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Research Article

Subacute cholinesterase inhibition and behavioral effects of chlorpyrifos experimentally supplied via drinking water in chicks

Banan K. Al-Baggou
Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract. Objective: Chlorpyrifos is a chlorinated organophosphate insecticide that might contaminate the water. The purpose of the study was to assess plasma and tissue cholinesterase inhibition and behavioral effects of chlorpyrifos in young chick model after exposure via the drinking water. Methods: Two-day old chicks were allocated randomly into two groups of 33 birds each. The control group was given free access to tap water, whereas the second group had free access to chlorpyrifos solution prepared in tap water (100 ppm) from the age of two days to the age of 27 days. At the ages of 8, 14 and 28 days, chicks of the control and chlorpyrifos groups (n=11/age group) were subjected to measurements of body weight, 3-min open-field activity, tonic immobility, followed by measurement of brain and liver weights as well as determination of plasma, brain and liver cholinesterase activities using an electrometric method. Results: chlorpyrifos did not significantly affect the growth and body weight of the chicks until the age of 28 days. In the chlorpyrifos group, the brain weight significantly increased on day 8, whereas liver weight significantly decreased on day 14 in comparison with respective control values. In the open-field behavioral paradigm, chicks exposed to chlorpyrifos in the drinking water showed significantly decreased locomotor activity at the age of 8 days as well as lower pecking score at the age of 28 days in comparison with respective control values. Significant prolongation of tonic immobility occurred in the chlorpyrifos group at the age of 28 days. Chlorpyrifos decreased cholinesterase activities in the plasma, brain and liver of the chicks at ages of 8, 14 and 28 in comparison with respective age-matched control values by 3-57%. Conclusion: the present findings suggest the sensitivity of the young chick model to examine subtle adverse effects of chlorpyrifos when given in the drinking water. These were expressed in the chicks behaviorally as decreased locomotion and increased tonic immobility and biochemically in the form of decreased cholinesterase activity in the plasma, brain and liver.

Key Words: organophosphate, chicken behavior, chlorpyrifos, cholinesterase, open-field.

Corresponding Author: B. K. Al-Baggou, bananalbaggou@yahoo.com

Introduction

Chlorpyrifos [O,O–diethyl–O–(3,5,6–trichloro–2–pyridyl) phosphorothioate] is a chlorinated organophosphate (OP) insecticide used in public health and on crops as well as livestock to control various pest infestations (Tomlin 2006; Watts 2012). It has been considered as an environmental pollutant because of its release to the environment contaminating the air, water, ice and snow (Davis & Ahmed 1998; WHO 2004; Albers et al 2007; Rose et al 2007; Palma et al 2008; Watts 2012). The mode of toxic action of chlorpyrifos in animals after systemic absorption and via its active oxon metabolite involves cholinesterase (ChE) inhibition in the nervous tissues causing the buildup of synaptic acetylcholine which stimulates muscarinic and nicotinic receptors (WHO 1986; Wilson 2005; Lotti 2010). The cardinal signs and symptoms of OP poisoning that result from the accumulated acetylcholine at synapses are muscarinic, nicotinic and central nervous system (CNS) effects in mammals and birds including the chicken (WHO 1986; Wilson et al 1988; Wilson 2005; Mohammad et al 2008; Lotti 2010).

Organophosphates induce various forms of behavioral changes in laboratory animals (Moser 2000; Carr et al 2001) as well as ChE inhibition in the blood or tissues which is an important biomarker endpoint to assess exposure of animals, including the birds, to such compounds (Wilson et al 1988; Wilson 1999; Cocker et al 2002; Jaga & Dharmani 2003; Wilson et al 2005; Worek et al 2005). Nonetheless, experiments are warranted to assess blood and tissue ChE inhibition and behavioral effects of chlorpyrifos in the young chick model after exposure via the drinking water. Unfortunately, limited investigations are available on the toxicological and behavioral effects of chlorpyrifos in the young chick model (Al-Badrany & Mohammad 2007), especially when the drinking water becomes the source of exposure.

Materials and Methods

Animals

Day-old Ross chicks were obtained from a certified hatchery in Mosul, Iraq. They were housed in a room conditioned at a temperature of 30–34○C, constant lighting; and the floor litter consisted of wood shavings. The chicks had free access to water and feed. A commercial insecticidal solution of chlorpyrifos (48% concentration, Chloodain 48 T.C., Sanliurfa, Turkey) was used. The concentrate solution of chlorpyrifos was further diluted in drinking tap water to reach the required concentration

which was 100 ppm for the experiment. At the age of two days, 66 chicks were divided randomly into two groups of 33 birds each. The control group was given free access to tap water, whereas the second group had free access to chlorpyrifos solution prepared in tap water (100 ppm) from the age of two days to the age of 27 days. The choice of chlorpyrifos concentration in the drinking water was based on a preliminary experiment in chicks in which overt signs of OP toxicosis were not seen and the birds were able to drink the treated water without aversion. Chlorpyrifos solution was freshly prepared each day. At the ages of 8, 14 and 28 days, chicks of the control and chlorpyrifos groups (n=11/age group) were subjected to the following measures which are summarized in table 1: measurement of body weight, 3-min open-field activity test as described before (Al-Baggou et al 1999; Mohammad & Faris 2006; Mohammad et al 2012b), tonic immobility test (Gallup Jr et al 1971; Mohammad & Faris, 2006), followed by determination of plasma, brain and liver ChE activities using a modified electrometric method described in the chicken (Mohammad 2007; Al-Badrany & Mohammad 2007; Mohammad et al 2012a,b). The weights of the brain and liver were also recorded.

Table 1: Design of the experiment in chicks and their treatment allocations

Age (day)	Action taken
1	Chicks were purchased and housed
2	66 chicks were divided into: Control group, treated with tap water, n=33 and chlorpyrifos-treated (100 ppm) group, n=33
8	Measurements: body weight, open-field activity, tonic immobility, cholinesterase activity (plasma, brain, liver), brain and liver weights
14	Measurements: body weight, open-field activity, tonic immobility, cholinesterase activity (plasma, brain, liver), brain and liver weights
27	Treatment ended
28	Measurements: body weight, open-field activity, tonic immobility, cholinesterase activity (plasma, brain, liver), brain and liver weights

The Scientific Committee of the College of Veterinary Medicine at the University of Mosul, Iraq approved the design of the present study which complied with our institutional animal care, use and housing regulations obtained originally from the guidelines of the National Research Council (2011).

Past Statistics Package (available at http://folk.uio.no/ohammer/past/index.html) was used to analyze the data statistically. Parametric data as multiple means were subjected to the one way analysis of variance followed by the least significant difference test and the non-parametric data were subjected to MannWhitney-U-test (Petrie & Watson 2006). The data of organ and body weights were subjected to the two-way analysis of variance followed by the least significant difference test (Petrie & Watson 2006). Frequency data were analyzed by the Fisher's exact probability test (Runyon 1977). The level of statistical significance was accepted at $p < 0.05$.

Results

Chlorpyrifos did not significantly affect the growth and body weight of the growing chicks up to the age of 28 days (Table 2). In the chlorpyrifos group, the mean brain weight significantly increased on day 8 (minimum-maximum, 0.9–1.26 g vs. control 0.81–1.21 g) whereas liver weight significantly decreased on day 14 (minimum-maximum, 3.17–4.9 g vs. control 3.49–6.4 g) in comparison with respective mean control values (Table 2). In the open-field behavioral paradigm, chicks exposed to chlorpyrifos in the drinking water showed significantly decreased locomotor activity (minimum-maximum lines crossed, 2–18 vs. control 5–31) at the age of 8 days as well as lower pecking score (minimum-maximum, 0–2 vs. control 0–3) at the age of 28 days in comparison with respective control values (Table 3). Furthermore, significant prolongation of tonic immobility occurred in the chlorpyrifos group (minimum-maximum, 17–185 vs. control 4–50 seconds) at the age of 28 days in comparison with control values (Table 3).

Exposure of chicks to chlorpyrifos in the drinking water significantly decreased their ChE activities in almost a generalized manner in the plasma, brain and liver at ages of 8 (19–46%), 14 (3–26%) and 28 (23–57%) days in comparison with respective age-matched control values (Table 4). The overall decreases in ChE activities ranged between 19–29% in the plasma, 3–46% in the brain and 23–57% in the liver in respective to control values (Table 4).

Discussion

The main action of chlorpyrifos seen in the chicks of the present study is its effect in the open-field behavioral paradigm which was represented by decreased locomotion- a form of CNS depression (Cory-Slechta 1989; Al-Zubaidy & Mohammad 2013) at the age of 8 days and decreased pecking at the age of 28 days. These findings are in accordance with the reported hypoactivity seen in chicks dosed orally with chlorpyrifos once at 2 and 4 mg/kg or dosed daily with the same doses for seven consecutive days (Al-Badrany & Mohammad 2007). In both the present study and the previous one (Al-Badrany & Mohammad 2007), the behavioral changes occurred in a subtle manner in the absence of overt signs of OP toxicosis which are characterized by muscarinic, nicotinic and CNS effects (Wilson 2005; Lotti 2010; Mohammad et al 2008). These behavioral changes, though not apparent until the tests were conducted and the birds were examined in a challenging environment, indicate the sensitivity of the young chick model in exploring the behavioral toxic effects of ChE inhibitors. Of particular interest, are the behavioral alterations which occurred without significant changes in the body weight of the growing chicks. Depressed body or organ weights are important toxic outcomes of OP poisoning in laboratory animals which usually occur in overtly poisoned animals (ATSDR 1997; Farag et al 2003).

Furthermore, tonic immobility which is a behavioral test sensitive to fear and CNS depressants (Gallup Jr et al 1971; Hennig et al 1984; Mohammad & Faris 2006) additionally supported the notion that chlorpyrifos might depress the central function since the duration of immotility was prolonged in chicks at the age of 28 days. The results of the present study also correlate with those reported in young rats in which oral chlorpyrifos dosing caused behavioral changes, mainly depressed locomotor activity (Moser 2000; Carr et al 2001).

Table 2: Body and organ weights (g) in chicks treated with chlorpyrifos in the drinking water (100 ppm) from the age of day two until the age of 27 days

Age (day)	Control (tap water)			Chlorpyrifos		
	Body	Brain	Liver	Body	Brain	Liver
8	68.64±1.11	0.98±0.04	1.30±0.06	63.82±2.25	1.13±0.04*	1.37±0.08
14	96.27±2.75[a]	0.94±0.06	4.57±0.26[a]	101.45±2.84[a]	1.00±0.04[a]	3.82±0.18*[a]
28	115.45±4.48[ab]	1.46±0.06[ab]	4.64±0.41[a]	113.36±3.59[ab]	1.47±0.06[ab]	4.44±0.39a[a]

* Significantly different from the respective control value, $p<0.05$.
[a] Significantly different from the respective value of day 8, $p<0.05$.
[b] Significantly different from the respective value of day 14, $p<0.05$.

Table 3: Three-min open-field activity and tonic immobility in chicks treated with chlorpyrifos in the drinking water (100 ppm) from the age of two days until the age of 27 days

Variable	Control	Chlorpyrifos
Age: 8 days		
Latency to move (seconds)	22.00±2.95	26.42±5.10
Lines crossed	14.25±2.11	7.67±1.34*
Escape jumps	0.42±0.15	0.67±0.36
Defecations	0.92±0.23	1.00±0.11
Pecking (scores)	0.00±0.00	0.17±0.11
Distress calls (scores)	2.92±0.08	2.58±0.29
Tonic immobility (seconds)	36.17±5.54	41.92±0.35
Age: 14 days		
Latency to move (seconds)	17.27±4.92	22.73±4.12
Lines crossed	11.09±2.23	11.18±1.94
Escape jumps	3.18±0.86[a]	2.18±0.66
Defecations	0.91±0.09	0.55±0.16
Pecking (scores)	1.73±0.38[a]	2.09±0.39
Distress calls (scores)	2.82±0.12	3.00±0.00
Tonic immobility (seconds)	58.82±26.70	57.45±18.30
Age: 28 days		
Latency to move (seconds)	24.55±6.10	20.00±4.93
Lines crossed	7.09±0.94[a]	7.55±1.27
Escape jumps	1.55±0.31[a]	1.55±0.53
Defecations	1.00±0.23	1.09±0.21
Pecking (scores)	2.00±0.40[a]	0.27±0.19*
Distress calls (scores)	2.64±0.15	2.64±0.24
Tonic immobility (seconds)	17.27±4.87[a]	52.64±16.52*

*Significantly different from the respective control value, $p<0.05$.
[a] Significantly different from the respective value of day 8, $p<0.05$.

Table 4: Cholinesterase (ChE) activity (delta pH/30 min) in chicks exposed to chlorpyrifos in the drinking water (100 ppm) from the age of 2 to 27 days

Age (day)	Control	Chlorpyrifos	% decrease in ChE activity
Plasma ChE			
8	0.37±0.02	0.30±0.01*	19
14	0.62±0.05[a]	0.46±0.04*[a]	26
28	0.58±0.03[a]	0.41±0.03*[a]	29
Brain ChE			
8	0.37±0.03	0.20±0.01*	46
14	0.31±0.02	0.30±0.02[a]	3
28	0.35±0.03	0.27±0.01*[a]	23
Liver ChE			
8	0.25±0.01	0.14±0.01*	44
14	0.13±0.004[a]	0.10±0.01*[a]	23
28	0.21±0.01	0.09±0.01*[a]	57

* Significantly different from the respective control value, $p<0.05$.
[a] Significantly different from the respective value of day 8, $p<0.05$.

Plasma, brain and liver ChE activities were decreased at the ages of 8, 14 and 28 days (which correspond to treatment days 7, 13 and 27). The enzyme inhibition occurred regardless of the coexistence of behavioral changes or the changes in organ weight which were not generalized in nature in the age groups mentioned above. Similar to present findings, no correlation was found between the extent of blood and tissue ChE inhibition and the behavioral changes in rats (Nostrandt et al 1997; McDaniel & Moser 2004). This is because overt behavioral signs of OP poisoning usually appear after reaching a high level (>50%) of ChE inhibition (WHO 1986; Nostrandt et al 1997; Marrs & Vale 2006) and neurobehavioral alterations could occur at even lower levels of enzyme inhibition. Several reports suggested rather non-ChE inhibition mechanisms to be involved in the deleterious effects of OP insecticides in laboratory animals including the chicks, such as oxidative stress and modulation of other non-cholinergic neurotransmission (Dam et al 1999; Lukaszewicz-Hussain, 2010; Lotti 2010; Al-Baggou et al 2011). Studies have described and assessed the acute toxicity, blood and tissue ChE inhibition and behavioral effects of chlorpyrifos after single or repeated oral administration via the gavage needle in 1-2 weeks-old chicks (Al-Badrany & Mohammad 2007; Mohammad et al 2008). This experimental chick model of acute OP poisoning depends on the acute animal response within less than two hours after oral dosing of the pesticide (Mohammad et al 2008; Al-Baggou et al 2011; Al-Zubaidy et al 2011; Mohammad et al 2012a). The young chick or adult chicken models of OP toxicosis have been widely advocated to examine acute and subacute toxicological profiles of OP insecticides (Farage-Elawar & Francis 1988; Wilson et al 1988; Wilson 1999; Al-Badrany & Mohammad 2007; Mohammad et al 2008; Al-Baggou et al 2011; Al-Zubaidy et al 2011; Mohammad et al 2012a). Moreover, adult chickens are excellent models of OP-induced delayed neuropathy (WHO 1986; Wilson et al 1988; Wilson 2005). Another toxicologic approach which is considered in the present study, regarding further toxicity assessment of rather subtle effects of chlorpyrifos, is the experimental exposure of chicks via the drinking water to this insecticide. Occurrence of chlorpyrifos in the drinking water is not an uncommon finding (ATSDR 1997; WHO 2004; 2009). The solubility of chlorpyrifos in water is around 1 mg/L and the acceptable daily intake of chlorpyrifos in drinking water was set at 30 ppp (WHO 2004; 2009).

Chlorpyrifos is metabolized in vivo into the active oxon metabolite which inhibits blood and tissue ChEs (Richardson 1993; ATSDR 1997). In the present study, chlorpyrifos depressed ChE activities in the plasma, brain and liver. Similarly, in the chicken dosed orally with chlorpyrifos or other OP insecticides, tissue ChE activity decreased concurrently with that of the plasma (Al-Badrany & Mohammad 2007; Mohammad et al 2008; Al-Baggou et al 2011; Al-Zubaidy et al 2011; Mohammad et al 2012a). Inhibited ChE is a biomarker of OP exposure, and regardless of the extent of enzyme inhibition, 20–30% decrease in plasma ChE activity usually suggests animal exposure to OP insecticides, and when the enzyme inhibition exceeds 50% it refers to the outcome of a serious adverse event (WHO 1986; Wilson 2005; Worek et al 2005). Several factors play roles in the extent of ChE inhibition, including the type of the insecticide, its formulation, concentration applied and route of absorption, duration of exposure, environmental temperature and temporal blood or tissue sampling (WHO 1986; Wilson 1999; Kwong 2002; Jaga & Dharmani 2003; Wilson 2005; Kachaiyaphum et al 2010; Świergosz-Kowalewska et al 2014).

Conclusions

The implication of the present findings is the sensitivity of the young chick model to examine subtle adverse effects of OP insecticides when it is present in the drinking water. These were expressed in the chicks behaviorally as decreased locomotion

and increased tonic immobility and biochemically in the form of decreased ChE activity in the plasma, brain and liver.

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Authors

•Banan K. Al-Baggou, Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, e-mail: bananalbaggou@yahoo.com

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