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Prediction of Acute Toxicity of Different Phthalate as Plasticizer on *Daphnia magna* as per Simulated Molecular Descriptors: An *in Silico* Approach

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Abstract: ***An organic compound, dimethyl phthalate (DMP) and diethyl phthalate (DEP) are well-known plasticizers used in plastic materials. The prediction of acute toxicity in daphnid (Daphnia magna) through in silico approach is potential examination because daphnids are fish food. The objective of the present study was to predict acute toxicity (median lethal concentration or LC_{50} at 48hrs exposure) of DMP and DEP on daphnids related to molecular descriptors by using toxicity estimation software tool (T.E.S.T.). The predicted LC_{50} (48 hrs) values of daphnid (D. magna) for DMP and DEP were predicted to be about 3.85 and 4.06 -Log10(mol/L) as per ≥0.5 (0.40 and 0.43) of mean absolute error (MAE) of 0.50. The regression curve represented on DMP and DEP predicted as per model equation in which Pearson's correlation coefficient (R^2) values were obtained 97% and 94%, respectively, which are significantly higher for these toxicity predictions. the prediction of toxicity of DMP and DEP in daphnids (Daphnia magna) for these compounds were easily predicted after simulation of different molecular descriptors within the T.E.S.T. software.***

Keywords: Acute toxicity prediction, Diethyl phthalate, Dimethyl phthalate, Daphnid toxicity prediction, *In silico* study, Plasticizer, T.E.S.T

1. Introduction

Generally, biological activity can be expressed quantitatively as the concentration of a chemical substance required to provide a certain biological response. The mathematical expression, if carefully validated can then be utilized to predict the modeled response of other chemical structures.[1]

At present, quantitative structure–activity relationship (QSAR) modeling is one of the basic tools of modern drug design and environmental sciences, especially toxicity prediction. [2,3] Models have developed into robust and reliable systems, at the same time, they became highly complex and non-interpretable: so-called "black boxes".

Conventionally, the mission of establishing toxicity of chemicals has been accomplished as per *in vivo* models, where a test model (organism) is exposed to a chemical compound for obtaining the toxic effects. But the approaches required animal harming, costly and time consuming,[4] and when required the hundreds of thousands of compounds related to toxicological screening, innovative alternatives are obtained for faster screening of chemicals. In recent decades, for predictive toxicity screening with large-scale chemicals, efforts have emerged by using QSAR modelling inbuilt in many tools.[2,5] The tool is called Toxicity estimation software tool (T.E.S.T), which helps toxicity screening of chemicals in different test models. [6,7]

Among several chemical compounds, Phthalates with a longer side chain are referred to as high molecular weight phthalates (HMWP), which are fat-soluble, and are used industrially as part of polyvenyl chloride (PVC), which may contain 50-80% phthalates by weight. [8,9] Several phthalates have already been established in which dimethyl phthalate (DMP), diethyl phthalate (DEP) and other derivatives.[9] Some investigators observed toxicity in animal studies. [10-12]

In the present study, it was attempted to predict acute toxicity (median lethal concentration or LC_{50} at 48hrs exposure) of dimethyl and diethyl phthalate on daphnid (*Daphnia magna*) related to molecular descriptors by using toxicity estimation software tool (T.E.S.T.).

2. Materials and methods

As per literature, the dimethyl phthalate (DMP) and diethyl phthalate (DEP) were selected (Fig 1a and b).[10,13,14] In this *in silico* study, the test model commonly called as daphnids (*Daphnia magna*) were selected as per the tool. The tool used as toxicity estimation software tool (T.E.S.T.) as per Martin.[6] For toxicity prediction, QSAR modelling was performed by using T.E.S.T (version, 5.1.1) for predicting LC_{50} value of studied daphnids as per different molecular descriptors.

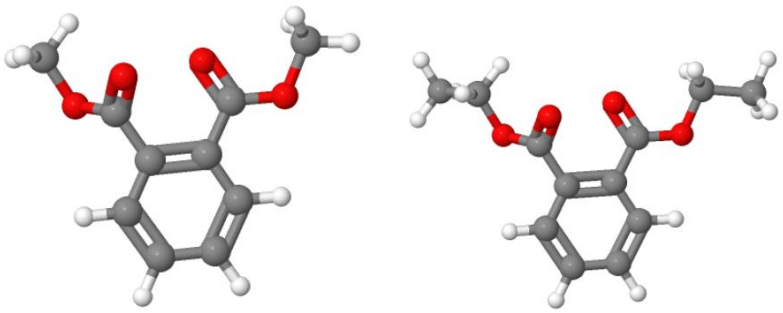


Figure 1: Three-dimensional structure of DMP and DEP

3. Results

Table 1 evaluates the prediction results of toxicity i.e., LC_{50} value as 3.85 -Log10(mol/L) and 4.06 -Log10(mol/L) for DMP and DEP as per consensus method.

Table 1: LC_{50} value of daphnids of DMP and DEP as per consensus method

Compounds	Endpoint	Predicted value
DMP	*Daphnia magna* LC_{50} (48 hr) -Log10(mol/L)	3.85
DEP	*Daphnia magna* LC_{50} (48 hr) -Log10(mol/L)	4.06

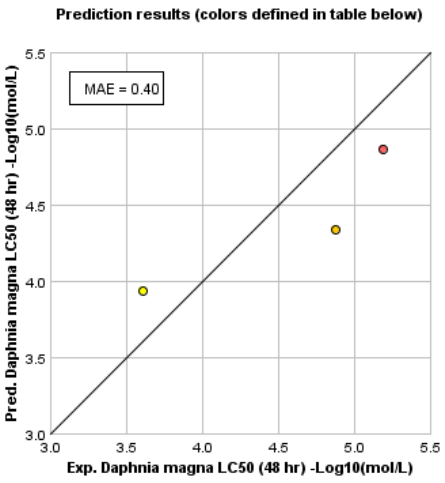
Table 2 evaluates the consensus method is the mean of different methods viz. Hierarchical clustering (3.86), Single model (3.55), Group contribution (3.43) and nearest neighbour (4.56), respectively for DMP and Hierarchical clustering (3.82), Single model (4.18), Group contribution (3.63) and nearest neighbour (4.61), respectively for DEP.

Table 2: LC_{50} value of daphnids of DMP and DEP based on different models

Methods	DMP	DEP
	Predicted value-Log10(mol/L)	Predicted value -Log10(mol/L)
Hierarchical clustering	3.86	3.82
Single model	3.55	4.18
Group contribution	3.43	3.63
Nearest neighbour	4.56	4.61

In Fig 2 and 3, the regression curve represented on DMP and DEP predicted with similar compound like Dibutyl 1,2-benzenedicarboxylate as per similarity coefficient ≥0.5 (0.40 and 0.43) of mean absolute error (MAE) of 0.50.

In Fig 4 and 5, the regression curve represented on DMP and DEP predicted as per model equation in which Pearson's correlation coefficient (R^2) values were obtained 97% and 94%, respectively, which are significantly higher for these toxicity predictions.



Results for entire set vs results for similar chemicals

Chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.40

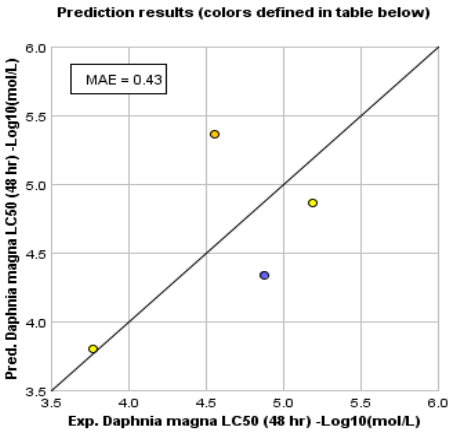
*Mean absolute error in -Log10(mol/L)

Color legend

Color	Range*
Green	SC ≥ 0.9
Blue	0.8 ≤ SC < 0.9
Yellow	0.7 ≤ SC < 0.8
Orange	0.6 ≤ SC < 0.7
Red	0.6 < SC

*SC = similarity coefficient

Figure 2: Regression curve represented on DMP predicted with similar compound like Dibutyl 1,2-benzenedicarboxylate as per SC value



Results for entire set vs results for similar chemicals

Chemicals	MAE*
Entire set	0.50
Similarity coefficient ≥ 0.5	0.43

*Mean absolute error in -Log10(mol/L)

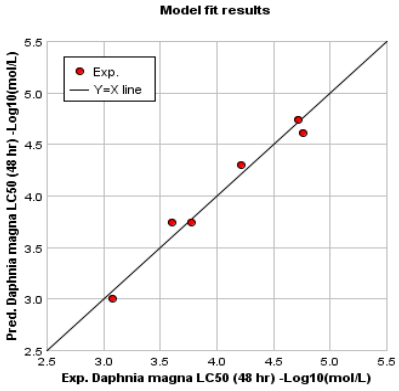
Color legend

Color	Range*
Green	SC ≥ 0.9
Blue	0.8 ≤ SC < 0.9
Yellow	0.7 ≤ SC < 0.8
Orange	0.6 ≤ SC < 0.7
Red	0.6 < SC

*SC = similarity coefficient

Figure 3: Regression curve represented on DEP predicted with similar compound like Dibutyl 1,2-benzenedicarboxylate as per SC value

Parameter	Value
Endpoint	Daphnia magna LC_{50} (48 hr)
r^2	0.975
q^2	0.938
Number of chemicals	6
Model	691



Model coefficients

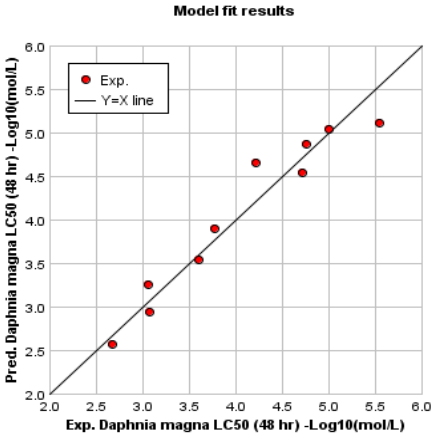
Coefficient	Definition	Value	Uncertainty*
GATS3p	Geary autocorrelation - lag 3 / weighted by atomic polarizabilities	-3.7462	0.6418
Model intercept	Intercept of multilinear regression model	7.9993	0.6887

* value for 90% confidence interval

Model equation:
Daphnia magna LC50 (48 hr) = -3.7462×(GATS3p) + 7.9993

Figure 4: Regression curve represented on DMP predicted as per model equation

Parameter	Value
Endpoint	Daphnia magna LC_{50} (48 hr)
r^2	0.938
q^2	0.870
Number of chemicals	10
Model	740



Model coefficients

Coefficient	Definition	Value	Uncertainty*
SssCH2	Sum of (– CH2 –) E-States (SssCH2)	-0.9978	0.2570
MATS1m	Moran autocorrelation - lag 1 / weighted by atomic masses	5.7035	1.3923
Model intercept	Intercept of multilinear regression model	5.0365	0.2441

* value for 90% confidence interval

Model equation:
Daphnia magna LC50 (48 hr) = -0.9978×(SssCH2) + 5.7035×(MATS1m) + 5.0365

Figure 5: Regression curve represented on DEP predicted as per model equation

4. Discussion

Phthalate compounds are well-known plasticizers, which pose adverse health impacts among aquatic biota and their surrounding environment. [11,12,15-21]

Recently, Lotfi et al. [22] conducted an *in silico* study of various chemicals by using quantitative structure-toxicity relationship (QSTR) models, which have been developed to predict the toxicity of a large dataset comprising 2106 chemicals toward *Daphnia magna*. They obtained R^2 values ranging from 0.9467-0.9607, which is similar to our study as per T.E.S.T. prediction.

It was recorded that phthalate compound at a concentration as low as 3.4 mg/L has been observed to cause detrimental effects in aquatic organisms, especially in *Daphnia magna*.[23]

5. Conclusion

It is concluded that the prediction of toxicity of DMP and DEP in daphnids (*Daphnia magna*) for these compounds were easily predicted after simulation of different molecular descriptors within the T.E.S.T. software. The toxicity screening is an important method for any chemical compound for the prevention of environment and adverse impacts in organisms. It is suggested that the prediction of other phthalate compounds on *D. magna* and another aquatic organisms like fish should be conducted in future.

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Conflict of interest
Authors declare no conflict of interest.

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References

[1] Tropsha A. Best practices for QSAR model development, validation, and exploitation. Molecular Informatics. 2010; 29:476-488.
[2] Roncaglioni A, Toropov AA, Toropova AP, Benfenati E. In silico methods to predict drug toxicity. Current Opinion in Pharmacology. 2013;13(5): 802-806.
[3] Romano JD, Hao Y, Moore JH. Improving QSAR Modeling for Predictive Toxicology using Publicly Aggregated Semantic Graph Data and Graph Neural Networks. Pac Symp Biocomput. 2022; 27:187-198.
[4] Raies AB, Bajic VB. In silico toxicology: computational methods for the prediction of chemical toxicity. Wiley interdisciplinary reviews. Computational Molecular Science. 2016; 6(2):147-172.
[5] Tice RR, Austin CP, Kavlock RJ, Bucher JR. Improving the human hazard characterization of chemicals: a Tox21 update. Environ Health Perspect. 2013 Jul;121(7):756-65. Roncaglioni et al., 2013).
[6] Martin, T. M. (2012). User's guide for T.E.S.T. Centre for Computational Toxicology and Exposure, Cincinnati, OH.
[7] Talapatra SN, Misra D, Banerjee K, Banerjee P, Swarnakar S. QSAR modeling for acute toxicity prediction of fluroquinolone antibiotics by using software. International Journal of Advanced Research. 2015;3(6):225-240.
[8] Wang Y, Zhu H, Kannan K. A review of biomonitoring of phthalate exposures. Toxics. 2019;7(2):21.
[9] Hlisníková H, Petrovičová I, Kolena B, Šidlovská M, Sirotkin A. Effects and Mechanisms of Phthalates' Action on Reproductive Processes and Reproductive Health: A Literature Review. Int J Environ Res Public Health. 2020;17(18):6811.
[10] Zhang Y, Jiao Y, Li Z, Tao Y, Yang Y. Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. Sci Total Environ. 2021;771:145418.
[11] Oliveira Pereira EA, Labine LM, Kleywegt S, Jobst KJ, Simpson AJ, Myrna J. Simpson MJ. *Daphnia magna* sub-lethal exposure to phthalate pollutants elicits disruptions in amino acid and energy metabolism. Aquatic Toxicology. 2023; 257:106432.
[12] Kang X, Wei J, Wang Y. Toxic effect of dimethyl phthalate on *Daphnia magna* through immune related enzymes activities. Fifth International Conference on Green Energy, Environment, and Sustainable Development (GEESD 2024), edited by Aghaei M, Zhang X, Ren H, Proc. of SPIE Vol. 13279, 132791D, 2024.
[13] Dutta S, Haggerty DK, Rappolee DA, Ruden DM. Phthalate exposure and long-term epigenomic consequences: A review. Front Genet. 2020;11:405.
[14] Wang Y, Qian H. Phthalates and their impacts on human health. Healthcare (Basel, Switzerland). 2021;9(5):603.
[15] Liu X, Shi J, Bo T, Zhang H, Wu W, Chen Q, Zhan X. Occurrence of phthalic acid esters in source waters: a nationwide survey in China during the period of 2009-2012. Environ Pollut. 2014;184:262-70.
[16] Gao X, Li J, Wang X, Zhou J, Fan B, Li W, Liu Z. Exposure and ecological risk of phthalate esters in the Taihu Lake basin, China. Ecotoxicol Environ Saf. 2019;171:564-570.
[17] Kotowska U, Kapelewska J, Sawczuk R. Occurrence, removal, and environmental risk of phthalates in wastewaters, landfill leachates, and groundwater in Poland. Environ Pollut. 2020 Dec;267:115643.
[18] Ai S, Gao X, Wang X, Li J, Fan B, Zhao S, Liu Z. Exposure and tiered ecological risk assessment of phthalate esters in the surface water of Poyang Lake, China. Chemosphere. 2021;262:127864.
[19] Sun C, Chen L, Zhao S, Guo W, Luo Y, Wang L, Tang L, Li F, Zhang J. Seasonal distribution and ecological risk of phthalate esters in surface water and marine organisms of the Bohai Sea. Mar Pollut Bull. 2021 Aug;169:112449.
[20] Guo W, Li J, Luo M, Mao Y, Yu X, Elskens M, Baeyens W, Gao Y. Estrogenic activity and ecological risk of steroids, bisphenol A and phthalates after secondary and tertiary sewage treatment processes. Water Res. 2022;214:118189.
[21] Huan-yu T, Jiawei Z, Bin L, Hui G, Mengtao Z, Wei G, Jianghong S, Xiao-yan L. Development of ecological risk assessment for Diisobutyl phthalate and di-n-octyl

phthalate in surface water of China based on species sensitivity distribution model. Chemosphere. 2022;307(3):135932.

[22] Lotfi S, Ahmadi S, Azimi A, Kumar P. *In silico* aquatic toxicity prediction of chemicals toward *Daphnia magna* and fathead minnow using Monte Carlo approaches. Toxicol Mech Methods. 2025;35(3):305-317.

[23] Slesha Sudharsan KJ, Sakthi DS, Kishore Kumar MS, Martin TM. Evaluating the combined toxicity of chlorhexidine and dibutyl phthalate on *Daphnia magna*: Implications for aquatic ecosystem safety and environmental risk management. Journal of Pioneering Medical Sciences. 2025;14(S1):293-301.