

Effect of Cadmium Chloride on the Development of *Chrysomya Megacephala* (Diptera:Calliphoridae) and its Importance to Postmortem Interval Estimate



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Abstract

Cadmium chloride is one of the highly toxic compounds among the heavy metals. It exerts a negative impact on the living organisms and accumulates in food chain and could lead to some serious problems, such as high rate of mortality, lower longevity, decreased fecundity and lower hatching ability of many insects and other arthropods. The present work dealt with the evaluation of cadmium chloride because there is lack of published entomotoxicological reports for cadmium chloride have evaluated their effect on the growth and development of this forensically important fly blow fly species, *Chrysomya megacephala* (Diptera: Calliphoridae). Larvae of *Chrysomya megacephala* (Diptera: Calliphoridae) reared on rat tissues that were previously exposed to Cadmium chloride (CdCl₂) in different concentrations: Half lethal (3.25mg/kg bw), Lethal (6.5 mg/ kg bw) and Twice Lethal (13 mg/kg bw) by intraperitoneal injection (i.p.). Development rate, larval body length, width, weight, pupal and adult weight and mortality were the observed parameters. Results demonstrated that the development rate of larvae between treated group and control group varied significantly. Development took longer time in the presence of high Cadmium concentration compared to control. Mortality results indicated greater mortality among the larvae with increased cadmium concentration as compared to control. It can be concluded that Cadmium chloride has negative effect on all the life stages of *Chrysomya megacephala*. Since cadmium chloride alters the rate of development in *Chrysomya megacephala*. There are chances of miscalculation of PMI if the presence of cadmium chloride is not taken into consideration. For example there could be wrong estimation of PMI by up to 18-86 hours if age of larvae is determined on the basis of its length ignoring the effect of cadmium chloride.

Keywords: Cadmium chloride; *Chrysomya megacephala*; Development rate; Postmortem interval

Introduction

With industrialization, the use of heavy metals has increased tremendously during the last few decades. Heavy metals have high toxicity and are non biodegradable. They can be accumulated by the organisms and affect the development and physiological state of the individual. Different studies have been conducted on the effect of heavy metals on insects [1-8] They have been reported to have resulted in alterations of respiratory [9] and metabolic processes [7,10-16].

Cadmium chloride is one of the highly toxic compounds among the heavy metals. It exerts a negative impact on the living organisms and accumulates in food chain and could lead to some serious problems, such as high rate of mortality, lower longevity, decreased fecundity and lower hatching ability of many insects and other arthropods [3,5,17-20]. AL- Misned [21] studied

the effect of cadmium on the development of *Chrysomya* I[22]. Studied the accumulation of cadmium and its effects on growth, development and hemolymph biochemical compositions in *Boettcherisca peregrina* larvae (Diptera: Sarcophagidae) and results demonstrated that cadmium had negative effects resulting in significant reduction both in larval body weight and length [23]. Studied the effect of cadmium on the Gypsy moth *Lymantria dispar* L and concluded that cadmium had no effect on the larval duration but shortened the pupal duration and reduced the pupal mass. The development of insects is dependent upon the ambient temperature [15,24-27]. Similarly the quantity and quality of food also plays a significant role in the rate of growth [28-37]. Several chemicals present in the food also interfere with the normal development of blow flies [38-49].

Chrysomya megacephala is a forensically important blow fly distributed in many parts of the world and available throughout the year in northwestern part of India [50-52]. Larvae of this species have been reported in association with human corpses in several cases [53-59]. The effects of cadmium chloride on the development rate, length, width and weight of larvae, pupal weight, adult weight and mortality of blow fly *Chrysomya megacephala* have been investigated in the present study.

Materials and Methods

Insect rearing and experimental group

Stock colony was populated from the wild type specimens *Chrysomya megacephala* collected with the help of sweeping net from Punjabi University Campus by using goat skeletal meat as bait. The culture was maintained out of phase such that each generation comprised a number of emergences separated over time to provide a mix of different aged gravid females. Generations were conserved in a way that no intergenerational mating occurred, except when newly trapped wild type individuals were added to the culture. This stock colony was repopulated, when required.

Animal Ethical Committee permission

Institutional Animal Ethical Committee (vide letter no. 107/99/CPCSEA-2012-12).

Cadmium chloride treatment

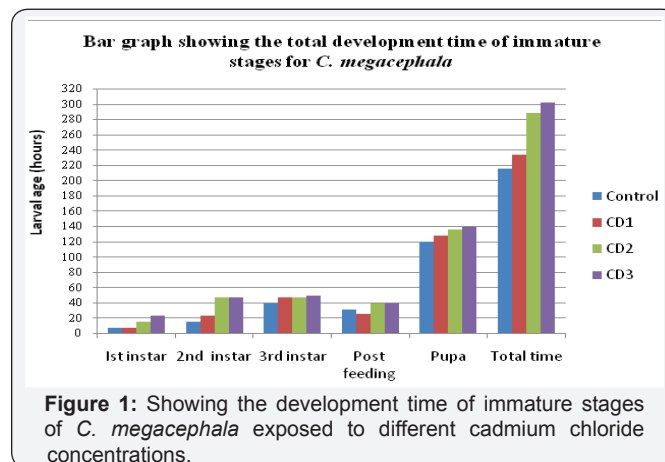
Female Sprague-Dawley laboratory rats (100-110 g) were used for experiments that were previously exposed to cadmium chloride (CdCl_2) in different concentrations: Half lethal (3.25mg/kg bw), Lethal (6.5 mg/kg bw) and Twice Lethal (13 mg/kg bw) by intraperitoneal injection (i.p.). Newly hatched larvae (250-300) were obtained from these laboratory breed colonies and allowed to feed upon the rat carcasses CD1 (Half Lethal), CD2 (Lethal) and CD3 (Twice Lethal). Controls were also maintained to compare with the treated group in order to study the effect of cadmium chloride only leaving the other factors unaltered (food, temperature humidity etc.). Two replicates were done and the results combined for analysis. Time of hatching was noted and subsequently development time was estimated for each larval instars (1st, 2nd and 3rd), post feeding larvae, pupa and time of emergence of adults. 15 larvae were randomly collected at 8 hour intervals. 5 larvae were used to determine growth based on weight increase and 10 larvae were measured to determine development based on increase in total length and width. Minimum and Maximum temperature and humidity were noted daily by using Electronic Thermo hygrometer (Maximum daily temp. 24.5 ± 2 °C, Minimum daily temp 22.5 ± 2 °C, Relative Humidity 68% - 72% and Photoperiod LD 10: 14) .

Data analysis

Data were analyzed using Arithmetic Mean, Standard deviation, Analysis of Variance (ANOVA) and Chi square test. Graphs and tables were prepared using Microsoft Excel 2007.

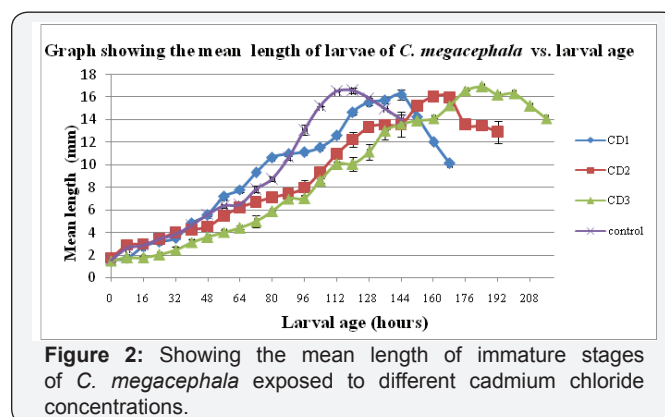
Results and Discussion

Larval development



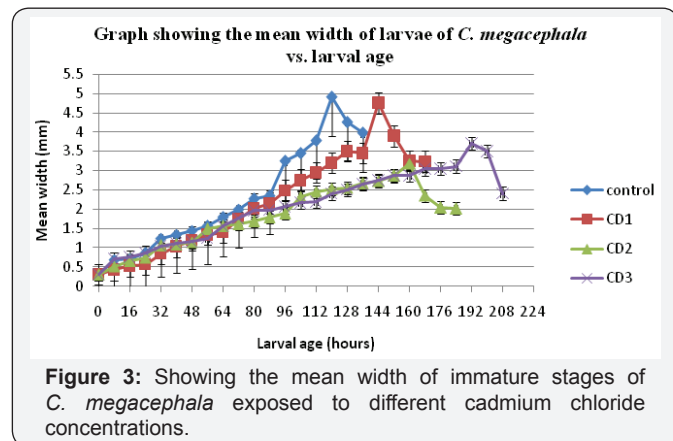
Development time from egg to adult was significantly different for *C. megacephala* reared on tissues with different dosages of cadmium chloride (Figure 1). There were highly significant differences between the mean larval development times among the different concentrations of cadmium chloride for first instar ($F=2,051.74$, $P=0.000$), second in star ($F=4,439.251$, $P=0.000$), third in star ($F= 6,924.611$, $P=0.000$), post feeding stage ($F=10,224.815$, $P=0.000$), pupa ($F= 5,907.292$, $P=0.000$) and total development ($F= 58,698.74$, $P=0.000$). It can be concluded that the presence of the cadmium chloride had a significant impact on larval growth and also delayed the pupation by larvae [22]. Shown that there is no effect of cadmium on the development of *Drosophila melanogaster* but adults died without egg lying [59]. Studied the effect of cadmium on *Chironomus riparius* and that caused the prolongation in the development of first and second instar stage [3,18]. Concluded that duration of nymphal stages of *Aiolopus thalasinus* was prolonged when exposed to cadmium up to 100 ppm in soil. *Al- Misned* [21] showed that development time of larvae of *Chrysomya albiceps* was extended with increasing cadmium concentration.

Larval length and width



Length has been one of the most frequently used parameters for successfully estimating larval age [57-58]. It is measured

between the head and the tip of the eighth abdominal segment [30]. The development curves created from the larval length are shown in (Figure 2). There were significant differences in the lengths of the larvae feeding upon the different doses of cadmium chloride and the time required to reach the maximum length ($F=6,713.356$; $p=0.000$). The maximum lengths for the larvae feeding upon CD1 ($16.2 \pm 0.05\text{mm}$), CD2 ($16.05 \pm 1.12\text{mm}$), CD3 ($16.09 \pm 0.07\text{mm}$) and control ($16.59 \pm 0.09 \text{ mm}$) were recorded at 144 hours, 160 hours, 184 hours and 120 hours respectively. This shows that larvae took more time to attain the maximum length as the concentration of the cadmium chloride increased.



Larval width has recently been regarded as a valuable parameter for age determination of larvae and consequently PMI estimation. The width of the larvae, viewed laterally, was measured between the ventral and dorsal surfaces at the junction of the fifth and sixth abdominal segment. [30]. the development curves created from the width data are (Figure 3). There are significant differences in widths of the larvae feeding upon the different doses of cadmium chloride and the time required to reach the maximum width ($F=263.864$; $p=0.001$). Larvae belonging to the group CD1 attained maximum width at 144 hours ($4.75 \pm 0.34 \text{ mm}$), CD2 at 160 hours ($3.18 \pm 0.14 \text{ mm}$) and CD3 at 184 hours ($3.11 \pm 0.24 \text{ mm}$) respectively. The control group took less time to attain maximum width i.e. 120 hours ($4.9 \pm 0.27 \text{ mm}$) as compared to treated groups [60]. Suggested that the differences in cadmium and mercury concentrations between sexes in mayflies may have resulted from different factors, including differences in body size and body composition [22]. Studied the effect of cadmium on the growth of *Boettcherisca peregrina* and evaluated the larval length. The larval body lengths were significantly shorter in comparison to control.

Larval and pupal weight

The mean larval weight varied significantly as the concentration of cadmium was increased (Table 1). The maximum weight of CD1 ($99.97 \pm 0.26\text{mg}$) was attained in 114 hours, while the maximum weight for the larvae feeding upon CD2 ($95.89 \pm 0.074\text{mg}$) and CD3 ($93.8 \pm 0.31\text{mg}$) was recorded at 160 and 184 hours respectively. Maximum weight of control group was $102.6 \pm 0.014\text{mg}$ attained at 120 hours. Pupal and

adult weight was also decreased with increased concentration (Table 1) [61]. Reported that there were no significant differences between the adult weight of control and cadmium treated groups [3] studied that cadmium was the reason for the loss of weight of adult of *Aiolopus thalassinus* [22]. Studied the effect of cadmium on *Chrysomya albiceps* and concluded that cadmium caused decrease in pupal and adult body weight. The results from the present study also demonstrate that increase in the concentration of cadmium affects the larval and pupal weight negatively.

Table 1: Maximum weight of larvae, pupae and adults exposed to the different concentrations of cadmium chloride.

Different concentration of cadmium chloride (mg/kgbw)	Maximum weight of larva	Maximum weight of pupa (Mean \pm SD) mg	Maximum weight of Adult (Mean \pm SD) mg
Control	102.6 ± 0.014	99.56 ± 1.27	74.56 ± 0.07
CD1	99.96 ± 0.26	95.05 ± 0.41	70.67 ± 0.11
CD2	95.89 ± 0.07	92.8 ± 0.25	65.02 ± 0.51
CD3	93.8 ± 0.31	89.85 ± 0.32	57.34 ± 0.47
Analysis of variance	$F=1,539.75$ $df= 3$ ($p < 0.001$)	$F= 346.79$ $df=3$ ($p < 0.01$)	$F= 1,688.21$ $df= 3$ ($p < 0.01$)

Larval mortality

Table 2: Showing the percentage of larval and pupal mortality in *C. megacephala* exposed to different Concentration of cadmium chloride.

Different concentration of cadmium chloride (mg/kgbw)	Larval mortality (%)	Pupal mortality (%)
Control	3.16	4.67
CD1	5.96	9.95
CD2	11.05	14.50
CD3	19.68	21.19

The larval stage showed more mortality due to cadmium than the pupal stage. The percentage of mortality was increased with the increase of cadmium chloride concentration (Table 2). In the control group there were no significant differences ($X^2 = [p = 0.05, df= 1,] = 3.84$), but with increase in the concentration of cadmium, larval and pupal mortality was increased i.e. CD1 ($X^2 = [p = 0.01, df= 3,] = 11.34$), CD2 ($X^2 = [p = 0.01, df= 9,] = 21.7$) and CD3 ($X^2 = [p = 0.01, df= 13,] = 27.7$). The effect of cadmium chloride on mortality rate observed in the present study is similar to the previous studies [62]. Studied the effect of cadmium on the alligatorweed flea beetle *Agasides hygrophila*. The mortality rates were increased with presence of cadmium [3,18]. Observed a significantly short life span due the effect of cadmium in *Aiolopus thalassinus*. The nymphs were more tolerant than eggs and adults to different concentrations of cadmium [63] also reported that the longevity of *Ceratitis capitata* and *Coptera occidentalis* was negatively affected by cadmium.

Conclusion

It can be concluded from this study that cadmium chloride has negative effect on every stage of the life cycle of *Chrysomya megacephala*. Larval development took longer time in the presences of high cadmium concentrations as compared to control. Larval, pupal and adult weights were decreased with its higher doses. Similarly there was greater mortality among the larvae and pupae under the influence of cadmium chloride. The time of death on the basis of insect evidence is generally determined by estimating the age of the maggots from a dead body. Since cadmium chloride alters the rate of development in *Chrysomya megacephala*. There are chances of miscalculation of PMI if the presence of cadmium chloride is not taken into consideration. For example there could be wrong estimation of PMI by up to 18-86 hours if age of larvae is determined on the basis of its length ignoring the effect of cadmium chloride. Hence the investigator must take into consideration the presence of chemical in the larval food that may affect the rate of development.

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