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Determination of malathion levels and the effect of malathion on the growth of *Chrysomya megacephala* (Fibricius) in malathion-exposed rat carcass

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Abstract. This study was conducted to examine the effect of malathion on the development of *Chrysomya megacephala*. A total of 12 adult Sprague-Dawley rats was divided into 4 groups. Each animal in the 4 groups was given orally 0 (control), 10, 25 and 50ml/kg body weight of malathion, respectively. *Chrysomya megacephala* larvae were then allowed to grow on the liver of carcass. Larvae development was estimated by means of weight and length, time of adult emergence and survival rate. Results indicated that for the first 6 to 30 hours, larvae from control group developed more rapidly than larvae feeding on tissue containing malathion. However, the 3 doses of malathion did not exhibit significant impact on larvae length and weight. The time required for adult emergence was significantly greater for malathion-treated colony which was 10 days compared to 7 days in control colony. Control larvae of *C. megacephala* had higher survival rate compared to larvae exposed to the three different doses of malathion. Analysis of the tissues indicated that all rats and fly samples were positive for malathion. Malathion concentration was highest in liver. It was concluded that the presence of malathion altered the development rate of *C. megacephala* and thus disrupted normal postmortem interval estimation.

INTRODUCTION

Among all the organophosphorous compounds, malathion had been reported to have low mammalian toxicity and considered to be one of the safest of the OP pesticides. However, there have still been numerous cases of either accidental or suicidal poisoning due to malathion (Nalin, 1973; Matsumura, 1975; Hayes, 1982; Karalliedde & Senanayake, 1988; Pannell *et al*., 2001). Malathion acts by binding to and phosphorylation enzymes such as red blood cells and plasma cholinesterase that prevents the breakdown of the neurotransmitter acetylcholine initially producing stimulatory and then inhibitory, effects at nerve synapses (Eto, 1974). Death due to malathion results from a combination of factors contributing to respiratory arrest including respiratory muscle weakness, bronchospasm and excessive bronchial secretion (Ford, 1993).

Some death that occurs by poisoning remains undiscovered until the body is wholly or partially skeletonised. In such cases, analysis of toxicology using body fluids and tissues are almost impossible. Recently forensic entomologist has introduced a procedure using insects as a silence witness interpreting information concerning death. Fly larvae (maggots) involved in processing the corpse tissues would likely ingest any chemical metabolites from the corpse into their own tissues. These insects can then be analyzed to detect those

substances (Anderson & Sherah, 1996; Goff *et al*., 1997). Maggot age also could be used in estimating the postmortem interval (Rodriguez & Lord, 1993).

The blowfly, *Chrysomya megacephala* (Fabricius) (Calliphoridae) is one of the dominant flies of forensic importance in Malaysia (Omar *et al*., 1994; Hamid *et al*., 2003; Lee *et al*., 2004). Flies are the first insects to colonize decomposing remains. Fly larvae from decomposing bodies not only can serve in the estimation of postmortem interval, but also can be used in qualitative identification of drugs or toxins (Rodriguez & Lord, 1993). Gunatilake & Goff (1989) have demonstrated that detection of malathion in larvae of *C. megacephala* and *Chrysomya rufifacies* (Macquart) (Calliphoridae) are possible provided certain conditions are met.

The purpose of this study was to determine the effect of malathion in poisoned rats on the development and growth of *C. megacephala* as well to verify the time of emergence of adults, that can affect the estimation of postmortem interval.

MATERIALS & METHODS

A total of 12 adult Sprague-Dawley rats weighing 190g to 220g were used as test animals for the administration of malathion. The rats were supplied by Animal House of Universiti Kebangsaan Malaysia. Lethal dose of malathion on rats was 1000mg/kg BW. The malathion was administered orally with three different doses which are 10 ml/kg BW, 25 ml/kg BW and 50 ml/kg BW. The chemical was given after the animals have being fully anaesthetized to comply with ethical requirements. Control group was fed with distilled water and killed by chloroform. Three replicates were performed for each group. Immediately after all the animals dead, samples of blood, liver, heart, lung and urine were taken for chemical analysis of malathion using gas chromatography.

The following procedure of Carvalho *et al*. (2001) was followed with some modifications. Sample of liver from the rats was exposed to 100 newly emerged *C. megacephala* larvae and incubated at 33 ± 1.5°C and relative humidity of 76 ± 3% (Mohd Iswadi *et al*., 2007). The colony of larvae was established from adult insects that have been reared at the Insectarium of Faculty of Allied Health Science, Universiti Kebangsaan Malaysia.

At 6 hours interval until the larvae started to pupate, total weight of larvae was individually recorded for 10 randomly selected larvae of each liver sample. At the end of third instar, 10 larvae were taken for chemical analysis. The rest were allowed to complete their development. The time required for pupation, emergence and longevity was also recorded. Samples of pupae and adults were collected for chemical analysis. SAS statistical package 9.1 was used to analyze the data of the life cycle of the flies. Analysis of variance (ANOVA) test was performed using the general linear models (GLM).

Chemical analysis

Solid phase extraction using Oasis HLB Cartridge equipped with a vacuum pump was used to extract malathion (Waters Corporation 2003). Procedure to extract malathion was in accordance with procedure supplied with the Oasis HLB Cartridge kit. Approximately 0.5g of tissues, fluid and insects samples were weighed and homogenized in 5 ml phosphate buffer saline. The mixture was centrifuged at 3000rpm for 10 min. The pellet was then discarded and supernatant was added with 10µl of 10mg/ml 1,4-diphenoxybenzene as an internal standard. One ml of the mixture was loaded into the HLB cartridge that have been conditioned and equilibrated. The cartridge was then washed with distilled water and eluted with 10:90 methanol/diethyl ether. The solution collected was dried with sodium sulphate and evaporated. A total of 2µl of the solution was injected into Gas Chromatography (GC).

The chemical analysis was performed on a Shimadzu model GC-2010, coupled to an electron capture detector, ECD-2010. Chromatographic separations were done on a DB-5MS 30m x 0.32mm x 0.25µm capillary column.

Calibration curves

Calibration curves were used as a method to quantify malathion obtained from rats and flies samples on GC. One mg of malathion standard was dissolved in 1ml acetonitril. The stock was diluted to a series of concentrations between 0.1µg/ml to 10µg/ml and 2µl of each aliquot were injected in the GC. Calibration curves were obtained by plotting the peak-area ratio of malathion. The curve obtained was: y= 13421x, R^2=0.9497

RESULTS & DISCUSSION

The comparison of internal organs between control rats and malathion-poisoined rat was shown in Figure 1. Internal examination revealed most of the organs especially the gastrointestinal tract from malathion-poisoned rats were swollen compared to the control rats. Pungent odor of malathion was present and generalized congestion was observed.

Larvae development was observed from larvae weight and length that were recorded through out the experiment (Table 1 and Figure 2). The differences of in larvae development were noticed at the first 6 hours of exposure. Control larvae developed faster than larvae exposed to malathion.

There was no differences of larvae weight at dose of 10ml/kg BW and 50ml/kg

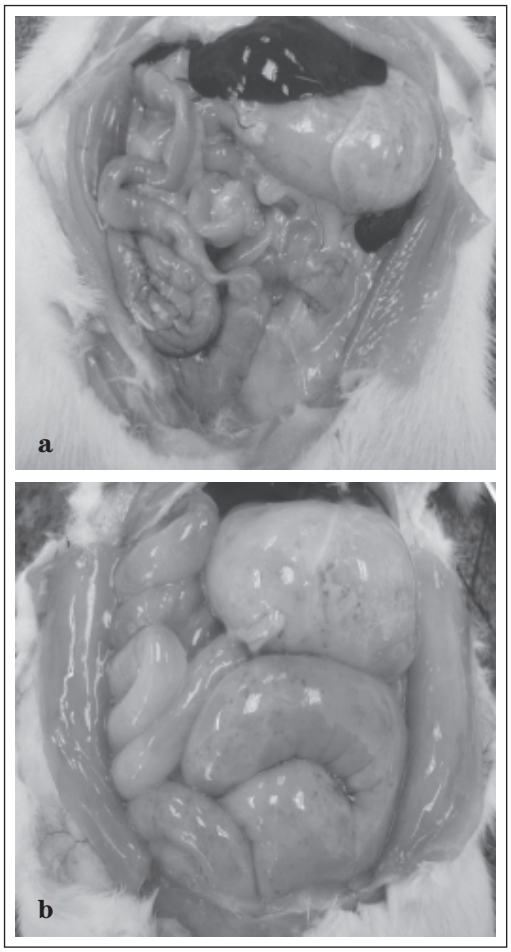

Figure 1. The differences in internal visceral organs between a) control rat and b) malathion-exposed rat.

Table 1: Larvae weight (mg) means of *C. megacephala* related to time of exposure (hours) on rat liver with or without (±S.D) malathion

Time	Control	Malathion dosage		
		10ml/kg BW	25ml/kg BW	50ml/kg BW
0	1.0 (±0)	1.0 (±0)	1.0 (±0)	1.0 (±0)
6	5.80 (±2.51)	4.782 (±2.77)	2.89 (±1.13)	4.77 (±1.81)
12	11.21 (±4.1)	10.00 (±3.17)	7.74 (±2.23)	6.40 (±1.63)
18	16.91 (±10.29)	17.55 (±4.44)	14.68 (±2.54)	8.24 (±1.17)
24	29.48 (±7.00)	27.82 (±5.76)	27.67 (±7.29)	19.02 (±7.27)
30	38.19 (±8.48)	38.78 (±9.16)	40.68 (±12.08)	35.54 (±15.69)
36	44.73 (±7.65)	42.56 (±6.56)	49.44 (±6.75)	48.27 (±12.10)

Except for 1 hr exposure, all means are significantly different ($p<0.05$)

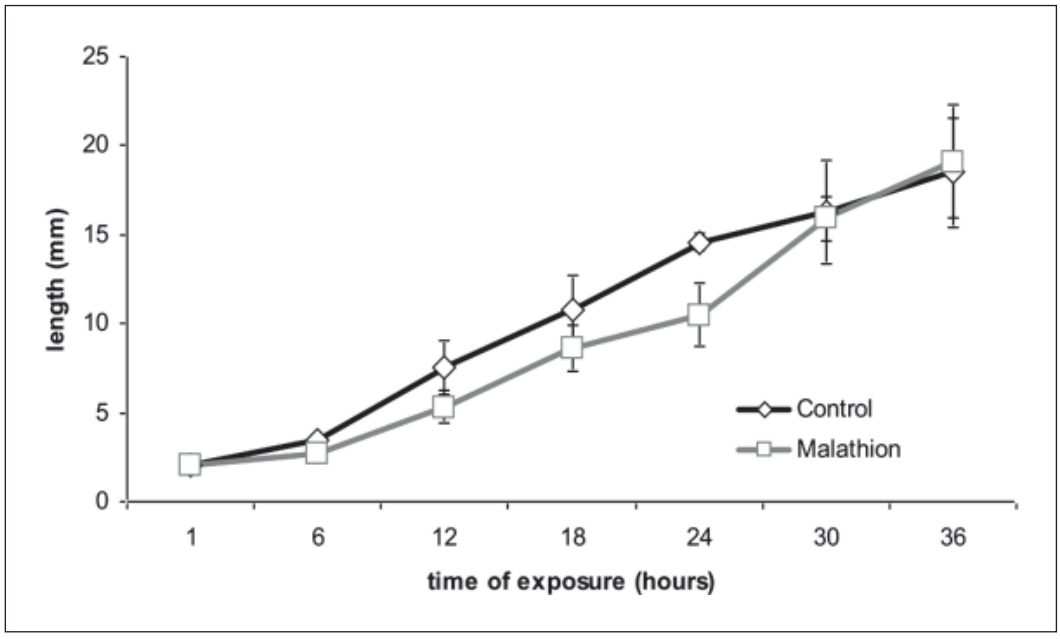

Figure 2. Comparison of means of larvae length (mm) vs time of exposure (hrs) between malathion exposed larvae and control.
Except for 1 hr exposure, all means are significantly different ($p<0.05$).

Table 2: Duration (hours) of each development stages of *C. megacephala* on rat liver with and without malathion

Stage	Control	Malathion
Larva Instar I	$x \leq 8$	$x \leq 12$
Larva Instar II	$8 \leq x \leq 18$	$12 \leq x \leq 24$
Larva Instar III	$18 \leq x \leq 36$	$24 \leq x \leq 48$
Prepupa	$36 \leq x \leq 72$	$48 \leq x \leq 90$
Pupa	$72 \leq x \leq 168$	$90 \leq x \leq 240$
Total duration	168hrs(±12) =7 DAYS	240hrs(±25) =10 DAYS

indicated from the larvae length. This was clearly seen in the early phase of the larvae development in which larvae exposed to malathion took an extended 12 hours to reach to the second stage. This trend was continued in every stages of the blowfly development where control larvae grew significantly faster than malathion-exposed larvae. Overall, the time required by control larvae to adult fly was 168 hours or 7 days, whereas larvae from malathion exposed group required 240 hours or 10 days to reach BW. However, the growth on both doses were two times more than larvae-exposed to 25ml/kg BW. From the larvae length recorded, there was no significant difference between the larvae exposed to three doses of malathion.

Figure 2 showed the comparison mean of larvae length between control and exposure to malathion at 50ml/kg BW. This suggests that larvae exposed to malathion might ingest and bioaccumulate the OP compound in their system and affect the development.

In addition, from 12 hours onwards, development of larvae exposed to malathion was still slower than control larvae until it reached 30 hours post exposure, the malathion exposed larvae boosted their development. This might be due to the malathion in the tissue has already been metabolised by the larvae.

The duration required for the larvae to complete each stage of development was recorded in hours (Table 3). Results indicated that larvae development into adulthood was much longer in the malathion group compared to control. Each stage was

Table 3: Percentage (%) of larval and pupal survival and non-emergence of *C. megacephala* adult

	Control	Malathion		
		10 ml/kg BW	25 ml/kg BW	50 ml/kg BW
Larvae survived	97.62 (±3.37)	91.62 (±2.57)	87.19 (±1.68)	66.09 (±3.28)
Pupae survived	98.01 (±1.05)	89.31 (±2.26)	69.55 (±9.56)	68.45 (±9.26)
Non-emerged adults	1.98 (±1.03)	10.68 (±2.26)	30.45 (±9.57)	32.89 (±9.19)

Table 4: Malathion quantitation (ug/mg) in samples of rat tissues and fly specimens from GC-ECD analyses

Sample	Quantitation
Liver	1.892 (±0.93)
Heart	0.305 (±0.682)
Lung	0.740 (±0.902)
Blood	0.402 (±1.282)
Urine	0.031 (±0.018)
Larvae	0.157 (±0.092)
Pupae	0.202 (±0.184)
Adult	0.045 (±0.021)

adult stage. Based on the result, it would suggest that the presence of malathion in the larvae system had significantly delayed the development of *C. megacephala* larvae to almost 3 days.

Table 4 shows survival rate of *C. megacephala* at different stages of development. The number of dead larvae on malathion exposed rat was much higher compared to control larvae. In addition, high percentage of malathion-exposed pupae did not survive to reach the adult stage. Larvae exposed to the highest concentration of malathion exhibited the lowest larvae and pupae survival rates.

Tissues and flies samples analyzed for malathion by GC were positive for malathion. Rat liver had the the highest concentration of malathion compared to others. This might be due to the metabolic process of malathion that takes place in the liver (Aizawa, 1982). Generally, malathion accumulates in the liver, intestine, kidney and lung of a mammal due to the presence of the metabolic enzyme, mixed function oxidase which later metabolises malathion to various metabolites excreted through urine (Rao, 2000). This study has also shown that malathion can be detected in various organs. The concentrations obtained from all organs were not a reflection of the concentrations given to the animals. A wide range of concentration were seen in which high concentration of malathion was detected in the lung but very low in urine. Despite liver's ability to accumulate the malathion, no correlation was found between malathion concentration in the liver and the larva that were feasting on the tissue ($p>0.05\%$).

Larvae of control group developed more rapidly than larvae fed on the liver containing malathion. This observation was supported by Carvalho *et al.* (2001) which had seen similar characteristics and had later suggested that larvae feeding on tissue laced with chemicals will either bioaccumulate or excrete the substance and its metabolites. Since malathion showed a significant effect in *C. megacephala* development, we concluded that malathion was bio-accumulated and present throughout the development stages. This conclusion was further supported by Introna *et al.* (2001) which had also suggested that a toxin can be detected in the larvae when its rate of absorption exceeds the rate of elimination.

In the present study, we had shown that malathion extended the development of *C. megacephala* to 10 days compared to 7 days in control. In addition, the process of estimation of larval age or estimation of

postmortem interval might be erronous if the presence of malathion in the larvae is not considered for the first 6 hours to 36 hours. This is supported by the case of malathion poisoning reported by Gunatilake & Goff (1989) in which the development stages of *C. megacephala* and *C. rufifacies* were indicative of a minimum postmortem interval of 5 days, whereas the victim had last been seen alive 8 days prior to the discovery of the body.

Entomological data are very useful tools for forensic investigation only if the investigation takes into account all aspects including metabolism and bioaccumulation of substances in insect sample.

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