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Is it Possible to Predict the ADI of Pesticides using the QSAR Approach?

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ABSTRACTS

Objectives: QSAR methodology was applied to explain two different sets of acceptable daily intake (ADI) data of 74 pesticides proposed by both the USEPA and WHO in terms of setting guidelines for food and drinking water.

Methods: A subset of calculated descriptors was selected from Dragon® software. QSARs were then developed utilizing a statistical technique, genetic algorithm-multiple linear regression (GA-MLR). The differences in each specific model in the prediction of the ADI of the pesticides were discussed.

Results: The stepwise multiple linear regression analysis resulted in a statistically significant QSAR model with five descriptors. Resultant QSAR models were robust, showing good utility across multiple classes of pesticide compounds. The applicability domain was also defined. The proposed models were robust and satisfactory.

Conclusions: The QSAR model could be a feasible and effective tool for predicting ADI and for the comparison of logADIEPA to logADIWHO. The statistical results agree with the fact that USEPA focuses on more subtle endpoints than does WHO.

Keywords: ADI, risk, QSAR, noncancer pesticides

I. Introduction

An increasing number of environmental effects of pesticide applications are now being taken into account by regulatory bodies, leading to increased restrictions on their use or even bans. The World Health Organization (WHO) and United States Environmental Protection Agency (U.S. EPA) have established an ADI for an actual risk management decision in the regulatory process of pesticides for setting safety standards. Therefore, EPA gave highest priority to pesticides in food and drinking water and all other non-occupational sources. The U.S. Department of Agriculture (USDA) and The Food and Drug Administration (FDA) have also develop statistically valid information on pesticide residues in foods for compliance with these residue limits.[1,2)]

Two safety standards, ADI (mg/kg/day) or tolerable intakes, referred as reference doses for noncarcinogens, are used to establish a level of pesticide residues on food products that will pose a negligible risk to human health. The ADI takes into account daily exposure of a substance over a lifetime. The ADI concept has often been used as a tool in reaching risk management decisions with an equation as follows:

Acceptable Daily Intake (ADI) = NOAEL (or LOAEL)/(UF*MF) (Eq. 1)
NOAEL = No observed adverse effect level
LOAEL = Lowest observed adverse effect level
UF = uncertainty factor
MF = modifying factor

In this equation the NOAELs (or LOAELs) that are derived directly from toxicological studies, may be modified by both an UF and MF. The NOAEL is scaled by a safety factor, conventionally of 100, to account for the differences between test animals and humans (factor of 10) and possible differences in sensitivity between humans (another factor of 10).

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The feature of the formula is that it provides a mechanism for viewing all the data simultaneously, resulting in an integrated profile of a compound's toxicity. In addition, exposure duration-responset rends, providing a possible strategy for estimating acceptable intakes for partial lifetime exposures. The formula using graphic method relies on a simple severity ranking system for data presentation (i.e., NOEL, NOAEL, etc).[31)]

However, the formula has potential methodological limitations. The main ones are that the value is dependent on the dose levels selected in the study, that the value will be higher for studies of low sensitivity, and that data from doses above the NOAEL are used only to define the nature of the hazard. The NOAEL approach is used to establish an intake with negligible risk such as an ADI but cannot be used to estimate the risk associated with intake levels above the ADI.[32)]

False or misleading ADI for scientific and policy guidelines adopted to guide risk assessments will affect the likelihood of under- or overestimation of the health risk.[3)] For example, typical human exposure at 1% of the ADI represents an exposure 10,000 times lower than levels that do not cause toxicity in animals. For most pesticide residues in food consignments, the measurement uncertainty is 50% of the maximum residue limit (MRL), which is fundamentally critical for comparing ADI guidelines.

The presence of statistical errors can lead to inflated error rates and substantial distortions of parameter and statistic estimates.[12)] The impact of exposure misclassification on relative risks using the range of correlation coefficients as the ratio of the between-subjects exposure variance to total variance is assessed in the risk management.[13,30)]

Because of the increasing need to alternative methods for toxicity testing, a variety of computational methods are being proposed for the assessment of toxicology of the pesticides.[5)] Among these methods, the quantitative structure-activity relationship (QSAR) modeling has attracted an increasing attention because of its high predictabilities to approximate ranking of chemical hazards.[6,7)] QSAR method is particularly useful for new substances where data from human or animal substances is limited and which are structurally related to other substances of known toxicological properties.

Linear and non-linear QSAR models are developed and validated with multiple linear regression (MLR), and nonlinear methods namely partial least square (PLS) or artificial neural network (ANN). The genetic algorithm-multiple llinear regression (GA-MLR) method was shown to be more powerful tool than other linear- or nonlinear methods.[23-25)]

QSAR is a widely used method to relate chemical structures to biological responses or properties. Traditional QSAR models on pesticides are built mainly based on lethal concentration 50% (LC50)[8)] or physico-chemical data[9-11)], which are then used to construct a regression model. However, no attempt has yet been made to predict QSAR models utilizing health-based guidance such as ADI due to uncertainty factors. Therefore, a full weight of the evidence including characterization of uncertainty has not yet been finalized in a preliminary human health risk assessment for certain pesticides.[3,4)] An approach using a robust QSAR technique to detect potential sources may provide critical information about uncertainty of ADI values in addition to the model development.

In these respects, the aims of this study were: (1) to validate and predict MLR models based on two sets of ADI values, and (2) to determine whether or not the robust QSAR models can be used in qualitative and quantitative risk assessments.

II. Materials and Methods

1. Data sets

Table 1 shows a data set including names, CASRN (CAS Registry Number), ADI_{EPA}, ADI_{WHO} and ADI_{WHO}/ADI_{EPA} of 74 pesticides which were selected from the literature.[14)]

Seven compounds with the highest ADI_{WHO}/ADI_{EPA} ratio (greater than 10) were selected to compare if the compounds would include the group of outliers obtained from QSAR models or vice versa (Table 2).

2. Optimization and descriptor calculation

By using Hyperchem software 7.0 (Hypercube, Inc., Gainesville, FL, USA), chemical structures were drawn and named by CAS-number. Molecular mechanic force field (MM+) was selected for the geometry optimization using Polak-Ribiere algorithm with a maximum cycle (10000) and a convergence limit of the 0.005 kcal/mol. After optimizing chemical structures from Hyperchem 7 software, Dragon 5.0

Table 1. Names, CASRN, ADI_{EPA}, ADI_{WHO} values and ADI_{WHO}/ADI_{EPA} of 74 pesticides (mg/kg/day)

	Compound	CASRN	ADI_{EPA}	ADI_{WHO}	ADI_{WHO}/ADI_{EPA}
1	Anilazine (Dyrene)	101-05-3	0.0004	0.1	7.5
2	Triforine(Funginex)	26644-46-2	0.025	0.02	3
3	Triadimenfon (Bayleton)	43121-43-3	0.04	0.03	0.25
4	Thiram	137-26-8	0.008	0.01	3.333
5	Thiophanate-methyl	23564-05-8	0.08	0.08	1.587
6	Thiodicarb (Larvin)	59669-26-0	0.03	0.03	4
7	Thiabendazole (+salt)	148-79-8	0.1	0.1	0.8
8	Terbufos	13071-79-9	0.0001	0.0002	1.333
9	Propiconazole (Banner/Tilt)	60207-90-1	0.013	0.04	714.286
10	Propargite (Omite)	2312-35-8	0.04	0.15	0.769
11	Profenofos (Curacron)	41198-08-7	0.0001	0.01	0.714
12	Prochloraz	67747-09-5	0.0075	0.01	5
13	Pirimiphos-methyl	29232-93-7	0.01	0.03	2
14	Phosphamidon	13171-21-6	0.0002	0.0005	1.5
15	Phosmet (Imidan)	732-11-6	0.01	0.02	3.333
16	Phosalone	2310-17-0	0.0025	0.001	1
17	Phorate (Thimet)	298-02-2	0.0005	0.0002	0.8
18	Permethrin	52645-53-1	0.05	0.05	20
19	Pentachloronitrobezene	82-68-8	0.003	0.007	1.25
20	Parathion (Ethyl pararthion)	56-38-2	0.0003	0.005	2.667
21	Oxydemeton-methyl	301-12-2	0.0005	0.0003	1
22	Oxamyl (Vydate)	23135-22-0	0.0002	0.03	0.8
23	Monocrotophos (Azodrin)	6923-22-4	0.0001	0.0006	1.2
24	Mevinphos (Phosdrin)	7786-34-7	0.0003	0.0015	1.667
25	Methyl parathion	298-00-0	0.0003	0.02	1
26	Methoxychlor	72-43-5	0.005	0.1	1
27	Methomyl	16752-77-5	0.008	0.03	20
28	Methiocarb (Mesurol)	2032-65-7	0.005	0.001	0.25
29	Methidathion	950-37-8	0.0015	0.001	0.667
30	Mthamidophos (Monitor)	10265-92-6	0.001	0.004	0.4
31	Metalazyl	57837-19-1	0.074	0.03	0.41
32	Maleic hydrazide	123-33-1	0.25	0.5	2
33	Malathion	121-75-5	0.02	0.02	1
34	Lindane (gamma BHC)	58-89-9	0.0047	0.008	1.786
35	Isofenphos (Amaze)	25311-71-1	0.0005	0.001	4
36	Iprodione (Glycophene)	36734-19-7	0.06	0.2	0.6
37	Imazalil	35554-44-0	0.025	0.03	3
38	Hexythiazox (Savey)	78587-05-0	0.025	0.03	2
39	Folpet	133-07-3	0.009	0.01	5
40	Fenvalerate (Pydrin)	51630-58-1	0.025	0.02	0.6
41	Fenthion	55-38-9	0.0007	0.001	3.846
42	Fensulfothion	115-90-2	0.003	0.003	1.2

Table 1. Names, CASRN, ADI_{EPA}, ADI_{WHO} values and ADI_{WHO}/ADI_{EPA} of 74 pesticides (mg/kg/day)

	Compound	CASRN	ADI_{EPA}	ADI_{WHO}	ADI_{WHO}/ADI_{EPA}
43	Fenitrothion (Sumithion)	122-14-5	0.0013	0.005	0.8
44	Fenamiphos (Nemacur)*	22224-92-6	0.0001	0.0005	1.429
45	Ethoprop (Ethoprophos)	13194-48-4	0.0001	0.0003	1
46	Ethion	563-12-2	0.0005	0.002	0.875
47	Endosulfan	115-29-7	0.006	0.006	0.25
48	Disulfoton	298-04-4	0.0003	0.0003	1.2
49	Diphenylamine	122-39-4	0.03	0.02	1
50	Dimethoate	60-51-5	0.0005	0.01	2
51	Dimethipin (Harvade)	55290-64-7	0.02	0.02	1
52	Difubenzuron (Dimilin)	35367-38-5	0.02	0.02	10
53	Dicofol (Kelthane)	115-32-2	0.0012	0.002	0.405
54	Dicloran (DCNA/Botran)	99-30-9	0.025	0.03	4
55	Dichlorvos (DDVP)	62-73-7	0.005	0.004	0.667
56	Diazinon	333-41-5	0.0001	0.002	0.2
57	Cyromazine (Larvadex)	66215-27-8	0.0075	0.02	3.75
58	Cypermethrin (Ammo)	52315-07-8	0.01	0.05	20
59	Cyfluthrin (Baythroid)	68359-37-5	0.025	0.02	66.667
60	Chlorpyrifos-methyl	5598-13-0	0.01	0.01	100
61	Chlorpyrifos	2921-88-2	0.003	0.01	6
62	Chlorothalonil	1897-45-6	0.02	0.03	1.2
63	Chlorobenzilate	510-15-6	0.01	0.02	0.6
64	Carbophenothion	786-19-6	0.0001	0.0005	1
65	Carbofuran	1563-66-2	0.005	0.01	0.4
66	Carbaryl	63-25-2	0.014	0.01	0.889
67	Captan	133-06-2	0.13	0.1	2
68	Bifenthrin (Talstar)	82657-04-3	0.015	0.02	2.5
69	Bentazon (Basagran)	25057-89-0	0.03	0.1	1.714
70	Bendiocarb	22781-23-3	0.005	0.004	2.857
71	Baygon (Propoxur)	114-26-1	0.005	0.02	100
72	Azinphos-methyl (Guthion)	86-50-0	0.0015	0.005	0.625
73	Aldicarb (Tern ik)	116-06-3	0.001	0.003	1
74	Acephate	30560-19-1	0.004	0.03	1.316

CASRN,

package (Milano Chemometrics and QSAR Research Group, University of Milano-Bicocca, Milan, Italy) was employed for the calculation of the Dragon molecular descriptors.[15,16]

3. Statistical methods using GA-MLR

The MLR models by using genetic algorithms (GA) for variable selection, in comparison with the result obtained by using calculated descriptors and R package, were developed with a training set of 29 compounds using the MobyDigs software (TALETE srl–Milano, Italy). To perform multilinear regression, GA was used to select, from among all the calculated Dragon descriptors, the most relevant in obtaining models that yielded the highest predictive power for the response. Reliability of a QSAR was

Table 2. Seven compounds with the highest ADI_{WHO}/ADI_{EPA} ratio

Compounds	ADI_{EPA}/ADI_{WHO} ratio
1. Propiconazole	714
2. Permethrin	20
3. Methomyl	20
4. Difubenzuron	10
5. Cypermethrin	20
6. Cyfluthrin (Baythroid)	67
7. Baygon (Propoxur)	100

estimated using the leave-one-out cross-validation (LOO_{cv}) method.

Model performance was described by means of parameters related to model predictive capability (Q^2_{cv} and Q^2_{boot}) and fitting power (R^2 and R^2_{adj}). Standard deviation error in prediction (SDEP), prediction sum of squares (PRESS), standardized regression coefficient (SRC), standard error of estimate (s), the F value of the Fisher's exact test, the inter-correlation of the selected descriptors (K_{XX}) and the correlation of the X block with response (K_{XY}) were also calculated. The Hat value was the measure of leverage, to verify the structural applicability domain. Influential compounds (influential points) were those with a leverage greater than the critical value (warning leverage) h*=3p'/n, where p' is the number of model variables plus one, and n the number of objects used to calculate the model. If a chemical has a hat value greater than the warning leverage (h*), it means that the chemical greatly influences the regression line, and therefore may be unreliable. The QUIK (Q under influence of K) rule was used to discard models with high predictor collinearity which might lead to chance correlation.[17]

4. External validation

For relatively small data sets, internal validation of prediction models by bootstrap techniques may not be sufficient and indicative for the model's performance in future patients. External validation is then essential before implementing prediction models.. We randomly split into a training (70%) and a test (30%) set out of 74 pesticides, respectively. QSAR models were developed using only chemicals in the training set. Results were then validated using the test set.

III. Results

1. QSAR model using GA-MLR

MobyDigs software was used to select descriptors and build QSAR models, as described in the Method section. Table 3 contains the list of descriptors used and outliers removed. Descriptors and outliers of a 5-descriptor MLR model. As was presented in Table 3, $logADI_{WHO}$ model had MATS2e and GATS2e as 2D orbital energy descriptors, JGI6 to evaluate the charge transfer between a pair of atoms, HATS6u and H051 while $logADI_{EPA}$ contains np, X5, MATS8m, JGI6 and MLOGP2 as a descriptor of hydrophobicity. The MATS2e and GATS2e are related to the atomic electronegativities of a molecule.[18] MATS8m belongs to 2D Moran autocorrelations of lag 8 / weighted by atomic masses.

2. Statistics on GA-MLR model

The real usefulness of QSAR models is not just their ability to reproduce known data, verified by their fitting power (R^2). For leave one out (LOO) cross-validation, a data point is removed from the set and the model is recalculated. The predicted activity for that point is then compared to its actual value to get Q^2_{LOO}. This is repeated until each data

Table 3. Selectided molecular descriptors and outliers of a 5-descriptor MLR model

Dependent variable	$LogADI_{EPA}$	$LogADI_{WHO}$
5 Descriptors selected	np, X5, MATS8m, JGI6, MLOGP2	MATS2e, GATS2e, JGI2. HATS6u, H051
Influential point or Outliers	Dicofol (No 14)	

nP, number of phosphorous atoms (Constitutional indices); X5, connectivity index of order 5 (Connectivity indices); MATS8m, Moran autocorrelation - lag 8 / weighted by atomic masses; JGI6- mean topological charge index of order6; MLOGP2, squared Moriguchi octanol-water partition coeff; MATS2e: Moran autocorrelation – lag 2 / weighted by atomic Sanderson electronegativities; GATS2e : Geary autocorrelation - lag 2 / weighted by atomic Sanderson electronegativities; JGI2, mean topological charge index of order 2; HATS6u. leverage-weighted autocorrelation of lag 6 / unweighted (Getaway descriptor); H-051: number of H attached to α-C

Table 4. Statistical results of internal and external validation.

No of descriptor	Descriptors	n	R^2	Q^2_{LOO}	Q^2_{boot}	R^2_{adj}	SDEP	K_{xx}	K_{xy}	F	SE	PRESS
$LogADI_{EPA}$												
3	nP MATS7m MATS7v	74	62.40	57.74	57.29	60.74	0.579	14.28	35.14	37.62	0.56	24.11
4	nP X5 JGI6 MLOGP2	74	70.19	65.93	64.9	68.41	0.52	36.59	42.36	39.44	0.5	19.44
5	nP X5 MATS8m JGI6 MLOGP2	74	76.96	72.50	71.49	75.22	0.467	32.78	38.38	44.1	0.45	15.69
External*	Five descriptors		70.22	63.48	64.2	69.41	0.484	30.02	31.62	26.72	0.46	11.69
$LogADI_{WHO}$												
3	X0Av nArOCON nPO4	74	57.49	52.16	51.74	55.59	0.53	32.04	42.97	30.21	0.51	19.92
4	MATS2e GATS2e JGI2 H-051	74	61.86	56.94	56.15	59.55	0.50	27.1	27.27	26.77	0.49	17.93
5	MATS2e GATS2e JGI2 HATS6u H-051	74	66.43	60.67	58.86	63.85	0.50	26.71	27.38	25.73	0.46	16.38
External*	Five descriptors		70.87	65.28	66.24	69.06	0.42	22.44	34.63	27.03	0.41	8.6

*External validation with five descriptors for training set (70% training set; 30% external set)

point has been omitted once. The internal predictive ability of the model was also verified using the bootstrap Q^2_{BOOT} procedure, as is strongly recommended for QSAR modeling. The robustness of the proposed model and its predictive ability was guaranteed by the high value of Q^2_{BOOT} based on the bootstrapping being repeated 5000 times. Q^2_{BOOT} values in the models were seen to have similar vales to Q^2_{LOO}. The cross-validation parameters are shown in Table 4. The cross-validation results confirmed that the obtained regression model has a good internal and external predictive power.

According to the QUIK rule, global correlations of the [X + y] block (K_{XY}) of the constructed QSAR models were greater than those of the global correlation of the X block (K_{XX}) variables (X being the molecular descriptors and y the response variable) and were considered acceptable. The predictive model with three- to five descriptors for internal and external validation distinctively had K_{XY} greater than multivariate correlation K_{XX} to fulfill the QUIK rule (K_{XX}=32.78, K_{XY}=38.38 for $logADI_{EPA}$; K_{XX}=26.71, K_{XY}=27.38 for $logADI_{EPA}$).

Also, The 5-descriptor QSAR of $logADI_{WHO}$ obtained by external validation also showed a far greater difference (ΔK) than $logADI_{WHO}$ model [(K_{XX}=32.78, K_{XY}=38.38 for $logADI_{EPA}$; K_{XX}=26.71, K_{XY}=27.38 for $logADI_{EPA}$)] for internal, and (K_{XX}= 22.44, K_{XY}=34.63, ΔK=12.19 for $logADI_{WHO}$; K_{XX}= 30.02, K_{XY}=31.62, ΔK=1.60 for $logADI_{EPA}$) for external].

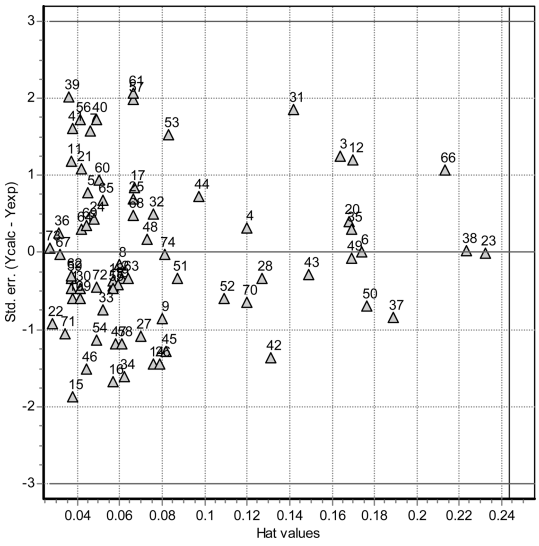

Fig. 1. Williams plot of standardized residuals (y-axis) *versus* leverages (hat values; x axis) for $logADI_{WHO}$.

3. Domain of applicability

The domain of applicability was verified by the

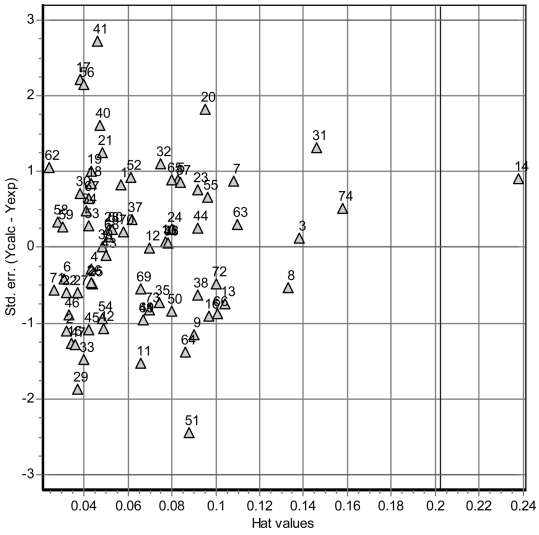

Fig. 2. Williams plot of standardized residuals (y-axis) *versus* leverages (hat values; x axis) for $logADI_{EPA}$.

leverage approach [a, b] and both the influential and the outlier chemicals were identified by the Williams plot (Figs.1 and 2).

The applicability domain was established for Model 2, determining the leverage values for each compound. Figs. 1 and 2 show the Williams plot; i.e. plot of standardized residuals (y-axis) versus leverages (x-axis) for each compound of the training set. From this plot, the applicability domain is established inside a squared area within ±2 standard deviations and a leverage threshold h* (h*=3p´/n, being p´ the number of model parameters and n the number of compounds). As seen in Fig. 1 for $logADI_{WHO}$ model, all the 74 compounds except no. 61 (weak outlier) are inside of this area (h*=0.243). Whereas, an influential chemical with leverage values greater than 3p/n (h*=0.202) within ±2 was identified in the $logADI_{EPA}$ model (Fig. 2) while four weak outliers (compound 17, 41, 51 and 56) were isolated between ±2 and ±3. We did not attempt further to delete weak outliers or influential data points to compare.

4. External validation

We performed an external validation of the proposed model to verify its performances on an independent population and obtained interesting results. Models 6 and 7 were considered for external validation. For this purpose, the whole set of 74 compounds was randomly splitted into a test set (30%) and a training set (70%). A training set composed of 52 compounds and test set comprised of 22 compounds for the $logADI_{EPA}$ and $logADI_{WHO}$, respectively. The same set of descriptors as used for external validation were used to frame a new

Table 5. Regression coefficients of final 5-descriptor QSAR models for $logADI_{EPA}$ and $logADI_{WHO}$

	$LogADI_{EPA}$			
	Regression Coeff.	Errors Reg.Coeff.	Conf.Intervals[a] (.95)	Std. Reg.Coeff.
Variable Intercept	1.985	0.193	0.386	-
X5	-0.302	0.079	0.157	-0.343
nP	0.720	0.144	0.288	0.433
JGI6	34.383	7.359	14.719	0.375
MATS8m	0.234	0.047	0.094	0.886
MLOGP2	-2.010	0.390	0.780	-0.939
	$LogADI_{WHO}$			
Variable Intercept	0.336	0.507	1.015	-
MATS2e	2.794	0.777	1.554	0.345
GATS2e	0.739	0.207	0.414	0.269
JGI2	0.59	0.184	0.367	0.259
HATS6u	0.633	0.132	0.264	0.424
H-051	0.341	0.128	0.256	0.197

a: minimum (low) value

equation for the test set (70% training set) to conclude if it still gives significant statisticals results. The statistical parameters obtained were R^2_{EXT}=70.2; Q^2_{EXT}=63.5; and F_{EXT}=26.72 for the $logADI_{EPA}$ test set, while R^2_{EXT}=70.8; Q^2_{EXT}=65.3; and F_{EXT}=27.23 for the $logADI_{EPA}$ test set (Table 4).

5. The final predictive QSAR model

Acceptability of the regression model was judged by examining cross-validated squared correlation coefficient (Q^2_{LOO}), squared correlation coefficient (R^2), fisher's value (F) and standard error. Performing multiple linear regression analysis results They are presented in Table 5. Best correlations with logADI and statistical results were noticed in 5-descriptor models. The predictive power of the best MLR model was then checked by the criteria. All these calculated criteria indicated a model with predictive power, respectively: $Q^2_{EXT} > 0.5$, $R^2 > 0.6$. The Δk (K_{XY}-K_{XX}) was greater than 5 in all models. According to the stdardized regression coefficient, MATS8m and HATS6u were most positively affecting variables in $logADI_{EPA}$ and $logADI_{WHO}$, respectively.

Scatter plots between experimental versus predicted $logADI_{EPA}$ and $logADI_{WHO}$ were presented in Figs. 1 and 2.

IV. Discussion

The GA-MLR for descriptor selection proved to be very efficient in generating QSAR models with a good predictive power, as indicated by the LOO cross-validation and external-validation statistics. The best set of the calculated descriptors was selected with the genetic algorithm. The statistical parameters of the built QSAR models were satisfactory, illustrating the high quality of the chosen descriptors.

In this work, five descriptors were selected including nP, X5, MATS8m, JGI6 and MLOGP2 (which have been presented for the $logADI_{EPA}$ values prediction, and including MATS2e, GATS2e, JGI2, HATS6u and H-051 for the $logADI_{WHO}$ (Table 3). No matching of selected molecular descriptors was found between $logADI_{EPA}$ and $logADI_{WHO}$ models. The finding seemed to imply that the QSAR models were built with a combination of specific molecular descriptors and UF and MF factors. Scientific judgment is required to determine the appropriate value to use for any given UF, MF and sources of error inherent in quantitative risk assessment for validation of QSAR models in the context of chemical regulation.[29)]

Table 4 shows the necessary statistics, including F-ratio, R^2, Q^2_{LOO}, and PRESS for $LogADI_{EPA}$ and $LogADI_{WHO}$. In general, a QSAR model is acceptable when it has an R^2 value greater than 0.6 (60%) and R^2_{LOO} greater than 0.5. High correlation coefficients (R^2=76.96 for $LogADI_{EPA}$ and R^2=66.43 for $LogADI_{WHO}$, respectively). Q^2_{LOO} value of 72.50 and 60.67 exhibited a good internal predictive power of two developed models, indicating that the model had high precision. The values of Q^2_{boot} for $logADI_{WHO}$ and $logADI_{WHO}$ were fairly close to Q^2_{LOO} confirming the internal predictability and stability of the model. The difference between R^2 and Q^2_{LOO} is not large (Table 4). In view of these observations, we conclude that the final QSAR model of equation is fairly robust.

As a general trend, both F and R^2_{LOO} increase with the number of descriptors indicating a significant increase in the predictive power of the QSAR models. Smaller the value of PRESS statistics indicates better prediction. High F values indicate that the model is statistically significant. It was also found that the R^2 and Q^2_{LOO} obviously increased when the model size increased from 3 descriptor term to 5 descriptor terms while the PRESS values decreased with increased R^2. The fact indicated that these QSAR models are of high stability and significance, namely higher predictability and correlation. The regression analysis of 5-descriptor regression model was clearly evidenced by the high correlation coefficients were obtained as 76.96 (R^2) and 72.50 (Q^2_{LOO}), and 66.43 (R^2) and 60.67 (Q^2_{LOO}), respectively, indicating that the QSAR models possess good internal consistency. The result by the QUIK rule indicated that logADIEPA model is a better robust model.

The negative sign of the corresponding regression coefficient between $logADI_{EPA}$ and X5 and MLOGP2 indicates the $logADI_{EPA}$ increases with the increase of five descriptors values (Table 5). The positive sign of the corresponding regression coefficient indicates the $logADI_{EPA}$ value increased with the value increase of the three remaining descriptors (nP, X5, JGI6), while $logADI_{WHO}$ was proportional to MATS2e, GATS2e,

JGI2, HATS6u and H-051. The contributions of each descriptor by standardized regression coefficients in the MLR models were determined, and are provided in Table 5. The standardized regression coefficients indicated that MLOGP2 (=-0.939) and MATS8m (=0.886) affected significantly higher than other descriptors for logADIEPA, while HATS6u and MATS2e were significant descriptors that affected logADIWHO model.

Additionally, a penta-parametric linear model of logADI has much better statistics than tri-and tetra-parametric models for external validation. For the majority of compounds, the residuals are small, showing that the penta-parametric model has a fairly good statistical quality. External validation for the set (70 training, 30% test) was accessed by Q^2_{EXT} (Table 4). The value of Q^2_{EXT} is 63.48, which is smaller than Q^2_{LOO} (=72.50%). The Q^2_{EXT} is acceptable because the difference is small. In the same manner, a penta-parametric model was shown to be superior to tri- and tetra-parametric models.

Moreover, the applicability domain of the developed model was assessed and visualized by the Williams plot (Figs. 1 and 2). All 74 compounds included test sets in the applicable domain. A number of statistical approaches to account for the applicability domain have been described.[19,20] For both the training set and test set, the suggested model matches the high quality parameters with good fitting power and the capability of assessing external data. Furthermore, almost all of the compounds was within the applicability domain of the proposed model and were evaluated correctly. The no 14 chemical (Phosphamidon) was seen as a weak outlier outside leverage (Fig. 2), but decided not to exclude it.

The highest ratio was calculated using a 10-fold uncertainty factor which accounts for the uncertainty in extrapolating from a lowest-observed-adverse-effect levels (LOAELs), and chose the chemicals as a group with highest variability in endpoints. The selected seven chemicals with highest ratio, however, did not coincide with those observed in a outlier list. (Tables 2 and 3). It suggests that each data set should be used or separately for comparative study. The ratio, ADI_{WHO}/ADI_{EPA}, alone does not seem to explain the differences among 74 compounds, possibly leading to false or misleading interpretation, Identification of influential outliers derived from the QSAR model. Furthermore, the

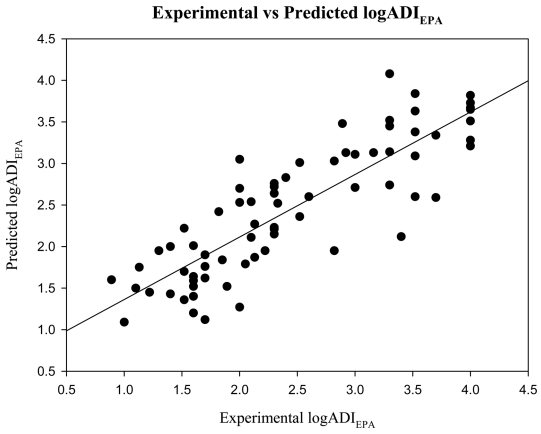

Fig. 3. A scatter plot between experimental and predicted $logADI_{EPA}$.

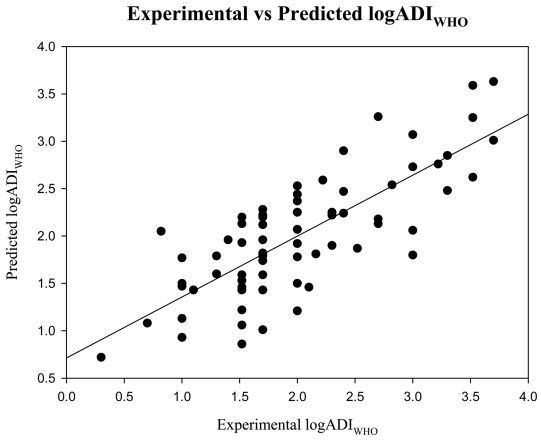

Fig. 4. A scatter plot between experimental and predicted $logADI_{WHO}$.

transformation of ADI_{WHO}/ADI_{EPA} as logRatio may be useful to derive a logarithmic regression model equation to represent the data In future study.

Figs. 3 and 4 show the plots of linear regression predicted *versus* experimental values of the $logADI_{EPA}$ and $logADI_{WHO}$ of 74 compounds. The closer the regression line comes to all the points on the scatter plot the better it is. Comparing two plots, logADIEPA showed smaller residual variations among data points than $logADI_{WHO}$. The dindings seem to support that USEPA focuses on more subtle endpoints than WHO even though there are many other factors in scientific judgement.[14] Clear differences are apparent in risk values set for the

same chemicals by the two organizations. Only 6 out of 38 (16%) of WHO values are lower than the EPA values. For noncarcinogens only 20 out of 74 (27%) WHO values are lower – more stringent – than those of EPA.[14)]

The results obtained in this study demonstrated that the simple linear quantitative structure-wavelength relationship model was robust and satisfactory. Additionally, higher levels of exposure or exposures to multiple pesticides may result in additional health effects.

V. Conclusions

From the results, it is concluded that: (1) Robust 5-descriptor QSAR prediction models for the ADIs of pesticides were constructed by using the MLR, (2) The predictive QSAR models provided useful information about molecular characteristics of 74 pesticides on the ADIs, and (3) EPA data gave more accurate and reliable QSAR models, with higher predictive ability, than WHO models, by the statistical validation. Seven compounds with the highest ADI_{WHO}/ADI_{EPA} ratio did not consistent with outliers or influential points isolated from the training set. The QSAR method provided an applicability of QSAR analysis to the evaluation of the ADIs of pesticides for a health risk assessment.

Regulatory decisions can leads to under- or overestimation of the actual risks for the least toxic pesticides for a specific subpopulation group. The collection of additional information of pesticides for scientific judgement is required to be updated or reassessed to meet the current scientific and regulatory standards and guidelines. The following specific issues need to be addressed for better assessment of ADI: (1) construction of requisite database, (2) establishment of assessment methodology based on existing data and the best available scientific knowledge, and (3) conducting basic research to reduce scientific uncertainties including gaps in the data and evidence base.[26,27)]

This QSAR approach was proven to useful to estimate endpoints or reference values in the risk evaluation process, given the high expense of monitoring pesticides.[21,22,28)] Very careful attention should also be paid to assessments of the toxicological, physiological and pathological parameters before rules are issued.

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