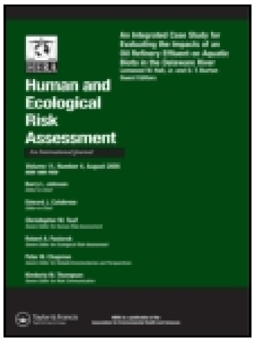

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Human and Ecological Risk Assessment: An International Journal

ISSN: 1080-7039 (Print) 1549-7860 (Online) Journal homepage: https://www.tandfonline.com/loi/bher20

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To cite this article: John Abbot & Jennifer Marohasy (2011) Has the Herbicide Diuron Caused Mangrove Dieback? A Re-Examination of the Evidence, Human and Ecological Risk Assessment: An International Journal, 17:5, 1077-1094, DOI: 10.1080/10807039.2011.605672

To link to this article: https://doi.org/10.1080/10807039.2011.605672

Human and Ecological Risk Assessment, 17: 1077–1094, 2011

ISSN: 1080-7039 print / 1549-7860 online
DOI: 10.1080/10807039.2011.605672

Has the Herbicide Diuron Caused Mangrove Dieback? A Re-Examination of the Evidence

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ABSTRACT

The claim that the herbicide Diuron in agricultural runoff caused dieback of the grey mangrove (*Avicennia marina*) in Central Queensland, Australia, has influenced government policies including programs to save the Great Barrier Reef. Several investigations on mangrove dieback in Central Queensland river estuaries have been published during the past decade. However, proof of a causal link between mangrove dieback and Diuron remains inconclusive. This study presents a systematic review of the evidence using Hill's Criteria of Causation. Our review shows that using concentrations of the chemical bound to sediment as a measure for biological availability in either glasshouse or field studies is inappropriate. The appropriate measure is Diuron concentration in solution and this parameter bears no simple relationship to concentration bound to sediment, and is not strongly correlated with mangrove health. Only when the herbicide is applied in experimental investigations at many orders of magnitude higher than measured in rivers has an impact on *A. marina* been demonstrated. Evidence from field studies suggests burial of pneumatophores, the plant's breathing roots, following flood events is a more likely causal factor in mangrove dieback, whereas any contribution from Diuron remains unproven.

Key Words: causation, cause, weight of evidence, Diuron, *Avicennia marina*, flooding.

INTRODUCTION

In June 2001 the World Wide Fund for Nature released the *Great Barrier Reef Pollution Report Card* claiming the beauty and biodiversity of the Great Barrier Reef was threatened by land-based pollution. This document and associated campaigning created political pressure to "save" the Great Barrier Reef. In response the Queensland government created the Reef Protection Taskforce. Based primarily on work of a botanist at the University of Queensland, Norman Duke (Duke *et al.* 2001; Shearer

Received 11 April 2010; revised manuscript accepted 16 July 2010.
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2004), the herbicide Diuron was identified by the taskforce as responsible for the death of mangroves within the Great Barrier Reef Marine Park, especially dieback of the grey mangrove *Avicennia marina* in the Mackay region of central Queensland (Marohasy and Johns 2003).

In 2002, the Australian Pesticides and Veterinary Medicines Authority began a reconsideration of approvals of the active constituent Diuron. At the time of writing this paper, 8 years after the review was initiated, the final report is still pending. The Queensland Government has introduced the *Great Barrier Reef Protection Amendment Act 2009* with the purpose of reducing the impact of agricultural activities on the quality of water entering the reef with new regulations, specifically targeting Diuron, and fines for noncompliance to be in place by October 2010.

Diuron is used in sugarcane production in catchments adjacent to the Great Barrier Reef (Hamilton and Haydon 1996) and has been detected in river and flood plume waters (Lewis *et al.* 2009). Schaffelke *et al.* (2005) claim strong correlative and causative evidence that herbicides used in sugarcane production, particularly Diuron, have seriously affected mangrove species in downstream estuarine habitats. Other researchers (Mitchell *et al.* 2005; Lewis *et al.* 2009) have assumed a causal relationship between Diuron and mangroves dieback, and have used this to support more general concerns about pesticides in coastal regions in Great Barrier Reef catchments and elsewhere.

The Pesticide Action Network has lobbied in the United Kingdom against the continued registration of Diuron on the basis of alleged impacts on the Great Barrier Reef (PAN-UK 2005). Diuron remains registered in the EU having passed the "harmonised EU safety assessment" in July 2008 (Europa Press Release 2009) and the U.S. Environmental Protection Agency approved continued registration in September 2003 (USEPA 2003).

The grey mangrove, *A. marina*, is common in estuarine areas, salt flats, and along the seaward margins in the east coast of Africa, southwest Asia, south and south-east Asia, and is the most widespread mangrove in Australia, growing as a multi-stemmed tree to 25 meters, but more usually 4 to 10 meters (Brock 1988). A distinguishing feature is the pencil-like aerial roots, pneumatophores, which protrude upwards from cable roots (Illustration 1). These assist with uptake of oxygen by the plant.

Dieback is a condition characterized by the yellowing and wilting of leaves, gradual dying of young shoots, with severely affected trees eventually losing all their foliage (Duke *et al.* 2001).

The herbicide Diuron (1,1-dimethyl, 3-(3′,4′-dichlorophenyl) urea) was developed in the United States and first produced commercially by du Pont de Nemours Co. in 1966. It is now used as a broad-spectrum residual herbicide and algaecide in many countries for pre- and post-emergent control of weeds in agriculture, and for weed control around buildings, railway lines, roads, and as a component of marine antifouling paints.

APPLICATION OF HILL'S CRITERIA OF CAUSATION

The causes of an alleged environmental impact can be difficult to determine with certainty. In the often emotive and politically charged area of environmental science

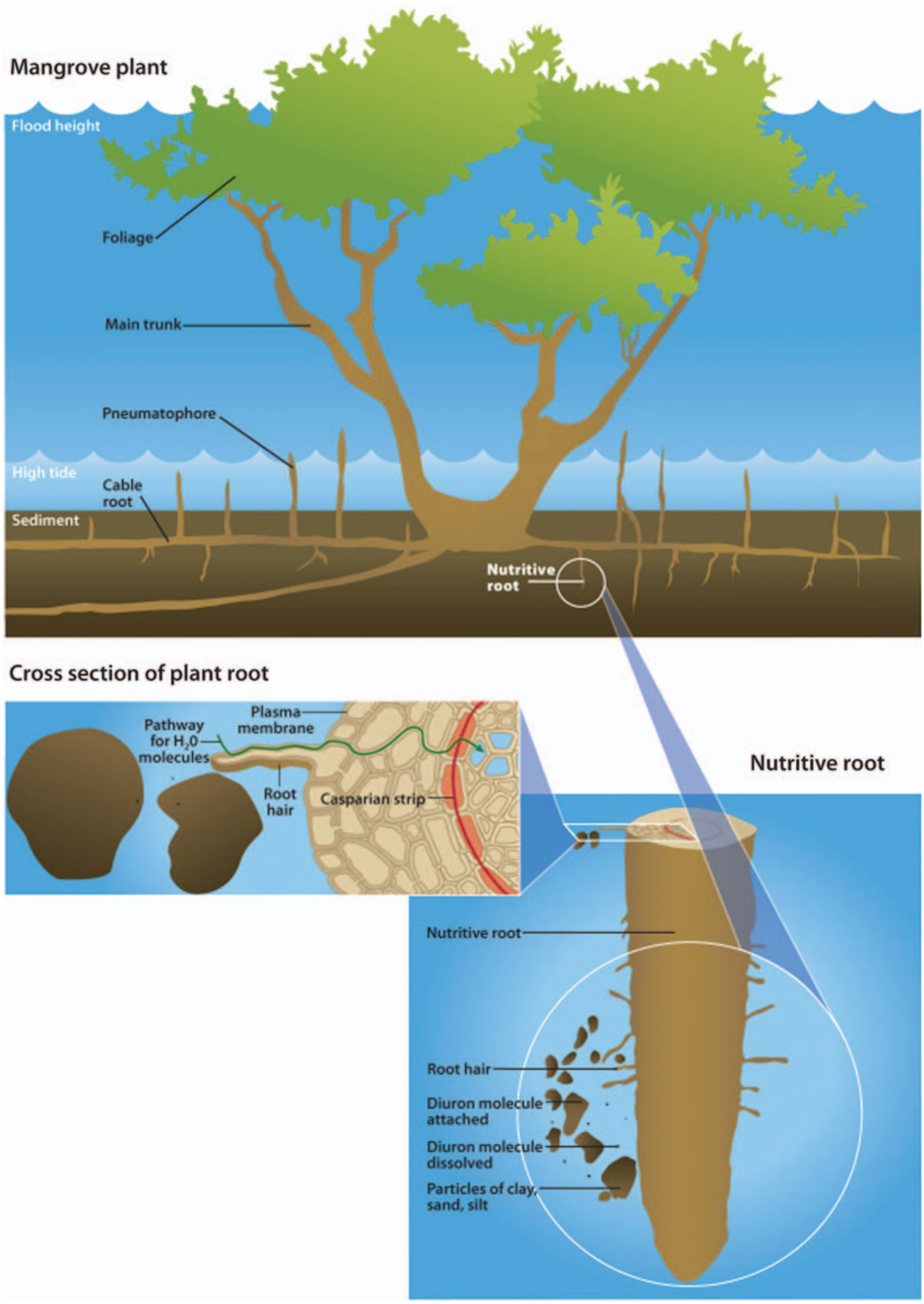

Illustration 1. The grey mangrove, *Avicennia marina*, grows in tidal areas and has pneumatophores, also known as breathing roots. Chemicals such as Diuron must be in solution for uptake by nutritive roots. Chemicals bound to sediment cannot cross cell membranes. (Color figure available online).

traditional approaches to environmental impacts can too often be purely descriptive in nature (Peters *et al.* 1997), lack supporting hypothesis-driven experimental research (Havens and Aumen 2000), and fail to differentiate between correlation and causation (Havens 1999).

British medical statistician Austin Hill (1965) outlined nine criteria that can be applied to determine the nature of an association: strength; consistency; specificity; temporality; biological gradient; plausibility; coherence; experiment, and analogy. These criteria now form the basis of modern epidemiological research recognizing they represent logical categories of evidence that can be used to organize information to evaluate a specific hypothesized-cause between two variables (Thygesen *et al.* 2005; Suter *et al*. 2010). More recently a subset of the criteria has been used to test causality between environmental stressors and effects in aquatic ecosystems (Adams 2003).

The Great Barrier Reef is one of the seven wonders of the natural world and for the last 50 years there has been growing concern about human impact with sometimes spectacular claims of its imminent demise from coral-eating starfish, oil pollution, overfishing, fertilizer runoff, pesticides, sewerage, anchor damage, and more recently ship groundings and global warming (Starck 2005).

The Great Barrier Reef Marine Park is an officially defined region that extends to the shoreline of parts of Queensland including areas of mangrove where Duke *et al.* (2001) claimed the worst mangrove dieback of its kind in the world. It was thus reported in advice to government and the popular press that Diuron was threatening the Great Barrier Reef (Marohasy and Johns 2003).

In this article we use Hill's nine original criteria to test the claim that Diuron, and in particular, Diuron from sugarcane cultivation caused mangrove dieback.

Plausibility

Diuron is a urea herbicide that inhibits electron transport in PSII of the photosynthetic pathway of plants (Percival and Baker 1991); there is therefore a reasonable biological basis for the association between Diuron and mangrove dieback.

Temporality

Flooding is common in eastern Australia following periods of heavy rainfall. Extensive mangrove dieback has followed severe flood events in the Pioneer and Fitzroy Rivers of Central Queensland. This is consistent with claims that Diuron may be a causal factor as concentrations of herbicides have been detected in flood waters (Lewis *et al*. 2009).

According to Kirkwood and Dowling (2002), from the Queensland Environmental Protection Agency, rainfall in the Pioneer Catchment in August 1998 was between four and eight times higher than the average rainfall for this area during August. Most of the rain fell over a 3-day period and stream heights during this period were the highest for 1998, with no other floods occurring that year.

An examination of aerial photographs of the Mackay region by Dowling and Kirkwood (2002) indicated that mangrove dieback was not visible in 1996, commenced sometime after June 1998, and was clearly visible from November 1999 with only *A. marina* affected. These observations are broadly consistent with those of Norman

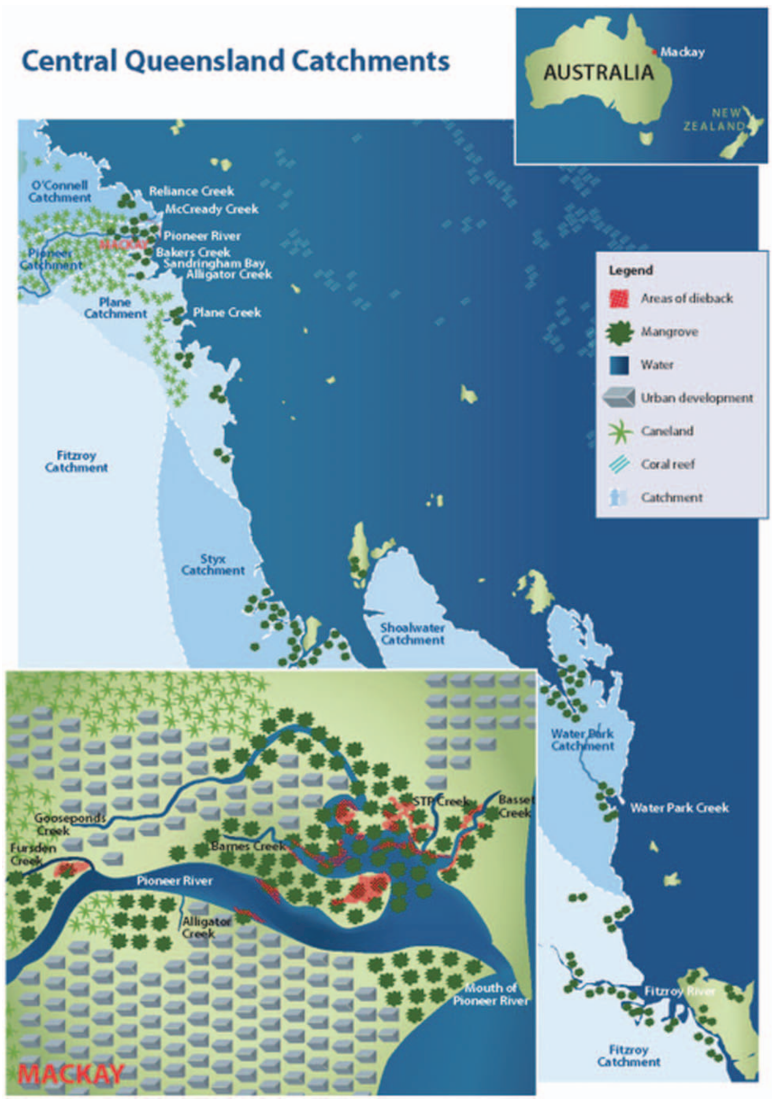

Illustration 2. Dieback of the grey mangrove, *Avicennia marina*, has been reported in the O'Connell, Pioneer, Plane, and Fitzroy Catchments of Central Queensland and considered most severe in the Bassett Basin at the mouth of the Pioneer River in the city of Mackay. Areas marked with dieback redrawn from Kirkwood and Dowling (2002). (Color figure available online).

Duke and coworkers (2001), from the University of Queensland, who surveyed a region extending from Reliance Creek in the O'Connell Catchment south to Alligator Creek in the Plane Catchment (Illustration 2). Both Kirkwood and Dowling (2002) and Duke *et al.* (2001) concluded the Bassett Basin, in the Pioneer catchment, was the area worst affected by dieback. This area is adjacent to the central business district of the city of Mackay and is located near the mouth of the Pioneer River.

In mid-January and early February 2008 a major flood event occurred in the Fitzroy River situated 350 kms south of the Pioneer River (Dowling 2008). Over a 4-week period, two peak flood heights of 7.5 m were recorded. During this period, the Fitzroy River did not drop in height below the flood level of 6 m.

Widespread mangrove dieback in the Fitzroy estuary was reported in the Rockhampton Morning Bulletin in September 2008 (Chapman 2008), and subsequently confirmed by Dowling during fields investigations for the Queensland Environmental Protection Agency (Dowling 2008).

Biological Gradient

Norman Duke and coworkers (2003; 2005) undertook research to establish a biological gradient, also known as a dose-response curve, for Diuron and the incidence of dieback in *A. marina*, based on the assumption that the concentration of Diuron bound to sediment, also known as sorbed Diuron, constitutes a measure of herbicide availability. Uptake of chemicals, including Diuron, by plants may occur either from soil solution through the roots or from the atmosphere or solution through aerial plant parts (Singh *et al.* 2002). Few studies compare these modes of uptake for the same species (Simpson *et al.* 2005). While accepting aerial plant parts would be exposed to Diuron in solution during flood events, following Duke we focus on uptake through the root system.

Water, nutrients, and chemicals are taken up through the nutritive roots of *A. marina* and must pass through at least one cell membrane before being able to access the vascular tissues of the plant (Trapp and McFarlane 1995; Lawton *et al.* 1981; Illustration 1). Diuron molecules can penetrate membranes. However, Diuron sorbed on the surfaces of sediment particles must first desorb into aqueous phase before crossing membrane barriers.

There is a significant technical literature dating from the 1950s explaining that once herbicides like Diuron become bound to sediment they are no longer available for uptake by plants. However, Duke and coworkers (2003; 2005), ignoring this literature, incorrectly used Diuron bound to sediment as a measure of herbicide availability. The questionable conclusions that followed have been widely and uncritically reported, including in the technical literature, but are based on a misunderstanding of how herbicides interact with soil, sediment, water, and plant roots.

When there is no soil, for example, under hydroponic conditions, uptake of non-ionic molecules, for example Diuron, from solution by the roots is controlled by two main factors: the concentration of the herbicide in solution and the rate of uptake of water by the roots (Trapp and McFarlane 1995; Sheets 1972; Walker and Featherstone 1973; Su *et al.* 2007).). This concentration dependence has been reported for Diuron by barley (Moyer *et al.* 1972), and for other non-ionic herbicides including uptake of Simazine by oats and cotton (Sheets 1961). It has also been reported for uptake of Atrazine by wheat (Walker 1972); uptake of Atrazine and Linuron by carrot, parsnip, lettuce, and turnip seedlings (Walker and Featherstone 1973), and for uptake of Atrazine by rice (Su *et al.* 2007).

The addition of solid to a solution will reduce the concentration of herbicide, for example Diuron, through sorption onto solid surfaces (Walker 1972). Different types of solids in soils and sediments will have very different sorption characteristics. Soils and sediments usually contain organic matter. And there is a strong correlation with the amount of organic matter and sorption of herbicides (Grover 1966; Wang and Keller 2009; Walker 1972) including Diuron (Harris and Sheets 1965; Gilchrist *et al.* 1993).

The distribution coefficient or soil-water partitioning coefficient (K_d) is the ratio of a chemical's sorbed concentration (mg/kg) to the dissolved concentration (mg/L) at equilibrium. K_d values ranging between 8.8 and 21.3 (L kg-1) have been reported for Diuron and K_d increases by approximately threefold as the soil carbon content (a measure of organic matter content) increased from approximately 1% to 6% (Ahangar *et al.* 2008a,b). This relationship between K_d and carbon content for Diuron is consistent with the findings of Hance (1965), Grover (1975), and Liyanage *et al.* (2006).

Not only the amount, but also the chemical composition, of organic matter influences the sorption properties, commonly described by K_{OC}, the C-normalized partition coefficient (Chiou 1989). K_{OC} values for an herbicide vary considerably between soils (Wauchope *et al.* 2002), indicating that sorption of non-ionic compounds cannot always be explained by simple partitioning between soil solution and a homogeneous organic phase. K_{OC} cannot be considered as a universal constant, but rather a measure of the affinity of the organic matter in a particular soil for the herbicide. For Diuron, K_{OC} values between 452 and 707(L kg^{-1}) have been reported (Ahangar *et al.* 2008b).

Nuclear Magnetic Resonance studies confirm that the chemistry as well as the amount of organic matter influences the sorption affinity of Diuron (Ahangar *et al.* 2008a) with aromatic carbon having the highest affinity for Diuron, consistent with previous studies (Ahmad *et al.* 2001; Xing 1997).

Healthier plants are associated with higher concentrations of the herbicide sorbed on the soil or sediment, as this reduces the concentration available for uptake (Doherty and Warren 1969; Harris and Sheets 1965; Rahman 1976). Uptake of non-ionic herbicides, for example, Diuron (Upchurch 1958; Upchurch and Mason 1962; Upchurch *et al.* 1966; Harris and Sheets 1965), is therefore, in general, inversely related to the concentration of herbicide sorbed on the soil.

Considering dieback in the Mackay area, Duke and coworkers report the organic content of mangrove sediments varied between 4% and 23% depending on the locality (Table 1) (Duke *et al.* 2003). Extrapolating from Angahar *et al.* (2008a) this six-fold variation in organic content can be calculated to correspond to a three-fold variation in K_d.

No characterizations of the organic matter associated with mangrove sediments were reported by Duke *et al.* (2003), so it is not possible to calculate K_{OC} values for the sites (Reddy *et al.* 1992; Oliver *et al.* 2005).

Components of a soil or sediment additional to organic matter content also influence sorption. For example, the mineral component of a soil or sediment will have a significant impact (Talbert and Fletchall 1965; Huang *et al.* 1984; Simone *et al.* 2006), with studies showing Diuron much more strongly sorbed to clay particles than sand (Wang and Keller 2009). Inoue *et al.* (2006) showed that Diuron sorption

Table 1. Percentage organic matter and Diuron concentrations in core water at Pioneer River sites, from Duke *et al.* (2003), (NR = not reported, ND = not detected).

Site location	% Organic matter	Diuron (μg/L $\times$ 10^3)
McCreadys Creek—MCS1	8	7.57
McCreadys Creek—MCM1	10	NR
Barnes Creek—M1	8	6.49
Barnes Creek—BH1	15	14.11
Barnes Creek—M2	4	9.14
Barnes Creek—BS1	7	12.92
Barnes Creek—BM1	4	NR
Bakers Creek—BCH1	23	ND
Bakers Creek—BCS2	22	8.98
Bakers Creek—BCM1	23	3.89
Bakers Creek—BCS1	6	8.23
Fursden Creek	NR	9.24

correlated with both the organic carbon and clay content. Angahar *et al.* (2008b) evaluated the effect of organic matter–mineral interactions for Diuron–soil sorption by comparing K_{OC} for demineralized and whole soils, finding a substantial depression of K_{OC} (by a factor of 2.5) due to the presence of minerals.

Different particle size distributions will also give rise to different surface areas per unit mass, and sorption phenomena are dependent on available surface area, rather than total mass of solid sediment. Diuron exhibits highly soil particle-size dependent sorption behavior due to the organic carbon associated with the different size fractions (Wang and Keller 2009). Particle size distributions were highly variable between sites in the Mackay area, with the median grain size in the root zone varying between 44 μm at Barnes Creek to 742 μm at STP Creek (Wake 2005).

In addition to the sorption characteristics of the solid matter itself, the water content of the sediment or soil has a significant impact on the uptake of non-ionic molecules by plants (Grover 1966; Walker 1971). The ratio water-to-solids for a soil or sediment will directly influence the herbicide concentration in solution at equilibrium with sorbed herbicide. However, none of the field studies in Queensland reported ratios of water-to-solids (Duke *et al*. 2003; 2005; Wake 2005; 2006).

Strength

If Diuron caused mangrove dieback in river estuaries adjacent to the Great Barrier Reef then it would be expected that a larger portion of plants would be affected in the areas with higher concentrations of Diuron in solution.

Core water samples taken in the relevant field studies were collected by taking samples from water that seeped into a hole created in the sediment after a core was removed to a defined depth. This procedure does not correspond to any of the four methods reviewed by Bufflap and Allen (1995) for extraction of core water. However,

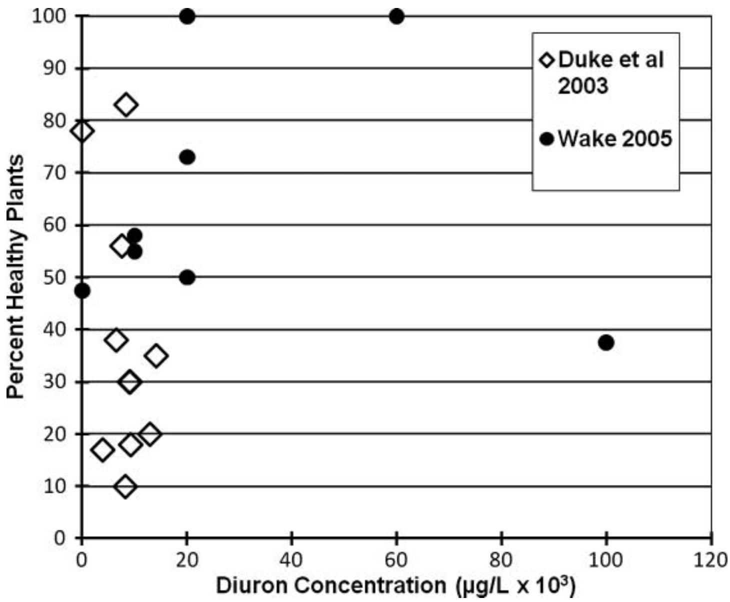

Figure 1. Percentage of healthy mangroves plotted against concentration of Diuron in root zone core water. Data from Duke *et al.* (2003) ($r = -0.401$) and Wake (2005) ($r = 0.025$).

these samples are all that are available to represent concentrations of Diuron in the aqueous phase.

Duke *et al.* (2003) report measured concentration ranges of 0.004 to 0.014 μg/L and a corresponding wide variation in mangrove health status with the percentage of healthy trees varying between 10% and 85% (Table 1 and Figure 1). We tested the correlation between the values reported by Duke *et al.* (2003) and the status of mangrove health (represented by percentage healthy trees) and found it was not statistically significant (Pearson correlation coefficient, $r = -0.401$). Wake (2005) reports higher concentrations of Diuron and 100% healthy trees at 60 μg/L Diuron (Figure 1).

Hill (1965) cautioned, however, not to dismiss a cause and effect hypothesis just because the observed association appears to be slight. He used, as an example, that relatively few persons occupationally exposed to rat's urine contract Weill's disease.

Consistency

There are 36 major river catchments along the east coast of Queensland and sugarcane is grown in 24 of these (Table 2). Eleven catchments within this area are routinely monitored for pesticide residue (Hunter *et al.* 2003). Estimated Diuron usage has only been calculated for catchments with sugarcane (Hamilton and Haydon 1996, 1997).

Table 2. Area of mangrove, sugarcane, and amounts of Diuron used in Queensland's east coast catchments (from Duke *et al.* 2001).

East Coast Catchments, Queensland	Mangrove Area (km^2)	Cane Crop (km^2)	Diuron Applied (kg/pa)	Diuron/ Mangrove (kg/km^2)
Baffle	94	13	234	2
Barron	12	40	835	68
Black	7	4	100	13
Boyne	15	0	n/a	2
Brisbane	7	0	n/a	0
Burdekin	49	230	3272	67
Burnett	13	174	3445	271
Burrum	11	198	3303	308
Caboolture	5	0	n/a	0
Calliope	8	0	n/a	0
Daintree	32	32	2378	75
Don	52	34	489	9
Fitzroy	200	0	n/a	0
Haughton	103	281	4123	40
Herbert	155	464	16618	107
Johnstone	15	252	17353	1123
Kolan	6	94	1761	299
Logan-Albert	19	47	766	41
Maroochy	3	60	9828	2823
Mary	27	80	9614	356
Mossman	10	46	3278	326
Mulgrave/Russell	45	206	4702	103
Murray	54	44	1252	23
Noosa	38	6	938	25
O'Connell	101	229	23896	236
Pine	14	0	n/a	0
Pioneer	8	219	23435	3084
Plane	108	448	52079	481
Proserpine	78	139	9281	119
Pumicestone	25	0	n/a	0
Ross	27	0	n/a	0
Shoalwater	119	0	n/a	0
South Coast	65	0	n/a	0
Styx	61	0	n/a	0
Tully	22	104	2768	125
Water Park	209	0	n/a	0

Over the last two decades mangrove dieback has been reported in the Fitzroy, O'Connell, Plane, and Pioneer Catchments. Sugarcane is not grown in the Fitzroy Catchment (Table 2). Mangrove dieback is thus not consistently associated with sugarcane production.

Working in the Pioneer Catchment, Judith Wake (2005; 2006) from Central Queensland University, developed a stress index for mangrove health status

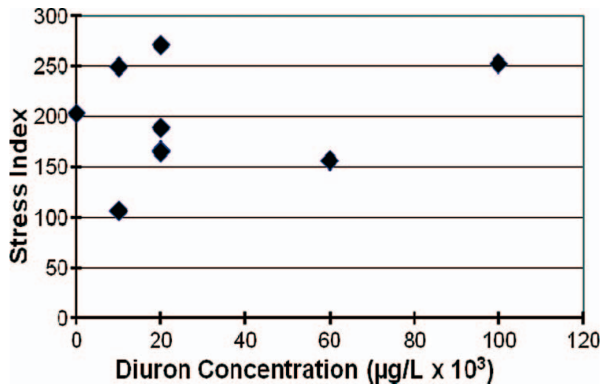

Figure 2. Stress index plotted against concentration of Diuron in core water. Data from Wake (2005), $r = 0.237$. (Color figure available online).

representing a composite of five factors including leaf colour, canopy density, and the proportion of the tree that was dead or alive. Each factor was expressed as a percentage and the composite stress index expressed as a sum of these values ranging between 0 and 500. Correlating this index with Diuron concentration, Wake (2005) found no consistency of association: Diuron concentrations varied from 0 to more than 0.10 μg/L but the stress index fell anywhere in a range between 100 and 260 (Figure 2).

Specificity

In both the Pioneer and Fitzroy catchments *A. marina* occurs as the dominant species with a mix of other mangroves including *Rhizophora stylosa* and *Ceriops Australia.* If Diuron was the cause of the mangrove dieback all species of mangrove would expect to be impacted. However, *A. marina* was the only species affected by dieback in these catchments (Duke *et al.* 2001, Kirkwood and Dowling 2002). Duke *et al.* (2001) also reported dieback of *C. australis* at Sandringham Bay south of Mackay in the Plane catchment, but did not investigate its cause and considered it a different kind of dieback.

Only mangroves of the genus *Avicennia* have pneumatophores, which are peg-like aerial roots containing a spongy material allowing the roots to breathe at low tide and to absorb oxygen for use by the submerged root system at high tides. Smothering of the pneumatophores for prolonged periods by silt, sediment, or water inhibits oxygen supply to the plant, and has been associated with mangrove dieback in Saudi Arabia, Singapore, Florida, Trinidad, French Guiana, and Australia (Ellison 1998).

Kirkwood and Dowling (2002) reported an absence or unusually low occurrence, of *A. marina* pneumatophores protruding above the substrate in the dieback-affected areas of Bassett Basin. When mud and silt were washed away from around the base of the dead mangroves, it was evident the pneumatophores had been buried by sediment (Kirkwood and Dowling 2002).

In the Fitzroy Catchment dieback occurred immediately adjacent to the main channel of the river with a clear line corresponding to flood height.

That *A. marina* is the only species with pneumatophores and the predominant species affected by dieback indicates smothering by water and/or sediment associated with flooding is a more likely cause of the dieback than poisoning by the herbicide Diuron.

Coherence

In order for an association to have what Hill called coherence, the cause and effect interpretation of the data should not be seriously in conflict with generally known facts. The claim that Diuron has caused dieback of mangroves is flawed in two key areas: (1) Evidence for a biological gradient is based on consideration of the wrong parameter (Abbot and McKillup 2010); that is levels of sorbed Diuron on sediment rather than concentrations in solution; and (2) Other potential causes of dieback, in particular burial and prolonged immersion of mangrove pneumatophores is more plausible (Dowling 2008).

Experiment

Experimental evidence can be used to test a causation hypothesis. Duke and coworkers undertook glasshouse experiments on potted *A. marina* seedlings (Duke *et al.* 2003; 2005) to determine whether high concentrations of Diuron would kill seedlings. The high concentrations were justified on the basis it was necessary to ensure a rapid response as part of their preliminary review of relative impacts. However, obviously great caution should be exercised in attributing causation when experimental concentrations are many orders of magnitude higher than field observations. Furthermore, while seedlings were specifically excluded in the field observations of dieback (Duke *et al.* 2003), all the experimental work was undertaken on seedlings and seedlings have a different physiology from mature plants (Xiao *et al.* 2009).

Potted seedlings of *A. marina* were treated with a range of initial dosages of Diuron, reported as 4000, 400, 40, and 4 μg/kg dw (dry weight) based on the total mass of solid sediment in each pot (1.7 kg dw). These dosages corresponded to total amounts of Diuron initially applied to the surface of sediments in individual pots, and do not reflect actual concentrations at any time during the experiments.

Only at the highest initial dosage of Diuron was any effect on the seedlings observed, with plants displaying chlorosis and necrosis along the mid-veins of the leaves. To achieve this effect, a solution containing 6800 μg Diuron in 0.6 L aqueous solution was prepared. This represents a Diuron concentration of 113,333 μg/L, which is approximately 10,000,000 times higher than measured concentrations at field sites, where the typical concentration of Diuron in core water samples was only 0.10 μg/L (Duke 2008).

Maximum concentrations of Diuron in flood waters in surface water of the Pioneer River have been recorded in the range 1–5 μg/L for short periods during flood events (Lewis *et al.* 2009). Mangrove roots are buried in the sediment and the transfer rates of herbicide from river surface waters to sediment layer are not known.

Concentration levels in the uppermost layer of the potted sediment would have declined over time and with diffusion into lower levels of the sediment and the associated aqueous phase, while also leaching into supernatant water. However, uptake of herbicides from solution by plant roots can occur very rapidly—within minutes of exposure (Su *et al.* 2007) and is highly dependent on herbicide concentration in the liquid phase (Trapp and McFarlane 1995; Su *et al.* 2007).

In order to "simulate natural run-off conditions," some clay (100 μg) was mixed with the prepared Diuron solution (Duke *et al.* 2003; 2005). It is not clear why this particular ratio of Diuron to clay was chosen. Addition of clay will reduce the concentration of Diuron in solution through surface sorption, but the resulting change in solution concentration was not reported.

The amount of Diuron sorbed onto the clay can be calculated using a reported value of a sorption coefficient (K_d) for Diuron on a soil with high (55.9%) clay content (Baskaran and Kenney 1999). Using a value of $K_d = 4$, the amount of sorbed Diuron on 100 μg clay would be 500 μg, or about 7% of the initially dissolved Diuron. In the glasshouse experiments, the maximum concentration of Diuron in the solution applied to the top layer of the sediment was therefore approximately 100,000 μg/L. This highly concentrated solution of Diuron was applied to the sediment surface by syringe, resulting in an "extremely patchy" herbicide distribution.

The experimental design reported by Bell and Duke (2005) provided for simulation of high and low tide water conditions by adjustment of the water level covering the surfaces of the sediments, with saline water pumped from a holding tank for one hour periods, twice daily. However, the circulated solution was common to the entire set of pots with differing initial Diuron dosages, creating a major confounding factor. As noted by the Australian Pesticides & Veterinary Medicines Authority (2005) the same simulated tidal water would have contained a composite of leached Diuron from all the samples.

The only reported experimental concentration of Diuron in solution, 13.73 μg/L, was measured at day 21 as a composite from all the samples. It is unclear what significance, if any, this measurement represents. This concentration exceed the highest levels found in surface waters during flood events, where high levels are typically maintained only for a few hours, then decline rapidly (Simpson 2002).

Bell and Duke (2005) acknowledged that the experimental concentration levels of Diuron were higher than encountered in field studies, the uncertainties in relating results for seedlings to impacts on mature trees, and also the need for appropriate long-term investigations. Taking these factors into account, it is impossible to justify their conclusion that: "Given the state of *A. marina* mangroves in the Mackay region, coupled with the presence of Diuron in the mangrove sediments, the results of this [glasshouse] investigation were consistent with the observation that herbicides were a major factor contributing to dieback in the Mackay region." (p. 305).

Analogy

There is widespread community concern about the potential impact of pesticides in environmentally sensitive areas. Duke *et al.* corroborate this concern by providing a value for the amount of Diuron applied to mangroves expressed as the amount of Diuron applied in a particular catchment divided by the area of mangrove in that catchment (Table 2). The relatively high application rates calculated were then reported for the Pioneer and Plane catchments compared to much lower application rates for the Burdekin, Herbert, and Tully catchments and reference made to the catchments with higher rates also having mangrove dieback. However, the analogy falters when also considering the high application rates for the Noosa and Mary catchments, where dieback was not reported (Table 2).

But given Diuron was not applied directly to the mangroves there is no justification for this analogy as only a fraction of the applied herbicide will be transported to the vicinity of the mangroves and numbers of mangroves will not affect concentration levels (Simpson 2002). To illustrate the inapplicability of the application rate concept, consider a situation where mangroves are growing on opposite sides of a river carrying a fixed concentration of Diuron. If all the mangroves growing on one side of the river were removed, this would not change the concentration of the herbicide affecting the remaining mangroves.

CONCLUSIONS

Government policies and funding allocations directed toward environmental protection should be based on sound science including the testing of hypotheses, the consideration of alternative causal factors, and an awareness of the relevant scientific literature. Suter *et al.* (2010), while acknowledging that no one method for identifying causation is completely reliable, stress the importance of being consistent, logical, and skeptical.

Dieback in the Bassett Basin of the Pioneer River estuary in Central Queensland, Australia, was reported to be the worst of its kind in the world and associated with the presence of Diuron in sediment (Duke *et al.* 2001; 2003; 2005). Reporting of the claim was extrapolated to justify additional regulations on the basis that Diuron from agricultural runoff has harmed the Great Barrier Reef (Marohasy and Johns 2003; PAN-UK 2005; Queensland Government 2009).

Kirkwood and Dowling (2002) and Dowling (2008) tested the claimed association between mangrove dieback and Diuron concluding that burial and/or smothering of breathing roots unique to *A. marina* was a more likely cause of mangrove dieback in the Pioneer and also the Fitzroy estuaries. In this study we have used Hill's Criteria of Causation, a framework developed in medical epidemiology, to test the causal association and conclude that burial and/or smothering is a more plausible explanation.

A key flaw in the evidence provided against Diuron by Duke and coworkers (2001; 2003; 2005) is their focus on correlations between concentrations of Diuron sorbed on sediment surfaces and mangrove health. Such correlations are not relevant to establishing a causal linkage because this parameter does not represent the availability of Diuron to the plant. It is clear from the technical literature that concentration of herbicides in the root zone solution is the key parameter.

Our analysis of Diuron concentrations in core water show no statistically significant correlation between Diuron and mangrove health (Pearson correlation coefficient $r = -0.401$). Furthermore the reported glasshouse experiments are of questionable value because seedlings rather than mature plants were used; a biological impact could only be demonstrated when Diuron was applied at concentrations millions of times greater than measured in the field; and the experimental design incorporated circulation of saline water common to tanks containing the entire range of initial dosages of Diuron.

Limitations described in the experimental studies are currently being addressed through investigations with mature *A. marina* mangrove plants at Central Queensland University. With the cooperation of Fisheries Queensland these experiments aim to establish the sensitivity of *A. marina* to Diuron, rates of transfer of Diuron from surface water to sediment and also the effects of foliar Diuron uptake.

Given the widespread belief that the herbicide Diuron has caused mangrove dieback, the pending Australian Pesticides and Veterinary Medicines Authority inquiry, and continuing campaigning against the use of Diuron, our re-examination of the evidence is timely and suggests flooding a much more likely cause of mangrove dieback in Central Queensland catchments than Diuron. This study also supports the increasing number of calls for an overhaul of how environmental impacts are assessed and advocates use of the Hill's Criteria of Causation to avoid popular but premature conclusions.

ACKNOWLEDGMENT

This work was supported by the B. Macfie Family Foundation.

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