Introduction:

One agricultural practice that carries significant ecological costs is the use of pesticides. Over past 60 years, pesticides have been increasingly used in the environment. According to a 2001 market estimate, approximately 5046 million pounds of pesticide active ingredients were used throughout the world (USEPA, 2004). An ideal pesticide should be toxic only to the target organisms, should be biodegradable and should not leach into the groundwater. Unfortunately, this is rarely the case, and the widespread use of pesticides in modern agriculture is of increasing concern. WHO reported that approximately 3 million human cases of pesticide poisoning occur annually, which result in 220000 deaths worldwide (Pandey et al., 2005). Pesticides are often considered quick, easy, and inexpensive solution for controlling pests in agriculture and domestic uses. Use of these products has played a significant role in raising the yields of crops from agricultural land around the world. Consumers, in turn, benefit from a food supply. Increased use of the pesticides, however, has its own disadvantages. Pesticides have contaminated almost every part of our environment. Pesticide residues are found in soil and air and in surface and ground waters across the world. Pesticide contamination poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms, to, insects, plants, fish, and birds. Some compounds are classified as non-persistent when they get degraded easily by microorganisms, while others are considered persistent since they do not get easily degraded and tend to remain in a segment of environment for longer time duration, causing more damage. There is possibility of bio-accumulation and bio-amplification of the pesticides, poisoning greater risk.

Definition of Pesticide

Pesticides are chemicals used to kill or control pests like, fungi, bacteria, insects, plant diseases, snails, slugs, or weeds among others.

Synthetic Organic Pesticides

Table below shows some more commonly used synthetic organic pesticides.

Sr.No.	Name of the Group	Name of the pesticides
1	Organochlorine	DDT, Lindane, Chlorpyrifos, Methoxychlor
2	Organophosphorous	Malathion, Phorate , Diazinon
3	Carbamates	Carbaryl, Methiocarb
4	Pyrethroids	Permethrin, Bifenthrin, Cypermethrin
5	Chloronicotynils and Neonicotynils	Imidacloprid

Pesticides act on pest by ingestion or by touch and death may occur immediately or over a long period of time. Insecticides are a type of pesticide that is used to specifically to target and kill insects. Some insecticides include snail bait, ant killer, and wasp killer etc. Herbicides are used to kill undesirable plants or “weeds”. Some herbicides will kill all the plants they touch, while others are designed to target one species. They are classified according to their chemical class or intended use. Insecticides act by poisoning the nervous system of target harmful insects.

Pesticides reach aquatic system through leaching, equipment washing, disposal of empty containers and direct dumping of wastes into the water systems (Yang et al., 2005). The distribution of various pesticides contaminants in the marine and estuarine environment depends on the physicochemical properties of the ecosystem as well as the partition coefficients of individual chlorinated compounds (Sarkar et al., 1997). For example OCPs (organochlorine pesticides) could distribute among the components of the ecosystem, such as water and sediment, accumulate in the biota. As a result of their persistence, OCPs in water can be transferred into the food chain and accumulate in aquatic organisms like plankton. Different pesticides pose varying degrees and types of risk to water quality. Anindita et al. 2013 reported that the application of pesticide should be restricted to recommended doses only for sustenance of soil health.

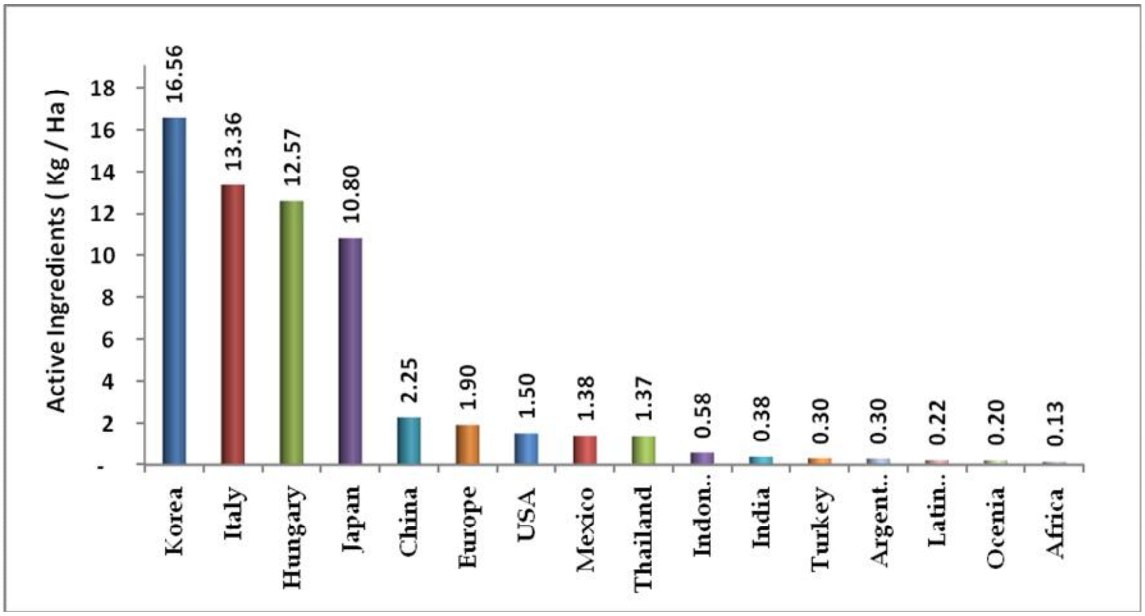
Why Need Pesticides.

As the human population continues to grow, more and more crops are needed to meet this growing demand. This has increased the use of pesticides to increase crop yield per acre.

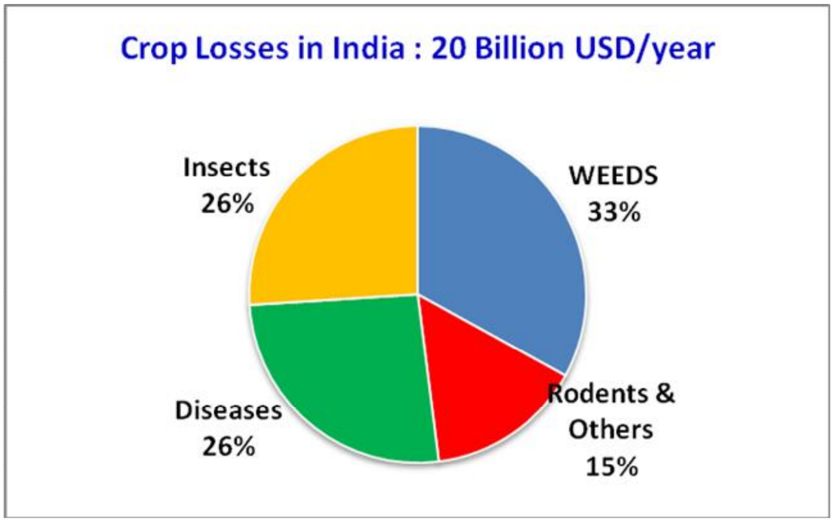
The tropical climate in India makes the country ideal for agriculture but also results in the occurrence of various pests that can cause significant damage to crops. Rapid economic and population growth have led to an intensification of agricultural practices and, consequently, heavy use of pesticides to increase yields. Since 1960, following the Green Revolution, increasing amounts and varieties of chemicals have been introduced into the country. The majority of insecticides currently in use are organophosphorus, carbamate, and synthetic pyrethroid compounds. Organophosphorus compounds are an economically important class of chemical compounds with numerous uses, such as in pesticides, industrial fluids, flame retardants, therapeutics, and nerve gas agents. Most modern synthetic organophosphorus compounds are tailor-made to inhibit acetylcholinesterase (AChE), an enzyme essential for life in humans and other animal species.

Even then, in India, pesticides consumption is just about 0.38% of the world average, as shown in figure below.

Pesticides Usage Global (2009)



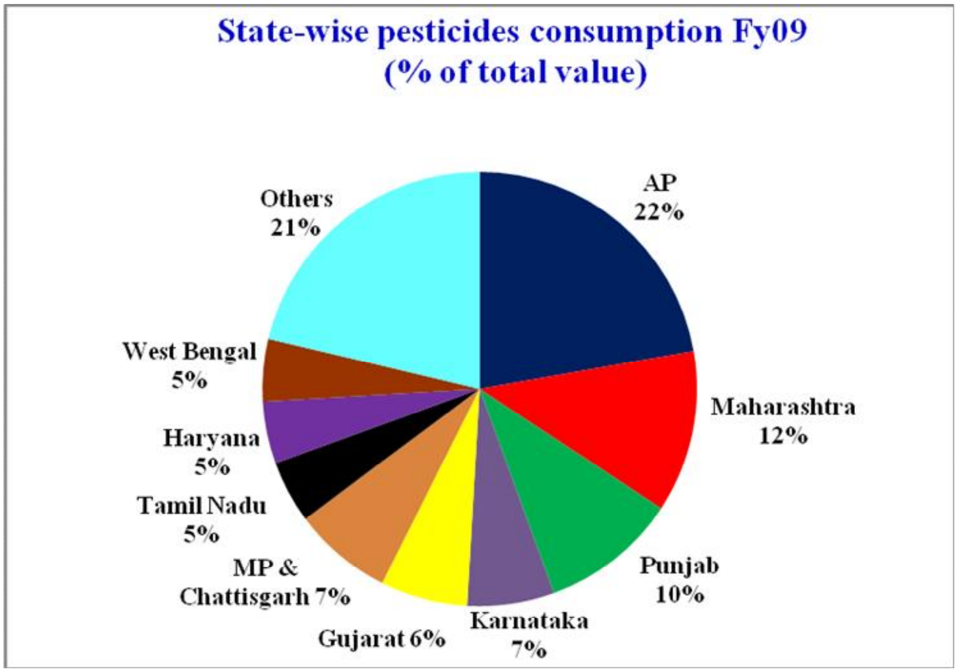
In fact, the available data suggests that India still suffers huge crop losses due to various reasons, including lower use of pesticides, as shown in figure below (2009-2010).



Source : 37th report of Dept. of Chemicals and Petrochemicals, Government of India.

Pesticides state –wise consumption of pesticides India data show in figure below. Andhra Pradesh tops the chart, being largest consumer of pesticides.

State –wise pesticides consumption FY09 (% of total value)



SOURCE: TATA STRATEGIC ANALYSIS AND INDUSTRY REPORTS

With ever increasing population, and growing need for increasing crop yields, pesticides consumption has been on rise in India as well. Pesticides significantly contribute to the food security in India and help to the crop loses. Pesticides use also helps farmers to benefit economically and help to ensuring food safety for the nation. Along with this development, there is also rise in pollution due to pesticides and associated health issues.

How do Pesticides Contaminate the Environment?

Pesticeds and their metabolites are increasingly being reported to be present in various environment segments, air, water and sediments. This is due to pesticide residues entering aquatic environments through effluent release, discharges of domestic sewage and industrial wastewater, atmospheric deposition, runoffs from agricultural fields, leaching, equipment washing, and disposal of empty containers and direct dumping of wastes into the water systems. Pesticides are inherently toxic molecules. Once they reach the water bodies, they adversely affect the aquatic environment. The pesticides used in controlling the target organisms have potential for adverse effects on non target organisms because these compounds are often non-selective.

How to Minimize the Contamination of Pesticides

The pesticides should be used proper recommended dose and proper monitoring of pesticides residue in soil, water, food, fruit and grain. Integrated Pest Management is considered a better option for optimizing pesticide consumption.

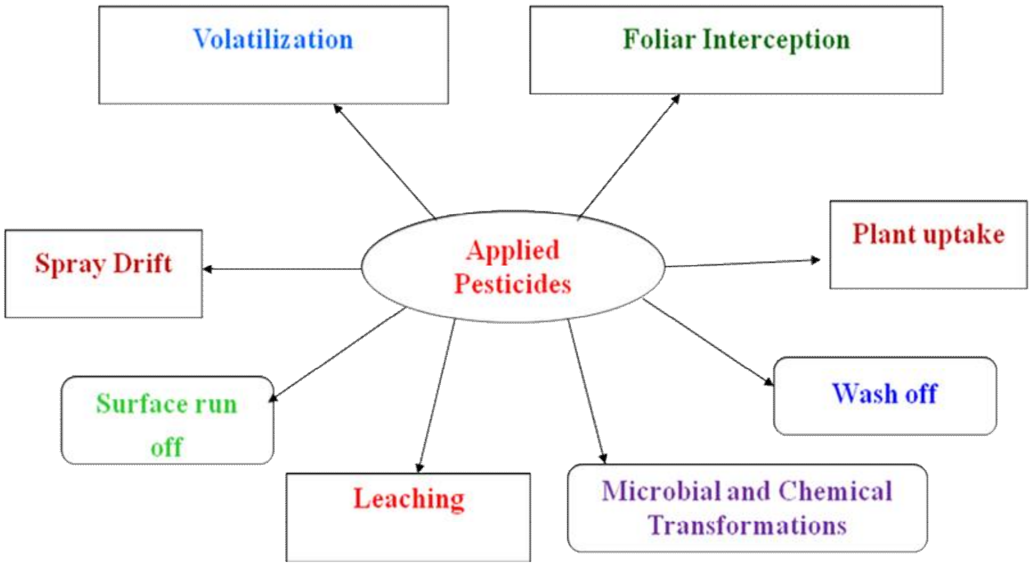
How do pesticides reach water bodies?

Soil, an important component of the environment, acts as a sink for the pesticides used in agriculture. The pesticides present in soil sometimes act as a source of contamination for succeeding crop. From soil, the pesticide residues can reach water bodies by leaching and run offs.

Pesticides degradation/Dissipation

Once it reaches an environmental segment like water or sediment, a pesticide is acted upon by biotic and / or abiotic factors prevalent and hence may undergo breakdown or change. Pesticide active ingredient and its depredates tend to accumulate in the environment. These may stay there affecting the system or may get transported to other segment. The environmental fate studies are designed to help identify which dissipation processes are likely to occur when a pesticide is released into the environment and to characterize the breakdown products that are likely to result from these degradation processes. The diagram below illustrates the potential dissipation pathways for a pesticide after it is applied.

Dissipation of Pesticide



The behavior and fate of pesticides in the soil environment in controlled by their physic-chemical properties and various complex dynamic physical chemical and biological processes, among which the most important are sorption- desorption (interaction of pesticide molecules with natural sorbents, soil organic matter and soil minerals), as well as pesticides degradation processes.

Pesticides participating in bio-physico-chemical processes can lead to a their transitory or permanent accumulation in soils or, on the contrary, to their elimination from the environment. They determine the pesticide concentration in the soil solution, and have a large influence on pesticide transfer toward ground or surface waters and on their ecotoxicological impacts on soil organisms as well. The main difficulties in studying and predicting the retention and degradation of pesticides in soils are the diversity of chemical structures and reactivities of pesticides, the high diversity of soils and their heterogeneous composition and structure. In addition, the pedoclimatic conditions, in particular soil temperature and water content, have a strong influence on retention and degradation because of their effect on soil biological, chemical and physical properties.

Fate and Transport Studies Needed

The type of environmental fate studies required depends on the indoor or outdoor use of the pesticide. In the laboratory studies, hydrolysis, photolysis, and soil metabolism, are routinely conducted for all outdoor use pesticides. The studies involve the following determinations:

- The half-life of the parent;
- Rates of formation and decline of breakdown products; and
- Mobility of the parent and breakdown products

Following are the Pathways of Degradation of Pesticides in Water and Soil.

Physicochemical Degradation

This includes hydrolysis and photo-degradation in water and soil. Hydrolysis studies determine the potential of the parent pesticide to degrade in water, while photo-degradation studies determine the potential of the parent pesticide to degrade in water and soil, when exposed to sunlight.

Biological Degradation

Biological studies include aerobic and anaerobic soil metabolism, and aerobic and anaerobic aquatic metabolism. The soil metabolism studies determine the persistence of the parent pesticide when it interacts with soil microorganisms living under aerobic and anaerobic conditions. The aquatic metabolism studies produce similar data that are generated by pesticide interaction with microorganisms in a water/sediment system. These studies also identify breakdown products that result from biological degradation.

Mobility of the Parent and Breakdown Product(s)

The adsorption/desorption study determines the potential of the parent pesticide and its degradation products to bind to soils of different types. The potential mobility of the parent pesticide and each breakdown product is determined by examining the data from both of these studies and may range from immobile to highly mobile.

Field Dissipation

These studies assess the most probable routes and rates of pesticide dissipation under actual use conditions at representative field sites. While laboratory environmental fate studies are designed to address one dissipation process at a time, field dissipation studies assess pesticide loss as a combined result of chemical and biological processes (e.g., hydrolysis, photolysis, microbial transformation) and off-site transport (e.g., volatilization, leaching, run-off) as well as loss from plant uptake.

This work focuses on biotic and abiotic degradation of Imidacloprid in water and soil under laboratory conditions.

Chemical Class and Type of Imidacloprid

Imidacloprid is a neonicotinoid insecticide in the chloronicotinyl nitro guanidine chemical family. The International Union of Pure and Applied Chemistry (IUPAC) name for the compound is 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine and the Chemical Abstracts Service (CAS) registry number is 138261-41-3. Neonicotinoid insecticides are synthetic derivatives of nicotine, an alkaloid compound found in the leaves of many plants in addition to tobacco. Imidacloprid was first registered for use in the U.S. by the United States Environmental Protection Agency (U.S. EPA) in 1994.

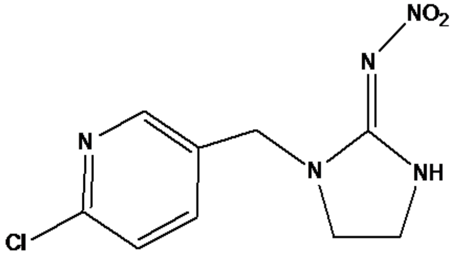
Regulatory Status

Imidacloprid is a general use Pesticide, and is classified by EPA as both a toxicity class II and class III agent, and must be labelled with the signal word "Warning" or "Caution" (223). There are tolerances for residues of imidacloprid and its metabolites on food/feed additives ranging from 0.02 ppm in eggs, to 3.0 ppm in hops (U.S. Environmental Protection Agency. 1995).

Physical and Chemical Properties of Imidacloprid

Molecular formula	$C_9H_{10}ClN_5O_2$
Molecular weight	255.7 g/mol
CAS Number	13826-41-3
IUPAC name	1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine.
C.A. name	1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine.
Form	Colorless crystals with a weak characteristic odor.
Melting point	143.8 °C (crystal form 1) 136.4 °C (crystal form 2)
Solubility in water:	0.51 g/L (20 °C)
Solubility in other solvents @ 20 ° C	dichloromethane - 50.0 - 100.0 g/L; isopropanol - 1.0-2.0 g/L; toluene - 0.5-1.0 g/L; n-hexane - < 0.1 g/L; fat - 0.061 g/100g
Vapor pressure	0.2 μPa (20 °C) (1.5×10^{-9} mmHg)
Specific gravity/density	1.543 (20 °C)
Stability	Stable to hydrolysis at *p*H 5-11.
Kow log p	0.57 (22 °C).

Structure of Imidacloprid



Imidacloprid is a systemic, chloronicotinyl insecticide with soil, seed and foliar uses for the control of sucking insects including rice hoppers, aphids, trips, whiteflies, termites, turf insects, soil insects and some beetles. It is most commonly used on rice, cereal, maize, potatoes, vegetables, sugar beets, fruit, cotton, hops and turf, and is especially systemic when used as a seed or soil treatment. Kidd et al. 1991 reported that the chemical works by interfering with the transmission of stimuli in the insect nervous system. Specifically, it causes a blockage in a type of neuronal pathway (nicotinergic) that is more abundant in insects than in warm-blooded animals (making the chemical selectivity more toxic to insects than in warm-blooded animals). This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis, and eventually death. It is effective on contact and via stomach action (Abbink 1991; Tomizawa and Yamatto 1992; Liu and Casida1993; Elbert et al.1998). Neonicotinoids interact with nicotinic acetylcholine receptors at the central and peripheral nervous system, resulting in excitation and paralysis, followed by death. Imidacloprid, acetamiprid and thiamethoxam interact with nAChR in a structure-activity relationship (Tomizawa et al. 1995a). Hence, it has been suggested that these compounds are called neonicotinoids (Yamamoto et al. 1995). Comparative toxicity was carried out, under laboratory conditions, of two neoicotinoids, acetamiprid and imidacloprid, against the whitefly *Bemisia tabaci* (Gennadius), using foliar and systemic applications on cotton seedlings (Horowitz et al. 1998). In other assays, it was found that the comparative toxicity of the neonicotinoids could be different when assayed on different host plants. Several neonicotinoids have been commercialized for controlling insect pests.

Imidacloprid Formulations

Imidacloprid based insecticide formulations are available as
Dunstable powder (DP), granular,
Seed dressing (Flowable Slurry Concentrate)
Soluble Concentrate
Suspension concentrates (SC)
Wettable Powder (WP)

Imidacloprid Application Rate

Typical application rates range from 0.05 - 0.125 pounds/acre. These application rates are considerably lower than older, traditionally used insecticides. It can be phytotoxic if it is not used according to manufacturer's specifications, and has been shown to be compatible with fungicides when used as a seed treatment to control insect pests.

Environmental Fate of Imidacloprid

Imidacloprid applied to the soil or seed treatment, imidacloprid is metabolized more or less completely depending on plant species, time, soil type and other environmental conditions (Araki *et. al.*1994). Due to greater solubility of the pesticide in water, it is expected to be present in water systems due to agricultural run offs. In water, degradation of the pesticide depends on *p*H and other organic and inorganic species present in water. (Suparna Pal *et. al.* 2006).

Determination of imidacloprid residues in soil and in water is necessary so that frequency of crop cycle can be established and harmful effects of the pesticide minimized. Das et al (2008) reported that soil plays a preponderant buffering role in the fate of the pesticide. Apart from volatilization, the main processes that control the fate of the pesticide in soil are retention on soil particles and degradation (biotic and abiotic).

Therefore, the objective of this chapter is to provide an overview of the factors involved in the retention and degradation of pesticides in soils and to discuss and clarify the needs of new integrated approach. In particular, this work will examine (i) retention and degradation studies (ii) soil structure (iii) the primordial role of water.

Burauel and Bassmann, 2005, have reported that soil works as a filter buffer and has a degradation potential for pesticides having the property of storage of pollutant, owing to the presence of organic carbon. It is recognized that the soil is a pathway for the pesticides' transportation to water, air and food through runoff and leaching. Pesticides are also transferred from soil to plants and animals and ultimately to the human being.

In the environment, the principal routes of dissipation for imidacloprid are aqueous photolysis, microbial degradation and uptake by plants. Imidacloprid was reported to photo degrade rapidly (half-life of 4 hours) in water, compared to soil (half-life of 171 days). It was hydrolytically stable at *p*H 5 and 7, but hydrolyzed slowly in sterile alkaline solutions (half-life of 355 days). The half-life of the imidacloprid degradation in anaerobic soil was found to be 27 days. Imidacloprid was persistent in aerobic soil under laboratory conditions (half-life of 188 to > 365 days). The presence of vegetation substantially increased the rate of imidacloprid degradation in the soil (half-life of 48 days). Studies on the imidacloprid mobility in soil revealed that imidacloprid residues leached into the 6-12 inch soil depth under field conditions (Sarkar MA et al 2001).

Pimentel et al. 1986 have reported that pesticide dissipation processes control the transport, retention and transformation of pesticides within the soil matrix and their transfer from the soil to other environmental compartments. Novarro et al. 2007 reported that while a pesticide is bound to particles in the soil, the main processes in the soil cause its loss and transformation. Significant losses of pesticides can occur during application, with the amount of loss affected by the nature of the pesticide formulation, atmospheric conditions and method of application.

Imidacloprid is currently listed by the Department of Pesticide Regulation (DPR) (US-EPA) as a potential ground water contaminant, based on its high solubility in water, mobility and persistence in soil. The low vapour pressure of imidacloprid indicates that its volatilization from soil and leaf surfaces may not be a major route of dissipation.

A soil adsorption/desorption study was carried out to characterize the mobility of imidacloprid in soil (Fritz, 1988). Aqueous solutions of [pyridinyl-14C-methyl]-imidacloprid were equilibrated for 48 h at 25ºC with four different soil types – sandy loam, silt loam, low-humus sandy soil and silt clay. The highest tested concentration of imidacloprid was approximately 290 mg/L. The soil to water ratio was 1:4. Based on the soil-carbon sorption constant (Koc), the mobility of imidacloprid could be classified as high in silt (Koc of 132) and medium in low-humus sandy soil, silt clay and sandy loam (Koc of 157, 212 and 256, respectively). In a subsequent study, aqueous solutions of [pyridinyl-14C-methyl]-imidacloprid were incubated at 25ºC with four different soil types – sand, loamy sand, slit loam and loam. The mobility of imidacloprid was classified as medium Koc of 277-411; (Hayes M. H. B. et al., 1970).

A soil adsorption/desorption study was carried out to characterize the sorption properties of the major metabolite of imidacloprid, desnitro-imidacloprid. Aqueous solutions of [14C]-desnitro-imidacloprid were equilibrated at 25ºC with four different soil types – sand, loamy sand, silt loam and loam. The highest tested concentration of desnitro-imidacloprid was approximately 250 ppm. The soil to water ratio was 1:3 for sand, loamy sand and 1:5 for silt loam. Desnitro-imidacloprid had a stronger sorption affinity for soil than the parent compound. Based on the Koc values, it can be classified as a medium mobility compound in sand (Koc 327) and low mobility compound in loamy sand, silt loam and loam (Koc of 833, 942 and 866, respectively). Therefore, desnitro-imidacloprid is less likely to leach through the soil than the parent chemical (Dyer et al., 1992). The persistence of imidacloprid in the aqueous environment depends on environmental factors including exposure to light, *p*H, temperature, and microbial community. Photolysis appears to be a major process for the degradation. DT_{50} value of 30, 130 and 160 days have been calculated in the absence of light and with variable sediment (Krohn and Hellpointer 2002). Similarly, Spiteller (1993) reported that the half life of imidacloprid under dark conditions was 129 days. Combining metabolic and photolysis processes reduces the DT_{50} value to the range of days (Heimbach and Hendel 2001).

Imidacloprid field dissipation rates are widely variable, and in some case it has been shown to degrade slowly in soil. Liu et al (2006) also reported that imidacloprid was stable in water at neutral and acidic *p*H and slowly degraded in alkaline solutions. Vegetation increased the rate of dissipation of imidacloprid. Biodegradation of pesticides is controlled by the bioavailability of the pesticides to pesticides – degradation microorganism and the activity of the microorganism. Jennifer C. et al 2007 have reported the isolated microbial degradation of imidacloprid. Imidacloprid is slowly mineralized to CO_2. Degradation of imidacloprid–guanidine or imidacloprid – urea proceed by breaking apart the imidazolidin ring at its two adjacent carbon or perhaps the methyl group is oxidatively cleaved producing 6-chloronicotinic acid and the imidazolidin ring as separate compound.

Field Dissipation

A series of field dissipation studies were performed at different sites (Georgia, Minnesota and California) with various soil types to evaluate the degradation and mobility of imidacloprid under actual field conditions. Imidacloprid formulation 240 FS (23.3% active ingredient liquid suspension) was applied to the soil at the highest recommended rate of 0.5 lb a.i./acre. Soil core samples were analyzed for imidacloprid immediately post-application through 18 months. Each core was sectioned into 6-inch segments. The half-life for imidacloprid dissipation in loamy sand and sandy loam was 12 days. Imidacloprid applied to a field planted with corn had a half-life of 7 days. Residues at or above the detection limit (10 ppb) were not detected below 0-6-inch soil depth (Rice et al., 1991a,b). Imidacloprid applied to a tomato plot dissipated with a half-life of 53 days. Residues above the detection limit (10 ppb) were detected below the 6-inch soil depth, indicating that the pesticide had leached into the 6-12 inch soil depth (Rice et al., 1991c).

The half-life for imidacloprid dissipation in the turf grass was 61-107 days with no leaching below the 0-6 inch soil depth (Rice et al., 1992a,b). The field dissipation studies revealed that imidacloprid had a shorter half-life under field conditions (7 to 146 days), when compared to the half-life observed under aerobic laboratory conditions (greater than 1 year, Anderson et al., 1991). This difference in the imidacloprid stability was attributed to the combined effect of various dissipation pathways under field conditions, including photolysis, hydrolysis, chemical and microbial degradation and plant uptake.

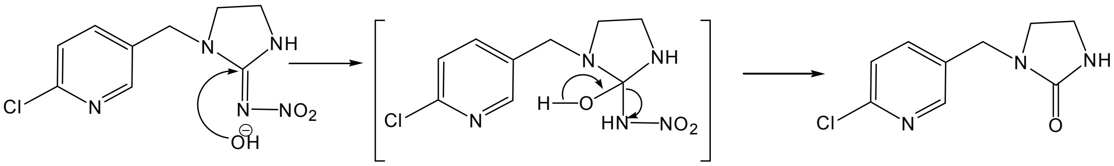
The soil metabolism has also been reported in sugar beet field crops (Rouchaud 1996). The study on sorption and desorption of imidacloprid indicated that organic carbon in soil has the most significant effect and hysteresis was found to occur in desorption process.

Hydrolysis of Imidacloprid

The hydrolysis of pesticides is an area that has received extensive study, since compounds entering the environment will at some stage come in contact with water or will get adsorbed on lipophilic media.

The hydrolysis pathway is studied to understand the stability of the pesticides and hydrolysed products. Koester 1992 has reported imidacloprid metabolites in plant cell culture viz., 1-[(6-chloro-3-pyridinyl) methyl]-5-hydroxy-4,5-dihydro-N-nitro-1H-imidazole-2-amine and 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-1H-imidazole-2-amine, which might decompose to 6-chloronicotinic acid. Wei Zheng et.al 1999 has reported the hydrolysis of imidacloprid in water at acidic, neutral and basic conditions. Little hydrolysis of imidacloprid in acidic and neutral conditions was observed. Under basic conditions hydrolysis of imidacloprid is highly *p*H and temperature dependent, higher the temperature and *p*H increases the hydrolysis of imidacloprid. They have reported only one hydrolysis product,1-[(6-chloro-3-pyridinyl) methyl]-2-imidazolidone.

Proposed mechanism for hydrolysis of imidacloprid



Photolysis of imidacloprid

Zheng Wai et al 2003 have reported that complete degradation of 20 mg/L imidacloprid in aqueous phase was observed in 40 min under ultraviolet (UV) irradiation system at 254 nm and 365nm. They suggested the ultraviolet radiation played significant role in direct photolysis of imidacloprid. The imidacloprid photo-degradation with proposed reaction is as follows.

$C_9H_{10}N_5O_2Cl + 17\ O_2 \xrightarrow{\hspace{2cm}} 9CO_2 + 5NO_3^- + Cl^- + 4H + 3H_2O$

Effect of Soil Characteristic on Persistence of Imidacloprid in Soil

A detailed study of the available literatures revealed that the majority of the pesticide degradation studies were carried out in normal agricultural soils. However, there are a large part of arable soils, which are naturally or anthropogenically saline. The degradation of pesticides on temperate soils cannot be extrapolated to tropical soils. Das *et al.*, 2007 reported the effect of novaluron on microbial biomass, respiration and fluorescein diacetate hydrolyzing activity in tropical soils.

The preceding sections thus show that use of imidacloprid as insecticide is increasing and along with that concerns about its transport and dissipation in environment are also increasing. Careful monitoring of the pesticide is very important to understand the fate of the insecticide. Suitable analytical methods are reported for the purpose, as described below.

Analytical methods for Deteramination of Imidacloprid

Sundaram Baskaran et al. (1997) reported extraction of imidacloprid residue in soil. The soil materials were mixed thoroughly before extraction of pesticide residues. Soil samples (25g) were weighed in centrifuge tubes and extracted with acetonitrile:water (80:20 by volume; 40mL) for imidacloprid and cleanup was done by SPE method. The methods were subjected to a thorough validation procedure. The mean recovery was in the range of 75 to 114% with repeatability below 20%. The limits of detection were below 2.5 μg/kg, while the limits of quantification did not exceed 4.0 μg/kg. The total uncertainty was evaluated taking the main independent uncertainty sources under consideration. The expanded uncertainty did not exceed 49% for the 10 μg/kg concentration level and was in the range of 16–19% for the 40 μg/kg fortification level.

Fernandez-Alba et al. (1996) have performed extraction of imidacloprid in water followed by HPLC-PDA (High-performance liquid chromatography-Photo diode array detector). They reported the detection limit of imidacloprid at the level of 0.01-0.60 mg/ kg in water. The limit of detection of imidacloprid in this method was relatively high.

Soudamini Mohapatra et al. (2011) describe the extraction procedure with acetonitrile, partitioning into dichloromethane, clean up with neutral alumina and estimation of residues with HPLC, equipped with UV-VIS detector maintained at wavelength of 270 nm. They reported the limit of detection as 0.05 mg/kg in grape berries and soil.

Ralf et al. (2003) have reported analytical method for determination of imidacloprid residues by LC MS/MS. This method allows determination of the parent compound imidacloprid and two toxicologically relevant plant metabolites of this systemic insecticide. The imidacloprid residues and its metabolites 5-hydroxy-imidacloprid and olefin-imidacloprid are extracted with methanol/water. The extracts are subjected to liquid-liquid partition on a column filled with diatomaceous earth and subsequent solid phase extraction on a silica gel column. Quantification is performed by reversed phase HPLC with electrospray MS/MS-detection. The overall recoveries for imidacloprid, 5-hydroxy-imidacloprid and olefin-imidacloprid from plant matrices were between 91 and 97% with a relative standard deviation between 6.0 and 8.5%. The detector linearity and the repeatability of the method proved to be very precise. The limits of quantification were 0.005 and 0.01 mg/kg for imidacloprid and 5-hydroxy-imidacloprid, and for the olefin-metabolite, respectively.

J. M. Bonmatin et al. (2003) have reported method for the determination of low amounts of imidacloprid in soils, plants (leaves and flowers), and pollens by using HPLC coupled to tandem mass spectrometry (APCI-MS/MS). The linear range of application is 0.5-20 μg/kg imidacloprid in soils, in plants, and in pollens, with a relative standard deviation of 2.9% at 1 μg/kg. The limits of detection and of quantification are LOD 0.1 μg/kg and LOQ 1 μg/kg, respectively

A number of other methods have also been employed to measure residues of imidacloprid in water or in soil, e.g., Photo chemical fluorimetric method (Vilchez *et al.* 1998), electrochemical method, enzyme-linked immune sorbent assays (Wantatable, *et al.* 2004), capillary electrophoresis, gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography HPLC (Zhou Q *et al.* 2003). Among these methods GC and LC are the most suitable methods. However, GC cannot be used directly to determine imidacloprid due to its poor volatility and polarity. In case of imidacloprid analysis in GC samples should be derivatised. HPLC is more suitable for analysis of imidacloprid.

Vilchez *et al.* (1998) have reported gas chromatograph method for the determination of imidacloprid in water. They carried out imidacloprid hydrolysis with basic medium to convert it to volatile imidacloprid urea. This hydrolyzed product was extracted with chloroform and this extract was subjected to GC analysis. The method detection limit is 0.16μg/L.

Srivastava Ashutosh K. et al. (2012) have reported gas chromatographic method for determination of imidacloprid residue in water. They converted imidacloprid into a volatile imidacloprid-urea by alkaline hydrolysis. The hydrolyzed product was extracted with dichloromethane and finally taken into 1 mL mixture of acetone and n-hexane (1:9 v/v). They separation of peaks was done on gas chromatography coupled with Electron Capture Detector (ECD) and Nitrogen Phosphorous Detector (NPD). The mean recovery of imidacloprid-urea in water was 92%. The limit of detection of imidacloprid-urea was found to be 20 mg/L with a signal to noise ratio of 3:1 and limit of quantification was found to be 75 mg/L with a signal of noise ratio 10:1.

The derivatization of imidacloprid is very difficult and does not give repeatability. In contrast to GC, HPLC is more effective and appropriate for the residual analysis of imidacloprid, and it has been successfully employed for assaying imidacloprid in the soils, water as well as in the vegetables (Sajjad Ahmad Baig *et al*. 2012). We have developed and validated the analytical method for the determination of low amounts of imidacloprid in soil and water by HPLC and the method was validated before use.

Analytical Method Validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results.

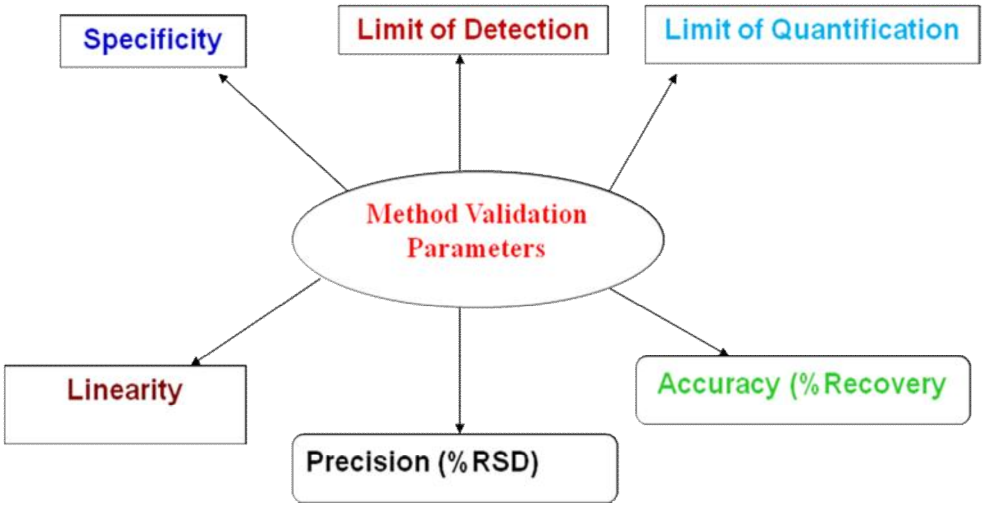
Analytical Methods need to be Validated

1) Before their introduction into routine analysis.

2) Whenever the conditions change for which the analytical method has been validated (e.g., an instrument with different characteristics or samples with a different matrix.)

3) Whenever the method is changed and the change is outside the original scope of the method.

The analytical method validation involves optimisation of following parameters that are commonly:



Method Validation Parameters for Imidacloprid

Analytical Method Validation for Determination of Imidacloprid in Soil and Water

We have developed and validated the analytical method for the determination of low amounts of imidacloprid in/on different soils viz. sandy loam soil, clay soil, red soil and black soil, and in water at different *p*H values, viz. 4, 7 and 9 using HPLC. The accuracy and precision of the method was evaluated on the basis of the recoveries obtained for fortified soil and water samples. The following parameters were undertaken for method validation.

- Specificity
- Linear Dynamic Range (LDR)
- Limit of Detetction (LOD)
- Limit of Quantisation (LOQ)
- Precision (%RSD)
- Accuracy (%Recovery)

Specificity

The standard sample and the control sample solutions in acetonitrile along with suitable mobile phase with optimized parameters were injected into a High Performance Liquid Chromatograph (HPLC). The interference (if any), in the determination of active ingredient in test substance was reported. Interference should not be more than 3% to the total peak area measured for target active content.

Linear Dynamic Range (LDR)

Five different concentrations of imidacloprid reference standard were prepared and injected into a HPLC in duplicate. The linear calibration curve was established by plotting the mean peak area against concentration (mg/L). The correlation coefficient (r), intercept (a) and slope (b) were estimated.

Limit of Detection (LOD)

Limit of detection of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated, under the stated experimental conditions.

Several approaches for determining the detection limit are used, depending on whether the procedure is a non –instrumental or instrumental.

In our study, the minimum quantity of imidacloprid, which could be detected by the HPLC with signal to noise ratio of 3 ± 0.5, was calculated as limit of detection (LOD).

Limit of Quantification (LOQ)

The minimum quantity of imidacloprid, which could be quantified by the HPLC with signal to noise ratio between 5 and 10, was calculated as limit of quantification (LOQ).

Precision and Accuracy

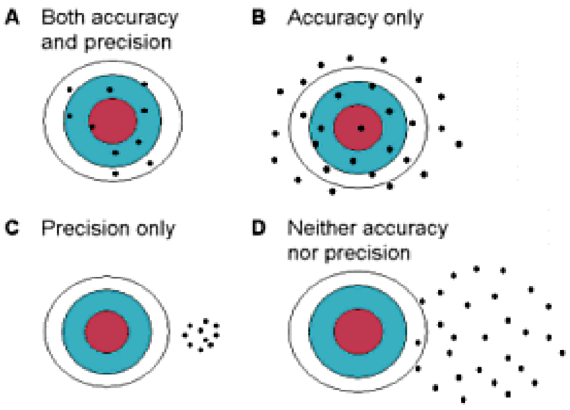
Accuracy and precision are two important factors to consider when taking data measurements. Both accuracy and precision reflect how close a measurement is to an actual value, but accuracy reflects how close a measurement is to a known or accepted value, while precision reflects how close reproducible measurements are among themselves, even if they are far from the accepted value.

Accuracy

Accuracy expresses the closeness of a result to a true value and is often described using two components, trueness and precision.

Precision

Precision is the degree to which several replicate measurements provide answer very close to each other. It is an indicator of the scatter in the data. The lesser the scatter, higher the precision. The pictures given below clearly describe Accuracy and Precision.



Determination of Repeatability

The five replicate injections of single sample solution of imidacloprid along with sequential injections of standard solution were injected into HPLC using the selected parameters. The active content in each sample injection, mean of active ingredient content and % RSD was calculated and reported.

Calculation of Precision (% RSD)

Precision is defined by the relative standard deviation (%, RSD). The precision (% RSD) is calculated as followed.

$$\text{Precision (\%, RSD)} = \frac{\text{Standard deviation}}{\text{Arithematic mean (a.i.content)}} \times 100$$

Precision and accuracy was reported at each fortification level i.e., at LOQ and 10 times LOQ along with two sets of control soil and water samples. Recovery of analytical method was determined by the method of test substance addition to the test media viz. soil and water. Five determinations were made at each fortification level. The active ingredient concentration in each replicate, mean of active ingredient concentration, SD and % RSD was calculated and reported. Each replicate of sample solution was injected in duplicate, mean of the two data was used for computations.

The fortified samples were extracted and analysed by HPLC. The imidacloprid residue in soil and water samples was calculated using the following formula:

$$Y = bX + a$$

$$X = \frac{Y - a}{b} \times D$$

Where,

Y = Peak area of the sample
a = Intercept
b = Slope
D = Dilution factor (if applicable)

Calculation of % Recovery (Accuracy)

The % recovery was calculated using the following formula:

$$\% \, Recovery \, (Accuracy) = \frac{Concentration \, Recovered}{Concentration \, Fortified} \times 100$$

Accuracy/recovery values should fall within 70 to 110%.

Soil Characterization

The soil characteristics were studied to determine the physico-chemical properties of soil viz., *p*H, organic carbon, Water Holding Capacity (WHC), sand content (particle size distribution), and moisture content. The soil samples were collected from different places of Gujarat.

a) Valvada
b) Bardoli
c) Umarsadi
d) Baroda

Analytical Method Validation for Determination of Imidacloprid in Vegetables using SPE and QuEChERS

Assessing the risk of pesticide residues in the commodities intended for human consumption is necessary. Several methods have been reported for the analysis of imidacloprid in foods, fruits and vegetables. The LC method gave good results for imidacloprid in groundwater, soils, and fruits and vegetables but the limit of detection (LOD) was still too high for the present purpose.

Anastassiades et al. 2003 have reported a new approach called "quick, easy, cheap, effective, rugged, and safe" (QuEChERS), which involves extraction of pesticides with acetonitrile partitioned from the aqueous matrix using anhydrous $MgSO_4$ and NaCl followed by a dispersive-SPE, cleanup with $MgSO_4$ and primary secondary amine (PSA). The QuEChERS method commonly uses GC–MS and LC–MS/MS to cover the wide range of pesticides for analysis. Furthermore, the required method must satisfy strict quality criteria to be classified as a trace levels determination and low levels extraction procedure. The QuEChERS method was applied to sample preparation in this study, because it has several advantages over most of the traditional extraction techniques.

The extraction procedure for method used depends on the nature of matrix. The vegetable samples are simply extracted by shaking with suitable solvent. Kapoor U**.** et.al (2013) have reported QuEChERS (quick, easy, cheap, effective, rugged, and safe) method of extraction procedure for imidacloprid in fruits, fruit juices, and baby foods followed by high-performance liquid chromatographic analysis, and imidacloprid residues were qualitatively confirmed by liquid chromatography-mass spectrometry.

Rapid and reliable multiresidue analytical methods were developed and validated for the determination of six neonicotinoids pesticides (acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam) in honey by Michele Proietto et al. in 2013. The modified QuEChERS method allowed a very rapid and efficient single-step extraction, while the detection was performed by UHPLC/MS-MS. The recovery studies were carried out by spiking the samples at two concentration levels 10 and 40 µg/kg fortification levels.

Lehotay et al. (2005) observed high recoveries for a wide polarity and volatility range of pesticides. They reported very accurate results, low solvent usage and waste, and high sample throughput. Their method is quite rugged, relatively inexpensive and fewer materials and glassware are needed. This method is nowadays the most applied extraction method for the determination of pesticide residues in food samples, providing acceptable recoveries for acidic, neutral and basic pesticides.

Solid phase extraction was used as a rapid and effective method for the extraction of imidacloprid from water for the determination (Baskaran et al. 1997).

SPE is currently the most popular technique for extracting pesticides from water. Early comparisons of SPE with liquid –liquid extraction, showed SPE to be as much as 20% better than accepted traditional extraction procedure (Krynitsky and Lehotay, 2003).

We developed a new extraction scheme with high recovery rates, coupled to a UFLC methodology in order to extract and quantify imidacloprid in soils, vegetables -

cabbage and spinach. The present study will concern extensively to trace level determination of imidacloprid in vegetables (cabbage and Spinach) and soil by validating and using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) and solid phase extraction method followed by Ultra Fast Liquid Chromatography (UFLC).

Comparison between (QuEChERS) extraction method and solid phase extraction for its efficiency and sensitivity were carried out. More generally, such a method can be easily adapted for the analysis of fruits and vegetables.

Objectives are undertaken

In view of the forgoing account, the present investigation was designed to understand the persistence of imidacloprid in different type of soil and water at *p*H 4, 7 and 9 in biotic and abiotic conditions. The following objectives are undertaken for study of persistence of imidacloprid in soil and water.

- Development of analytical method for determination of trace level Imidacloprid residue in/on Soil and Water by HPLC
- Method Validation
- Soil Characterisation
- Biotic and Abiotic Degradation of Imidacloprid in/on different soils.
- Impact of concentration of imidacloprid on persistence
- Impact of soil characteristic in persistence of imidacloprid
- Degradation of imidacloprid in water at different *p*H
- Recent Techniques SPE and QuEChERS Method Applied for Trace level Determination of Imidacloprid in Vegetables

Biotic factors are living or once-living organisms in the ecosystem. These are obtained from the biosphere and are capable of reproduction. Examples of biotic factors are animals, birds, plants, fungi, and other similar organisms. A detailed study of the available literatures revealed that the majority of the pesticide degradation studies were carried out in normal agricultural soils.

Abiotic factors refer to non-living physical and chemical elements in the ecosystem. Abiotic resources are usually obtained from the lithosphere, atmosphere, and hydrosphere. Examples of abiotic factors are water, air, soil, sunlight, and minerals.

Role of biotic and abiotic factors other than photo-degradation in attenuating imidacloprid residues in soil was also studied.

In the present work, the research efforts were directed to study the degradation behaviour of imidacloprid in different type of Gujarat soils under laboratory.

References

Anindita Bhattacharya and Sanjat Kumar Sahu, (2013), Environmental impact of Imidacloprid on soil fertility: a case study on Drawida willsi earthworm. Department of Forestry, Wildlife & Environmental Sciences, Guru Ghashidas University, Chhattisgarh, India. The Clarion , 2 (1), 21-29.

Abbink, J., (1991), The biochemistry of imidacloprid. Pflanzenschutz-Nachur Bayer (44), 183–94.

Ashutosh Srivastava K., Srivastava M.K., Patel D.K., Mudiam M.K.R. and Srivastava L.P. (2012), Gas chromatographic determination of imidacloprid in water, J. of Environ. Res. and Develop. 7- 2.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D. & Schenck, F. J., (2003), Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "Dispersive solid-phase extraction" For the determination of pesticide residues in produce, J. AOAC Int, 86, (2), 412-431.

Araki Y, Bornatsch W, Brauner A, Clark T, Drager G, Urogochi S, Sakamoto H, Vogeler K, (1994), Metabolism of imidacloprid in plants. - In: Proceedings of IUPAC Congress, Washington D. C., 2B, 157.

Bonmatin J. M, I. Moineau, R. Charvet, C. Fleche, M. E. Colin and E. R. Bengsch. (**2003),** A LC/APCI-MS/MS Method for Analysis of Imidacloprid in Soils, in Plants, and in Pollens, Anal. Chem. 75, 2027-2033.

Burauel P., and Bassmann, F., (2005), Soil as filter and buffer for pesticides – experimental concept to undersatand soil function. Enviroment Pollution, (133), 11-16.

Baskaran, S., Kookana, R.S., and Naidu R. (1997), Determination of the insecticides imidacloprid in water and soil using HPLC, J. of Chromatography A 787, 271-275.

Dyer, D., Williams, M.D. and Berghaus, L., (1992), Soil/sediment adsorption-desorption of NTN 33823. Miles Inc. (Mobay) Premise Termiticide. Study No. 103817. DPR Vol. 51950-0029 # 119545.

Das P., R. Pal A. Chowdhury, (2008), Influence of biotic-abiotic factors on the degradation of novaluron in tropical soil. Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India, available online.

Das AC, Chakravarty A, Sukul P, Mukherjee D., (2003), Influence and persistence of phorate andcarbofuran insecticides on microorganisms in rice field. Chemosphere (53), 1033-1037.

Elbert, A., R. Nauen, and W. Leicht., (1998), Imidacloprid, a novel chloronicotinyl insecticide: Biological activity and agricultural importance. In Insecticides with novel modes of action: Mechanism and application, ed. I., 50–73. Berlin: Springer

Fritz, S.J., (1988), A comparative study of gabbro and granite weathering.Chemical Geology (68), 275-290.

Fernandez-Alba., Valverde A. R., Agiiera A., Contreras A. and Chiron S., (1996). Determination of imidacloprid in vegetables by high-performance liquid chromatography with diode-array detection, *J. Chrom.* A., 721(1), 97-105,

Hayes M. H. B., (1970), Adsorption of triazine herbicides on soil organic matter, including a short review on soil organic matter chemistry. Residue Rev. (32), 131-174.

Horowitz, A. R., (1998), Comparative toxicity of foliar and systemic application of acetamiprid and imidacloprid against the cotton whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae). Bull. Entomol. Res., (88), 437–42.

Heimbach and Hendel, (2001), Unpublished study bayer crop science AG. Monheim an Rhein , Germany.

Jennifer C. Anhalt, Thomas B moorman and William C., (2007), Biodegradation degradation of imidacloprid by isolated soil microorganism. J.Environ. Sci. Helth, (42), 509-514.

Kapoor U, Srivastava MK, Srivastava AK, Patel DK, Garg V, Srivastava LP, (2013), Analysis of imidacloprid residues in fruits, vegetables, cereals, fruit juices, and baby foods, and daily intake estimation in and around Lucknow, India, Environ Toxicol Chem. 32, (3):723-7.

Krynitsky A.J., and Lehotay S.J., (2003), Overview of analytical technologies available to regulatory laboratory for the determination of pesticides residues, 752-755. In P. W. Lee. Handbook of Residue Analytical Methods for Agrochemicals. John Wiley & Sons.

Kidd, H. and James, D. R., Eds., (1991),The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK.

Krohn J., and E. Hellpointer, (2002), Environment fate of imidacloprid ., Pflanzenschutz Nachrichten Bayer Volume (55), www.byercropscience/bayer/cropscience.

Koester J., (1992), Proceeding Crop Prot. Conf.- Pests Dis., (2), 901-906.

Liu, M.-Y., and J. E. Casida., (1993), High affinity binding of [3H]-imidacloprid in the insect acetylcholine receptor. Pest. Biochem. Physiol., (46), 40–46.

Lehotay SJ., (2005), Quick, easy, cheap, effective, rugged and safe (QuEChERS) approach for determining pesticide residues. In: Vidal JLM, Frenich AG, editors. Methods in biotechnology. Totowa: Humana, . p. 239.

Liu W.P., Zheng W. and Y. Liu., (2006), Sorption and degradation of pesticides imidacloprid in soil and water. J.Environ. Sci. Helth, (41), 623-634.

Michele Proietto Galeano, Monica Scordino, Leonardo Sabatino, Valentina Pantò, Giovanni Morabito, Elena Chiappara, Pasqualino Traulo, and Giacomo Gagliano, (2013), UHPLC/MS-MS Analysis of Six Neonicotinoids in Honey by Modified QuEChERS: Method Development, Validation, and Uncertainty Measurement., Intern. J. of Food Sci. (2013)-7

Novarro S., and Vela N., (2007), Review an overview on the environmental behavior of pesticides residues in soils, Spanish J. Agri. Research. 5, (3), 357-375.

Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK and Verma MS, (2005), Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish Channa punctatus (Bloch). Ecotoxicol Environ Safety, (61), 114-120.

Pal, R.; Chakrabarti, K.; Chakraborty, A.; Chowdhury, A., (2006a), Degradation and effects of pesticides on soil microbiological parameters a review. Int. J. Ag. Res., 1 (3), 240-258.

Pimentel D., and Levitan L., (1986), Pesticides amount applied and amount reaching pests., Bio Science, 36, (2), 86-89.

Ralf SCHÖNING and Richard SCHMUCK, (2003), Analytical determination of imidacloprid and relevant metabolite residues by LC MS/MS , Bulletin of Insectology 56 (1): 41-50.

Racke, K. D.; Skidmore, M. W.; Hamilton, D. J.; Unsworth, J. B.; Miyamoto, J.; Cohen, S. Z., (1997), Pesticide fate in tropical soils. Pure and Appl. Chem., 69 (6), 1349-1371.

Rice, F., Judy, D., Koch, D. and Cain, K., (1991c),. Terrestrial field dissipation for NTN 33893 in California soil. Study No. 101989. DPR Vol. 51950-0032 # 119548.

Rice, F., Schwab, D., Noland, P. and Cain, K., (1992a), Terrestrial field dissipation in Turf for NTN 33893 in Georgia. Study No. 102603. DPR Vol. 51950-0033 # 119549.

Sarkar, A., Nagarajan, R., Chaphadkar, S., Pal, S and Singbal, S.Y., (1997), Contamination of Organochlorine Pesticides in Sediments from the Arabian Sea along the West Coast of India. Water Research, 31, (2), 195-200.

Sarkar MA[1], Roy S, Kole RK, Chowdhury A, (2001), Persistence and metabolism of imidacloprid in different soils of West Bengal. Pest Manag Sci., 57, (7), 598-602.

Suparna Pal, Surajit Dutta, Pramanik S K, Manojit Ghosh, Anjan Bhattacharyya, (2006),

Effect of pH on dissipation of pyrazosulfuron-ethyl in water. Pest. Res. Journal., 18, (1) 98-100.

Soudamini Mohapatra, Ashok Kumar Ahuja, Debi Sharma, Manthirachalam Deepa, Gondakar Seshagirirao Prakash & Sampath Kumar (2011). Residue study of imidacloprid in grapes and soil, Quality Assurance and Safety of Crops & Foods, 3, 24–27.

Sajjad Ahmad Baig1, Niaz Ahmad Akhter, Muhammad Ashfaq, Muhammad Rafique Asi and Umair Ashfaq, (2012), Imidacloprid residues in vegetables, soil and water in the southern Punjab, Pakistan J. Agri. Tech., 8, (3), 903-916.

Spiteller M., (1993), Aerobic metabolism of imidaclorid ^{14}C – NTN 33893 in an aquatic model ecosystem., Bayer AG. Institute for metabolic research, Germany Report No. PF-3950.

Tomizawa, M., and I. Yamamoto., (1992), Binding of nicotinoids and the related compounds to the insect nicotinic acetylcholine receptor. J. Pest. Sci., (17), 231.

Tomizawa, M., (1995a), Pharmacological characteristics of insect nicotinic acetylcholine receptor with its ion channel and the comparison of the effect of nicotinoids and neonicotinoids. J. Pest. Sci. (20), 57–64.

Tomizawa, M., (1995b), Pharmacological effects of imidacloprid and related compounds on the nicotinic acetylcholine receptor with its ion channel from the Torpedo electric organ. J. Pest. Sci., (20), 49–56.

USEPA, (2004), Pesticide Industry Sales and Usages: (2000 and 2001) Market Estimates. (Online). Pesticides industry sales and usage report, pp. 8.

USEPA, (1999), Spray drift of pesticides. Washington, DC: Office of Pesticide Programs December). http://www.epa.gov/pesticides/citizens/spraydrift.htm#1.

Vilchez J.L., El-Khattabi R., Fernandez J.,Gonzalez-Casado and Navalon A., (1996). Determination of imidacloprid in water and soil sample by gas-chromatography-mass.

spectrometry., J. Chrom. A., 746 (2), 289-294.

Vilchez, J.L., Vilchez J.L., El-Khattabi R., Blanc, R. and Navalon A., (1998), Photochemical-flurimetric method for the determination of the insecticide imidacloprid in water samples, Anal. Chemica. Acta., (371), 247-253.

WHO/UNEP, (1990), Public Health Impact of Pesticides used in Agriculture. WHO, Geneva.

Yang, R., Ji, G., Zhoe, Q., Yaun, C and Shi, J., (2005), Occurrence and Distribution of Organochlorine Pesticides (HCH and DDT) in Sediments collected from East China Sea, Environment International, (31), 799-804.

Zhu G, Wu H, Guo J, Kimaro FME, (2004), Microbial degradation of fipronil in clay loam soil. Water, Air, and Soil Pollution (153), 35-44.

Zhou, Q., Ding, Y., and Xiao, J., (2003), Sensitive determination of thiamethoxam, imidacloprid and acetamiprid in environmental water samples with solid-phase extraction packed with multiwalled carbon nanotubes prior to high-performance liquid chromatography., Anal. and Bioanaly. Chemistry., (385), 520-1525.

Zheng W, Liu W, Lee S J, (2003), Sorption and degradation of pesticides imidacloprid in soil J. Chemosphere.

Zheng W, Liu W, (1999), Kinetic and mechanism of the hydrolysis of imidacloprid. J. Pesticides Sci. (55), 182-485.