ESTIMATION OF ECO-TOXICITY OF NEUTRAL ORGANIC CHEMICALS BY DEVELOPING AND EVALUATING A NEW TARGET PASSIVE SAMPLER MODEL (TPSM).

By

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DEDICATION

This thesis is dedicated to (Late) Dr. Anwar Baig.

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ACRONYMS INDEX

Abbreviation	Meaning
ASM	Abraham Solvation Model
TLM	Target Lipid Model
WBM	Wang Baseline Model
TPSM	Target Passive Sampler Model
$\mathbf{K_{POM-w}}$	Polyoxymethylene-Water Partition Coefficient
$\mathbf{K_{PE-w}}$	Polyethylene-Water Partition Coefficient
$\mathbf{K_{PA-w}}$	Polyacrylic-Water Partition Coefficient
$\mathbf{K_{PDMS-a}}$	Polydimethylsiloxane-Air Partition Coefficient
PS	Passive Sampler
MDS	Multidimensional Scaling
RMSE	Root Mean Square Error
QSARs	Quantitative Structure Activity Relationships
LC50	Lethal Concentration

Abstract

Various Neutral Organic hydrocarbons exert toxic effect via narcosis (baseline) mode of action. Baseline toxicity is the minimum toxicity, which a chemical offer by disrupting the normal function of biological membranes. Irrespective of the nature of chemical and of species, the critical membrane concentration (C_{-mem}) required to kill 50% of tests organism is fairly constant at 100 mmol/kg (membrane lipid). Exploiting the relationship between bio-membrane-phase concentration (C_{-mem} = 100 mmol/kg) and aquatic-phase concentration (LC50) of chemical ratioed by membrane-water partition coefficient (Km-w) of chemical, scientists successfully estimated the baseline toxicity for broad set of chemicals and diverse aquatic organism. However, this approach requires the knowledge of Km-w, the available experimental dataset of which is thin, and estimation approaches for this property are cumbersome. This limitation motivated us to evaluate the hypothesis that in above relationship, Km-w can be replaced with partition coefficient between passive sampler and water (Kps-w). We referred to the resultant model as Critical Passive-sampling-phase Concentration (CPC) model.

For 596 organic chemicals our model (CPC)'s predictions exhibited a minimum Root Mean Square Error (RMSE) of 0.33 log unit for various types of passive sampler such as Poly Dimethyl Siloxane (PDMS), Poly Oxymethylene (POM), Poly Acrylate (PA) and Poly Ethylene (PE) when compared to the experimental values of median lethal concentration (96-hr LC50 for fish) reported in literature. Whereas, previous model of critical membrane concentration (CMC) returned approximately similar or higher rmse

value (0.35 log unit) when compared to the same experimental dataset. The experimental dataset used for this evaluation was chemically diverse, and represented with typical pure hydrocarbons including acyclic aliphatic, cyclic aliphatic, aromatic hydrocarbon chemicals, Oxygenated hydrocarbons, Nitrogen and Sulphur containing compounds.

Passive sampling is low-cost and low-tech approach, which is known to provide cleaner extracts, better detection limits, easy storage, indexing and archiving. Our study shows that passive samplers can be used as alternative to animal testing for measuring the ecotoxicity. Our model is a useful add-on to passive sampling technique.

Chapter 1

Introduction

1.1 Chemical Footprint of Human Society

In daily life, we need to manage a dramatically expanding number of various chemicals and their mixtures. For the last few decades, the chemical manufacturing industry has flourished worldwide. The global production of chemicals compounds has increased from 1million tons in 1930 to 400 million tons in the 21st century. Nearly, 100,000 different chemicals are enrolled in the European market out of which 10,000 are synthesized in volumes of more than 10 tons, and a further 20,000 chemicals are marketed at 1–10 tons per year. (European Union (EU) white Paper, 2001).

Globally, the quantity of enrolled synthetic compounds is around 65 million, including food coloring and additives, medications, stains and paints for wearing and items, pesticides and numerous others. It is all around perceived that chemical may present high danger to climate and to people, and thus their toxicity must be evaluated. (OECD,2001).

Bio-active substances interact with bio-membranes, setting off explicit mechanisms like initiation of a protein course or opening of a particle channel, which at last prompts an organic reaction. These chemical and physical mechanisms, derived by the chemical composition of the active substances, are unfortunately largely unknown, and therefore it requires to study toxicity via experimental testings and associated procedures. (Fröhlich *et al.*,2014)

1.2 Toxicology and its Assessment

Toxicology (from Greek. Toxicos and Logos) is the study of the harmful effects of chemical products on living organisms. It is the study of symptoms, mechanisms, treatment methods and poisoning detection, especially the study in terms of human and environmental pollution. Substances that can damage the organism and cause the deterioration of its basic life functions, disease or death. If the body absorbs sufficient concentrations, almost any chemical substance can be harmful or fatal. Toxicology stipulates: "Everything is toxic, and nothing is non-toxic, only a dose can be non-toxic." In other words, substances that are generally regarded toxic may be non-toxic or beneficial in small concentrations. On the contrary, if overdose, generally harmless substances can be fatal.

1.3 Lethal Concentration (LC50)

One of the most used indexes is LC50 (Lethal Concentration), which is the dose (mg/kg bodyweight) that is responsible of the death of the 50% of the animals exposed to the different chemical agents. (OECD). The term LC50 is often interchanged with Lethal Dose (LD50).

If a chemical agent or one among its metabolites contains unhealthful effect, it should act with specific locations within the body and be present in decent concentration for a sufficient amount of time. It's thus necessary to grasp what effects an exact substance may have caused, data concerning its chemical structure, the properties of exposure, type

of administration, time and speed of exposure, and also the properties of the organism. In particular, the toxic effects of all substances are caused by ever-changing their organic chemistry and physiological properties. Many toxic reactions result in cellular death and the loss of organ efficiency, which affects the functionality of the entire tissue of the organism, however, there are totally different reactions that don't seem to be due to the cell death, but are supported by the imbalance of physiological and organic chemistry processes. Chemicals influence these processes through numerous mechanisms of action, which might be synthesized as follows:

i. Disruption of traditional ligand-receptor interactions;

ii. Disruption of membrane functions;

iii. Disruption of cellular energy production;

iv. Binding / influence on biomolecules;

v. Toxicity through specific cell death;

vi. Non-fatal genetic modification of body cells

1. 4 Target Lipid Model (TLM)

Target Lipid Model is an estimation model that estimates critical body exposures of a chemical. The model relates toxic endpoints with the accumulation of toxicants in target tissues such as target lipids mainly in membranes, depending on the threshold of the critical effects.

$$\log LC50 = -\log K_{L\text{-}W} + \log C_{\text{-mem}} \quad \textbf{(1.1)}$$

The Cmem (μmol / g lipid) is the concentration of the chemicals in the target lipids that are the sites of action of these types of organic chemicals, and K_{L-W} is the target lipid-water partition coefficient. which is a function of the test species and the endpoint.

K_{L-W} was estimated using the linear solvation energy (LSER) relationship of the lipid-water-poly (LSER) parameter (e.g., LSER-based TLM). The general form of the

LSER model of the parameter poly is given;

$$\log K = eE + sS + aA + bB + vV + c \qquad \textbf{(1.2)}$$

where the lower-case letter parameters (e, s, a, b and v) correspond to the solvent system (e.g., target lipid−water) and parameters in capital letters (E, S, A, B, and V) are chemical interaction terms for solute. The parameter E is the excess molar power, S is the polarization, A is the ability to donate a hydrogen bond, B is the ability to take up a hydrogen bond, and V is the molar volume. The term c is an adaptation constant and takes into account the unit conversions between the various phases and the uncertainties in the partitioning process, which are not explicitly described in the model in equation 1.2. This general LSER modeling approach is widely applied to partition data (AD Redman et al., 2018). neutral chemicals.

According to the values reported in the literature, the mean value for C-mem is approx. 100 mmol / kg (membrane lipid) for a large number of 42 aquatic organisms, including 333 different organic chemicals (Bittermann et al., 2017).

1.5 Partition Coefficient

Equilibrium Partition coefficient (P) is the ratio at equilibrium of compound concentration in a mixture of two immiscible phases. It is therefore a measure of differences in solubility of compound present in two phases. (Kwon, 2001).

The transport and distribution of chemicals in the environment are strongly influenced by the equilibrium distribution properties (Schwarzenbach et al., 2002). Therefore, fateful models for the environmental behavior and environmental impact assessment of chemicals often include partitioning properties, which are defined below;

$$Pxy, i = \left\{\frac{Cx,i}{Cy,i}\right\}_{\text{equilibrium}} \qquad \textbf{(1.3)}$$

Where Pxy,i is partition coefficient between two phases x and y, and Cx,i and Cy,i are the concentrations of toxicant in at partitioning equilibrium present in these phases. Thus, to assess the chemical exposure and transport in the environment, Equilibrium partition coefficients are required.

The Abraham Solvation Model (ASM) has been extensively researched in environmental chemistry to estimate various distribution and transport properties. The model parameterizes the cavity model of the solution and is based on the transition of neutral molecules from the one phase to another. Methods are available for estimating partition coefficients, but there are limitations as discussed earlier for the single parameter LFER. Abraham's solvation model has its own limitations. It has a limited database; only 8000 descriptors for dissolved Abraham substances are available for chemicals and are not available for many existing and emerging non-polar

environmental pollutants such as polychlorinated n-alkanes (PCA), polyhalogenated dibenzopdioxins (PHDD), dibenzofurans (PHDF) (Greim et al., 1997; van den Berg et al., 2013).

The aim of the study is to find a simple and inexpensive estimation method, by incorporating passive samplers, that uses a few important and easily accessible descriptors.

1.6 Use of animals for testing to assess chemicals toxicities

For the assessment of toxicities of organic pollutants in environment, living organisms are being used for centuries in scientific research. The most important target sites of organic pollutants (Hydrophobic and hydrophilic) are membranes of these test organisms. The persistent hydrophobic pollutants are uptake by hydrophilic tissues and lipids which cause disturbance in the structure and function of membrane including expansion, fluidity and ion permeability of the body membrane. Membrane toxicity is also affected by physiological traits of test-organisms as specie sensitivity, exposed time, bio concentration potential, and environmental conditions (Tchounwou,2014). Mode of action (MOA) is also a critical factor in determining toxicity of organic pollutants in organisms which can be calculated by bio concentration potential of an organic pollutant (Kienzler,2017).

Data collection and assessment using living organisms needed large chemical management programs, therefore Development of methods for replacement of test organisms is very important. Alternative methods can be used to save test animals and a considerable reduction in test animals is possible if these techniques would be applied

intensely. Different methods are developed, validated, and adopted such as QASR, in vitro testing and read cross techniques for the reduction of test animals use and cost. Use of passive samplers as an alternative of test animals is an effective way to reduce the use and adverse impacts of test methods of animals (AgataKot-Wasik,2007).

1.7 Problem statement

Previously, Toxicity Models (TM) are based on pure estimation of toxicities by the use of different descriptors e.g., ASM, QSAR Models etc. Furthermore, to get experimental values of toxic chemicals, arduous efforts are required by dissecting and killing the test organisms in order to evaluate the phospholipid layers and tissues. Therefore, by using passive samplers as surrogate for lipids and as they mimic the test organism, Target Lipid Model (TLM) is replaced in this study with the Target Passive Sampler Model (TPSM), which correctly estimates the non-polar narcosis, polar narcosis and reactive toxicants.

Furthermore, the toxicities of Organic Chemicals are determined using different test organisms which are discourage in the new legislations across the world. This calls for an animal alternative approach.

1.8 Objectives of the study

Objectives of this study are;

i. To estimate toxicities of neutral organic chemicals through TPSM using literature data for fish. The data from fish include experimental LC50 values.

ii. To evaluate widely-used passive sampling phases as alternatives to fish testing. This objective is accomplished by comparing various already established models

with my TPSM.Furthermore, LC50 values obtained from TPSM are compared with the experimental data and results are obtained as residuals.

iii. To correlate different types of passive samplers with organic chemicals based on their functional groups. This objective helps us to discriminate and correlate various types of passive samplers used in this study with the various groups of chemicals based on their structural and functional properties.

1.9 Scope of Study

The scope of this study is established based on the type of chemicals, literature LC50 values for test organism, partition coefficient experimental and computation data for 04 types of passive samplers and toxic endpoint.

Table 1. Scope of study

Sr#	Parameter	Details
1.	Type of Chemical	Neutral Organic Chemicals
2.	Test Organisms:	Passive Samplers and Fathead Minnow (Fish) literature data (a new model to evaluate fish-alternative approach for toxicity)
3.	Toxicity Endpoint	Lethal: Median Lethal Concentration, LC50

1.10 Hypothesis

The toxicity of organic chemical in water is due to its water-soluble hydrocarbons which are easily bioavailable. Compounds with lower molecular weight are less persistent in the environment due to their volatility while higher molecular weight compounds are

persistent and have long term impact on environment. Acute toxicity is based on the hypothesis that the concentration of a toxicant in aqueous medium to exert a toxic endpoint, such as median lethal concentration (LC50) can be predicted from the critical body burden in the target lipid of an organism (Cmem), which can be calculated from the target lipid to water partition coefficient $K_{L\text{-}W.}$ The critical body burden in the target lipid of an organism (Cmem) is calculated at 100 mmol/kg for a broad variety of 42 aquatic organisms using 333 different chemicals. (Bittermann *et al*.,2017).

$$LC50 = K_{L-w} \times Cmembrane \quad \textbf{(1.4)}$$

$$Cmembrane = 100 \text{ mmol}/kglipid \quad \textbf{(1.5)}$$

$$-\log LC50 = 1 + \log K_{L-w} \quad \textbf{(1.6)}$$

The hypothesis is if we replace membrane with passive sampler,

$$\text{Log } LC50 = 1 + \log K_{L-w} \quad \textbf{(1.7)}$$

$$-\log LC50 = 1 + \log K_{PS\text{-}w} \quad \textbf{(1.8)}$$

The uptake of chemical by a passive sampler will be similar to that of phospholipid and we are able to capture the toxicity in a good way.

REVIEW OF LITERATURE

2.1 Toxicity Classification of Neutral Organic Chemicals

Verhaar *et. al.* proposes a scheme that allows organic pollutants to be classified into four categories, namely:

i. Inert chemicals,

ii. Less inert chemicals,

iii. Reactive chemicals

iv. Specifically acting Chemicals,

For chemicals categorized and belong to one of the four categories, the expected exposure toxic concentration for toxic end point such as LC50 or the expected range of possible exposure concentration can be obtained from the correlation/ratio of octanol/water partition coefficient (Log Kow). Without knowing the Log Kow, it is impossible to predict if it does fall into any of these four categories or prioritize the use of chemicals for further testing. In addition, these assessments are very important for assessing risks and hazards. (Verhaar *et al.*,1992).

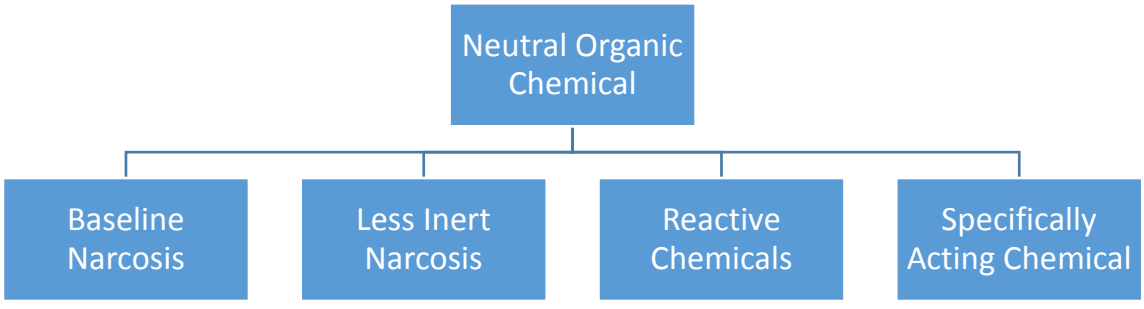


Figure 1: *Verhaar classification of toxic chemical*

In the field of aquatic toxicology, quantitative structure-activity relationship (QSAR), a modeling technique, has been developed as a scientifically effective model for predicting the toxicity of chemicals when very little or no empirical data is readily available. Recently, the development and use of QSAR has evolved to a chemical category closer to the hypothetical mode of toxic action of a chemical. The reason of this study in specific is to develop a method to correlate the toxicity regime of fathead minnow (Pimephales Promelas) with the chemical structure. Toxicity study of effects, creation of toxicology curves, and interpretation of behavior and dose effects of the 96-hour LC50 test. Through these efforts and the early toxicology inventory, nearly six hundred chemicals have been classified as drugs in 03 different groups;

i. oxidative phosphorylation uncoupling agents, and respiratory inhibitors,
ii. electrophiles/electrophiles, acetylcholinesterase inhibitory Drugs,
iii. central nervous system (CNS) epilepsy drugs.

On the basis of the data set, a computerized originated specialized system was created in which the chemical structure is associated with possible toxicity modes. (Russom et al.1997)

2.2 Toxic Endpoints

The understanding of chemical toxicity is usually confined to easy and generalized effects, such as lethality or inhibition of growth or reproduction, and expressed as LC50, which is the concentration of an aqueous solution that produces 50% lethality in acute toxicity, or NOEC (No-Observed-Effect Concentration on sublethal toxicity determination) (Maki and Bishop, 1985).

Additionally, there are three categories of toxicity endpoints that can be observed for a test organism exposed to a certain chemical:

1. Exposure level at which a particular percentage of the effect occurs, commonly referred to as ECx., where x can vary from 0 to 100%, but it is generally 10, 20 or 50 in percentage. The calculation of an ECx, endpoint needs the observation of a dose-response graph.
2. Exposure level at which the minimum detectable effect concentration is observed, commonly referred to as LOEC (lowest observed effect concentration).
3. The exposure limit for which no effect is observed is usually called NOEC (No Observed Effect Concentration). NOEC is the highest concentration tested, at which no statistically significant treatment-related effects have been observed (usually at $p<0.05$).

For all of these categories of endpoints, the outcome of the effects of organism in the replicates, exposed to the chemical is compared with the reaction of the organism in the control. (Amy C. Brook *et.al.*,2019)

2.3 Predicting Toxicities from Abraham Solvation Model

Abraham Solvation Model (ASM) is a multi-parameter equation that can be used to estimate various transmission characteristics and distribution coefficients in environmental chemistry. It is based on cavity model parameters and other intermolecular interactions. It is written as

$$\log SP = c + e\mathbf{E} + s\mathbf{S} + a\mathbf{A} + b\mathbf{B} + l\mathbf{L} \qquad \textbf{(2.1)}$$

Depending upon different type of intermolecular forces and interactions the parameters can be selected for example in case of transfer between two phases.It is written as

$$\log P = c + e\mathbf{E} + s\mathbf{S} + a\mathbf{A} + b\mathbf{B} + v\mathbf{V} \qquad \textbf{(2.2)}$$

The letters that are written in lower case are system constants whereas the capital letters are solute descriptors. Partition constant and other free energy terms describe the solute property which is log P. The complimentary interactions between the solute and solvent are described by lowercase letters. The solute descriptors are **E** describing the solute polarizability, **S** describing the solute polarity, solute's hydrogen bond interactions are represented by **A** and **B** where **A** represents hydrogen accepting capacity and **B** is hydrogen bond donating capacity. **V** tell us about the cavity formation by solute in solvent and **L** is the solute's hexadecane-air partitioning constant at 25^{o}C (Bradley *et al*., 2015).

The Abraham Solvation model is employed to constitute mathematical equations to describe the non-specific aquatic toxic outcomes of organic compounds to bluegill, fathead fish, guppy, golden orfe, goldfish, and high-eye medaka. The resulting mathematical correlation describes the published toxicity data observed within a total average standard deviation of app. 0.28 log units. (Hoover *et al*.,2005)

Likewise, by incorporating ASM, neutral organic chemicals are divided based on toxicity and MOA, there is positive correlation shared between vibrio fishery and fish when treated for different chemicals with excess toxicity(Toxic Ratio) is given by;

$$TR = \frac{T \text{ predicted (Baseline)}}{T \text{ experimental}} \qquad \textbf{(2.3)}$$

$$\log TR = \log \frac{1}{T\ exp} - \log \frac{1}{T\ predicted(baseline)} = Residual \quad \textbf{(2.4)}$$

A log TR value from -1 to 1 is regarded as baseline or less inert narcosis. A log TR value fairly greater than 1 is assumed as excess toxicity due to the presence of reactive or specific mode of toxic action. (Wang et al.,2016)

2.4 Passive Sampling

Passive sampling is a sampling technique. Since the chemical potentials of the analytes in the two environments are different, a sampling device is used to capture free-flowing analyte molecules from the sampling environment. A balance is established or until the sampling stops (Mackay et al., 1997).

Passive sampling determines the time-weighted average (TWA), so the response rate depends on the duration of the time-weighted average over time (Gorecki and Namiesnik, 2002). Whether the sampler collects the analyte depends on two things: one is the concentration of the analyte in the medium sample, and the other is the exposure time. If we have information about the relationship between sampling frequency and analyte concentration, we can easily calculate the time-weighted average TWA. Know what they are, even if the analyte concentration reaches zero, the captured molecules will not be released. This is called "Zero Sink." The sampling rate must remain constant over time. This is quite simple when the analyte is absorbed or chemically adsorbed, but problems arise when the physical adsorption process is involved when collecting the analyte.

2.5 Types of Passive Samplers

There is a vast range of passive sampling devices present that can be used for sampling of different pollutants that are pre-dominant in different media of environment. It is of great significance to select an appropriate passive sampling device for a specific and particular sampling environment. Different materials are used as absorbents/sorbents in passive sampling devices as it provides the device with a specific property which is good such as polyethylene passive samplers which are ethylene sheet based passive samplers are great when it comes to capturing of hydrophobic compounds. Sampling devices such as low density polyethylene (LDPE) film (Carls et al., 2004; Cornelissen et al., 2008; Zhu et al., 2015),semipermeable membrane devices (SPMD) (Turgut *et al*., 2017), , polyoxymethylene (POM) devices (Beckingham and Ghosh, 2013), polyacrylic (PA) plastic sorbent (DiGiana et al., 1988; Namies´nik et al., 2005) and polydimethylsiloxane (PDMS) fibers (Zhang *et al*., 2014)can be used in different as well as more than one environment depending upon what type of compounds under investigation.

A **B**

C

Figure 2: *A) High Density Polyethylene (HDPE) Strip, B) Cage of HDPE used for the deployment of passive sampler, C) Deployment of Polyethylene(PE) passive sampler at a water site by IESE,NUST team using BBQ grill as a cage.*

2.6 Use of Passive Sampler as an Alternative Technique

Passive sampling is an alternative environmental monitoring tool. It is economical, reliable, and provides representative quality data that is comparable between time and place. Passive samplers can be used for long periods ranging from days to months and provide a time-weighted average pollution level. Compared with traditional methods, passive angle sampling has many advantages because it greatly simplifies the sampling procedure by eliminating sample preparation and storage, provides cleaner extracts with less solvent consumption, speeds up processing time, and eliminates power/ Current input is significantly reduced. It is more environmentally friendly technology with less

matrix effects, because it provides better detection and quantification limits than other methods. This method is particularly suitable for determining time-weighted pollutant concentrations, and has great potential due to its simple functional and structural principles and low cost (Górecki and Namieśnik, 2002). The use of passive samplers requires certain calibration parameters, such as partition coefficients, sampling rate, and loss rate constant, which are usually determined in the laboratory or sampling location (Phi e Hiramatsu, 2012). In order to obtain useful information from the passive sampler, we need the separation and diffusion coefficients used in the field.

Chapter 3

METHODOLOGY

3.1 Phase 1: Development of TPSM for the estimation of PS to water partition coefficients by using ASM descriptors.

3.1.1 Acquisition and Curation of Data:

This phase of this study was the most difficult one and was conducted very carefully via following steps;

i. The experimental values for Fathead Minnow (96-hr LC50 for fish) are collected from the literature (want *et al*., 2016). Data sets retrieved from literature for 596 neutral organic chemicals.

ii. Partition Coefficient of PS to water data for 4 types of passive samplers is calculated via Abraham Solvation Model (ASM) by retrieving data of Abraham Solvation Descriptors (E, S, A, B, V, L, B0) from LSER Database-UFZ.

iii. Incorporation of equations from ASM, Phospholipid Model, and Wang Model and PS to Water Partitioning Coefficient experimental (Kps-w) data is retrieved from literature. Likewise, experimental data for Partition Coefficient of PS to water is also retrieved from literature. Experimental database is thin and it is a limiting factor in this study.

iv. Equations from 3 models including Target Lipid Model, Abraham Solvation Model and Wang Baseline Model are incorporated in the excel formulas and are compared as shown in the table 2. Hypothesis is applied to the data for the 4 passive

samplers and the calculated LC50 is compared with the experimental LC50 Value to get the values of Root Mean Square Errors (RMSE)

Table 2. Data Compiled and Used to Calculate Partition Coefficient for Phospholipid, Abraham Solvation Model and Wang Baseline Model and Comparison with passive sampler for LC50 values.

Reference	Target Contaminant*	Passive Sampler **	Organism/Interactive site	*Equation*
1. Satoshi Endo et al. (2011) (Phospholipid)	B, L, R	-----	fathead minnow (P. promelas.)	$-\log LC50 = 0.290 + 0.740E - 0.720S + 0.110A - 3.630B + 3.330V$
2. Hoover et al. (2005) (ASM)	B, L, R	-----	Storage and membrane lipids	$-\log LC50 = 0.996 + 0.418E - 0.182S + 0.417A - 3.574B + 3.377V$
3. Wang et al. (2016) * (Baseline Model)	B, L, R	-----	Predominantly fathead minnow (P. promelas.)	$\log 1/LC_{50} = 0.883 \log K_{OW} + 1.16$
4. Sprunger et al. (2007)	B, L, R	PDMS	PDMS Surface	$-\log LC50 = 0.27 + 0.60E - 1.41S - 2.52A - 4.11B + 3.64V$
5. Satoshi Endo et al. (2011)	B, L, R	POM	POM Surface	$-\log LC50 = -0.37 + 0.39E - 0.28S - 0.46A - 3.98B + 2.98V$
6. Satoshi Endo et al. (2011)	B, L, R	PA	PA Surface	$-\log LC50 = -0.12 + 0.50E - 0.16S + 0.16A - 4.0B + 3.53V$
7. Deedar et al. (2020)	B, L, R	PE	PE Surface	$-\log LC50 = 1.002E - 1.296S - 1.82A - 4.037B + 3.399V$

*: B: Bassline: L: Less Inert, R: Reactive.

**: Experimental data for the available chemicals for 4 types of PS is collected form literature.

3.2 Phase 2: Categorization of data into 12 Classes /sub-classes based on Functional Groups and Mode of Actions (MoAs) and Dimensionality Analysis.

During this phase of the study, chemicals are grouped and are divided into 12 classes and sub-classes on the basis of Functional Groups and Mode of Toxic Action. The obtained groups include pure hydrocarbons, oxygenated hydrocarbons, aromatics, nitrogen and Sulphur containing compound.

Mode of Toxic Action is determined for various classes depending on their RMSE/Toxic ratios. Chemicals are divided into 3 categories such as Baseline Narcosis, Polar or less Inert Narcosis and Reactive/Excess toxicity (Russom et al.,1997).

In phase 2, the following steps are followed;

i. Groups are divided into BL Chemicals, Less-Inert, Reactive, Oxygen containing compounds including alcohols, ketones, aldehydes, acids, esters, Pure Hydrocarbons, Nitrogen containing compounds including Amines, nitrobenzenes, Nitriates, etc.

ii. MoA is determined for all the groups and sub groups based on the RMSE/Toxic Ratios.

iii. One to One plot, Multidimensionality Scaling (MDS) are conducted for the following set of chemicals;

Table 3. ***Categorization of Data into 12 classes and sub-classes***

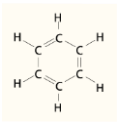
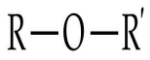
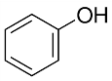
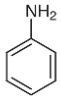
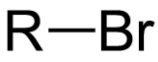
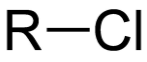
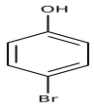
Group	Class/Sub-class	MoA	No. of Chemicals in each group	Chemical Structures
1.	Compounds used in baseline model (Cycloalkanes, alkanes, benzenes, ethers with alkyl, fluoro or chloro groups)	Baseline(B)	**68**	 
2.	Compounds used in less inert model (anilines with alkyl, fluoro or chloro groups and phenols)	Less Inert	**73**	 
3.	Alkanes with bromo group	Baseline(B)	**09**	
4.	alkynes with chloro group,Alkenes, dienes, triene	Baseline(B)	**15**	
5.	Phenols with bromo group*,alkoxy or ester, and cyano group	---	**09**	
6.	Pure Hydrocarbons (3-ring-terphenyl, alkanes, alkane cyclo, alkenes, dienes, benzenes, benzene cyclo, biphenyl, dienes, naphthalene, PAH, phenyl ethenes or acetylene, triene)	Baseline(B)	48	Pure Hydrocarbons (Compounds with Carbon and Hydrogen)

Table 3. ***Categorization of Data into 12 classes and sub-classes (Continued…)***

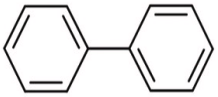
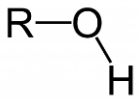
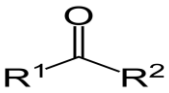
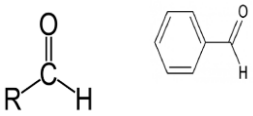
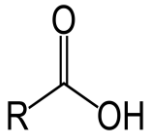
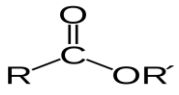
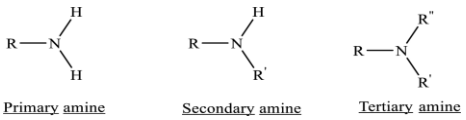
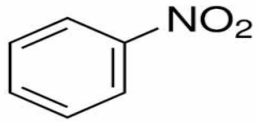
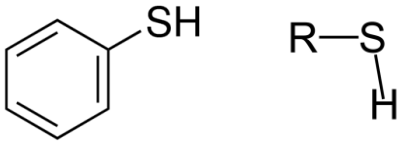
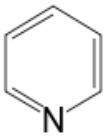
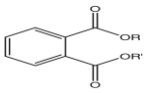
Group	Class/Sub-class	MoA	No. of Chemicals in each group	Chemical Structures
7.	Biphenyl Derivatives (,Biphenyls with hydroxy or amino group, , alcohols, ethers, ketones, esters, amines or amides, Biphenyls with alkyl and chloro group Diphenyl alkanes,)		**21**	
8(i).	Pure Alcohols, cyclo alcohols	Baseline(B)	**23**	
8(ii).	Alcohols derivatives (α-HaIogenated alcohols, α, β-Unsaturated alcohols, and Alcohol-ethers)	α, β-Unsaturated alcohols show reactive[R] mode of action with some halogenated alcohols	**24**	Alcohols Derivatives.
8(iii).	Ketones, cyclo ketones, and α, β -Unsaturated ketones	Baseline(B)	**21**	
8(iv).	Aldehydes and derivatives (α, β -Unsaturated aldehydes, Benzaldehydes with alkyl, halogen or alkoxy group)	[Reactive]	**24**	
8(v).	Carboxylic Acids and derivatives (Unsaturated aliphatic acids, aromatic acids and diacids)	Unsaturated acids, acid chloride and diacids show reactive[R] mode of action	**14**	

Table 3. ***Categorization of Data into 12 classes and sub-classes (Continued…)***

Group	Class/Sub-class	MoA	No. of Chemicals in each group	Chemical Structures
8(vi).	Esters and derivatives (cyclo esters and diesters, α-HaIogenated esters, α, β-Unsaturated esters, epoxides Esters, and phthalates)	Unsaturated esters follow reactive[R] mode of action	**31**	
9(i)	Amines: (Primary mono amines*, Secondary mono amines*,Tertiary amines*, Diamines and poly amines),	Primary mono amines*(L,R) Secondary mono amines (B) Tertiary amines (B) Diamines [R]	**25**	
9(ii).	Nitro Benzene derivatives (Mono nitrobenzenes with alkyl or halogens, Dinitrobenzenes with halogen, alkoxy, hydroxy, alcohol, and cyano group)	Mono nitrobenzenes with alkyl or halogens (L) Dinitrobenzenes with halogen, alkoxy, hydroxy, alcohol, cyano group [R]	**34**	
10	Sulphur containing OCs (Thiols, thioethers, dithioethers and disulphides, Thiophenols and aromatic thio compounds)	Thiols, thioethers, dithioethers and disulphides ,Thiophenols [R]	**24**	
11.	Pyridines and derivatives (Pyridines with alkyl, Pyridines with alkoxy, aldehyde, ketone group ,Amino pyridines*, and cyano pyridine)	Pyridines with alkyl (L)	**16**	
12.	Phthalates (saturated and unsaturated)	Unsaturated Phthalate[R]	**5**	

3.2.1 One-To-One Plot

One-to-One Plot is commonly called as Identity Line and is extremely beneficial in statistical analysis. In this thesis, One-to-One Plots are 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 1:1 are 1:2 lines are usually used as reference (upper and lower limits) in a two-dimensional(2D) scatter plot when comparing two data sets expected to be similar. When the data points from the two data groups are correlated significantly to each other, the corresponding scatters fall accurately within the identity line. In this study, data points of LC50 for passive samplers are compared with the experimental LC50 Values.

In this research, I have initially used PCA for the analysis of dimensionality of 3 models with 4 types of passive samplers. However, the results of the components are very close to each other and it is impossible to segregate them for effective understanding. Therefore, Multidimensional Scaling (MDS) technique is employed to evaluate and cross validate my data.

3.2.2 Multidimensional Scaling (MDS)

Multidimensional Scaling (MDS) is a mapping tool for creating a scaling chart that shows the relative dimensions and positions of various data points, considering only the distance table between them. A map can have many dimensions (1,2,3 or more). The distance table is called the proximity matrix, and it is created directly from experiments or indirectly as a correlation matrix.

MDS provides a way to envisage the degree of similarity, dissimilarity or correlation of specific cases individually in a data set (Mead *et. al.*,1992).

By drawing the data sets of passive samplers with 3 models (TLM, ASM and WBM), the distances based on dissimilarities are calculated and relationships among TPSM, TLM, ASM, WBM and experimental data are graded.

3.2.3 Goodness of Fit

Goodness-of-fit test is a statistical hypothesis test to assess how accurately sample data fit a distribution from a population with a normal distribution.

With data analysis issues, an explanation is required to explain how well a data set is represented by a model based on the analysis. With MDS, we try to model the distances. Hence the most obvious option for goodness of fit statistic is based on the differences between actual distances and their predicted values. Such a measure is called stress or raw-stress.

MDS with stress number near zero are considered as the best statistically. According to Kruskal (1964), the following stress values corresponds to goodness of fit;

Table 4: Criterion for Kruskal Stress and Goodness-of-fit

Kruskal Stress	Goodness-of-fit
0.200	poor
0.100	fair
0.050	good
0.025	excellent
0.000	perfect

Chapter 4

RESULTS AND DISCUSSION

4.1 Phase 1- Performance of TPSM with respect to experimental values

4.1.1 Comparison of Results based on Root Mean Square Error (RMSE)

Root Mean Square Error, which is the difference between the experimental LC50 and predictive LC50 on logarithmic scale for already established 3 models including Abraham Solvation Model (ASM), Target Lipid Model (TLM) and Wang Baseline Model (WBM) are compared with the 4 types of passive samplers (PDMS, POM, PA and PE). The RMSE values are shown in the graph;

Table 5. The number of classified chemicals in each class (N), number of outliers(N') and Root Mean Square Error Values (RMSEs) for residuals of 4 type of Passive Samplers (PS) with experimental LC-50 data of fish.

Group	Classes /Sub-classes	Mode of Action			Residuals-RMSE (Prediction Error)						
		(MOA)(ref.)	N	N'	Lipid	ASM	PDMS	PA	POM	PE	Wang-BL
1	Compounds used in baseline model (Compounds used in baseline model (Cycloalkanes, alkanes, benzenes, ethers with alkyl, fluoro or chloro groups)	B	68	0	0.33	0.33	0.48	0.39	0.91	0.53	0.38
2	Compounds used in less inert model (Anilines with alkyl, fluoro or chloro groups and Phenols)	L	73	0	0.60	1.71	2.15	0.70	1.57	1.87	0.74
3	Alkanes with bromo group	B	9	1	0.20	0.21	0.43	0.39	0.97	0.58	0.34

4	Alkenes, dienes, triene alkynes with chloro group	B	15	1	0.27	0.36	0.37	0.54	1.06	0.51	0.24
5	Phenols with bromo group*,alkoxy or ester, and cyano group		9	0	0.44	2.00	2.46	0.49	1.42	1.98	0.59
6	Pure Hydrocarbons (3-ring-terphenyl, alkanes, alkane cyclo,alkenes, dienes, benzenes, benzene cyclo, biphenyl, dienes, naphthalene, PAH, phenyl ethenes or acetylene, triene)	B	48	2	0.39	0.34	0.33	0.41	0.89	0.39	0.31
7	Biphenyl Derivatives (Biphenyls with alkyl and chloro group,Biphenyls with hydroxy or amino group,Diphenyl alkanes, alcohols, ethers, ketones, esters, amines or amides)		21	1	1.06	2.33	2.42	1.12	1.63	2.58	1.98
8	**Oxygenated hydrocarbons**										
8-(i)	Pure Alcohols, cyclo alcohols	B	23	0	0.28	1.57	1.09	0.49	1.18	1.17	0.32
8-(ii)	Alcohols derivatives (α-HaIogenated alcohols, α, β-Unsaturated alcohols, and Alcohol-ethers)	α, β-Unsaturated alcohols show reactive[R] mode of action with some halogenated alcohols	24	0	1.66	1.44	2.79	1.88	2.45	2.74	1.52
8-(iii)	Ketones, cyclo ketones, and α, β -Unsaturated ketones	B	21	0	0.35	0.26	0.73	0.54	1.18	1.03	0.26
8-(iv)	Aldehydes and derivatives (α, β -Unsaturated aldehydes, Benzaldehydes with alkyl, halogen or alkoxy group)	[R]	24	0	1.91	1.80	2.57	2.10	2.65	2.63	1.90

8-(v)	Carboxylic Acids and derivatives (Unsaturated aliphatic acids, aromatic acids and diacids)	Unsaturated acids,acid chloride and diacids show reactive[R] mode of action	14	0	1.11	2.59	3.19	1.17	2.00	2.87	1.41
8-(vi)	Esters and derivatives (cyclo esters and diesters, α-HaIogenated esters, α, β-Unsaturated esters, epoxides Esters, and phthalates)	Unsaturated esters follow reactive[R] mode of action	31	0	1.50	1.30	1.98	1.69	2.37	2.22	1.50
9	**Nitrogen containing Organic Chemicals**										
9-(i)	Amines: (Primary mono amines*, Secondary mono amines*,Tertiary amines*, Diamines and poly amines),	Primary mono amines*(L,R) Secondary mono amines (B) Tertiary amines (B) Diamines [R]	25	0	1.51	1.01	2.30	1.93	2.56	2.41	1.10
9(ii)	Nitro Benzene derivatives (Mono nitrobenzenes with alkyl or halogens, Dinitrobenzenes with halogen, alkoxy, hydroxy, alcohol, and cyano group)	Mono nitrobenzenes with alkyl or halogens (L) Dinitrobenzenes with halogen, alkoxy, hydroxy, alcohol, cyano group [R]	34	0	0.90	0.75	1.85	0.97	1.59	1.73	1.22
10	Sulphur containing OCs (Thiols, thioethers, dithioethers and disulphides, Thiophenols and aromatic thio compounds)	Thiols, thioethers, dithioethers and disulphides ,Thiophenols [R]	24	0	1.68	1.47	2.41	1.88	2.49	2.38	1.69
11	Pyridines and derivatives (Pyridines with alkyl, Pyridines with alkoxy, aldehyde, ketone group, Amino pyridines*, and cyano pyridine)	Pyridines with alkyl (L)	16	0	1.73	1.32	2.75	1.98	2.59	2.63	1.59
12	Phthalates (saturated and unsaturated)	Unsaturated Phthalate[R]	5	1	1.11	0.80	1.64	1.17	2.07	1.83	0.93

4.1.2 Baseline Narcosis/Non-polar/Inert Toxicants

Neutral Organic chemicals that exert baseline or non-polar narcosis consists of Cycloalkanes, alkanes, ethers, benzenes with alkyl, fluoro or chloro groups. Total of 68 chemicals are included in this particular class and the results for various models and comparative passive samplers is shown in the bar chart.

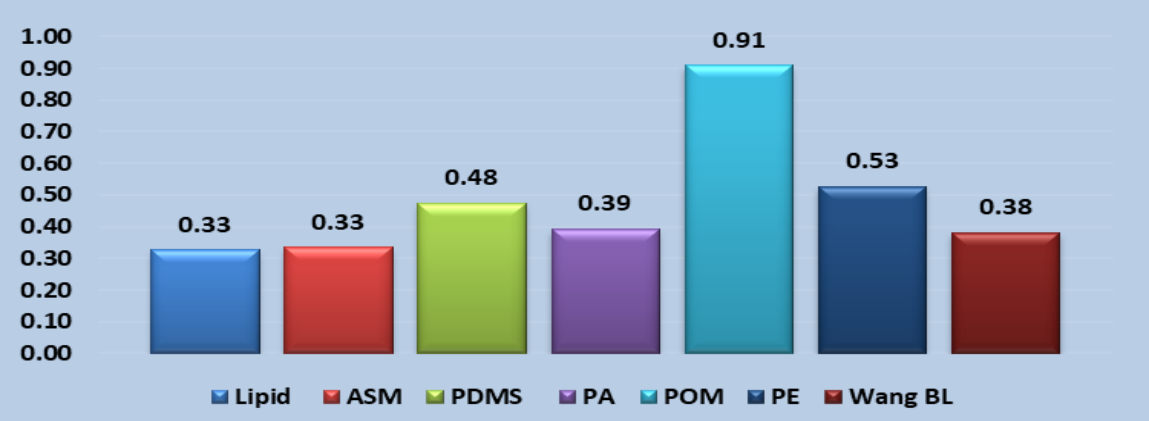


Figure 3: *Residual-RMSE (Prediction Error) comparison of TPSM with other Models and Bar Chart Representation for Baseline toxicants.*

The bar chart shows that Target Lipid Model (TLM) and Abraham Solvation Model are in close compliance with the experimental values for the 68 baseline chemicals with RMSE value of 0.33 log unit. Out of the 4 passive samplers, Polyacrylate (PA) represents the best results when compared with the experimental LC50 values. Results from Wang Baseline Model also gave considerably better results, however, there is an ambiguity in the wang model due to the fact that the experimental data represented in this study is a sub-set of the Wang's data. Polyoxymethylene (POM) passive sampler

performed poorly compared to the other passive samplers with RMSE value of 0.91 log units.

4.1.3 Polar Narcosis/Less Inert Toxicants

Toxicants that are polar/less inert in nature are included in this class. This class consists of Anilines with alkyl, fluoro or chloro groups and Phenols. There are 73 chemicals in this class and is referred as group no. 2. Bar Chart for less inert toxicants is shown below;

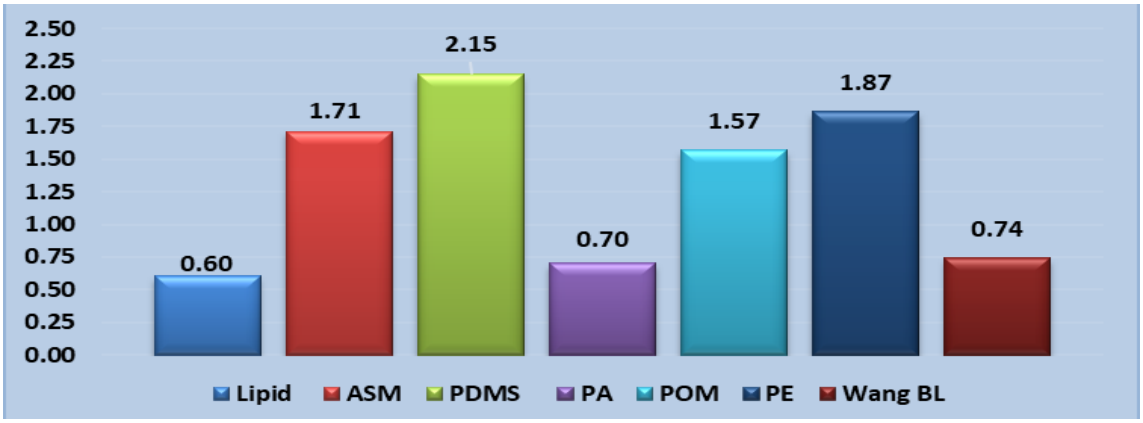


Figure 4: *Residual-RMSE (Prediction Error) comparison of TPSM with other Models and Bar Chart Representation for Less Inert toxicants.*

Bar representation shows better prediction for TLM, and WBM.ASM predicted toxicity poorly for the less inert chemicals when compared with the experimental LC50 Values.PA passive sampler outperformed all the other passive samplers for non-polar chemicals with residual value of 0.70 log unit.

4.1.4 Pure Hydrocarbons

Pure hydrocarbons without any functional group are categorized as group no. 6. This class consists of 3-ring-terphenyl, alkanes, alkane cyclo, alkenes, dienes, benzenes,

benzene cyclo, biphenyl, dienes, naphthalene, PAH, phenyl ethenes or acetylene, and triene. Total numbers of chemicals included in this class are 48. Two chemicals, Dodec-1-ene (Smile Code: C(=C)CCCCCCCCCC, Cas No. 112-41-4) and m-Terphenyl (Smile Code: c1cc(cc(c1)c1ccccc1)c1ccccc1, Cas No. 92-06-8) are removed as outliers due to the fact that the predicted error is very high within the range 2.03 and 1.97 log unit respectively.

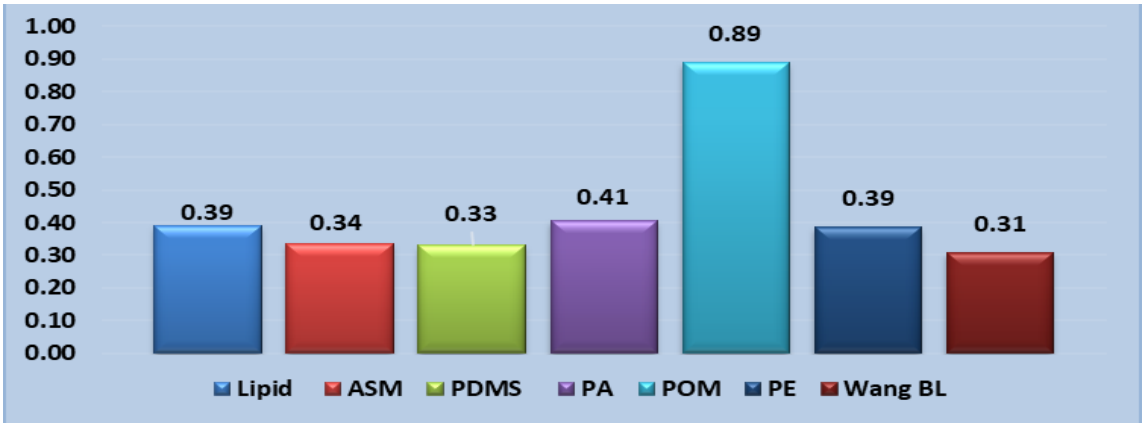


Figure 5: *Residual-RMSE (Prediction Error) comparison of TPSM with other Models and Bar Chart Representation for Pure Hydrocarbons.*

PDMS outperformed all the predictions by all the other models (except Wang Baseline Model) and passive samplers by rmse value of 0.33 log unit. Results from Wang Baseline Model also gave considerably better results, however, there is an ambiguity in the wang model due to the fact that the experimental data represented in this study is a sub-set of the Wang's data. POM predicted the toxicity poorly for pure hydrocarbons with rmse value of 0.89 log unit, when compared with other passive samplers.

4.2 Phase 2- Discrimination of Mode of Toxic Action

4.2.1 Findings Based on Correlation of RMSE, MoA and Type of Chemical Class

Chemicals are classified in to different types based on their mode of toxic action as mentioned in Verhaar *et. al*, 1992.Similarly, chemicals are divided into three mode of actions and a criterion defined for the excess toxicity;

1. B: identified as baseline compounds (or non-polar narcotics);
2. L: identified as less inert compounds (or polar narcotics);
3. R: identified as reactive or specific reactive compounds

Criterion for baseline, less inert and excess toxicity for organic chemicals is given as;

- RMSE/Toxic Ratio (TR) ≤ 1 (non-polar and polar narcosis for Fish)
- RMSE/Toxic Ratio (TR) >1 (excess or specific toxicity for Fish)

On the basis of aforementioned criteria, the following findings are obtained from rmse values;

Pure Hydrocarbons follow baseline Narcosis and includes only those organic compounds which have carbon and hydrogen atoms in their chemical structure. Similarly, oxygenated compounds are categorized separately and includes alcohols, ketones, aldehydes, organic acids and esters. Alcohols follow baseline toxicities for ASM, TLM and PA (passive sampler). Ketones show baseline toxicity for all the models and passive samplers except POM and PE. Aldehydes and Carboxylic Acids exert excess toxicities for all the models and passive samplers.

Likewise, Phenols and Anilines based on the toxic ratios follows less inert narcosis and lower root mean square error for poly acrylate passive sampler. Nitrobenzenes exhibit baseline toxicity for ASM and TLM only.

4.3 Graphical Comparison of predicted and experimental LC50 values

4.3.1 Phase 2 - One-To-One plots

4.3.1.1 One-To-One Plot for Baseline Toxicants

One- to-One plots are based on the upper and lower limits of 1 log unit which is equal to the acceptable experimental error or in other words, it is equal to the measurement uncertainty of the experimental data.

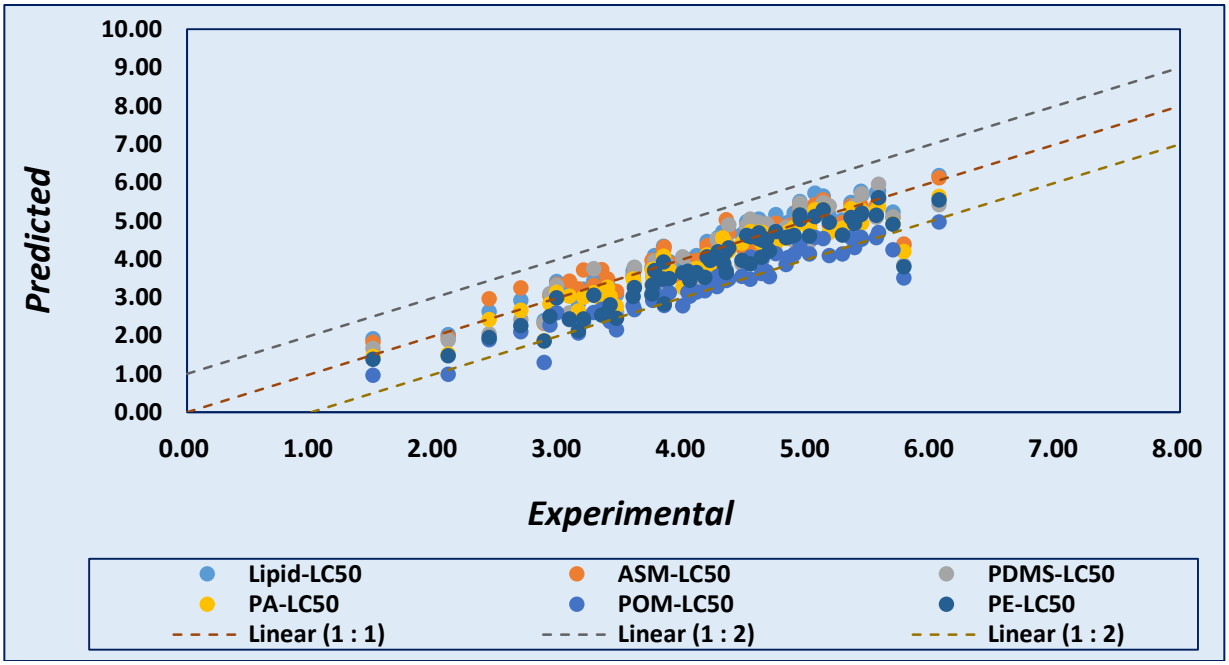


Figure 6: *One-To-One Plot for Non-polar Toxicants (Baseline Narcosis).*

One-To-One plot is drawn to compare all the models with the TPSM. For nonpolar chemicals, the 3 models performed precisely as do the TPSM. The minimum rmse value

is given by TLM and ASM (0.33 log unit) and maximum by POM passive sampler (0.91 log unit).

4.3.1.2 One-To-One Plot for Less Inert Toxicants

Chart distribution of polar narcosis is depicted below. In this representation, resultant values are taken i.e., trendlines are calculated for the models and are compared with each other and 4 types of passive samplers.

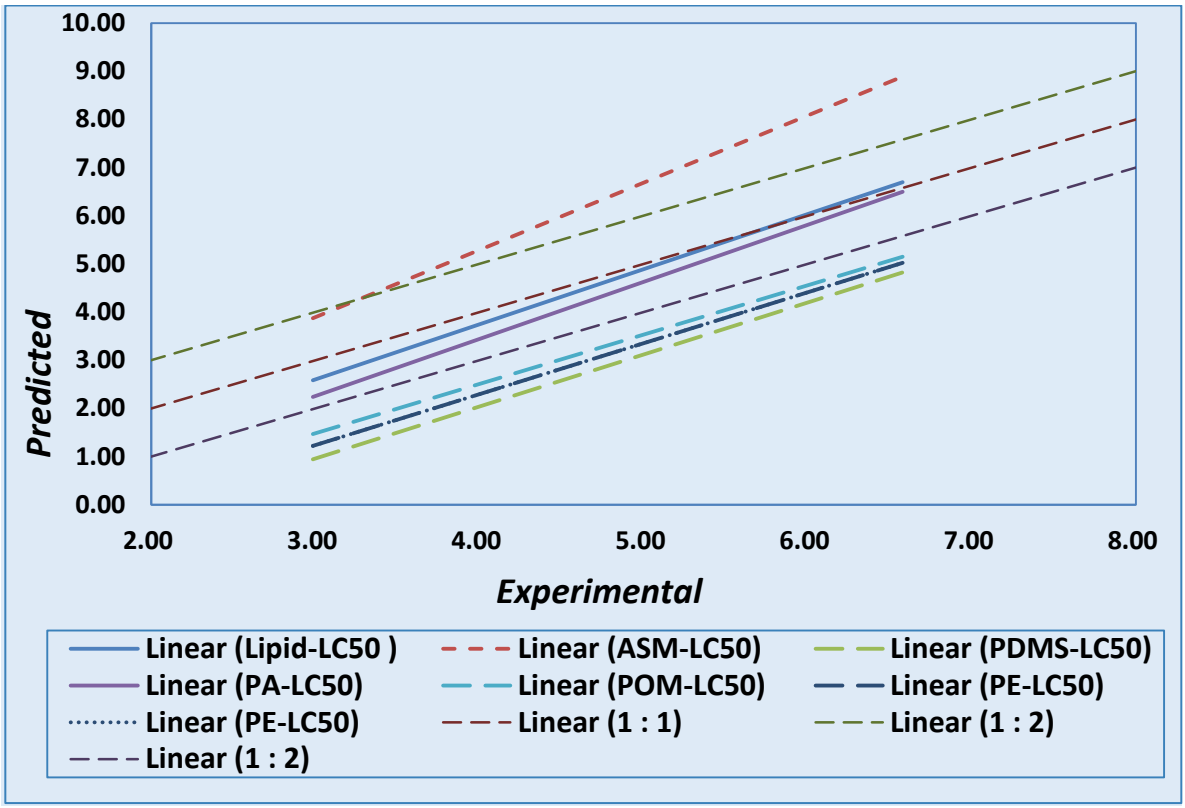


Figure 7: *One-To-One Plot for Polar Toxicants(Less Inert Narcosis).*

One-to-One chart for less inert narcosis represents that TLM and PA passive sampler fall within the upper and lower limit with a difference of 1 log unit. Thus, the data of the 2 models are closer with the experimental LC50 data.

Some of the common members of this class include phenol and aniline. For phenol, although, chronic or continuous exposure of the chemical may have harmful effects on the liver and kidneys, but there is no evidence that phenol is carcinogenic in humans.

4.3.1.3 One-To-One Plot for Pure Hydrocarbons

Graphical representation of pure hydrocarbons is shown as under;

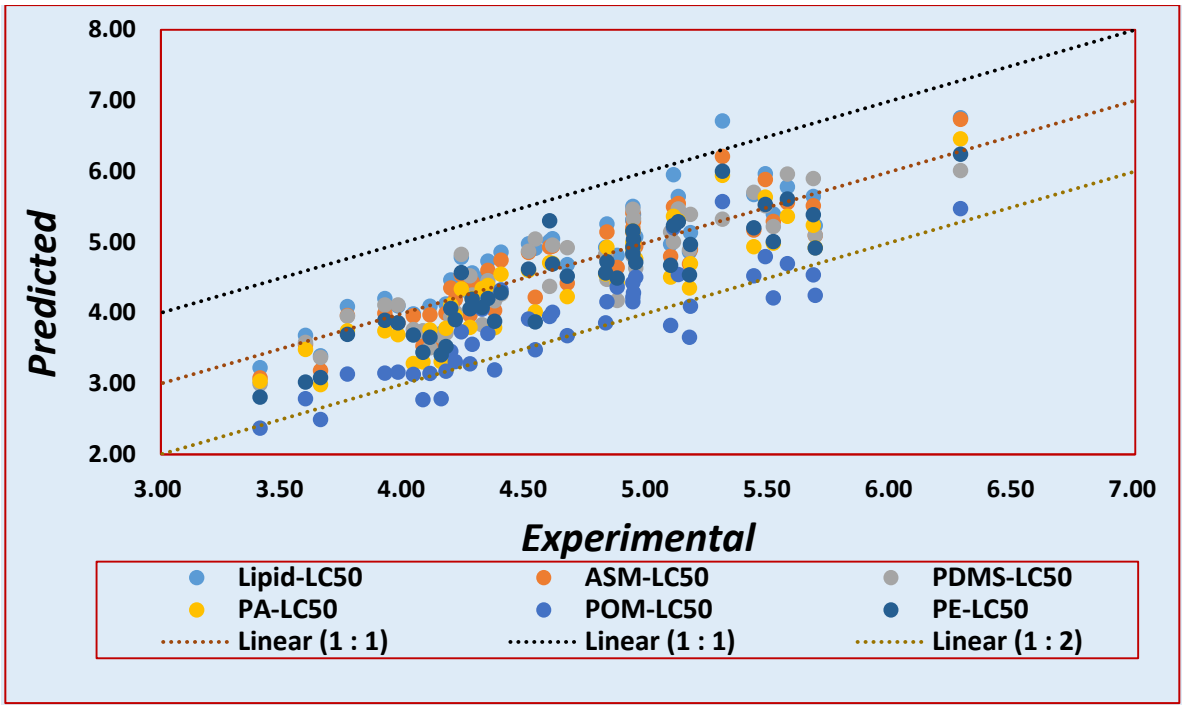


Figure 8: *One-To-One Plot for Pure Hydrocarbons.*

In Pure hydrocarbons, PDMS predicted LC50 value far better than the other models and passive samplers with rmse value of 0.33 log unit. This shows that if pure hydrocarbons are desired to be monitored in the environment, the PDMS will perform better than other passive samplers.

4.3.1.4 Oxygenated Hydrocarbons

This category of neutral organic compounds includes alcohols, aldehydes, ketones, carboxylic acids and esters.

4.3.1.4(I) One-To-One Plot for Alcohols

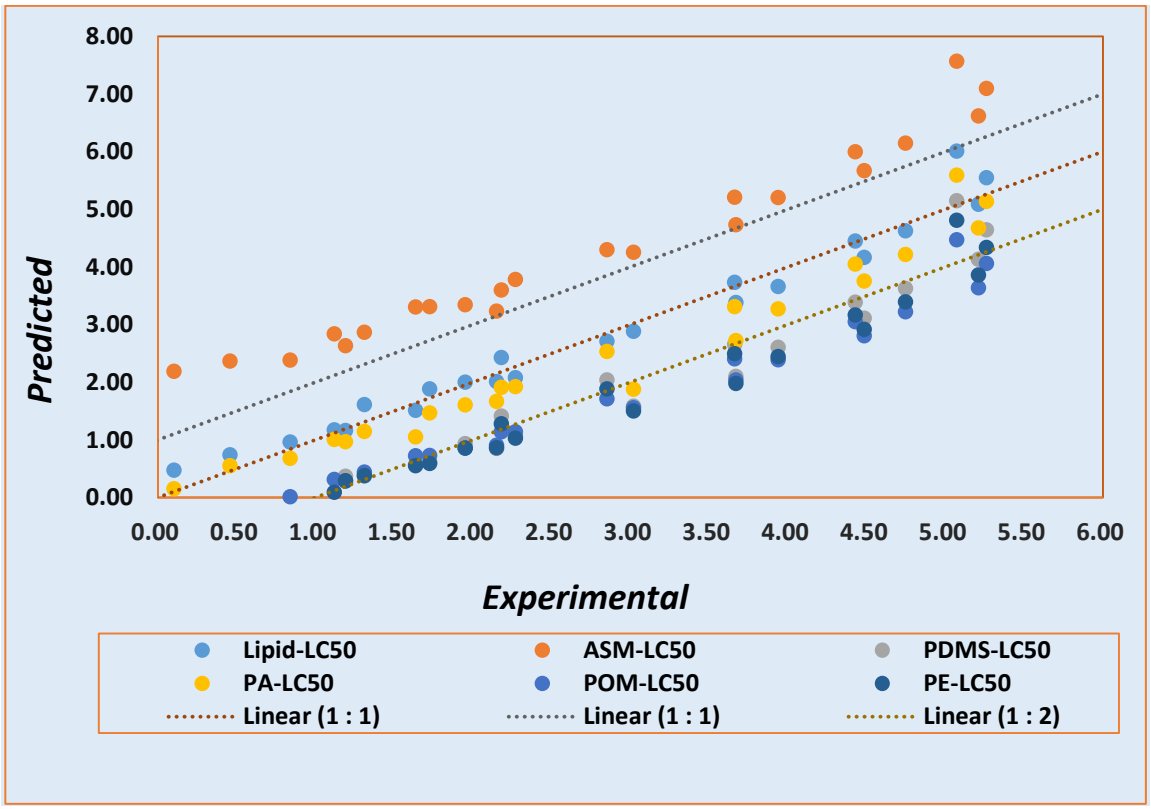


Figure 9: *One-To-One Plot for Pure Alcohols.*

Oxygenated organic compounds includes alcohols in group no. 8(i). For alcohols, TLM predicted LC50 much accurately with rmse value of 0.28 log unit, followed by WBM (0.31 log unit) and PA passive sampler with rmse value of 0.41 log unit. It means that when alcohols are the target compounds in the environment then PA can be helpful in predicting the toxicities more effectively compared with the other 3 passive samplers.

4.3.1.4(II) One-To-One Plot for Ketones

Graphical representation of Ketones is shown as under;

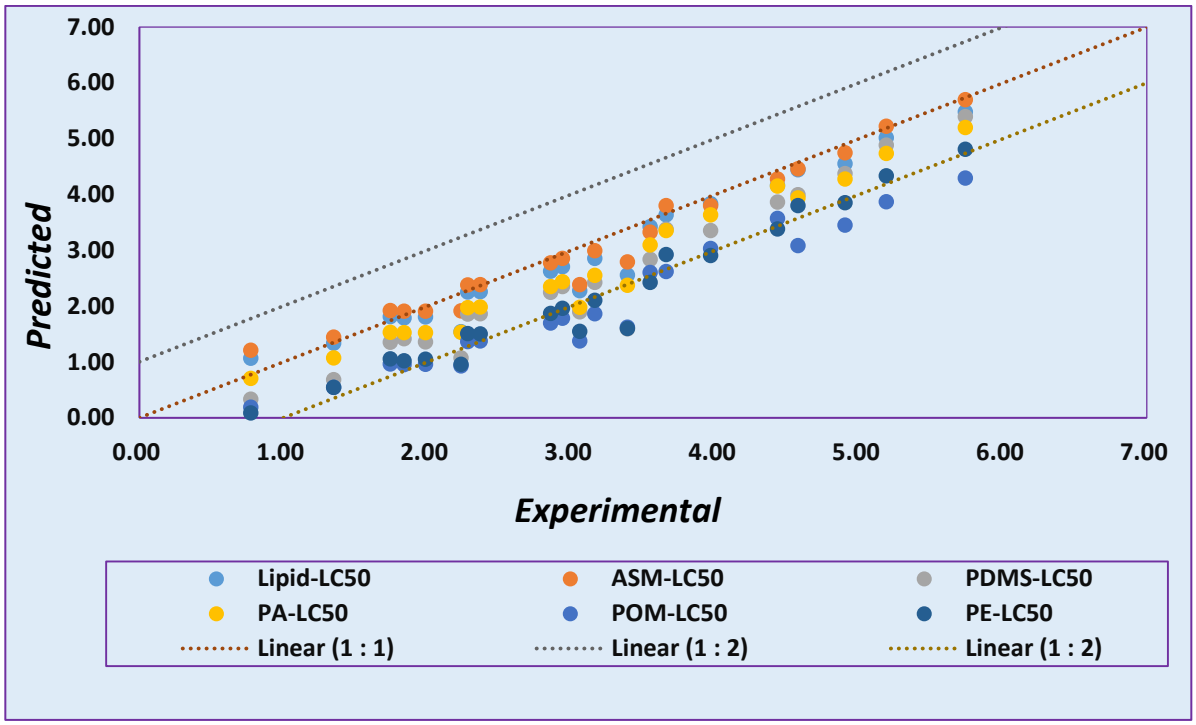


Figure 10: *One-To-One Plot for Ketones.*

Toxicities exerted by Ketones are precisely predicted by all the models except POM passive sampler which gives higher rmse value of 1.18 log unit.

4.3.1.4(III) Excess Toxicity for Aldehydes, Acids and Esters

Aldehydes, Acids and Esters are the classes of chemicals which usually are excessively toxic and have harmful impacts on the environment. Aldehydes are electrophilic compounds that humans often come into contact with. Although exposure can bring significant health risks, little is known about the toxicity mechanism of aldehydes (Richard M. LoPachin,2014). Similarly, Esters act via the nonpolar narcosis mechanism

of toxic action (JS Jaworska,1995), however, the real mechanism of MoA is not yet known.

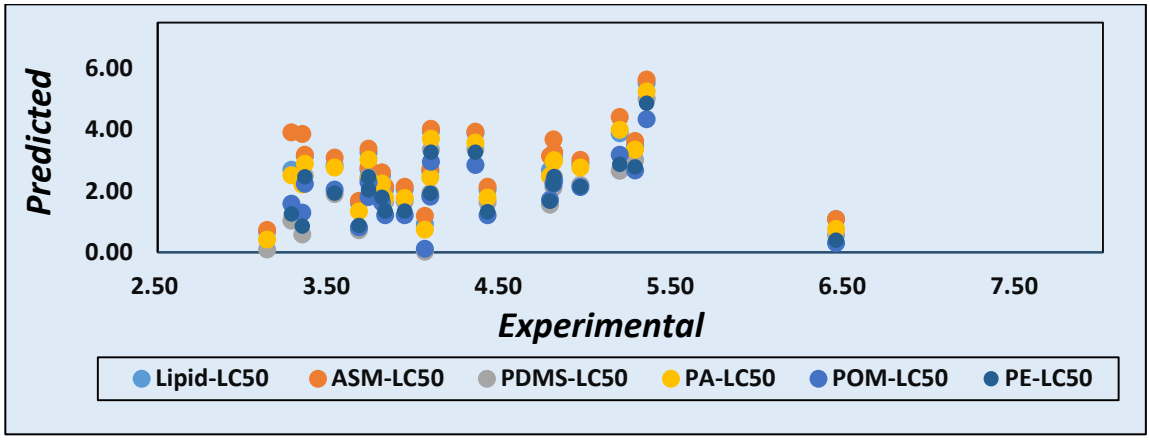


Figure 11: *One-To-One Plot for Aldehydes.*

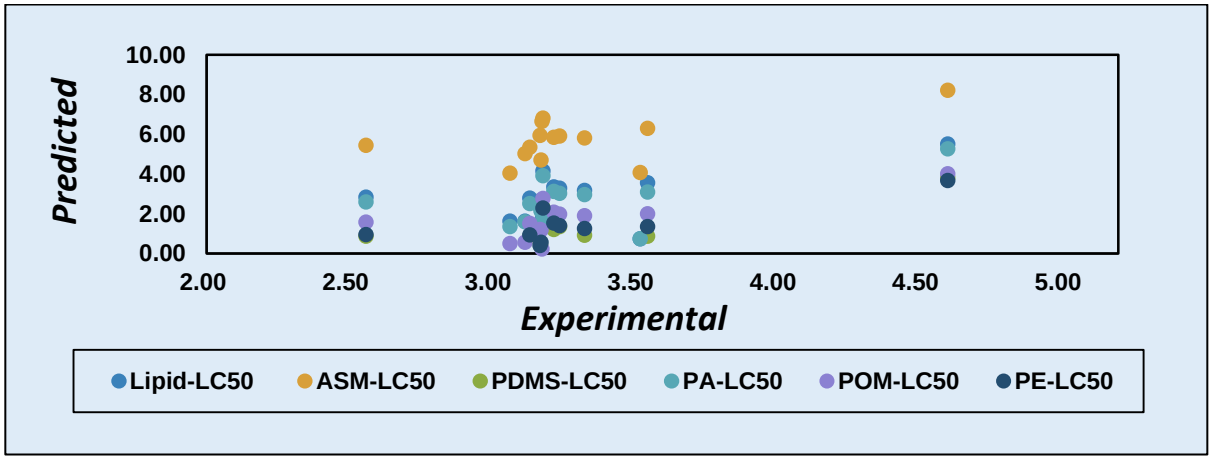


Figure 12: *One-To-One Plot for Organic Acids.*

The toxic effects of organic acids are significant as it is an essential part of many lifesaving drugs. The effects of different organic acids on the growth of different bacteria were examined, and are characterized by a two-dimensional equation combining the basic principles of the dose-effect theory with the growth kinetics of microorganisms

(Secondary model). The toxicity of carboxylic acids decreases as the molecular weight of these chemicals increases. (José Antonio Vázquez,2011)

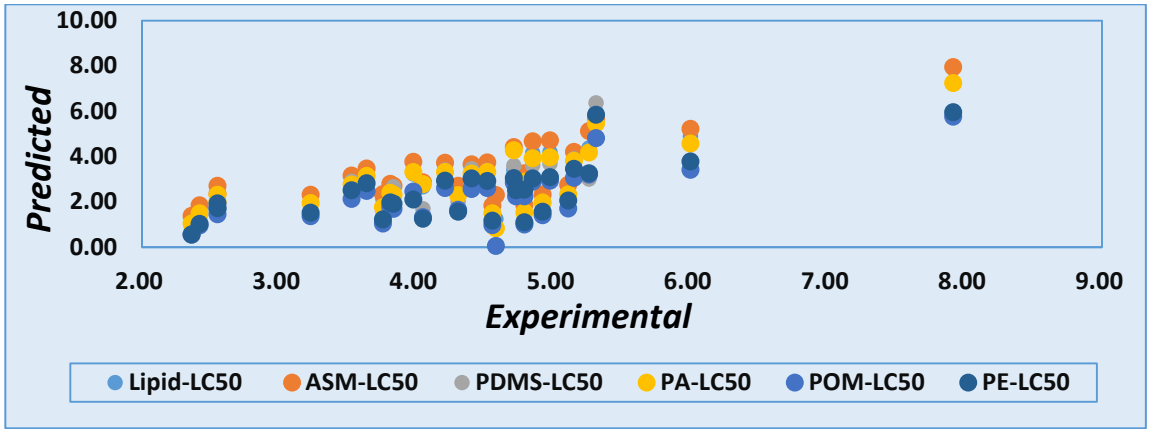


Figure 13: *One-To-One Plot for Esters.*

TPSM evaluates the toxicities of aldehydes, acids and esters and mode of action is predicted to be reactive or specific in nature. This model is unable to predict the excess toxicity of aldehydes, acids and esters effectively. This fact is even similar for the already established TLM, ASM and WBM.

4.3.2 Phase 2 - Multidimensional Scaling (MDS)

Multidimensional scaling may be a wide used method in spatiality reduction and manifold learning.The strategy takes in an exceedingly difference matrix and outputs a low-dimensional configuration matrix supported by a spectral decomposition.

4.3.2.1 MDS plot for Baseline toxicants/Narcosis

Euclidean distance of Poly Acrylate is closer to the data of Experimental and Lipid Model when compared to the other passive samplers for BL Toxicants. It gives me the idea that if you want to predict Baseline toxicants in an environment, PA is more effective compared to the other passive samplers.

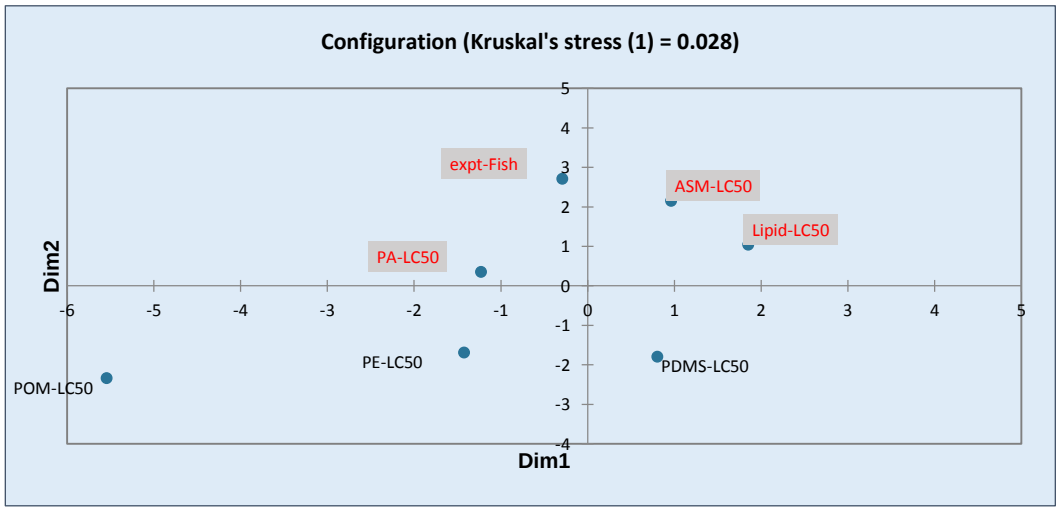


Figure 14: *MDS for Baseline toxicants.*

4.3.2.2 Goodness-of-Fit and Shepard Diagram for Baseline toxicants/Narcosis

Shephard Diagram validates my MDS for BL through goodness of fit. where the abscissas are the observed dissimilarities, and the ordinates, the distance on the configuration generated by the Multidimensional Scaling. The disparities are also

displayed. The more the points are spread, the less the Multidimensional Scaling map is reliable. If the ranking of the abscissa is respected on the ordinates, then the chart is reliable. If the points are on the same line, then the quality is perfect which is obvious in the represented diagram.

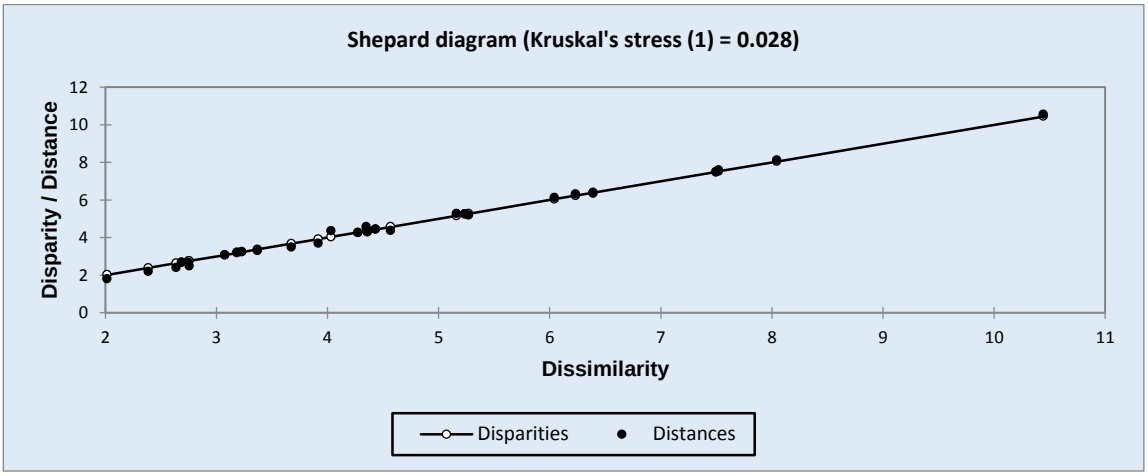


Figure 15: *Shepard Diagram for Baseline toxicants.*

4.3.2.3 MDS plot for Less Inert toxicants

Euclidean distance of PA is closer to the Experimental data and Lipid Model when compared with other passive samplers for Less Inert Toxicants. It means that as per this model, PA passive sampler will demonstrate better results for those Neutral Organic Chemicals which follow Less Inert mode of toxic action as shown in the graph below;

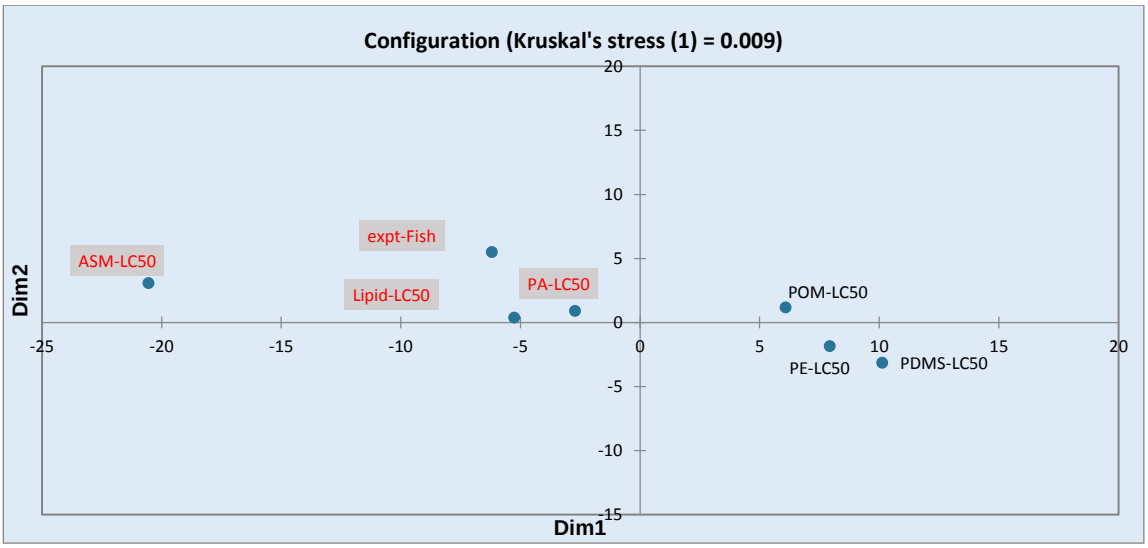


Figure 16: *MDS for Less Inert toxicants.*

4.3.2.4 Goodness-of-Fit and Shepard Diagram for Less Inert toxicants

TPSM shows goodness-of-fit for Less Inert toxicants and it is validated in the shepherd diagram with all the points are closed to the line of equality and stress value of 0.009.

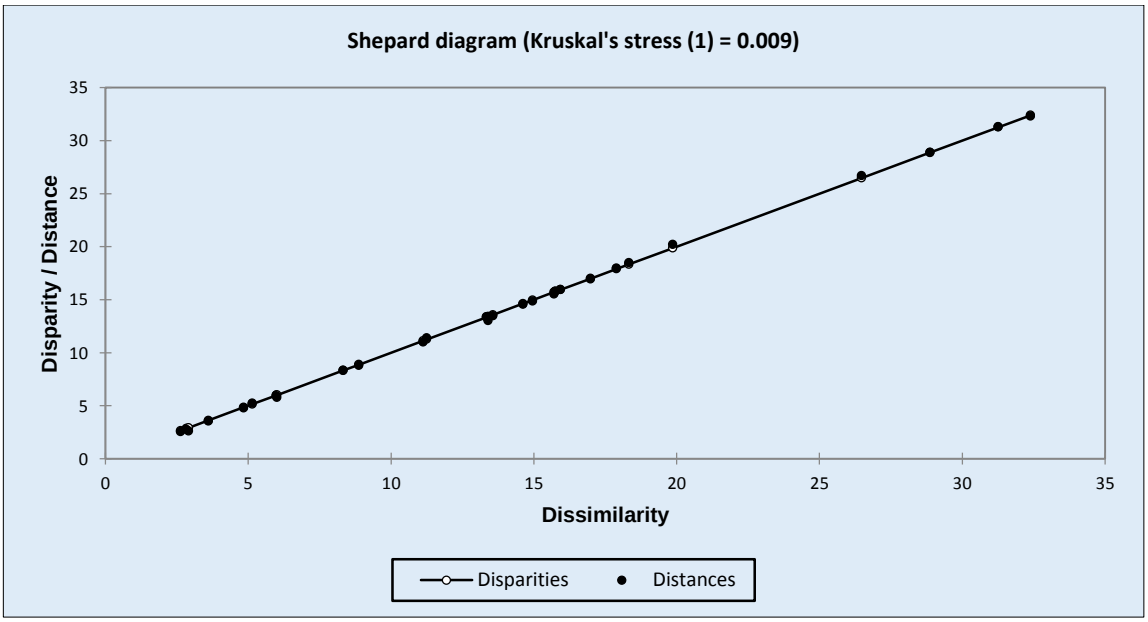


Figure 17: *Shepard Diagram for Less Inert toxicants.*

4.3.2.5 MDS plot for Pure Hydrocarbons

For Pure Hydrocarbons, Euclidean distance of PDMS is closer to the Experimental data when compared with other passive samplers. It means that as per this model, PDMS passive sampler will demonstrate better results for pure hydrocarbons.

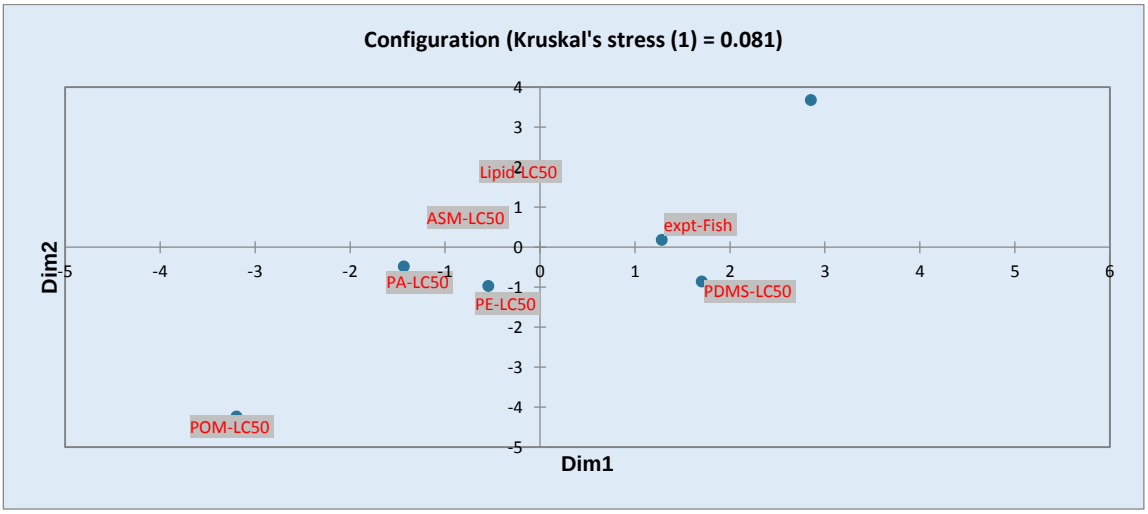


Figure 18: *MDS for Pure Hydrocarbons*

4.3.2.6 Goodness-of-Fit and Shepard Diagram for Pure Hydrocarbons

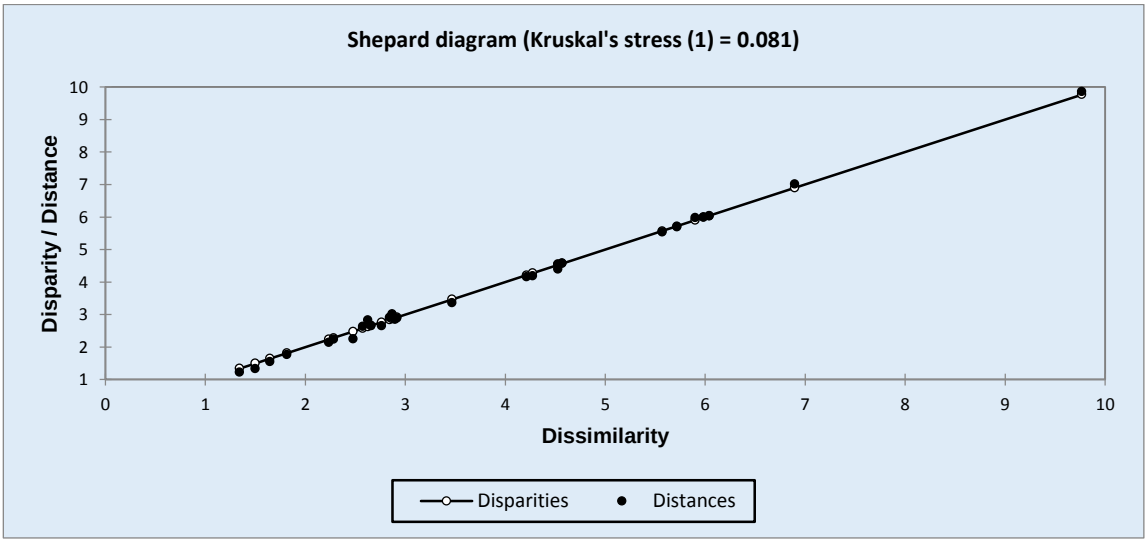


Figure 19: *Shepard Diagram for Pure Hydrocarbons*

LIMITATIONS, CONCLUSION AND RECOMMENDATION

5.1 Limitations and Outlook

Further studies and investigations are required for ionizable organic compounds as my TPSM failed to predict the toxicities of ionizable chemicals. Recently, commercial passive samplers such as o-DGT, POCIS and chemcatcher are recommended for ionizable compounds. Furthermore, lack of experimental passive sampler to water Partition Coefficient (KPS-W) data for Organic Chemicals is required to be calculated for better understanding of Mode of toxic actions which can be achieved via high-throughput methods to widen experimental data.

5.2 Conclusion

Passive Sampler (PS) can be substituted easily as an alternate to Target Lipid Model (TLM) as the results are close to the experimental data. Target Passive Sample Model (TPSM) strongly differentiates Mode of Toxic Action (MoA) for different types of PS. Polyacrylate (PA) outperforms all the other passive samplers based on the RMSE values for baseline (BL) and less-inert (LI) Narcosis, such as for BL and LI, RMSE for PA is lowest (RMSE=0.39 & 0.70 respectively) among the PS. The estimation errors for TPSM falls within the range of experimental error (usually by magnitude of 1 log unit) which shows that passive sampler can be a good alternative to the test organisms.

5.3 Recommendations

i. Legislations are required by regulators such as REACH, US-EPA etc. to discourage animal testing by providing alternatives such as Passive Samplers.

ii. Passive samplers are low-tech and low-cost and can be used as an effective tool for environmental monitoring system whether it is Air, water or soil based environmental system. Similarly, efficiency of passive samplers can be evaluated as it provides cleaner extracts and by developing and redefining the Limits of Quantification (LoQs), Limits of Detections (LoDs), it can be further enhanced in terms of effectiveness.

iii. Capacity building and development programs should be planned by Pak-EPA on how to use and install passive samplers in various environmental medium.

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