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Dimethoate Induced Behavioural Anomalies in Juvenile Carps, *Cyprinus carpio* var. *communis*

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A B S T R A C T

Present study was aimed to investigate the effects of sub lethal concentrations of dimethoate, an organophosphate pesticide in juvenile common carps (*Cyprinus carpio* var. *communis*) (10 ± 2 g). Fishes were exposed to the sub lethal concentration of the pesticide available as per the literature under acute toxicity tests. Various behavioural responses like excessive mucus secretions, convulsions, uncoordinated movements, imbalanced swimming, scale erosion and reflex response were measured qualitatively as feeble (+), progressive (++), intense (+++) and severe (++++). Results indicated alterations in the behaviour of fishes with excessive mucus secretion, un-coordination, imbalanced swimming pattern and hemorrhages. Results indicated that dimethoate is a potent toxicant to common carps which induces profound behavioural toxicity.

Introduction

Organophosphate compounds have largely replaced organochlorine compounds on account of their less persistent nature in the environment. Although less persistent in the environment, they are perhaps the most pervasive compounds with characteristically more toxic to mammals than organochlorines. Organophosphates are highly toxic to fish and are powerful nerve poisons, since they inhibit acetylcholinesterase activity. This enzyme inhibition results in the accumulation of acetylcholine in nerve tissues and effector organs, with the principle site of action being peripheral nervous system (PNS). Some organophosphorous compounds have been associated with delayed neurotoxicity, known as Organophosphorous Induced Delayed

Neuropathy (OPIDN) (Hodgson, 2004). Delayed neurotoxicity associated with organophosphate compounds is primarily reflected in the behaviour of fishes. Behavioural deficits have been shown for some agents at doses well below those that cause anatomical alterations (Needleman, 1995). The physiological basis for behavioural responses in fish is complex and thus understanding how behaviour is affected by contaminants at the physiological level is a complex process. Disruption of memory, perception (i.e., sense organs) and locomotion could all play roles in the observed effects of toxicants on behaviour (Rao, 1999). The earliest expression of behavioural toxicity in fishes could be found by altered behaviour, disturbed swimming pattern, body discoloration, convulsions, mucus secretion and various other parameters. Thus, the study of fish behaviour with respect to the toxicological insults can act as a sensitive and ecologically relevant tool for monitoring thresholds of effect due to pesticides (Rand, 1985; Beitinger, 1990). In this connection, present study was carried out to evaluate various qualitative behavioural responses in juvenile carps, *Cyprinus carpio* var. *communis* against the sub-lethal exposure of organophosphorous compound-dimethoate. This study can lay a base to study the molecular mechanisms involved behind behavioural anomalies in fishes associated with pesticide toxicity.

Materials and Methods

Test organism

Experiment was carried out as per Reish and Oshida (1987) with the ethical guidelines opted by Canadian Department of Fisheries and Oceans Animal user training template (CDFOA, 2004). Juveniles of common carp, *Cyprinus carpio* var. *communis* weighing of 10±2 gwere brought from hatchery of Faculty of Fisheries SKUAST-K to the laboratory in plastic bags with adequate water, avoiding any physical injury to them. Care was taken that the length of the largest fish was not more than 1.5 times the length of the smallest fish.

As soon as fishes were brought to laboratory, they were disinfected by giving bath in 0.05% $KMnO_4$ solution for two minutes to avoid any infection and transferred to glass aquaria measuring 60×30×40 cm. Fishes were acclimatized for two weeks and fed with artificial diet during that period.

Test concentration and dose

Technical grade of dimethoate was used in the present study having purity of 98%. The stock solution of pesticides was prepared in methanol and subsequent concentrations in deionised water. Five concentrations of dimethoate, i.e. 20%, 40%, 60%, 80% and 100% of LC_{50} were chosen on the basis of already available literature (Qayoom *et al.*, 2016 a&b) and fishes were subjected to various sub lethal concentrations of dimethoate pesticide for the calculation of behavioural responses.

It is important to note that the fishes of same size to which LC_{50} values have been determined, were chosen in the present study as well. The calculated concentrations for dimethoate are given in Table 1.

Bioassay

Static type of bioassay for 96 hours was carried out during which no food was given to fishes. Feeding was stopped 24 hours before the start of experiment. During the experiment also, no food was given to fishes. Test organisms were introduced to the test chamber (aquaria) within one hour after the toxicant was added to the dilution water. Mortality counts were recorded in each concentration at 6, 12, 24, 48, 72 and 96 hours. The five

concentrations of toxicant in definitive test were given and each concentration was replicated thrice with 10 specimens per replicate. Control was also run in which same amount of solvent added as to the treated one. Addition of solvent to the control was to rule out the mortality caused, if any, due to its toxic effects in the fishes. Fishes were treated dead if any sign of immobilization, loss of equilibrium, lack of opercular movement or morbidity was seen.

This reflected an indication of pending death. Dead test organisms were removed from aquaria as soon as observed. After the experiment was over, test solution was disposed of and container scrubbed and washed thoroughly with 10% HCl (Reish and Oshida, 1987).

Calculation of behavioural responses elicited in fishes

Fishes were observed in different concentrations and qualitative behavioural responses were calculated. Fishes were treated dead if there is immobilization, loss of equilibrium, lack of opercular movement or morbidity as this an indication of pending death. Qualitative behavioural response like excess mucus secretion, convulsions, uncoordinated movements, imbalanced swimming, erosion of scales, hemorrhagic patches, body colour, surfacing and gulping, reflex responses and change in swimming pattern were analyzed visualized and marked as feeble (+), progressive (++), intense (+++) and severe (++++) as adopted from Qayoom *et al.*, (2016 a & b).

Results and Discussion

During present investigation, three trials were carried out for every parameter represented by T_1, T_2 and T_3 for Trial 1, Trial 2 and Trial 3 respectively. The results of the present study are described below:-

Excessive mucous secretion

Excessive mucous was shown to be secreted by fishes in small quantities when exposed to dimethoate. In dimethoate exposed fishes excessive mucous secretion was merely found and could be seen in the highest concentration C_4 & C_5 and ranged from feeble (+) to progressive (++) stage. At low concentrations of C_1, C_2 & C_3 excessive mucous secretions were not recorded during any period of the experiment. The excessive mucous was secreted by fishes which started late within 72h to the fishes in dimethoate. In all the trails no excessive mucous secretion was recorded in the control samples both in dimethoate trails (Table 2).

Convulsions

Fishes exposed to various concentrations of dimethoate suffered convulsive fits in higher concentrations which started within 48h till the termination of experiments. The convulsions increased with the increase in concentration and exposure time of dimethoate to the fishes. The convulsive fits ranged from feeble (+) to progressive (++) in C_3 & C_4 while as those exposed to LC_{50} concentrations (C_5, 1.1ppm) depicted intense (+++) convulsive fits as compared to the control (Table 3). The fishes in control were not found to elicit fits in any stage of the bioassays.

Uncoordinated movements

As fishes started to show convulsive fits when exposed to dimethoate, the uncoordinated movements were simultaneously recorded in them. Although, these movements were found dose and time dependent yet in some experiments, they were recorded early within 24h of start of experiment and progressed with the termination of bioassay. In LC_{50} (C_5) concentration, these movements were found to

commence even earlier within 12h while as in low concentrations they were found progressive with the termination of experiments in C_4 & C_5. Uncoordinated movements intensified at the end of the trials (Table 4).

Imbalanced swimming

Fishes elicited imbalanced swimming behaviour when exposed to dimethoate. In dimethoate exposed fishes, the imbalanced swimming was not found in C_1 (0.22ppm) while as in rest of the concentrations it was found to progress with the increase in duration of exposure of pesticides. In C_3, C_4 & C_5 of dimethoate experiments, the imbalance in the swimming of fishes increased from feeble (+) to progressive (++) and remained constant in all the trails of dimethoate bioassay. However, in C_5 a feeble (+) imbalance in the swimming was recorded within 12h of experiment which turned intense (+++) in one of the trails as compared to control (Table 5).

Erosion of scales and hemorrhagic patches

A very less incidence of erosion of scales and hemorrhagic patches were recorded in dimethoate bioassays. Only feeble (+) to progressive (++) erosion of scales were seen in the highest concentration (C_5) in dimethoate exposed fishes (Table 6) usually left over with hemorrhagic patches on the eroded portion. The fishes with no or feeble (+) erosion of scales were merely found to develop hemorrhages along their bodies (Table 7). Apart from the skin, the hemorrhages were also noticed at the base of fins near operculum and at the caudal peduncle.

Reflex response

The reflex response of the fishes exposed to dimethoate showed progressive degeneration with the passage of time and lasted up to the termination of experiment. The reflex of fishes showed dose and time dependent and increased with the increase in concentration of pesticide or contact of pesticide with fishes. The reflex response dropped down from severe (++++) to feeble (+) in the fishes that escaped death in C_5. In C_4 & C_5, the alteration in the fish reflexes started earlier as compared to lower concentrations (C_1, C_2& C_3) (Table 8). The degeneration in the reflex responses is indication of causing paralysis and ultimately death of fishes due to pesticide toxicity.

Surfacing & gulping, swimming patterns and body colour

In dimethoate trails, fishes were found to come on surface and gulping for inhalation of oxygen due to the suffocation caused by the pesticide toxicity. Although a very feeble (+) gulping and surfacing was noticed in dimethoate trials (Table 9). Exposure of fishes to pesticides which caused convulsions, imbalanced swimming and uncoordinated movements led to a change in the swimming pattern of fishes as well. The change in swimming pattern was also found dose and time dependent and vividly noticed in the C_4 & C_5 trails of dimethoate exposed fishes where it reached to progressive (++) stage with the termination of experiments (Table 10).

The change in body colour of the fishes found altered in dimethoate treated fishes. Changes were feebly (+) found to change their body colour at 48 hours and remained so till the end as compared to the control (Table 11).

Toxic insults due to pesticides, metals and xenobiotics are known to induce behavioural changes in fishes in terms of swimming behaviour, feeding activities predation, competition, reproduction and species-species social interactions such as aggression (Banaee *et al.,* 2011). Particularly, organophosphate

pesticides are known to induce behavioural toxicity in fishes by targeting specific physiological systems and exert their effects on behaviour via physiological pathways. In most of the studies, the behavioural changes may be detectable before the occurrence of apparent physiological alterations as studied by various researchers (Nagaraju *et al.,* 2011, Devi and Mishra (2013) and Qayoom *et al.,* 2016 a&b). Most insecticides influence the behavioural patterns of fish by interfering with nervous systems and sensory receptors and this incident may impair the identification of situation and development of appropriate response by the fish exposed to insecticide.

Among qualitative behavioural responses, mucous secretion was found to get increased with the increase of pesticide concentration and exposure time. In dimethoate exposed fishes, it started late at 72 (Table 2). Our results are in consonance with the results of Rao *et al.,* (2003) who reported excessive mucous secretions in the fish *Oreochromis mossambicus* against CPF intoxication. Jindal and Jha (2005) also reported excessive mucous sections in *Orechromis mosambicus* and *Cyprinus carpio* exposed to chlorpyrifos and monocrotopos respectively. Similar findings were reported by Ramesh and Saravanan (2008) in *Cyprinus carpio* against chlorpyrifos intoxication and Ramesh and Munniswamy (2009) in *Cyprinus carpio* exposed to chlorpyrifos. Pandey *et al.,* (2009) and Singh (2013) reported copious secretion of mucus all over the body in, *Heteropneustes fossilis and Colisa fasciatus* exposed to dimethoate. Wast *et al., (*2015) reported higher mucous secretions in *Poecilia reticulata* respectively against chlorpyrifos intoxication. Hussain *et al.,* (2015 & 2016) also reported excessive secretion of mucus against dimethoate intoxication in *Catla catla* and *Labeo rohita.* Qayoom *et al.,* (2016 b), who reported higher mucous secretions in juvenile common carps exposed to dimethoate while as similar findings are reported by Verma *et al.,* (2017) who reported copious secretion of mucus all over the body in *Heteropneustes fossilis* exposed to Hilban, Khan *et al*. (2018) also reported the higher mucous secretions in juvenile common carps exposed to dichlorovos. Banjara and Singh (2019) reported the same results in *Mystus singhala* exposed to endosulphan, carbofuran, dichlorvos, dimethoate and phorate. The excessive secretion of mucus has been reported in the fishes exposed to pollutants as a defending mechanism against the irritation caused by the toxicant (Mezin and Hale, 2000). Mucous secretions are the first line defense mechanism against infections, stress or toxicity in fishes to neutralize the effect of toxicant and to avoid it. The mucous produces coagulates with the toxicant and prevents its cutaneous entry into the body (Bisht and Agarwal, 2007).

Convulsions in fishes were recorded in, dimethoate exposed fishes and got increased with the increase in concentration as well as the exposure time of the pesticides. In dimethoate exposed fishes, convulsive fitsstarted within 48 hours of experiments (Table 3). The results obtained in the present study are in accordance with the findings of Dogan and Can (2011) who reported convulsive fits in *Oncorhynchus mykiss* exposed to the sub lethal concentrations of dimethoate. Their findings suggested that dimethoate exerts its toxic action even in sublethal concentrations and causes abnormal behavior which may be sensitive indicator to evaluate pesticide intoxication. Devi and Mishra (2013) reported convulsive fits in *Channa punctatus* against chlorpyrifos intoxication. Hussain *et al.,* (2015 & 2016) also reported convulsions in common carp *Catla catla* and *Labeo rohita* exposed to dimethoate. Convulsions were also reported by Qayoom *et al.,* (2016 a&b) in juveniles of *Cyprinus carpio* var. *communis* exposed to the

median lethal concentrations of dimethoate and chlorpyrifos along with khan *et al*. (2018) who reported the convulsive fits in juveniles of *Cyprinus carpio* var. *communis* exposed in dichlorovos (76% EC). Their study suggested that dimethoate to is toxic to common carps which is in consonance with the present study.

The uncoordinated movements were simultaneously depicted as fishes started to show convulsive fits when exposed to dimethoate. Although, these movements were found dose and time dependent yet in some experiments, they were recorded early within 24 hours of start of experiment and progressed with the termination of bioassay. These movements were seen to get intensified at the end of the experiments (Table 4). Our results are in accordance with Pandey *et al.,* (2009) and Srivastava *et al.,* (2010) who worked on acute toxicity bioassay of dimethoate on freshwater air breathing catfish *Heteropneustes fossilis* and reported strong uncoordinated movements in the experimental animals under sublethal exposure of dimethoate. They reported alterations in various behavioural indices which included increased opercular movement, sluggish, lethargic and abnormal swimming, loss of buoyancy, muscular tetany and fading of body colour in fishes exposed to dimethoate. Narra *et al.,* (2012) while working on sub-acute toxic effects of chlorpyrifos on acetylcholinesterase activity in *Barytelphusa guerini* reported many behavioural changes in the freshwater crabs exposed to CPF including loss of equilibrium, uncoordinated movements and increase in the levels of respiratory frequency. Same findings were reported by Banaee *et al.,* (2013) who worked on sub-lethal toxicity of chlorpyrifos on common carp. Kaur *et al.,* (2013) while working on dyeing industrial effluent induced behavioral and morphological changes in *Cirrhinus mrigala* reported various behavioral responses like erratic movements, gulping air on the surface or jumping out of water, opercular movements, loss of equilibrium, hitting against the wall, restlessness, sluggishness, fishes lied on the water surface before death and morphological changes like loosening of scales, redness in eyes, profuse mucous secretion, bleeding from gills, ballooning and belly upward pigmented patches on the abdomen. Dey and Saha (2014) who worked on *Labeo rohita* under the toxic exposure of dimethoate and reported the same results that is in accordance with the present study. Qayoom *et al.,* (2016 a&b) in *Cyprinus carpio* var. *communis* also reported uncoordinated movements under CPF and dimethoate exposure. Banjara and Singh (2019) who worked on behavioural changes induced in *Mystus singhala* due to the exposure of endosulphan, carbofuran, dichlorvos, dimethoate and phorate and reported disrupted schooling behaviour in the fishes which depict the uncoordinated movements in fishes.

Imbalanced swimming behavior in *Cyprinus carpio* var. *communis* was observed when they were exposed to dimethoate. Imbalance in swimming was noticed to get increased with the increase in exposure time of dimethoate (Table 5). Our findings are in agreement with Woke and Aleleye-Wokoma (2009) who reported imbalance in the swimming pattern of*Clarias gariepinus* under chlorpyrifos-ethylon intoxication. Pandey *et al., (*2009) and Srivastava *et al.,* (2010) also reported alterations in the behavioural responses including swimming pattern of *Heteropneustes fossilis* exposed to dimethoate insecticide. Qayoom *et al.,* (2016a&b) also reported imbalance in the swimming pattern of *Cyprinus carpio var. communis* exposed to dimethoate and CPF. Similar findings were reported by Banaee *et al.,* (2013); Singh (2013); Dey and Saha (2014) Qayoom *et al.,* (2016 a&b) and Adewumi *et al.,* (2018) in various fishes under exposure of various insecticides. Imbalance in swimming was also

reported by Hussain *et al.,* (2015 & 2016) in *Catla catla* and *Catla catla* & *Labeo rohita* respectively under dimethoate intoxication. The primary target of organophosphate (OPs) compounds is the neuro-inhibitory nature of acetylcholinesterase (AChE) enzyme in the synaptic clefts which leads to paralysis in acute toxicity exposures. However, the OP toxicity in natural water bodies where fishes are exposed chronically to these xenobiotics induces irreversible long term effects leading to physiological dysfunctions in them. Like other organophosphates, CPF and dimethoate inhibits acetylcholinesterase (AChE) which is present in mammals, fish, birds and insects. AChE is a class of enzymes which initiate the hydrolysis of acetylcholine (ACh), a neurotransmitter, into inactive choline and acetic acid (Hoegberg & Cassaday 1951). The inhibition creates a buildup of acetylcholine at the nerve synapses disabling the enzyme cholinesterase which is vital for a functioning central nervous system (Verma *et al.,* 1979). The continuous accumulation of acetylcholine leads to loss of balance, convulsions, paralytic symptoms and eventually death. The same results have been observed in the present investigation.

Erosion of scales and haemorrhagic patches were seen with very less incidence in dimethoate bioassays. Only feeble to progressive erosion of scales were seen in the highest concentration (C_5) of dimethoate (Table 6) which was left over with haemorrhagic patches usually on the eroded portion. The fishes with no or feeble erosion of scales were merely found to develop haemorrhages along with their bodies (Table 7. Apart from the skin, the haemorrhages were also noticed at the base of fins near operculum and at the caudal peduncle which are in agreement to the results obtained by Woke and Aleleye-Wokoma (2009) who also reported scale erosion in*Clarias gariepinus* against chlorpyrifos-ethylon intoxication. Devi and Mishra (2013) also reported erosion of scales in *Channa punctatus* against chlorpyrifos intoxication while as Kaur *et al.,* (2013) for obtained the same results in industrial effluent exposed *Cirrhinus mrigala*. Qayoom *et al.,* (2016 b) reported profound scale erosion in juvenile common carps exposed to dimethoate. The erosion of scales and haemorrhagic patches over the body of fishes could be due to the dermal absorption of the pesticide after long exposure (Qayoom *et al.,* 2016 a&b).

The reflex of any organism is a response to external stimulus. Under intoxication of pesticides, the reflex of fishes is known to get altered with the increase in the intoxication time or concentration of pesticide. In this study, the reflex of dimethoate exposed fishes showed progressive degeneration up to the termination of experiment. From C_1 to C_5 the reflex of fishes was also found diminishing from severe to feeble (Table 8). The loss of reflex is the indication of paralysis and pending death of fishes due to pesticide toxicity. Our findings are in accordance with Adewumi *et al.,* (2018) who reported loss of reflex in *Clariasgariepinus* juveniles against chlorpyrifos and DDforce. The diminishing reflex response of fishes against CPF and dimethoate is due to the induction of paralysis due to the inhibition of AChE enzyme which leads to the onset of sluggish movements, lethargy, alternate quiescent and frenzied movements and signs of pending death in fishes (Qayoom *et al.,* 2015). The inhibition of AChE disables the cholinesterase activity and hence leads to the dysfunction of CNS. Same results have been observed in the present investigation.

Surface and gulping of fishes was witnessed indimethoate exposed fishes. In dimethoate trails, fishes were found to come on surface and gulping for inhalation of oxygen due to the suffocation caused by the pesticide toxicity

(Table 9). Our results are in accordance with Santhankumar *et al.,* (2000) who also reported surfacing in *Anabas testudineus* against sublethal concentrations of monocrotophos. Ural and Simsek (2006) obtained the same results in *Silurus glanis* against dichlorvos intoxication where fishes were seen gulping right from the day of the exposure of pesticide up to the termination of experiment. Woke and Aleleye-Wokoma (2009)obtained same results in *Clarias gariepinus* exposed to chlorpyrifos-ethylon while as Pandey *et al., (*2009) and Verma *et al.,* (2017) also reported sufacing and gulping of *Heteropneustes fossilis* against dimethoate and CPF exposures respectively. Surfacing phenomenon i.e., significant preference of upper layers in pesticide exposed fishes might be a demand for higher oxygen level and gulping of surface water appears to avoid breathing of fish in the poisoned water during the exposure period (Katja *et al.,* 2005, Qayoom *et al.,* 2015).

Swimming pattern of the fishes was found to get altered with the dimethoate intoxication and was found dose and time dependent. In dimethoate bioassays, the loss of normal swimming pattern was found more profound in the higher concentrations which grew intense with the termination of experiments (Table 10). The results obtained from present study are in agreement with Rao *et al.,* (2003) who reported loss of balance and disturbed swimming pattern in chlorpyrifos exposed *Oreochromis mossambicus*. Patil and David (2008) and Ramesh and Saravanan (2008) obtained the same results in *Labeo rohita* and *Cyprinus carpio* exposed to malathion and chlorpyrifos respectively.

Halappa and David (2009) and Ramesh and Munniswamy (2009) reported similar findings in *Cyprinus carpio* against chlorpyrifos toxicity. Cong *et al.,* (2009) worked on *Channa striata* under diazinon exposure while as Dey and Saha (2014) worked on *Labeo rohita* against dimethoate also reported the same results. The swimming pattern was found to get altered due to AChE inhibition which enabled fishes to go sluggish, leaving their swimming behaviour disturbed (Halappa and David, 2009).

The body colour of common carps was found to get changed when exposed to dimethoate. In dimethoate challenged fishes, a feeble change in the colour was observed (Table 11). Same observations have been reported by Vasait and Patil (2005) in *Nemacheilus botia* challenged with sublethal concentrations of monocrotophos where they reported a paler shade of intoxicated fishes compared to the controls. Pandey *et al.,* (2011) also reported discolouration of *Channa punctatus* against investon intoxication while as Devi and Mishra (2013) also reported discolouration of body of *Channa punctatus* challenged with sublethal concentrations of chlorpyrifos. Dey and Saha (2014) and Qayoom *et al.,* (2016 a&b) also obtained the same results in *Labeo rohita* and *Cyprinus carpio* var. *communis* exposed to dimethoate and CPF. The pale colouration of the fishes under pesticide exposure is related to the fact that stimulation of adrenal glands and hyper secretion of epinephrine during stress condition inhibits the action of MSH (melanocyte stimulating hormones), which results in pale body colour (Tyagi 2004).

A similar mechanism may be operable in the present study indicating an influence of the pituitary as well. Depigmentation has been attributed to dysfunction of pituitary gland under a stress conditions; this causes changes in the number and area of chromatophores (Pandey *et al.,* 1990). Pituitary dysfunction has also been reported in *Channa punctatus* following exposure to endosulfan for 120h (Agarwal, 2003).

Table.1 Calculation of dose for dimethoate

LC_{50} value of dimethoate (Qayoom *et al.*, 2016b)	20% of LC_{50} value	40% of LC_{50} value	60% of LC_{50} value	80% of LC_{50} value	100% of LC_{50} value
1.1 ppm	0.22ppm	0.44ppm	0.66ppm	0.88ppm	1.1ppm

Table.2 Excessive mucous secretion in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-
96	-	-	-	-	-	-	-	-	-	++	+	+	+++	++	+	-

Table.3 Convulsions recorded in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
72	-	-	-	-	-	-	+	+	+	+	++	++	++	++	++	-
96	-	-	-	-	-	-	++	+	++	++	++	++	+++	+++	+++	-

Table.4 Uncoordinated movements in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
48	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
72	-	+	-	+	+	-	+	+	+	+	++	+	++	++	++	-
96	-	+	-	+	+	+	++	+	++	++	++	+++	+++	+++	+++	-

Table.5 Imbalanced swimming in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
24	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
48	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
72	-	-	-	+	+	-	+	+	+	+	++	+	++	++	++	-
96	-	-	-	+	+	+	++	+	+	++	++	++	++	++	+++	-

Table.6 Erosion of scales in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
48	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
72	-	-	-	-	-	-	-	-	-	-	+	+	+	+	++	-
96	-	-	-	-	-	-	-	+	-	+	+	+	++	++	++	-

Table.7 Hemorrhagic patches developed in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
72	-	-	-	-	-	-	-	-	-	-	+	-	+	+	++	-
96	-	-	-	-	-	-	-	-	-	-	+	+	++	++	++	-

Table.8 Reflex response in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	+++	++	++	++	++	++++
12	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	+++	++	++	++	++	++++
24	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	++	+	+	+	++	++++
48	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	++	+	+	+	+	++++
72	++++	++++	++++	++++	++++	++++	++	+++	++	+	+	+	+	+	+	++++
96	++++	++++	++++	++++	++++	++++	++	++	++	+	+	+	+	+	+	++++

Table.9 Surfacing & gulping in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-
72	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
96	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-

Table.10 Change in swimming pattern in *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-
24	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-
48	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	-
72	-	-	-	-	-	-	+	-	+	+	+	+	+	++	++	-
96	-	-	-	-	-	-	+	-	+	++	++	++	++	++	++	-

Table.11 Change in body color of *Cyprinus carpio* var. *communis* exposed to different concentrations of dimethoate

Time in hours	C_1			C_2			C_3			C_4			C_5			CT
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
72	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
96	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-

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