Australian Government

Australian Pesticides and Veterinary Medicines Authority

DIURON REVIEW

Volume 3 of 4

Supplemental Environmental Assessment Report

Additional Data

This Report was prepared for the APVMA by

Department of Sustainability, Environment, Water, Population and Communities

9 July 2012

ISBN 978-1-922188-02-1 (electronic)

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Environment Protection Branch

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Additional Data - Diuron

V3.1 Introduction

The evaluation relates to additional data provided as part of submissions, or obtained independently, following the publication or the previous diuron environmental assessment (APVMA, 2011). Consequently, not all aspects of environmental fate or toxicity are being assessed in this report. Where additional data or arguments were not received, the outcomes of APVMA (2011) remain.

The additional data considered have been rated using our data rating system as follows:

1	**Fully reliable:**	GLP compliant and fully compliant with the Test Guideline specified.
2:	**Reliable with restrictions:**	GLP compliant but not fully compliant with the Test Guideline specified, but nevertheless judged to provide a reliable basis for regulatory decision-making. An asterisk is to be added to identify studies that are not standard that are judged to be reliable for the purpose conducted (*e.g.* mechanistic studies)
3	**Not reliable:**	Not GLP compliant and/or not compliant with the Test Guideline specified, and judged to not provide a reliable basis for regulatory decision-making.
4	**Not assignable:**	Insufficient information provided to allow the reliability of the test or study report to be assessed (*e.g.* published literature).

It should be noted, these ratings are derived from the OECD. Australia does not have mandatory GLP and consequently some allowances need to be made in addressing the validity of a study. For example, non-GLP studies cannot be considered unreliable on these grounds alone. Therefore, a degree of expert judgement has been used in applying the validity rankings associated with studies assessed.

V3.2 Additional Environmental Fate Data

V3.2.1 Mobility

Many new papers were provided, or have been obtained, addressing the issue of diuron mobility. Where these results were provided in submissions, they were done so primarily as an argument for increasing the Kd of diuron used in the runoff assessment, and to demonstrate the increased sorption of diuron with increasing time after application.

It is our view that the Kd data referred to in the following publications, unless specifically part of an adsorption experiment, are actually measuring a desorption Kd. This is reflected in some papers by referring to the measure of sorption as the "Kp" rather than the Kd, Kp being the adsorption in runoff, which is likely higher than Kd due to being associated with desorption and greater water-solid ratios and longer times of soil pesticide contact (Silburn and Kennedy, 2007).

Title	Lessons for achieving effective management from field research on agrochemicals
Authors	Simpson
Date	2007
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in this assessment.

Studies on field persistence and mobility of the pesticides (including diuron), sites were established with devices to quantify off-site losses via runoff, leaching or preferential flow pathways. The study described in this paper considers fate characteristics such as soil half-lives, Kd and concentrations in run-off. Further, an assessment of increase in Kd over time is made.

The main study sites were established in subtropical (approx. 1000 mm/year) Bundaberg area ($S25^{0}06'$, $E152^{0}18'$) of SE-Queensland, the dry tropics (approx. 780 mm/year) area of Atherton Tableland ($S17^{0}06'$, $E145^{0}16'$) in North Queensland and in the wet tropical (>4000 mm/year) Valetta area ($S20^{0}16'$, $E57^{0}33'$) of Mauritius. Both of the study areas in Queensland were supplemented by irrigation whereas the Mauritian sites were non-irrigated, similar to many of the wet tropical sugar production areas of Queensland.

Details were kept on all farming operations as well as irrigation and rainfall events. Most hydrological data collection was automated and logged so that useful time-series data were collected. Such data were useful when interpreting runoff from rainfall events and for calculating export loads of sediments and pesticides.

Surface and sub-surface soil samples (0-2.5, 2.5-5, 5-10, 10-20, 20-30 and 30-50 cm) were taken immediately before spray applications and for regular intervals following each application to determine dissipation rates (DT50). It should be noted that the field soils were subject to the normal farming practices of irrigation, cultivation and rainfall etc., with the soil profile subjected to some disturbance. Despite this, the data collected were considered statistically sound and provided 'real life' scenario information, rather than 'controlled' laboratory of glasshouse-generated data. First order kinetics were used to determine DT50 and associated risk periods.

Soil water partition coefficients (Kd) were also determined for a number of the key pesticides on different of soil types at various time intervals after pesticide application.

DT50*:* Unfortunately, no data on residue levels over time was provided. The paper reports for this study, diuron showed considerable variation in field half-lives, ranging from 6.5 days to >250 days. The mean DT50 in the 0-2.5 cm layer (excluding the site with the longest half-life) was 12 days.

The site with the longest half-life was a red ferrosol soil in SE Queensland. On this site, diuron had not been previously used, and pre-application soil sampling showed no diuron residues present. Sampling of the soil (surface and sub-surface) from immediately after application showed that there was some initial dissipation and downward movement (to 30 cm), but afterwards, the remaining diuron was highly persistent (DT50 >200 days) and 'immobile'. Such a 'two-phase' dissipation suggested that after the initial period of 'higher mobility', diuron was then adsorbed to, and protected within the soil matrix.

Kd: Soil/water partition coefficients (Kd) were determined on the four key soil types in the study area. All soils had typically low organic carbon, generally less than 1% in the surface layer and lower at depth. The findings indicated considerable variability in (Kd), with reduced pesticide adsorption at depth. It should be noted that these data were generated using a 30 minute soil/water equilibration (20 g/L), two days after pesticide application. The application rate is not reported.

To measure the effect of field ageing of pesticide residues on soil adsorption, further laboratory studies were undertaken, with Kd values being determined at intervals over a 0-56 days. Data show considerable increase in soil adsorption following pesticide application, until a period of stability (in adsorption) is reached.

Apart from information provided above, there are no details of these studies. The rate of application is not provided and there is no detail on the soil characteristics (% clay, % OC, CEC etc), although % OC can be calculated from the reported Koc values. The author was contacted to obtain further information. While the raw data were unable to be provided, advice was that the laboratory methodology involved treating the soil with pesticide and then storing for a range of days after treatment. At selected days the soil was shaken in water and the pesticide measured in both the water and soil phase. Kd values were then calculated. Many experiments were previously conducted on different soil water ratios to ensure that the procedure was meaningful and reproducible. GC/MS and HPLC/MS were used depending on the pesticide. Field treated soils (from known normal agricultural practices) was also examined and sampled at various times after application. It is our view that this process is measuring the desorption Kd, which will be different (and expected to be higher) that the adsorption Kd determined in the available regulatory studies and used in the runoff modelling.

The following results were obtained for diuron in the four selected sugarcane soils for the top 0-2.5 cm soil horizon:

Table V3.1: Desorption Kd and Calculated %OC, Simpson (2007).

Soil	Yellow chromosol	Grey chromosol	Red ferrosol	Redoxic hydrosol
Kd (desorption), L/kg	12.1	27.1	27.3	39.3
Calculated %OC	0.95	0.80	1.23	0.72

The ageing experiment was conducted on the redoxic hydrosol. The following results were obtained:

Table V3.2: Increase in Desorption Kd over Time After Applicaiton, Simpson (2007).

Days after application	0	3	7	42	56
Kd (desorption), L/kg	19	67.9	93.6	144	210
Increase from Day 0	-	3.57	4.93	7.58	11.1

Runoff: Different intensity events would produce higher mobilisation of sediment and thus produce different concentrations in runoff. Over the three 'wet' seasons on the Bundaberg site, the highest measured concentrations of diuron were 140, 66 and 11 µg/L at block (4 ha), farm (45 ha) and catchment-scale (790 ha) respectively. Whilst concentrations in the runoff were quite high at times, the annual amount of pesticides exported in runoff events from this site was less than 1% of applied. Unfortunately, no information on important aspects such as slope, application rate or rainfall intensity and time following application to the runoff event are available for any further analysis of these results. It is worth noting, however, that the levels of diuron are very high. The DSEWPaC model, even though it relies on the standard laboratory based guideline measure of Kd for input, would under predict these maximum levels. For example, with an application rate of 1800 g/ha, a slope of 5%, a rainfall event of 50 mm occurring 3 days after application and a Kd of 6.2 (1% OC), the DSEWPaC model would predict an edge of field concentration of 69.9 µg/L, which is around half the block level found in this field experiment.

In the dry-topics study area (Atherton Tableland, North Queensland) hydrology during the wet season is mainly surface driven. In this location, the highest concentrations of diuron in the stream draining the catchment were detected in the low-flow conditions late in the dry season when most of

the herbicides were applied (65%), but by far the highest pesticide export (92%) was during the wet season. The actual concentrations were not reported.

Conclusions from this study: The Kd results from this study, while confirming a trend of increased sorption over time, can't be used in the risk assessment. The Kd results from this study relate to a desorption Kd, not an adsorption Kd that is used in runoff modelling. The values derived here can't override the data available through accepted regulatory studies, the results of which show a good agreement with each other in terms of measured adsorption Kd and %OC. There are insufficient details in this test to allow a comparison between them and the regulatory studies already provided.

The risk assessment and associated modelling needs to be performed for a very wide range of climatic and geographic conditions. There is only limited data that can be used in such a model as it is not (and can't practicably be made to be) site specific. DSEWPaC agrees that sorption is likely to increase over time, but the modelling is undertaken to account for a runoff event 3 days after application. The model results in this case still under predicted maximum concentrations found in the study.

Title	Evidence of the role of climate control and reversible aging processes in the fate of diuron in a Mediterranean topsoil.
Authors	Saison et al
Date	2010
Test Guideline	None identified – field study
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in this assessment

The study aimed to characterise the change in distribution of diuron residues in different soil chemical fractions (labile, sorbed and non-extractable) with increasing residence time in the soil for up to 1 year, and *in situ* with varying environmental conditions. The work was undertaken on undisturbed soil micro-lysimeters that were exposed to natural climatic conditions for 1 year starting in the spring in the French Mediterranean region. ^{14}C-labelled diuron was applied at the equivalent of a field rate of 1.8 kg/ha.

The soils in the study were noted as having 1.02% OC with 27.1% sand, 54.4% silt and 18.5% clay. The ^{14}C-diuron was applied by a spiked solution prepared in dichloromethane and was dripped onto the soil surface with a micropipette such that an "application rate" of 1.8 kg ac/ha was used. Soil samples were taken at 0, 0.5, 1, 2, 3, 4, 5, 18, 31, 45, 61, 80, 105, 129, 159, 180, 221, 283, 347 and 376 days after application and runoff and infiltration waters (when present) were collected after each rainfall event.

Residues in water extractable, methanol only extractable and non-extractable fractions and in runoff waters were traced. Mass-balance calculations were fitted to the observed data to determine the quantities degraded or transferred within and between each of the compartments studied. Losses of diuron residues in runoff declined exponentially from 473 to 4 µg/L in 1 year.

Findings: Diuron residues were first measured in runoff waters 7 days after the beginning of the experiment, after a cumulative 74 mm rain in 1 week. The concentration in runoff was 74 µg/L. The maximum concentration of diuron residues recorded for the whole experiment was 473 µg/L, and this was found 20 days after the beginning of the experiment. During this event, 0.53% of the radioactivity initially applied was found in the water. After this time and throughout the rest of the experiment, diuron residue concentrations in the surface runoff water showed a general exponential decline with time.

The ageing processes were shown to be important with an increased sorption of diuron residues, but it was also found that the increase was not constant and depended on climatic conditions, especially

rainfall. After 80 days, sorption of diuron residues in the soil was significant, as was degradation in the water only and methanol only pools. Losses in runoff were small because of sparse rainfall. The following 140 days corresponded to a humid period with increased degradation in both water only and methanol only pools. A redistribution from the methanol only pool towards the water only pool occurred, probably because of intense rainfall at that time, leading to an apparent desorption of diuron residues. This was the most important result, suggesting that ageing processes should not be considered irreversible over time.

Conclusions with respect to this study

The results found here have been considered in the context of the DSEWPaC runoff model. Organic carbon of 1.02% indicates a Kd of 6.2 L/kg (Appendix 3, Volume 1). Runoff from the DSEWPaC model (Appendix 1, Volume 1) will be predicted based on application to loamy soils. Application was assumed to be to a dry soil based on information in the paper. No slope data are available in the report which makes it difficult to compare figures. However, the following results for concentration **in runoff water** (edge of field) for a range of slopes are obtained at 1.8 kg ac/ha, based on a 74 mm rain event (realising this value was for a week's rainfall, but the first runoff was measured after 7 days):

Table V3.3: DSEWPaC runoff model calculations based on input parameters from Saison et al (2010).

Slope (%)	Runoff %	Concentration (µg/L)
3	0.32	24.6
5	0.59	45.6
7	0.92	70.2

This indicates the DSEWPaC model works relatively well as a tool for predicting runoff given the closeness in runoff % and runoff water concentrations at 5 and 7% slopes. However, it is emphasised that this is based on runoff 1 week after application while the Kd is based on the regulatory studies and supporting information from Section 4.3.

Interestingly, in this study the highest runoff levels were found after 20 days. While levels found in runoff water are only presented graphically, it is shown that following the day 20 peak, residues declined exponentially over the course of the study, but it took until around 100 days before levels fell to those found after the first 7 days. Such a result makes it very difficult to develop and use a time dependency factor for increasing Kd in this assessment, particularly where such an argument must be able to be confidently applied over a wide range of climatic conditions.

Title	Aging Effects on the Availability of Herbicides to Runoff Transfer
Authors	Louchart and Voltz
Date	2007
Test Guideline	None identified – field study
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in this assessment

The study was undertaken to examine the temporal change of an effective partition coefficient (Kd^{eff}) for simazine, diuron and oryzalin from a 0.12 ha field experiment during 7 vineyard growing seasons. Over this period, herbicide concentrations in soil and runoff water were observed. The test site was in France. The soil properties (0-5 cm) included bulk density of 1.44 g/cm^3, pH 8.5, 18.6% clay and 1.06% OC. Diuron was applied over the entire soil surface at varying application rates and on different dates. Application was in either March or April and rates ranged from 0.49 kg/ha in 2002 to 1.59 kg/ha in 1999. Diuron applications occurred in 1995, 1997, 1998, 1999, 2002, 2003 and 2004.

The content of herbicide in the topsoil (0-2 cm) was regularly measured to study dissipation. Samples were taken weekly after application and then monthly. During all runoff events, the herbicide concentrations in runoff water at the outlet of the field were monitored. In all, 80 runoff events were observed with a number of water samples ranging from 3 to 47 according to the duration and intensity of the event.

The Kd^{eff} was calculated for the time of each runoff event as the ratio of solvent extractable herbicide in the topsoil (0-2 cm) to the average concentration in runoff water.

Findings: The dissipation exhibited two distinct phases. The first lasted 90-100 days and corresponded to an initial loss of at least 90% applied, noting only the top 2 cm soil was assessed, so no consideration of diuron at >2 cm soil depth is made. The second phase exhibited a significantly slower dissipation. Dissipation half-lives of diuron in the top 0-2 cm soil ranged from 15 days in 2004 to 49 days in 1995. The average of the 7 seasons where measurements were taken was 26 days.

The effective Kd (considered to represent a desorption Kd) increased rapidly (10 times within 1 month after the first runoff event) and steadily from application onwards. The minimum Kd^{eff} values were those calculated for the first runoff event. These happened between 9 and 60 days after application depending on the year of application, and the values ranged from 1.5 L/kg (30 days after application, 2002) to 10.7 L/kg (9 days after application, 2004). The average minimum Kd^{eff} was 5.85 L/kg. This is not dissimilar to standard adsorption Kd values obtained in laboratory batch equilibrium studies, and based on the soil 1.06% OC, a Kd of 6.5 L/kg would be calculated using the DSEWPaC regression equation. Given these runoff events occurred at up to 2 months after application, this indicates that ageing on soil will not necessarily result in increased sorption prior to the first runoff event with the mobile fraction of diuron remaining available.

Following initial and subsequent runoff events, the Kd^{eff} increased over time. The average maximum Kd^{eff} was 143.7 L/kg (an increase of over 24 times the minimum Kd), with the last runoff events in the years of application ranging from six months (179 days) to 304 days after application. This increase is to be expected as it is considered there will be less mobile fraction of diuron available for runoff with each subsequent event, and degradation processes will play an increasing role over time for removal of the chemical from the soil profile.

The study authors described the seasonal variation of Kd^{eff} between years by a model taking into account the cumulative rainfall since application.

Conclusions with respect to this study: The results are acceptable and demonstrate that with subsequent rainfall events, the available diuron for runoff will decrease. It is noted that there was little difference in the initial Kd based on soil topsoil concentrations and runoff water concentrations as would be expected from the standard laboratory batch equilibrium studies, but importantly, this value was found whether the first runoff event occurred around 1 week or 2 months after application. Based a single field or area of application, it would be expected, following rain events, to produce lower diuron concentrations in runoff water as the mobile fraction would be removed either in earlier runoff waters or downwards through the soil profile. The difficulty in applying this in the overall assessment is that on a wider spatial scale, different areas may receive applications at different times.

Title	Rainfall Effect on Dissipation and Movement of Diuron and Simazine in a Vineyard Soil
Authors	Alister and Kogan
Date	2010
Test Guideline	None identified – field study
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in this assessment

From 2003 to 2007, a field study was performed in a vineyard in Chile to investigate diuron and simazine soil behaviour and the effect of additional rainfall. Diuron was applied at a rate of 2 kg ac/ha to bare soil in an experimental area (1.4 ha) in August of each year using a backpack sprayer. The 0-15 cm soil layer had 20.79% clay, 1.28% OC, density of 1.56 g/cm^3 and pH of 6.1.

Soil samples were collected at 0, 10, 20, 40, 90 and 340 days after application (DAA) in the 0-15 and 15-30 cm soil layers. In the 30-60 cm and 60-90 cm layers, samples were taken at 90 and 340 DAA and at 90-120 cm samples were taken in 2005 and 2006. At sampling times, soil samples were extracted and analysed with HPLC with a diode array detector (DAD).

In addition, sorption and desorption coefficients were determined in batch equilibrium studies. In these, 6 mL aqueous 0.01 M $CaCl_2$ solutions at six concentrations were added to 3 g air dried soil. These were shaken for 8 h then centrifuged. 1 mL supernatant was filtered and quantified using HPLC-DAD. The amount of diuron adsorbed was calculated as the difference between the amount in the initial solution and that remaining in solution after centrifugation.

Findings: The adsorption Kd of diuron in the top 0-15 cm soil layer was 6.75 L/kg. This corresponds relatively well with a value that would be predicted of 7.75 L/kg based on the regression analysis used by DSEWPaC in the refined assessment.

Dissipation half-lives for diuron differed according to rainfall conditions. In all seasons, soil samples taken from irrigated plots showed reduced DT50 values, which cannot be explained only by the soil moisture content during this period, but because twice more diuron was mobilised below the 15 cm soil layer at 40 DAA under natural rainfall than irrigated in the three study seasons (data not shown). The following summary of results is provided:

Table V3.4: Summary of findings, Alister and Kogan (2010)

Year	Natural rainfall			Irrigated		
	DT50 (days)	*k* (/day)	r^2	DT50 (days)	*k* (/day)	r^2
2003	42.9	0.048	0.89	24.6	0.088	0.89
2004	35.8	0.054	0.82	25.8	0.075	0.85
2005	68.0	0.021	0.86	56.9	0.026	0.88
2006	69.2	0.021	0.89	46.4	0.031	0.90

Diuron soil residues, at the end of each study season, decreased each year from 2003 to 2007. After the first year of application the residue represented 28% of the initial amount compared to 16-18% after the second year and 5-7% after the third year, with no effect of rainfall conditions.

Diuron movement in soil was very similar during the 2004-05 and 2005-06 seasons. However, in the 2003-04 season, diuron was only detected in the first 30 cm soil. This limited movement could be explained considering the herbicide was applied for the first time in this soil in August 2003. In the other seasons, diuron had downward movement up to 90 cm regardless of the rainfall that occurred during the first 90 DAA. Movement was more pronounced in soils subject to natural rainfall, and the reason for this is not theorised. However, there is no information on times following application to either the first rainfall or irrigation event, and it may be the more mobile portion of diuron was removed through irrigation if this occurred shortly after application.

Conclusion from this study: The study does not provide any additional information that allows refinement of values used in the risk assessment. The field half-lives, which a little shorter than the 79 days currently used, still approached 70 days in two of the years tested. The adsorption Kd value obtained is in agreement with the Kd model used by DSEWPaC based on the %OC in the field soil.

Title	Environmental Behaviour of Metolachlor and Diuron in a Tropical Soil in the Central Region of Brazil
Authors	Dores et al
Date	2009
Test Guideline	None identified – field study
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment.

The environmental behaviour of metolachlor and diuron was studied in the Central-western region of Brazil, by means of a field study where six experimental plots (5 X 10 m) were installed. In each plot, one lysimeter, one run-off collector system and one monitoring well were installed. The soil was classified as a Latosol and the top soil horizon had 1.55% OC. The plots had slopes ranging from 3.6-4.7%.

Diuron was applied at a rate of 2000 g ac/ha. Field sampling started at the application date. Topsoil (0-10 cm) samples for field dissipation calculations were taken at 0, 1, 2, 4, 8, 16, 32, 64 and 128 DAA. Percolated water from lysimeters, runoff water and sediment were collected per event. Water samples from monitoring wells were collected monthly. For diuron analysis was performed by HPLC.

Findings: The dissipation half-life for diuron was calculated to be 15 days, and while soil was only sampled to 10 cm so may not account for movement down the soil profile, it is reported the first runoff event did not occur until 20 days after application, so downward movement over this period may not have been significant.

Diuron concentrations in water collected in the lysimeters varied from 0.02 to 6.29 µg/L in detected samples. The average mass of leached diuron in the lysimeters was 9.013 µg, representing 0.08% of the amount applied. In the water table, diuron was detected only once at 2.32 µg/L

In runoff water, diuron was transported in high quantities (average total of 193,800 µg during the experiment) with much higher concentrations measured at the beginning of the sampling period. The mass of diuron transported off-site dissolved in water averaged 3.9% of the applied quantity. The total mass of diuron associated with the runoff sediment was 488,900 µg representing 10% of the applied quantity.

Based on values read from a graph in the report, the concentration (considered edge of field) in the runoff water following the first runoff event was about 45 µg/L. A second runoff event three days later resulted in water concentrations approaching 80 µg/L. From this time though, concentrations in runoff water decreased exponentially for the remainder of the study.

Conclusions from this study: The field half-life under the tropical conditions of this study is shorter than the DT50 value of 79 days used in the refined assessment. Unfortunately, there is no information on the rainfall patterns leading to runoff (rainfall intensity, runoff volume) to allow a better comparison between the results and those that would be obtained using the DSEWPaC model for validation. However, based on the slope (3.6-4.7%, modelled using a slope of 4.2%), and using the application rate of 2000 g ac/ha, field half-life of 15 days and observing a rain event 20 days after application, DSEWPaC model outcomes can still be predicted. The %OC in the topsoil was reported as 1.55%, and DSEWPaC predicts a Kd of 9.26 L/kg (compared to the value of 14.7 L/kg measured in this study). Using a rainfall intensity of 10 mm/d and 25 mm/d, the DSEWPaC model

would predict edge of field concentrations of 89.5 µg/L and 35.8 µg/L respectively, which is in good agreement with the measured values.

V3.2.2 Australian Field Studies

Title	Paddock to Sub-catchment Scale Water Quality Monitoring of Sugarcane Management Practices: Interim Report 2009-2010 Wet Season. Mackay Whitsunday Region.
Authors	Rohde and Bush
Date	2011
Test Guideline	None identified – Field studies
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment.

Under the Paddock to Reef program, paddock scale monitoring of water quality from various levels of management practices was implemented in selected Great Barrier Reef catchments and agricultural industries. As part of this program, two sugar cane blocks in the Mackay Whitsunday region are being used to measure levels of herbicides, nutrients and sediments in runoff under different cane management strategies with the emphasis on improving water quality with improved management practices.

The Victoria Plains site (uniform cracking clay) was divided into two treatments of soil, nutrient and herbicide management practices. The Marian site (duplex soil) was divided into five treatments. Each treatment was instrumented to measure runoff and collect samples for water quality analyses (total suspended solids, total and filtered nutrients, and herbicides).

Victoria Plains site: The selected block had a slope of 1.1% and the soil had organic carbon of 2.56% (top 10 cm). The block was divided into two treatments of 30 rows (Treatment 1: 1.5 m row spacing) and 25 rows (Treatment 2: 1.8 m row spacing, controlled traffic). Row lengths across the entire block ranged from around 225-300 m. Application of diuron is noted as being a directed interspace application at 1872 g/ha.

Cane was planted on both treatments on 2 August 2009. Diuron and other herbicides were applied in mid-January 2010, but diuron was only applied to the treatment 1 paddock.

Marian site: The selected blocks had a slope of 0.4% and organic carbon of 1.35% (top 10 cm). The block was divided into five treatments of 18 rows each with an approximate row length of 260 m. Of the five different treatments, two assessed residual herbicides and consisted of either a 1.5 m row spacing, or a 1.8 m row spacing with controlled traffic. Diuron was applied as a directed interspace application at 1980 g/ha.

All treatments were planted on 15 August 2009. Diuron was applied in late October.

Multi-block and Multi-farm scale: In addition to the farm paddock scale sites, assessment was undertaken on a multi-block scale and multi-farm scale. At the multi-block scale, runoff was measured within a farm drain (catchment area approximately 53.5 ha) using a 1 in 40 flat vee crest weir, with depth of flow again being recorded by a pressure transducer at one minute intervals. It is unclear what percentage of this catchment would have received diuron treatment, or when this treatment occurred. As with the paddock sites, rainfall (amount and intensity) was measured using a Hydrological Services TB4 tipping bucket rain gauge.

At the multi-farm scale runoff was measured within a natural drain (catchment area approximately 2965 ha) using a 1 in 20 flat vee crest weir, with depth of flow again being recorded by a pressure

transducer at one minute intervals. Again, it is unclear what percentage of this catchment would have received diuron treatment, or when this treatment occurred.

No information is available on average slopes at either the multi-block or multi-farm scale.

Herbicide analysis: All water samples from all sites were analysed using liquid chromatography mass spectrometry.

Findings – Diuron in runoff waters:

At the Victoria Plains site, there were 15 runoff producing rain events. The data of diuron application is only stated as "mid-January", so the time between application and the first runoff event on 25 January is unclear, but would appear to be around 7 to 10 days after application. The following table indicates the runoff events at treatment site 1 with shaded cells indicating days of herbicide sampling:

Table V3.5: Summary of findings, Rohde and Bush (2011)

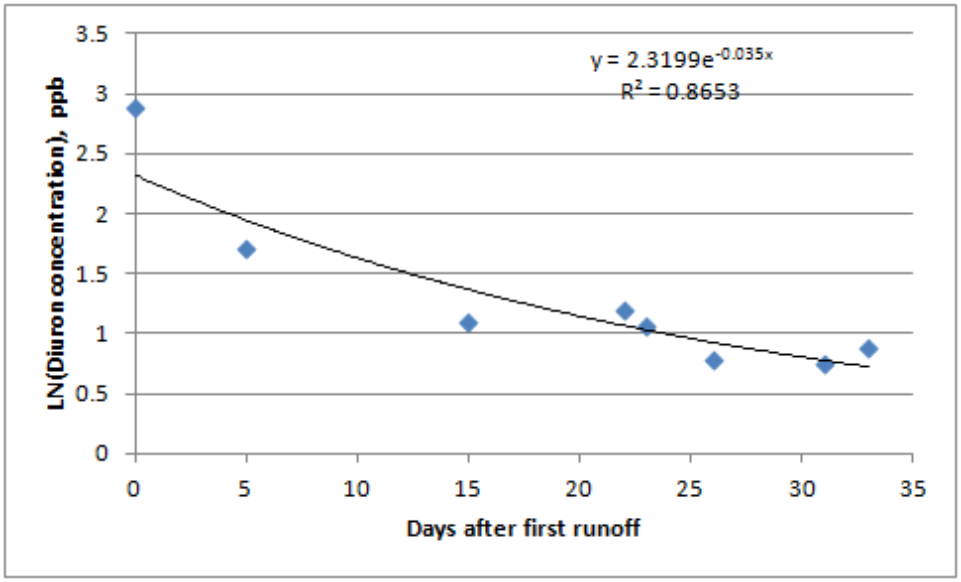
Event	**Start of Runoff**	Days after 1st runoff	**Rainfall Total (mm)**	**Treatment 1 Runoff (mm)**	Diuron conc. (µg/L)[1]
1	25/01/2010 0:00	0	152.4	77.0	18
2	26/01/2010 9:00	1	56.4	12.2	
3	30/01/2010 12:00	5	209.4	142.0	5.5
4	9/02/2010 16:00	15	69.6	21.8	3.0
5	10/02/2010 9:00	16	51.6	46.3	
6	16/02/2010 12:00	22	34.4	22.3	3.3
7	17/02/2010 0:00	23	97.4	79.6	2.9
8	18/02/2010 9:00	24	8.2	5.1	
9	20/02/2010 0:00	26	13.2	4.5	
10	20/02/2010 9:00	26	96.8	59.1	2.2
11	25/02/2010 0:00	31	97.0	55.8	2.1
12	27/02/2010 7:00	33	127.8	98.9	2.4
13	20/03/2010 18:00	54	161.4	126.3	
14	22/03/2010 0:00	56	39.0	26.7	
15	22/03/2010 12:00	56	53.6	32.2	

1) Actual levels not reported. The values have been read from a graph.

A total of eight (event integrated) runoff herbicide samples were collected from Treatment 1 in the 2009/2010 wet season. Diuron was detected in all samples. The study authors report concentrations decreasing exponentially with time over the two month sampling period ($r^2 = 0.78$). Based on these equations, the runoff-available half-life of diuron was determined to be 13 days, which is presumably dependent on the number and intensity of rainfall events that occur over the period.

The following graph shows the decline of diuron in runoff waters over this two month period based on DSEWPaC estimation of water concentrations from the graph in the report:

Figure V3.1: DSEWPaC estimated decline of diuron in runoff waters, data based on Rohde and Bush (2011).



Interestingly, there is little change in residues in runoff water from events 15 days after the first runoff to 33 days after (final sampling day). DSEWPaC modelling based on the slope, organic carbon (Kd = 12.4 L/kg), application rate and rainfall event at 7 days after application predicted an edge of field concentration of 2.2 µg/L, which was 8 times lower than that detected, but of a similar order to detections found 15 to 33 days after the first application.

The Marian site proved somewhat problematic for accurate water quality sample collection during the 2009/2010 wet season due to persistent flooding at the lower end of the site (primarily Treatments 4 and 1). The first runoff event caused these treatments to flood and remain submerged for several days. Subsequent runoff events caused site flooding several additional times, with at least one event submerging all monitoring sites. Given the slope of the paddock, it is likely that some of the flood waters originated from other cane paddocks and this may have been reflected in the water quality results.

Thirteen rainfall events causing paddock runoff occurred in the 2009/10 wet season at the Marian site. A rainfall and runoff event was defined as rainfall that caused enough runoff for samples to be collected (>3 mm of runoff). Not all plots ran off for each event. As expected more rainfall was required to produce a runoff event at the beginning of the wet season than later in the wet season when the soil moisture had significantly increased.

The highest recorded concentrations of diuron (0.12 and 0.04 µg/L) were found in runoff from Treatment 1 on two occasions, late in January and late in February (data not shown). All other sites recorded concentrations of 0.02 µg/L or less. Diuron was found in the first five of eight samples analysed for herbicides from Treatment 2, however in each case the concentration was at the limit of detection (0.01 µg/L).

There were difficulties with determining flow rates through the Multi-block and Multi-farm weirs when there was sufficient runoff to overtop the drains and spread out into nearby cane paddocks. This problem was more prevalent at the Multi-farm site which overtopped its banks several times throughout the wet season and would remain that way for days at a time. On at least one occasion (the first runoff event) the volume of water flowing through the Multi-farm site drain was so great that it flooded into the Multi-block drain, further confounding flow estimates. During several flow events, water would back up across the Multi-block weir after the downstream dam and channel filled; causing significant flow rates to be recorded when there was virtually no flow across the

weir. It was therefore not possible to determine accurate volumes of runoff for events, and consequently loads could not be calculated.

Concentrations of diuron were always greater at the Multi-block site rather than the Multi-farm site with the three highest recorded concentrations being from a single runoff event (the first for Multi-block with sufficient sample volume for herbicide analysis) over an 11 hour period. Again concentrations of diuron generally decreased throughout the wet season. At the Multi-block site concentrations ranged from 1.1-43 µg/L, with a mean concentration of 11 µg/L and a median of 2.7 µg/L, while Multi-farm recorded a range in concentrations of 0.23-8.3 µg/L, a mean concentration of 2.9 µg/L and a median of 1.8 µg/L.

Findings – Impact of management practices:

While diuron was not applied to the treatment 2 sites (1.8 m rows, controlled traffic) at Victoria Plains, the report provides good data to assess how this change in management practice from conventional (1.5 m rows) may affect the amount of runoff waters leaving the site. During the test period, there were 15 rain events recorded ranging from 8.2 mm to 209.4 mm. Total runoff from the two different management systems were recorded. Although not always the case, there was clearly a reduction in runoff waters leaving the 1.8-CT plots. Using the data from all 15 runoff events, the mean runoff from the conventional plots equalled 60.4% of rainfall moving off site as runoff compared to 51.6% (average) leaving the site from the 1.8-CT plots. This represents an overall reduction in runoff waters (total water leaving the site) of 14.6%, and while concentrations of diuron in the runoff waters may be similar, the overall load reaching receiving waters would be reduced accordingly. In addition, it was calculated by the study authors that runoff from the 1.8-CT treatment area was delayed by ~6 minutes compared to conventional treatments and the peak runoff rate was ~2% lower.

These results can be compared to those found in Rhode et al (2011) below.

Title	Paddock to Sub-catchment Scale Water Quality Monitoring of Sugarcane Management Practices: Interim Report 2010/2011 Wet Season. Mackay Whitsunday Region.
Authors	Rohde et al
Date	2011
Test Guideline	None identified – Field studies
Data Validity	2*
Data Relied On	Yes – The data were considered critical and relied on in this assessment.

The same sites and treatments were as identified above in Rohde and Bush (2011). At the Victoria Plains site, cane was machine harvested on 3rd September 2010. The cane was harvested green and the trash blanket left on the soil surface. No cultivation was undertaken. Diuron was only applied in Treatment 1 on 13 September 2010 at a rate of 1778 g ac/ha. Application was as a boom blanket spray. The first ratoon cane crop was machine harvested then on 10 August 2011. The cane was harvested green, the trash blanket was left on the soil surface and no cultivation was undertaken.

At the Marian site, all treatment plots were burnt prior to machine harvesting on 29 October 2010. This was a decision made by the grower due to the season outlook and the high risk of the ratoon cane being waterlogged if a trash blanket was left. A single cultivation (two-row multiweeder) was undertaken on 30 October 2010 to remove some of the compaction effects of the machine harvesting operations. No cane trash remained on the soil surface.

Due to the continuing wet weather post-harvest, the herbicide treatments could not be applied according to the original project design. On 14 December 2010 and 26 January 2011, herbicide

treatments were applied as a directed spray to the interspace and base of the cane stool. However, for this year diuron was not included in the herbicide treatments at the Marian sites.

Victoria Plains site: Surface soil (0-2.5 cm) and cane trash samples were collected for herbicide analysis prior to herbicide application, and on eight occasions (0.3-100 days) after application. During this sampling period, 1090 mm of rainfall was recorded.

Findings – Diuron in runoff waters

Diuron was detected (0.17 mg/kg) in the surface soil prior to application this season (239 days after previous application). After application, peak concentrations were not recorded in the surface soil until ~10 days after application as the herbicide was applied to the cane trash blanket. During this 10 day period, 143 mm of rain was recorded (first rain was recorded seven days after application). Using the field dissipation data of 10-100 days after application, the calculated half-life of diuron in soil was 199 days.

Similar to the surface soil, diuron was detected at 0.096 mg/kg on the cane trash blanket prior to application this season. Peak concentrations of all herbicides were detected at the first sampling after application and rapidly declined within 10 days of application. Using this field dissipation data, the calculated half-life for diuron on the cane trash blanket was 11 days.

Diuron was detected in relatively high concentrations in runoff from Treatment 1 in the first runoff event, which was seven days after application. There was no rainfall during that period (prior to the event that caused runoff). Concentrations rapidly declined, but there was an increase in the diuron concentration detected on 20 December 2010. By mid-October (within one month of herbicide application), ~92% of the total loss of diuron and hexazinone in runoff had occurred for the wet season, despite only 6% of runoff having been experienced. The data provided in the report are summarised below. The concentrations of diuron in runoff water have been calculated based on the runoff water volume and loadings (kg/ha) provided in the report:

Table V3.6: Summary of findings, Rohde et al (2011)

Runoff (days after application)	Rainfall			Runoff		Diuron Concentration (ppb)
	Start date	Total mm	Max intensity (mm/h)	Total mm	Max mm/h	
7	20/09/10	131.4	96	73.7	19.1	239.9
12	25/09/10	32.4	48	12.9	4.1	37.0
16	29/09/10	45.8	120	32.7	10.8	27.0
29	12/10/10	35.2	96	5.4	2.1	10.0
55	07/11/10	30.0	108	11.7	5.6	4.6
58	10/11/10	21.4	36	7.5	2.8	2.7
63	15/11/10	48.6	48	32.4	14.9	2.7
66	18/11/10	23.0	36	14.4	2.7	1.6
67	19/11/10	72.8	60	65.5	9.8	1.5
69	21/11/10	38.0	72	29.4	10.8	1.3
70	22/11/10	30.2	36	13.5	3.9	1.3
71	23/11/10	362.4	132	320	48.0	1.2
84	06/12/10	52.6	120	41.5	29.1	0.5
90	12/12/10	23.8	60	16.5	14.2	0.3
97	19/12/10	23.8	108	3.6	5.7	2.8
98	20/12/10	21.6	96	18.8	25.3	7.1
101	23/12/10	53.2	84	26.7	13.9	1.4
102	24/12/10	495.2	144	462	61.2	1.8
115	06/01/11	20.8	96	13.9	24.5	1.1
115	06/01/11	38.4	144	36.6	46.2	0.7
122	13/01/11	37.0	144	24.8	35.8	0.5
139	30/01/11	144.2	72	68.2	30.3	0.2
142	02/02/11	49.0	24	24.6	27.6	0.3
145	05/02/11	18.0	12	20.5	21.8	0.3
150	10/02/11	70.4	168	44.9	39.5	0.2
153	13/02/11	43.6	96	14.1	17.0	0.2
155	15/02/11	24.8	96	17.4	21.7	0.2
163	23/02/11	28.4	60	18.1	6.6	0.2
169	01/03/11	45.6	72	21.8	23.6	0.1
180	12/03/11	296.8	180	197	71.1	0.1
188	20/03/11	22	120	7.4	5.4	0.0
192	24/03/11	160.8	33	94.7	33.2	0.0
196	28/03/11	362.0	108	212	34.9	0.0
200	01/04/11	50.0	60	20.5	8.0	

These initial levels of diuron (7 to 29 days after application) are high, and the DSEWPaC runoff model under predicts the maximum level by more than 100 times. However, it is stressed these are edge of field concentrations. The impact of the trash blanket is expected to reduce overall runoff (water loss) from the plot, and with this water, a proportionately lower percent of applied chemical could be expected to be lost. Despite this, the actual concentrations in the runoff water could be expected to be similar (see Masters et al, 2012 below). As an example, the day 7 event with a rainfall intensity of 96 mm/h resulted in maximum runoff of 19.1 mm/h (trash blanket). The DSEWPaC model predicts runoff from a rain event this heavy of 42 mm. The model predicts in both cases (3% slope, application rate of 1800 g ac/ha), the concentration of diuron in the runoff waters will be 14.6 µg/L. However, percent loss with runoff of 19.1 mm/h is predicted to be 0.15% while with runoff of 42 mm/h, it increases to 0.34%.

At the Multi-block site, initial diuron concentrations were low (0.16 µg/L). By the next sample (19 November 2010), the concentration had increased to 5.9 µg/L even though diuron was not applied in the catchment area. Concentrations then declined rapidly to 0.49 µg/L by mid-December and 0.09 µg/L by mid-February.

At the Multi-farm site the first diuron sample was collected in mid-November (2.8 µg/L). Concentrations then declined, with increases in late December and again in late January. These increases and subsequent decline could be attributed to multiple application times throughout this catchment. This is an important observation, and is a major reason why it is difficult to apply an argument of increasing sorption over time when assessing over a spatial scale larger than an individual farm. Concentrations from the multi-farm site showed several peaks. One of around 3 µg/L was observed in mid-November, the second of around 1 µg/L in late December and then the third of around 3 µg/L in late January. This corresponds to continual peaks in concentration found in water monitoring for diuron, and is expected to be due to differences in application times within a catchment.

Overall, diuron concentrations at the Multi-block site ranged from 0.09-5.9 µg/L (mean 0.95 µg/L) and 0.24-3.1 µg/L for the Multi-farm site (mean 1.1 µg/L). These concentrations (range and mean) are much lower than those detected in the 2009/10 season, which were a mean of 11 µg/L and 2.9 µg/L for the Multi-block and Multi-farm sites, respectively.

Findings – Impact of management practices:

While diuron was not applied to the treatment 2 sites (1.8 m rows, controlled traffic) at Victoria Plains, the report provides good data to assess how this change in management practice from conventional (1.5 m rows) may affect the amount of runoff waters leaving the site. During the test period, there were 34 rain events recorded ranging from 18 mm to 495.2 mm. Total runoff from the two different management systems were recorded. Although not always the case, there was clearly a reduction in runoff waters leaving the 1.8-CT plots. Using the data from all 34 runoff events, the mean runoff from the conventional plots was 60.5% of rainfall moving off site as runoff compared to 50.1% (average) leaving the site from the 1.8-CT plots. This represents an overall reduction in runoff waters of 17.2%, and while concentrations of diuron in the runoff waters may be similar, the overall load reaching receiving waters would be reduced accordingly. In addition, it was calculated by the study authors that runoff from the 1.8-CT treatment area was delayed by ~11 minutes compared to conventional treatments and the peak runoff rate was ~33% lower.

The implications of these findings, along with those from Rhode and Bush (2011) above are considered in the runoff risk assessment, Volume 2, Section V2.11.7.

Title	Reducing the risk of herbicide runoff in sugarcane farming through controlled traffic and early banded application
Authors	Masters et al
Date	2012
Test Guideline	None stated
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment

Residual herbicide runoff and infiltration was measured using a rainfall simulator in a replicated trial on a brown Chromosol with 90–100% cane trash blanket cover in the Mackay Whitsunday region, Queensland. Management treatments included conventional 1.5 m spaced sugarcane beds with a single row of sugarcane (CONV) and 2 m spaced, controlled traffic sugarcane beds with dual sugarcane rows (0.8 m apart) (2mCT). The aim was to simulate the first rainfall event after the application of ametryn, atrazine, diuron and hexazinone, by broadcast (100% coverage, on bed and furrow) and banding (50–60% coverage, on bed only) methods.

These events included heavy rainfall 1 day after herbicide application, considered a worst case scenario, or rainfall 21 days after application. The trial was situated on a 5.13 ha block with 0.25% slope. Diuron was applied as the Velpar formulation (co-formulated with hexazinone). The whole hectare application rate was 1404 g ac/ha diuron (396 g ac/ha hexazinone). Runoff simulator plots

were 7 m long and centred over the row extending each side from the furrow to a total width of 1.5 m or 2 m. The edges of the plots were bound by metal plates driven approx 0.05 m into the soil and extending 0.05 m above the soil. Runoff was routed through a metal gutter (protected with a canopy) and a 0.3 m long outlet pipe, cut into the top of the bed (approximately level with the bottom of the furrows).

Each plot received rainfall at an average intensity of 100 mm/h for >50 min, generating 40 min of runoff. This simulates a large storm equivalent to a 1:20 average recurrence interval (ARI) for the region. Prior to the final day of simulations on day 21, 71 mm of natural rainfall occurred. Despite plots being covered with tarpaulins, rainfall was sufficient to penetrate underneath the tarpaulins via runoff from the furrow up-slope. Therefore due to increased discharge from these plots, results were excluded from statistical analysis. No irrigation was applied during the monitoring period.

The trial was a split–split–plot design with three replicate blocks of two main plots (assigned to herbicide application treatments) split into two plots (assigned to row configuration treatments) and further split into two plots (assigned to days post-application of rainfall simulation treatments). Additionally there were six plots, one in each replicate of the CONV and 2mCT plots, measured prior to herbicide application to give baseline measurements.

During each simulated rainfall event, runoff rate was measured manually. Runoff water samples were collected at regular intervals and analysed for total sediment and herbicides. For the second and third simulation plot replicates of each treatment, discrete herbicide sampling was limited to the initial, peak and tail of flows. Prior to simulated rainfall events, samples of cane trash (0.25 × 0.25 m) and soil (0–0.025 m) were collected at four locations immediately outside of plots and composited for herbicide analysis. Soil cores were also taken from the centre, furrow and mid-section of the beds and composited for gravimetric moisture and herbicide analysis (0–0.10, 0.20–0.30 and 0.45–0.60 m only for herbicides). This sampling was then repeated the following day (after rain) from within plots.

Findings: The 2 m controlled traffic plots (2mCT) showed a significant reduction in runoff compared to the CONV plots. For both, around 932 mm rain was applied. However, total runoff from the 2mCT plots was 17.1 mm compared with 27.4 mm from the CONV plots. Conversely, infiltration was greater (72 mm/h) in the 2mCT plots compared to the CONV plots (52 mm/s).

The following results are summarised:

Table V3.7: Summary of findings, Rainfall simulation, Paddock Scale Trial, Masters et al (2012)

Treatment	Mean concentration (µg/L)	Total load (g/ha)	Percent applied
Broadcast	312	62	4.5
Banded	137	36	4.5
Conventional (CONV)	241	64	5.8
2 m, controlled traffic	208	34	3.2
Day 1 (CONV)	295	67	6.1
Day 21 (CONV)	154	31	2.8

The concentrations in the above table are edge of field. DSEWPaC recognises that edge of field concentrations can be very high and dependent on the actual field characteristics where sampling occurs. As expected for herbicide concentrations, these were higher initially and decreased over time. Following broadcast application, the mean concentration of diuron in runoff waters over the application of rain (~93 mm at 100 mm/h) was 312 µg/L. This was reduced by 56% in the banded application with a mean concentration in the first runoff waters of 137 µg/L. In both cases, the percent runoff in terms of applied chemical was 4.5%. The difference between CONV and 2mCT plots was not statistically significantly different with mean concentrations of 241 µg/L and 208 µg/L respectively, although, it is noted that this difference was significant for some other herbicides

tested. Despite concentrations in edge of field waters not being significantly different, it is important to put the overall impacts of this management practice into perspective. The impact of moving from conventional to 2m-CT was to reduce overall runoff water leaving the site. The paper reports peak runoff rates of 49.4 mm/h from the conventional plot compared to 28.4 mm/h from the 2m-CT plot. Therefore, while similar concentrations are found, the actual amount of chemical leaving the site is significantly reduced, and this is demonstrated in the percent applied column of the above table where there was a reduction of around 45% of the applied diuron leaving the site in runoff water.

The comparison of diuron concentrations in runoff waters where runoff occurred at 1 day or 21 days after application (CONV plots) showed mean levels of 295 µg/L and 154 µg/L respectively. This represents a decrease of <50% over three weeks, noting there was significant natural rainfall prior to the day 21 simulation. This corresponded to an almost 70% reduction in the soil concentration (top 0-10 cm) over the same period.

These results were validated with a paddock scale trial monitoring runoff from natural rainfall events over the following wet season. Each treatment consisted of three beds 180 m long. This resulted in catchments of 810 m^2 and 1080 m^2 for CONV and 2mCT treatments, respectively. Storm events measured at the paddock scale throughout this wet season ranged from 25-80 mm/h. Total runoff ranged from 5 to 73% of rainfall and commenced approximately 5–10 min later on 2mCT rows than on CONV rows. The 2mCT rows averaged 30% less total runoff and 37% lower peak runoff rate than CONV rows. The first rain on day 21 was somewhat similar in scale to rainfall simulations. In this event, total runoff was 61% of rainfall on 2mCT rows with banded treatments. The following table summarises the results of this paddock scale trial with respect to diuron:

Table V3.8: Summary of findings, Natural rainfall runoff events, Paddock Scale Trial, Masters et al (2012)

Days after application	Rainfall (mm)	Runoff (mm)		Diuron conc. (µg/L) (event mean concentration)	
		CONV	2mCT	CONV	2mCT
21	70.5	-	43	-	107
66	80.2	67	40	6.60	2.10
69	24.5	1.1	1.4	1.70	0.58
72	41.2	30	26	1.70	0.54
122	45.4	28	21	-	-

Where both CONV and 2mCT concentrations are available, the decrease in diuron concentration from the 2mCT plots was approaching 70%, which is a significant reduction through this management practice. 21 days after application the mean concentration of diuron in the paddock runoff waters was 107 µg/L. This compares to a concentration of 154 µg/L for the 2mCT plots in the simulator test, which is in relatively good agreement. In terms of percent reduction in runoff waters leaving the sites under the two different management practices from these natural events, the average reduction from a move from conventional to 2m-CT was around 13%, which compares favourably to the findings from Rhode and Bush (2011) and Rhode et al (2011) above.

Conclusions from this study: Based on an application rate of 1400 g ac/ha, 1.4% OC (Kd = 7.4 L/kg), and a slope of 0.25%, the DSEWPaC model predicts an edge of field concentration of 1.13 µg/L, well below the >100 µg/L measured in this study. This again highlights the potential for this model to underpredict runoff from heavier soils noting the surface soil in this study contained 18% silt and 17% clay.

While these results show that changes to management practices can reduce runoff and increase infiltration, the results with respect to diuron remain of concern. Edge of field levels were elevated for extended periods following application. The implications of these findings, along with those from Rhode and Bush (2011) and Rhode et al (2011) above are considered in the runoff risk assessment, Volume 2, Section V2.11.7.

Title	Large-scale pesticide monitoring across Great Barrier Reef catchments – Paddock to Reef Integrated Monitoring, Modelling and Reporting Program.
Authors	Smith et al
Date	2011
Test Guideline	None identified
Data Validity	4
Data Relied On	No – the data were considered information only and not relied on in this assessment

This paper reports on the initial findings from the first year (2009/2010) of monitoring for the GBR pesticide monitoring program. The objectives for reporting these initial findings were to: (1) provide a spatial overview of pesticide inputs to the GBR lagoon and end-of-system aquatic habitats associated with GBR catchments; (2) assess the degree of contamination (the number and types of pesticides, the concentration of pesticides and duration of exposure); and (3) assess the potential toxicity of pesticides occurring as a mixture.

Manual grab samples (1 L) were collected at each site (11 in total) over at least two flow events from the 2009/2010 wet season. Samples were collected from approximately 0.3 m below the water surface in an area of high flow, in close proximity to deployed passive samplers. Samples were collected directly into solvent rinsed, 1 L glass bottles, transported on ice and stored in the dark at ~4^{o}C before analysis. The number of grab samples collected and the timing of collection was based on the occurrence of large flow events at each site. The objective was to sample at least two events such that approximately four samples were collected on the rise of an event and three samples were collected on the fall of an event, but more were collected if possible. This was not always possible at some sites as logistical issues (e.g. flooding) made it impossible to do so. A total of 268 grab samples were collected and chemically analysed across all sites. The monitoring data have been provided separately to DSEWPaC by the Queensland Department or Environment and Resource Management (DERM) and are not repeated here. The study authors calculated the 95th percentile values (µg/L) from the monitoring data at the following sites:

Table V3.9: 95th percentile diuron levels (Smith et al, 2011)

Barratta Ck	Tully River	S. Johnstone River	Sandy Ck	Pioneer River	Burdekin River	Fitzroy River	Burnett River
5.78 µg/L	0.58 µg/L	0.14 µg/L	4.70 µg/L	3.40 µg/L	0.02 µg/L	0.02 µg/L	0.13 µg/L

In the Burdekin River, of 21 samples only one showed a detectable level of diuron. In the Fitzroy River, out of 59 samples, only 5 showed positive detection (0.02-0.04 µg/L). In addition, at other sites (Belyando River, Suttor River and Comet River) no diuron was detected.

These monitoring results were converted to atrazine toxicity equivalent quotients (TEQs). Toxicity equivalence was based on the relative toxicity of the PSII herbicides to atrazine, and the relativities in toxicity were based on previous research undertaken by Ma (2002) where EC50 concentrations from an acute 96 h growth bioassay of two species of freshwater microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa* were used. Based on this approach, toxicity equivalent factors of 32.7 and 120.2 for these two species respectively were determined.

The authors calculated diuron concentration (and the other chemicals considered in this paper) in terms of atrazine equivalents by multiplying the chemical specific detected levels by the toxicity

equivalence factor. For example, based on *Scenedesmus obliquus*, the 95th percentile diuron level of 4.7 µg/L in Sandy Creek would be multiplied by 32.7 to give an atrazine equivalent level of around 154 µg/L. This was then compared to the atrazine ANZECC guideline of 13 µg/L to conclude on times herbicide concentrations exceeded this toxicity value.

The study concluded there was widespread contamination based on the suite of chemicals considered (atrazine, diuron, ametryn, simazine and prometryn), stating this was characterised by frequent and widespread occurrences of pesticides including PSII herbicides and the presence of complex pesticide mixtures.

Conclusions from this study: DSEWPaC is not relying on the results of this study. The individual monitoring data for diuron have been supplied separately and they are considered elsewhere in this report. Mixture toxicity assessment in terms of a suite of chemicals found in receiving waters is outside the scope of this assessment. DSEWPaC considers the actual approach used here for determining potential risk based on toxicity equivalence is somewhat misleading. The toxicity equivalence was established based on two short term toxicity tests, but exceedences were based on a 95th percentile SSD of chronic results. This appears inappropriate. For example, the diuron toxicity equivalence factor to atrazine for *Chlorella pyrenoidosa* based on the test relied on was around 120. Therefore, a diuron level of 1 µg/L would be considered equal to 120 µg/L atrazine equivalents. This was compared to the 95th percentile for atrazine in the ANZECC guidelines of 13 µg/L, so the water level would be considered 9.2 times higher than the toxicity value.

However, the 96 h EC50 for atrazine that was used to establish equivalence was 144 µg/L, meaning based on this, the atrazine equivalent level for diuron in water did not exceed that toxicity value. If the 95th percentiles were used to establish equivalence (13 µg/L for atrazine; 1.56 µg/L for diuron based on this assessment), the toxicity equivalent factor would be 8.3. It is then more appropriate to compare water levels to the ANZECC guideline.

Title	Dynamics of herbicide transport and partitioning under event flow conditions in the lower Burdekin region, Australia
Authors	Davis et al
Date	2011
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and were relied on in this assessment

This study examined the temporal variability in herbicide delivery to the Great Barrier Reef (GBR) lagoon from one of the catchment's major sugar cane growing regions, the lower Burdekin. Data were collected from a five year wet season intensive monitoring program. Water quality sampling from one wet season (2009-10) also investigated the transport partitioning of key pesticides, specifically those transported in dissolved versus particulate bound forms. Pesticide presence in benthic sediments from freshwater, estuarine and marine environments across the study area were also considered.

The water quality sampling focussed on two major sub-catchments being Barratta Creek and the Haughton River. These are the only two gauged sub-catchments of the lower Burdekin, and the data from these gauge stations were required for contaminant load calculations. One sub-catchment (Upper Barratta Creek) and three end-of-catchment sites (Haughton River, East and West Barratta Creek) were selected for water quality monitoring.

The paper presents the temporal dynamics of herbicide concentration over the duration of wet season flood events occurring at Upper Barratta Creek during the 2008-09 and 2009-10 wet seasons. Invariably, the highest concentrations occurred during the initial stages of early wet season flow events. By the time the very high flow rates for these seasons were recorded, diuron

concentrations appear to have declined to very low levels. The graph in the paper is somewhat difficult to read, but the elevated levels appear to be obtained from the start of November (2008-09) and towards the end of December (2009/10) and be largely diluted or removed by the end of January in both seasons. The issue of temporal exposure is considered elsewhere in this assessment.

Comparison of filtered versus unfiltered concentrations for samples collected during the 2009-2010 wet season showed diuron was transported predominantly in the dissolved rather than particulate-bound form. Nine samples were taken from the three Barratta Creek sites over the 26-29 January 2010 period. The percentage of particulate bound diuron was ranged from 21.7% to 40.7% with an average of 33.4% bound to particulate matter.

For the sediment sampling, five samples were taken from four Barratta Creek sites for freshwater samples. At two of these sites (Upper Barratta and West Barratta Creek), diuron was not detected in the sediments. At a third site (Barratta Creek at Allen Road), diuron was measured at 9.4 µg/kg while at the fourth site (East Barratta Creek), two samples returned diuron concentrations of 1.3 and 28 µg/kg.

Of the nine estuarine/marine samples taken at different sites, diuron was not detected at three sites, while five sites had sediment levels ranging from 0.12 to 0.31 µg/kg. One site, a Barratta Creek site in a Ramsar Wetland area showed diuron at 1.62 µg/kg.

Conclusions from this study: The temporal trends of diuron concentrations in runoff waters are considered in more detail elsewhere in this assessment.

This is first monitoring data DSEWPaC has received that addresses the difference between diuron levels in the dissolved phase compared to the particulate phase. Measurements of sediment concentrations are not common for diuron in freshwater environments, and the results provided here show that diuron can be found in sediments. Given that results showed around 33% diuron in runoff is bound to particulate matter, this is not surprising.

V3.2.3 Impact of reduced tillage

Several submissions referred to the benefits of reduced tillage systems in reducing runoff. DSEWPaC has obtained the following literature study to further assess this aspect.

Title	Comparison of runoff, soil erosion and winter wheat yields from no-till and inversion tillage production systems in northeastern Oregon
Authors	Williams et al
Date	2009
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and were relied on in this assessment

The objectives of this study were to compare a conventional, intensively tilled winter wheat–fallow system versus a no-till four-year cropping rotation system in terms of runoff, soil erosion, and crop yields. The experiment was undertaken within a small watershed to provide results that would be representative of conservation effectiveness at the field scale. Two neighbouring drainages, 5.8 ha (tillage) and 10.7 ha (no tillage) in the 340 mm/year precipitation zone of northeastern Oregon, were instrumented to record rainfall, runoff, and erosion over a four-year period (2001 through 2004). One drainage was cropped to a winter wheat–fallow rotation and received inversion tillage (tillage fallow). The second drainage was cropped in a four-year no-till rotation: winter wheat–chemical fallow–winter wheat–chickpea (no-till fallow).

In crop years 2003 and 2004, six metal runoff collectors were placed on backslope positions in each watershed during the typical erosion season (November through March) for this region. The metal frames consisted of a 9.5 mm thick by 254 mm wide steel plate bent into a rectangle about 800 mm wide and 1,200 mm, with the bottom side formed into a slight V-shaped funnel. The total surface area circumscribed by the frame was 1 m^2. The frame was placed with the funnel pointing down-slope. Runoff was measured at the mouth of each drainage.

Findings: Based on values read from a graph, the residue soil cover in the no-till field was almost 70% compared to around 5% in the tilled field. From the 1 m^2 plots runoff was significantly less in the no-till system compared to the inversion tillage system, where 3.4 times more runoff (79 mm versus 23 mm) were produced than in the no-till system. A total of 13 runoff events from the inversion tillage system and 3 from the no-till system were recorded. These results were corroborated by results from two sites within 10 km of this research site that effectively bracket the size and slope conditions of the research site used in this experiment. Runoff and soil erosion were monitored through the same set of weather events at a 1.5 ha hillslope (23% slope) and a second paired drainage (18 ha and 25 ha). Both sites were managed as no-till systems, and neither runoff nor erosion was observed during crop years 2001 to 2004.

Conclusions: The no-till rotation was substantially more effective in conserving soil and water in this field-scale comparison. The no-till cropping system was more effective in reducing runoff and soil erosion.

Title	The Effect of Tillage on Soil Surface Properties and the Water Balance of a Xeralfic Alfisol
Authors	Hamblin
Date	1984
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and were relied on in this assessment

Two long term tillage trials were established in 1977 on a xeralfic alfisol. The surface soil is a clay-depleted horizon with 10-20% clay content whereas the B horizon has a clay content 2 to 3 times this with a high proportion of very fine clay. The soil at the test site had an average 25% clay, 13% silt and 62% sand in the A horizon. Organic carbon content in the 0-10 cm soil layer was 1.04% at the start of the experiment and the pH (1:5 in water) was 6.25. Subsoil pH rises to 8.8 at 25 cm. The site was Merredin in Western Australia with an annual average rainfall of 287 mm, 75% of which occurs in the growing season from May to October. Growing season maximum and minimum mean temperatures were 20.5^{o}C and 7.1^{o}C.

Each trial occupied an area of 1.4 ha. Three tillage treatments were compared, 1 in a continuous cropping sequence, 1 in a 1 year crop, and 1 year pasture rotation. The rotation trial had 3 replicates in crop and 3 in pasture each year. Each treatment plot occupied 4 drill widths.

The tillage treatments, which were monitored on the continuously cropped trial over the period 1977-1982 were a) disc ploughed, b) direct drilled; and c) no-till.

Findings: Yields and soil physical conditions did not differed markedly in the first 3 years between tillage treatments, although the ploughed soil had the lowest soil strengths and highest porosities. However, by the fifth and sixth years, soil physical conditions were much poorer for crop growth on the ploughed treatments and those yields on both trials were only half that of the direct drilled crops. Because the site was nearly level, no significant runoff occurred and the 40-70 mm less soil water stored in the ploughed treatments at seeding were ascribed to increased evaporation from wetter soil surfaces. A relative reduction in organic matter in the 0-2.5 cm zone of the ploughed soil was found by the third year, and by the sixth year this had become a significant reduction. After

six years, the %OM in the 0-2.5 cm layer had been reduced to 0.91% (compared with 1.04% at the start of the test), while in the direct drilled and no-till soils, levels remained at starting values.

Water infiltration was apparently much greater in the direct drilled and no-till plots. For example, at seeding (mid June) in 1982, total soil water was 26% to 41% greater in the no-till and direct seeded plots, respectively, than in the disc ploughed plots. The depth to June wetting front was 26.5 cm in the no-till crop compared to less than 20 cm in the disc ploughed crop.

Conclusion: These results demonstrate that no-till cropping should, over time, allow for increased infiltration and at least maintain soil organic carbon levels, both of which are important measures for decreasing the runoff potential of diuron.

V3.2.4 Additional Australian Monitoring Data

Title	Diuron Concentration Data from QLD Catchments
Authors	QLD Department of Environment and Resource Management
Date	9 October 2011
Test Guideline	None – monitoring data
Data Validity	2*
Data Relied On	Yes – the data were considered critical and were relied on in this assessment

The most recent (2009-10 and 2010-11) monitoring data from the Great Barrier Reef Catchment Loads Monitoring Program have been supplied to the APVMA as Excel spreadsheets by the Queensland Department of Environment and Resource Management (DERM).

The following table provides summary details of sites sampled during either 2009-10 or 2010-2011.

Table V3.10: Summary details of DERM sampling sites, 2009-10 and 2010-11

Catchment	Site name	Gauging Station ID	Catchment/Sub Catchment	Pesticide site		Mean Monthly Flow (all months, ML/d)
				(09-10)	(10-11)	
Johnstone	North Johnstone R @ Tung Oil	112004A	Sub-Catchment		**Yes**	4970
Johnstone	South Johnstone R @ Upstream Central Mill	112101B	Sub-Catchment	**Yes**		2191
Tully	Tully R @ Euramo	113006A	Catchment	**Yes**	**Yes**	8366
Herbert	Herbert R @ Ingham	116001F	Catchment		**Yes**	21340
Haughton	Upper Barratta @ Northcote	119101A	Catchment	**Yes**	**Yes**	453
Burdekin	Burdekin R @ Home Hill (Inkerman Bridge)	120001A	Catchment	**Yes**	**Yes**	24956
Burdekin	Belyando R @ Gregory Development Road	120301B	Sub-Catchment	**Yes**		1800
Burdekin	Suttor R @ Bowen Development Road	120310A	Sub-Catchment	**Yes**		2270
Pioneer	Pioneer R @ Dumbleton Pump Station	125013A	Catchment	**Yes**	**Yes**	3714[1]
Plane	Sandy Ck @ Homebush	126001A	Catchment	**Yes**	**Yes**	464
Fitzroy	Fitzroy R @ Rockhampton	1300000	Catchment	Yes	**Yes**	14566
Fitzroy	Comet R @ Comet Weir	130504B	Sub-Catchment	**Yes**	**Yes**	
Fitzroy	Dawson R @ Taroom	130302A	Sub-Catchment		**Yes**	
Burnett	Burnett R @ Ben Anderson Barrage Head Water	136014A	Catchment	**Yes**	**Yes**	2295[2]

1) Data from Pioneer River at Dumbleton Weir; 2) Streamflow data for Site 136014 does not appear available. The flow data for the Burnett River is reported for site 136007A, Burnett River at Figtree Creek.

The limit of detection for both years was 0.01 µg/L. The monitoring program appeared to differ somewhat between the years with the 2009-10 results focussing on discreet events, while the 2010-2011 monitoring included the bulk of measurements through the wet season, but also several results from outside this season.

In order to put the sizes of these rivers into some perspective, the mean monthly (all months) flow data for some of the sites have been included. These values are the means of all months over the course of available monitoring, which could extend several decades. In the assessment chapter for sugar cane, a more detailed analysis of river flow characteristics throughout the various sugar growing regions had been undertaken. However, for this purpose, flow rates could be used to indicate the expectation of residues IF the sampling stations are located in sugar growing catchments. For example, the much lower flow rates of Barratta Creek and Sandy Creek suggest higher concentrations should be found than in the very high flow rate rivers of the Burdekin River and the Herbert River.

2009 – 2010 data

This is largely supported in the monitoring. Over the 2009-10 monitoring period, only limited detections were found in the Fitzroy River (noting this is not really in a major sugar production region) with a maximum level detected of 0.04 µg/L. In the Burdekin River, only one positive detection (0.02 µg/L) was recorded. Diuron was not detected in the Suttor or Belyando Rivers.

Table V3.11: Findings from DERM monitoring, 2009-10

Site number	Event dates	n	% > 0.01µg/L	Mean µg/L	Max µg/L
112101B	26/1/2010 to 2/2/2010	9	78	0.02	0.05
	19/2/2010	1	100	0.04	0.04
	23/3/2010	1	100	0.14	0.14
	28/3/2010 to 30/3/2010	4	0	<0.01	<0.01
113006A	10/1/2010	1	100	0.59	0.59
	23/1/2010 to 3/2/2010	12	100	0.14	0.28
	19/2/2010	1	100	0.09	0.09
	10/3/2010 to 15/3/2010	9	100	0.14	0.57
	28/3/2010/3/4/2010	8	100	0.01	0.02
119101A	29/12/2009 to 19/1/2010	5	100	2.90	6.5
	25/1/2010 to 8/2/2010	11	73	0.62	1.8
	12/2/2010 to 15/2/2010	3	100	0.05	0.09
	19/2/2010 to 23/2/2010	3	100	0.02	0.03
	19/3/2010	1	100	0.12	0.12
	24/3/2010 to 8/4/2010	3	100	0.03	0.04
125013A	25/1/2010 to 29/1/2010	6	100	2.07	3.4
	31/1/2010 to 26/3/2010	6	83	0..20	0.55
126001A	25/1/2010 to 30/1/2010	7	100	3.35	4.7
	31/1/2010 to 2/2/2010	2	100	1.7	1.9
	10/2/2010 to 13/2/2010	5	100	0.61	0.91
	18/2/2010 to 20/2/2010	3	100	0.38	0.54
	21/2/2010 to 24/2/2010	4	100	0.26	0.32
	26/2/2010 to 27/2/2010	2	100	0.23	0.26
	22/3/2010 to 26/3/2010	3	100	0.09	0.11
136014A	02/02/2010 to 8/3/2010	22	86	0.06	0.14

In the Burnett River (site 136014A), a further 17 samples were taken from 9/3/2010 to 24/5/2010. Diuron was only detected once at 0.01 µg/L during this period. Not surprisingly, the highest findings were in the creeks with the lowest likely flow rates, Barratta Creek and Sandy Creek. Data from these sites showed that the highest levels were associated with the first event, and at this time, exposure could last for many days at elevated concentrations. For example, the first event at Barratta Creek was recorded over the period 29/12/2009 to 19/1/2010, or 21 days. The average concentration was approaching 3 µg/L during this period. However, after that for the next 5 events, concentrations were very much lower, seldom exceeding 0.1 µg/L despite being found in almost all samples. Similarly, at Sandy Creek the first event lasted 5-6 days with average concentrations over this period being 3.35 µg/L. They fell progressively with the next six events and only exceeded 1 µg/L in one sample over the sampling from the end of January to the end of March.

2010 – 2011 data

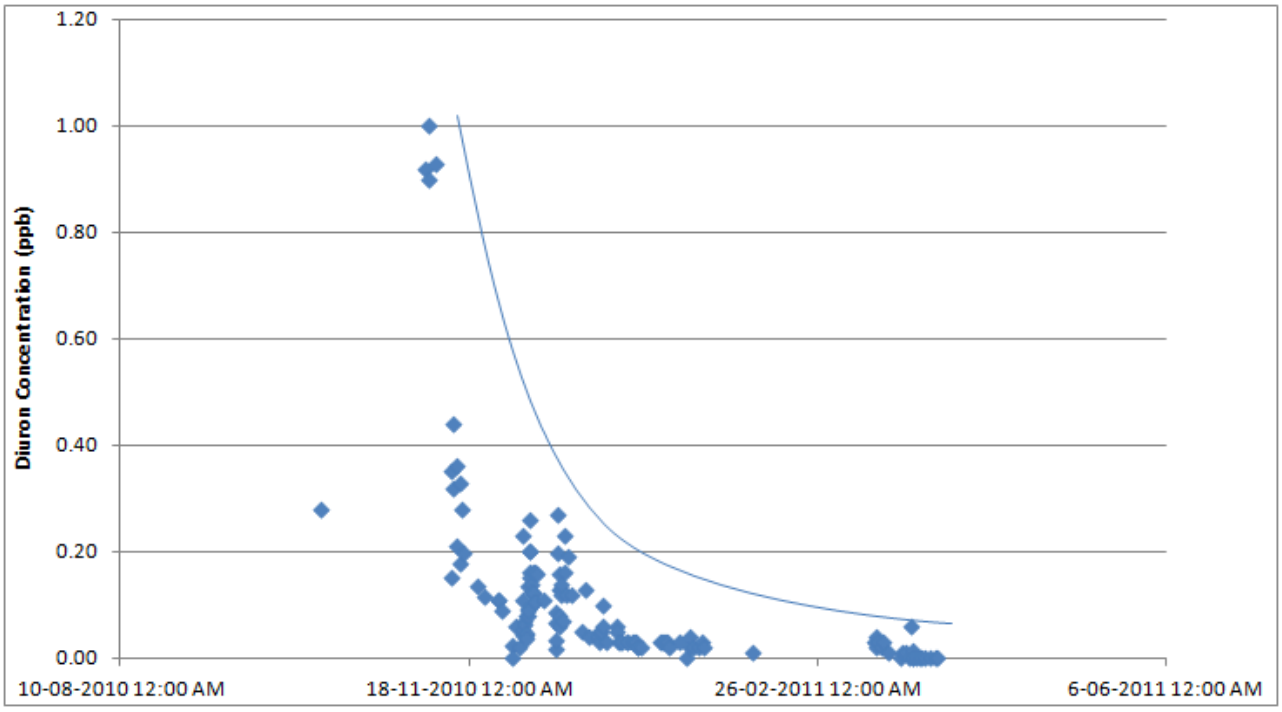
Diuron was largely not detected in the Burdekin, Comet, Fitzroy, Dawson or North Johnstone Rivers through the monitoring period. In the Burnett River, out of 159 samples, diuron was only detected in 19 samples (12%). The bulk of these came in the period 10/12/2010 to 15/12/2010 (n = 10), where the mean concentration was 0.05 µg/L (range 0.013 to 0.096 µg/L).

Interestingly, in the Tully River (which has a relatively large flow rate), diuron was found in 90% of the 30 samples taken from 24/11/2010 to 3/4/2011, with a maximum level of 0.27 µg/L and a mean level over this period of 0.05 µg/L.

The Pioneer River, which also has a relatively high flow rate (certainly compared with smaller creeks such as Barratta Creek and Sandy Creek), showed positive detections of diuron in 93% of 202 samples taken over the period 5/7/2010 to 29/6/2011. Positive detections commenced on 20-9/2010 and essentially continued all the way through this monitoring period. During this time, the peak concentration was 1.74 µg/L and the mean was 0.21 µg/L.

As with 2009/10, the highest detections, and longest periods of exposure, were found in the Barratta and Sandy Creek sites. A plot of the Barratta Creek levels is as follows:

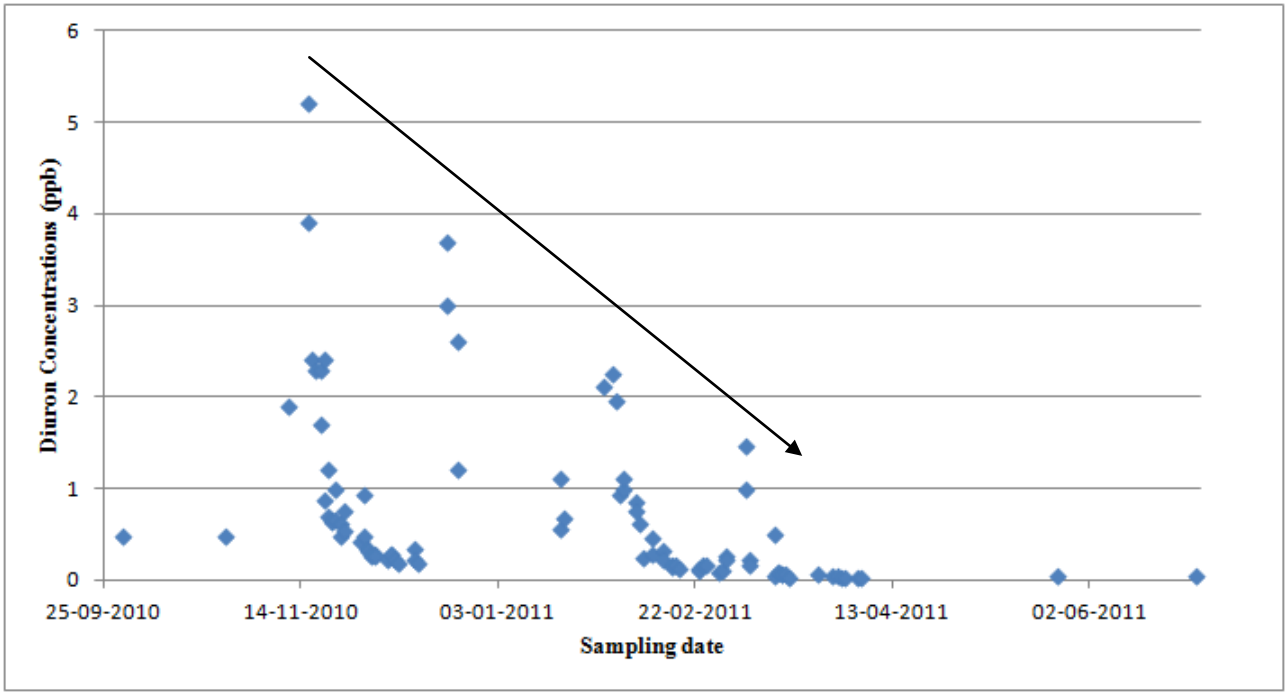
Figure V3.2: 2010-11 DERM diuron detections in Barratta Creek



This shows a peak of 1 µg/L at 6/11/2010, although one level of 15 µg/L was reported for 14/10/2010 but was not included in the above plot. There were essentially three peaks in the above plot, but peak levels decreased each time. Following the 6/11/2010 peak, the next highest cluster of detections was from 13/12/2010 to 22/12/2010 where the concentrations ranged from 0.05-0.27 µg/L and averaged 0.14 µg/L. The final small peak of 0.06 µg/L was measured on 24/3/2011.

A more distinct set of peaks was observed in the Sandy Creek monitoring results as shown in the following diagram:

Figure V3.3: 2010-11 DERM diuron detections in Sandy Creek



This shows 4 distinct peaks, each subsequent one being lower than that before it. The first set of elevated levels are found between 11/11/2010 and 20/12/2010 where concentrations ranged from 1.7-5.2 µg/L (mean 2.8 µg/L over this 9 day period). The second peak occurred on 21/12/2010 (3.7 µg/L) and levels were >1 µg/L from here until 19/1/2011 in the 5 samples taken over this period. The third peak (2.11 µg/L on 31/1/2011) was associated with a series of measurements ranging from 0.93 to 2.11 µg/L over a 5 day period (mean 1.56 µg/L) while the last peak of 1.47 µg/L was measured on 7/3/2011.

In fact, over the period 11/11/2010 to 14/3/2011 (a period of 4 months), the mean concentration was 0.9 µg/L while the 75th and 90th percentile concentrations were 1.08 and 2.3 µg/L respectively. This is a very long period of elevated concentrations, and as shown in the sugar cane chapter of this assessment, there are likely many more waterways more at risk than Sandy Creek due to small flow volumes, and locations within cropping areas.

Title	Final Report: Monitoring of organic chemicals in the Great Barrier Reef using time integrated monitoring tools (2009-2010)
Authors	Kennedy et al
Date	2010a
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in the assessment
Title	Monitoring of organic chemicals in the Great Barrier Reef Marine Park and selected tributaries using time integrated monitoring tools (2008-2009)
Authors	Kennedy et al
Date	2010b
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in the assessment

Monitoring has been conducted at sites within five major Natural Resource Management Regions (Cape York, Wet Tropics, Burdekin, Mackay Whitsunday and Fitzroy) with results obtained from the 2008-09 and 2009-10 sampling period. The following methodology describes that used during 2009-10. Baseline concentrations of specific pesticides/herbicides were estimated using passive sampling techniques. Specifically, diuron was measured for using SDB-RPS Empore^{TM} Disk based passive samplers.

The monitoring year for pesticide sampling was from May 2009 to April 2010. The 2009-2010 monitoring year was divided into "Dry 09" (May 2009 to October 2009) and "Wet 09-10" (November 2009 – April 2010) periods for reporting purposes. Within each dry period samplers were deployed for two months (maximum of three periods) and within each wet period samplers were deployed for one month (maximum of six monitoring periods).

All Empore Disk (ED) samples were extracted and one replicate analysed for each location. All replicate sample extracts were stored at 1 mL in glass vials in freezers with selected replicates (10%) subsequently analysed. Replicates from the river sites were extracted and analysed separately. The results are summarised in the following table:

Table V3.12: Inshore Diuron Findings, Kennedy et al (2010a, b)

Region	Site	Monitoring period	Findings (**ng/L**)				
			Samples/detects	Min	Mean	Max. Dry season	Max
Cape York	Pixies Garden	08/09	6/3	n.d.	0.47	0.43	1.7
		09/10	5/0	n.d.	n.d.	n.d.	n.d.
Wet Tropics	Low Isles	08/09	9/8	n.d.	1.8	3.5	5.0
		09/10	9/7	n.d.	1.4	1.5	5.7
	Green Island	09/10	9/6	n.d.	0.99	0.39	6.2
	Fitzroy Island	08/09	8/8	0.88	3.8	3	15
		09/10	10/10	0.90	3.5	5.0	12
	Normandy Island	08/09	10/8	n.d.	2.2	3.0	7.8
		09/10	8/5	n.d.	1.2	n.d.	3.6
	High Island	08/09	2/2	1.2	1.8	2.3	2.3
	Dunk Island	08/09	4/3	n.d.	1.9	n.d.	3.2
		09/10	7/7	0.57	2.6	1.8	5.9
	Tully River[1]	09/10	4/4	3.0	13	5.0	22
Burdekin	Orpheus Island	08/09	7/5	n.d.	0.94	1.7	1.7
		09/10	4/4	1.5	27	100[2]	100[2]
	Magnetic Island	08/09	9/9	1.0	2.1	4.4	4.4
		09/10	4/3	n.d.	2.6	-	6.9
	Cape Cleveland	08/09	10/8	n.d.	1.2	0.92	4.5
		09/10	7/6	n.d.	1.8	0.42	6.7
Mackay Whitsunday	Outer Whitsunday	08/09	3/2	n.d.	1.6	3.9	3.9
		09/10	5/4	n.d.	6.2	0.44	27
	Daydream Island	08/09	3/3	4.4	44.7	120	120
		09/10	2/2	1.5	26	-	51
	Pioneer Bay (Inner Whitsunday)	09/10	6/6	3.6	23	43	43
	Pioneer River[1]	09/10	5/5	0.62	156	7.3	761
	Sarina Inlet[1]	09/10	7/7	0.46	70	1.2	429
Fitzroy	North Keppel Island	08/09	6/6	0.46	0.82	1.1	1.1
		09/10	6/2	n.d.	2.0	n.d.	6.4

1) River site – not considered as inshore levels; 2) Kennedy et al (2011) reports this level as an outlier, and the maximum level detected as 8.4 ng/L.

Passive sampling techniques provide a time averaged estimate of the concentrations in water of analytes detected in samplers after a defined exposure period in the environment. These concentration estimates are derived using the concentration of analytes sequestered in the sampler within this exposure period and calibration data obtained in laboratory or field studies. This calibration data is typically comprised of sampling rates (volume of water sampled per day).

The duration of the deployment period can be a critical factor in determining whether time integrated sampling or equilibrium phase sampling are occurring for a given analyte in a given sampler. During the dry season monitoring period EDs were routinely deployed in a 2-disc configuration to increase the capacity of the sampling phase and hence theoretically the time required to achieve equilibrium. Time integrated sampling is assumed for these samplers in all monitoring periods. A sampling rate of 0.08 L/day has been assumed for all target chemicals in ED samplers since monitoring commenced in 2005. This sampling rate is equivalent to the sampling

rate of diuron in this sampler at nominal flow velocity of 0.14 m/s, determined in a previous tank calibration study.

In order to achieve meaningful results with passive sampling techniques, it is necessary to understand the techniques and their limitations and consider site specific factors that may influence the uptake of chemicals into samplers in-situ. It should be noted that the uptake of chemicals into the sampler is expected to be primarily via the dissolved phase. Consequently the total concentration in water may be underestimated for extremely hydrophobic chemicals or chemicals which partition significantly into suspended particulate matter. Furthermore, an assumption is made that chemicals are not degraded in the passive samplers. However, for passive samplers deployed in shallow and very clean water, degradation may be an issue for compounds susceptible to photo-transformation. When samplers are deployed for extended periods both reversibility of sorption and degradation may be confounding factors which influence estimated water concentrations.

Conclusions from these monitoring results: The advantage of passive sampler results is they provide a longer term average concentration, and through comparison with earlier data, can identify how these chronic concentrations may change over time. The problem is, however, they can't identify peak concentrations, or the likely time higher concentrations have existed. The results from 2008-09 and 2009-10 are not dissimilar to the earlier 2005-06 results previously reported in APVMA (2011). Long term (1 to 2 months) concentrations in the in-shore areas tested are generally remaining below 10 ng/L (0.01 µg/L), which is over 100 times less than the 99th percentile protection level determined by DSEWPaC (APVMA, 2011). However, some higher findings did occur with the sites at Daydream Island determining a mean wet season (1 month) concentration in 2009-10 of 51 ng/L (no dry season sampling), and a mean dry season (2 months) concentration of 120 ng/L in 2008-09. This average concentration is 10 times below the 99th percentile trigger level.

While there were many cases where wet season concentrations exceeded dry season, this was not always the case.

V3.3 Additional Ecotoxicity Data

V3.3.1 Higher tier tests

Title	Evidence for adaptation of riverine sediment microbial communities to diuron mineralization: incidence of runoff and soil erosion
Authors	Pesce et al
Date	2010
Test Guideline	None stated (soil sampling to ISO 10381-6)
Data Validity	2*
Data Relied On	Yes – the data were considered critical and were relied on in the assessment

Test system: A microcosm study was undertaken to assess the impact of runoff and erosion processes on the adaptation of riverine-sediment microbial communities to diuron mineralisation. The set-up consisted in aquariums filled with natural riverine sediments and water that were supplemented or not, in triplicate, with diuron (10 µg/L, final concentration) to simulate surface water runoff and/or diuron-treated vineyard soil (350 g) to simulate erosion following a strong rainfall event. Microcosms were run in triplicate and the resulting effects were estimated by assessing and comparing (a) the fate of diuron and diuron partitioning between sediment and water phases, (b) the evolution of sediment-based bacterial community structure (density and community structure), and (c) the evolution of diuron mineralization potential in sediment samples.

To prevent the occurrence of diuron and of pesticide residues in initial samples, sediments and water were collected in February 2008, before the pesticide application period, at an unpolluted and forested upstream reference site.

Twelve 35-L aquariums were filled with 4 kg (about 3 kg dry weight) of sediment and 7 L of water. At the start of the experiment, three microcosms (called "Diuron" microcosms) were contaminated with a diuron solution to simulate surface water runoff leading to an initial nominal concentration of 10 µg/L to mimic the high diuron levels detected in the Morcille River. Three other microcosms ("Soil" microcosms) were contaminated with 350 g (about 300 g dw) of the diuron-treated vineyard soil to simulate erosion following a strong rainfall event. Diuron was present in the soils at a nominal 1.2 mg/kg, taken to represent an application rate of 1.2 kg ac/ha distributed in the top 10 cm. Three more microcosms ("Diuron + Soil" microcosms) were contaminated with diuron (10 µg/L final concentration) and diuron-treated soil (350 g). The remaining three microcosms were used as replicate controls ("Control" microcosms). The microcosms were kept at ambient temperature (~20^{o}C) under an artificial 10 h day/14 h night light regime. For each parameter, samples were collected on day 0 and after 2, 4 and 9 weeks.

Diuron and its main metabolites (DCPMA and DCA) were quantified in water, and diuron concentrations in soil and sediment samples were measured, using LC/MS/MS. The DT50 of total diuron was estimated using a log-normal regression model for each treatment.

Flow cytometry was used to estimate sediment bacterial densities. Bacterial community structure in soil and sediments was assessed by terminal restriction fragment length polymorphism analysis. The ability of soil and aquatic microbial communities to mineralise diuron was determined by radiorespirometry.

Findings: Diuron was not detected at the initial sampling time in the upstream sediment used to inoculate the microcosms. In the "Diuron" microcosms, diuron concentrations in the sediment fraction remained below the LOQ (1 µg/kg) throughout the study. Diuron in the water phase decreased during the first four weeks (DT50 = 13 days) and was below the LOQ (0.02 µg/L) at week 9. In "Soil" and "Diuron + Soil" microcosms, a diuron release from sediment to water was recorded from the first hour of the experiment. Diuron in the water phase increased ("soil) or remained stable ("Diuron + Soil") over a 2 week period and then decreased to levels lower than detection limits by the end of the study.

Diuron concentrations in the sediment fraction decreased significantly throughout the experiment. It should be underlined that there was variance between microcosm replicates in the rate of diuron disappearance. At the end of the study, the total amount of diuron in all the soil-contaminated microcosms was diminished by 91% to 98% with DT50 of 5.6 (±0.9) days in "Diuron + Soil" microcosms and 18.3 (±4.2) days in "Soil" microcosms. At weeks 2 and 4, the metabolite DCPMU was detected in the water phase in contaminated microcosms at concentrations varying from 0.42 to 1.09 µg/L (data not shown). Additionally, low levels of DCA (<0.1 µg/L) were also detected in "Soil" microcosms at week 2.

Bacterial densities remained relatively constant throughout the study. However, analysis of the global structure of the bacterial community revealed a marked distinction between non-treated soil, diuron-treated soil, and sediment bacterial diversities, as shown by principal component analysis (PCA). This difference appeared to occur at 2 weeks, but at the 4 and 9 week measurements, the bacterial community structure in the four microcosms was very close to that found in the initial sediment community.

For the diuron mineralisation results, upstream sediments, assumed to be non-exposed to diuron, exhibited a low diuron mineralization potential (about 5% $^{14}CO_2$ resulting from the mineralization

of ^{14}C-diuron after 12 weeks) with a diuron DT25 higher than 600 days and a maximal mineralization rate >0.1% per day. Conversely, diuron-treated and non-treated soils could be easily distinguished according to their diuron mineralization potential. After 12 weeks of incubation, soil exposed to diuron showed a 2.5-fold higher ability to mineralize diuron (53.3± 0.9%; no initial lag-phase) than non-treated soil (20.7± 2.7%; initial lag-phase of about 2 weeks). Furthermore, the comparison of diuron DT25 values (about 18 days in treated soil versus 98 days in non-treated soil) and maximal mineralization rates (about 1.9% per day in treated soil versus 0.4% per day in non-treated soil; obtained from radiorespirometry studies statistically confirmed that the diuron-treated soil microbiota had indeed adapted to rapidly mineralize diuron.

Two weeks after the microcosms were set-up, there were no significant differences between the four treatments in terms of mineralization kinetics, diuron DT25, and maximal mineralization rates.

After 4 weeks, diuron mineralization potentials increased in all the microcosms supplemented with diuron and/or diuron-treated soil. After respective initial lag phases of about 3 and 4 weeks, mean cumulative percentages of $^{14}CO_2$ resulting from diuron mineralization by sediments from "Soil" and "Diuron" microcosms reached over the 50% mark after 12 weeks of incubation, without significant difference ($p > 0.05$) between the two treatments for DT25 values (about 50 days) and maximal mineralization rates (about 1.4% per day). Diuron mineralization potential was also clearly stimulated in the "Diuron + Soil" microcosms although this treatment showed strong between-replicates variation in mineralization kinetics and maximal mineralization rates.

After 9 weeks of incubation, the diuron mineralization potentials in the "Control," "Diuron," and "Soil" microcosms were quite similar to those observed after 4 weeks. Sediments from "Diuron + Soil" microcosms showed very similar mineralization potentials to the "Soil" microcosm, with low between-replicate variation. No statistical difference was observed between the three treatments for DT25 values but maximal mineralization rate were significantly lower ($p < 0.05$) in "Diuron" microcosms in comparison to the two other treatments.

Conclusions from this study: This study helps demonstrate that diuron contamination via runoff and/or erosion may stimulate the diuron mineralization capacities of the sediments. The resulting mineralization potentials provide evidence for increased diuron biodegradation potential of benthic microbial communities in chronically exposed watersheds. The results suggest that interrelations in soil–water–sediment systems involve not only chemical functions but also biological functions.

Long term impacts on sediment microbiota would appear unlikely based on the results of this study, however, short term (2-4 weeks) effects on bacterial community structures could occur.

Title	Chronic herbicide exposures affect the sensitivity and community structure of tropical benthic microalgae
Authors	Magnusson et al
Date	2012
Test Guideline	None stated
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment

Test System: The potential for pollution induced community tolerance (PICT) to develop in tropical periphyton (benthic microalgal) communities was investigated by exposing naturally seeded communities to four concentrations diuron for 4 weeks in laboratory microcosms. A multi-faceted approach was used to detect effects on toxicological (dose–response), functional (photosynthetic quantum yield) and structural (community composition) levels. Phyto-PAM fluorometry was used to explore algal class-specific sensitivities to diuron and subsequent changes in community structure, alongside analysis of algal class-specific pigments using HPLC and direct

cell counts of preserved material. Furthermore, the ability of the community to recover following a prolonged herbicide-exposure was tested.

Sediment and mangrove root scrapings were collected from a local tidal creek near Townsville, tropical Queensland, Australia to colonize microcosms with a natural biofilm. The sediment samples were homogenized by gentle stirring of the slurry. The slurry was allowed to settle and excess water decanted before dispensing 10 mL sediment in 10 cm diameter Petri-dishes and placing two dishes in each of 10 microcosms (10 L aquaria) custom built as raceways with a small propeller mounted in one corner driving the circulation of the water. White fluorescent lights provided an average light intensity of 51 ± 3 µmol photons/s/m^2 across all aquaria with a 12:12 h light:dark cycle. A peristaltic pump system delivered water to this flow-through system from a header tank, fed by 1 µm filtered sea water at 24^{o}C, at a flow-rate of 10 mL/min.

After a 2-week acclimation period of the seeding community, glass microscopy slides were vertically suspended hanging from Perspex rods into the water column to encourage fouling/benthic species to colonize for 4 weeks. Any variability in colonization between microcosms was minimized by randomly transferring the slides between aquaria during the colonisation period. Subsequently, a 4-week continuous herbicide exposure was initiated, using diuron as a model PSII-inhibiting contaminant, followed by a 2-week recovery period in uncontaminated water. Four different diuron concentrations (1.5, 3.1, 6.5 and 13.4 µg/L measured concentrations) were delivered from stock solutions. Two additional microcosms received DMSO-control solution in an identical manner.

Glass slides were sampled weekly for 4 weeks, including day 0. Five slides per microcosm were sampled in each analysis by scraping the biofilm off the surface of the slide using a razor blade and suspending the biomaterial in 15 mL of water taken from the sampled aquarium. Separate aliquots (2 mL) of the suspension was used for Phyto-PAM readings or preserved in 10% formaldehyde/ filtered sea water (v/v) for later cell counts, and the remainder gently filtered and immediately stored -8^{0}C for HPLC pigment analysis.

For the recovery experiment at the end of the 28-d exposure, the remaining slides in each microcosm-aquarium were temporarily moved to a separate container while the contaminated water was pumped out and replaced by uncontaminated water before the slides were re-inserted into the same aquaria. Final sampling was carried out as described above after 2 weeks in uncontaminated flow-through water.

Findings: The biofilms developed a tolerance to diuron during the four week exposure period as measured by Phyto-PAM fluorometry. The overall results are summarised as follows:

Table V3.13: Summary of effect-concentrations (µg/L) for all end-points and sampling occasions.

	Sampling day					
	0	7	14	21	28	14 d recovery
IC50 (Phyto-PAM)	11.4	14.0	17.0	25.6	NA	NA
IC10 (Phyto-PAM)	1.1	0.96	1.4	1.2	NA	NA
LOEC (Phyto-PAM)	1.6	1.6	1.6	1.6	1.6	13.4
LOEC community structure	NS	3.1	1.6	1.6	1.6	NS
LOEC Diatom abundance	NS	NS	NS	13.4	6.6	NS
LOEC (PICT)[1]	NS	NS	6.6	6.6	6.6	NA

1) PICT was only formally tested in an acute bioassay after the initial 24 h recovery, however, communities from the two highest exposure concentrations showed significantly less inhibition based on Phyto-PAM on days 14-28 than on days 0-7 in the same treatment.; NS = Not significant; NA = Not applicable.

The PAM measured IC10 values above were for the whole community. Lower or higher values were obtained for different algal classes. For example, at day 7, the IC10 for cyanobacteria was 1.7 µg/L while that for Ochrophytes was 0.63 µg/L. The whole community IC10 was 0.96 µg/L.

The combination of techniques used in this study enabled the identification of PICT in tropical estuarine periphyton in response to chronic diuron exposures. However, microscopy and pigment analysis revealed that this decrease in sensitivity was accompanied by a shift in species composition towards communities dominated by diatoms. Community composition changed compared to controls at 1.6 µg/L while development of PICT was evident at 6.5 µg/L, with no recovery over 2 weeks in uncontaminated water, indicating chronic pollution induced shifts in community structure.

Conclusion: While there were lower IC10 values obtained for individual algal classes, the community LOEC is a good measure from this study to consider diuron effects under more real-world situations. While there was a demonstration of PICT, the accompaniment of this by a shift in community structure should not be ignored. A $LOEC_{community}$ of 1.6 µg/L is in very close agreement with the 95^{th} percentile protection level of 1.56 µg/L used for freshwater surface waters in the diuron assessment.

Title	Co-tolerance of phytoplankton communities to photosynthesis II inhibitors
Authors	Knauer et al
Date	2010
Test Guideline	None stated
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment

In the previous assessment (APVMA, 2011) the work of Knauer et al (2008) is described where an outdoor mesocosm study was conducted for a period of seven months and included a six week pre-exposure, five week constant exposure and five month post exposure period. In the diuron only experiment, the test concentration for diuron was a nominal 5 µg/L, which was held constant for around 34 days before being able to dissipate (half life of 43 days). While the results from this study did show recovery in algal communities, the time period for recovery was long although it was recognised the exposure concentration was high and the period was long compared to what may be expected in the environment.

This study follows on from the outdoor study, whereby samples of the algal community during the diuron dissipation period from the mesocosms were further exposed to a range of doses of diuron (and other PSII herbicides) in a short term experiment (3 h exposure). Phytoplankton assemblages from the untreated control ponds as well as from the treated mesocosms were sampled on days −8, 5, 12, 19, 26, 40, 54, 82 and 110 after application for taxonomic determination and community analysis.

Water samples were collected at four locations in each mesocosm. The sampling tube was lowered to the sediment surface, then lifted several centimetres above the sediment surface to avoid sample contamination with sediment and closed with a plastic plug to collect a sample of the entire water column. Of the total water volume gained by this procedure, 10 L was then filtered over an Apstein plankton net with a mesh size of 55 µm. From the filtrate, a subsample of 50 mL was preserved with 2 mL Lugol iodine solution for further taxonomic determination.

The remaining filtrate was used for short-term inhibition tests in the laboratory to determine dose–response relationship between the herbicides and photosynthetic efficiency of the algal assemblage.

To investigate tolerance and co-tolerance pattern of the treated phytoplankton in short-term laboratory, experiments phytoplankton was sampled on days 40, 54, 61, 68, and 96 after the first application from all 15 mesocosms. These were exposed to diuron at 1.25, 2.5, 5.0 and 10 µg/L.

One algal sample served as medium control receiving only untreated filtered pond water. Another sample served as a solvent control. Exposure lasted for 3 hours. For the taxonomic determination, aliquots of the fixed phytoplankton samples from days 40, 54, 82, and 110 were taken and cells were allowed to settle in a sedimentation chamber for at least 24 h. Quantitative evaluation was done by using an inverted microscope with a 400× magnification

Findings: According to the PCA from the outdoor mesocosm component of the study, the algal composition was comparable in all mesocosms before start of the exposure period (day −8). On day 40, at the end of constant exposure (mid June), the community structure of phytoplankton from the mesocosms treated with the single herbicides and the mixture were all statistically significantly different from the control. However, community structure and species composition of the diuron treated mesocosm recovered and was similar to that of the control ponds from day 54 onwards with the exception of day 82.

In this study, as acute toxicity endpoints, effects on the photosynthetic efficiency determined as *in vivo* chlorophyll fluorescence of the phytoplankton were evaluated. The following results for diuron using algae from the control mesocosm and from the pre-exposed diuron mesocosm were reported as follows:

Table V3.13: Summary of findings, Knauer et al (2010)

Phytoplankton pre-exposed to:	LOEC (µg/L)				
	Day 40	Day 54	Day 61	Day 68	Day 96
Control	1.25	5	1.25	1.25	1.25
Diuron	5	1.25	5	10	2.5

With the exception of the day 54 sample, the algae that had been pre-exposed to diuron under chronic exposure conditions in the mesocosms had higher LOECs ranging from 2.5-10 µg/L. The day 54 sample algae had a LOEC of 1.25 µg/L.

Conclusions from this study: These results indicate that the algal community was less susceptible to adverse effects of diuron exposure after it had been pre-exposed. Similar findings were made for atrazine, isopropturon and a mixture of these three compounds in that the algal communities pre-exposed to the herbicides showed less sensitivity to re-exposure than algal samples that had not previously been exposed.

Title	Phytotoxicity of atrazine, isoproturon and diuron to submersed macrophytes in outdoor mesocosms.
Authors	Knauert et al
Date	2010
Test Guideline	None stated
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment

This study assessed the effects atrazine, isoproturon, diuron and of their mixture on photosynthetic efficiency and growth of the three submersed macrophytes *Elodea canadensis*, *Myriophyllum spicatum* and *Potamogeton lucens* in an outdoor mesocosm experiment. The first objective was to determine species-specific sensitivities of the three macrophytes to the single and mixture herbicide treatments. A second objective was to compare effects from the single herbicide exposure to the mixture treatment with respect to additive toxicity. Only the diuron alone treatment is reviewed here.

Exposure started on 3 May 2006 (day 0). After the first application (day 0), herbicide concentrations in the single and mixture treatments were kept rather constant over a period of five weeks, i.e. the herbicides were supplemented to the ponds if necessary to maintain target

concentrations at approximately ±20%. The herbicides were applied with a sprayer on the water surface of the mesocosms. The water column was than stirred for approximately 5 min with a polyethylene tube to achieve a homogenous distribution of the test compound. Separated sampling equipments such as tubes, buckets etc. were used for each individual treatment to avoid cross contamination.

Sampling for chemical water analysis to control herbicide concentrations started on the day of application. For chemical analysis depth-integrated water samples were taken from the four quadrants of each mesocosm using a polyethylene tube, 120 cm long and 4.5 cm in diameter. Samples were taken by lowering this tube close to the mesocosm sediment surface to avoid sample contamination due to sediment perturbation. Concentrations to be re-applied were calculated based on these measurements.

Two 7 cm-cuttings of the apical part of *M. spicatum* were planted together in one plastic pot of 5 cm-diameter filled with rock wool (artificial substrate) to assure robust fixation. The same procedure was performed with *E. canadensis. P. lucens* was delivered as potted plants. The substrate for *P. lucens* comprised 28% pumice, 24% fractional expanded clay, 8% sand, 12% clay, 16% bark humus and 12% white peat. The *P. lucens* plants had not developed any leaves from the overwintering rhizomes in early spring (April) when they were introduced into the mesocosms. In each mesocosm, twelve pots with *M. spicatum*, four pots with *E. canadensis* (four pots to measure length only) and ten pots with *P. lucens* were arranged on the top of plastic boxes which were then introduced into the mesocosms. The macrophytes were put in a depth of 70 cm below the surface and allowed to acclimate for three (*P. lucens, M. spicatum*) and two weeks (*E. canadensis*).

The twelve replicates of *M. spicatum* were used to study natural variability of plant growth. The four pots with *E. canadensis* were used for growth measurements only whereas photosynthetic efficiency was measured from the plants growing in the sediment.

Effects on photosynthetic efficiency (PE) of the three submersed macrophytes were determined with a MINI PAM fluorometer by measuring *in vivo* chlorophyll fluorescence using the saturating pulse method. For that purpose, plants were lifted out of the ponds. Prior to the fluorescence measurements, leaves and shoots were carefully sprayed and washed with filtered pond water to reduce the amount of attached periphyton. Subsequently, one leaf in the case of *P. lucens* or one shoot in the case of *M. spicatum and E. canadensis* was placed in the leaf clip holder of the PAM device. Ten measurements on different leaves or shoots were performed for each species always at the same time of the day on sampling days 1, 2, 5, 12, 19, 26, and 34 in the various treatments and control.

To determine growth rates of *M. spicatum* and *E. canadensis*, lengths of the main and the side shoots of the potted plants were measured on day 1, 5, 12, 19, 26 and 34. Total plant length values, i.e. the sum of main and side shoots, were transformed to ln(length) values and plotted versus time to calculate relative growth rates (RGR) according to a least square linear regression.

Findings: The target concentration of diuron in the mesocosms was 5 µg/L and the time weighted average measured concentration was 4.9±3.8 µg/L with re-application occurring on days 12 and 20.

Diuron significantly reduced PE of *E. Canadensis* on days 2 and 5 resulting in 57 and 80% of control, respectively. PE of *P. lucens* was significantly reduced to 55% by diuron on day 5 only.

The relative growth rates of *E. canadensis* and *M. spicatum* over the 34 day exposure period were 0.019/d and 0.008/d respectively. These were not significantly reduced compared to those in control treatments of 0.017/d and 0.009/d respectively.

Conclusions for this study: Diuron concentrations around 5 µg/L, while having a transitory effect on photosynthetic efficiency of submerged macrophytes, did not have any effect on their growth rate over the 34 day exposure period of the test.

Title	Responses of chronically contaminated biofilms to short pulses of diuron: An experimental study simulating flooding events in a small river
Authors	Tlili et al
Date	2008
Test Guideline	None stated
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on for this assessment

The purpose of this study was to investigate the impact of diuron in river biofilms (autotrophic communities named as periphyton, and heterotrophic communities), and especially to study the sublethal and long-term effects of diuron pulses on an indoor microcosm mimicking different exposure scenarios resulting from flood events.

Water and stones containing biofilms were collected from the Morcille River (eastern France) at an upstream and unpolluted reference site. The acute diuron concentrations used in the microcosm study were similar to those at the polluted downstream site (vineyard area). Two 35 L aquariums were filled with river water from the reference site (filtered through a 500 µm mesh to remove most of the grazers). Two stones from the same site, which were coated with biofilms, were brought to the laboratory and one was placed at the bottom of each aquarium to act as a natural inoculums. The aquaria were connected to each other to ensure homogeneity of the microbial communities. After 3 days, this connection was cut off, the stones removed and 70 artificial substrates (microscope slides) installed. At this point, one aquarium was contaminated with a nominal 1 µg/L diuron to represent a chronic exposure level. The other was used as a control.

During the experiment (32 days) the aquarium water was replaced weekly and in the contaminated aquaria, diuron was re-dosed. No replicates were used. To assess short-term contamination scenarios, two nanocosms were filled with 3.5 L filtered water from the reference site and were contaminated with a nominal concentration of either 7 or 14 µg/L diuron. On day 21 of the experiment, biofilm samples from the control and chronically contaminated microcosm were subjected to short-term (3 h to simulate average duration of a flood event in a small stream) pulses of diuron by plunging the colonised microscope slides into the highly contaminated nanocosms. After each pulse, the slides were gently rinsed and returned to the microcosm. Some slides that had been subjected to the first 7 µg/L pulse were subjected 4 days later to a second 7 µg/L pulse. In all, the following exposure scenarios were therefore considered:

Table V3.14: Summary of findings, Tlili et al (2008)

	Non-pulsed	Day 21	Day 21; Day 25*	Day 21
Control	No exposure	3 h pulse, 7 µg/L	3 h pulse, 7 µg/L	3 h pulse, 14 µg/L
Diuron exposure	1 µg/L chronic	3 h pulse, 7 µg/L	3 h pulse, 7 µg/L	3 h pulse, 14 µg/L

* Some only

On days 21, 28 and 32, biofilms were sampled and analyses performed. Total biomass and *in vivo* chlorophyll-α fluorescence analysis and bioassays (carbon incorporation) were performed immediately after sampling while others (chemical, molecular and pigment analysis) were performed subsequently on deep frozen biofilm. Diuron concentrations in both water and biofilm samples was determined by ESI-LC-MS-MS. Total biomass was evaluated by calculating the ash free dry weight (AFDW). Algal biomass was estimated by *in vivo* chlorophyll-α fluorescence measurements.

To test the concept of pollution induced community tolerance, the effects of increasing concentrations on biofilm using ^{14}C photosynthetic assimilation was monitored. A semi-logarithmic series of concentrations was prepared with final test concentrations ranging from 0.737 to 737.12 µg/L, five blanks and three replicates for each of the seven increasing concentrations. Photosynthesis activity was measured by ^{14}C incorporation.

Findings: *Biomass parameters*: AFDW increased in both contaminated (1 µg/L) and control microcosms throughout the 32 days. Chronically exposed biofilms were characterised by having higher biomass than the control ones, especially at day 32. At day 28 of growth, the effects of chronic exposure or chronic plus pulse exposure interaction on AFDW were significant. No significant differences in AFDW biomass were reported between single-pulsed and non-pulsed biofilms at day 28 of growth apart from the higher diuron pulse (14 µg/L) on control biofilms, whereas on day 32 of growth, the effect of chronic and pulsed exposures on AFDW were significant and the biomasses of the double pulsed biofilms were significantly lower than that of the non-pulsed ones.

In vivo fluorescence was significantly higher in chronically exposed biofilms at the three sampling dates. At day 28 of growth, the effect of chronic exposure, pulses and chronic pulse exposure were all significant. At the same date, no inhibition of *in vivo* fluorescence was found for single pulsed and chronically exposed biofilms, but significant inhibition was found for single pulsed control biofilms.

Carbon incorporation and EC50 values: Carbon incorporation by the biofilm was significantly higher in the chronically exposed microcosm during the first 28 days of growth. At day 28 growth, chronic exposure, pulsed exposure and chronic + pulsed exposures all had a significant effect on carbon incorporation. Single and double pulses significantly inhibited carbon incorporation by the biofilm communities, especially in the control biofilms. The short term inhibition of photosynthesis (EC50) fluctuated throughout the experiment, but was never significantly different in exposed and non-exposed biofilm.

Community structure: Bacterial structural differences were observed between single-pulsed and non-pulsed biofilms, but not between double-pulsed and non-pulsed biofilms. The different pulses affected the eukaryotic community structures in the control biofilms, but not of the chronically exposed ones. Unlike the bacterial communities, the control eukaryotic communities were structurally different from the chronically exposed ones.

Conclusions from this study: This is a complex study measuring a number of end-points and exposure scenarios. The results tend to indicate an increased tolerance to pulse exposures to diuron from chronically (1 µg/L) exposed periphyton communities. However, where periphyton communities were not continually exposed to low level diuron concentrations, there were adverse impacts with, for example, significantly lower biomasses and significant inhibition of *in vivo* fluorescence when exposed to pulses of diuron.

Title	Impact of chronic and acute pesticide exposures on periphyton communities
Authors	Tlili et al
Date	2011
Test Guideline	None stated
Data Validity	4
Data Relied On	No – the data were considered for information only and not relied on in this assessment

This study was undertaken with two aims. The first aim was to study the sublethal and long-term effects of successive pulses with the same pesticide mixture on periphytic communities, mimicking different flood-event exposure scenarios. The second aim of this study was to gain deeper insight into the relationship between toxicant dynamics, either in the water column or in the periphyton matrix, and the impacts on periphytic communities.

The study used an outdoor mesocosm platform using four stainless steel channels (4 m long, 0.4 m wide and 0.35 m deep). The channels were run in a semi-open mode and were continuously supplied with 36 m-deep incoming water from Lake Geneva, ensuring an adequate supply of nutrients and natural seeding of periphytic communities. The entire volume of water in the system (2.2 m^3 per channel) was replaced 4 times a day. Water flow and velocity in the channels were controlled using a valves-and bypass system. At the start of the experiment, artificial substrates (279 1.5-cm^2 frosted glass disks and 168 16-cm^2 frosted glass slides) were installed horizontally in each channel. The outdoor mesocosms were subject to the seasonal variations of the natural environment (light, temperature, day length and water quality) during the 3-month exposure.

Diuron and tebuconazole, were used in mixture during the chronic and successive pulse exposures. Two channels were chronically contaminated with this mixture at a ratio of 2:1 for diuron and tebuconazole respectively, and at a nominal concentration of 1 µg/L of diuron and 0.5 µg/L of tebuconazole. The two remaining channels were used as controls. During the experiment, chronic contamination of channels was controlled by a peristaltic pump delivering a constant supply of pollutants.

After 28 days of growth, periphyton from one control and one chronically-contaminated channel were subjected to three successive short-term pulses, controlled by the peristaltic pump, with the same compositional mixture as for chronic exposure at the same ratio (2:1) but at higher concentrations (20 µg/L of diuron and 10 µg/L of tebuconazole) simulating the sudden increase in pesticide concentrations during a flooding event. To simplify the study, impacts of hydrodynamics on periphyton were not considered. Each pulse was followed by a 7-day recovery period with a return to initial exposure conditions. These 3 short-term exposures were performed at day 28, day 35 and day 42 of the experiment. Total duration of each pulse was 17 h consisting of a 5-hour increase in pesticide concentration, a 6-hour period of peak exposure, and a 6-hour decrease. During pulses, the 4 channels were turned from semi-open to closed systems in order to facilitate the control of pollutant concentrations and pulse exposure periods.

Periphytons were sampled randomly in each mesocosm just before the first pulse on day 28, 1 week after each pulse on day 35, day 42, day 49 and 3 weeks after the third pulse on day 63. Total biomass, carbon incorporation and photosynthetic efficiency were analyzed immediately on the periphyton communities on small frosted-glass disks, while molecular fingerprinting was performed later on using deep-frozen samples (−80°C) scraped off big frosted glass slides. Furthermore, periphyton were also sampled during each pulse at just before the pulse, then at 5, 11 and 24 h after the pulse to monitor photosynthetic efficiency on the periphyton communities on small frosted-glass disks and pesticide bioaccumulation on the periphyton communities on big-frosted glass slides.

Tebuconazole, diuron and its main metabolites DCPMU and DCA were measured in water and periphyton samples by ESI-LC-MS/MS at days 28, 35, 42, 49, 63 and during each pulse (at 0, 5, 11 and 24 h). Biological analysis considered of assessment of the periphyton collection, total and autotrophic biomass, and photosynthetic efficiency monitoring. Short term bioassays were conducted to assess pollution induced community tolerance (PICT) whereby a semi-logarithmic series of concentrations was freshly prepared by serial dilution of a stock solution in 0.2-µm filtered incoming water. Final test concentrations in the test vessels ranged from 0 µM to 3.2 µM (~750 µg/L; 3 blanks without toxicant and 3 replicates for each of the 5 increasing diuron concentrations). One week after the first and third pulses and 3 weeks after the third pulse, periphyton samples (n=3 per test concentration) were exposed to increasing concentrations of diuron during 3 h, under exposure to light and continuous gentle shaking. Measurements were performed on a PhytoPAM fluorometer. The relative inhibition of photosynthetic yield at 665 nm in relation to controls was calculated to model concentration–response relationships and plot a concentration–response curve to determine photosynthetic EC50 values for each channel and period.

Findings: In the pulsed control, mean diuron concentrations in water at 0, 5, 11 and 24 hours after the pulse were <0.05, 13.7, 13.3 and 0.9 µg/L respectively while in the pulsed chronic channel, they were 0.69, 15.5, 15.4 and 2.13 µg/L respectively. Thirteen hours after pulse cessation, low pesticide concentrations were still detectable in the pulsed-control and pulsed-chronic channels.

Eleven hours after pulse starts, diuron was detected in the periphyton matrices in both pulsed channels. In the pulsed chronic channel, accumulated diuron amounts increased as periphyton underwent increasing successive pulse exposures. Consequently, one week after the last pulse, diuron concentration in the periphyton was still high (reported as 0.48 µg/L, but possibly should be 0.48 µg/g as calculated for the ash free dry weight - AFDW), unlike in the pulsed control channel where diuron was not detected. However, three weeks after the last pulse, diuron concentration decreased back to a more or less similar value to that measured before the pulses started.

Total and Autotrophic biomass: In measurements of total and autotrophic biomass, AFDW was relatively constant in both pulsed chronic and control mesocosms throughout the 63 days of the experiment. Control periphyton were characterized by a significantly higher biomass than chronically-contaminated periphyton except at days 49 and 63. Otherwise, 1 week after the first pulse, the effects of chronic exposure and pulse exposure taken separately were significant. Periphyton from the pulsed chronic channel showed lower AFDW than periphyton from the non pulsed-chronic channel one week after each pulse. At day 42, one week after the second pulse, the additive effect of chronic and pulse exposures was significant and there was no significant effect of chronic or pulse exposure when they were considered separately. After the second pulse, periphyton from the pulsed-chronic channel was characterized by significantly lower biomass than periphyton from the non pulsed-chronic channel whereas biomasses of periphyton from the non pulsed- and double pulsed-control channels were not different. At day 49, one week after the third pulse, there was no significant effect of the different modalities of exposure on the AFDW of the pulsed and non pulsed periphyton, whatever the original channel. However, at day 63, three weeks after the last pulse, periphyton from the triple pulsed channels (pulsed-control and pulsed-chronic) showed higher biomasses than periphyton from non pulsed channels (control and chronic).

Primary production: Periphyton from the non pulsed-chronic channel were characterized by higher primary production than periphyton from the non pulsed-control channel at the beginning of the experiment (days 28 and 35), and this trend reversed at days 49 and 63. In fact, while there was a slight increase in primary production from the non-pulsed control channel over the course of the experiment, there was a large decrease in primary production from the pulsed chronic channel between days 35 and 42, after which primary production remained relatively constant.

Based on measurements of carbon incorporation, at day 35 and day 42, the additive effect of chronic and pulse exposures was significant. Pulses inhibited carbon incorporation by chronically-exposed periphyton but not by control periphyton. At day 49, chronic exposure, pulse exposure and chronic-pulse exposure interaction all had significant effects on carbon incorporation. Three successive pulses significantly inhibited carbon incorporation by control periphyton but stimulated carbon incorporation by chronically exposed periphyton. Three weeks after the third pulse (day 63), only the chronic exposure still had a significant effect on carbon incorporation, whereas no significant differences were observed between the periphyton from pulsed-chronic and non pulsed-chronic channels or from pulsed-control and non pulsed control channels.

Photosynthetic efficiency during pulses: The photosynthetic efficiency of periphyton was first inhibited by each pulse before recovering after pulse cessation. During the first pulse, at peak pulse exposure, which was from t0+5 h to t0+11 h, the photosynthetic efficiencies of periphyton were significantly inhibited by 43±6% in the pulsed-control channel and 58±4% in the pulsed-chronic channel compared to the non pulsed channels. However, during the second pulse, the pulsed-control periphyton was more affected than the pulsed-chronic periphyton (58±4% and 49±1%, respectively) and during the last pulse this tendency was confirmed (59±6% and 39±5%, respectively). Generally, as pulsed-chronic periphyton were subjected to successive pulse exposures, the inhibition of photosynthetic efficiency progressively weakened compared to non pulsed-control periphyton, especially at the third pulse.

Diuron induced tolerance: The short-term bioassays of photosynthetic efficiency gave relatively stable EC50s throughout the experiment, but were never significantly different between periphyton from non pulsed chronic and control channels. At day 35, the effects of the chronic exposure and pulse exposure were taken separately, and interaction effect of both were not significant. Single pulsed periphyton (chronic and control) showed similar sensitivities to diuron as non-pulsed periphyton (chronic and control). In contrast, at day 49, only the interaction effect of chronic and pulse exposures was significant. Periphyton from the triple pulsed-chronic channel showed a significantly higher EC50 than periphyton from the triple pulsed-control channel and from the non pulsed periphyton (chronic and control). At the end of the experiment, there were no significant differences between periphyton sensitivities to diuron whatever the previous exposure history (chronic, control, pulsed or non pulsed). Pearson product moment correlation analysis showed that phototrophic community tolerance to diuron was positively and exclusively correlated to diuron concentration in the periphyton matrices.

Conclusions from this study: There is a lot of information provided from this test. During the first pulse, photosynthetic efficiency was correlated with pesticide concentration in the water phase, and there was no difference between periphyton from chronically contaminated channels and control channels. However, during the second and third pulses, the photosynthetic efficiency of periphyton chronically exposed to pesticides appeared to be less impacted by the acute pulsed exposure of pesticide. These changes were consistent with the acquisition of induced tolerance to diuron since only after the third pulse that periphyton from chronic channel became tolerant to diuron. However, the findings on primary production appear to counteract this. While photoshythetic efficiency appears to adapt to continued low level exposure with pulses at higher levels, the primary production of the periphyton community did not. While there was increased primary production in exposed channels earlier in the experiment, this was significantly diminished by the second pulse, and did not appear to recover to control levels throughout the course of the experiment.

In general, the results of this study are complex and difficult to interpret. This is particularly so as the results are confounded the presence of tebuconazole. While the study appears to lend support that pre-exposure to diuron will not lead to increased effects following repeat exposure, these results are considered information only as there is no way of distinguishing between effects resulting from diuron or tebuconazole exposure.

V3.3.2 Non-Standard laboratory tests

As explained in APVMA (2011), DSEWPaC is not using toxicity tests based on impacts on chlorophyll fluorescence in the standard risk assessment framework. However, where results have become available, they are reported for completeness.

This methodology is becoming well developed, and for interpretation, the following definitions have kindly been provided by Dr. Andrew Negri, Australian institute of Marine Science:

> **PAM fluorometry summary:** The photosynthetic efficiency, expressed as the maximum quantum yield (measured after dark adaptation) and effective quantum yield (achieved under experimental light conditions) of algae, seagrasses and symbiotic algae in corals can be estimated from chlorophyll fluorescence measurements taken with a pulse amplitude modulated (PAM) chlorophyll fluorometer (Maxwell and Johnson, 2000).
>
> **Light adapted yield = effective quantum yield**: The effective quantum yield in an illuminated plant ($\Delta F/Fm$) provides an estimate of the efficiency of photochemical energy conversion within PSII under a given light intensity (Genty et al., 1989). The reversible binding of PSII herbicides to the D1 protein in PSII results in an acute reduction $\Delta F/Fm$ (Jones and Kerswell, 2003) and reduced energy acquisition by the host coral (Cantin et al., 2009).
>
> **Dark adapted yield = maximum quantum yield**: The maximum quantum yield ($Fv/F'm$) is equivalent to the proportion of light used for photosynthesis by chlorophyll when all reaction centres are open (Genty et al., 1989). A reduction Fv/Fm indicates photooxidative damage to PSII (chronic photoinhibition) and is observed in bleaching corals due to temperature stress (Jones et al., 1998), herbicide exposure (Negri et al., 2005) or combinations of these pressures (Negri et al., 2011).

Title	Symbiont-specific responses in foraminifera to the herbicide diuron
Authors	Van Dam et al
Date	2011
Test Guideline	None identified
Data Validity	2*
Data Relied On	Yes – the data were considered critical and relied on in this assessment

The effects of diuron were assessed tropical foraminifera hosting diatom, dinoflagellate, red or green algae endosymbionts. As explained in the paper, benthic foraminifera are mobile, single-celled calcifying protists and widely distributed through the world's oceans; however, most symbiotic species are found in tropical waters. Symbiotic foraminifera can contribute significantly to total calcification on coral reefs and in contrast to corals that only host dinoflagellates of the genus *Symbiodinium*, foraminifera can host a variety of microalgal groups, including dinoflagellates (*dinophyta*), diatoms (*bacillariophyta*), red algae (*rhodophyta*) and green algae (*chlorophyta*).

The foraminifera were collected by hand using SCUBA from various inshore sites of the GBR between August 2008 and April 2010. Rapid light curves (RLCs) were established for 6 different species utilizing pulse-amplitude modulated (PAM) chlorophyll fluorescence techniques to calculate optimal light intensities for maintenance and toxicity testing.

Maintenance and dosage experiments were conducted under 12 h:12 h diurnal light–dark cycles. A total of 13 different species of foraminifera were used in dose–response toxicity bioassays. These species host at least four different taxa of endosymbiotic microalgae, with one species tested

carrying retained chloroplasts. *Marginopora vertebralis* was collected at both inshore and midshelf reefs, while *Heterostegina depressa* exists in two different reproductive stages. These varieties were tested individually.

Experimental organisms were exposed to concentrations of 0.3, 1, 2, 3, 10, 30 and 100 µg/L diuron in aerated 0.5 µm filtered seawater for up to 48 h. PSII yield was measured in exposures and tested against a solvent control (carrier only). Two sets of dose–response experiments were performed. One set was aimed at investigating diuron uptake, transport, effect on PSII and recovery over time. A subset of 7 species of foraminifera (*H. depressa*, *Amphistegina radiata*, *Calcarina mayorii*, *Alviolinella quoyi*, *M. vertebralis* – collected inshore and offshore – and *Peneropolis planatus*) were exposed to diuron for 48 h, followed by a 48 h recovery period after washing and transfer to uncontaminated seawater.

Chlorophyll fluorescence parameters were measured immediately before transfer to herbicide solutions and following 2, 6, 24, 48, 72 and 96 h incubation to determine effective quantum PSII yield Δ*F/F'm* and potential quantum PSII yield *Fv/Fm* after 30 min dark adaptation.

The following results were reported (95th CI in parentheses):

Table V3.15: Summary of findings, Van Dam et al (2011)

Species	Exp. light conditions[1]	24 h IC20 (Δ*F/F'm*), µg/L	24 h IC25 (F_v/F_m), µg/L
Diatoms			
H. depressa macro	5	2.9 (2.6-3.6)	20.4 (17.6-23.6)
	10	-	4.7 (4.1-5.4)
A. radiata	5	3.5 (3.2-3.9)	25.9 (16.7-44)
	10	-	7.1 (5.8-8.9)
A quoyi	5	11.8 (10.8-12.9)	53 (46-60)
	10	-	11.1
C. mayorii	5	6.6 (4.9-9.4)	53
	10	-	11.3 (10.1-12.5)
Operculina ammonoides	10	-	3.0 (2.7-3.4)
H. depressa micro	10	-	6.0 (4.9-7.4)
Dinoflagellates			
M. vertebralis - inshore	5	6.3 (4.7-8.8)	49.8 (37-67)
	20	-	11.9
M. vertebralis – off shore	5	6.6 (6.0-7.3)	54 (36-87)
	20	-	7.3 (5.3-10.1)
Sorites. orbiculus	20	-	5.4 (4.9-6.1)
Red algae			
Peneropolis. planatus	5	20.3 (17.6-23)	>100
	10	-	12.4
Peneropolis. antillarum	10	-	49.1
Green algae			
P marginalis	10	-	8.6 (7.3-10.4)
Retained plastids			
Eiphidum sp.	10	-	32.6

1) µmol photons/m^2/s photosynthetically active radiation (PAR)

For all species, 25% inhibition of *Fv/Fm* (photodamage) was observed at higher diuron concentrations for specimens incubated at 5 µmol photons/m^2/s PAR than at higher irradiation densities.

All species demonstrated a similar general pattern of diuron-induced PSII inhibition over the 48 h exposure and recovery periods, although the response times and the extent of inhibition varied somewhat. PSII yields of control organisms remained within 5% of initial values over the course of the experiments. Maximum inhibition of PSII yield was reached after 24–48 h incubation for most species, with specimens exposed to concentrations ≥10 µg/L diuron exhibiting the most rapid and pronounced responses.

For *H. depressa* and *A. radiata*, maximum inhibition of PSII yield was reached after 24 h incubation, while *C. mayorii* exhibited an initial slower response in the high concentrations but was comparably affected after 48 h. *Fv/Fm* in these three species recovered quickly (90–100% within 24 h) following washing and transfer to uncontaminated seawater. *A. quoyi* proved somewhat of an exception in comparison among diatom bearers, in that it exhibited a slower response, was less vulnerable to PSII inhibition by diuron and did not fully recover after 48 h in uncontaminated seawater. Like *A. quoyi*, both varieties of dinoflagellate hosting *M. vertebralis* demonstrated relatively slow response and recovery kinetics, with maximum inhibition observed after 48 h for the lower (<10 µg/L) diuron concentrations and incomplete recovery after 48 h. *P. planatus*, hosting red algae, was only affected at concentrations ≥10 µg/L diuron and quickly recovered after transfer to clean medium. Diuron-induced inhibition of *Fv/Fm* elicited comparable, although less pronounced, response over time for all species tested.

Conclusions from this study: For the foraminifera tested under this test method, inhibition of photosynthesis (reduced Δ*F/F'm*) by diuron depended on both symbiont type and test ultrustructure, with greatest sensitivity observed for diatom and chlorophyte hosting species (24 h IC50 2.5-4 µg/L). Damage to PSII was light dependent and occurred at low light intensities indicating limited photoprotective capacity. DSEWPaC considers the results here, while obtained with non-standard test methodology, show the ecotoxicity end-point used in the risk assessment should remain protective to foraminifera.

Title	Phytotoxicity induced in isolated zooxanthellae by herbicides extracted from Great Barrier Reef flood waters
Authors	Shaw et al
Date	2012
Test Guideline	None identified
Data Validity	4
Data Relied On	No – the data were considered information only and not relied on in this assessment

The study aimed to obtain information about the potential effects on corals of PSII herbicides reaching the Great Barrier Reef by sampling a 'first flush' flood plume using an environmentally relevant bioassay. The flood plume sampled occurred in late January 2005, with sampling stations based on the plume reaching inshore areas from the O'Connell River and the Pioneer River. Water samples were collected in 1 L solvent washed brown glass bottles and refrigerated immediately until extraction. Simultaneously, 1 L water samples were collected and later analysed (separately, Rohde et al, 2006) by HPLC/MS/MS, to quantify herbicide concentrations. The herbicide concentrations, in samples where diuron was detected, were reported in Rohde et al (2006) as follows:

Table V3.16: Summary of diuron levels, O'Connell River and Pioneer River January 2005 Flood Plumes (Rohde et al, 2006).

	PSII Herbicide levels in plume samples (µg/L)			
Site	Diuron	Atrazine	Hexazinone	Tebuthiuron
PSC1	0.07	0.02	0.01	0.02
PSC2	0.08	0.01	0.01	n.d.
PSC4	0.44	0.10	0.09	0.08
PSC5	0.18	0.04	0.02	0.02
PSC6	0.24	0.06	0.04	0.05
PSC7	0.13	0.04	0.03	0.03
PSP1	0.33	0.05	0.07	n.d.
PSP3	0.31	0.06	0.07	n.d.
PSP8	0.06	n.d.	0.01	n.d.
PSP12	0.05	n.d.	0.01	n.d.

In all, there were seven sampling sites associated with the O'Connell River ("PSC" sites), with diuron sampled for, and found in six of these. There were 12 sites monitered in association with the Pioneer River plume ("PSP" sites). Diuron was sampled for at five sites and found in 4 of these. Where diuron was found, it was the dominant PSII herbicide found, but was always detected with other PSII herbicides as well.

For the bioassay, biomaterial selected was endosymbiotic dinoflagellates (zooxanthellae, *Symbiodinium* sp.) isolated from the coral *Heliofungia actiniformis*. *H. actiniformis* colonies were collected from Heron Island. This site was chosen because it is located approximately 80 km offshore, and has relatively pristine water quality.

For the dose/response tests, methanol-based sample (flood plume water) extracts were serially diluted with methanol to produce concentrations that were 0.3, 1, 3 and 10 times those present in the flood plume (concentration factors from 0.3 to 10). Diuron standards of 0.3, 1, 3 and 10 µg/L were also formulated in 99% pure methanol for use as positive controls in the dose responses. Each assay was performed in triplicate, and effects determined based on chlorophyll fluorescence analysis.

The relationships between reduction in photosynthetic yield and conductivity, and diuron concentration were analysed using simple linear regressions in SPSS™ statistical program. The equation to determine predicted diuron concentration from given values of photosynthetic inhibition was determined by running a logistic curve estimation in SPSS™ of diuron standard concentration verses observed inhibition of photosynthesis.

Dose/response experiments for reduction of photosynthetic yield were carried out for sites PSP1, 3 and 5; and PSC1, 4 and 6. The choice of PSP5 is unclear, as Rohde et al (2006) report this site was not actually sampled for diuron or other PSII herbicides. The results of the dose/response tests are only provided graphically, so the following interpretations of values by DSEWPaC should be treated as a guide only.

The greatest effects came from the diuron control test where around 30% inhibition was found at 3 µg/L, and inhibition was approaching 60% effects at 10 µg/L. At concentrations found within the flood plumes (concentration factor of 1), there did not appear to be any significant inhibition of photosynthesis. However, as zooxanthellae were exposed to increased concentrations, effects did appear. Around 10% inhibition was found at 3X the PSC4 concentration (diuron concentration of around 1.3 µg/L), with almost 50% inhibition at 10X the PSC4 concentration (~4.4 µg/L). While this appears more sensitive than diuron alone, there were several other PSII herbicides in the flood plume water. For the other sites, inhibition remained <10% at 3 X the flood plume concentrations.

Only 1 site (PSC1) remained at <10% inhibition at 10X the flood plume concentration, while inhibition of between 15% and 40% was found for the other sites at 10X the flood plume concentration.

Conclusions from this study: The flood plume data were previously assessed in APVMA (2011). Those values above, and other plume measurements resulted in a 90th percentile flood plume level of 0.28 µg/L, which was used in the risk assessment. The 99th percentile protection level for diuron was established to be 1.19 µg/L, and an acceptable risk to aquatic organisms in coastal environments was subsequently concluded. The results of this new study do not provide information that will result in a change in this conclusion. With diuron concentrations in flood plumes ranging from 0.05 to 0.44 µg/L in the above study, and the use of chlorophyll fluorescence bioassays, it was shown that these levels did not inhibit photosynthesis, but inhibition >10% was found when the diuron concentration (in mixture with other PSII herbicides) increased to around 1.3 µg/L. This is still a value higher than the 99th percentile value (1.19 µg/L) used in the diuron risk assessment.