

# University of Wollongong - Research Online

## Thesis Collection

Title: Pesticide exposure in free-living native birds and the effects of acute dosing of fenitrothion and fipronil on physiological performance in selected species

Author: Karen J Fildes

Year: 2008

Repository DOI:

### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.**

Research Online is the open access repository for the University of Wollongong. For further information contact the UOW Library: [research-pubs@uow.edu.au](mailto:research-pubs@uow.edu.au)

2008

## Pesticide exposure in free-living native birds and the effects of acute dosing of fenitrothion and fipronil on physiological performance in selected species

Karen J. Fildes

*University of Wollongong*

Follow this and additional works at: <https://ro.uow.edu.au/theses>

### University of Wollongong

#### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

### Recommended Citation

Fildes, Karen J, Pesticide exposure in free-living native birds and the effects of acute dosing of fenitrothion and fipronil on physiological performance in selected species, PhD thesis, School of Biological Sciences, University of Wollongong, 2008. <http://ro.uow.edu.au/theses/856>

## **NOTE**

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

## **UNIVERSITY OF WOLLONGONG**

### **COPYRIGHT WARNING**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**School of Biological Sciences**

**Pesticide exposure in free-living native birds and the effects of acute dosing of fenitrothion and fipronil on physiological performance in selected species**

**Karen Fildes BABSc (Hons)**

**"This thesis is presented as part of the requirements for the  
Award of the Degree of Doctor of Philosophy  
of the  
University of Wollongong"**

**September 2008**

**CERTIFICATION**

I, Karen Josephine Fildes, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

## **ABSTRACT**

### **Chapter 1**

This chapter introduces the main classes of pesticides with a brief overview of their mode of toxic action. Cholinesterase inhibiting chemicals and their known effects on birds are reviewed in detail. The benefits and limitations of the use of lethal compared to sublethal toxicological endpoints to assess pesticide impacts on avian species in toxicological research are discussed. It is argued that there is a critical need for investigations of sublethal effects to consider biochemical and physiological components of ecologically relevant traits. The use of pesticides for locust control, particularly fenitrothion and fipronil, by the Australian Plague Locust Commission, is discussed. The chemical properties and mode of toxic action of these two pesticides are reviewed and the potential impact of their application on Australian native bird species is assessed.

### **Chapter 2**

Cholinesterase (ChE) inhibiting pesticides are applied throughout Australia to control agricultural pests. Blood plasma ChE activity is a sensitive indicator of exposure to organophosphorus insecticides in vertebrates. To aid biomonitoring and provide reference data for wildlife pesticide-risk assessment, plasma acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were characterised in nine native bird species: brown songlarks (*Cinclorhamphus cruralis*), budgerigars (*Melopsittacus undulatus*), clamorous reed warblers (*Acrocephalus stentoreus*), double-barred finches (*Taeniopygia bichenovii*), king quails (*Coturnix chinensis*), Richard's pipits (*Anthus novaeseelandiae*), white-plumed honeyeaters (*Lichenostomas penicillatus*), willie wagtails (*Rhipidura leucophrys*) and yellow

throated miners (*Manorina flavigula*). The plasma of all species contained AChE and BChE except in king quail where no AChE was present. The lowest detectable plasma AChE activity was 0.10  $\mu\text{mol}/\text{min}/\text{ml}$  in budgerigars and the highest was 0.86  $\mu\text{mol}/\text{min}/\text{ml}$  in clamorous reed-warblers. BChE activity in the plasma ranged from 0.37 in double-barred finches to 0.90  $\mu\text{mol}/\text{min}/\text{ml}$  in white-plumed honeyeaters and clamorous reed-warblers. The lowest proportion of AChE was found in budgerigars (12.8%) and highest in willie-wagtails (67.8%). Apart from king quail AChE activities in all species were within the range reported for other avian species. The absence of AChE in king quail has not previously been reported for any bird species.

The effect of sampling time on plasma ChE was assessed in budgerigars and zebra finches (*Taeniopygia guttata*) and seasonal effects were examined in zebra finches. No diurnal variation in ChE activity was found at any time of day in either species although there was a significant difference in all ChE activity between seasons in zebra finches.

### **Chapter 3**

Huge aggregations of flightless locust nymphs pose a serious threat to agriculture when they reach plague proportions, but provide a very visible and nutritious resource for native birds. Locust outbreaks occur in spring and summer months in semiarid regions of Australia. Fenitrothion, an organophosphate pesticide, is aerially sprayed to control locust plagues. To evaluate fenitrothion exposure in birds attending locust outbreaks, we measured total plasma cholinesterase (ChE), butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activities in four

avian species captured pre- and post-fenitrothion application and ChE reactivation in birds caught post spray only. Eleven of 21 plasma samples from four species had ChE activity below the diagnostic threshold (two standard deviations below the mean ChE activity of pre-spray samples). Granivorous zebra finches (*Taeniopygia guttata*) and insectivorous white-winged trillers (*Lalage sueurii*) had significantly lower mean plasma total ChE, BChE, and AChE activity post-spray, while two other insectivores, white-browed (*Artamus superciliosus*) and masked woodswallows (*Artamus personatus*), did not. Cholinesterase was reactivated in 19 of the 73 plasma samples collected, and in one of three brain samples. We conclude that native bird species are exposed to fenitrothion during locust control operations. This exposure could have detrimental impacts as locust outbreaks and avian reproductive events are both stimulated by heavy summer rainfall, leading to co-occurrence of locust-control and avian breeding activities.

## Chapter 4

The effect of fenitrothion exposure on birds was examined by measuring aerobic metabolism, blood haemoglobin (Hb) content, plasma cholinesterase activities and body mass for up to 21 d post-dose. Peak metabolic rate (PMR) was measured in a flight metabolic chamber in three dose groups of house sparrows (*Passer domesticus*) (100 mg/kg = high, 60 mg/kg = medium, 30 mg/kg = low), and one dose group each of zebra finches (*Taeniopygia guttata*) (3 mg/kg) and king quails (*Coturnix chinensis*) (26 mg/kg). Aerobic metabolism was measured during a 1 h exposure to sub-freezing thermal conditions in low dose house sparrows and king quails (26 mg/kg). Fenitrothion had no effect on metabolic rate during cold exposure or on Hb at any time. By contrast, aerobic performance during exercise in sparrows was



reduced by 58% (high), by 18% (medium), and by 20% (low) 2 d post dose. House sparrows (high) had the longest recovery period for PMR (21 d) and plasma cholinesterase (ChE) activity (14 d). House sparrows (high) and treated king quails had significantly lower body weight at 48 h post-dose whereas body mass was invariant in zebra finches and house sparrows (medium and low). Cholinesterase was maximally inhibited at 6 h post dose, and had recovered within 24 h, in house sparrows (low), king quails and zebra finches. Exercise PMR in zebra finches and king quails was reduced by 23% at 2 and 3 d post-dose, respectively, despite these birds being asymptomatic in both behaviour and plasma ChE activities.

## Chapter 5

We examined the sublethal effects of the fipronil based pesticide Adonis 3UL ® insecticide on birds by measuring exercise induced peak metabolic rate (PMR) in zebra finch (*Taeniopygia guttata*) and king quail (*Coturnix chinensis*), and during a 1-h exposure to sub-freezing conditions in king quail. Exercise induced peak metabolic rate was measured in zebra finch pre-dose and at one, two, ten and twenty days after treated birds ingested 17.5 mg/kg Adonis 3UL ® mixed with canola oil and control birds received canola oil alone. Peak metabolic rate measurements were taken during exercise pre-dose and two, six and fourteen days after king quail received 30 mg/kg Adonis 3UL ® or canola oil alone. Peak metabolic rate after was not affected by fipronil in Adonis 3UL ® or by sham treatment in birds of both species. We conclude that the administered sublethal dose of fipronil did not affect exercise performance in zebra finch or in king quail nor was there evidence of fipronil induced thermoregulatory effects in king quails.

## ACKNOWLEDGEMENTS

There are a number of people who I would like to acknowledge for their assistance and support throughout this project. First my supervisors, Professor Bill Buttemer and Professor Lee Astheimer at the University of Wollongong, as well as my PhD committee member, Associate Professor Mike Hooper at the Institute of Environmental Health at Texas Tech University. Their advice on various aspects of this study and comments on earlier drafts of this thesis were invaluable. I would like to thank the Australian Plague Locust Commission for supporting this project, and acknowledge their staff member Paul Story, without whose commitment to environmental management, this project would not have happened.

It is with much appreciation and gratitude that I thank my work colleagues, Megan Jones, Wendy Russell, Harry Battam, Malsha Kitulagodage and Judit Szabo for their friendship and encouragement as well as for their field, technical and intellectual support.

I would also like to thank Dave Fildes, Joan Powell, Jodie Molinia, Mathew and Jenny O'Donnell as well as Susan and Darrel Saberton. All were members of the 'PhD support team' that carried me most of the way. Their faith, generosity, patience and child-care was much appreciated and without their help I would never have made it to the finish line. Finally, thank you to Dave, Dylan and Eliana Fildes for helping me to keep this in perspective.

## TABLE OF CONTENTS

1	Introduction .....	1
1.1	Pesticides .....	1
1.2	Pesticide effects on birds .....	6
1.2.1	Avian mortality as an endpoint in toxicological research .....	7
1.3	Considerations of sublethal effects in toxicological research .....	12
1.4	Locust control, pesticide use and implications for native birds in Australia	18
2	PLASMA CHOLINESTERASE CHARACTERISTICS IN NATIVE AUSTRALIAN BIRDS: implications FOR MONITORING AVIAN SPECIES FOR PESTICIDE EXPOSURE in avifauna .....	40
2.1	Introduction .....	41
2.2	Materials and Methods .....	44
2.3	Results .....	49
2.4	Discussion .....	53
3	CHOLINESTERASE RESPONSE IN NATIVE BIRDS EXPOSED TO FENITROTHION DURING LOCUST CONTROL OPERATIONS IN EASTERN AUSTRALIA .....	64
3.1	Introduction .....	65
3.2	Materials and Methods .....	67
3.2.1	Pesticide application .....	67
3.2.2	ChE analysis .....	69
3.2.3	Plasma ChE characterisation .....	70
3.2.4	Reactivation analysis .....	70
3.2.5	Summary of statistical tests .....	71
3.3	Results .....	72
3.3.1	Plasma ChE characterization .....	72
3.3.2	Cholinesterase activity levels - inhibition, reactivation, and diagnostic thresholds .....	73
3.4	Discussion .....	78
3.4.1	ChE inhibition and reactivation .....	78
3.4.2	Significance of exposure .....	80

4	THE EFFECT OF ACUTE FENITROTHION EXPOSURE ON A VARIETY OF PHYSIOLOGICAL INDICES, INCLUDING AVIAN AEROBIC METABOLISM DURING EXERCISE AND COLD EXPOSURE .....	89
4. 1	Introduction .....	90
4. 2	Materials and Methods .....	91
4.2.1	Experimental animals .....	91
4.2.2	Pesticide administration .....	92
4.2.3	Metabolism during cold exposure .....	93
4.2.4	Metabolism during exercise .....	95
4.2.5	Blood haemoglobin concentration and body mass .....	96
4.2.6	ChE analysis .....	97
4.2.7	Summary of statistical tests .....	98
4. 3	Results .....	98
4.3.1	Dosing toxicity .....	98
4.3.2	Metabolism during cold exposure .....	99
4.3.3	Metabolism during exercise .....	100
4.3.4	Blood haemoglobin and body mass .....	104
4.3.5	Cholinesterase activity .....	105
4. 4	Discussion .....	106
4.4.1	Metabolism during cold exposure .....	106
4.4.2	Blood haemoglobin and body mass .....	107
4.4.3	Metabolism during exercise .....	108
5	THE EFFECT OF AN ACUTE SUBLETHAL EXPosURE TO FIPRONIL-BASED ADONIS 3UL® INSECTICIDE (BASF) ON AVIAN AEROBIC METABOLISM DURING FLIGHT AND COLD EXPOSURE .....	116
5. 1	Introduction .....	117
5. 2	Materials and Methods .....	119
5.2.1	Experimental animals .....	119
5.2.2	Experimental protocols .....	120
5.2.3	Pesticide administration .....	120
5.2.4	Metabolism during exercise .....	120
5.2.5	Metabolism during cold exposure .....	121
5.2.6	Blood haemoglobin concentration and body mass .....	122
5.2.7	Summary of statistical tests .....	123

5. 3	Results .....	123
5.3.1	Blood haemoglobin and body mass .....	124
5. 4	Discussion .....	128
5.4.1	Metabolism during cold exposure.....	128
5.4.2	Metabolism during exercise .....	129
5. 5	Conclusion .....	132
6	SUMMARY OF MAJOR FINDINGS AND DIRECTIONS FOR FUTURE RESEARCH.....	138

## LIST OF FIGURES

Figure 2-1	Mean plasma cholinesterase activity at four different times of day ( $\pm$ standard error) in (A) budgerigars (number of individuals = 10) and (B) zebra finches (number of individuals = 58; Time A = 6 am; Time B = 10 am; Time C = 2 pm; Time D = 6 pm). There were no significant differences in any enzyme activity at any time point.....	52
Figure 2-2	Seasonal changes in mean plasma cholinesterase activity in zebra finches ( $\pm$ standard error; number of individuals: summer = 58, winter = 34). All plasma ChE activities in winter were significantly lower than summer.....	53
Figure 4-1	Mean maximum metabolic rate (MMR) and integrated (Int.) oxygen consumption ( $\text{VO}_2$ ) as a percentage of pre-dose levels during cold exposure after dosing with 30 mg/kg fenitrothion in house sparrows and 26 mg/kg fenitrothion in king quails. ( $\pm$ 1 standard error; number of individuals sampled = eight treated and eight control birds for both species) .....	99
Figure 4-2	Mean peak metabolic rate as a percentage of pre-dose levels during exercise in house sparrows before and after dosing with fenitrothion in pesticide-exposed and unexposed birds. ( $\pm$ 1 standard error; number of individuals sampled = eight treated and eight control birds, except where specified as ( <i>n</i> ) beside data-points). .....	101
Figure 4-3	Mean percentage change in peak metabolic rate during exercise in zebra finches before and after dosing with fenitrothion in exposed and unexposed birds ( $\pm$ 1 standard error; <i>n</i> = number of individuals). .....	103
Figure 4-4	Mean percentage change in peak metabolic rate during exercise in king quails before and after dosing with fenitrothion in exposed and unexposed birds. ( $\pm$ 1 standard error; number individuals sampled = eight treated and eight control birds) .....	104
Figure 5-1	Mean percentage change ( $\pm$ standard error) in maximum metabolic rate (MMR) during cold exposure after king quail received 30 mg/kg fipronil (number of treated birds = 7; number of control birds = 8) .....	125
Figure 5-2	Mean percentage change ( $\pm$ standard error) in integrated oxygen consumption ( $\text{VO}_2$ ) during cold exposure after king quail received 30 mg/kg fipronil (number of treated birds = 7; number of control birds = 8) .....	126

- Figure 5-3 Mean percentage change ( $\pm$  standard error) in peak metabolic rate (PMR) during exercise after zebra finch received 17.5 mg/kg fipronil (number of treated birds = 7; number of control birds = 8)..... 127
- Figure 5-4 Mean percentage change ( $\pm$  standard error) in peak metabolic rate (PMR) during exercise after king quail received 30 mg/kg fipronil (number of treated birds = 7; number of control birds = 7)..... 128

## LIST OF TABLES

Table.1-1	Organophosphate and carbamate (anticholinesterase) pesticides used for agricultural purposes that have resulted in mass mortalities of bird species worldwide ( <i>n</i> = number of individuals found dead) .....	11
Table 1-2	Studies on captive birds demonstrating the effects of anti-cholinesterase pesticides on behaviour .....	17
Table 2-1	Plasma cholinesterase activity from selected Australian avian species (AChE = acetylcholinesterase, BChE = butyrylcholinesterase, SD = standard deviation, <i>n</i> = sample size, ND = not detected) .....	50
Table 3-1	Plasma cholinesterase activity in species caught pre- and post-fenitrothion application (SD = standard deviation, <i>n</i> = sample size, DT = diagnostic threshold, * = significantly lower post-spray ChE activity than pre-spray samples).....	76
Table 3-2	Summary of species caught with breeding and/or moulting status and reactivating ChE.....	77
Table 3-3	Frequency and magnitude of plasma ChE reactivation in avian species sampled during the first 5 days post-fenitrothion application.....	77
Table 4-1	Mean plasma cholinesterase activity in birds exposed and unexposed to fenitrothion (* = significantly lower activity compared to unexposed samples)	