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Analysis of sugarcane herbicides in marine turtle nesting areas and assessment of risk using *in vitro* toxicity assays

Hannah L. Allan [a], Jason P. van de Merwe [a, *], Kimberly A. Finlayson [a], Jake W. O'Brien [b], Jochen F. Mueller [b], Frederic D.L. Leusch [a]

[a] *Australian Rivers Institute & Griffith School of Environment, Griffith University, Parklands Drive, Gold Coast, QLD 4222, Australia*
[b] *Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, 39 Kessels Rd, Coopers Plains, QLD 4108, Australia*

HIGHLIGHTS

- Herbicides were detected in sand samples taken from turtle nest depths ranging from 60 to 100 cm.
- In sand, photosystem II inhibiting herbicides were detected in the highest concentrations.
- Turtle cell bioassays indicated a low risk of acute toxicity at concentrations detected on nesting beaches.

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ABSTRACT

Agricultural processes are associated with many different herbicides that can contaminate surrounding environments. In Queensland, Australia, herbicides applied to agricultural crops may pose a threat to valuable coastal habitats including nesting beaches for threatened loggerhead turtles (*Caretta caretta*). This study 1) measured concentrations of herbicides in the beach sand of Mon Repos, an important marine turtle nesting beach in Australia that is adjacent to significant sugarcane crops, and 2) investigated the toxicity of these herbicides to marine turtles using a cell-based assay. Samples of sand from turtle nest depth and water from surrounding agricultural drains and wetlands were collected during the wet season when herbicide runoff was expected to be the greatest and turtles were nesting. Samples were extracted using solid phase extraction and extracts were analysed using chemical analysis targeting herbicides, as well as bioanalytical techniques (IPAM-assay and loggerhead turtle skin cell cytotoxicity assay). Twenty herbicides were detected in areas between sugarcane crops and the nesting beach, seven of which were also detected in the sand extracts. Herbicides present in the nearby wetland were also detected in the beach sand, indicating potential contamination of the nesting beach via the river outlet as well as ground water. Although herbicides were detected in nesting sand, bioassays using loggerhead turtle skin cells indicated a low risk of acute toxicity at measured environmental concentrations. Further research should investigate potentially more subtle effects, such as endocrine disruption and mixture effects, to better assess the threat that herbicides pose to this population of marine turtles.

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1. Introduction

Pesticides used in agriculture have the potential to contaminate groundwater and surrounding environments. Once in the environment, there are many different mechanisms by which pesticides can impact non-target species, including behaviour alterations (Saglio and Trijasse, 1998), cytotoxic and genotoxic responses (Çavaş and Könen, 2007; de Campos Ventura et al., 2008), DNA mutations (de Campos Ventura et al., 2008), and endocrine disruption (Sumpter, 2005).

A wide range of herbicides have physical-chemical properties (including soil-sorption properties and relatively high water solubility) that result in high mobility and associated off-site transport (Nachimuthu et al., 2016; Stover and Hamill, 1994). This off-site transportation has been observed for herbicides applied to sugarcane crops, which resulted in contamination of groundwater and the coastal waters of the World Heritage listed Great Barrier Reef (Lewis et al., 2009; Southwick et al., 1995). Although runoff

* Corresponding author.
E-mail address: j.vandemerwe@griffith.edu.au (J.P. van de Merwe).

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Fig. 1. Water (W) and sand (S) sample locations. Markers of sand samples (with stars) represent two samples, one each at 60 cm and 1 *m* depth.

assessments of sugarcane crops indicate the loss of applied herbicide is usually less than 1% (but up to 18% after extreme rainfall), significant amounts of herbicides can leach into groundwater (Devlin et al., 2015; Southwick et al., 1995). In fact, concentrations of sugarcane herbicides in groundwater have been reported as high as 403 μg/L (Southwick et al., 1992). Given this potential for contamination, important ecosystems and habitats adjacent to sugarcane crops could be at risk.

In Queensland, Australia, approximately 360,000 ha of land is assigned to sugarcane production with crops heavily concentrated in coastal areas. Although the Great Barrier Reef has specific protection measures in place to help control herbicide input (Environmental Protection Act, 1994), other important coastal habitats may also be impacted by herbicide contamination. For example, marine turtles that nest along the Queensland coastline could be affected by herbicide use in these areas. Although it is recognized that herbicides may impact marine turtles indirectly by affecting their food sources (e.g. dieback of seagrass, a major food source for the herbivorous green turtle), the direct effects of herbicides accumulating in marine turtles are largely understudied (Devlin et al., 2015; Finlayson et al., 2016). This is primarily due to logistical and ethical issues associated with whole animal toxicity testing on marine turtles. Cell-based toxicity bioassays have recently been proposed as a suitable alternative in large, threatened species (Finlayson et al., 2016; Webb et al., 2014).

Of the few studies that have investigated the effects of herbicides in turtles, atrazine has been found to act as an endocrine disruptor in both freshwater (de Solla et al., 2006) and marine turtles (Keller and McClellan-Green, 2004). In other reptiles and amphibians, diuron and alachlor have been identified as endocrine disruptors in lizards (Bicho et al., 2013; Cardone et al., 2008), and atrazine was found to cause feminization in frogs (Hayes et al., 2009). Endocrine disruptors are particularly problematic for marine turtles during embryonic development as the natural sex ratio can skew towards the production of female (if estrogenic) or male (if androgenic) hatchlings (Merchant-Larios et al., 1997; Russart and Rhen, 2016). Pesticides have been found to readily penetrate turtle eggs, especially if they have high water solubility, low soil sorption, and low lipophilicity (de Solla and Martin, 2011). The accumulation of herbicides in beach sand may therefore pose a threat to marine turtle embryonic development. Overall, due to their potential to contaminate groundwater and reach surrounding environments, as well as their capacity to penetrate eggs, herbicides could pose a threat to incubating marine turtle eggs via nesting sand contamination.

The beach at the Mon Repos Regional Park (near Bundaberg, Queensland) is adjacent to extensive sugarcane crops, and serves as a major nesting area for the critically endangered South Pacific population of loggerhead (*Caretta caretta*) turtles (Limpus and Casale, 2015), as well as small numbers of flatback (*Natator depressus*) and green turtles (*Chelonia mydas*). Understanding the potential transfer of herbicides from the sugarcane crops to the turtle nesting beach and the subsequent effects on marine turtles is therefore important for the conservation and management of these threatened species.

This study investigated both the potential for exposure of nesting sea turtles at Mon Repos to herbicides commonly used on the sugarcane crop, and subsequent toxicological risk, using a sea turtle cell-based bioassay to assess the adverse effects of herbicide mixture exposures.

2. Materials and methods

2.1. Sample sites and sample collection

Sand and water samples were collected from within and adjacent to the Mon Repos Regional Park (24 °48′ S, 152 °26′ E), near Bundaberg, Queensland. Sampling was carried out over a three-day period in late February 2016, soon after the main herbicide application period (April–December) for sugar cane (Davis et al., 2013), and during the wet season when runoff was expected to be greatest and turtles were still nesting. Agricultural land, including pasture and a sugarcane crop covering ~55 ha, is located behind the beach. Water samples (W1– W7) were collected at seven sites from wetland and agricultural drainage areas (Fig. 1). Water sample sites were strategically selected to represent all water bodies near the beach, as well as upstream and downstream positions of agricultural drainage areas. At each of the seven sites, paired 1 L water samples were collected in amber glass bottles. Samples were immediately acidified to pH 2 with hydrochloric acid (12 M) and refrigerated for transport and storage. Sand samples (S1– S5) were collected from five sites along Mon Repos beach (Fig. 1). These sites were chosen to systematically cover the extent of turtle nesting areas on the beach. All sand samples were taken under permit guidelines set by the Department of Environment and Heritage Protection and the Department of National Parks, Sport and Racing. At each site, paired ~5 kg samples were taken - one from 60 cm to 1 *m* depth - the typical depth range of turtle nests. Samples were collected in pre-washed polypropylene containers and refrigerated

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