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A Survey of Pesticide Accumulation in a Specialist Feeder, the Koala (*Phascolarctos cinereus*)

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Abstract To maintain profitability in Australia's agricultural and urban landscapes pesticides are used throughout the range of koala habitats. The koala is a specialist feeder, reliant on metabolic enzyme capacities to utilise a toxic diet of eucalypt leaves and is potentially prone to adverse effects when xenobiotic interactions between dietary and anthropogenic xenobiotics occur. The aim of this study was to investigate accumulation of frequently used pesticides in wild koalas in 4 areas of New South Wales and Queensland. Liver samples of 57 deceased koalas were collected from care facilities and analysed using a modified QuEChERS extraction method followed by GCMSMS, HRLCMS and LCMSMS. No accumulation of any of the 166 investigated pesticides was found. Data indicate hepatic accumulation of pesticides in this species is uncommon even with close interactions with intensive land use. Despite the lack of hepatic bioaccumulation, this study cannot exclude a direct effect on hepatocellular metabolic pathways.

Keywords Pesticide · Survey · Koala · Cytochrome P450 · Xenobiotic metabolism

Exposure to pesticides in Australian wildlife has been investigated most extensively in birds and to a lesser extent in mammals. In the 1990s in the Sydney region of New South Wales (NSW), the crop contents of 90% of birds tested (11 species of raptors, insectivores, granivores and nectivores) were positive for DDE, DDT, diuron, endosulfan and parathion-methyl (Sánchez-Bayo et al. 1999). Residues of organochlorine pesticides were found in fatty tissue of birds, mammals, reptiles, and fish species in the Northern Territory (Best 1973); and DDT, DDE and Dieldrin were found in liver, visceral fat and eggs of Australian bird species in South-Australia (Falkenberg et al. 1994) and the tissues of birds in the North Coast region of New South Wales (McDougall et al. 1989). Years after the ban in 1987, DDT and DDE was found in the liver, brain and visceral fat of southern bent-wing bats (Falkenberg et al. 1994; Allinson et al. 2006) and in liver and body fat of Australian freshwater crocodiles (Yoshikane et al. 2006). Organochlorines and organophosphates were found in platypus (Munday et al. 2002; Science and Waterhouse 1972), in ringtail and brushtail possums (Bolton and Ahokas 1997; Science and Waterhouse 1972), and macropod species (NRS 1989–1997; Best 1973; Science and Waterhouse 1972).

Very few studies have measured the impacts of pesticides on marsupial species. The insectivorous fat-tailed and stripe-faced dunnarts were reported to be 10–14 times more susceptible to Fenitrothion (an important locust control pesticide) compared to mice (Story et al. 2011) and the herbivorous brushtail possum was found to be 20 times more susceptible to Malathion (Eason et al. 1994).

The *n*-octanol/water partition coefficient (KOW) is an indicator for lipophilicity and tendency of chemicals to bio-accumulate in organic tissue (Mackay 1982). The lipophilic nature of many pesticides makes fatty tissues a standard sample to investigate. With a fat content of 3%–20% (Nakata et al. 1998; Adiels et al. 2006; Fry 1937; Hammon et al. 2009) liver was used in a range of species (Nakata et al. 1998; Tomza-Marciniak et al. 2014; Walker et al. 1967) and for most pesticide classes it is the most or second preferred sample (Berny 2007). Koalas have no free body

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fat, even when in good body condition, necessitating the use of liver.

The second half of the twentieth century saw an 836% increase in use of agricultural and horticultural pesticides in Australia (Radcliffe 2002) to 35,400 tonnes in the last available statistic (FAOStat 2006). The absence of a publicly available record system of specific pesticide usage (Radcliff 2002) renders assessment of potential impacts on wildlife difficult.

Across its large range in eastern Australia, from the tropical north to the southernmost part of the mainland, koala habitat is now predominantly in close proximity to agricultural, horticultural and urban land use (Martin and Handasyde 1999). Farmland makes up 61% of the Australian landmass (NFF 2014) and much koala habitat overlies this land-use.

Fragmentation of habitat may further increase potential for exposure due to edge-effects. Airborne particles transported horizontally through the landscape are shown to get trapped by forest edges (Weathers et al. 1992, 1995) and deposition of pollutants was found highest in those areas (Hasselrot and Grennfelt 1987; Weathers et al. 2000; Beier and Gundersen 1989; Draaijers et al. 1988; Lindberg and Owens 1992).

With increasing trophic level, biomagnification can occur (Connolly and Pedersen 1988), therefore species at high trophic levels are considered most vulnerable to accumulation of toxins. However, the koala, despite being a folivore, is of potential interest. The koala's exclusive food source of eucalypt leaves is of low nutritional value and potentially highly toxic due to plant secondary metabolites (PSM), including terpenoids and phenolics (Eberhard 1972). Exposure to dietary PSMs has driven the evolution of increased activity of metabolizing enzymes in koalas (Jones 2000; Liapis et al. 2000; McLean and Foley 1997). Several cytochrome P450 isoforms were identified including Cyp 2C47/48 (Jones et al. 2008), Cyp 3A78 (El-Merhibi et al. 2011), and CYP 4A15 (Ngo et al. 2006). The affinity of koala microsomes to tolbutamide, a cytochrome 2C8/2C9 inhibitor, was found 12 times higher than in possums and inhibitory actions of 1.8-cineole towards tolbutamide hydroxylation indicated the importance of those specific cytochrome P450 subsets in terpenoid metabolism (Liapis et al. 2000). Enhanced metabolic capacity was thought to be the major reason for rapid clearing in vitro and in vivo of eucalypt terpenoids (Pass et al. 2001) as well as drugs (Pass and Brown 1990; Pass et al. 2001; Kimble et al. 2013, 2014; Black et al. 2014a, b). The cytochrome P450 enzymes are also critical to convert a wide range of other environmental xenobiotics (Lewis 2001), and the majority of pesticides impact cytochrome P450 enzyme activities significantly in other species (Abass and Pelkonen 2013; Badawi et al. 2000; Buratti et al. 2002, 2007; Mehmood et al. 1996; Reidy et al. 1987; Schulz-Jander and Casida 2002). If competitive or non-competitive Cytochrome P450 inhibition due to pesticides were present in the koala, dietary intake and survival could be significantly affected as high exposure levels to eucalypt PSMs were found to decrease or even cease food intake in eucalypt folivores (Boyle et al. 2005; Marsh et al. 2006; McLean et al. 2007).

This study screened liver samples from 57 koalas from four different regions of New South Wales and South-East Queensland (SE Qld) across a range of habitats, for the presence of pesticides.

Materials and Methods

Liver tissue was taken between January and October 2015, during necropsy of 57 koalas that had died of natural causes or been euthanized for humane reasons (NSW National Parks Scientific Licence SL101290; University of Sydney Animal Ethics Approval 565). Specimens from 3 animals found in close proximity (1–5 km radius) were pooled (3 animals per pooled sample, 19 pooled samples in total). Animal sampling targeted koala habitats impacted by horticultural, urban and agricultural industries. Koala habitat impacted mainly by horticultural but also by agricultural pesticide use, was represented by the Lismore Shire, NSW (a landscape consisting mainly of orchards and nut farms, with 5 pooled samples, 15 animals). Koala habitat impacted by mainly agricultural and, to a lesser extent, horticultural industry was represented by Byron Shire (Montecollum, 1 pooled sample), Ballina Shire (Tintenbar, 1 pooled sample), Richmond Valley (Coraki, 1 pooled sample), Kyogle Shire (Kyogle, 1 pooled sample) and Sydney area (Richmond, 1 pooled sample), all in NSW. Urban land use was represented by pooled samples from Lismore City Council area (1 pooled sample), Port Macquarie City Council, NSW (1 pooled sample), South of Sydney area (Campbelltown and Southern Highlands, NSW, 1 pooled sample each), and Southeast Queensland (n = 5 pooled samples, 15 animals). In Southeast Queensland, a substantial disruption of koala habitat and population decline have been documented (Adams-Hosking et al. 2016). The current study sought to sample from this region of population decline to try to assess the potential role of pesticide exposure in this process.

Liver tissue was transported and stored at −20 °C until analysis, within 8 months. Pooled liver samples of 150 g total (3 × 50 g) were analysed for the specified pesticides (Table 1) by Symbio laboratories (NATA accreditation number 2455, Eight Mile Plains, Qld). Samples were extracted using a modified QuEChERS method and analysed by GC/MSMS, HRLCMS and LCMSMS (Fillion

Table 1 Information on screened pesticides in liver samples of koalas (n = 57, pooled samples = 19)

Group of pesticide	Name of pesticide	Detected amount in µg/kg
Organophosphates	Acephate, Azinphos methyl, Chlorpyrifos, Chlorpyrifos methyl, Chlorfenvinphos, Diazinon, Dichlorvos, Dimethoate, Ethoprophos, Fenamiphos, Fenitrothion, Fenthion, Fenthion oxon, Fenthion oxon sulphone, Fenthion oxon sulphoxide, Malathion, Methamidophos, Methidathion, Mevinphos, Monocrotophos, Omethoate, Parathion ethyl, Parathion methyl, Phorate, Phosmet, Pirimiphos methyl, Profenofos, Prothiofos, Terbufos	<LOD
Herbicides	Chlorthal dimethyl, Linuron, Metribuzin, Oxyfluorfen, Pendimethalin	<LOD
Carbamates	Carbaryl, Pirimicarb	<LOD
Phenols	*o*-Phenylphenol	<LOD
Acaricides	Bifenazate, Bifenazate diazene, Chlorfenapyr, Chlofentezine, Etoxazole, Propargite, Tebufenpyrad, Tetradifon	<LOD
Synthetic Pyrethroids	Bifenthrin, Bioresmethrin, Cyfluthrin,, Cyfluthrin-beta, Cyhalothrin, Cyhalothrin-lambda, Cypermethrin, Cypermethrin-alpha, Deltamethrin, Esfenvalerate, Fenvalerate, Fluvalinate, Fluvalinate-tau, Permethrin, Phenothrin, Pyrethrins	<LOD
Organochlorines	Aldrin, BHC (-alpha, -beta, -delta, -delta), DDD, DDE, DDT, *o,p*-Dicofol, *p,p*-Dicofol, Dieldrin, Endosulfan-alpha, Endosulfan-beta, Endosulfan-sulphate, Endrine, keto-Endrin, HCB, Heptachlor, Heptachlor Epoxide, Trichlorfon	<LOD
Fungicides	Benalaxyl, Bitertanol, Captan, Chlorthalonil, Cyproconazole, Cyprodinil, Dicloran, Difenoconazole, Dimethomorph, Diphenylamine, Fenarimol, Fenarimol, Fludioxonil, Flusilazole, Hexaconazole, Imazalil, Iprodione, Kresoxim-methyl, Metalaxyl, Myclobutanil, Paclobutrazol, Penconazole, Penthiopyrad, Piperonyl Butoxide, Prochloraz, Procymidone, Propiconazole, Pyrimethanil, Quintozene, Tebuconazole, Tolclofos methyl, Triadimefon, Vinclozolin	<LOD
Others	Abamectin, Buprofezin, Fenoxycarb, Fipronil, Fipronil sulphenyl, Fipronil sulfone, Fipronil trofluoromethyl, Hexythiazox, Indoxacarb, Pyriproxyfen, Sulfoxaflor, Azoxystrobin, Benomyl, Boscalid, Carbendazim, Chlorantraniliprole, Dithianon, Diuron, Fenhexamid, Fenpyroximate, Flubendiamide, Imidacloprid, Methomyl, Methomyl oxime, Pymetrozine, Pyraclostrobin, Spinetoram (-A&-B), Spinosad (-A&D), Spirotetramat, Tebufenozide, Thiabendazole, Thiacloprid, Thiamethoxam, Trifloxystrobin	<LOD

et al. 1995; Gilvydis and Walters 1990; Sheridan and Meola 1999).

Results and Discussion

For all samples analysed pesticide levels fell below the limit of detection (LOD <50 µg/kg tissue), Table 1.

The inability to detect pesticides in the livers of wild koalas across a fairly large area of its natural range is a surprising result given the close association of the koala with disturbed habitat and agricultural and horticultural industry across most of its natural range. Although pooling of specimens within each region may have diluted individual animal residue results, the detection limit (50 µg/kg) of the assays means that, at worst, a level of 150 µg/kg in one animal of the pooled sample may have not been detected. The levels of pesticides found in other mammals, birds, reptile and fish have been from 40 µg/kg to around 3 mg/kg of tissue in mammals (Allinson et al. 2006; Best 1973; Falkenberg et al. 1994). Liver tissue was chosen for this study due to the scarcity of fat tissue in this species, and liver being a suitable organ for pesticide screening (Berny 2007). For some pesticides in this study the logKOW is low and bio-accumulation is therefore considered less likely to occur, therefore the study may be limited to examining short term exposure rather than accumulation. Potential rapid detoxification in this species could also prevent detection of some pesticides.

This study is the first investigation into the exposure of koalas to pesticides. Data suggest that an chronic ongoing or acute exposure to pesticides is uncommon in this species. Even though the study is not sufficiently comprehensive to allow pesticide exposure to be completely disregarded, it suggests a more rational approach is to focus our investigations in this species towards exposure to ubiquitous natural plant secondary metabolites in eucalypt food tree species, potentially habitat quality and animal health.

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Compliance with Ethical Standards

Conflict of interest The authors certify that they have no conflict of interest.

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