced with chloro group (or chemical formula: $C_8H_{15}N_5S$ and $C_7H_{12}ClN_5$ respectively). Both S5 and S6 gave semi identical results in Caco-2, intestinal absorption, skin permeability, CNS, max. tolerated dose, oral rat acute (LD50) with the same response to herbicide activity, honey bee and avian, p- glycoprotein, cytochrome P450, renal OCT2, Ames, hERG I and II, hepatotoxicity, skin sensitization, and general anticancer activity as an inactive material. In reverse, BBB, total clearance, and oral rat chronic (LOAEL) of S5 and S6 were in different values especially LOAEL. These results briefly confirm chemical structure influences upon prediction outcomes.

References

[1] V. Beasley,"Direct and indirect effects of environmental contaminants on amphibians" in *Reference model in earth system and environmental sciences*, Elsevier, USA, 2020.
[2] P. Gupta, "Herbicides and fungicides," Chapter 39, in Reproductive and development toxicology, R. Gupta (Editor), Academic Press, Elsevier, USA, 2011.
[3] C. Martins-Gomes, T. Silva, T. Andreani, and A. Silva, "Glyphosate vs. Glyphosate-based herbicides exposure: a review on their toxicity," *Journal of Xenobiotics*, vol. 12, no. 1, pp. 21-40, 2022.
[4] C. Peillex and M. Pelletier, "The impact and toxicity of glyphosate and glyphosate – based herbicides on health and immunity," *Journal Immunotoxicology*, vol. 17, no. 1, pp. 163-174, 2020.
[5] J. Lehel, G. Szemerédy, R. Szabó, L. major, A. Grúz, and P. Budai, "Reproductive toxicological changes in avian embryos due to pesticide and an environmental contaminant," *Acta Veterinaria Hungarica*, vol. 64, no. 4, pp. 363-371, 2021.
[6] Science, Safe Food, and Sustainability – European Union [Online] available: https://www.efsa.europa.eu/.
[7] U.S. Environmental Protection Agency | US EPA [Online] available: https://www.epa.gov/.
[8] World Health Organization (WHO) [Online] available: https://www.who.int/.

[9] European Union Law [Online] available: https://eur-lex.europa.eu/.
[10] Occupational Safety and Health Administration (OSHA) [Online] available: https://www.osha.gov/.
[11] K. Gandhi, S. Khan, M. Patrikar, A. Markad, N. Kumar, A. Choudhari, P. Agar, S. Indurkar, " Exposure risk and environmental impacts of glyphosate: highlights on the toxicity of herbicide co-formulants,'' *Environmental Challenges* vol. 4, article number 100149, *2021*.
[12] D. Pires, T. Blundell, and D. Ascher, "pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures," *Journal of medicinal chemistry*, vol. 58, no. 9, pp.4066–4072, 2015.
[13] D. Pires, K. Stubbs, J. Mylne, and D. Ascher, " cropCSM: designing safe and potent herbicides with graph-based signatures, "*Briefings in Bioinformatics*, vol. 23, no. 2, article number bbac042, 2022.
[14] R. Al-Jarf, A. de Sa, D. Pires, and D. Ascher, "pdCSM-cancer: Using Graph-Based Signatures to Identify Small Molecules with Anticancer Properties," *Journal of Chemical Information and Modeling*, vol. 61, no. 7, pp.3314-3322, 2021.
[15] T. Lea, "Caco-2 cell line, in The impact of food bioactive on health: *in vitro* and *ex vivo* models,"Chap. 10, K. Verhoeckx, p. Cotter, I. López-Expósito, C. Kleivland, T. Lea, A. Mackie, T. Requena, D. Swiatecka, and H. Wichers (editors), Springer, USA, 2015.
[16] L. Lim and M. Rogers (editors), " Food applications of nanotechnology," in Advances in food and nutrition research, book series, volume 88, Elsevier, USA, eBook ISBN: 9780128160749, 2019.
[17] N. Darling, C. Mobbs, A. Gonzàlez –Hau, M. Freer, and S. Przyborski, "Bioengineering novel in vito co-culture models that represent the human intestinal mucosa with improved Caco-2 structure and barrier function," *Frontiers in Bioengineering and Biotechnology*, vol. 8, pp. 992, 2020,
[18] E. Istifili, M Hüsunet, and H Ila, "Cell division, cytotoxicity, and the assays used in the detection of cytotoxicity, cytotoxicity-definition, identification, and cytotoxic compounds," IntechOpen, UK, 2019.
[19] D. Dahlgren and H. Lennernäs, "Intestinal permeability and drug absorption: predictive experimental, computational and *in vivo* approaches," *Pharmaceutics*, vol. 11, article number 411 (18 pages), 2019.
[20] M. Macedo, E. Martinz, C. Barrias, and B. Sarmento, "Development of an improved 3D *in vitro* intestinal model to perform permeability studies of paracellular compounds," *Frontier in Bioengineering and Biotechnology*, vol. 8, pp. 1076, 2020.
[21] Y. Kamiya, R. Yamada, C. Akase, Y. Abe, Y. Sekiguchi, N. Murayama, M. Shimizu, M. Kitajima, F. Shono, K. Funatsu, and H. Yamazaki, "Determination and predication of permeability across intestinal epithelial cell monolayer of a diverse range of industrial chemicals/ drugs for estimation of oral absorption as a putative marker of hepatotoxicity," *Toxicology Reports*, vol. 7, pp. 149-154, 2020.
[22] N. Ball, R. Bars, P. Botham, A. Cuciureanu, M. Cronin, J. Doe, T. Dudzina, T Gant, M. Leist, B. van Ravenzwaat, "A framework for chemical safety assessment incorporating new approach methodologies within REACH," *Archives of Toxicology*, vol. 96, pp. 743-766, 2022.
[23] E. Erhirhie, C. Ihekwereme, and E. Ilodiqwe, " Advances in acute toxicity testing: strengths, weaknesses, and regulatory acceptance," *Interdisciplinary Toxicology* vol. 11, no. 1, pp. 5-12, 2018.
[24] S. Supe and P. Takudage, "Methods for evaluating penetration of drug into the skin: a review," *Skin Research and Technology*, vol. 27, no. 3, pp. 299-308, 2021.
[25] Y. Wu, G. Ta, Y. Lung, C. Weng, and M. Leong, "*In Silico* prediction of skin permeability using two-QSAR approach, " *Pharmaceutics* vol. 14, article number 9612, 2022.
[26] K. Srivalli and P. Lakshmi, "Overview of P-glycoprotein inhibitors: a rational outlook, *'' Brazilian Journal of Pharmaceutical Sciences*, vol. 48, no. 3, pp. 353-367, 2012.
[27] A. Seelig, "p-Glycoprotein: one mechanism, many tasks and the consequences for pharmacotherapy of cancers, " *Frontiers in Oncology*, vol. 10, article number 576559, 2020.
[28] S. Defeme and P. Augustijns (2003). The effect of food components on the absorption of P-gp substrates: a review," *Journal of Pharmacy and Pharmacology* vol. 55, pp. 153-162, 2003.
[29] C. Karthika and R. Sureshkumar, "p-Glycoprotein efflux transporters and its resistance its inhibitors and therapeutic aspects," IntechOpen, UK, 2020.
[30] A. Nanayakkara, P. Vogel, and J. Wise, "Prolonged inhibition of P- glycoprotein after exposure to chemotherapeutics increase cell mortality in multidrug resistant cultured cancer cells, *PLoS ONE* vol. 14, no. 4, article number e021794, 2019.
[31] H. Kadry, B. Noorani, and L. Cucullo, "A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. Fluids and Barriers of the CNS," vol. 27, article number 69 (24 pages), 2020.

[32] S. Bagchi, T. Chhibber, B. Lahooti, A. Verma, V. Borse, and R. Dev Jayant, " *In vitro* blood- brain barrier models for drug screening and permeation studies: an overview," *Drug Design, Development, and Therapy*, vol. 13, pp. 3591-3605, 2019.
[33] S. Defeme and P. Augustins, "The effect of food components on the absorption of P-gp substrates: a review, " *Journal of Pharmacy and Pharmacology*, vol. 55, pp. 153-162, 2003.
[34] T. Beck, K. Beck, J. Morningstar, M. Benjamin, and R. Norris, "Descriptors of Cytochrome inhibitors and useful machine learning based methods for the design of safer drugs," *Pharmaceuticals*, vol. 14, pp. 472, 2021.
[35] R. Ward and S. Kern, "Principles of pharmacokinetics," Chapter 19, Fetal and neonatal physiology, R. Polin, S. Abman, D. Rowitch, W. Benitz, and W. Fox (Editors), 5th edition, volume 1, Elsevier, USA, 2017.
[36] B. Feng, J. La Perle, G. Chang, and M. Varma, "Renal clearance in drug discovery and development: molecular descriptors, drug transporters and disease state," *Expert Opin Drug Metabol Toxicology*, vol. 6, no. 7, 15 pages, 2010.
[37] T. Cressey, O. Siriprakaisil, R. Kubiak, V. Klinbuayem, P. Sukrakanchana, J. Ouame-Amaglo, H. Okochi, Y. Tawon, R. Cressey, J. Baetene M. Gandhi, P. Drain, "Plasma pharmacokinetics and urinary excretion of tenofovir following cessation in adults with controlled levels of adherence to tenofovir disproxil fumarate," *International Journal of Infectious Diseases*, vol. 97, pp. 365-370, 2020.
[38] B. George, X. Wen, E. Jaimes, M. Joy, and L. Aleksunes, " *In vitro* inhibition of renal OCT2 and MATE1 secretion by antiemetic drugs,*" International Journal of Molecular Sciences*, vol. 22, no. 6439, pp. 1-13, 2021.
[39] A. Noorlander, S. Wesseling, I. Rietjens, and B. Ravenzwaay, "Incorporating renal excretion via the OCT2 transporter in physiologically based kinetic modeling to predict in vivo kinetics of mepiquat in rat," *Toxicology Letters*, vol. 343, pp. 34-43, 2021.
[40] Z. Zazuli, N. Duin, K. Jansen, S. Vijverberg, A. van der Zee, and R. Maserruw, "The impact of genetic polymorphisms in Oragnic Cationic transporters on renal drug disposition," *International Journal of Molecular Sciences*, vol. 21, article number 6627, 2020.
[41] H. Koepsell, "Update on drug – drug interaction at organic cation transporters: mechanisms, clinical impact, and proposal for advanced *in vitro* testing," *Expert Opinion on Dug Metabolism and Toxicology*, vol. 17, no. 6, pp. 635-653, 2021.
[42] K. Hacker, R. Maas, J. Kornhuber, M. Fromm, and O. Zolk, "Substrate –dependent inhibition of human Organic Cationic Transporter OCT2: a comparison of metformin with experimental substrates," *PLoS ONE* vol. 10, no. 9, article number e0136451, 2015.
[43] S. Wright, "Molecular and Cellular physiology of organic cation transporter 2," *American Journal of Physiology and Renal Physiology*, vol. 317, pp. F1669 - F1679, 2019.
[44] S. Zhou, S. Zeng, and Y. Shu, "Drug- drug interactions at organic cation transporter 1," *Frontiers in Pharmacology*, vol. 12, no. 628704, 2021.
[45] Chemical Abstract Service (CAS) [Online] available: https://www.cas.org.
[46] K. Mortelmans and E. Zeiger, "The Ames Salmonella / microsome mutagenicity assay," *Mutation Research*, vol. 455, pp. 29-60, 2000.
[47] M. Honma, "An assessment of mutagenicity of chemical substances by (quantitative) structure – activity relationship," *Gene and Environment*, vol. 42, no. 23, 2020.
[48] A. Hakura, T. Awogi, T. Shiragiku, A. Ohigashi, M. Yamamoto, K. Kanasaki, H. Oka, Y. Dewas, S. Ozawa, K. Sakamoto, T. Kato, and E. Yamamura, "Bacterial mutagenicity test data: collection, by the task force of the Japan pharmaceutical manufacturers association," *Gene and Environment*, vol. 43, no. 41, pp. 1-16, 2021.
[49] Y. Fan, Y. Fu, Y. Zhou, Y. Liu, B. Hao, and R. Shang, "Acute, subacute oral toxicity and Ames test of pymulin: an antibacterial drug candidate," *BMC Pharmacology and Toxicology*, vol. 23, no. 2, pp. 1-12, 2022.
[50] C. Smith and T. Perfetti, "Statistical treatment cytotoxicity in Ames bacterial reverse mutation assays can provide additional structure – activity relationship information," *Toxicology Research and Application*, vol. 4, pp. 1-5, 2020.
[51] H. Stampfer, G. Gabb, and S. Dimmitt, "Why maximum tolerated dose?," *British Journal of Clinical Pharmacology*, vol. 85, no. 10, pp. 2213-2217, 2019.
[52] B. Priest, I. Bell, and M. Garcia, "Role of hERG potassium channel assays in drug development," *Channels* vol. 2, no. 2, pp. 87 – 93, 2008.

[53] S. Krishna, A. Borrel, R. Huang, J. Zhao, M. Xia, and N. Kleinstreuer, "High- throughput chemical screening and structure – based models to predict hERG inhibition," *Biology* vol. 11, pp. 209, 2022.
[54] S. Guth, A. Roth, B. Engeli, D. Lachenmeier, A. Cartus, S. Hüser, M. Baume, P. Diel, G. Eisenbrand, J. Hengstler, H. Humpf, H. Joost, A. Lampen, M. Leist, D. Marko, P. Steinberg, A. Mally and J. Zarn, "Comparison of points of departure between subchronic and chronic toxicity studies on food additives, food contaminants and natural food constituents," *Food and Chemical Toxicology*, vol. 146, no. 111784, 2020.
[55] C. Araujo-Lima, R. Nunes, R. Carpes, C. Ajub, and I. Felzenszwab, " Pharmacokinetic and toxicological evaluation of zinc gluconate- based chemical sterilant using *in vitro* and *in Silico* approaches." *BioMed Research International*, vol. 2017, Article ID 5746768, pp. 1-8, 2017.
[56] D. Zageer and S. Hantoosh, "Insight drugs induce liver toxicity," 1st edition, B P International, UK, 2021.
[57] D. Singh, W. Cho, and G. Upadhyay, "Drug – induced liver toxicity and prevention by herbal antioxidants: an overview," *Frontiers in Physiology*, vol. 6, no. 363, pp.1-18, 2016.
[58] A. Tabernilla, B. Rodrigues, A. Pieters, A. Caufriez, K. Leroy, R. Van Campenhout, A. Cooreman, A. Gpmes, E. Arnesdotter, E. Gijbels, and M. Vinken, "*In vitro* liver toxicity testing of chemicals: a pragmatic approach," *International Journal of Molecular Sciences*, vol. 22, no. 5038, pp. 1-45, 2021.
[59] A. Rehfeld, M. Nylander, and K. Karnov, "Compendium of Histology. The integumentary Syste," Springer Nature, UK, 2017.
[60] A. Lee and J. Thomson, "Drug – induced skin reactions,*'' Adverse Drug Reactions*, vol. 262, pp. 125 -156, 1999.
[61] A. Gomolin, E. Netchiporouk, R. Gniadecki, and I. Litvinov, "Artificial intelligence applications in dermatology: Where do we stand?" *Frontier in Medicine*, vol. 7, pp. 100, 2020.
[62] G. Ta, C. Weng, and M. Leong," *In Silico* prediction of skin sensitization: Quo vadis?" *Frontier in Pharmacology*, vol. 12, no. 655771, 2021.
[63] D. Pires, K. Stubbs, J. Mylne, and D. Ascher, "cropCSM: designing safe and potent herbicides with graph – based signatures," *Briefings in Bioinformatics*, vol. 23, no. 2, pp. 1-9, 2022.
[64] E. Clarke and J. Delanet, "Physical and molecular properties of agrochemicals: an analysis of screen inputs, hits, leas, and products," *CHIMIA International Journal of Chemistr*y, vol. 57, pp. 731-734, 2003.
[65] L. Weihua and T. Yun, " *In Silico* prediction of terrestrial and aquatic toxicities for organic chemicals," *Chinese Journal of Pesticide Sciences*, vol. 12, no. 4, pp. 477 -488, 2010.
[66] D. Pires and D. Ascher, "mCSM – AB: a webserver predicting antibody – antigen affinity changes upon mutation with graph – based signatures, " *Nucleic Acid*, vol. 44, pp. W469 –W473, 2016.
[67] G. Seralini and G. Jungers, "Toxic compounds in herbicides without glyphosate," *Food Chemical Toxicology*, vol. 146, pp. 111770, 2020.
[68] Z. Zhang, J. Guan and S. Zhou, "FraGAT: a fragment – oriented multi – scale graph attention model for molecular property prediction," *Bioinformatics*, vol. 37, pp. 2981-2987, 2021.
[69] R. Al-Jarf, A. de Sá, D. Pires, and D. Ascher, " pdCSM-cancer: using graph – based signatures to identify small molecules with anticancer properties," *Journal of Chemical Information and Modeling*, vol. 61, pp. 3314 – 3322, 2021.
[70] World Health Organiztion (WHO), Cancer, fact sheet, Https://Www.Who.Int/En/News-Room/Fact-Sheets/Detail/Cancer, 2003.
[71] H. Singh, R. Kumar, S. Singh, K. Cgaudhary, A. Gautam, and G. Raghava', "Prediction of anticancer molecules using hybrid model developed on molecules screened against Nci-60 cancer cell lines," *BMC Cancer*, vol. 16, pp. 77, 2016.
[72] S. Hoelder, P. Clarke, and P. Workman, "Discovery of small molecule cancer drugs: successes, challenges, and opportunities," *Molecular Oncology*, vol. 155, 2012.
[73] M. Menden, F. Iorio, M. Garnett, U. McDermott, C. Benes, P. Ballester, and J. Rodriguez, "Machine Learning prediction of cancer cell sensitivity to drug based on genomic and chemical properties," *PLoS One*, vol. 8, no. e61318, 2013.
[74] B. Zagidullin, J. Aldahdooh, S. Zheng, W. Wang, Y. Wang, J. Saad, A. Malyutina, M. Jafari, Z. Tanoli, A. Pessia, and J. Tang, "Drugcomb: an integrative cancer drug combination data portal," *Nucleic Acids Research*, vol. 47, pp. W43. – W51, 2019.
[75] Public repository for information on chemical substances and their biological activities started in 2004, Molecular Libraries Roadmap Initiatives, US National Institutes of Health (NIH) [Online] available: https://pubchem.ncbi.nlm.nih.gov/.