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2,4-DICHLOROPHENOXYACETIC ACID : A REVIEW

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Physical and chemical properties, mode of action, toxicity, environmental hazards, health hazards and biodegradation of 2,4-D has been reviewed in the present communication.

Kebwords : Biodegradation; 2, 4-D; Growth regulator; Herbicide; Hormone; Toxicity.

Introduction

Modern agriculture and industry depend on a wide variety of synthetically produced chemicals, including insecticides, fungicides, herbicides, and other pesticides. However, excessive use of such chemicals without any restrictions causes pollution in soil and water and they reach to human body *via* food chain or pesticide contaminated fruits and vegetables[1,2]. Applied herbicides reach aquatic environment *via* rain fall and this causes pollution in aquatic areas that damages microorganisms, plankton, fish and indirectly human beings through the food chain[3,4].

2,4-Dichlorophenoxyacetic acid (2,4-D) is a common systemic phenoxyalkanoic herbicide used in the control of broadleaf weeds and for defoliation[5]. 2,4-D is actually an important synthetic auxin (plant hormone). It is absorbed through the leaves and is translocated to the meristems of the plant. Uncontrolled, unsustainable growth ensues, causing stem curl-over, leaf withering, and eventual plant death. 2,4-D is typically applied as an amine salt, but more potent ester versions exist as well. The widely used herbicide 2,4-D was first synthesized in 1941, commercially marketed in the U.S. in 1944[6], and has been produced throughout the world since the 1950s[7]. 2,4-D is registered for use on a variety of food/feed sites including field, fruit, and vegetable crops. 2,4-D is also registered for use on turf, lawns, aquatic and forestry applications. Residential homeowners may use 2,4-D on lawns.

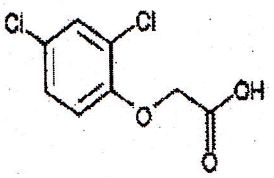

Fig.1. Structure of 2,4-D

Physical and Chemical Properties: - Pure 2,4-D can be found as flakes, powder, crystalline powder and solid material. It is white to light tan in color and may be odorless or have a phenolic aroma. 2,4-D is soluble in most organic solvents and is insoluble in benzene and petroleum oils[8]. However, the esters are soluble in oils[9] and are generally formulated as emulsions. Esters of low molecular weight alcohols have relatively high vapor pressures and will readily volatilize[10]. The 2,4-D salts are formulated as aqueous solutions.

The manmade herbicide 2,4-D can be found in a variety of water soluble amine salts and in the acid form, but it can also be produced with ester derivatives which strongly enhances its diffusion properties through organic matrices. Because of their chemical characteristics the common commercial 2,4-D formula dimethyl-amine salt (DMA) and 2-ethylhexyle ester (EHE) accounted for approximately 90% of global use in the decades proceding this century[11]. According to the International Programme on Chemical Safety (IPCS), alkali or amine salts or esters of 2,4-D are used as herbicides against broadleaf weeds in cereal crops, as well as on pastures and lawns, at rates of about 0.2-2.0 kg ha^{-1} active ingredient (acid equivalent). Esters are also used at rates of upto 6 kg ha^{-1} (acid equivalent) to suppress weeds, brush and some trees. Granular formulations are used as aquatic herbicides at rates of 1-122 kg ha^{-1}. At very low foliar application rates (20-40 mg 2,4-D L^{-1} spray water), 2,4-D can also be used as a growth regulator (http://www.inchem.org/documents/hsg/hsg/hsg005.htm).

Mode of action : Plants absorbed 2,4-D through their roots and leaves within 4-6 rain free hours after application[7]. Following foliar absorption, 2,4-D progressed through the plant in the phloem, most likely moving with the food material. If absorbed by the roots, 2,4-D moves upward in the transpiration stream[8]. 2,4-D mimics the effect of auxins, or other plant growth regulating hormones, and thus stimulates growth, rejuvenates old cells, and over stimulates young cells leading to abnormal growth patterns and death in some plants[12]. Plants treated with 2,4-D often exhibit malformed leaves, stems, and roots. 2,4-D affects plant metabolism by stimulating nucleic and protein synthesis which affects the activity of enzymes, respiration, and cell division[8].

Synthesis of DNA, RNA and protein increased in plants due to action of 2,4,-D especially in the meristematic tissues of broad leaf weeds, with some indication of affecting lipid metabolism[13,14]. To induce the process of cell de-differentiation the compound can also be used in plant cell as a complementary hormone[15]. Accelerated foliar scenescence, chloroplast damage and chlorosis with following disruption of vascular systems are the common symptoms of affected plants. Although a protein binding receptor specific for 2,4-D has not yet being characterized, a molecular signaling is assumed to induce a series of events such as aberrations in RNA synthesis, alterations in cellular membrane and intracellular calcium concentrations. Grossmann[16,17] suggested a metabolic pathway causing phytotoxic effect resulting in cellular death. An induction by such auxin-like herbicides of 1-aminocyclopropane-1-carboxylic acid synthase (ACC-synthase), which is a key enzyme during the production of ethylene is reported by the author. Cyanide is a co-product of ethylene biosynthesis in higher plants *via* the ACC pathway. To form ethylene ACC is oxidized by ACC oxidase generating CO_2 and cyanide. Cyanide is toxic if it accumulates in plant tissues. Therefore, it is suggested that cyanide, as the co-product of ethylene biosynthesis, causes phytotoxic effects on plants subjected to auxin-type herbicide treatments. Therefore, despite the uncontrolled production of ethylene which in itself is also a phytotoxic compound capable of causing early tissue senescence, phytotoxicity would be firstly caused by the accumulation of cyanide. Wei *et al.*[18] pointed out that ACC-synthase might not be the ultimate metabolic pathway targeted by auxin-like herbicides once such compounds would also inhibit root elongation, but the nature of this inhibition is not related to ethylene biosynthesis pathway. Thus 2,4-D may affect more than one metabolic pathway in sensitive organism.

Toxicity :- Toxicity is defined as the adverse effects caused by the interference of specific agents to the structure and/or process which are essential for survival and proliferation of a particular organism[19]. Extensive use of pesticides has a potential adverse impact on human health and the environment. 2,4-D is toxic to plants, microflora, and human beings[20-22]. Many reports have indicated that herbicides are toxic to different algae[23-25]. Plant and algae show some similarities in symptomatic responses to exposure to 2,4-D. It is also reported[26] that 2,4-D at concentrations ranging from 0.02 to 2 mg l^{-1} is not toxic to algae in general, but it starts to inhibit algal growth at concentrations higher than 200 mg l^{-1}.

According to Hayes and Laws[27] phenoxyherbicides have been placed in the third and fourth class in a list of toxic compounds and their effect may be acute to animals between concentration doses of 100 to 1200 mg kg^{-1}. Depending on the organisms, concentration and time of exposure, 2,4-D may produce a toxic effect ranging from embryotoxicity and teratogenecity to neuro, immune- and hepatotoxicity[28].

Environmental Hazards : - Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments, and food[29]. The primary source of 2,4-D in air is drift from spray applications of the herbicide[30]. In the aqueous environment, 2,4-D is most commonly found as the free anion[31]. Residues of 2,4-D can enter ponds and streams by direct application or accidental drift; by inflow of herbicide previously deposited in dry streambeds, pond bottoms, or irrigation channels; runoff from soils; or by leaching through the soil column[32]. Groundwater contribution of 2,4-D residues into ponds and streams is dependent upon soil type, with coarse-grained sandy soils with low organic content expected to leach 2,4-D into groundwater[30]. The amount of pesticide that migrates from the intended application area is influenced by the particular chemical's properties: its propensity for binding to soil, its vapour pressure, its water solubility, and its resistance to being broken down over time[33].

The effect of 2,4-D on fish depends on the formulation and the age and species of fish. 2,4-D when applied as the acid shows little tendency to bioconcentrate in fish while if applied as the isooctyl ester it is expected to bioconcentrate in the absence of metabolization. In fish, accumulated 2,4-D is rapidly broken down into hydrocarbon fragments which are utilized by the fish for synthesis of normal body tissue and/or eliminated[34]. A current environmental concern is the contamination of aquatic ecosystem due to pesticide discharges from manufacturing plant, agricultural runoff, leaching, accidental spills and other sources.

Health Hazards :- 2,4-D enters mammals via inhalation, ingestion, or through the skin. 2,4-D is not metabolized in mammals but is rapidly eliminated via the kidneys and excreted with the urine as the parent compound. Due to its high solubility, 2,4-D is carried in blood and interstitial tissues through the gut and kidneys, but does not accumulate in any tissues[7].

In the late 1970s and early 1980s, Hardell and colleagues suggested that 2,4-D was associated with Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), and soft tissue sarcoma (STS)[35-38]. These reports were followed by studies of the risks of lymphomas and sarcomas in other groups of agricultural workers and in Vietnam veterans who had been exposed to herbicides[39-43]. An additional study by scientists at the U.S. National Cancer Institute in 1986 pointed toward an association between NHL and 2,4-D use. Prompted by these reports, additional original research in animal models was performed to clearly define the potential cancer risks related to 2,4-D exposure. The toxicity of 2,4-D relating to non-cancer outcomes has also been studied extensively in animals, and these results confirm the lack of adverse observations made in humans.

Biodegradation : In soil, 2,4-D esters and salts are first converted to the parent acid prior to degradation. The fate of 2,4-D may be affected by several processes including runoff, adsorption, chemical and microbial degradation, photodecomposition, and leaching. 2,4-D does not persist for long in the environment (half-life in soil, 1 to 6 weeks) because it is susceptible to microbial degradation[6,44,45]; however, adverse conditions such as low pH and low temperature are known to promote its longevity[46]. Microbial degradation is considered to be the major route in the breakdown of 2,4-D in soil.

2,4-Dichlorophenoxyacetic acid was the first xenobiotic compound demonstrated to be biodegradable to innocuous products of the tricarboxylic acid cycle[47] and one of the first to provide the molecular basis for the organization of catabolic genes[48]. The most important mechanism of microbial degradation involves the removal of the acetic acid side chain to yield 2,4-DCP. This is followed by ring cleavage and degradation to produce aliphatic acids such as succinic acid[34]. The *tfdAB* genes encode the respective cleavage of the ether linkage to 2,4-dichlorophenol (2,4-DCP) and the subsequent monooxygenation to 3,5-dichlorocatechol. Plasmid pJP4 in *Alcaligenes eutrophus* JMP134 contains the *tfd CDEF* genes, which encode the complete chlorocatechol pathway downstream from the central metabolite, 3,5-dichlorocatechol[48].

Han and Peter[49] found that sandy loam soil containing 2,4-D degrading single-celled bacteria, filamentous bacteria (actinomycetes), and fungi had the lowest degradation rates at a low water potential of -5.5 MPa (megapascals), with -0.1MPa corresponding to soils at or below field capacity. An increase in water potential resulted in increased rates of breakdown up to an optimum at -0.1 MPa[49]. Dry soil conditions contribute to the inhibition of 2,4- D mineralization by restricting solute mobility, reducing the herbicide degrading activity of organisms, and suppressing the 2,4-D degrading microorganism populations. Under dry conditions the addition of organic matter may enhance degradation by simulating the co-metabolizing fungal and actinomycete communities[49]. The rate of microbial degradation is also dependent on soil depth and temperature, with rates of degradation decreasing with increased depths and lower temperatures[50].

Biodegradation of 2,4-D by microorganisms has received considerable attention lately[51-53], not only because of its extensive use but also because it serves as a model compound for understanding the mechanism of biodegradation of other, structurally related, environmentally significant haloaromatic compounds[45]. Dejonghe *et al.*[54] have reported that a great variety of soil microorganisms (mostly aerobic or facultative) such as species of *Pseudomonas, Alcaligenes, Ralstonia, Bordetella, Arthrobacter, Sphingomonas* and *Burkholderia*, among others, can degrade the 2,4-D.

Conclusions : Farmers use various types of pesticides. Extensive use of pesticides is inevitable since pesticides provide a sure cover to the farmers in protecting their investment in seeds, fertilizers, irrigation and his own hard labour from the insects and other pests.

Pesticide manufacturing industries release waste water in water bodies or in land. Although industries treat their waste water by activated sludge process, no attention is paid to removal of specific pesticides or their metabolites which exert toxicity at very low concentrations. Various regulatory authorities worldwide have introduced control measures to address pesticide application[55]. Contamination of the environment, however, due to pesticide discharges from manufacturing plants (*i.e.*, untreated or partially treated effluents), storage sites, accidental spills, surface runoff, and inefficient application technologies still remains a particular concern. Use of specific microflora adapted to these pesticides, in treatment of industrial effluents is not in practice. Therefore, research should be concentrated to develop economical but effective microbial processes for treatment of industrial effluents containing these pesticides and take them to field.

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