Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 5, No. 1, p. 111-120, 2014
http://www.innspub.net

REVIEW PAPER OPEN ACCESS

Environmental exposure and health risks of the insecticide monocrotophos – a review

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Article published on July 08, 2014

Key words: Monocrotophos, toxicity, decomposition, isomers.

Abstract

Monocrotophos is a organophosphate based insecticide used for crop protection. Monocrotophos use has induced heath issues and water pollution. From the ecotoxicology, human health and regulatory aspects, it is essential to restrict the emissions and release of the highly acutely toxic chemical from the industrial processes and agricultural applications. In this review, we present the toxicity and decomposition in media such as vegetables, human tissues, animal tissues and rations, synthesis of the analytical procedures and materials used to determine the monocrotophos and identification of *cis* and *trans* isomers of monocrotophos. Also the main physical spectroscopic methods have been discussed in this review. The analytical techniques which are presented permit to select the best analytical conditions to detect monocrotophos. These methods are widely applicable for remaining organophosphate and other polar pesticides.

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Introduction

Monocrotophos [(E)-dimethyl-1-methyl-3-(methylamino)-3-oxo-1-propenyl phosphate ($9C_l$); dimethyl phosphate ester with (E)-3-hydroxy- *N*-methylcrotonamide (8 C_l)], trade names include Nuvacron and Azodrin, is a broad-spectrum organophosphate insecticide, acaricide, and termiticide against gall midge, cutworms, corn rootworms, cockroaches, leaf folder, and leaf hopper, etc (Bhadbhade *et al*., 2002). Approximately 30,000 tons of MCP are used annually. As per data's Asia is the top user of MCP as; India (43%), South America (26%), China (15%), and Southeast Asia (9%) account for 90% of the use, internationally. In India Andhra Pradesh and Punjab are the chief consumers of MCP (WHO, 2009).

Studies, forced us to write about monocrotophos are (1) regarding the unfortunately use of monocrotophos in oil use for the mid-day meal (Bihar India) cooking process for school student as result more than 30 student reported died (http://www.hindustantimes.com, 2013), (2) a hospital toxicity cases of monocrotophos, in India where the proportion of identified pesticides responsible for all deaths reported at MGM hospital, Warangal, 1997-2001 approximately 54% death case by the use of monocrotophos only has been occurred and among the others column maximum were organophosphates (WHO, 2009) (Fig. 1). Study regarding monocrotophos become most important by knowing the fact that, Asia is the top most consumer of it and among Asian countries India (43%) is top most consumer of monocrotophos and in India Andhra Pradesh and Punjab are the chief consumers of monocrotophos (Roberts *et al.,* 2003; WHO, 2009).

Fig. 1.

One of the main agents for farmer suicides in India, where the annual average reported cases was 17,366 in 2007, but thought to be up to 126,000 annually, because it is the most commonly consumed insecticide in India, with a case fatality rate of 35% recorded (Roberts *et al.,* 2003; WHO, 2009). In Sri Lanka, the ban of monocrotophos and endosulfan has reduced the number of deaths from suicide. Monocrotophos has been linked to significant occupational poisoning in Indonesia, Philippines, Egypt, Brazil, and Central America (Roberts *et al.,* 2003; WHO, 2009). Monocrotophos (MCP) (Fig. 2) was introduced in 1965 Ciba AG and Shell Chemical Co. USA Company for widespread use as a foliar insecticide for important crops such as rice, cereals, cotton, tobacco, fruits, vegetable crops, pasture, ornamental horticulture plants, and soil applications to control termites (WHO, 2009). It exists in two chemical or geometrical forms *cis* and *trans*, among them cis is considered as active form (Ismail *et al.,* 2000). It has moderate to high toxicity, high water solubility (1 kg/litre) and medium soil sorption (organic carbon partitioning coefficient log Koc -0.20 l/kg) (Ismail *et al.,* 2000; Tomlin C, 1995; WHO, 2009). The logarithm of the octanol–water partitioning coefficients (log Kow) for monocrotophos is -0.20 (Ismail *et al.,* 2000; Tomlin C, 1995).

Fig. 2.

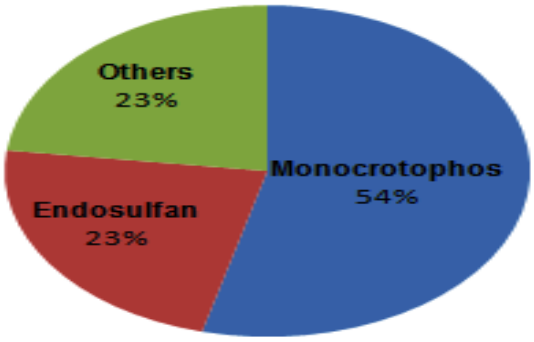

Fig. 1. Pie deaths reported at MGM hospital (India), Warangal, 1997-2001 by the application of monocrotophos.

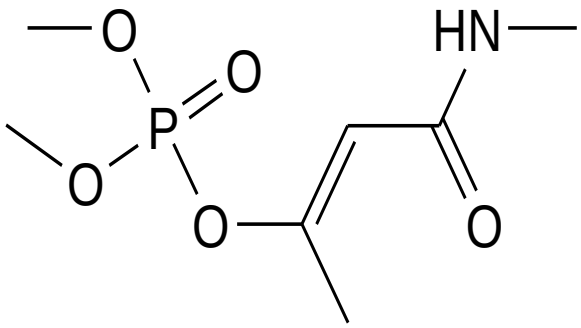

Fig. 2. Structure of Monocrotophos.

Toxicity of Monocrotophos

Monocrotophos is a direct acting cholinesterase inhibitor capable of penetration through the skin, LD_{50}, is 17-18 mg/kg for male rats and 20 mg/kg for female rats. The LD_{50} for dermal exposure is 126 mg/kg for male rats, 112 mg/kg for female rats, and 354 mg/kg for rabbits. Monocrotophos is an extremely toxic to aquatic invertebrates, birds and mammals. It is toxic to most organisms and in particular to birds (Swamy *et al.,* 1992). The most likely route of exposure to monocrotophos for the public is via residues in food. The prolonged or continued use of monocrotophos in plant protection may lead to significant dermal exposure with an impact on cholinesterase, genotoxicity and cardiotoxicity activities (Bhunya SP and Jena GB. 1993; Schulze-Rosario C. and Loosli R. 1994; Swamy *et al.,* 1992). The toxicologically relevant mode of action is the inhibition of **acetylcholinesteras**e (AChE) activities (Rao JV *et al.,* 1992; Swamy *et al.,* 1992). The basic mechanism of AChE inhibition with monocrotophos is analogous to the reaction of enzyme with ACh, excluding for the reaction in which the leaving group of the monocrotophos is lost, so the enzyme becomes phosphorylated in-stead of acetylated. Phosphorylation is a two-step addition-elimination reaction in which the addition step is rate-determining, while the elimination process is faster. It should be noted that phosphorylation occurs via a *trigonal bipyramidal* intermediate, whereas in the case of acetylation is anticipated through a *tetrahedral* intermediate. The irreversibly inhibited phosphorylated enzyme can no longer hydrolyze acetylcholine. This leads to an accumulation of acetylcholine in cholinergic receptors and consequent continuous stimulation of the nerve fiber. Phosphorylated AChE having the stable bonding and forces than acetylated one, but it can undergo a possible secondary process. Among these first one is the reactivation-hydrolysis of phosphorylated enzyme. But the rate of hydrolysis is much slower than in the case of an acetylated enzyme. Another mechanism is the breaking of the PO-C bond the inhibited enzyme known as “aging” (Kumar *et al.,* 2013). Monocrotophos is neurotoxic in nature and caused a significant inhibition of the brain acetylcholinesterase activity. There are age-related differences in the inhibition of brain, but not necessarily red blood cell and AChE. The sensitivity of the tissue AChE was in the order gill>brain>muscle (Moser, 2011; Rao *et al.,* 2005).

Hyperglycemic and stressogenic

effects of monocrotophos have been observed, hyperglycemia and hyperlactacidemia induced by monocrotophos were abolished by pretreatment with atropine (Joshi and Rajini, 2012). Higher concentrations of monocrotophos caused acute toxicity, which marked increase in mobility of cells exhibiting rocking movements within two mins of exposure but were decreased after 30 mins and cytotoxic effects of monocrotophos revealed non-repair of necrosis (Amanchi and Hussain, 2010). Monocrotophos is genotoxic on meretrix ovum and also induces a pollution stress related retardation of somatic growth of this mussel (Revankar and Shyama, 2009). Genotoxic mechanism in fish taken placed by DNA damage and measured in the gill, kidney and lymphocytes as the percentage of DNA in comet tails of fish exposed to different sublethal and nonlethal concentrations of monocrotophos (Jamil *et al.,* 2004).

Monocrotophos insecticide during sexual development causes the feminization/demasculinization of the reproductive traits. Reproductive toxicity caused by organophosphates (monocrotophos) at cellular and molecular level in the ovaries of rat. Reproductive toxicity of monocrotophos also has been observed in bobwhite quail (Tian *et al.,* 2012).

Monocrotophos has histopathological effects in liver, kidney and muscles of normal, protein malnourished and diabetic as well as both protein-malnourished, diabetic albino rats and fish. Histopathological effects of monocrotophos on the gill, kidney and intestine tissues of the *Cirrhinus mrigala* were determined by

light microscopy (Srinivasulu *et al.,* 2012; Velmurugan *et al.,* 2007).

Persistent and Contamination by Monocrotophos

Excessive use of monocrotophos has contaminated several ecosystems. It has been suggested the ability of monocrotophos, and other organophosphorus pesticides to accumulate in living tissues may create a potential risk to humans and other organisms (WHO, 2009). Combinations with other chemicals and higher concentrations of monocrotophos have been considered innocuous or inhibitory to the phosphatase activity (Velmurugan *et al.,* 2007). On soils-black vertisol soil and red alfinsol soil under laboratory conditions, interaction responses the persistent of monocrotophos even for 30 days. Monocrotophos have adverse effects on nontarget soil microorganisms and their activities. It was expected to undergo *E*/*Z* isomerization and cleavage of the P-O vinyl linkage (Gundi *et al.,* 2007; Vig *et al.,* 2008). The fate of monocrotophos in the aqueous and soil environment was examined. Hydrolysis rates for monocrotophos are pH-dependent and follow first-order kinetics. The half-lives of monocrotophos in pH 3 and 9 buffer solution at 25°C are 131 and 26 days, respectively. Its half-life is less than 7 days in soil exposed to natural sunlight (Lee *et al.,*1990; Vig *et al.,* 2008).

Decomposition of Monocrotophos

The main mechanism of biotransformation of monocrotophos was hydrolysis of the P-O vinyl linkage, to give dimethyl phosphate and *N*-methylacetoacetamide as major metabolites. The mechanisms involved in the absorption, distribution, metabolism, and elimination of monocrotophos seem to be largely species independent. In the initial biotransformation, three different metabolic reactions occur: hydroxylation of the *N*-methyl group, demethylation of the *N*-methyl group, and hydrolysis of the phosphate-vinyl linkage (Gundi *et al.,* 2006; Lee *et al.,*1990; Vig *et al.,* 2006). The degradation of monocrotophos in black vertisol and red alfinsol soils was rapid accounting for 96-98% of the applied quantity and followed the first-order kinetics with rate constants of 0.0753 and 0.0606 day^{-1} and half-lives ($t_{1/2}$) of 9.2 and 11.4 days, respectively. Degradation of monocrotophos in soils proceeded by hydrolysis with formation of N-methylacetoacetamide. Monocrotophos show the dissipation and leaching behavior in soil and water, ^{14}C-monocrotophos dissipated faster, up to 45% in first 90 days in columns treated with only monocrotophos compared to 25% in columns that received monocrotophos along with other insecticides. After 180 days of treatment, 46% radio labeled residues were observed, which reduced up to 39.6% after 365 days (Gundi *et al.,* 2006; Lee *et al.,*1990; Vig *et al.,* 2006). Leaching of ^{14}C-monocrotophos to 15-30 cm soil layer was observed in both the experimental setups. In the 15-30 cm soil layer of both soil columns, up to 0.19 mg ^{14}C-monocrotophos kg^{-1}d wt. soil was detected after 270 days (Gundi *et al.,* 2006; Lee *et al.,*1990; Vig *et al.,* 2006). Study reveal about the persistence of monocrotophos in soils at different temperatures and decay in the microbial activities in the presence of less organic substances in soils. Monocrotophos is unlikely to undergo photochemical reactions on soil due to lack of chromophores in their molecules (Gundi *et al.,* 2006; Lee *et al.,*1990; Vig *et al.,* 2006).

Analytical Procedures to Determine the Monocrotophos

In past there have been different analytical procedures and materials used to determine the monocrotophos including other pesticides in media such as soils, vegetables, human tissues, animal tissues and rations (Hu *et al.,* 2013; Kumar *et al.,* 2013; Prasad *et al.,* 2011; Velmurugan *et al.,* 2007). But there is variability among the analytical techniques which are presented permit to select the best analytical conditions to detect monocrotophos. On the basis of facilities of these techniques we divided this section into two main parts i.e best analytical techniques and procedure developed or used to detect monocrotophos in its active form and secondly in its fragments or metabolites detections.

Active form Determination Techniques

There are two best way to determine active form of monocrotophos, firstly by using the analytical methods and secondly by enzymatic approach. In first one, monocrotophos can be determined by the general procedure using the flame photometric detector. Specific methods for monocrotophos using this detector have been developed and allow analyses of crops down to a limit of determination of 0.01ppm (Hu *et al.,* 2013). The following procedure has proved satisfactory in analyzing crops: samples are extracted by maceration with chloroform; low water content crops are first dampened with water. The monocrotophos-containing extract is analyzed using the flame photometric detector. Using this procedure, mean recoveries are 75 - 120% from crops at the 0.05 - 0.20 ppm level (Hu *et al.,* 2013). The thermionic detector has also been employed in the analyses of crops for residues of monocrotophos. Mean recoveries with this method are 80 - 120% for crops, with a limit of determination of 0.03 - 0.05 ppm. The study made use of the gas liquid chromatography for the analysis of monocrotophos in extracts. The result of the study shows that there is no degradation of monocrotophos occurred in the dark while 72.8% of the applied monocrotophos could be recovered when exposure to sunlight for eight hours is done. Cold drinks samples were filtered, degassed (if carbonated), and analyzed using liquid chromatography with tandem mass spectrometry, in chromatogram monocrotophos and phorate were observed at 101(25.7%) and 86.6 (20.7%) (Miller and Milne, 2008). A method combining hydrophilic interaction liquid chromatography with tandem mass spectrometry was developed for the determination of polar organophosphorus pesticides, method was validated at 0.05, 0.5, and 5µg/L levels in water samples, and the recoveries of polar OPPs were between 76.4 and 98.6%. The limits of detection were between 0.13 and 1.0 pg on-column, and the limits of quantification were between 0.43 and 3.4 pg on-column. The method can be applied to the determination of trace amounts of organophosphate pesticides in environmental water samples (Hayama *et al.,* 2008).

Enzymatic Methods

In enzymatic inhibition specific enzymatic methods for the detection and determination of monocrotophos in crops, vegetables and milk have been developed. The samples for analysis are extracted with chloroform, followed by a solvent exchange to hexane and concentration of the extract. Column chromatography using a gradient column elution technique with hexane/dichloromethane removes co-extractives. The separated monocrotophos is transferred to water and determined by enzyme inhibition spectrophotometric methods. Using this procedure, the limit of determination of monocrotophos is about 0.10 ppm in crops and 0.01 ppm in milk. Recoveries are in the range of 70 - 125% (Rao *et al*., 2002; Wu *et al*., 2011). The other method the inhibition of monocrotophos was proportional to its concentration ranging from 0.001 to 1 µg/ml, 2 to15 µg/ml with the correlation coefficients of 0.9930 to 0.9985 respectively. The detection limit was 0.6 nano g/ml at a 10% inhibition (Rao *et al*., 2002; Wu *et al*., 2011). Methods for analyses of monocrotophos breakdown products, the analytical data for the N-methylol and for the un-substituted amide reported in this summary were developed using the method described for monocrotophos. The conjugate of the N-methylol was determined using the method, which depends on converting the conjugate to the sec-butyl thio-ether of the methylol and subsequent determination by T.L.C. with detection limit lower than 0.2 mg and enzyme inhibition (Janghel *et al.,* 2007).

Isomeric Identification of Monocrotophos

As earlier mentioned monocrotophos exists in two chemical or geometrical forms *cis* and *trans*, among them cis is considered as active form (Ismail and Rao, 2000). A simple reversed-phase LC method has been developed for the determination of *cis* and *trans* isomers of MCP and its process related impurities using a C_{18} bonded silica with water–acetonitrile

(87:13, v/v) as eluent and UV detection at 218 nm at an ambient temperature has been described (Ismail and Rao, 2000). In this method samples (100 mg) were dissolved in small volumes of acetonitrile and diluted with eluent (25 ml) and diluted to 10 ml and a 20-ml volume, injected and analyzed under the above conditions. Analyses were carried out under isocratic conditions at a flow-rate of 1.0 ml/min and a chart speed of 5 mm/min at 278°C. All the chromatograms were recorded at 218 nm. Excellent linearities between the amounts taken and found by LC were observed with regression coefficients greater than 0.9950. The contents of *cis* and *trans*-MCP were found to decrease from day 0 to 30 from 72.5 to 71.760.4% RSD and 6.9 to 3.961.58% RSD, respectively. These results clearly indicate that the rates of decomposition of *cis*- and *trans*-MCP are different. On decomposition both compounds yielded a product which was identified as 4-hydroxy *N*-methylcrotonamide by mass spectrometry.

Spectroscopic Methods

Ultraviolet-Visible Spectroscopy

A highly sensitive spectrophotometric method is developed for the determination of parts per million levels of widely used organophosphorus pesticide MCP. The method is based on alkaline hydrolysis of MCP to N-methylacetoacetamide followed by coupling with diazotized 2, 4- dinitrophenyl hydrazene in alkaline medium. The absorption maxima of the yellow coloured compound formed is measured at 490 nm (Sharma and Rajput 2012). A simple reversed-phase column liquid chromatographic method for the determination of *cis* and *trans* isomers of MCP (MCP) using a C column, aqueous acetonitrile as eluent and UV detection at 218 nm was developed. The method was used for quality assurance and to study the relative stabilities of *cis* and *trans* isomers in technical products of MCP (Ismail and Rao, 2000).

Nuclear Magnetic Resonance Spectroscopy

A Molecular imprinting method based on noncovalent interaction was used for the synthesis of a MCP-specific polymer. The selective binding characteristics of the template polymer were evaluated by ^{1}H NMR and ultraviolet spectrometry (Zhu *et al.*, 2005). The polymer obtained was found to interact specifically with MCP by cooperative hydrogen bonding. The infrared spectrometry of the polymer further indicated that there were some functional groups in the molecularly imprinted polymer which could interact on the template. Because of polymeric nature the life span raised 15 time more as copared to MCP and the total number of binding sites of the polymer were 4.046 μmg^{-1} (Zhu *et al.*, 2005).

Sensor Based Detection

A simple, low cost, viable and sensitive enzymatic method was developed, based on the inhibition of enzyme, succinate dehydrogenase. The developed enzymatic method was successfully applied for detection, separation and identification of MCP from environmental samples (Uma and Prameela, 2011). To detect the MCP a sensitive amperometric acetylcholinesterase (AChE) biosensor was fabricated based on mesocellular silica foam (MSF), which functioned as both an enzyme immobilization matrix and a solid phase extraction (SPE) material for the preconcentration of target molecules. A sensitive, fast and stable amperometric sensor for quantitative determination of organophosphorous insecticide was developed using MCP as a model compound, Developing the acetylcholinesterase (AChE) on silica sol-gel film assembling gold nanoparticles the conditions for detection of the insecticide were optimized. The inhibition of MCP was proportional to its concentration ranging from 0.001 to 1 microg/ml and 2 to 15 µg/ml, with the correlation coefficients of 0.9930 and 0.9985, respectively. The detection limit was 0.6 ng/ml at a 10% inhibition (Du *et al.,* 2007; Uma and Prameela, 2011).

Thin Layer Chromatography

TLC based method of separation of MCP from dichlrovos was developed, phenolic compounds and hydrolysed product of carbamate insecticides may interfere and differentiate from MCP and dichlrovos

by Rf values. The lower limit of detection is 0.2 mg for MCP and 0.1 mg for dichlorovos. The absorption maxima of the reddish-violet and red colour by diazotized with p-amino acetophenone formed by MCP and dichlrovos, are measured at 560 nm and 540 nm respectively (Patil and Shingare, 1994; Janghel *et al.,* 2007).

Mass Spectroscopy

The study made use of the gas liquid chromatography (GLC) for the analysis of MCP in extracts. The result of the study shows that there is no degradation of MCP occurred in the dark while 72.8% of the applied MCP could be recovered when exposure to sunlight for eight hours is done.[49] Cold drinks samples were filtered, degassed (if carbonated), and analyzed using liquid chromatography with tandem mass spectrometry, in chromatogram MCP and phorate were observed at 101(25.7%) and 86.6 (20.7%) (Hayama *et al.,* 2008; Miller and Milne, 2008; Paske *et al.,* 2007; Rao *et al.,* 2002). A method combining hydrophilic interaction liquid chromatography with tandem mass spectrometry was developed for the determination of polar organophosphorus pesticides, method was validated at 0.05, 0.5, and 5 µg/L levels in water samples, and the recoveries of polar OPPs were between 76.4 and 98.6%. The limits of detection were between 0.13 and 1.0 pg on-column, and the limits of quantification were between 0.43 and 3.4 pg on-column (Hayama *et al.,* 2008; Miller and Milne, 2008; Paske *et al.,* 2007; Rao *et al.,* 2002). The method can be applied to the determination of trace amounts of organophosphate pesticides in environmental water samples. A simple and rapid GC-MS method for separation, identification and quantitative determination of combustion products of organophosphorus and chlorine pesticides viz; MCP, chloropyriphos, butachlor and benzenehexachloride has been developed (Hayama *et al.,* 2008; Miller and Milne, 2008; Paske *et al.,* 2007; Rao *et al.,* 2002).

Summary of Methods including other Pesticides

Determination of sorption of hydrophilic, weakly sorbing organic compounds in soil by conventional batch methods using a slurried suspension is often prone to considerable errors because small changes in the solution concentration on equilibration must be accurately determined. The unsaturated transient flow method, which enables determination of sorption at sufficiently small solution to soil ratios was employed to check the sorption of pesticides and compared with traditional method. The sorption coefficient (K_d = 0.10 L kg-1) obtained for MCP was slightly lower than that by batch method (K_d =0.19 L kg-1) (Ahmad *et al.,* 2005).

Till date most efficient method to detect the MCP exclusively or in combination with other pesticides is the gas chromatographic/mass spectrometric method. Phorate's residues have been qualitatively confirmed at the 1 ppb level in fortified water samples from a variety of sources. Apparent residues in control water were less than 0.1 ppb (Singh *et al.,* 2009). Residues of MCP among the other pesticides have been observed in apple, cucumber, green vegetables (Mansour *et al.,* 2009; Singh *et al.,* 2009), honey bees from the sunflowers (44%), citrus groves (10%) and cotton field (35%), milk and potato (Mansour *et al.,* 2009; Pagliuca *et al.,* 2006; Singh *et al.,* 2009). There are number of detection available among them High-performance liquid chromatography (HPLC), Gas Chromatography (GC) and Thin layer chromatography (TLC) are considered as major methods.

Conclusion

We conclude that monocrotophos is insecticide of Asian countries especially India and having high toxicity level for living beings especially to birds. Monocrotophos cause the histopathological, genotoxic, acute, hyperglycemic and stressogenic effects and significant dermal exposure with an impact on cholinesterase, genotoxicity and cardiotoxicity activities.

The most common technique used in the determination of the monocrotophos and its metabolites is flame photometric detector followed by liquid chromatography with tandem mass

spectrometry and gas chromatography with electron capture detector. In most cases, this technique is coupled with mass spectrometry.

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