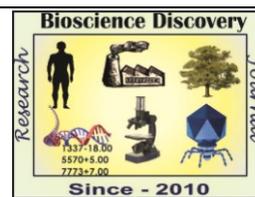


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Review Article



A review on the toxicity and other effects of Dichlorvos, an organophosphate pesticide to the freshwater fish

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Abstract

Organophosphorus pesticides are the most widely and commonly used insecticides worldwide. Toxicity and impact of Dichlorvos, an organophosphate pesticide polluting aquatic ecosystems as a potential toxicant was reviewed. This article summarized the LC₅₀ values of dichlorvos to various fish species and significant findings pertaining to its lethal and sublethal toxic effects in various aspects of ecotoxicological perturbations in fish which can be viewed as biomarkers of pesticide toxicity. These biomarkers reported due to toxic effect of the dichlorvos can be used to monitor pollution risk assessment in aquatic ecosystems.

INTRODUCTION

Pollution of aquatic ecosystems by extensive and indiscriminate use of toxic chemicals by drift, runoff, drainage and leaching (Cerejeira *et al.*, 2003) has become one of the most important problems worldwide. Among various toxic pesticides, organophosphate pesticides have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds due to their low-persistent nature in the environment (Oruc *et al.*, 2006) and rapid biodegradability (Ye *et al.*, 2010). Dichlorvos (dimethyl-2,2-dichlorovinyl phosphate) is one of the most widely used organophosphorus insecticides in the control of various pest that infect domestic animals, stored grains and in tropical aquaculture to control ectoparasitic infections. It is also used to combat outdoor and in-home mosquito vectors of several tropical diseases (Assis *et al.*, 2007). Organophosphates like dichlorvos are highly toxic to fish and other non-target aquatic organisms and are powerful nerve poisons, since they inhibit AChE activity in the nervous system by blocking

synaptic transmission in cholinergic neurons with disruption of the nerve function causing parasympathetic disorders and death of the organism (Nguyen *et al.*, 2008). Fishes are the most important inhabitants of the aquatic ecosystems which are more frequently exposed to and affected by these toxic pesticides (Scott and Sloman, 2004), because it is believed that regardless of where the pollution occurs, it will eventually end up in the aquatic environment (Firat *et al.*, 2011). Pesticides can accumulate in fish and affect human health too via ecological cycling and biological magnification (Chebbi and David, 2011). Ecotoxicological investigations are needed to determine the toxicity and potential risk of these toxic chemicals using various biomarkers in fish for monitoring the quality of the aquatic environment thereby health of organisms inhabiting those aquatic ecosystems. Against this background, the present review was aimed at various ecotoxicological aspects of dichlorvos reported in fish including behavioural, histopathological, haematological, biochemical alterations, and other toxic effects.

ACUTE TOXICITY OF DICHLORVOS TO FISH

Several authors evaluated the toxicity and other effects of dichlorvos as a potential chemical contaminant on various commercially and ecologically important fish species. 96hLC₅₀ values of dichlorvos to different fish species have been reported: *Anabas testudineus* - 2.35mg/L (Patar *et al.*, 2015); *Aphanius iberus* - 3.17mg/L (Varó *et al.*, 2008); *Channa punctatus* - 0.024ml/L (Kumar, 2014; Kumar and Gautam, 2014), 2.3mg/L (Verma *et al.*, 1981); *Cirrhinus mrigala* - 20mg/L (Srivastava *et al.*, 2014), 9.1ppm (Velmurugan *et al.*, 2009); *Clarias batrachus* - 0.07ml/L (Gautam *et al.*, 2014), 4.4mg/L (moderately toxic) (Verma *et al.*, 1983); *Clarias gariepinus* - 275.2µg/L (fingerlings) and 492µg/L (juveniles) (Omoniyi *et al.*, 2013), 0.184ml/L (0.105-0.240) (Ashade *et al.*, 2001); *Clupia harengus* larvae - 0.12mg/L (highly toxic) (McHenery *et al.*, 1991); *Cyprinodon variegatus* - 7.5ppm (Jones and Davis, 1994); *Cyprinus carpio* - 0.95mg/L (Tak *et al.*, 2014), 2.51mg/L (Günde and Yerli, 2012), 9410µg/L (Ural and Calta, 2005), 0.34ppm (Verma *et al.*, 1981); *Dicentrarchus labrax* - 3.5mg/L (Varó *et al.*, 2003); *Etrophus suratensis* - 0.09mg/L (Sobhana *et al.*, 2006); *Gambusia affinis* - 5.3mg/L (WHO, 1989, Jhonson and Finley, 1980); *Heterobranchus longifilis* - 1.32mg/L (Ekpo and Okorie, 2004); *Heteropneustes fossilis* - 19ppm (Deka and Mahanta, 2015), 6.4mg/L (Ahmad and Gautam, 2014), 6.6mg/L (Verma *et al.*, 1982); *Labeo rohita* - 16.71ppm (Bhat and Bhat, 2016); 0.11mg/ml (Giridhar *et al.*, 2015), 16.71ppm (Bhat *et al.*, 2012); *Lepomis macrochirus* - 0.48mg/L (Kenaga, 1979), 0.9mg/L (Jhonson and Finley, 1980); *Leiostomus xanthurus* - 0.55mg/L (Kenaga, 1979); *Liza parsia* - 0.482mg/L (Mohapatra and Noble, 1991); *Mugil cephalus* - 0.2mg/L (Verschueren, 1983); *Mystus vittatus* - 0.5mg/L (Verma *et al.*, 1980); *Poecilia reticulata* - 1.84mg/L (Günde and Yerli, 2012); *Pimephales promelas* - 12mg/L (WHO, 1989, Jhonson and Finley, 1980); *Salvelinus namaycush* - 0.18ppm (Mayer and Ellersieck, 1986), 0.2mg/L (Jhonson and Finley, 1980).

Saha *et al.*, 2016 reported 24, 48, 72 and 96hLC₅₀ values of dichlorvos to *Oreochromis mossambicus* as 3.84 (3.46-4.2), 3.5 (3.07-3.87), 3.12 (2.69-3.56) and 2.9mg/L (2.51-3.31), respectively using a static-renewal bioassay. Ashwini *et al.*, 2015 reported pH dependant variations in LC₅₀ values in *Rasbora daniconius*. The 24, 48, 72 and 96hLC₅₀ values of nuvan were

0.16, 0.12, 0.1 and 0.06ppm under normal laboratory conditions, 0.1, 0.12, 0.14 and 0.16ppm at pH 6.5, 0.16, 0.14, 0.12 and 0.08 at pH 7.5 and 0.2, 0.16, 0.12 and 0.08 at pH 9. Mishra and Poddar (2014) calculated 48hLC₅₀ value for *Channa punctatus* was 1mg/L. Calculated 24, 48, 72 and 96hLC₅₀ values of dichlorvos to *Cirrhinus mrigala* during a static-renewal test were 31.07 (30.33-31.83), 24.99 (24.29-25.71), 21.49 (20.89-22.1) and 20.72 (20.09-21.37)mg/L, respectively (Srivastava *et al.*, 2012). Al-Jowari, 2011 reported 48hLC₅₀ of dichlorvos to *Gambusia affinis* as 2µg/L. Sisman, 2010 reported 24hpf (hours post fertilization) LC₅₀ value of DDVP in semi-static test was 39.75mg/L for embryos of *Danio rerio*. Zhang *et al.* (2010) found 24 and 96hLC₅₀ values of DDVP for *Danio rerio* as 51.3 and 13mg/L, respectively.

Tilak and Swarna Kumari (2009) reported 24, 48, 72 and 96hLC₅₀ values of dichlorvos to *Ctenopharyngodon idella* as 13.1, 10.9, 9.8 and 6.5mg/L, respectively in static and 10.7, 9.5, 8 and 7.5mg/L, respectively in continuous flow-through system. Ural and Köprücü (2006) reported the toxicity of dichlorvos to fingerlings of *Silurus glanis* and calculated LC₅₀ values for 1, 24, 48, 72, 96h using static bioassay as 33.27 (25.11-34.2), 29.45 (24.96-32.07), 25.24 (22.72-27.06), 18.85 (16.61-20.63) and 16.67mg/L, respectively using a static bioassay. The 24, 48, 72 and 96hLC₅₀ value of trichlorofron (forming DDVP) in a static test were 92, 45.2, 29.5 and 17.6mg/L, respectively for *Oryzias latipes* (Yoshimura and Endoh, 2005). According to the Office of Pesticide Programs (2000), 24LC₅₀ values of dichlorvos to *Poecilia reticulata* and *Menidia menidia* were 5.81 (moderately toxic) and 9.6mg/L (moderately toxic), respectively, and 0.1 (highly toxic), 3.2 (slightly toxic) and 14.4 (slightly toxic) for *Oncorhynchus mykiss*, *Cyprinodon variegatus* and *Fundulus heteroclitus*, respectively for 96h. For marine fish, dichlorvos toxicity was estimated to be more than 4mg/L for adults and pre-adults of Atlantic salmon (*Salmo salar*) as opined by Roth (2000).

Calculated 24, 48, 72 and 96hLC₅₀ values of dichlorvos to *Abramis brama* were 33.05, 26.18, 21.11 and 16.66mg/L (slightly toxic), respectively (Chuiko and Slynko, 1995). 48hLC₅₀ value of dichlorvos to *Clarias batrachus* was 8.8mg/L (moderately toxic) (Benerji and Rajendranath, 1990). Perschbacher and Sarkar (1989) reported 24hLC₅₀ value of dichlorvos to *Channa punctata* using static-renewal test as 6mg/L (moderately toxic). For freshwater and estuarine fish, dichlorvos

is moderate to highly toxic and 96hLC₅₀ values range from 0.2 to 12mg/L (WHO, 1989). Calculated 24, 48 and 72hLC₅₀ values of dichlorvos to the fingerlings of *Cyprinus carpio* and *Tilapia mossambica* were 10.23 (slightly toxic), 8.99 and 8.21mg/L (moderately toxic), and 16.82, 16.03 and 15.57mg/L (slightly toxic), respectively (Dutt and Guha, 1988). USEPA (1988) reported LC₅₀ values of dichlorvos to *Pimephales promelas*, *Lepomis gibbosus*, *Gambusia affinis*, *Fundulus heteroclitus* and *Anguilla rostrata* as 11.6 (slightly toxic), 0.9 (highly toxic), 5.3 (slightly toxic), 3.7 (slightly toxic) and 1.8mg/L (moderately toxic), respectively for 96h, and 1mg/L for *Lepomis gibbosus* for 24h.

Yokoyama *et al.*, 1988 reported 24 and 48hLC₅₀ values of dichlorvos to *Anguilla japonica* as 11 (slightly toxic) and 1.5mg/L (moderately toxic), respectively. Devillers *et al.* (1985) found 24hLC₅₀ value of dichlorvos to *Danio rerio* as 35mg/L (slightly toxic). Koesoemadinata (1983) calculated 24, 48 and 96hLC₅₀ values of dichlorvos in *Cyprinus carpio* as 3.8, 2.7, 2.3mg/L, respectively and 4.1, 4 and 3.7mg/L, respectively for *Puntius gonionotus*. In *Tilapia mossambica* with three size groups, 96hLC₅₀ values were found to be 1.4 to 1.9mg/L, the smaller sizes being more sensitive (Rath and Mishra, 1981). Calculated 24, 48, 72 and 96hLC₅₀ values of dichlorvos to *Heteropneustes fossilis* were 8.13, 7.66, 7.24 and 6.61mg/L (moderately toxic), respectively (Verma *et al.*, 1982). According to Nishiuchi (1981), 48hLC₅₀ values of dichlorvos formulations to carp to be 0.5-10mg/L. Verma *et al.*, 1981 reported 24, 48, 72 and 96hLC₅₀ values of dichlorvos to *Mystus vittatus* as 0.73, 0.65, 0.51 and 0.45mg/L (highly toxic), respectively. The 24hLC₅₀ for dichlorvos to *Cyprinus carpio* was 20mg/L (Yamane *et al.*, 1974). The calculated 24 and 48hLC₅₀ values of dichlorvos in *Lepomis gibbosus* were 1 and 0.7mg/L, respectively (Pimentel, 1971). Alabaster (1969) reported 24 and 48hLC₅₀ value as 12 and 7.8mg/L, respectively in *Trigonostigma heteromorpha*.

MORPHOLOGICAL ALTERATIONS

Patar *et al.*, 2015 observed discoloration in *Anabas testudineus* exposed to dichlorvos. Bleached body with lesions was observed in *Clarias gariepinus* exposed to dichlorvos at different concentrations (fingerlings: 250, 275, 300 and 325µg/L; juveniles: 400, 450, 500 and 600µg/L). These external changes were more pronounced in the fingerlings at higher concentrations (Omoniyi *et al.*, 2013). Ashade *et al.* (2011) observed caudal bending and

discolouration in *Clarias gariepinus* exposed to dichlorvos (0.16, 0.32, 0.4 and 0.52ml/L). Zhang *et al.* (2010) reported greying of the natural colour of *Danio rerio* exposed to dichlorvos.

RESPIRATORY ALTERATIONS

Dose dependant tail fin beats were observed in *Oreochromis niloticus* exposed to dichlorvos at 0.5, 1, 1.5 and 2µg/L at 0, 24, 48, 72 and 96h (Mallum *et al.*, 2016). There was an increment in tail fin beats/m at 0 and 12h in exposed specimens of control and 0.5µg/L (2µg/L>1.5µg/L>1µg/L>0.5µg/L>control). Tail fin beats/m decreased with highest concentrations at control>0.5µg/L>1µg/L>2µg/L. *Ctenopharyngodon idella* exposed to lethal (13.1mg/L) and sublethal concentrations (1.31mg/L) of nuvan for 24h in static system showed decrease in oxygen consumption (Tilak and Swarna Kumari, 2009). Decreased rate of respiration was observed in *Heteropneustes fossilis* exposed to dichlorvos for 30 days at a concentration of 0.44mg/L (Verma *et al.*, 1984). Exposure to sublethal concentrations (0.5-1mg/L) of dichlorvos for 21 days found to decrease the respiratory rates in *Tilapia mossambica* in 3 different age groups (Rath and Misra, 1979).

BEHAVIOURAL ALTERATIONS

Most authors while describing toxicity of commercial formulations of dichlorvos, reported altered behavioural responses in various fish species. Mallum *et al.*, 2016 reported behavioural alterations in *Oreochromis niloticus* exposed to dichlorvos (0.5, 1, 1.5 and 2µg/L for 96h). Agitated movement was observed at all the concentrations. Loss of equilibrium was evident as dose dependent behavioural reaction after agitated movement which occurred from 1 to 2µg/L. Period of quiescence, air gulping and death occurred under higher doses of 1.5 and 2µg/L, while copious accumulation of mucus and blood on gill filaments finally occurred only in the highest dose 2µg/L. *Oreochromis mossambicus* showed various behavioural abnormalities exposed to dichlorvos (Saha *et al.*, 2016). Fish showed excess mucus secretion at (4.1mg/L) 72h and (3.1mg/L) 96h and hyper-excitability (3.6mg/L) at 24h. With the progress of time of exposure, the hyper-excitability of fish gradually decreased with the increasing concentration and it was almost absent at the lower concentrations (2.6-3.1mg/L at 72h and 2.6-3.6mg/L at 96h). Frequent vertical hanging posture was recorded especially at higher concentrations (4.1 and 4.6mg/L) during 72 and 96h. The opercular

movement was increased significantly ($p < 0.05$) over the control with the increasing concentrations of dichlorvos. On the other hand, the rate of opercular movement was significantly ($p < 0.05$) decreased at all the treatments with the progress of time of exposure.

Patar *et al.*, 2015 observed behavioural changes in *Anabas testudineus* exposed to 0.47mg/L of dichlorvos such as increase in surfacing and gulping. Erratic movements and abnormal swimming, gradual loss of equilibrium and drowning were also triggered by the toxicant. Mishra and Poddar (2014) observed vigorous swimming across the aquarium along with disruption in schooling behavior within a few minutes of exposure in *Channa punctatus* exposed to (0.5, 1, 1.5 and 2mg/L) dichlorvos. Within 1-2h of exposure, they calmed down and started swimming slowly. While, surfacing frequency and gulping of surface water with occasional coughing was increased remarkably in exposed fishes. Opercular movement was observed to decrease with increasing concentration of the toxicant. The exposed fishes exhibited heavy mucus secretion along with imbalance in posture and loss of equilibrium. Finally they succumbed to the toxicant with mouth and operculum wide open and body slime covered. At lower concentrations, however changes in behavior were not as conspicuous. The fish secreted copious mucus in order to neutralize the adverse effects of a large amount of the toxicant. Irregular and darting swimming movements, hyper excitability, loss of equilibrium and sinking to bottom were also observed. Obvious abnormal behavioural responses such as restlessness, quick circular movements, rolling on the back, excessive mucus productions on the body surface were observed in *Clarias gariepinus* exposed to sublethal concentrations (0.3, 0.4, 0.5 and 0.6ppm) of dichlorvos for 96h (Ogamba *et al.*, 2014). Dichlorvos induced severe behavioural changes in fingerlings (at 250, 275, 300 and 325 μ g/L) and juveniles (at 400, 450, 500 and 600 μ g/L) of *Clarias gariepinus* such as lateral and upward bending of the body, erratic and spiral swimming, spontaneous air gulping at different rates, sudden quick movement/jumping, respiratory distress and calmness (Omoniyi *et al.*, 2013).

After exposure to dichlorvos (16.71ppm for 96h), *Labeo rohita* showed aggregation at one corner of aquarium, irregular, erratic and darting swimming movements and loss of equilibrium. Fish slowly became lethargic, hyper excited, restless and

secreted excess mucus all over their bodies. The fish exhibited peculiar behavior of trying to leap out from the pesticide medium. They often spirally rolled at intervals and finally sank to bottom with their least opercular movements and died with their mouth opened (Bhat *et al.*, 2012). Günde and Yerli (2012) reported abnormal behavioural responses in *Poecilia reticulata* and *Cyprinus carpio* exposed to dichlorvos. The behavioural changes in guppy started 30m after dosing. Loss of equilibrium, erratic swimming and staying motionless at a certain location generally at mid-water level for prolonged periods were observed. Fish exposed to 1mg/L showed less general activity with occasional loss of equilibrium, which was intensified at 3mg/L. Fish at 5mg/L, stayed motionless close to the water surface and later fell to the aquarium bottom in an uncontrolled manner. At 8mg/L, all these responses were at high intensity. The behavioural changes in carp started 1h after dosing. Fish exposed to 2mg/L showed less general activity. The 3mg/L concentration group stayed motionless close to the water surface and later fell to the aquarium bottom in an uncontrolled manner. The highest concentration group showed the loss of equilibrium, hanging vertically in water, after long periods of motionlessness lying down on the aquarium bottom and suddenly starting to move.

Srivastava *et al.*, 2012 observed behavioural dysfunctions in relation to the toxicity of nuvan (10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, 42.5 and 45mg/L) at different intervals (4, 8, 12, 24, 36, 48, 60, 72 and 96h) in *Cirrhinus mrigala*. Altered swimming behaviour, opercular beat rate and surfacing behaviour were observed as sensitive indicators of nuvan induced stress. Ashade *et al.* (2011) observed the behavior of *Clarias gariepinus* exposed to dichlorvos (0.16, 0.32, 0.4 and 0.52ml/L). At 0.16ml/L, normal swimming was observed in the first 24h. Fingerlings became agitated and restless, swam to the surface for air and assumed vertical position before death. Some fingerlings were still active after 96h. At 0.32ml/L after 48h, fish showed erratic movement, increased opercular activities and sudden quick movement. Mucus secretion from gills was observed after 96h. At 0.4ml/L, response of fingerlings was immediate; they tried to jump out of the test medium, showed quick sudden movements, loss of equilibrium, and decreased opercular movements as exposure time increased. Fish became sluggish and remained at the bottom of the aquaria and excessive mucus secretion from

gills after death was also noticed. At 0.52ml/L, fish showed incessant jumping, loss of equilibrium, swimming to the surface for air, quick and fast swimming movement. Fish became weaker as exposure time increased and assumed vertical position before death with excessive mucus secretion. Sisman (2010) observed the behavior of the larvae of *Danio rerio* exposed to 10 and 25mg/L of DDVP at days 6 and 9 after fertilization. 25mg/L dose caused significant slowing of swimming activity on day 6 and 9 after fertilization. The early post-hatching swimming activity measurements were sensitive to the early functional effects of DDVP exposure level caused clearly discernible motor hypoactivity on day 6 after fertilization, 5 days after the end of DDVP exposure. This effect continued to be evident 3 days later on day 9 after fertilization. Zhang *et al.* (2010) reported toxicosis symptoms of *Danio rerio* after treatment with DDVP included abnormal gill movement, less general activity, loss of equilibrium, remaining motionless on the aquarium bottom, greying of the natural colour, fins becoming hard and stretched, and sinking in the water. Ural and Köprücü (2006) observed behavioural alteration in *Silurus glanis* exposed to different concentrations (8, 16, 24, 32, 40, 48 and 56mg/L) of dichlorvos. Abnormal behaviors such as less general activity and loss of equilibrium were observed after 16mg/L. Initial changes in behavior were observed 30m after exposure to five highest concentrations. Loss of equilibrium, hanging vertically in the water, erratic swimming, swimming near to the surface, or staying motionless at the bottom of the test chamber were the behavioural responses observed at all concentrations higher than 16mg/L. Reduced feeding was observed in an omnivorous fish, *Abramis brama* exposed to DDVP (1.87mg/L) during 4 days (Povlov *et al.*, 1992). Hence, monitoring of fish behaviors is a promising diagnostic tool for screening and differentiating toxic chemicals such as dichlorvos according to their mode of action as opined by Drummond *et al.* (1986).

ACETYLCHOLINESTERASE INHIBITION

AChE activity is a good biomarker of exposure to organophosphate pesticides (Varó *et al.*, 2008). Patar *et al.*, 2015 observed the effects of dichlorvos exposure (0.47, 0.047, 0.0047mg/L) on the AChE activity in different tissues of *Anabas testudineus*. After 40 days of exposure, AChE activity in brain, liver, kidney and gills were reduced at all concentrations compared to the control with

increasing dichlorvos concentration. At 0.47mg/L, the AChE activity reduced to 18% in brain, 33% in liver, 49% in kidney and 37% in gill. After 20 days withdrawal to untreated water, the AChE activity in brain, liver, kidney and gill restored up to 75, 83, 88 and 89% respectively at lowest concentration of dichlorvos (0.0047mg/L). Significant inhibition of AChE activity was recorded in head and body tissues of both sexes of *Aphanius iberus* exposed to 0.5, 1, 2 and 4mg/L of dichlorvos (Varó *et al.*, 2008). Fish was able to tolerate high concentrations of dichlorvos, and resist high levels of brain and muscle ChE inhibition without mortality. Both ChE inhibition and recovery followed a similar time-course pattern in response to sublethal exposure to the toxicant (1mg/L), and response to sublethal exposure to dichlorvos (1mg/L), and the ChE activity did not return to control levels after 96h in clean water. Exposure of *Sparus aurata* fingerlings to dichlorvos caused an inhibition of ChE activity (Varo *et al.*, 2007). Assis *et al.*, 2007 reported that dichlorvos was capable of inhibiting AChE extracted from *Colossoma macropomum* even at concentrations as low as 0.005ppm where 18% of inhibition was detected. An exponential decay of activity was recorded when the enzyme activity was measured after incubation with increasing concentrations of dichlorvos. Dichlorvos has been shown to inhibit the activity of ChE significantly in the brain and muscles of *Dicentrarchus labrax*, both in vitro and in vivo conditions (Varo *et al.*, 2003).

Chuiko, 2000 observed dichlorvos induced inhibition in brain and serum AChE in 11 freshwater teleost fish (*Cyprinus carpio*, *Abramis brama*, *Abramis ballerus*, *Blicca bjoerkna*, *Rutilus rutilus*, *Alburnus alburnus*, *Leuciscus idus*, *Perca fluviatilis*, *Stizostedion lucioperca*, *Esox lucius* and *Coregonus albula*). Yamin *et al.* (1994) found that when carps were exposed to a concentration of 25mg/L of dichlorvos for 45m, AChE activity of many tissues was inhibited or totally lost. Exposure of *Abramis brama* to DDVP (1.87mg/L) for 4 days led to marked inhibition of brain AChE activity. After 12h of recovery the enzyme activity remained significantly less than in control fish (Povlov *et al.*, 1992). Rath and Misra (1981) reported concentration and exposure period dependant inhibition in AChE activity of brain and liver with increasing size and age in *Tilapia mossambica* after exposure to sublethal concentrations of dichlorvos. Brain exhibited a

higher degree of enzyme inhibition in all age groups of fish as compared to liver. Small fish were more susceptible to the insecticide with respect to AChE activity. When transferred to clean water most of the exposed fish recovered their AChE activity and the recovery was greater in liver than in brain. Small fish exhibited comparatively a high level of recovery in the AChE activity. The degree of recovery followed an inverse relationship with the time of exposure.

BIOCHEMICAL ALTERATIONS

Deka and Mahanata (2015) investigated the effect of dichlorvos (76% EC) at their sublethal level (2.5 and 5ppm) on serum ammonia, serum urea, activity of SGOT and SGPT of *Heteropneustes fossilis* to assess the hepato-renal function for 10, 20 and 30 days. Mean values of the serum ammonia were recorded to be in decreasing trend whereas serum urea in increasing trend with the increased exposure time and the sublethal concentrations. There was a steady increment in the mean values of the SGOT and SGPT with the increase of exposure time of sublethal dose. Mean value of SGOT after 10 days exposure time was observed to be the highest among all exposures followed by 30 and 20 days. Mean value of the SGPT after 20 days exposure was observed to be the highest among all exposures followed by 10 and 30 days exposure. Giridhar *et al.*, 2015a observed alterations in blood glucose, glycogen levels in muscle and liver of *Labeo rohita* exposed to 0.011mg/ml of nuvan for 1, 7, 15 and 30 days. Blood glucose level was elevated at 1st day exposure and decreased gradually on 7th and 15th day. From 15th day onwards their levels gradually elevated and came near to control at 30th day. Levels of liver and muscle glycogen declined at 1st day, gradually elevated on 7th and 15th day and from 15th day onwards gradually declined and came near to control on 30th day. Giridhar *et al.*, 2015b observed alterations in the levels of structural proteins, total proteins, protease activity and free amino acids in brain, liver, gill, kidney and muscle of *Labeo rohita* exposed to 0.011mg/ml of nuvan for 1, 7, 15 and 30 days. The levels of structural proteins and total proteins declined in all organs of fish at 1st day exposure and continued its declination up to 15th day exposure period. From 15th day onwards their levels gradually elevated and came nearer to control at 30th day exposure period whereas the levels of protease activity and free amino acids followed an opposite trend on exposure periods.

Ahmed and Gautam (2014) observed decreased total protein and albumin and increased creatinine, bilirubin and urea in serum of *Heteropneustes fossilis* exposed to nuvan (0.26, 0.32 and 0.43mg/L) for 7, 15, 30 and 60 days. There was a decrement in total protein at all the exposure periods which was significant at 0.26mg/L, highly significant at 0.32mg/L and very highly significant at 0.43mg/L. Decreased levels of albumin were recorded at 0.26mg/L (significant) and at 0.32mg/L (highly significant) at all exposure periods. Elevated levels of bilirubin were observed at 0.26mg/L on 7th, 15th, and 30th day (significant) and highly significant increment on 60th day of exposure. At 0.32mg/L, highly significant and very highly significant elevated levels of bilirubin were recorded on 7th and 15th day, 30th and 60th day respectively. Creatinine and urea levels recorded significant increment at 0.26mg/L on 7th, 15th and 30th day, and very highly significant elevation at 0.32mg/L and 0.43mg/L. Gautam *et al.* (2014) investigated the toxic effect of nuvan on blood biochemistry of *Clarias batrachus* at 24, 48, 72 and 96h. There was a significant reduction in cholesterol, significant higher increment in blood glucose and blood urea, significant increase in SGOT and SGPT levels in exposed to nuvan, as compared to the control group. Kumar, 2014 reported significant decrease in liver glycogen, protein, lipid, ALP, ACP levels and increase in SGOT and SGPT levels in the fish *Channa punctatus* on exposure to nuvan (0.024ml/L) for 24, 48, 72 and 96h. Kumar and Gautam (2014) observed nuvan induced alterations in *Channa punctatus* exposed to dichlorvos (0.024ml/L) for 24, 48, 72 and 96h. There was a significant decrease in glycogen content, total protein and lipids in kidney as the concentration and exposure time increased.

Lakshmanan *et al.* (2013a) assessed the impact of sublethal doses of dichlorvos (0.00375, 0.0075 and 0.015ppm) on tissue glycogen, total protein and albumin content in gill, muscle and liver tissues of *Oreochromis mossambicus* after 7th, 14th and 21st day exposure period. They observed depleted levels of glycogen, total protein, albumin content in all the tissues and exposure periods. Sukirtha and Usharani (2013) examined the acute effect of dichlorvos on adult *Danio rerio* exposed to various concentrations (5, 10 and 25mg/L) for 24 and 48h. The total protein and LPO contents were increased except SOD, catalase in the brain tissue of the treated fish. There was no significant decrease

in the GPX activity at 5ppm. The GPX activity decreased significantly in test group treated with 10ppm and a significant difference were found between 5 and 10ppm test groups. Mastan and Shaffi (2010) reported sublethal effects of dichlorvos on phosphate activated glutaminase and L-Keto acid activated glutaminase in different regions of brain of *Labeo rohita* after 12, 24 and 36h (acute studies) and 15, 30 and 45 days (chronic studies). Phosphate glutaminase and X-ketoacid glutaminase registered significant changes in different brain regions under both acute and chronic studies. Mastan and Ramayya (2009) reported biochemical alterations in *Channa gachua* exposed to sublethal doses of dichlorvos (0.012mg/L) for 16, 24 and 48h (acute) and 15, 30 and 45 days (chronic). Exposure of pesticide to *Channa gachua* led to an increase in cholesterol, alkaline phosphatase in plasma, triglyceride in plasma, serum bilirubin, serum creatinine, SGPT and SGOT parameters, in both acute and chronic studies.

Rani *et al.* (2008) reported declined level of glycogen and very significant decline in protein and lipids in *Labeo rohita* due to nuvan toxicity. Koul *et al.*, 2007 observed increase in levels of SGOT and SGPT activities under sublethal effect of dichlorvos in *Channa gachua*. Exposure of *Sparus aurata* fingerlings to dichlorvos caused an increase in lipid peroxidation and a decrease in the RNA/DNA ratio. In contrast, no significant changes in GST and HSP70 were found (Varo *et al.*, 2007). Srinivas *et al.* (2001) reported that *Catla catla* exposed to dichlorvos showed increased blood glucose level. Arasta *et al.*, 1996 reported significant decrease in protein and lipid content in *Mystus vittatus* exposed to sublethal concentrations of nuvan at 0.0005, 0.00025 and 0.000125ppm. Sublethal exposure (0.24ppm) to nuvan for 3 days in *Catla catla* caused a significant hypoglycemic and hypoproteinemic response and an increase in AST and ALT (Medda, 1993). Medda *et al.*, 1992 observed that nuvan exposure resulted in a decrease in total protein level in *Labeo rohita* and *Cirrhinus mrigala*. Exposure of *Mystus vittatus* to sublethal concentrations of dichlorvos for 30 days increased the ALP, ACP and glucose-6-phosphatase levels in serum (Verma *et al.*, 1984). Dalela *et al.*, 1981 observed hyperglycemia in *Mystus vittatus* exposed to dichlorvos. Verma *et al.* (1981) recorded decline in ALP and ACP activities in liver, gills and kidneys of *Mystus vittatus* following long term exposure to dichlorvos. Rath and Mishra (1980) reported

alterations in nucleic acids and protein content in liver, muscle and brain of *Tilapia mossambica* exposed to a sublethal concentration (0.5mg/L) of dichlorvos for 21 days. Fish showed a lower brain and liver somatic indices. Post-exposure studies revealed a significant decline in DNA, RNA and protein contents of liver, muscle and brain. The liver exhibited a greater loss in DNA, RNA and protein contents than those of muscle and brain in the exposed fish. They also found that the RNA/DNA ratio decreased in exposed fish and it showed a positive correlation with protein.

CHROMOSOMAL ABERRATIONS AND CARCINOGENIC EFFECTS

Exposure of *Channa punctatus* to dichlorvos (0.01ppm) caused chromosomal aberrations in the form of chromatid gaps, centromeric gaps, attenuation, chromatid breaks, pycnosis, extra fragments, and stubbed arms etc. in the kidney cells (Rishi and Grewal, 1995) after exposure periods of 24, 48, 72 and 96h.

HISTOPATHOLOGICAL CHANGES

Histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Bernet *et al.*, 1999). Bhat and Bhat (2016) assessed the histological damage in intestine of *Labeo rohita* exposed to acute (4 days) and chronic (30 days) sublethal concentration of 1/10th (1.671ppm) and 1/15th (1.114ppm) of 96hLC₅₀ of dichlorvos. At acute exposure, intestine showed atrophy in the muscularis, pycnosis of nuclei, vacuolation in the muscularis, erosion of mucosal layer and necrosis. At chronic exposure, intestine showed erosion of brush border and lamina propia, degeneration of villi with severe necrosis in the absorptive columnar epithelial cells including brush border, aggregation of necrotized cells in the intestinal lumen, clumping of cytoplasm, infiltration of lymphocytes, broken villi tips, increase in intervilli space, catarrhal extrude, pycnosis of nuclei, necrosis, vacuolation, shortening of villi, necrosis in the mucosal layer and atrophy in muscularis.

Kumar (2016) observed histopathological changes in the liver of *Channa punctatus* after exposed to sublethal concentration of nuvan. Liver showed increase in sinusoidal spaces, cirrhosis, mild necrosis, fat accumulation, accumulation of cytoplasmic granules and shrinkage leading to damage of the cytoplasmic material in the liver cells after 24h exposure. After 48h exposure, liver showed lesions, necrosis, and inflammation in the sinusoidal tissue and ischemic condition.

Cloudy swelling, extension of sinusoids, fibrosis, cirrhosis in hepatic lobules, nuclear necrosis and hepatic vacuolization were evident after 72h exposure. After 96h, loss of polygonal shape of hepatocytes, hepatocyte degeneration, focal necrosis and loss of cell boundaries of giant cells were observed. In kidney after 24h of exposure, cloudy swelling and focal necrosis of renal tubules, variations in size and cellularity in glomeruli to normal were reported. After 48h, several hyper cellular glomeruli were seen with much vascular degeneration of the tubular cells and displacement of nucleus in renal cells. The renal tubules were shown mild necrosis of interstitial haematopoietic tissue and hyperchromatic nuclei and widening of the renal tubular lumen. While after 21 days of nuvan toxicity kidney showed pathogenesis with many lesions, hypertrophy in glomeruli, loss of haemopoietic tissue and lack of blood supply in tissue and nephrosis. After 28 days, kidney showed chronic inflammation of interstitial tissue, internal hemorrhage, fibrinoid necrosis as well as ischemic brinkling of glomeruli.

Kumar and Gautam (2014) observed severe histopathological changes in the liver of *Channa punctatus* exposed to sublethal concentration of nuvan after 7, 14, 21 and 28 days. After 7 days of exposure fish showed an increase in sinusoidal spaces, cirrhosis, mild necrosis and fat accumulation, accumulation of cytoplasmic granules and shrinkage, damage of the cytoplasmic material in the liver cells. After 14 days, liver showed lesions, necrosis and inflammation in the sinusoidal tissue and ischemic condition. After 21 days, cloudy swelling and extension of sinusoids, fibrosis and cirrhosis in hepatic lobules and nuclear necrosis were observed. The hepatocytes showed hepatic vacuolization after 28 days with loss of polygonal shape of hepatocytes, degeneration, focal necrosis and loss of cell boundaries of giant cell. Srivastava *et al.*, 2014 observed dose dependent alterations in the epithelium of the gill filaments and the secondary lamellae of the gills of *Cirrhinus mrigala* exposed to two sublethal concentrations (5 and 15mg/L) of nuvan. Increase in thickness of epithelium covering secondary lamellae, merger of epithelium of gill filaments and adjacent secondary lamellae, and aneurysm were observed. A significant decline in the density and area of the mucous goblet cells in the epithelium of the gill filaments and the secondary lamellae were also reported. These histopathological changes took longer time to recover in the fish exposed to

15mg/L than those exposed to 5mg/L indicating that the changes in fishes exposed to higher concentration are more severe than those at lower concentration of the insecticide.

Sukirtha and Usharani (2013) examined the acute effect of dichlorvos on adult *Danio rerio* exposed to various concentrations (5, 10 and 25mg/L) for 24 and 48h. Neural degeneration in some places, astrocytes affected with partial degenerative changes, oedema in the stroma of the brain tissue and collections of inflammatory cells predominantly lymphocytes were reported at 5ppm. Degeneration of the neurons and astrocytes, oedema of the stroma with a few collections of lymphocytes and plasma cells were observed at 10ppm. In *Cirrhinus mrigala* exposed to sublethal concentrations (0.91 and 1.82ppm) of dichlorvos for 10 days, gill showed hyperplasia, desquamation, and necrosis of epithelial, epithelial lifting, oedema, lamellar fusion, collapsed secondary lamellae and curling of secondary lamellae as the most common changes. At 0.91ppm, severe aneurism in the secondary lamellae with the rupture of the pillar cells was observed in gill. In liver, cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis, karyohexis, dilation of sinusoids and nuclear hypertrophy were observed at both 0.91 and 1.82ppm. (Velmurugan *et al.*, 2009). Shukla *et al.* (2005) observed that *Clarias batrachus* exposed to 0.16mg/mL of nuvan, the hepatocytes exhibited reduction in their size and peripheral accumulation of cytoplasm. The nuclei of the hepatocytes lost their rounded appearance and the cell boundaries became obliterated at places after 20 days of pesticide exposure. The hemorrhage in liver was evident by increased volume of sinusoidal space. Benarji and Rajendranath (1991) studied cyto-architectural changes in the oocytes, including pronounced vacuolation, degeneration and deformation, clumping of the cytoplasm and karyohypertrophy in *Clarias batrachus* exposed to lethal and sublethal levels of dichlorvos.

HAEMATOLOGICAL ALTERATIONS

Exposure of *Oreochromis niloticus* to dichlorvos for 96h showed significant lower percentages of packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC), neutrophil, monocyte and lymphocyte count ($P < 0.05$). In the test fish, PCV value at the lowest concentration of 0.5 μ g/L was 28.62%, while the highest concentration of 2 μ g/L had percentage value of 18.35 (Mallum *et al.*, 2016). Tak *et al.*, 2014 observed reduction in the number of RBC, PCV, mean corpuscular

hemoglobin (MCH) in *Cyprinus carpio* exposed to 0.48, 0.66 and 0.85mg/L of dichlorvos for 24 and 96h. Lakshmanan *et al.*, 2013b evaluated the impact of washed off dichlorvos on fingerlings of *Oreochromis mossambicus*. Total erythrocytes count (TEC), total leucocytes count (TLC), Hb and haematocrit (Hct) levels declined in all the sublethal concentrations and exposure periods.

Ashade *et al.* (2011) observed the haematological profile of *Clarias gariepinus* exposed to sublethal concentration of dichlorvos (0.0184mg/L). There was a slight reduction in the RBCs count, PCV, Hb, MCH and MCHC values. The T-test and analysis of variance for control and test media with ($P>0.05$) implies that there is no significant statistical difference in the haematology of the control and sublethal test medium exposed for 14 days which could be as a result of the length of exposure days or concentration of the test medium. Sobhana *et al.*, 2006 observed immune-suppressive action of a sublethal dose of dichlorvos (0.01mg/L) in juveniles of *Etroplus suratensis* for 4 weeks and evaluated its effect on hematology and humoral immune response. There was significant reduction in Hb content and total serum protein. The TEC, PCV and erythrocyte sedimentation rate (ESR) values were lowered. TLC was significantly higher in nuvan treated fish compared to control fish. Das and Mukherjee (2001) observed haematological alterations in fingerlings of *Labeo rohita* exposed to two sublethal concentrations (10.3 and 2.06ppm) of nuvan. There was an increase in blood glucose level (7.63-27.02%) over the control and elevation of TLC from 5.51 to 40.92% over 45 days period in comparison to control. The serum protein value decreased from -2.21 to -22.51%, the TEC decreased from -3.35 to -8.49% and Hb percentage decreased from -21.49 to -35.74. The variation of mean length, breadth and surface area reduction of erythrocytes in 15 days exposure was insignificant whereas it was significant ($p<0.05$) for 30 days and 45 days test period.

An increased level of RBC and Hb and reduction in the values of eosinophils and monocytes was reported by Benariji and Rajendranath (1990) in *Clarias batrachus* exposed to dichlorvos. Verma *et al.*, 1982 evaluated the effects of dichlorvos on selected haematological parameters of *Mystus vittatus*. He reported that 30 day exposure to the toxicant produced a decrease in the prothrombin time, WBC count, Hct and mean corpuscular volume (MCV) while clotting time, Hb,

RBC count, ESR, MCH and MCHC. Sublethal exposure (0.24ppm) to nuvan for 3 days in *Catla catla* caused a reduction in RBC and WBC counts, Hb content, and MCHC, and an increase in MCV (Medda, 1993).

REPRODUCTIVE AND DEVELOPMENTAL ALTERATIONS

Mir *et al.*, 2012 studied the effects of sublethal concentrations of dichlorvos (0.65, 0.9 and 1.17mg/L) on the gonadosomatic index of *Cyprinus carpio*. GSI decreased with the increase in concentration and exposure time. Reduction in GSI values was maximum at 1.17mg/L with histomorphological disorders in ovaries. Sisman (2010) observed DDVP induced developmental abnormalities in embryos and larvae of *Danio rerio*, such as no blood flow, cardiac edema, delayed hatching, and vertebra malformations when exposed to 5, 10, 25, 50, and 100mg/L of DDVP for 96h. Embryos exposed to 100mg/L were dead 24h post fertilization. The no-effect concentration for all sublethal endpoints was 5mg/L. The median effect concentrations were 70.05mg/L for no blood flow at 32hpf, 66.78mg/L for cardiac edema at 72hpf, and 20.81mg/L for delayed hatching at 72 hpf. Embryos exposed to sublethal concentration of DDVP (25mg/L) showed vertebra deformations. Al-Jowari, 2011 investigated the effect of dichlorvos (0.3, 0.6 and 0.8 μ g/L) pesticide on the weight of body and ovary as well as gonadosomatic index in female *Gambusia affinis*. There was a significant ($P<0.05$) decrease in the body and ovary weights. The body weight means were 251, 234, 196 and 346mg for concentration 0.3, 0.6, 0.8 μ g/L and control treatment respectively. While the ovary weight means were 4.62, 4.13, 3.35 and 6.39mg, respectively in the three treated groups and the control. There was no significant ($P<0.05$) difference in gonadosomatic index.

Hence, dichlorvos is proved to be toxic to fish by various findings and cause mortality even at lower concentrations as evidenced by acute toxicity results. In contrast to lethality and mortalities of fish on account of lethal concentration of dichlorvos, sublethal concentrations, even though they are not fatal, lead to subtle changes in biology of fish and consequently effect their survival. The toxicological findings presented in this review constitute an important reference to assess the hazard of any toxic pesticide in general and organophosphate pesticide in particular.

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