FROM PARTITION COEFFICIENTS TO MEDIAN LETHAL CONCENTRATION OF ORGANIC POLLUTANTS: EVALUATION OF PASSIVE SAMPLERS AS PROXY OF LIPID IN THE TARGET MODELS FOR FISH AND DAPHNIA

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A thesis submitted in partial fulfilment of requirements for the degree of Master of Science in Environmental Science

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Dedication

'This thesis is dedicated to my affectionate parents, without whom

I would not have been able to complete my research work'

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(Mahnoor Iftikhar)

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List of Abbreviations or Keywords

ASM	Abraham Solvation Model
TLM	Target Lipid Model
WBM	Wang Baseline Model
TPSM	Target Passive Sampler Model
K_{POM-w}	Polyoxymethylene-Water Partition Coefficient
K_{PE-w}	Polyethylene-Water Partition Coefficient
K_{PA-w}	Polyacrylic-Water Partition Coefficient
K_{PDMS-a}	Polydimethylsiloxane-Air Partition Coefficient
PS	Passive Sampler
WLIM	Wang Less Inert Model
RMSE	Root Mean Square Error
QSARs	Quantitative Structure Activity Relationships
LC	Lethal Concentration

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ABSTRACT

The number and volume of chemicals around us have increased significantly since industrialization. The main challenge in ecotoxicity is to accurately identify and distinguish the risks of tens of thousands of chemicals on aquatic life. We put forward a new approach to estimate median lethal concentrations (LC_{50}) of organic compounds by establishing target passive sampler models. In this study, we demonstrated that passive samplers are good proxies for the bio-membranes in organisms. Hence, passive samplers can be substituted in the target lipid model to predict the reliable baseline toxicities of chemicals accumulated on passive samplers. Our new approach sheds light on the mode of toxic actions of organic chemicals. We evaluated and categorized four types of passive samplers *viz.*, polyacrylic (PA), polyethylene (PE), polydimethylsiloxane (PDMS) and polyoxymethylene (POM). The analyses showed that PA works well for most of the chemical groups such as baseline compounds. However, for a few chemical groups such as aldehydes and alpha, beta unsaturated ketones, the PDMS displayed a good agreement between estimated and experimental values. Our approach worked well for fish but showed significant systematic deviations from experimental values for daphnia. Further investigation indicated that the assumption of a critical burden of 100 mmol/kg in bio-membrane is suitable for fish but needs to be revisited for daphnia. The predictive performance of our new approach is at par with previous approaches. However, our method is simple and offers the opportunity of interfacing the measurement of environmental levels of organic pollutants with modeling of LC_{50} values of those chemicals.

Keywords: Passive sampler; Target lipid model; Animal alternate testing; Lethal concentration

CHAPTER 1

1. INTRODUCTION

1.1. Background

More than 350000 chemicals and their mixtures are registered for commercial production worldwide (Richardson et al., 2021). The main challenge in ecotoxicity is to accurately identify and distinguish the risks of tens of thousands of chemicals on aquatic life (McElroy et al., 2011). Information on aquatic toxicity is necessary for determining the toxicity caused by organic chemicals in freshwater and marine organisms (Wang et al., 2016). Organic chemicals (e.g., phenols, anilines, and nitrobenzene) are extensively used in industrial processes and are regularly discharged into the environment. Once the chemicals released into the environment, extensive information on aquatic toxicity is required to assess risks and hazards caused by the chemical substances (Zhang et al., 2010).

Passive sampling is a technique in which a sampling device captures freely flowing analyte molecules from the sampling medium due to the analyte chemical potential difference in the two media (Mackay et al., 1997). Passive sampling comprised of three steps: analyte isolation, and pre-concentration, simplifying the need to pre-treatment the sample. It also requires no or minimal solvent. Passive sampling determines time-weighted average (TWA) concentrations, the response speed is the duration for which the time-weighted average is determined over time (Górecki & Namieśnik., 2002).

The quantity of analyte being collected by sampler depends upon two things; firstly, the concentration of analyte in sample medium, and the other is the exposure time. Time-weighted average TWA can easily be calculated if we have information about the relationship between the sampling rate and the analyte concentration. Nevertheless, certain conditions must meet: the sampling rate must be constant throughout the time. Those conditions can be achieved when the analyte is absorbed. However, some problems exist when a process of physical adsorption is involved in the collection of analytes.

1.2. Partition Coefficient:

Appropriate calibration is required to quantify the data for a passive sampler. There are some specific calibration parameters which are necessary in order to use passive samplers, such as partition coefficients, sampling rates, and loss rate constants, usually determined in the laboratory or at the sampling site (Phong et al., 2012).

The partition coefficient (P) is the ratio of the equilibrium of compound concentration in a mixture of two immiscible phases. It is a measure of differences in compound solubility in two phases. The equilibrium distribution properties strongly influence the transport and distribution of chemicals in the environment (Schwarzenbach et al., 2003). Therefore, fateful models for the environmental behavior and environmental impact assessment of chemicals often include partitioning properties,

$$Pxy, i = \left\{\frac{Cx,i}{Cy,i}\right\}_{\text{equilibrium}} \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

In equation 1, $P_{xy,i}$ represents the partition coefficient between two phases x and y. The $C_{x,i}$ and $C_{y,i}$ are the concentrations of toxicant at partitioning equilibrium present in these phases. Thus, to assess the chemical exposure and transport in the environment, equilibrium partition coefficients are required.

1.3. Use of animals for testing:

Living animals have been utilized in scientific studies for centuries to test the toxicities of organic contaminants in the environment. The membranes of these test organisms are the most critical target areas for organic contaminants (both hydrophobic and hydrophilic). Persistent hydrophobic pollutants are absorbed by hydrophilic tissues and lipids, causing changes in the structure and function of the body membrane, such as expansion, fluidity, and ion permeability. The membrane toxicity is affected by the physiological characteristics of test organisms. These characteristics include sensitivity of specie, exposure time, and bio-concentration potential.

Data collection and analysis using living organisms require extensive chemical management procedures. Alternative approaches can be utilized to save test animals. If these techniques are used extensively, a significant reduction in test animals can be achieved. Using passive samplers instead of test animals is an effective strategy to limit the use of animal testing methods and adverse effects. Many approaches, such as QASR

and in vitro testing methodologies, have been developed, tested, and accepted to decrease test animal use and cost.

Physiological characteristics of test organisms such as specie sensitivity, exposure time, bioconcentration potential, and ambient variables influence the membrane toxicity. The bioconcentration potential of an organic pollutant can be used to calculate the mode of action (MOA), which is a significant component in assessing the toxicity of organic pollutants in organisms.

1.4. Lethal Concentration (LC_{50}):

LC_{50} (Lethal Concentration) is the dose (mg/kg body weight) at which the death of 50% of the animals exposed to the various chemical agents (OECD). The term LC_{50} is often interchanged with Lethal Dose (LD_{50}).

Suppose a chemical/metabolite has a detrimental effect. In that case, the chemical must target specific areas of the body and be present in a significant quantity for an extended period. As a result, it is essential to understand what impacts a specific substance may have had. There is also a need of data on the chemical structure, exposure properties, kind of administration, time of exposure, and speed of exposure.

Many toxic reactions result in cellular death and the loss of organ efficiency, which affects the functionality of the entire tissue of the organism. Chemicals influence these processes through numerous mechanisms of action, which might be synthesized as follows:

(a) Disruption of traditional ligand-receptor interactions; (b) Disruption of membrane functions; (c) Disruption of cellular energy production; (d) Binding / influence on biomolecules; (e) Toxicity through specific cell death; (f) Non-fatal genetic modification of body cells.

1.5. Target Lipid Model:

The TLM is a model that anticipates critical body burden, which relates toxicity to accumulation in target tissues (e.g., target lipids primarily in membranes) relative to a critical effects threshold.

$$\log LC_{50} = -\log K_{LW} + \log CTLBB \quad \ldots\ldots\ldots\ldots \quad (2)$$

In equation no.2, the CTLBB (μmol/g lipid) is the chemical concentration in target lipids. K_{LW} is the target lipid−water partition coefficient. The CTLBB depicts tolerances and thresholds for the effect of chemicals that are a function of test species and endpoint. The K_{LW} was estimated using lipid-water's poly parameter linear solvation-energy relationship (LSER) (e.g., LSER-based TLM). The equation 3 shows the general form of the poly parameter LSER model:

$$\log K = eE + sS + aA + bB + vV + c \quad \ldots\ldots\ldots\ldots \quad (3)$$

The parameters in the equation no. 3; e, s, a, b, and v correspond to the solvent system. The uppercase parameters E, S, A, B, and V correspond to the chemical interaction terms for solutes. The letter E represents excess molar refractivity, S shows the polarizability, A represents the ability to donate hydrogen bond, B shows the ability to accept hydrogen bond, V is a molar volume and c is a fitting constant and accounts for unit conversions.

1.6. Passive sampler's good proxies for bio-membrane (Hypothesis):

Passive samplers are good bio-membrane proxies and can be substituted in the target lipid model to predict the reliable baseline toxicities of detected chemicals. Compounds with lower molecular weight are less persistent in the environment because of the volatility. In contrast, higher molecular weight compounds are persistent and have prolong impact on the environment. Passive samplers are good bio-membrane proxies and can be substituted in the target lipid model to predict the reliable baseline toxicities of detected chemicals.

The toxicity is formulated on the hypothesis that the concentration of a chemical/toxicant in an aqueous medium to exert a toxic endpoint, such as median lethal concentration (LC_{50}), can be predicted from the critical body burden in the target lipid of an organism (C_{mem}). That can be calculated from the target lipid to water partition coefficient $K_{L\text{-}W}$. The critical body burden in the target lipid (C_{mem}) is calculated at 100 mmol/kg for a wide variety of 42 aquatic organisms using 333 different chemicals (Escher et al., 2017).

$$K_{\text{bio-membrane-water}} = \frac{Concentration\ in\ biomembrane}{Concentration\ in\ water} \quad \ldots\ldots\ldots\ldots \quad (4)$$

Concentration in water when it equals to LC_{50}.

$$K_{L\text{-}w} = \frac{Concentration\ in\ biomembrane}{LC_{50}} \quad \ldots\ldots\ldots\ldots \quad (5)$$

The concentration in bio-membrane taken as 100 mmol/kg (Critical Target Lipid Body Burden). It becomes:

$$LC_{50} = \frac{100\ mmol}{K_{L-W}} \quad \ldots\ldots\ldots\ldots \quad (6)$$

If we replace membrane (mentioned in eq. 5) with passive sampler, it becomes:

$$-\log LC_{50} = \log K_{L-W} + 1 \quad \ldots\ldots\ldots\ldots \quad (7)$$

$$-\log LC_{50} = \log K_{PS\text{-}W} + 1 \quad \ldots\ldots\ldots\ldots \quad (8)$$

The chemical uptake by a passive sampler will be similar to that of a phospholipid.

1.7. Significance of the study:

Passive samplers are simple polymers deployed in the environmental phase for a certain period for the sorption of contaminants. They are significant in determining organic contamination in different environmental compartments. In contrast, to use organisms, passive sampling methods are easy to use and avoid laborious procedures in order to extract data. Using passive samplers as an alternate to testing animals reduces the use and adverse impacts of test methods on animals. Passive sampling is a method of environmental monitoring that is less intrusive. It is cost-effective, durable, and delivers representative data comparable across time and space. These samplers provide a time-weighted average pollution level over long periods, ranging from days to months. Passive sampling has numerous advantages over traditional methods since it substantially simplifies the sampling operation by removing sample preparation and storage, produces cleaner extracts with less solvent usage, reduces processing time, and removes power/current input. The primary benefit of passive samplers is the cost savings resulting from reduced sampling time and waste generation. Moreover, passive samplers do not require pumps/power supplies; less on-site time requirement; and convenient site operations.

1.8. Problem Statement

For LC_{50} measurement, there is shortage of resources, time and several ethical implications. A very large number of chemicals require risk assessment for experimental approach that might kill hundreds of test organisms. Hence, we need new alternate approaches to measure LC_{50}.

1.9. Objectives

This study was designed based on the problem statement. The objectives of the study are:

1. To dissect the target lipid model and reassemble target passive sampler model for organic pollutants.
2. To evaluate four types of passive sampling in the target passive sampler model using experimental data for 1952 individual experimental values.
3. To find the inter-specific differences in toxicities for Fish and Daphnia.

CHAPTER 2

2. LITERATURE REVIEW

A few industrial chemicals cause toxicity by forming irreversible covalent bonds between sulfur and nitrogen-containing amino acids within the toxic molecule (Enoch et al., 2008). Bulk of industrial chemicals causes aquatic toxicity through two non-covalent mechanisms: polar narcosis and nonpolar narcosis. In environmental risk assessment, knowledge about the chemical's emission, behavior, fate, and acute/chronic toxicity is required for risk assessment.

Behavior of chemicals in the environment comprises its partitioning and diffusion between two different phases. The chemical uptake by different living organisms leads to bioconcentration and bioaccumulation. The quantitative estimation of a life cycle of chemical substance (from its emission to its environmental fate) helps in its modeling and assessing its concentration in different environmental compartments. The methods to assess the toxicity of a chemical substance are limited and simple such as expressed in LC_{50} (Lethal Concentration 50), which is the concentration of a substance that produces 50% lethality. Moreover; another approach, Tolerable Maximum Concentration (MTC), is also used to determine the environmental risk assessment. Maximum Tolerable Concentration is a concentration of a pollutant at or below which a particular percentage of a specie would be affected in an ecosystem (Verhaar et al., 1992).

Verhaar conducted a study (Verhaar et al., 1992). He classified chemicals into groups. The classification method by Verhaar is a well-known decision tree built utilizing a series of structural alerts that allows essential organic molecules to be classified into one of four groups/categories (Verhaar et al., 1992). A classification system has also been devised based on mechanistically relevant structural signals. They are believed to be mechanistically interpretable and hence tend to be used in regulatory risk assessment. However, it is not necessary that they offer the same classification performance.

Table 1. Classification of chemicals

Chemical	Description
Non-polar narcotics or inert compounds	This group of compounds consists of baseline toxicity QSAR. The toxicity is dependent upon hydrophobicity.
Less inert compounds and polar narcotics	Chemicals that are not reactive but are slightly more hazardous than hydrophobicity.
Reactive chemicals	Chemicals create irreversible covalent bonds with amino acid protein residues and have considerably higher toxicity than predicted by hydrophobicity alone
Chemicals that have a specific action	This type of chemical class performed particular actions.
Chemical that does not belong in classes 1–4 is labeled "no decision can be made for this compound."	Before a mechanism of action could be given to such substances, additional research on alternative methodologies would be required.

Table 1 represents the four Verhaar classes. A test compound is evaluated according to alerts that define class 1. If a chemical fails to trigger an alert in class 1, it is screened using the alerts that define classes 2, 3, and 4. If an alarm is triggered in any class, the chemical is allocated to the class where the first warning was triggered. If a chemical does not trigger an alert for any of the four classes, it is placed in class 5, which means "no decision can be made for this chemical."

Many groups have coded the Verhaar classification method computationally because of its importance and perceived utility for regulators and risk assessors. The classification scheme; in particular, is presented in the OECD QSAR Application Toolbox and is available via the Toxtree software from the European Chemicals Bureau website. Despite its enormous popularity, few attempts to analyze the classification scheme have been made. Additional details are available in Verhaar et al. (2000), which qualitatively comment on the scheme's weaknesses but do not make firm recommendations for improvement. Since Verhaar's publication in 1992, there has been significant progress in understanding structural boundaries of a chemical (Enoch et al., 2008).

2.1. Organic Pollutants and their mode of action:

The toxicological effects of organic pollutants are predicted using their structural properties. It is necessary to associate a compound with a specific mode of action to develop quantitative structure-activity relationships (QSARs) (Enoch et al., 2008). Developing the QSARs model requires information about the mode of action necessary to develop models to predict toxicological effects. Numerous MOAs exist, such as nonpolar narcosis, oxidative uncoupling phosphorylation, respiratory inhibition, electron transport chain inhibition, inhibition of acetylcholinesterase (AChE), and neurotoxicity. Various structural rules are also provided in the literature, which classifies compounds based on mechanisms and modes of action. There is a difference of opinion on the separation methods of the two mechanisms of action. Baseline compounds (nonpolar narcotics) are inert and do not interact with specific receptors. These non-reactive and halogen-substituted hydrocarbon partitions at biological membranes consequentially disturb the integrity and functioning of the cell. Moreover, polar narcotics are less inert chemicals, slightly more reactive and toxic than the baseline compounds. Hence, these compounds possess the characteristics of hydrogen bond donor acidity, for instance, phenols and anilines. Various regression equations indicate a difference in mechanisms of action for polar and nonpolar compounds (Verhaar et al., 1992). However, some of the researchers reported that no difference exists, and the distinction is because of the uneven distribution of organic contaminants across the target and non-target lipids. Reactive chemicals evinced high toxicity with lower LC_{50} and EC_{50} values than estimated from the hydrophobicity. According to Verhaar and Enoch, these compounds form irreversible covalent interactions with the residues of protein amino acids. However, the toxic ratio can distinguish between the baseline narcotic compounds and the reactive compounds.

2.2. Target Lipid membranes:

Lipids are known as a significant source of energy in the aquatic organisms. A study conducted by McElroy et al., 2011 explained that the polar membrane lipids are the sites where toxic effect take place. The chemicals acting as contaminants via baseline toxicity cause the toxic effect by associating with polar membrane lipids (McElroy et al., 2011). The lipid-related tolerance and resistance to chemical exposure were analyzed in fish decades ago. However, the process was not deduced. Mostly, octanol is an acceptable

proxy for total lipids. Within an organism, hydrophobic organic chemicals are significantly partitioned into lipids. Subcellular dispersion is determined by the compound's chemical potential and is significantly affected by the lipid content, water, and protein of the tissues (McElroy et al., 2011). Nonetheless, it may be unable to describe toxicodynamic processes involving particular lipid groups (McElroy et al., 2011). A study was conducted by (Wang et al., 2016) he did comparison of toxicities of Vibrio fischeri and fish based on discrimination of excess toxicity from baseline level. I did comparison between fish and daphnia. Daphnia, a freshwater invertebrate species, was used to compare with the fish in this study. Daphnia is widely used as a test species worldwide to determine the ecotoxicological effect of industrial chemicals released into water bodies. It has been used for many years, and recently it has been used for developing quantitative models. Deneer developed QSAR models to predict acute toxicity in Daphnia using fifty chemical substances in this context. Another model was developed by Tao et al. to assess the EC_{50} values of approximately 217 organic compounds in Daphnia using fragment-based QSARs. Furthermore, Zvinavashe et al. developed a QSAR model to analyze the toxicity of organo-thiophosphate pesticides in Daphnia. Another QSAR model was developed using the log of octanol-water partition coefficient as an independent variable (Kar & Roy, 2010).

2.3. Animal experimental research:

In developing drugs, animal toxicology testing and experimental research are increasingly being challenged due to their poor association with in-human outcomes (van Norman, 2020). Evaluating the potential risk to human health and the environment posed by a chemical requires a combined quantitative analysis of exposure and the hazards, including uncertainty. During the past decades, assessing toxicological hazards concerning regulatory risk evolution has mainly relied on animal research (Sturla et al., 2014). There is a strong need to develop and promote alternate test methods to reduce and replace animal testing. In Europe, the requirement of alternative approaches has been directed by legislation such as REACH, the UK Animal Welfare Act (2006), the 7th Amendment to the European Union Cosmetics Directive (European Union 2009), the European Union Animal Protective Directive (European Commission 2010), and the German Legislation (Federal Law Gazette 2009, 2016) (Norberg-King et al., 2018). An adequate, robust model was built for 297 structurally diverse chemicals. A study by Kar

et al., 2010 suggests that higher lipophilicity and electrophilicity values significantly increase toxicity. (Kar et al., 2010). The research on comparison of toxicities between V. fischeri and fish proposed the chemical-specific species sensitivity between fish and V. fischeri. Baseline or less inert compounds share the same MOAs and bio-uptake process between fish and V. fischeri (Wang et al., 2016). A study conducted by Bittermann proposed the experimental data for many compounds and a critical body burden 100 mmol for fish (Bittermann et al., 2017). We used that experimental data and we developed models by which experiments on animals can be reduced.

2.4. Passive sampling

Passive sampling devices have been extensively used in environmental monitoring. They are used to measure chemical concentrations in environment for instance air, water and sand sediments (Kirchhelle, 2018). Because of its simple deployment; cleaner extract, simpler procedures, and time-integrated character, passive sampling devices (PSD) are widespread (Vrana et al., 2005). Passive sampling used to measure toxicity (Smith et al., 2010). Bioconcentration (Adolfsson-Erici, Kerman and Mclachlan, 2012) and exposure to organic substances have been started in laboratory experiments which favors passive dosing approaches (Bera et al., 2018).

Passive sampling is necessary because it mimics the passive intake of dissolved chemical concentrations in the environment. Traditional sampling returns various pollutants concentrations but no information on the dissolved or bioavailable fraction (BAF) of contaminants. PSDs are equal to finding the chemical activity of contaminants because they measure C_{free} at various ambient levels (Allan et al., 2021). The portability, weight, size, and electrical power of sampling devices limit the sample methods that can be used to measure the global-atmospheric transport of contaminants. To summarize, active and spot/grab sampling methods provide essential and trustworthy information about total airborne or waterborne pollutant concentrations in a short period but no information about time-weighted average (TWA) contaminant concentrations. This flaw severely restricts their use in organism exposure assessments. Environmental chemists choose low-cost and low-tech PSDs to address this limitation (Taylor et al., 2007). There are a variety of passive sampling devices available that can be used to sample various pollutants in environment. Selecting a suitable passive sampling instrument for a specific sampling setting is critical. Different materials are used as sorbents in passive sampling

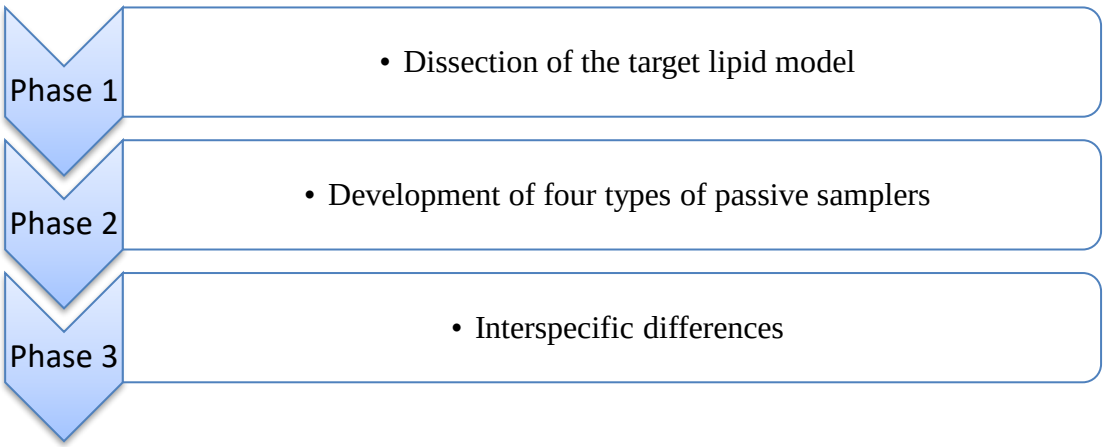
devices as it provides the device with a specific property. For instance; polyethylene passive samplers which are ethylene sheet based passive samplers are substantial when it comes to capturing hydrophobic compounds. A study conducted by Kirchhelle, 2018 explained that various types of passive samplers have been used to measure contaminants. Semipermeable membrane devices (SPMD), low-density polyethylene (LDPE) film, polyacrylic (PA) plastic sorbent, polyoxymethylene (POM) devices, polydimethylsiloxane (PDMS) fibers, and polyurethane foam (PUF) are examples of passive samplers that are used in a variety of situations. Study shows that there are passive sampling devices that have been extensively used in environmental monitoring to measure contaminant (Kirchhelle, 2018). Passive samplers are popular because they are simple to deploy, they have simple procedures and time-integrated character (Vrana et al., 2005).

CHAPTER 3

3. Methodology

3.1. Methods and Materials

The procedure for this study was organized on the bases of three phases. The first phase involved the dissection of the target lipid model. This work aims to reassemble the target passive sampler model for organic pollutants. In second phase, four types of passive samplers in the target passive sampler model developed using experimental data of individual experimental values. The third phase was performed to find the inter-specific differences in toxicities for fish and daphnia.



Data acquisition:

The data of one thousand and fifty-two compounds were taken from the literature (Wang et al., 2016). Table 2 displays the compounds which were classified into 87 groups based on different functional groups. Table 2 shows compound names from group 1 to group 40 (other 41-87 groups and classes of compounds are shown in supporting information). Data covered a very wide chemical range including several families of organic compounds. In regard to the chemical domain, the data set includes following compounds:

Table 2 Classification of compounds based on 87 different classes

Compound	**Compound**	**Compound**
Compounds used in the baseline model (Group 1)	Alpha beta-unsaturated ketones (Group 14)	Amides, alpha-chloro amides (Group 28)
Compounds used in the less inert model (Group 2)	Esters, bromo esters and diesters (Group 15)	Ureas (Group 29)
Alkanes with Bromo group (Group 3)	Alpha halogenated esters (Group 16)	Epoxides (Group 30)
Alkenes (Group 4)	Alpha beta unsaturated esters (Group 17)	Thiols, thioesters, dithio-ethers (Group 31)
Allylic and propargyl halogens (Group 5)	Carboxylic acids with fluoro or chloro group (Group 18)	Thioureas (Group 32)
Beta-halogenated alcohols (Group 6)	Diacids (Group 19)	Thiosulphates (Group 33)
Diols (Group 7)	Alpha beta-unsaturated carboxylic acids (Group 20)	Phosphates and phosphonic acids (Group 34)
Alpha, beta-unsaturated alcohols (Group 8)	Primary monoamines (Group 21)	Bromo or indo benzenes (Group 35)
Alcohol-ethers (Group 9)	Secondary mono amines (Group 22)	Benzyl chlorides and bromides (Group 36)
Aldehydes (Group 10)	Tertiary amines (Group 23)	Phenyl ethenes or acetylenes (Group 37)
Alpha beta-unsaturated aldehydes (Group 11)	Diamines and polyamines (Group 24)	Phenyl alcohols (Group 38)
Alpha halogenated ketones (Group 12)	Nitrates, chloro nitrates and cyclo nitriates (Group 26)	Alkoxy benzenes (Group 39)
Diones (Group 13)	Alkyl hydrazine (Group 27)	Benzaldehydes with alkyl, halogen or alkoxy group (Group 40)

The list of compounds along with the RMSE values calculated through target passive sampling model is shown in Table 1 in supporting information. The models were developed by using the equations of partition coefficients. Table 3 displays the equations and sources of partition coefficients used in this study.

Table 3 Equations and references for each category of the partition coefficient

Partition coefficient	Equation	References
Phospholipid-water	0.26 + 0.85E − 0.75S + 0.29A − 3.84B + 3.35V	Poole et al., 2013
Polydimethylsiloxane-water	0.268 + 0.601E − 1.416 S −2.523 A − 4.107 B + 3.637 V	Rabia et al., 2022
Polyoxymethylene-water	-0.37 + 0.39 E + 0.28 S - 0.46 A - 3.98 B + 2.98 V	Endo et al., 2011
Polyacrylic-water	-0.12 + 0.50E -0.16S +0.16A – 4.0B+3.53V	Endo et al., 2010
Polyethylene-water	-0.943 S- 2.945 A -4.060B + 2.035V+0.459	Khawar and Nabi., 2021

3.2. Phase 1: Dissection and reassembling of target passive sampler model:

In this phase, the experimental values for 1952 chemicals were collected from the literature Wang et al., 2016. The partition coefficients for different types of passive samplers to water were calculated by using equations (listed above in Table 2). These equations were taken from the literature (PA-water from Endo et al., 2010, PE-water from Khawer & Nabi, 2021, phospholipid-water from Poole et al., 2013, PDMS-water from Rabia et al., 2022, POM-water from the research paper of Endo et al., 2011). The data for Abraham Solvation Descriptors (E, S, A, B, V, L) was taken from LSER Database-UFZ (www.ufz.de/lserd/).

3.3. Phase 2: Evaluation of four types of passive samplers:

In this phase, the target passive sampler model was formed from target lipid model as mentioned in hypothesis. Then, the compounds are divided into 87 groups (shown in

Table 5 in supporting information).

Residuals and one-to-one plot:

In this phase, the root mean square for each group was calculated. Bar charts were formed on the rmse values. Then, the one-to-one plots for every group were constructed based on rmse values. Charts and plots were constructed to see the difference among model values. Residuals were calculated by subtracting predicted value from experimental values as shown in equation 8:

$$\text{Residual} = \text{Experimental value} - \text{Predicted value} \quad \ldots\ldots\ldots\ldots \quad (8)$$

The values obtained after applying methodology of Phase 1 and Phase 2 are mentioned in the Table 6 in supporting information. One-to-One Plots were constructed in the results section of phase 2. These plots are commonly called Identity Line and are extremely beneficial in statistical analysis. In this thesis, One-to-One Plots were drawn for different classes of chemicals and mode of actions. These plots define the upper and lower limits for the data sets by ±1 log unit. The best results shown by compounds used in baseline model (Alkanes, cycloalkanes alcohols, ethers, ketones, benzenes with alkyl, flouro or chloro groups.

3.4. Phase 3: Inter-specific differences in toxicities for fish and daphnia:

In this study, the dataset for daphnia was formulated. Then, it was divided on the basis of different groups. The data sheet divided into groups based on compounds containing Phosphorus, Oxygen, Chlorine, Sulphur, Nitrogen and Hydrocarbons. The tables 7, 8, 9 and 10 in appendices show the values of residuals (experimental – predicted value) and RMSE of chemicals for Daphnia. Then, the comparison was done between fish and Daphni

CHAPTER 4

4. Results and Discussions

The results for every phase are shown separately. In phase 1, the validation of 100 mmol is represented by box plot and by the validation insight of regression analyses for four models. Then, the computation of LC_{50} was done from target lipid model to passive sampler model. In phase 2, comparison of results was carried out by the root mean square error. That comparison has been done on the basis of chemical classes of 87 groups; every group has specific number of chemicals. Bar-charts were drawn on the rmse values of those chemicals and one-to-one plots were formulated to check agreement between experimental and predicted values. In phase 3, I did comparison between fish and daphnia. Interspecific difference for critical target lipid body burden was analyzed with the help of box plots. Then, the comparison of hydrocarbons for fish and daphnia was carried out with the help of calculated rmse values.

4.1 Phase 1: Validation of 100 mmol

The validation of the study has been done by box plot. Figure 1 shows the box plot which is used to display a reaction pattern of data. The plot offers a practical method for visualizing the range and other traits of responses for a group of chemicals.

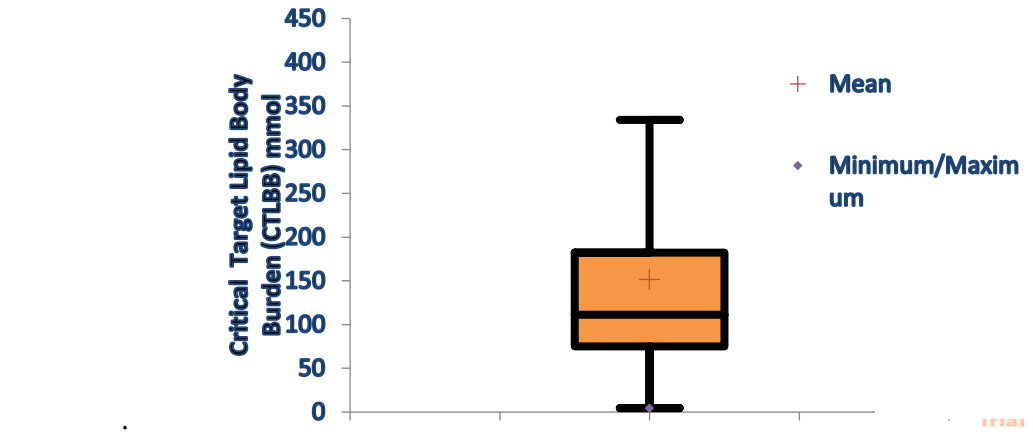


Figure 1 Figure above shows the box plot for 156 observations of Group 1. Total 156 chemicals were taken from Group 1 (shown in Table no. 6 in supporting information). The median marks the mid-point of the data and is shown by the line that divides the box into parts. The median is 114.8 which was close to the 100. The first and third quartiles are the boundaries of the red area, which is known as the inter-quartile range, while the black center line is the median for each dataset (IQR). The extreme ends are the points where the lines extending from the IQR. The plus sign (+) shows the mean value. Scores outside the middle 50% are represented by the upper and lower whiskers. Compared to the middle quartile groups, whiskers span a broader range of scores.

The total number of observations/chemicals in Group 1 are 156, the classes of these chemicals are alkanes, cyclo, chloro, flouro, alcohol, ether, ketone, unsaturated, benzene and trifluoromethyl. It is shown (fig. 1) that the median is 114.8 which is close to 100. It means that the study proves that the assumption of 100 mmol is valid for fish. The 100 mmol is the concentration in bio-membrane. The concentration was taken as 100 mmol/kg (Critical Target Lipid Body Burden). Figure above exhibits the minimum value 4.5, maximum value 1075.9, 1st quartile 75.0 and the 3rd quartile 182.24.

4.1.1 Validation insight from regression analysis

Regression analysis is a set of statistical methods used for the estimation of relationships between a dependent variable and one or more independent variables.

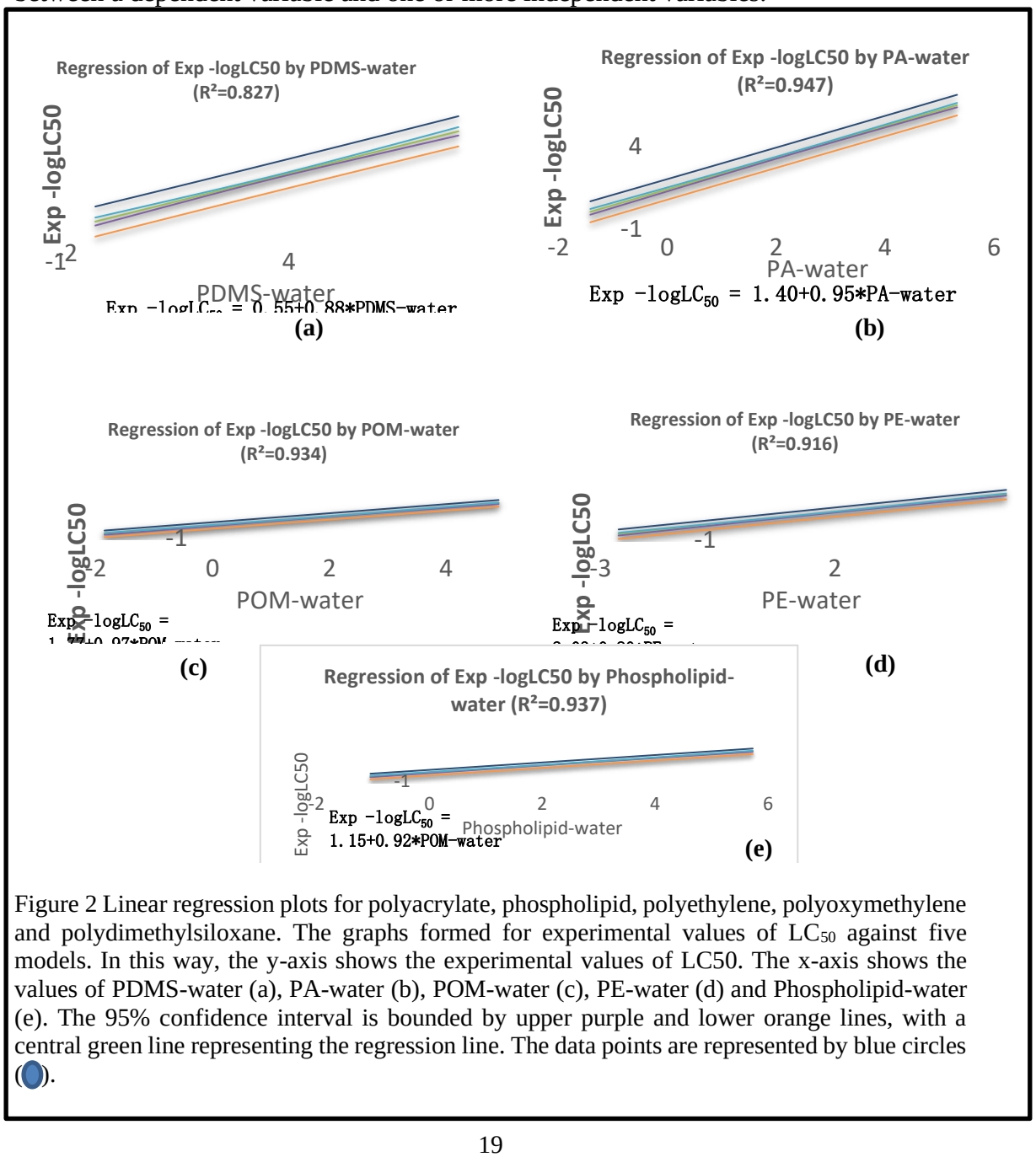


Figure 2 Linear regression plots for polyacrylate, phospholipid, polyethylene, polyoxymethylene and polydimethylsiloxane. The graphs formed for experimental values of LC_{50} against five models. In this way, the y-axis shows the experimental values of LC50. The x-axis shows the values of PDMS-water (a), PA-water (b), POM-water (c), PE-water (d) and Phospholipid-water (e). The 95% confidence interval is bounded by upper purple and lower orange lines, with a central green line representing the regression line. The data points are represented by blue circles ( ).

Regression analyses can be utilized to assess the strength of the relationship between variables and for modeling the future relationship between them. The observations are 115 chemicals of group 1 comprised of different classes: alkanes, cyclo, chloro, flouro, alcohol, ether, ketone, unsaturated, benzene and trifluoromethyl (shown in Table 6). The equations for all models show intercepts. The values of intercepts are close to 1. Meanwhile, the R2 value for PA (Polyacrylate) is 0.947 (a), the R2 value of PDMS (Polydimethylsiloxane) is 0.827 (b), for POM (Polyoxymethylene) it is 0.934 (c), and for PE (Polyethylene) it is 0.916 (d). Given the R2, 95% of the variability of the dependent variable (Exp-logLC50) is explained by the explanatory variable. The information brought by the explanatory variables is significantly better. Hence, it can be validated from regression analyses that the models are valid for prediction purposes, as shown by the good agreement of the results of all the models.

4.1.2 Computation of LC_{50} from Target lipid model to Passive sampler model

The table below shows the computation of Lethal concentration from Target Lipid model to Passive sampler. There are 1952 chemicals from which only few are shown here, only some data of PA-water.

Table 4: The table displays names, experimental values of chemicals, the value of PA-water, predicted values, residuals, values taken from Wang models and ECOSAR residual values.

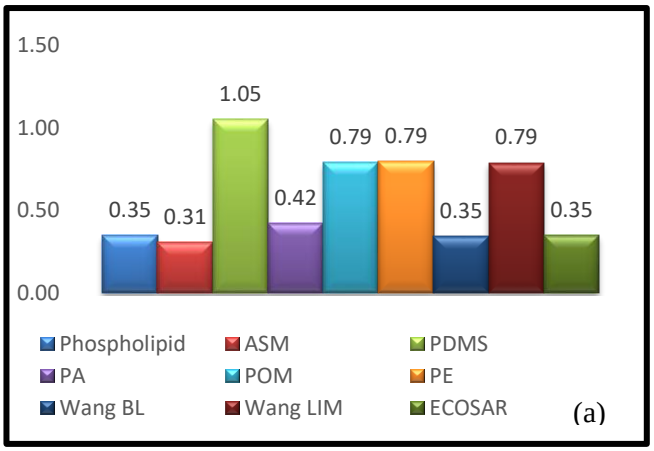
Name	Exp - logLC50	PA-water	Model PA - logLC50	Residual Exp-PA	Wang BL	Wang LIM	ECOSAR Residual
n-Octane	5.43	3.93	4.93	0.50	-0.30	-0.37	0.31
Methylcyclohexane	4.67	3.22	4.22	0.45	0.32	-0.13	0.15
decalin	5.57	4.36	5.36	0.21	0.70	0.39	0.51
cis-1-Isopropyl-4-methylcyclohexane	5.87	4.63	5.63	0.24	-0.20	-0.18	0.16
1-Chlorobutane	2.98	2.13	3.13	-0.15	-0.51	-1.20	-0.61
1-Chlorooctane	5.38	4.01	5.01	0.37	0.23	0.00	0.03
Dichloromethane	2.44	1.42	2.42	0.01	0.17	-0.86	-0.06
1,1-Dichloroethane	2.69	1.66	2.66	0.03	-0.05	-0.95	-0.18
1,3-Dichloropropane	3.08	1.83	2.83	0.25	0.16	-0.69	-0.29
1,1,1-Trichloroethane	3.28	2.12	3.12	0.15	-0.08	-0.81	-0.42
1,2,3-Trichloropropane	3.46	1.76	2.76	0.70	0.30	-0.49	-0.08
Tetrachloromethane	3.84	2.47	3.47	0.36	0.18	-0.47	0.35
1,1,2,2-Tetrachloroethane	3.84	2.49	3.49	0.35	0.57	-0.18	0.59

The table no. 3 shows: experimental value of LC_{50}, the value of PA-water (after applying equation), the values after applying model, the residual value, the Models of Wang (for comparison) and the residual values of ECOSAR. This table is shown as an example, all the other remaining values calibrated from target lipid model to passive sampler model are present in Table 5 in supporting information.

4.2 Phase 2: Comparison of results based on Root Mean Square Error (RMSE)

The values of root mean square error shown in the table 2 in supporting information. RMSE is an error that reveals the difference between the experimental LC_{50} and predictive LC_{50} values on logarithmic scale for established models (PDMS, POM, PA and PE). The comparison has also been done with Abraham Solvation Model (ASM), Wang (Baseline Model), Wang (Less inert model) and ECOSAR. ECOSAR is known as the Ecological Structure Activity Relationship. It is a tool for predicting toxicity of aquatic industrial chemicals developed by U.S Environmental Protection Agency and Syracuse Research Corporation.

Figures below exhibit the root mean square error for four predictive passive samplers i.e., POM, PA, PE, PDMS, and calibrated models Wang (BL) and Wang (LIM). They display the difference among the values of various models. Out of the four types of passive samplers, mostly the Polyacrylate (PA) exhibits the best results when compared with the experimental LC50 values. PA shows the RMSE value of 0.42, other passive samplers such as PDMS shows value 1.05. PDMS, PE and POM do not perform greatly for this model, they have high RMSE values. Polyacrylate have ability to withstand most chemicals and solvents. The comparison among passive models and other models are representing in figures 3 to figure 10:



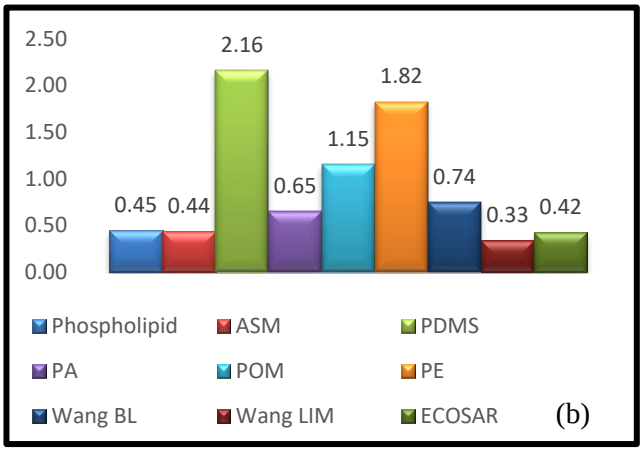


Figure 3(a) Baseline models (Alkanes, cycloalkanes alcohols, ethers, ketones, benzenes with alkyl, flouro or chloro group (Group 1)). (b) Less inert models (phenols and anilines with alkyl, fluoro, or chloro groups) (Group 2). RMSE values of the compounds shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for all respective categories. In this figure, the vertical axis shows RMSE values.

Figure 3 (a) exhibits that in group 1, there are total 159 chemicals available but the data to calculate RMSE value is applicable for 115 chemicals. Hence, 115 chemicals in Group 1 show RMSE values. The figure above also illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models. Figure 3 (b) shows the RMSE value of compounds used in less inert models (phenols and anilines with alkyl, fluoro or chloro groups) (Group 2). There are 73 chemicals in Group 2 which show RMSE values. Figure (b) illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models.

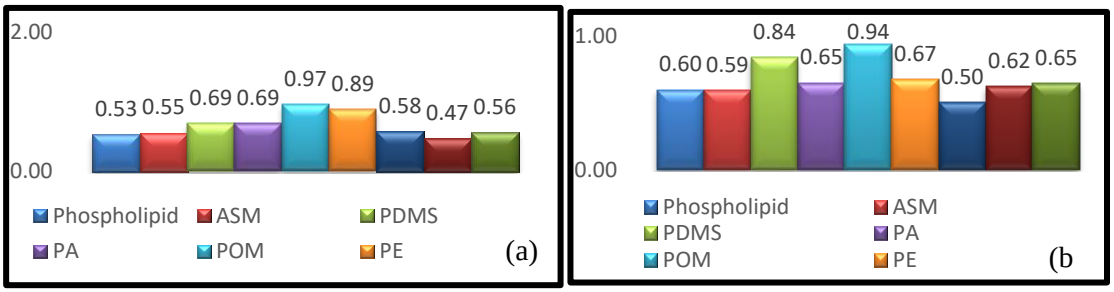


Figure 4 (a) Alkanes with bromo group (Group 3). (b) Alkenes, dienes, alkynes with chloro group (Group 4). RMSE values of the compounds shown in figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portray POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents value for respective categories. In this figure, the vertical axis shows RMSE

Figure 4 (a) represents that in group 3, there are 9 chemicals available which show RMSE values. The figure above illustrates that the RMSE value of polyacrylate (PA) and PDMS are less than other predicted passive sampler models. Figure 4 (b) shows the RMSE values of Alkenes, dienes, alkynes with chloro group (Group 4). There are 15 chemicals in Group 4 which show RMSE values. Figure 4 (b) above illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models.

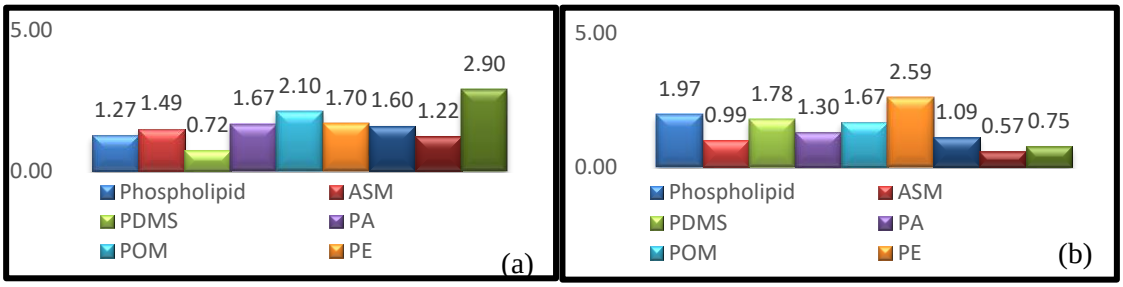


Figure 5 (a) Allylic and propargyl halogens (Group 5). Beta-halogenated alcohols (Group 6). RMSE values of the compounds are shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for respective categories. In this figure, the vertical axis shows RMSE values.

Figure 5 (a) represents rmse values of allylic and propargyl halogens in group 5, there are 3 chemicals available in this group which show RMSE values. Figure 5(a) above illustrates that the RMSE value of PDMS is less than other predicted passive sampler models. Figure 5 (b) shows the RMSE values of beta-halogenated alcohols (Group 6). There are 6 chemicals in Group 6 which show RMSE values. The figure above illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models.

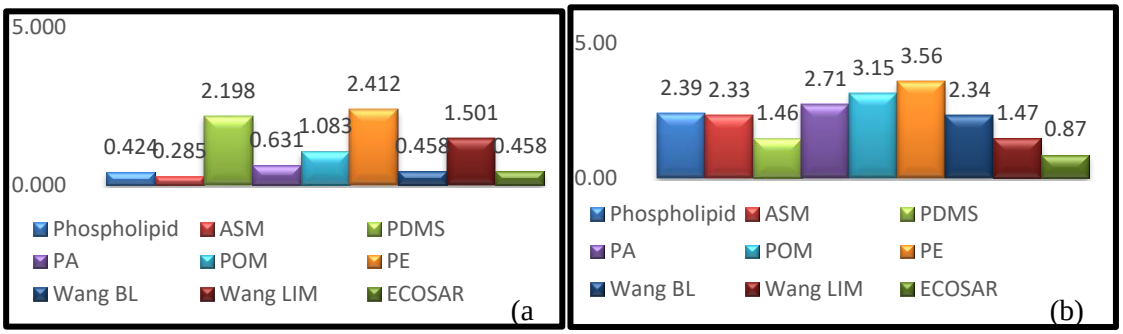


Figure 6 (a) Diols (Group 7). (b) Alpha, beta-unsaturated ketones (Group 8). RMSE values of the compounds are shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for those categories. In this figure, the vertical axis shows RMSE values.

Figure 6 (a) exhibits the RMSE values of Diols (Group 7). In group 7, there are 9 chemicals available but the data to calculate RMSE value is applicable for 3 chemicals. Hence, 3 chemicals in Group 7 show RMSE values. Figure 6 (a) above also illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models. Figure 6 (b) shows the RMSE value of alpha, beta Unsaturated ketones (Group 8). There are 8 chemicals in Group 8 which show RMSE values. Figure 6 (b) above also illustrates that the RMSE value of PDMS is less than other predicted passive sampler models.

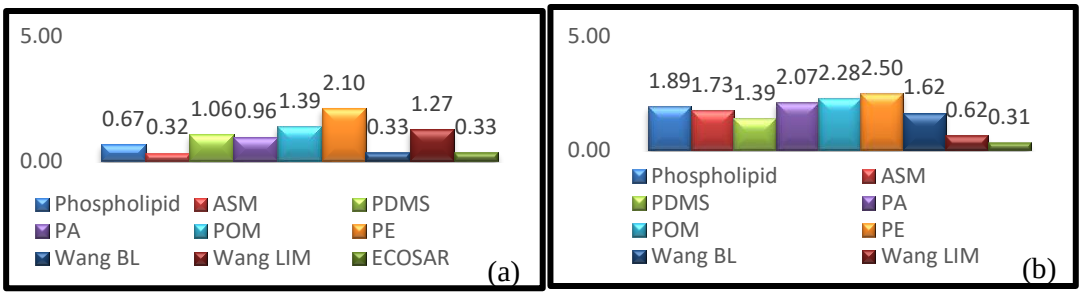


Figure 7 Alcohols-ethers (Group 9). (b) Aldehydes (Group 10). RMSE values of the compounds are shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for all respective categories. In this figure, the vertical axis shows RMSE values.

Figure 7 (a) represents the RMSE values of Alcohols-ethers (Group 9). In group 9, there are 9 chemicals available which show RMSE values. The figure above illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models. Figure 7(b) exhibits the RMSE values of Aldehydes (Group 10). There are 8 chemicals in Group 10 which show RMSE values. The figure above illustrates that the RMSE value of PDMS is less than other predicted passive sampler models.

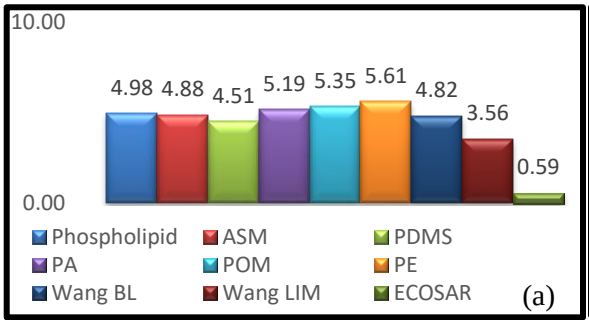


Figure 8 (a) Alpha beta-unsaturated ketones (Group 11). (b) Alpha halogenated ketones (Group 13). RMSE values of the compounds are shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for all respective categories. In this figure, the vertical axis shows RMSE values.

Figure 8 (a) exhibits the RMSE values of Aldehydes (Group 10). There are 8 chemicals in Group 10 which show RMSE values. Figure 8 (a)above illustrates that the RMSE value of PDMS is less than other predicted passive sampler models. Figure 8(b) represents that the RMSE value of alpha, beta-unsaturated ketones (Group 11). There are 2 chemicals in Group 11 which show RMSE values. Figure 8 (b) above illustrates that the RMSE value of PDMS is less than other predicted passive sampler models.

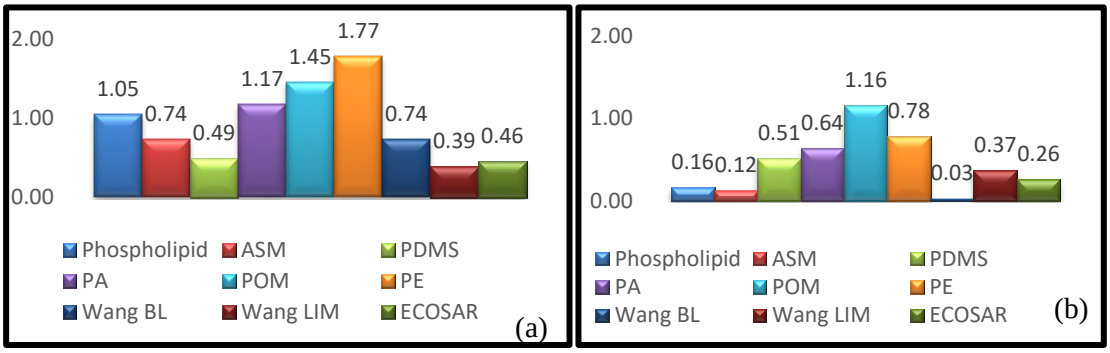


Figure 9 (a) Alpha-halogenated ketones (Group 13), (b) Alpha, beta-unsaturated ketones (Group 14). RMSE values of the compounds are shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for all respective categories. In this figure, the vertical axis shows RMSE values.

Figure 9 (a) shows the RMSE value of alpha-halogenated ketones (Group 13). There are 2 chemicals in Group 13 which show RMSE values. Figure 9(a) above illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models. Figure 9 (b) exhibits that the RMSE value of alpha, beta-unsaturated ketones (Group 14). One chemical in Group 14 show RMSE values. Figure 9(b) above also illustrates that the RMSE value of PDMS is less than other predicted passive sampler models.

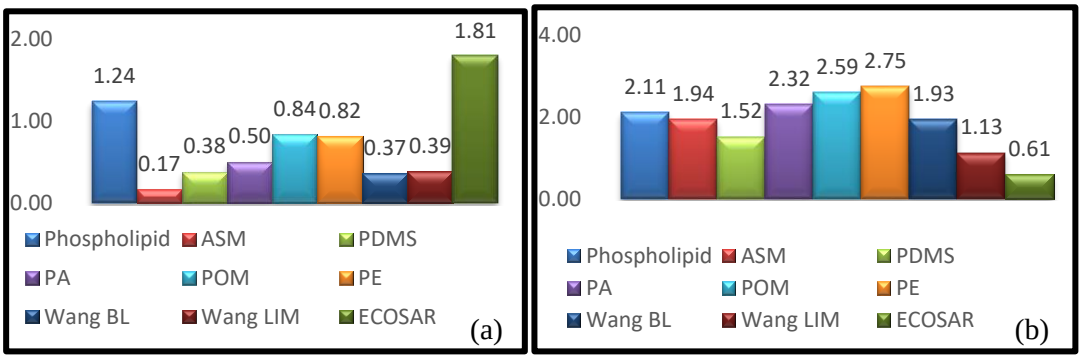


Figure 10 (a) Alpha-halogenated esters (Group 16), (b Alpha, beta-unsaturated esters (Group 17). RMSE values of the compounds are shown in the figures above. The horizontal axis (x) of the bar chart represents the Wang models, passive samplers, Abraham solvation model, and ECOSAR. Blue color represents phospholipid, red color illustrates Abraham solvation model, green color shows PDMS, purple color shows PA, light blue color portrays POM, orange color illustrates PE, dark blue color represents Wang baseline model, maroon color shows Wang less inert model and dark green denotes ECOSAR. The vertical (y) axis represents the value for all respective categories. In this figure, the vertical axis shows RMSE values.

Figure 10 (a) above illustrates the RMSE value of alpha-halogenated esters (Group 16). Only one chemical in Group 16 shows RMSE values. Figure 10 (b) above also illustrates that the RMSE value of PDMS is less than other predicted passive sampler models. Figure 10 (b) represents the RMSE value of alpha, beta-unsaturated esters (Group 17). There are 13 chemicals in Group 17 which show RMSE values. Figure 17 (b) above illustrates that the RMSE value of polyacrylate (PA) is less than other predicted passive sampler models.

4.2.1 Comparison between experimental and predicted values:

Figure 11 to figure 24 represent the graphical comparison between experimental values with predicted values obtained by inputting calculated values of four models. The dotted line in the middle shows 1:1 agreement, upper and lower dotted lines indicate 1:2 agreement between experimental and predicted values. It is analyzed that the best results are shown by compounds used in baseline model (Alkanes, cycloalkanes alcohols, ethers, ketones, benzenes with alkyl, flouro or chloro groups).

Graphical representation for compounds in baseline models:

Baseline model compounds in Group 1 composed of Alkanes, cycloalkanes alcohols, ethers, ketones, benzenes with alkyl, flouro or chloro groups.

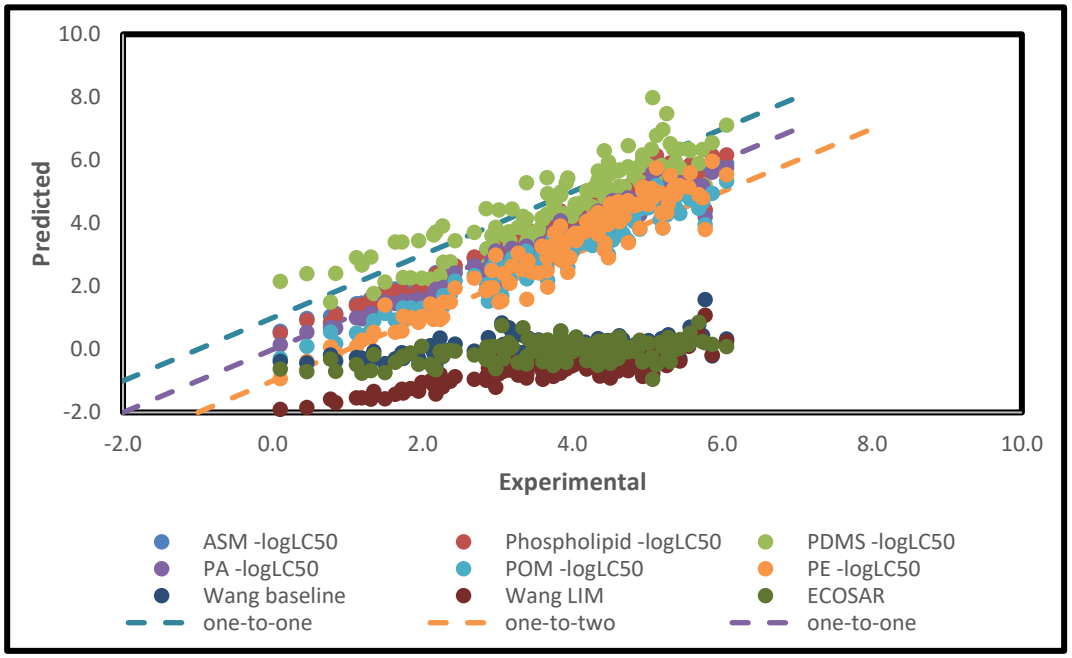


Figure 11 Compounds used in baseline model (Alkanes, cycloalkanes alcohols, ethers, ketones, benzenes with alkyl, flouro or chloro groups). The horizontal axis (x) indicates the experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against the experimental values.

Figure 11 exhibits that the polyacrylate and polyethylene fall within the upper and lower limit with a difference of 1 log unit. The data of the 2 models is very closer to the experimental LC_{50} data.

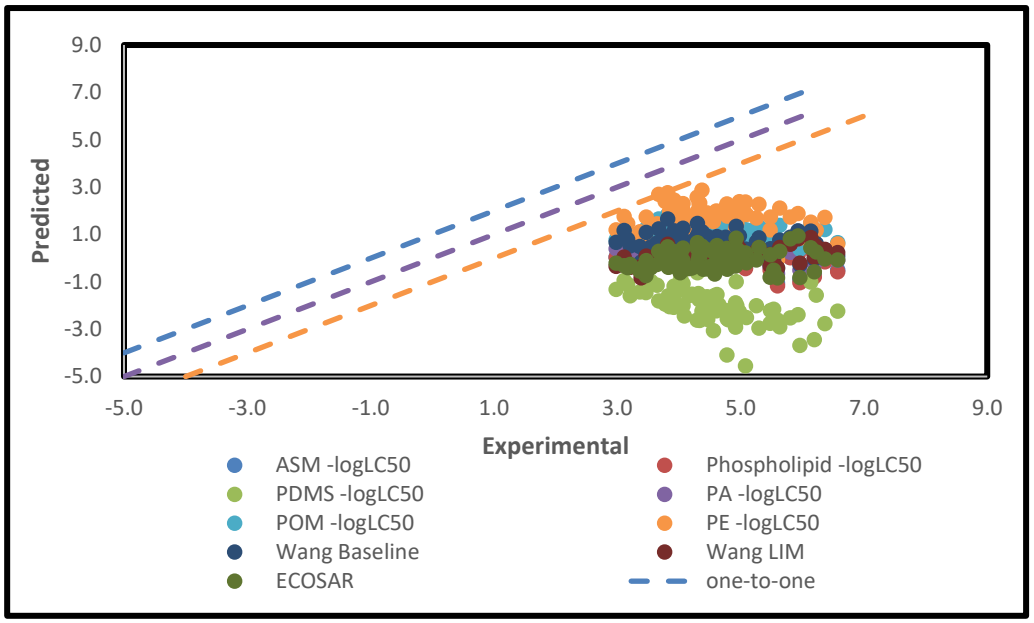


Figure 12 Compounds used in less inert model (Phenols and anilines with alkyl, fluoro or chloro groups). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 12 shows the comparison of compounds in less inert model (phenols, anilines with alkyl, fluoro or chloro group. Figure 12 represents that most of the models not fall within the upper and lower limit in the plot. Only some dots of model polyethylene touch the lower limit of the graph.

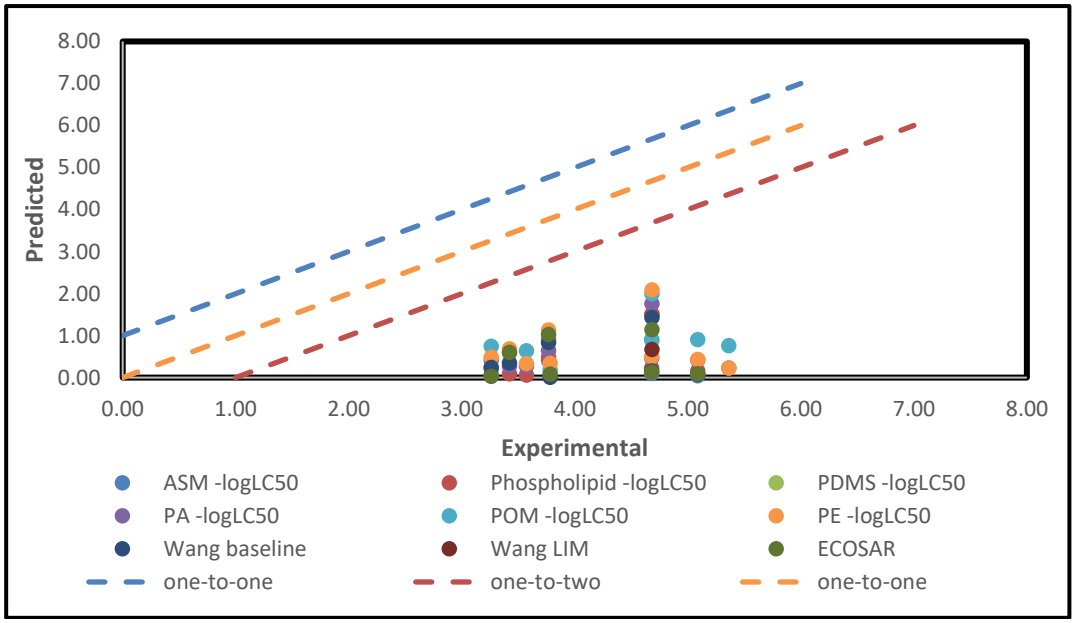


Figure 13 Alkanes with bromo group (Group 3). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 13 represents that the values of alkanes with bromo group (Group 3) do not fall within 1:1 and 1:2 agreement.

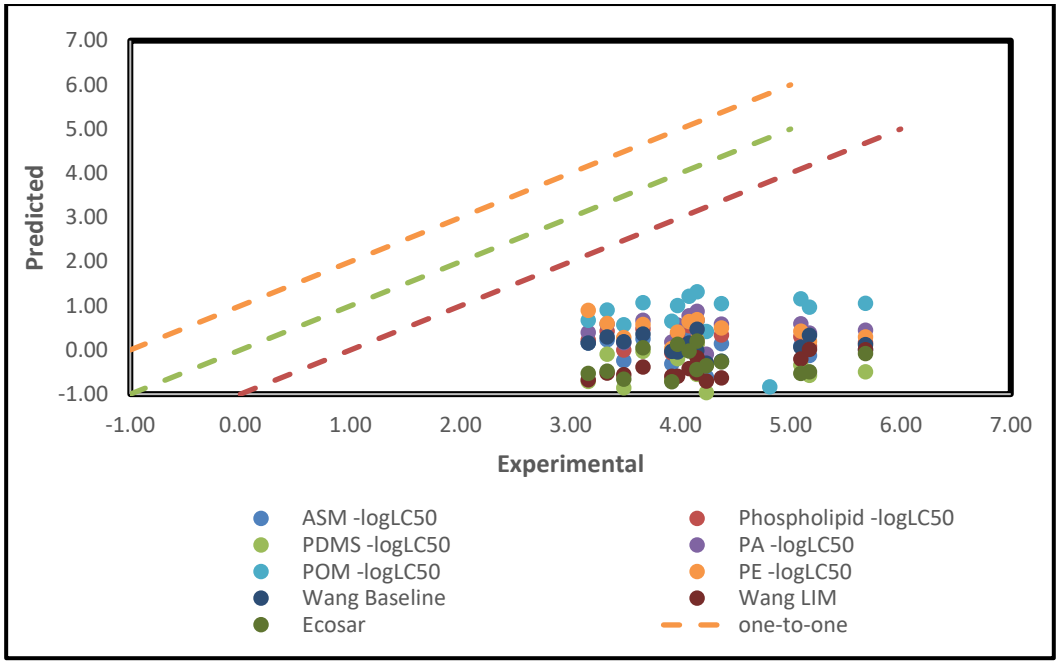


Figure 14 Alkenes, dienes, alkynes with chloro group (Group 4). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 14 shows that the values of Alkenes, dienes, alkynes with chloro group (Group 4) do not fall within 1:1 and 1:2 agreement.

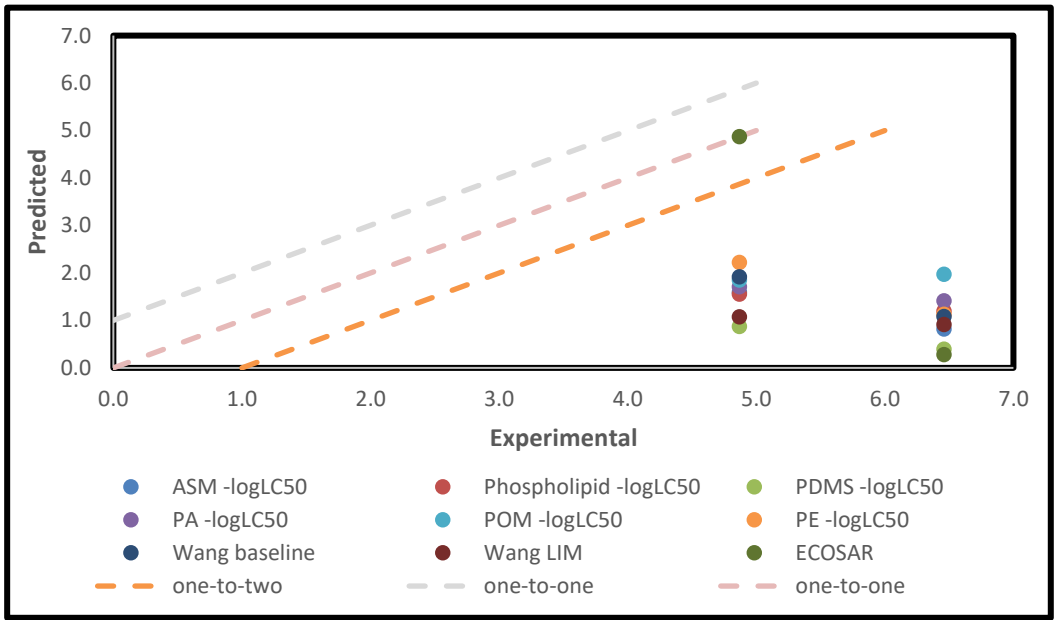


Figure 15 Allylic and propargyl halogens (Group 5). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 15 represents that the values of Allylic and propargyl halogens (Group 5) do not fall within 1:1 and 1:2 agreement.

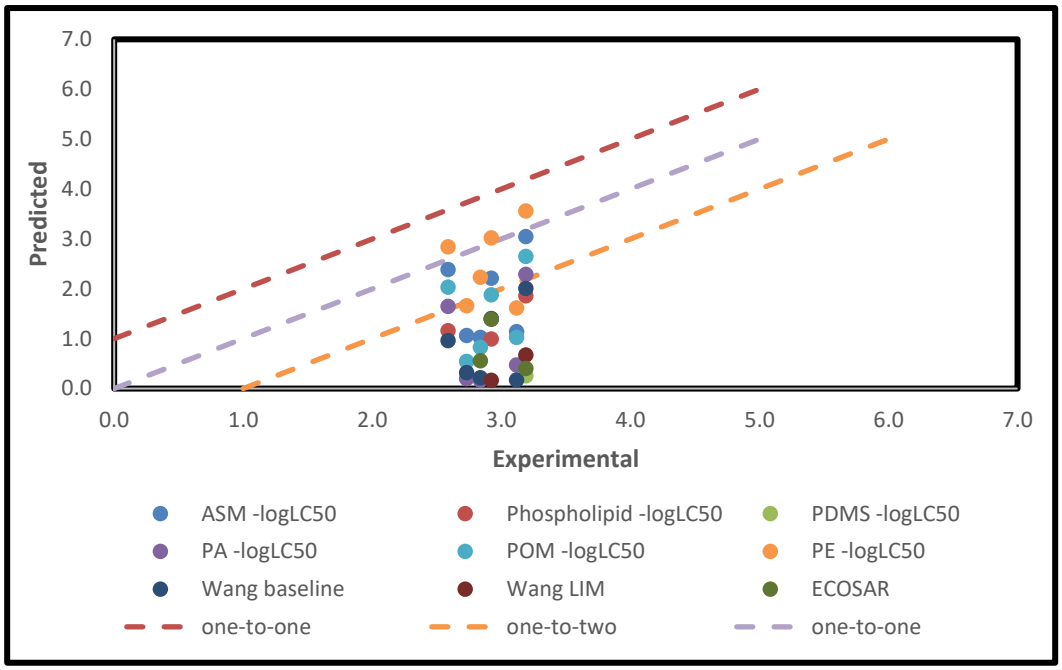


Figure 16 Beta-halogenated alcohols (Group 6). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 16 represents that some values of PE and Wang Baseline compounds of beta-halogenated alcohols falls within 1:1 and 1:2 agreement.

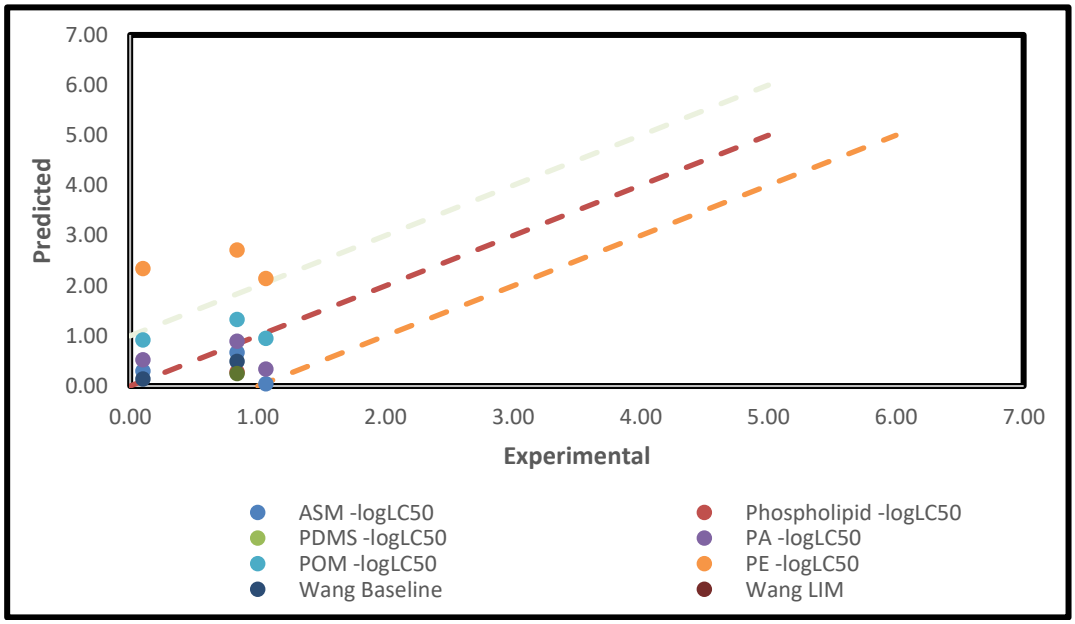


Figure 17 Diols (Group 7). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

The figure shows that some values of model PA and POM compounds of diols fall within 1:1 and 1:2 agreement.

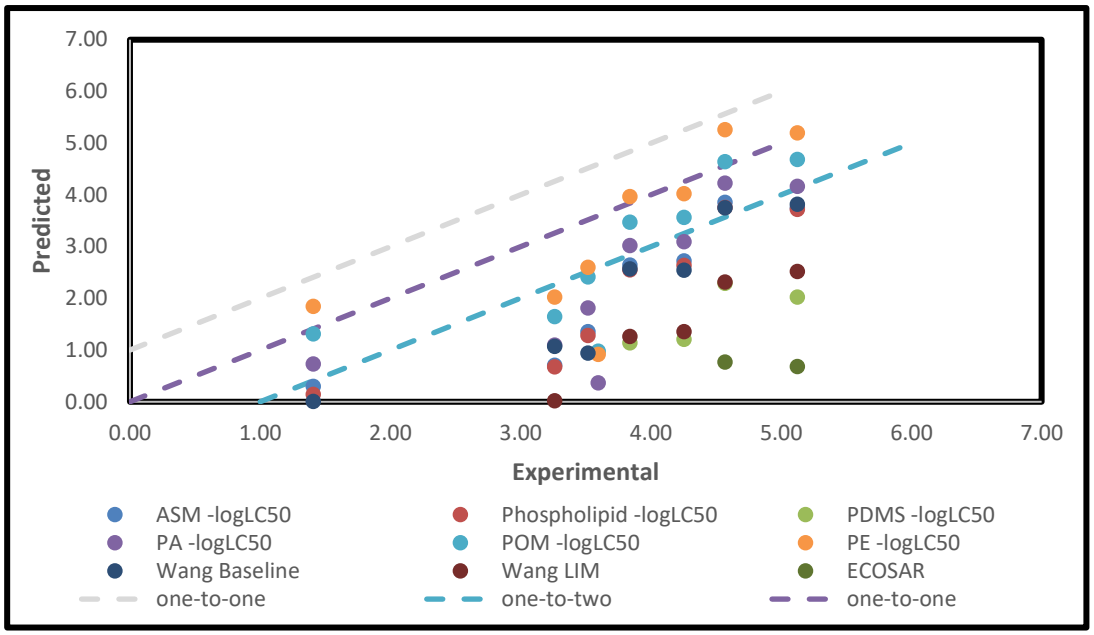


Figure 18 Alpha, beta-unsaturated alcohols (Group 8). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 18 represents that some values of model PE and POM of compounds Alpha, beta-unsaturated alcohols fall within 1:1 and 1:2 agreement.

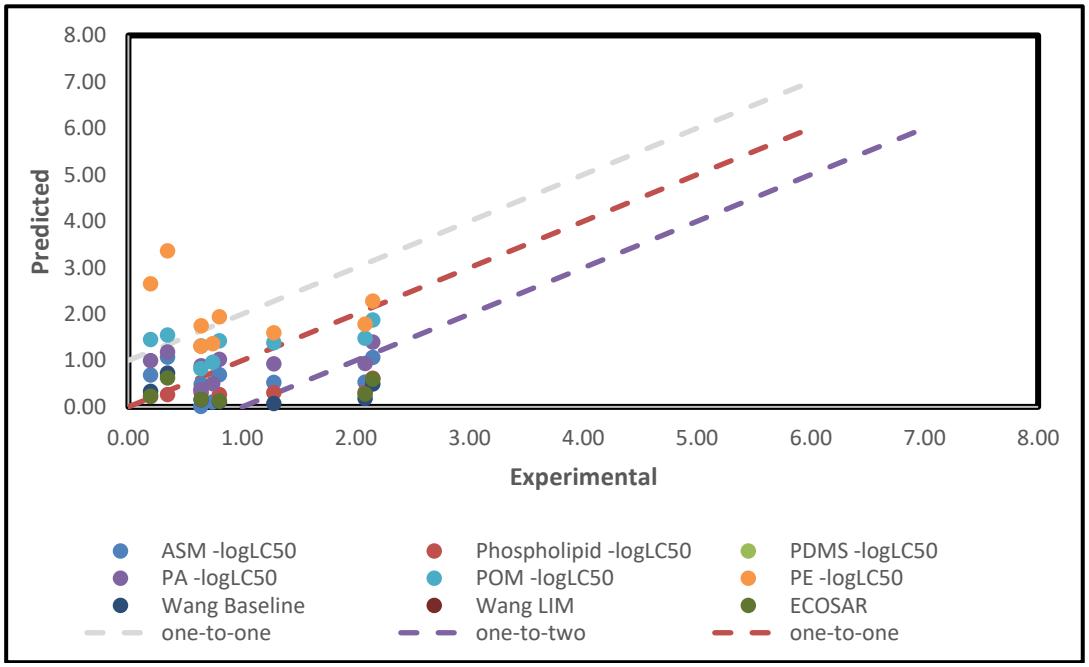


Figure 19 Alcohols-ethers (Group 9). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 19 illustrates that some values of model PE and PA of compounds Alcohols-ethers fall within 1:1 and 1:2 agreement.

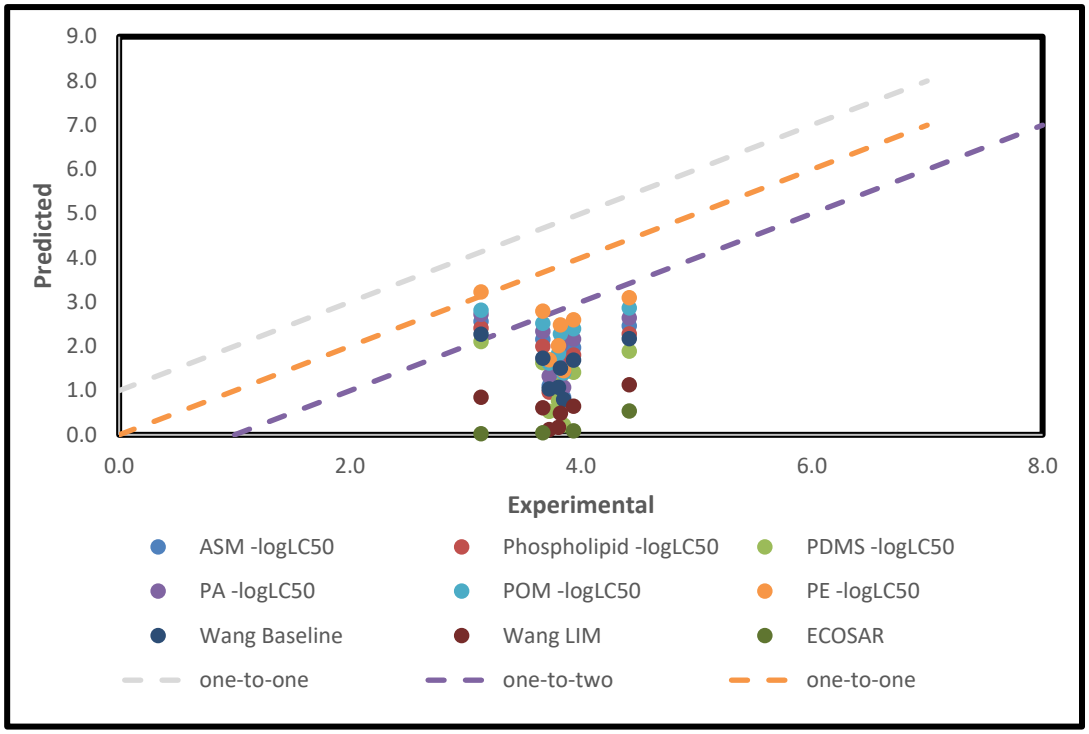


Figure 20 Aldehydes (Group 10). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 20 shows that some values of model PE of aldehydes fall within 1:1 and 1:2 agreement. However, most of the values fall out of the 1:1 and 1:2 agreement.

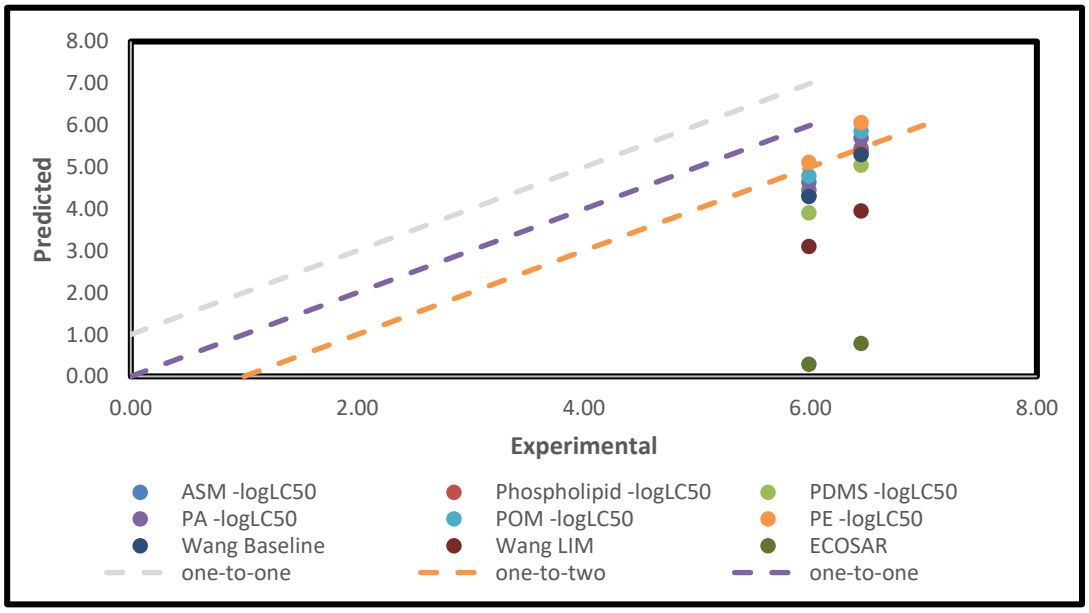


Figure 21 Alpha, beta-unsaturated aldehydes (Group 11). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 21 represents that no values of any model fall within 1:1 and only few touches the 1:2 agreement.

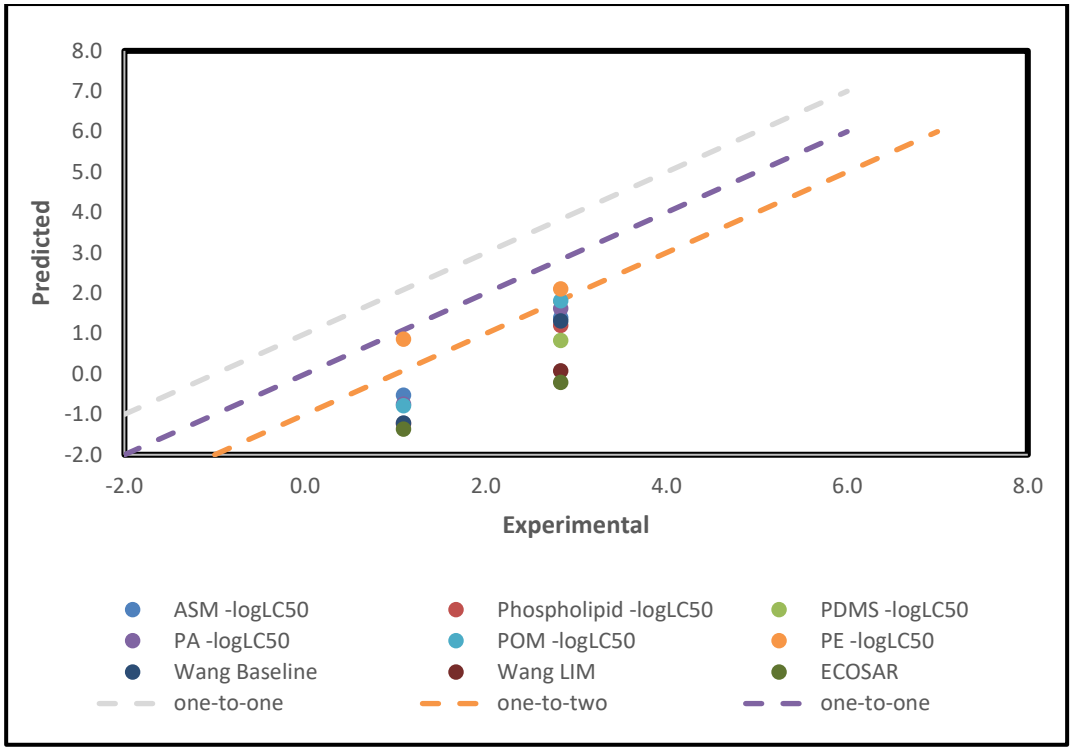


Figure 22 Diones (Group 13). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 22 shows that most of the values of group 13 do not fall within 1:1 and 1:2 agreement.

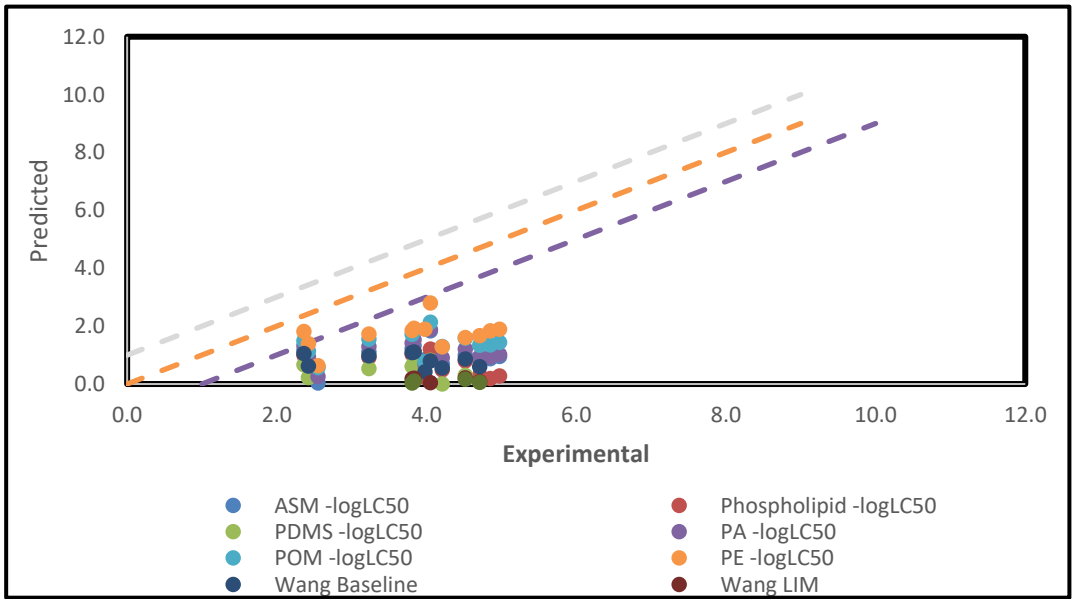


Figure 23 Esters, bromo esters and diesters (Group 15). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 23 shows that most of the values of group 15 do not fall within 1:1 and 1:2 agreement.

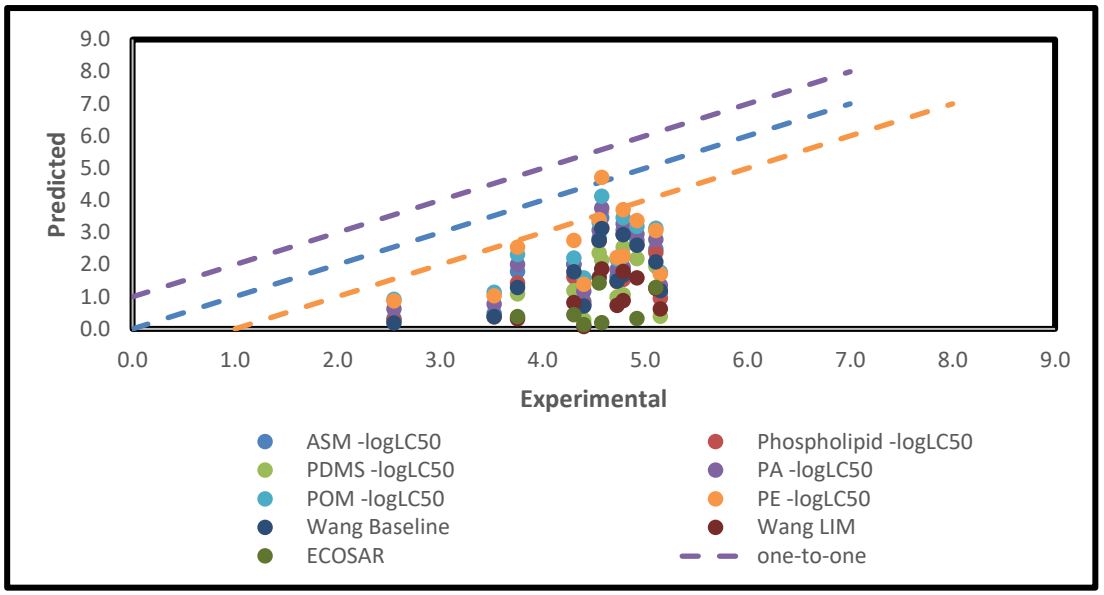


Figure 24 Alpha, beta-unsaturated esters (Group 17). The horizontal axis (x) shows experimental values and the vertical axis (y) shows the predicted values. Predicted values plotted against experimental values.

Figure 24 shows that most of the values of Alpha, beta-unsaturated esters do not fall within 1:1 and 1:2 agreement. (Other plots for rest of the data is shown in the supporting information). It is analyzed that from all 87 groups, only baseline compounds show the best results.

4.3 Phase 3: Comparison between fish and Daphnia

4.3.1 Interspecific difference

The Critical Target Lipid Body Burden is the backbone of this study. Results show that CTLBB (mmol) is valid for organic chemicals of fish but not for chemicals of Daphnia.

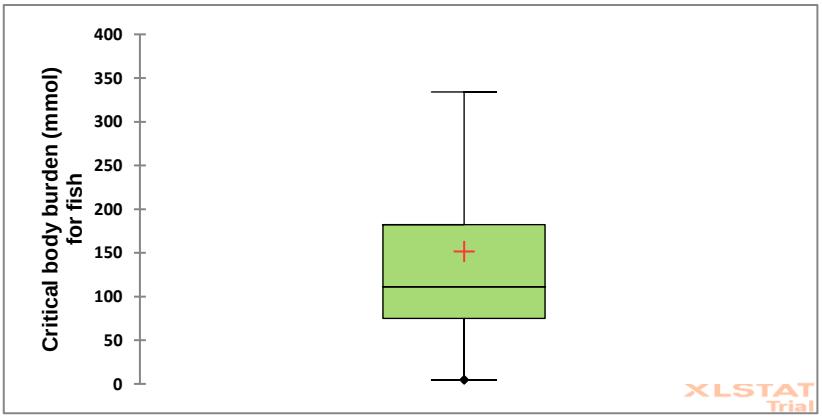


Figure 25 Boxplot for organic chemicals (Fish). Figure displays the box plot for 156 observations of Group 1 (shown in Table no. 6 in Supporting information). Total 156 chemicals were taken from Group 1. The median marks the mid-point of the data and is shown by the line that divides the box into parts. The median is 114.8. The first and third quartiles are the boundaries of the green area, which is known as the inter-quartile range, while the black center line is the median for each dataset (IQR). The extreme ends are the points where the lines extending from the IQR. The plus sign (+) shows the mean value. Scores outside the middle 50% are represented by the upper and lower whiskers. Compared to the middle quartile groups, whiskers span a broader range of scores.

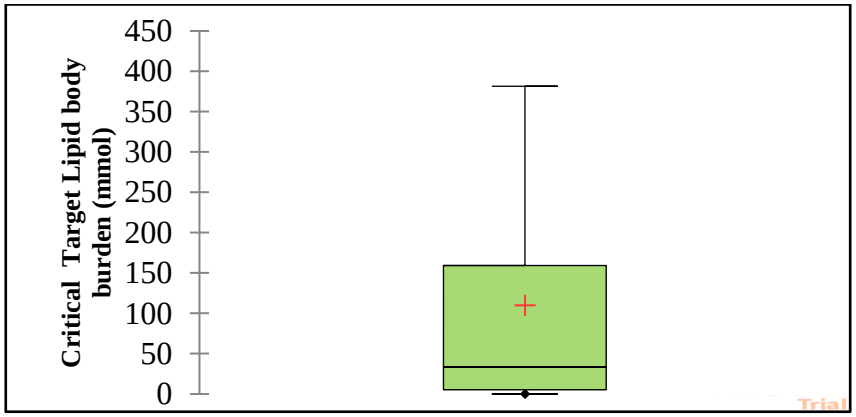


Figure 26 Boxplot for organic chemicals (Daphnia). The median marks the mid-point of the data and is shown by the line that divides the box into parts. The median is 33.51. The first and third quartiles are the boundaries of the green area, which is known as the inter-quartile range, while the black center line is the median for each dataset (IQR). The extreme ends are the points where the lines extending from the IQR. The plus sign (+) shows the mean value. Scores outside the middle 50% are represented by the upper and lower whiskers. Compared to the middle quartile groups, whiskers span a broader range of scores.

Figure 25 and figure 26 show the comparison between fish and Daphnia. Total 156 chemicals/observations were taken for the formation of box plots. The assumption of 100 mmol works fine for fish as the median is 114 closes to 100 mmol but that assumption doesn't work well for Daphnia.

4.3.2 Comparison based on RMSE values

Figure 25 displays the comparison between RMSE values of Fish and Daphnia. The RMSE value of PA is 0.51 for fish and 1.69 for Daphnia, meanwhile the RMSE value of phospholipid is 0.56 for fish and 1.41 for Daphnia, the RMSE values of PDMS is 0.97 for fish and 1.16 for Daphnia, for POM the RMSE value is 0.79 for fish and 1.99 for Daphnia, meanwhile for PE it is 0.53 for fish and 1.76 for Daphnia.

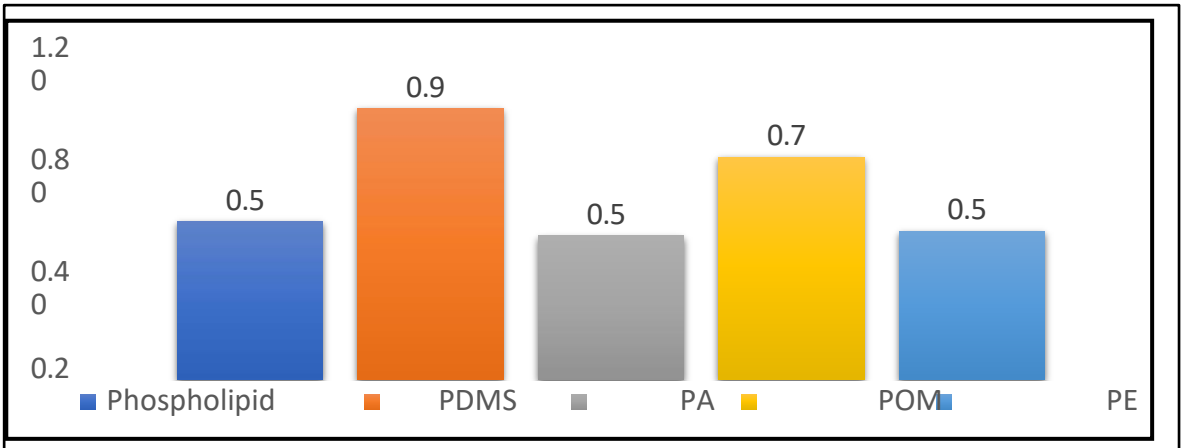


Figure 27 RMSE values of Hydrocarbons (Fish). The horizontal (x) axis shows passive samplers. The vertical axis shows the RMSE values. Dar blue color indicates phospholipid, orange color displays PDMS, grey color denotes PA, yellow color shows POM and light blue color shows PE. For fish, 50 chemicals were taken to construct this bar-chart. These chemicals only have hydrogen and carbon in them. They are pure hydrocarbons.

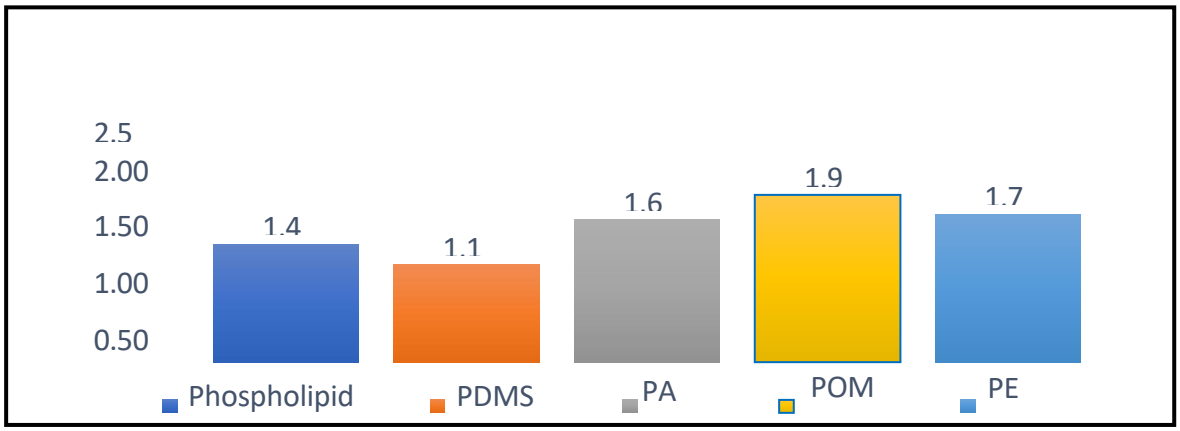


Figure 28 RMSE values of Hydrocarbons (Daphnia). RMSE values of Hydrocarbons (Daphnia). The horizontal (x) axis shows passive samplers. The vertical axis shows the RMSE values. Dark blue color indicates phospholipid, orange color displays PDMS, grey color denotes PA, yellow color shows POM, and light blue color shows PE. For daphnia, 16 chemicals were taken to construct this bar chart. These chemicals only have hydrogen and carbon in them. They are pure hydrocarbons.

These results illustrate that for hydrocarbons, polyacrylate (PA) outperformed all the other passive samplers for organic chemicals with residual value of 0.51 log unit. We can deploy polyacrylate where there is oil spill, this can be done to check the chemical concentration and toxicity.

CHAPTER 5

Conclusion and Recommendations

5.1 Conclusion

It is concluded that the passive sampler can be substituted as an alternative to the target lipid model. Mostly, polyacrylates proved great passive samplers; they have excellent transparency, resistance to breakage, and elasticity. Estimation errors for TPSM fall within the range of experimental error for fish. The analyses related to hydrocarbons show that the models can also be deployed at places such as oil spills. The models can also be used to predict toxicity, safety and risk assessment of chemicals to achieve better ecotoxicological management and prevent adverse health consequences. The assumption of 100 mmol concentration of organic pollutants in the bio-membrane provides reliable results for fish but not Daphnia.

5.2 Limitations and recommendation

There is a need for further investigations. The model is only applicable to neutral organic compounds. It applies to chemicals that show baseline toxicity, not those with excess toxicity. It is recommended that an investigation should be done on ionizable organic chemicals. The description of reactive toxicants can be added. This study also recommends that passive samplers should be used as an alternate to animal testing and experimentation.

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Schwarzenbach, R. P., Gschwend, P. M., & Imboden, D. M. (2003). *Chapter 4 VAPOR PRESSURE 4.1 Introduction 4.2 Theoretical Background Aggregate State and Phase Diagram: Normal Melting Point (Tm), Availability of Experimental Vapor Pressure Data and Estimation Methods Experimental Data Vapor Pressure Estimation Methods for Liquids Entropy of Fusion and the Vapor Pressure of Solids Box 4.1 : Parameters Used to Estimate Entropies of Phase Change Processes 4.5 Questions and Problems*.

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Supporting Information

Supporting information of my thesis comprises of all tables, plots, figures and other relevant detailed description. Supporting information of my thesis can be access by the following link:

https://drive.google.com/file/d/1e7057yXlbiuJ3Ub-K071IBQqq6u4D5dZ/view?usp=sharing