BioMed Research Journal
BMRJ, 2(1): 18-27
www.scitcentral.com

Original Research: Open Access

Human Skin Sensitizing Properties, Mutagenicity and Blood-Brain Barrier Penetration of Organotin Compounds Using *in silico* Approaches

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Received December 18, 2017; Accepted January 26, 2018; Published April 17, 2018

ABSTRACT

Tributyltins belong to the trialkyl organotin compound group which is widely used as plastic stabilizers and biocides. Several unwanted organism groups are controlled by these biocides. We investigated here 32 TBT compounds for their toxic effects on humans. As for skin sensitization Toxtree predicts that none of the TBTs tested is causing human skin irritation or skin corrosion. All TBT compounds can penetrate the blood-brain barrier as predicted by the LAZAR web server tool. TBTBe is a non-sensitizer according to the activation test of human cell lines. But, it is a human skin sensitizer according to the *in silico* Keratino Sens™ tool. TBTG and TBTPh both are non-sensitizers to human skin based on the Pred-Skin *in silico* assessment. The pKCSM tool (Keratino Sens TM model from Pred-Skin web server) predicts TBT, TBT2E, TBTA, TBTAc, TBTAz, TBTBr, TBTCA, TBTCl, TBTCl-d27, TBTCN, TBTEO, TBTF, TBTH, TBTI, TBTIA, TBTIC, TBTIPS, TBTIt, TBTL, TBTMc, TBTMO and TBTPr as skin sensitizers. Other TBTs are non-skin sensitizers. TBTAz, TBTIC, TBTCA, TBTPAZ are predicted as mutagens; other TBTs studied here as non-mutagens. Our results show that *in silico* approaches can provide a fast, reliable, and economical way to explore the toxicological effects of emerging contaminants like tributyl compounds by chemo informatic tools.

Keywords: Tributyltin, Computational chemistry, Computational biology, Human toxicity, Skin sensitization, Mutagenicity, Blood-brain barrier, SMILES.

Abbreviations: ADMET: Absorption, Distribution, Metabolism, Excretion, Toxicity; BBB, Blood-brain Barrier; LAZAR: Lazy Structure-activity Relationships; LLNA: Murine Local Lymph Node Assay; QSAR: Quantitative Structure-activity Relationship; SMILES: Simplified Molecular Input Line Entry System; TBT: Tributyltin.

INTRODUCTION

The principal use of organotins or Tributyltin compounds (TBTs) is primarily as stabilizers in the manufacturing of plastics. In addition are TBTs used as biocides, such as insecticides, fungicides, and bactericides. This way they preserve electrical equipment, leather, textiles, wood, and paper. Underwater structures, such as pipelines or ship hulls are typically prevented from fouling (settlement of macroorganisms) and corrosion by TBT oxides or methacrylate [1]. TBTs are also used as biocides in cooling systems [2].

Generally, TBT compounds are moderately toxic to rodents and humans. Although its extent is not known, human skin is

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Citation: Arulanandam C D, Madar I H, Ponnusamy V K, Rathinam A J & Dahms H-U. (2018) Human Skin Sensitizing Properties, Mutagenicity and Blood-Brain Barrier Penetration of Organotin Compounds Using in silico Approaches. Biomed Res J, 2(1): 18-27.

sensitive to TBTO. Inhalation of TBTs may cause headache, incoordination, and weakness, and may interfere with breathing [3-4].

For the assessment of chemicals, computational methods such as the quantitative structure-activity relationship (QSAR) models is increasingly required or encouraged, in order to increase the reliability and efficiency of risk assessments for environmental and human health and to replace and minimize the reliance on animal testing [5].

Chemically induced skin sensitization that can be induced by chemicals can substantially affect the working ability and the quality of life. Due to high expenditures for experimental testing, there is some necessity to develop alternative ways of toxicity testing that can replace the costly and time-consuming ones. These include computational approaches that use computer software to evaluate toxicological end points such as mutagenicity [6], blood-brain barrier penetrability, and skin sensitization.

In the present study, we examined TBT compound toxicity by *in silico* tools such as toxtree, lazar Toxicity Predictions; Pred-Skin[7], and pKCSM. The objectives of this study were to screen for toxicological effects of TBTs on human skin and other toxicological end points in a reliable and economic way.

MATERIALS AND METHODS

Data collection and retrieval of molecular structures

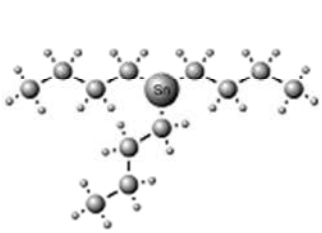
Test compound names, abbreviations and PubChem compound identifier numbers (PCIDs) are provided in **Table 1**. The molecular two-dimensional structures of TBT compounds were drawn (**Figure 1**) using Marvin Sketch 17.6, based on available molecular data from PubChem. This Java-based chemical drawing tool allowed to create and edit molecules in different file formats available from chemaxon [8,9].

Table 1. Simplified molecular-input line-entry system (SMILES) of TBTs.

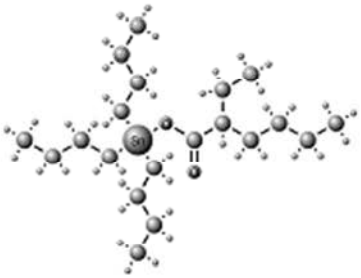
TBTs	SMILES	PubChem CID
TBT	CCCC[Sn](CCCC)CCCC	3032732
TBT2E	CCCCC(CC)C(=O)O[Sn](CCCC)(CCCC)CCCC	16682812
TBTA	CCCC[Sn](CCCC)(CCCC)OC(=O)C	16682741
TBTAc	CCCC[Sn](CCCC)(CCCC)OC(=O)C=C	16683072
TBTAd	CCCC[Sn](CCCC)(CCCC)OC(=O)CCCCC(=O)O[Sn](CCCC)(CCCC)CCCC	16683087
TBTAz	CCCC[Sn](CCCC)(CCCC)N=[N+]=[N-]	4984872
TBTBe	CCCC[Sn](CCCC)(CCCC)OC(=O)C1=CC=CC=C1	16682834
TBTBr	CCCC[Sn](CCCC)(CCCC)Br	305767
TBTCA	CCCC[Sn](CCCC)(CCCC)OC(=O)CCl	16683286
TBTCl	CCCC[Sn](CCCC)(CCCC)Cl	15096
TBTCl-d27	CCCC[Sn](CCCC)(CCCC)Cl	12228730
TBTCN	CCCC[Sn](CCCC)(CCCC)C#N	200512
TBTEO	CCCC[Sn+](CCCC)CCCC.CC[O-]	22042004
TBTF	CCCC[Sn](CCCC)(CCCC)F	16120
TBTG	CCCC[Sn](CCCC)(CCCC)OC(=O)CO	16684364
TBTH	CCCC[Sn](CCCC)CCCC.O	6327813
TBTI	CCCC[Sn](CCCC)(CCCC)I	23765
TBTIA	CCCC[Sn](CCCC)(CCCC)OC(=O)CSCC(C)(C)C(C)(C)C	16682826
TBTIC	CCCC[Sn](CCCC)(CCCC)N=C=O	120323
TBTIPS	CCCC[Sn](CCCC)(CCCC)OC(=O)CCC(=O)OC(C)C	16683081
TBTIt	CCCC[Sn](CCCC)(CCCC)OC(=O)CC(=C)C(=O)O[Sn](CCCC)(CCCC)CCCC	16684180

TBTL	**CCCCCCCCCCCC(=O)O[Sn](CCCC)(CCCC)CCCC**	**16683295**
TBTM	**CCCC[Sn](CCCC)(CCCC)OC(=O)C=CC(=O)O[Sn](CCCC)(CCCC)CCCC**	**16682777**
TBTMc	**CCCC[Sn](CCCC)(CCCC)OC(=O)C(=C)C**	**16682828**
TBTMO	**CCCC[Sn](CCCC)(CCCC)OC**	**16683411**
TBTO	**CCCC[Sn](CCCC)(CCCC)O[Sn](CCCC)(CCCC)CCCC**	**16682746**
TBTPAZ	**CCCC[Sn](CCCC)(CCCC)OC(=O)C1=CC=C(C=C1)NC(=O)C**	**16684361**
TBTPh	**CCCC[Sn](CCCC)(CCCC)OC(=O)C1=CC=CC=C1C(=O)O[Sn](CCCC)(CCCC)CCCC**	**16683955**
TBTPr	**CCCC[Sn](CCCC)(CCCC)OC(=O)CC**	**16683278**
TBTS	**CCCC[Sn](CCCC)(CCCC)S[Sn](CCCC)(CCCC)CCCC**	**16682974**
TBTSa	**CCCC[Sn](CCCC)(CCCC)OC(=O)C1=CC=CC=C1O**	**16683071**
TBTSt	**CCCCCCCCCCCCCCCCCC(=O)O[Sn](CCCC)(CCCC)CCCC**	**16683280**
TBTSCO	**CCCC[Sn](CCCC)(CCCC)OC(=O)O[Sn](CCCC)(CCCC)CCCC**	**16684480**

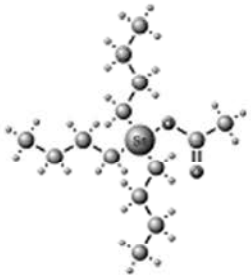
SMILES of TBTs obtained from PubChem.



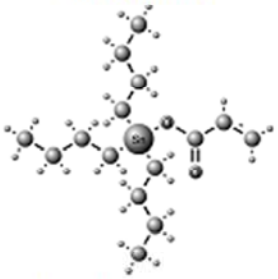
Tributyltin-TBT



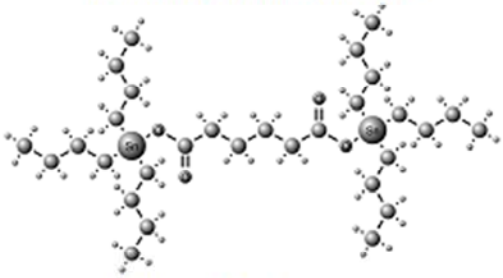
Tributyltin 2-ethylhexanoate-TBT2E



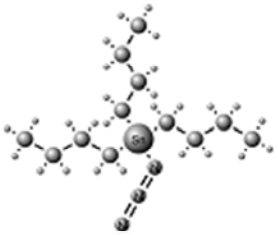
Tri-n-butyltin acetate-TBTA



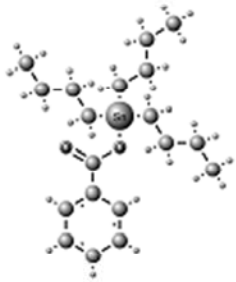
Tributyltin acrylate-TBTAc



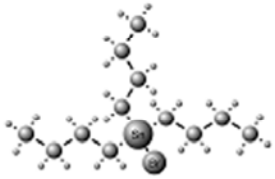
Bis(tributyltin) adipate-TBTAd



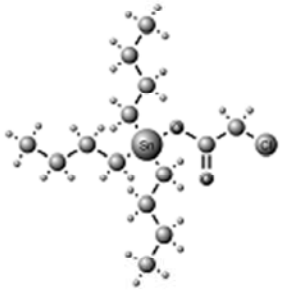
Tributyltin azide-TBTAz



Tributyltin benzoate-TBTBe



Tributyltin bromide-TBTBr

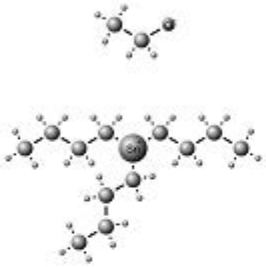


Tributyltin chloroacetate-TBTCA

Tributyltin chloride-TBTCl

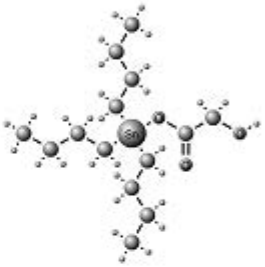
Tributyltin chloride-d27 (TBTCl-d27)

Tributylstannanecarbonitrile (TBTCN)



TBTEO

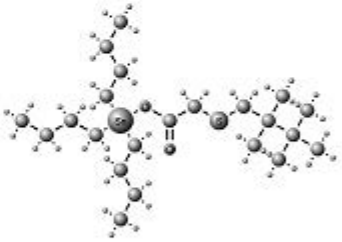
TBTF



Tributyltin glycolate-TBTG

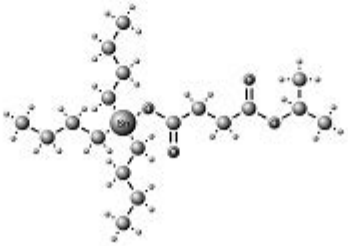
TBTH

Tributyltin iodide-TBTI

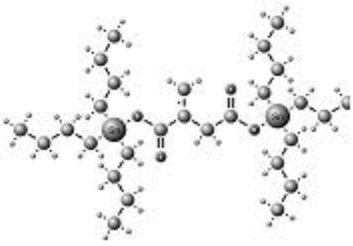


TBTIA

Tributyltin isocyanate-TBTIC



Tributyltin isopropyl succinate-TBTIPS



Bis(tributyltin) itaconate-TBTIt

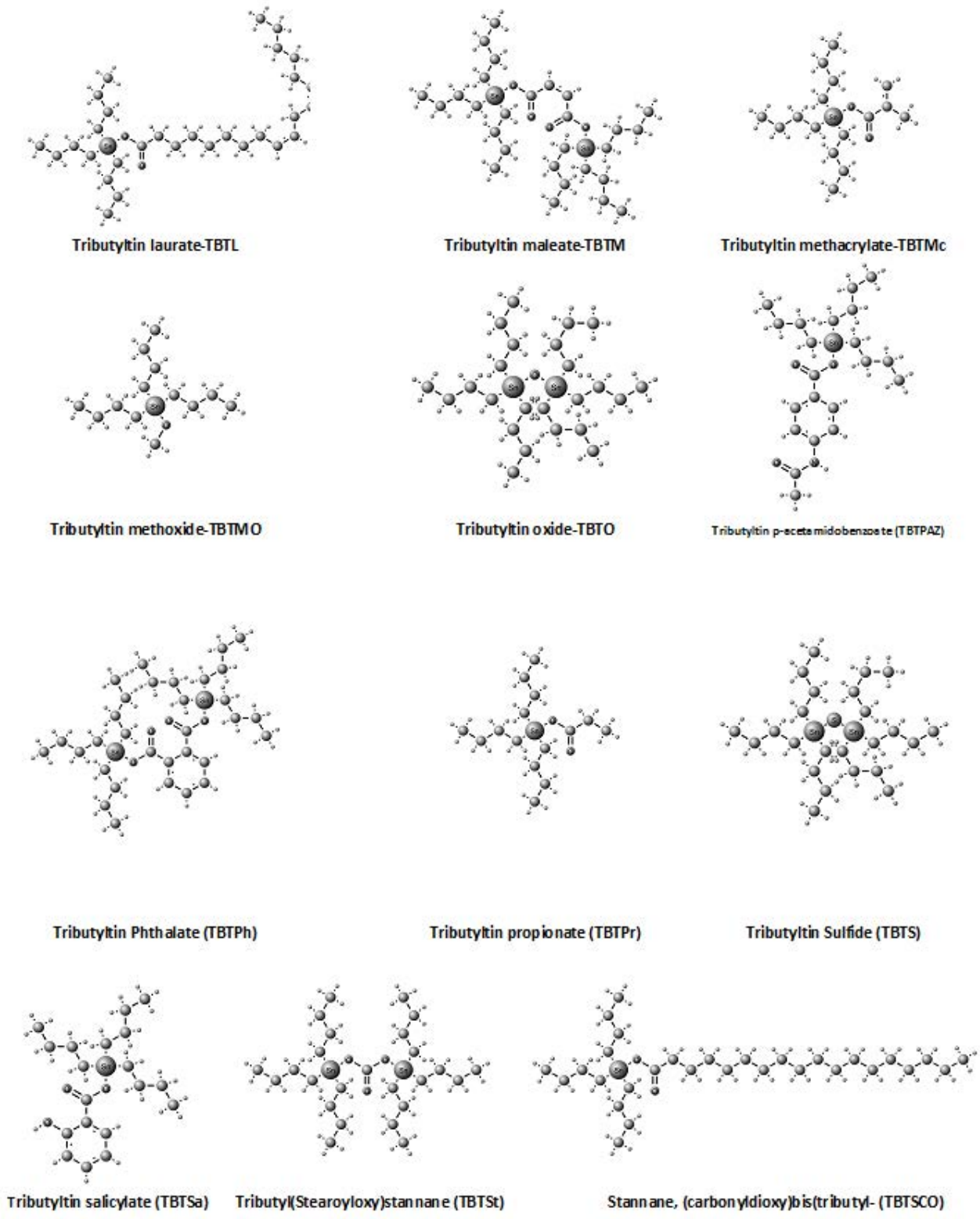


Figure 1. Chemical structures of TBTs provided as a ball and stick model by MarvinSketch 17.6.

SMILES based input format for compounds

Molecular structures are represented as strings of special characters using a simplified molecular input line entry system (SMILES) and such kernel strings are used for our computational toxicity prediction. SMILES based similarity functions are computationally more efficient [10]. SMILES of the test compounds were obtained from PubChem and shown in **Table 2**.

Table 2. Prediction of skin sensitizing properties of TBTs by Pred-Skin and pKCSM tools.

TBTs	Pred-Skin 2.0			pKCSM	Toxtree
	KeratinoSens™ (%)	h-CLAT (%)	Human skin sensitization (%)	Sensitization (Yes/No)	Irritation/corrosion (Yes/No)
TBT	80^{S}	70^{S}	60^{S}	Yes	No
TBT2E	80^{S}	80^{S}	60^{S}	Yes	No
TBTA	70^{S}	80^{S}	50^{S}	Yes	No
TBTAc	90^{S}	90^{S}	70^{S}	Yes	No
TBTAd	60^{S}	80^{S}	70^{S}	No	No
TBTAz	90^{S}	80^{S}	70^{S}	Yes	No
TBTBe	70^{S}	50^{NS}	60^{S}	No	No
TBTBr	80^{S}	80^{S}	70^{S}	Yes	No
TBTCA	70^{S}	80^{S}	70^{S}	Yes	No
TBTCl	80^{S}	80^{S}	60^{S}	Yes	No
TBTCl-d27	80^{S}	80^{S}	60^{S}	Yes	No
TBTCN	80^{S}	80^{S}	60^{S}	Yes	No
TBTEO	80^{S}	80^{S}	60^{S}	Yes	No
TBTF	80^{S}	70^{S}	60^{S}	Yes	No
TBTG	60^{S}	70^{S}	50^{NS}	No	No
TBTH	80^{S}	80^{S}	60^{S}	Yes	No
TBTI	80^{S}	80^{S}	70^{S}	Yes	No
TBTIA	80^{S}	80^{S}	60^{S}	Yes	No
TBTIC	80^{S}	80^{S}	70^{S}	Yes	No
TBTIPS	60^{S}	70^{S}	70^{S}	No	No
TBTIt	80^{S}	80^{S}	70^{S}	No	No
TBTL	70^{S}	80^{S}	80^{S}	Yes	No
TBTM	80^{S}	90^{S}	80^{S}	No	No
TBTMc	90^{S}	90^{S}	70^{S}	Yes	No
TBTMO	80^{S}	70^{S}	70^{S}	Yes	No
TBTO	70^{S}	80^{S}	70^{S}	No	No
TBTPAZ	80^{S}	80^{S}	60^{S}	No	No
TBTPh	70^{S}	50^{S}	70^{NS}	No	No
TBTPr	80^{S}	70^{S}	60^{S}	Yes	No
TBTS	80^{S}	80^{S}	70^{S}	No	No
TBTSa	70^{S}	70^{S}	60^{S}	No	No
TBTSt	70^{S}	80^{S}	80^{S}	No	No
TBTSCO	70^{S}	70^{S}	60^{S}	No	No

*^{NS}/**No**=Non-sensitizer, ^{S}/**Yes**=Sensitizer, %=Probability in percentage.*

Skin sensitization prediction

pKCSM: The pKCSM tool is a freely accessible web server tool using graph-based signatures to indicate ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of drugs or drug candidates [11]. It is used to predict the skin sensitization of TBT compounds and is available at http://biosig.unimelb.edu.au/pkcsm/prediction (**Table 2**).

Pred-Skin 2.0: Pred-Skin QSAR model screens for the sensitization potential on human skin. This tool was developed from human (109 compounds) and murine local lymph node assays (LLNA, 515 compounds). Experimental data by Braga and co-workers (2017) included a multiclass skin sensitization potency model based on LLNA data. When a user evaluates a compound in the web app, the outputs are (i) binary prediction of human and murine skin sensitization potential; (ii) a multiclass prediction of murine skin sensitization; and (iii) probability maps illustrating the predicted contribution of chemical fragments. The Pred-Skin web app version 1.0 is freely available from the web, iOS at the LabMol web portal, in the Apple Store, and on Google Play, respectively [7]. Such a tool is used to predict the skin sensitization of TBT compounds and this tool is available at Lab Mol [12].

Toxtree: Characteristics of TBTs: mutagenicity and protein binding alert

In order to predict the mutagenicity of chemicals, we used the bacterial reverse mutation assay (Ames test) [13]. To reduce the time and the expenditure of bacterial culture media, we used the Toxtree *in silico* tool to predict the mutagenicity of TBTs. The Toxtree toxicity prediction tool is available from http://toxtree.sourceforge.net/. The toxicological endpoints predicated by this program include mutagenicity, carcinogenicity, and protein binding alert, DNA binding alert, and biodegradability of chemical substances [14]. In this study, we assessed TBT compound-protein binding alert, mutagenicity and biodegradability.

LAZAR: TBTs penetration of blood-brain barrier (BBB)

The lazar toxicity prediction tool is available from https://nano-lazar.in-silico.ch/predict. LAZAR provides toxicological predictions by the analysis of compound structures. This holds for mutagenicity, blood-brain barrier penetration, rodent carcinogenicity, and maximum recommended doses [14]. In this study, we used the LAZAR tool to predict the BBB penetration. Predicted results are shown in **STable1**.

STable 1. TBTs potency for Blood Brain Barrier Penetration (human) according to LAZAR prediction.

TBTs	Penetrating probability	Non-penetrating probability	Prediction
TBT	**0.333**	**0.0**	**penetrating**
TBT2E	0.233	0.0167	**penetrating**
TBTA	0.2	0.0	**penetrating**
TBTAc	0.182	0.0	**penetrating**
TBTAd	0.182	0.0	**penetrating**
TBTAz	0.222	0.0	**penetrating**
TBTBe	0.153	0.0132	**penetrating**
TBTBr	0.286	0.0	**penetrating**
TBTCA	0.182	0.0	**penetrating**
TBTCl	0.286	0.0	**penetrating**
TBTCl-d27	0.286	0.0	**penetrating**
TBTCN	0.25	0.0	**penetrating**
TBTEO	0.222	0.0	**penetrating**
TBTF	0.286	0.0	**penetrating**
TBTG	0.182	0.0	**penetrating**

TBTH	0.286	0.0	**penetrating**
TBTI	0.286	0.0	**penetrating**
TBTIA	0.133	0.0	**penetrating**
TBTIC	0.222	0.0	**penetrating**
TBTIPS	0.143	0.0	**penetrating**
TBTIt	0.133	0.0	**penetrating**
TBTL	0.253	0.02	**penetrating**
TBTM	0.2	0.0	**penetrating**
TBTMc	0.167	0.0	**penetrating**
TBTMO	0.25	0.0	**penetrating**
TBTO	0.286	0.0	**penetrating**
TBTPAZ	0.291	0.0245	**penetrating**
TBTPh	0.153	0.0132	**penetrating**
TBTPr	0.182	0.0	**penetrating**
TBTS	0.286	0.0	**penetrating**
TBTSa	0.234	0.0604	**penetrating**
TBTSCO	0.222	0.0	**penetrating**
TBTSt	0.253	0.02	**penetrating**

RESULTS AND DISCUSSION

Human skin sensitization

Toxtree predicts that all the tested TBT compounds do not have skin irritation nor skin corrosion properties. All the TBT compounds investigated here can penetrate BBB as predicted by the LAZAR web server tool (**Table 2)**. The Pred-Skin 2.0 tool predicts all TBT compounds - except TBTBe - as skin sensitizers.

According to the human cell line activation test by Pred-Skin TBTBe is a non-sensitizer. But, it is a sensitizer according to *in silico* human skin sensitization and Keratino Sens™.

TBTG and TBTPh both are non-sensitizers to human skin based on the Pred-Skin *in silico* assessment.

The pKCSM tool predicts TBT, TBT2E, TBTA, TBTAc, TBTAz, TBTBr, TBTCA, TBTCl, TBTCl-d27, TBTCN, TBTEO, TBTF, TBTH, TBTI, TBTIA, TBTIC, TBTIPS, TBTIt, TBTL, TBTMc, TBTMO and TBTPr as skin sensitizers. Other TBTs are non-skin sensitizers (**Table 3)**.

Table 3. TBTs mutagenicity and protein-binding-alert predicted by Toxtree-v2.6.13.

TBTs	Mutagenicity	Protein-binding-alert
TBT	-	-
TBT2E	-	-
TBTA	-	-
TBTAc	-	-
TBTAd	-	-
TBTAz	+	-
TBTBe	-	+
TBTBr	-	-

TBTCA	+	+
TBTCl	-	-
TBTCl-d27	-	-
TBTCN	-	-
TBTEO	-	-
TBTF	-	-
TBTG	-	-
TBTI	-	-
TBTIA	-	-
TBTIC	+	-
TBTIPS	-	-
TBTIt	-	+
TBTL	-	-
TBTM	-	-
TBTMc	-	-
TBTMO	-	-
TBTO	-	-
TBTPAZ	+	+
TBTPh	-	+
TBTPr	-	-
TBTS	-	-
TBTSa	-	+
TBTSt	-	-
TBTSCO	-	-

+ = Yes (mutagen), - = No (non-mutagenic).

Mutagenicity

Toxtree predicts TBTAz, TBTIC, TBTCA, TBTPAZ as mutagens. Other TBT compounds are predicted as nonmutagens. Toxtree tool shows protein binding alerts for TBTPh, TBTSa, TBTBe, TBTCA, TBTL, TBTIt, and TBTPAZ. Other TBTs do not have protein binding effects (**Table 4**). Earlier reports on the prediction of mutagenicity often used toxicophores rather than whole-molecules as predictive tools [15].

LAZAR: TBTs penetration of the blood-brain barrier (BBB)

The lazar toxicity prediction tool provides predictions about the blood-brain barrier penetration. According to this tool are all TBT compounds screened here penetrating the human BBB. See also **STable 1**.

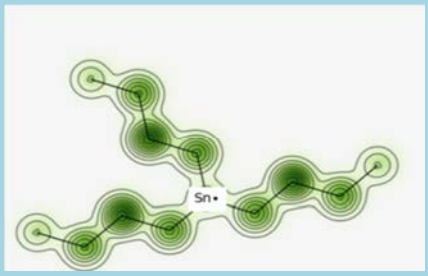
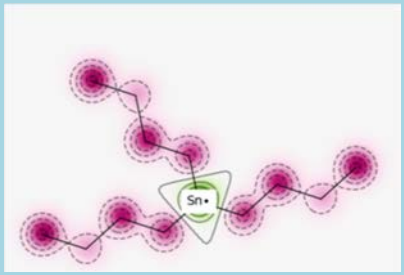
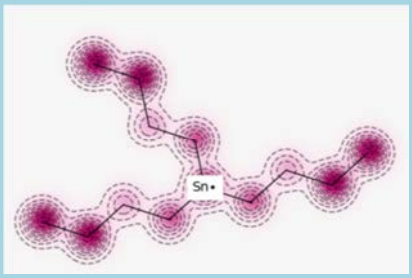
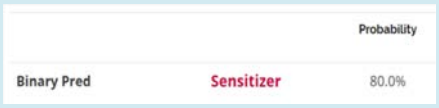
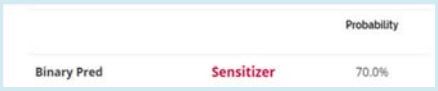
CONCLUSIONS

In silico predictive models provide fast and economic screening tools for desirable and other compound properties. **C**omputational approaches as demonstrated here can also demarcate chemicals for their toxicological evaluation in order to reduce the amount of costly *in vivo* and *in vitro* toxicological testing and also provide early alerts for newly developed substances.

ACKNOWLEDGMENTS

HUD and **VK** acknowledge the support of the Research Center of Environmental Medicine, Kaohsiung Medical University (KMU), and **CDA** acknowledges the Kaohsiung Medical University scholarship for international students. We thank Ms. Revathi Gurunathan, Mrs. Sravya Kosuru, and Mr. Cheng-Han Liu for their assistance in this computational study.

STable 2. Example of TBT raw data from Pred-Skin toxicity prediction.

KeratinoSens™	Human cell line activation test (h-CLAT)	Human skin sensitization
		
		

*Screen capture from http://www.labmol.com.br/predskin/ during *in silico* toxicological assessment.

COMPETING INTEREST STATEMENT

The authors declare no competing interests associated with this manuscript.

REFERENCES

1. Sekizawa J, Suter II, Lenn G, Birnbaum L (2001) C. Tributyltin and triphenyltin compounds. World Health Organ 1-25.
2. Benson R (1999) Tributyltin Oxide. Concise International Chemical Assessment Document 14. World Health Organization, Geneva.
3. Gehring PJ, Nolan RJ, Watanabe PG, Schumann AM (1991) Solvents, fumigants, and related compounds. Handb Pestic Toxicol. Hayes, WJ.
4. Registration Eligibility Decision for the Tributyltin Compounds: Bis(tributyltin) oxide, Tributyltin benzoate, and Tributyltin maleate Case 2620 (2008) https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/red_G-79_30-Jun-08.pdf
5. Worth A, Lapenna S, Lo Piparo E, Mostrag-Szlichtyng A, Serafimova R (2011) A Framework for assessing in silico Toxicity Predictions: Case Studies with selected Pesticides. JRC Rep EUR24705 46.
6. Xu C, Cheng F, Chen L, Du Z, Li W, et al. (2012) In silico prediction of chemical ames mutagenicity. J Chem Inf Model 52: 2840-2847.
7. Braga RC, Alves VM, Muratov EN, Strickland J, Kleinstreuer N, et al. (2017) Pred-Skin: A Fast and Reliable Web Application to Assess Skin Sensitization Effects of Chemicals. J Chem Inf Model 57: 1013-1017.
8. Chemaxon provides MarvinSketch17.6 Freeware for academic use.
9. Evans MJ, Moore JS (2011) A collaborative, wiki-based organic chemistry project incorporating free chemistry software on the Web. J Chem Educ 88: 764-768.
10. Frenzel F, Buhrke T, Wenzel I, Andrack J, Hielscher J, Lampen A (2017) Use of in silico models for prioritization of heat-induced food contaminants in mutagenicity and carcinogenicity testing. Arch. Toxicol 91: 3157-3174.
11. Pires DEV, Blundell TL, Ascher DB (2015) pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem 58: 4066-4072.
12. LabMol: Pred-Skin 2.0, available at http://labmol.com.br/predskin/
13. Modi S, Li J, Malcomber S, Moore C, Scott A, et al. (2012) Integrated in silico approaches for the prediction of Ames test mutagenicity. J Comput Aided Mol Des 26: 1017-1033.
14. Maunz A, Helma C (2008) Prediction of chemical toxicity with local support vector regression and activity-specific kernels. SAR QSAR Environ Res 19: 413-431.
15. Seal A, Passi A, Abdul Jaleel UC (2012) In-silico predictive mutagenicity model generation using supervised learning approaches. J Cheminform 4: 1-11.