Pesticides are economic, labor-saving, and efficient tool of pest management with great popularity in most sectors of the agricultural production. In spite of their popularity and extensive use, health risks arising from the exposure of farmers when mixing and applying pesticides or working in treated fields and from residues in food and in drinking water for the general population have been raised, making it a necessary evil in modern agricultural and pest management practices. The duration of 1960-2013 has witnessed tremendous developments in pesticide research that has enabled reduced usage rates. The introduction rate of new molecule has lowered while the cost to bring a new molecule to market has elevated from U.S. $152 million in 1995 to $256 million in 2005, as the number of compounds synthesized to deliver one new market introduction rose from 52,500 in 1995 to 140,000 in 2005 (Lamberth \textit{et al.}, 2013). New active ingredient registrations with the US Environmental Protection Agency (EPA) over the 1997–2010 period included biological (B), natural product (NP), synthetic (S) and synthetic natural derived (SND) substances. Combining conventional pesticides and biopesticides, NPs accounted for the majority of registrations, with 35.7%, followed by S with 30.7%, B with 27.4% and SND with 6.1% (Cantrell \textit{et al.}, 2012).

Although the environmental and health issues advocate the use of biopesticides but the success history and simple application of synthetic pesticides has always given the later an uncompetitive edge over the former. There has been a slow declining trend in the synthetic pesticide market share but still possess over 92% of the market share of pest control agents (Figure 1.1). Synthetic pesticides are chemically classified into four categories namely organochlorine (OC), organophosphate, carbamate and pyrethroid pesticides. OC pesticides are the oldest developed pesticides which mainly affect sodium channels by preventing the deactivation or closing of channels after activation and membrane depolarization. The hyper excitability results in repetitive discharges in neuron after a single stimulus (Coats, 1990). Many of OC pesticides (DDT, BHC etc.) were commonly used in the past, but have been removed from the
market because of environmental and health risks (U.S.E.P.A., 2002). As far as their biological activity is concerned against target organisms, OCs showed very good results but were banned worldwide because of their toxic effects due to biomagnification in food chain and their persistence in environment.

Figure 1.1: Global biopesticides and synthetic pesticides market, 2003-2010

DDT: A wonder pesticide

DDT, an OC pesticide, was first synthesized by Zeidler in 1874 (ATSDR, 2002) but it was used as an insecticide in the year 1939. DDT is 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane with chemical formula C₁₄H₉Cl₅. So, for every molecule of DDT, there are 14 carbon atoms, 9 hydrogen atoms and 5 chlorine atoms (Fig. 1.2). In its pure form, DDT is a white, crystalline powder with little odour. DDT has long life and is due to its low solubility in water and its relatively high solubility in fats (ATSDR, 2002).

After the discovery that DDT can be used against insects, it was extensively used by almost all the countries around the world for agriculture and health programs. DDT played heroic role not only during green revolution in India but also in other countries in increasing the agricultural productivity and in controlling vector borne epidemics. DDT is the most recognized insecticide all over the world due to the fact that its use has helped to reveal the numerous hazards coupled with the use of synthetic pesticides.
DDT is a colorless, odorless, and insoluble toxic pesticide which contains about fourteen chemical substances in it. This chemical is known for eradicating and destroying harmful insects like flies, mosquitoes, and lice. It is also widely used to kill agricultural pests. The many benefits of DDT include:

- **Disease control**
  
  DDT has been used to kill organisms that are responsible for causing malaria, filariasis and dengue fever. The use of this disinfectant has probably saved the lives of about 50 million people from these diseases (Van den Berg, 2009).

- **Effective for long periods**
  
  DDT is highly persistent and continues to be effective in killing disease causing mosquitoes for months after being applied (Service, 1977).

- **Beneficial for plants**
  
  As water spray or crop dust, DDT has been routinely used on orchards, fields, gardens, and forests. There was a time when the use of this chemical had increased so much that it was registered to be used on more than 300 agricultural crops all over the world (Mitra and Raghu, 1989).

- **Durable in nature**
  
  DDT is highly durable in nature and in some of its applications it is effective for more than 12 years. This chemical cannot be easily washed by water and it also resists breakdown by air and light (NPIC, 2000).

- **Low price**
  
  This chemical is quite cheap and can be afforded by all to kill any kind of insects and pests (Curtis, 2007).

- **Low toxicity to humans**
  
  The chemical is highly toxic to bugs and insects, but has very low toxicity to all mammals including humans. Thus, this chemical can be directly applied to the human skin for killing parasites without causing harm to the person using it (Curtis, 2007; NPIC, 2000).
Introduction

DDT-A tonic...or toxin?

Since Rachel Carson's *Silent Spring*, conservationists in rich and developed countries have waged decades-long campaign, no less persistent than DDT itself, to convince governments and citizens that DDT is an irredeemable pollutant. They have been very successful: Every industrial country, without exception, has ceased using DDT (Attaran *et al.*, 2000).

In this kind of 'balance of risks' paradigm, the evidence must be scrupulously weighed. Although the International Agency for Research on Cancer rates DDT as a possible human carcinogen (along with, notably, several pharmaceutical drugs), not one case-control study of DDT's human carcinogenicity has been affirmatively replicated. Breast cancer furnishes the clearest example: the first study to correlate DDT exposure with statistically elevated risk (Krieger *et al.*, 1994) has now failed to be replicated at least eight times (Schechter *et al.*, 1997; Hunter *et al.*, 1997; Longnecker *et al.*, 1997; Lopez-Carrillo *et al.*, 1997; van’t Veer *et al.*, 1997; Moysich *et al.*, 1988; Zheng *et al.*, 1999; Helzlsouer *et al.*, 1999) and of these later studies, some found exposure to significantly reduce risk. Much the same can be said of studies indicating involvement of DDT in multiple myeloma, hepatic cancer and non-Hodgkin lymphoma1 (Baris *et al.*, 1998; Gladen and Rogan, 1995).

![Space fill model and Ball & Stick model of DDT](image)

**Figure 1.2:** Space fill model [a] and Ball & Stick model [b] of DDT
Indeed, if precaution is relevant, it favours spraying houses with DDT, because it is affordable or effective where other interventions may not be. Cost data from India show that, even using DDT alone, the entire national malaria-control budget is sufficient to protect only 65% of high-risk persons. Switching to Malathion, the next-cheapest alternative reduces that coverage to 21%, which leaves 71 million more people unprotected (Goodman et al., 1999). House spraying also has the advantage that it protects whole families, which is sometimes overlooked in comparing it with insecticide-treated bed-nets, which protect only one or two people at a time (Goodman and Mills, 1999). To put simply, there are too few economic studies to determine with certainty whether bed-nets are more or less cost-effective than DDT house spraying (Gallup and Sachs, 2007).

The debate about the rationality of DDT or any other pesticide’s usage may continue further, but a fact remains unquestioned at its place that it is the most economic synthetic pesticide which has changed the perception of uncontrollable vector borne diseases and agricultural yield increase, because of its mode of action. Also, the fact remains true that ‘everything can wait but hunger cannot’. Therefore, keeping debate aside, the scientific pursuit demands for next generation pesticide having pesticidal potential comparable to DDT but with lower or negligible toxicity and persistence.

New Pesticide Development

Every pesticide in the market is the result of ten or more years of research and testing. Discovery of new compound (pesticide) is still needed for a variety of reasons like development of resistance to older compounds, lack of good solutions to some existing problems of generation of genetic resistance in target population, the changes in insect populations, and the elimination of older compounds with an insufficient safety margin for non-target organisms. The process of pesticide development starts with the discovery of a new chemical entity (NCE) and then evaluation of its activity against pests and toxicology screening.

The process by which a new chemical entity (NCE) is discovered, developed and formulated into a pesticide is tenuous and risky. Only one or two from million molecules tested for pesticidal properties ever becomes a registered product. As the discovery process is so costly and time consuming, scientists try to eliminate
questionable molecules as early as possible and focus their attention on marketable compounds (Saini and Kumar, 2014a).

Pesticide design is an iterative and costly process. The designed NCE must meet multiple criteria of being active, bioavailable, environmentally-/bio-degradable, non-bioaccumulative and non-toxic for being a good pesticide. Computational chemistry has been in the central stage of new drug and pesticide design from recent past. The computational methods can be either structure-based or ligand-based approach, as briefly depicted in figure 1.3 and 1.4. Target characterization and deciphering the reaction mechanism involved are the initial steps of structure based approach while structure-activity relationship (SAR) becomes the important step of ligand based approach of pesticide design (Saini and Kumar, 2014a).

*In-silico* tools in general and QSAR in particular, play a vital role in modern pesticide design, since they represent a much cheaper and rapid alternative to *in-vitro* and *in-vivo* assays (Price and Watkins, 2003) in order to pursue the ‘3Rs’ policy of reducing, refining and replacing animal testing. The major *in-silico* tools applied in computational pesticide design are combinatorial library generation, homology modelling, molecular mechanics simulation, molecular docking simulation, pharmacophore modelling, artificial technique based algorithms, etc. QSAR methodologies are the fore-runners and important tool in computational approach of pesticide design. We can say that nowaday no pesticide is developed without previous QSAR analyses. QSAR relates the biological activity of molecule to some selected features of their physicochemical structure by means of a statistical tool. Biological activity of congeneric molecular structures is related to specific molecular features (descriptors) by using regression techniques to estimate the relative importance of descriptors and their contribution to the biological activity. QSAR models are regarded as a scientifically efficient tool for predicting and classifying the biological activities of untested chemicals in both drug discovery and environmental toxicology (Perkins *et al.*, 2003). QSAR decreases the number of compounds to be synthesized by facilitating the selection of most promising candidates. If an active compound is detected by biological tests, then several of its derivatives are prepared. Computerized mathematical processing of data can reveal QSAR models, on the basis of which the efficiency of compounds not yet prepared, can be predicted with greater probability of upto 75-80% accuracy (Price and Watkins, 2003).
Figure 1.3: Structure based pesticide design process
(Adopted and modified from Young, 2009)
**Figure 1.4:** Ligand based pesticide design process

(Adopted and modified from Young, 2009)
Introduction

During the design and development of pesticides, toxicity of compounds is also tested in experiments with animals in addition to preliminary screening for minimal requirement as pesticides. As a result of this screening almost 90% of compounds are rejected due to low efficiency or high toxicity. Toxicological tests include investigation of the effect of active substance, administered continuously in small doses over a longer period (e.g. 2 years) on experimental animals of several species including rats, birds, fishes and bees etc. QSARs for toxicity i.e. QSTR (Quantitative Structure Toxicity Relationship) can help researchers to investigate the relationship of structural properties with toxicity. The QSAR methodology when applied to chemical compounds for their biodegradability is termed as Quantitative Structure Bioremediation Relationship (QSBR). The development of QSBRs has been relatively slow compared with proliferation of QSARs because of the nature of the biodegradability endpoint. QSBR is very complex because of environmental conditions and bioavailability of the chemical. QSBR relate the molecular structure of compounds to biological degradability and help in prediction of environmental fate. Thus, there is an increase in interest in QSBRs for development of pesticides (Damborsky, 1996).

Therefore, the present work was carried out to look for QSAR insights into design of OC molecule with good pesticidal activity, lower toxicity and biodegradability potential compared to the most successful OC pesticide i.e., DDT. The QSAR model generated was integrated with QSTR and QSBR models of EPI Suite of USEPA. A set of new DDT based compounds were designed and generated through virtual combinatorial library, evaluated and screened for their bioactivity, toxicity and degradability using QSAR models. A few screened OC compounds of virtual combinatorial library were also evaluated for in-vitro analysis, after chemical synthesis, for their pesticidal activity, toxicity and biodegradability alongwith natural degradation profile. The study ultimately attempted to develop QSAR model for use in ligand based pesticide (OCs) designing. Also, the validation of computational methods of QSAR was done using in-silico and in-vitro methods to evaluate the reliability of computational tools in pesticide design process. With these targets in mind, the present study was undertaken with the major objectives as listed next page.
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**Major Objectives**

- To develop QSAR models for bioactivity (pesticidal), toxicity and degradability of organochlorine compounds related to DDT.
- To generate combinatorial library based on designed and optimized lead molecule and screen the library for 5-best predicted pesticide candidates.
- *In-vitro* validation of the developed QSAR models after synthesis of 5-predicted pesticide candidates.