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Impact of Diuron contamination on blood cockles (*Tegillarca granosa* Linnaeus, 1758)

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ABSTRACT

Examination of the impact of Diuron contamination on blood cockles (*Tegillarca granosa*) was conducted by combining field screening at three sampling events and a toxicity test. Diuron was extracted using the liquid–liquid extraction (LLE) technique and analyzed using HPLC-UV. The median lethal concentration (LC_{50}) of Diuron on *T. granosa* was tested under a 72-h exposure. Diuron in water samples ranged from not detected (ND) to 3910 ppb, which was the highest concentration detected in samples after the irrigation water was discharged from the paddy plantation. Diuron was not detected in sediment samples. Mortality of *T. granosa* ranged from 4.74 to 38.33% with the highest percentages recorded after the release of the irrigation water. The LC_{50} value of Diuron was 1.84 ppm. This study suggests that irrigation water from paddy plantation that drifts to coastal areas containing Diuron harms *T. granosa* at the study area.

1. Introduction

Malaysia is the largest exporter of adult *Tegillarca granosa* in Southeast Asia, and Selangor is known to be the largest producer in the country due to its favorable habitat for juveniles (Department of Fisheries, 2012). The total amount of land used for *Tegillarca granosa* farms in Selangor in 2016 was 4613 ha (Department of Fisheries, 2016). However, *T. granosa* harvested in Selangor decreased to about 25,000 t in 2011 and further declined to about 2000 t in 2016 (Department of Fisheries, 2016; Yurimoto et al., 2014). The decline in *T. granosa* production was reportedly caused by many factors such as pollution (Ramli et al., 2013, 2014; Dahiya and Ahlawat, 2013), lack of farm management practices (Lokman, 1992), or predators (Broom, 1985). However, no concrete evidence is available to support these potential reasons. A field survey conducted by Yurimoto et al. (2014) along the Selangor coastline estimated that the mortality rate of *T. granosa* was over 30%, thought to be caused by high river flooding of farms (Yurimoto et al., 2014). However, there is limited insight into the stressor in the river water that led to the degradation of the *T. granosa* cultivation area; a highly limited report identified biocide contamination at the *T. granosa* cultivation area despite the affected areas on the Selangor coast being fringed by plantations.

About 37,500 ha of land in Selangor have been used for paddy plantation, situated mainly in the north-western and coastal areas of the state including Kuala Selangor, Sekinchan, and Sabak Bernam Districts (Ministry of Agriculture, 2015). The coastal areas of these districts are also known as shellfish producers. Paddy plantations in the area plant and harvest twice a year; the first cycle starts in March and the harvesting season begins in June, while the second season begins in September and ends in December. In this two-season practice, farmers are likely to use intensive biocides to produce a high yield of crops each season to optimize the paddy growth (Sow et al., 2012). This practice led to an extreme dependency on herbicides to control weeds in plantations without considering the effects on human health and the environment (Dilipkumar et al., 2017; Jabran and Chauhan, 2015).

Diuron (3-[3,4-dichlorophenyl]-1,1-dimethylurea) was investigated

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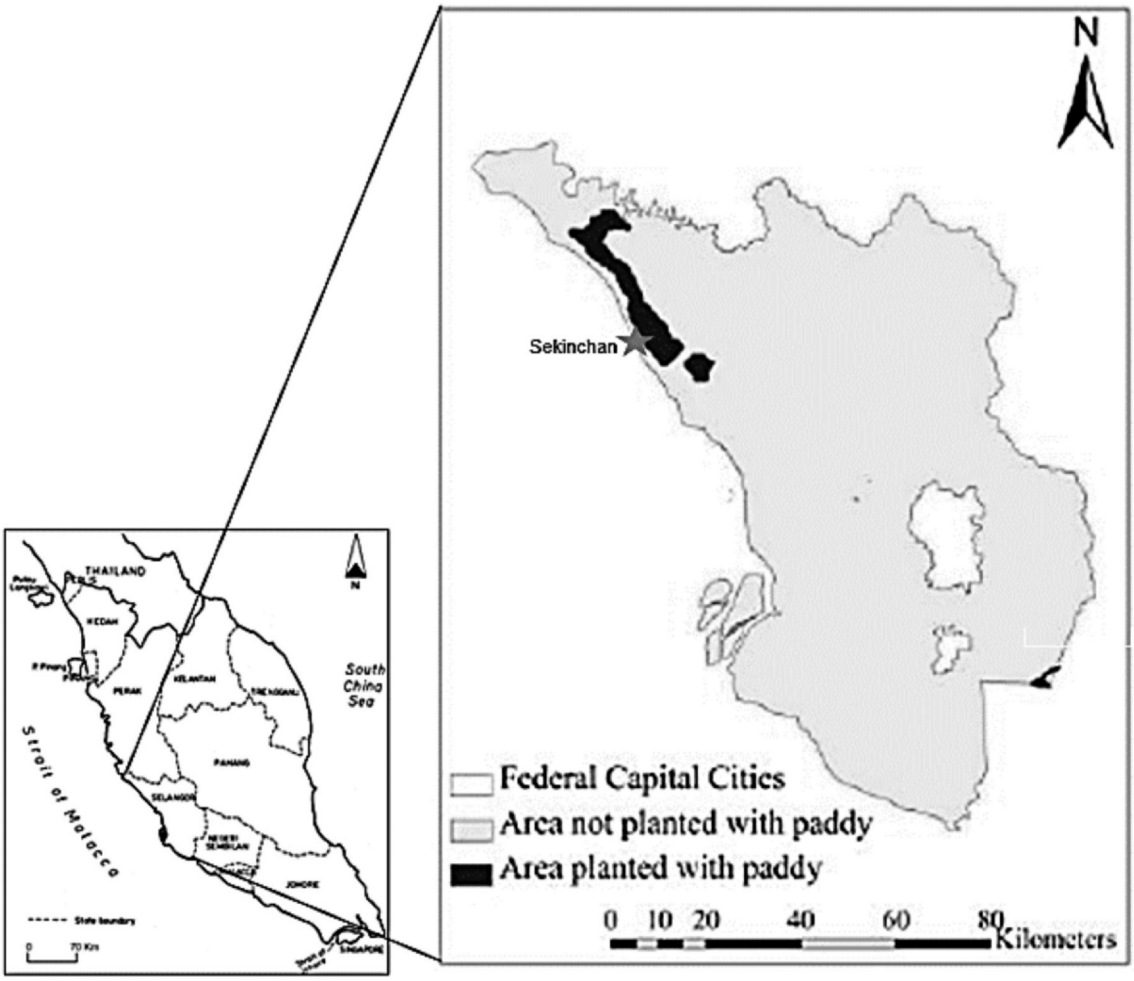


Fig. 1. Agricultural land use for paddy plantation in Selangor state (modified from Olaniyi et al. (2015)).

in the current study because it is a common herbicide used in Malaysia by plantations; others include Paraquat, Glyphosate, Glufosinate–ammonium, and 2,4-dichlorophenoxyacetic acid (Abdul Rani et al., 2003). The Diuron compound effectively inhibits photosynthesis in plants to control most broad-leaf and grassy weeds (Mou et al., 2008), and has also been applied excessively for non-agricultural uses. It is supposed that Diuron is also applied in paddy plantations in the Sekinchan area. Besides, Diuron application as an active agent in ship paints for controlling harmful antifouling systems on the ship hulls and marine vessels are escalating since the worldwide banning of organotin (OT) compound in the year 2008. Thus the concentration of Diuron is significantly increased in the marine environment, mainly in marinas and harbors with heavy shipping activities. The mean concentration of Diuron in the sediment samples from Port Klang which is a nearby area of Sekinchan was between the range 2.24 to 19.25 μg/kg (Hanapiah et al., 2017). Higher concentration was reported in other nearby areas in recent studies with the mean concentration in the range 9.56–55.56 μg/kg in the sample from Kuala Sungai Buloh, and between 21.40 and 72.80 μg/kg in Kapar (Mukhtar et al., 2020). Although Diuron is manufactured as less toxic than OT compounds specifically in comparison to tributyltin (TBT), the compound is toxic to microalgae, thus potentially to destruct the primary producer on the base of the food web (Bao et al., 2011).

Diuron is listed as a priority hazardous substance by the European Commission (Malato et al., 2002). It is highly persistent in the soil and poses risks to water bodies and sediment when leaching occurs (Gooddy et al., 2002). It was found by Sakugawa et al. (2010) that the half-life of Diuron was 9–38 days when natural sunlight was exposed to the water samples. The 7-day and 10-day no observed adverse effect level (NOAEL) values of Diuron in freshwater aquatic organisms such as *Daphnia pulex*, amphipods (*Hyalella azteca*), midges (*Chironomus tentans*), worms (*Lumbriculus variegatus*), and snails (*Physa gyrina*) have been found to range from 1.8 to 13.4 ppm (Nebeker and Schuytema, 1998). Mukhtar et al. (2020) reported Diuron concentrations of 5.0 to 9.8 ppm are detected in marine fish species (*Otolithus ruber*, *Polydactylus sextarius* and *Thryssa dussumieri*) caught from seagrass area of Johor Straits. Acute toxicity tests of Diuron in other types of aquatic life have been performed on zebrafish by Velki et al. (2017), Javanese medaka by Kamarudin et al. (2020), brine shrimp by Shaala et al. (2015), and oyster gametes and embryos by Akcha et al. (2012); the latter test showed that Diuron had embryotoxic and genotoxic effects on the oyster sperm which could cause developmental defects. Moreover, Diuron is reported causing negative effects to reproduction process of aquatic organisms such as fertilization inhibition and permanent damage transmissible to offspring of sea urchin (Manzo et al., 2006) and alterations in transcriptome and global DNA mehylation levels among Pacific oyster offsprings after parental Diuron-exposures (Bachère et al., 2017). The available toxicity data on Diuron indicates the potential hazard of the compound once it is present in the environment.

Considerable accumulation of Diuron in the marine environment occurs via the transport pathway from rivers and irrigation systems. However, limited information exists regarding Diuron contamination in the water surrounding the *T. granosa* cultivation area. A study of water pollution caused by paddy plantations was conducted in Kedah by Sapari and Ismail (2012). Hence, this study aimed to evaluate Diuron contamination along the Sekinchan coastline and its association with

the high mortality of *T. granosa* in areas adjacent to farms. The objectives were to determine the mortality rate of *T. granosa* before, during, and after the release of irrigation water from paddy plantations at Sekinchan; to determine the level of concentration of Diuron in the water and sediment samples in the period of before, during, and after the release of irrigation water discharged from the paddy plantations; and to determine the median lethal concentration (LC_{50}) of Diuron on *T. granosa*.

2. Material and methods

2.1. Study site

The sampling activities for the field screening works were conducted at Sekinchan, Selangor. The study site was situated in the northwestern area of Selangor, a fishing village in an area of substantial paddy fields and coastal *T. granosa* cultivation lots. Fig. 1 shows the land used for paddy plantation in the state of Selangor. This site was selected because it was included in a *T. granosa* cultivation project in 2008. However, the production has consistently decreased due to a shortage of *T. granosa* spats and mass mortality of adult *T. granosa*.

2.2. Sample collection

Samples of the field screening works (water, sediment, and *T. granosa*) were collected at three sampling events from May to August 2018, with 30 day intervals between each sampling. The sampling events represented the periods before, during, and after the discharge of irrigation water from the paddy planting activities of a complete paddy planting season. As shown in Fig. 2, sampling points covered the water channel, the paddy fields, and the receiving area of irrigation water at the coastal area, which is also the *T. granosa* cultivation area. The sampling points are described in Table 1. Sediment samples were collected using an Ekman Grab and scoop. A multi-parameter water quality meter (YSI 556 MPS, USA) was used to measure the water quality parameters. Salinity, turbidity, pH, and dissolved oxygen (DO) of water samples were recorded at each sampling point.

T. granosa samples were collected from the cultivation plots in the coastal area of Sekinchan, which were at S5 and S6 (Fig. 2). A handheld dredge (basket size: 45 cm length, 10 cm high, 30 cm deep) with a 1 mm mesh was used to collect the *Tegillarca granosa*. Hauling was performed several times horizontally to completely cover the hauling area of one sampling point. The hauling time and the velocity of the boat were used to estimate the hauling area. In order to minimize bias, the sampling protocol was retained during each sampling series, using the same dredging net, hauling time, velocity of the boat, and dredging mode.

Mortality observations of *T. granosa* were conducted by recording the number of alive and dead individuals to determine the mortality rate. The mortality rate was measured as the percentage of the number of dead individuals divided by the number of living individuals, while the population density of *T. granosa* was equal to the number of living individuals per sampling area for each sampling event (Yurimoto et al., 2014). The number of dead individuals was represented by empty shells and those that did not close upon stimulation.

2.3. Extraction procedures

The extraction of Diuron residues from the sediment and water samples was performed using the liquid–liquid extraction (LLE) technique (Harino et al., 2012). Samples of wet sediment weighing 1 g from each site were analyzed with acetone (analytical reagent; R&M Chemicals; UK) in a glass centrifuge tube. The samples were shaken by a mechanical shaker and then centrifuged. The supernatant formed in the sample was re-extracted with 50 mL distilled water, 0.5 g of celite (Sigma-Aldrich; USA), and zinc acetate dihydrate (analytical reagent; R &M Chemicals; UK). It was then filtered using Whatman paper into a separating funnel, 10 mL dichloromethane (DCM) (HPLC grade; Friendemann Schmidt Chemical, Australia) was added, and it was dried with a sodium sulfate anhydrous set (Merck; Germany). The analyte in the DCM was then combined with 20 mL hexane (analytical reagent; R& M Chemicals; UK) prior to evaporation to 2 mL using a rotary evaporator, then transferred into a vial, and combined with 100 μL (1 ppm) of internal standard (atrazine d-5) (Sigma Aldrich, USA). Then, the analyte was adjusted to 1 mL with nitrogen gas before analyses using HPLC-UV.

For the water samples, 200 mL of sample was combined with 10 mL of DCM and shaken with a shaker. Then the analyte in the DCM was transferred to a flask through a sodium sulfate anhydrous set. The aliquot was combined with 20 mL hexane and evaporated to 2 mL. It was then transferred to a vial and combined with 100 μL (1 ppm) of internal standard (atrazine d-5) (Sigma Aldrich, USA). Finally, the analyte was adjusted to 1 mL with nitrogen gas before analyses using HPLC-UV.

Fig. 2. Map of sampling points at Sekinchan, Selangor.

Table 1
Description of sampling points in Sekinchan, Selangor.

GPS coordinate	Points		Description	Water Body
N 03°33.532′, E 101°07.822′	P1	P1(A)	Located at the start of the water channel of the paddy plantation and from the main irrigation from Sungai Tengi.	Water channel
		P1(B)	Located in a trench near to Sungai Tengi and next to a paddy field.	Water channel
N 03°32.749′, E 101°06.654′	P2	P2(A)	Located along the water channel of the paddy plantation.	Water channel
		P2(B)	Located inside the paddy field.	Paddy field
N 03°31.989′, E 101°05.509′	P3	P3(A)	Located along the water channel of the paddy plantation.	Water channel
		P3(B)	Located inside the paddy field.	Paddy field
N 03°31.874′, E 101°04.980′	P4 (A)		Located at the end of water channel and in the vicinity of watergate of Pintu Kawalan Air Sg Leman.	River estuary
N 03°32.065′, E 101°04.766′	S5		Located at a cockle cultivation plot in the coastal area.	Littoral Zone
N 03°31.762′, E 101°05.003′	S6		Located at a cockle cultivation plot in the coastal area.	Littoral Zone

2.4. Preparation of stock standard solution

A stock of standard solution was prepared at 200 ppm concentration by dissolving 0.1 g of Diuron with 500 mL methanol in a 500 mL volumetric flask. Then, serial dilutions of the standard solution were prepared by diluting the stock solution with methanol to obtain five calibration standard solutions (0.5, 1.0, 5.0, 10.0, and 50.0 ppm). All standard solutions were kept in vials at 4 °C in the refrigerator until further analysis.

2.5. Chromatographic conditions

High-performance liquid chromatography was performed using an ultraviolet detector (HPLC-UV) (Dionex Ultimate 3000) to analyze the prepared samples. Analyte separation was carried out on a C18 column (4.6 × 250 mm, 5 μm), with column temperature of 25 °C. The mobile phases used were acetonitrile and methanol (4:6) in gradient mode. A sample volume of 10 μL was injected into the instrument. The flow rate of eluent was maintained at 1 mL/min. The UV detector of the instrument for detecting Diuron used a wavelength of 254 nm. The analysis runtime was 15 min. The average retention time for Diuron standard was 5.928 ± 0.5 min. Fig. 3 shows a chromatogram of one of the concentrations of Diuron standards used (1.00 ppm) for calibration in the HPLC-UV instrument and a histogram of Diuron in the sample. The limit of detection of the instrument was 0.5 ppm, respectively.

2.6. Toxicity test

2.6.1. Preparation of seawater

Seawater was collected from Batu Pahat, Johor. Immediately after sampling, seawater was sieved to eliminate debris and microorganisms using a 0.2 mm membrane filter before any further experiment.

2.6.2. Tegillarca granosa sample handling and acclimatization

Wild breed adult *T. granosa* samples with length ranging from 2 to 3 cm were collected in October 2018 from a well-established *T. granosa* farm at Batu Pahat, Johor. The sample area was isolated from paddy plantations in the southern part of Peninsular Malaysia. Samples were acclimatized for 24 h in an aerated tank filled with seawater and sediments taken from the sampling site. The water parameters were monitored according to their natural habitat and as in the Organisation for Economic Co-operation and Development (OECD) Environment Health and Safety Publication (2005). Water characteristics of salinity, temperature, and pH were maintained at 27 °C, 30 ppt and 7.5, respectively, throughout the study period. *T. granosa* were not fed because they were able to obtain sufficient nutrients from the detritus contained in sediment.

2.6.3. Tegillarca granosa exposure

A group of 45 *T. granosa* was selected randomly and used for the toxicity test. Three individual *T. granosa* were placed in a 1 L beaker and exposed to different concentrations of Diuron (0.6, 1.2, 1.8, and 2.4 ppm) including a control group (0 ppm) for 72-h. Concentrations selected in the study were based on a 24-h range-finding test method. *T. granosa* were exposed to fresh Diuron twice per day to maintain the test concentration and simulate the cockles' natural environment. A static renewal water delivery system was applied throughout the exposure. Exposure tanks were not aerated to minimize loss of Diuron content in the test solution due to Diuron oxidation (Wetzel, 2001; Simmons et al., 2012). Observations were recorded daily. Cockle death was death by its inability to close its valve upon mechanical stimulus, such as by touching with a glass rod, and was then immediately removed from the treatment tank (Reddy and Menon, 1979). The experiment was conducted in three replicates with a control tank containing methanol and seawater. Sediments were not included since they can affect the Diuron concentration in water. *T. granosa* were not fed during observation to reduce nitrogen excretion, maintain water quality, and prevent other interference (Silberberg, 2004).

2.7. Statistical analyses

Analysis of variance was performed on data between sampling sites and sampling times. Differences among the results were statistically significant when the p-value was < 0.05. The LC_{50} values (with 95% confidence limits) were calculated with the Probit Analysis Statistical Method in MS Excel 2013. To find the regression equation (Y = mortality; X = concentrations), the LC_{50} was derived from the best-fit line obtained.

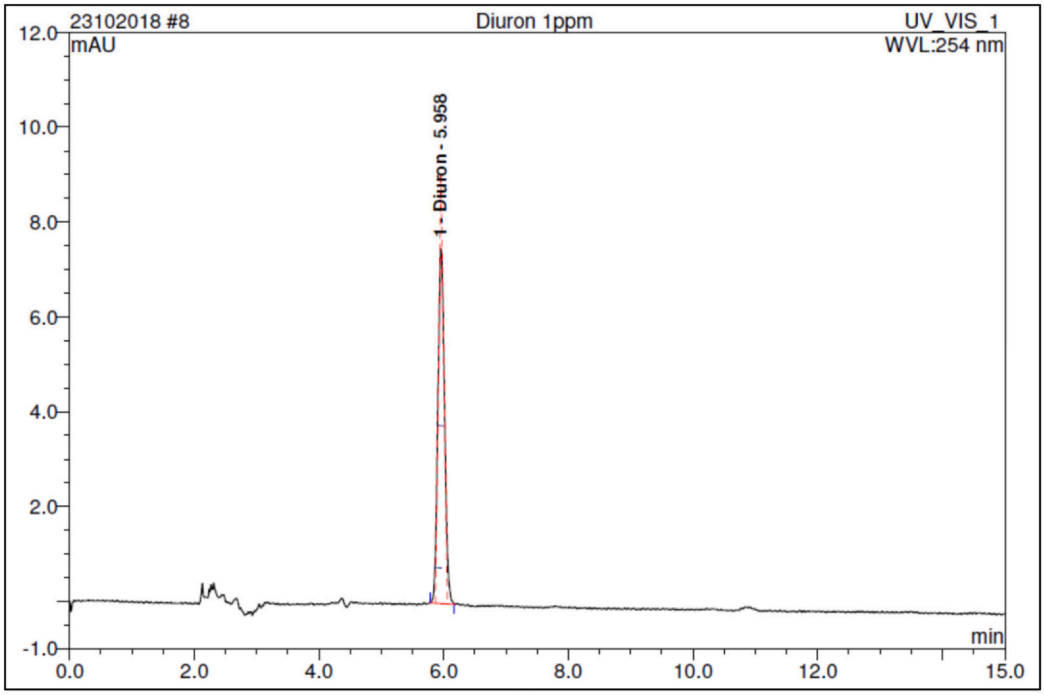
3. Results and discussion

3.1. Mortality and the population density of Tegillarca granosa

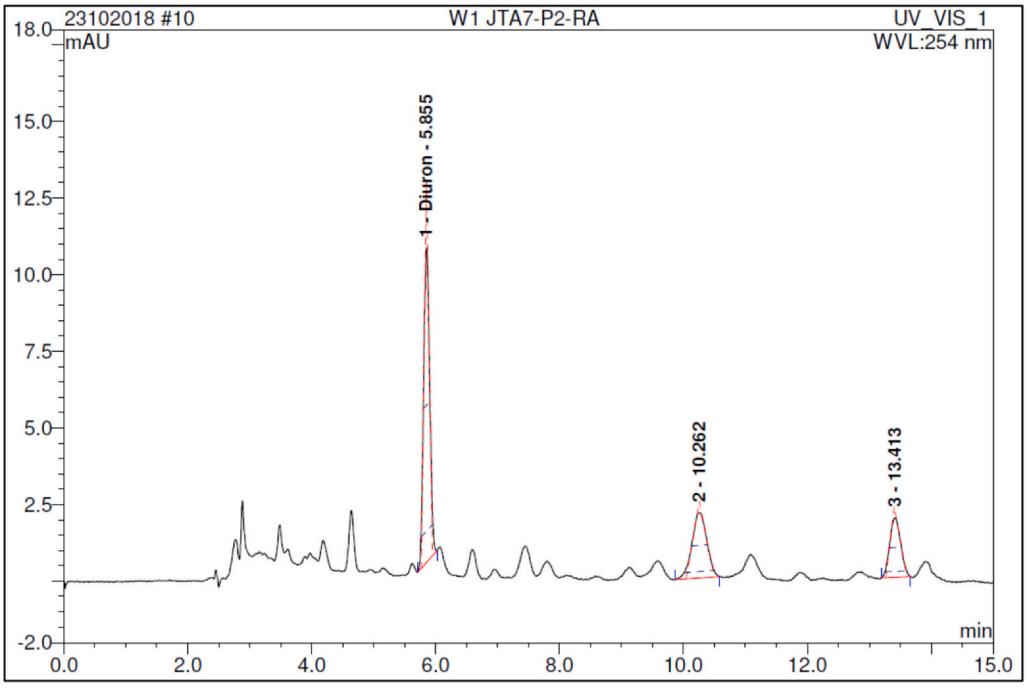
In the present study, the collected *T. granosa* were in a size range of 0.2–1.4 cm, indicating that the individuals were still *T. granosa* spats. The population density for each sampling event is shown in Table 2. The *T. granosa* mortality rate ranged from 4.74% to 38.33% (Table 2). ANOVA analysis revealed that there was a significant difference in the mortality rate of *T. granosa* between the sampling events with $p < 0.05$. The results showed that the mortality rate increased over the three sampling events. The highest mortality rate of 38.33% was found in samples collected after the irrigation water was discharged, and the lowest mortality rate of 4.74% was found in samples collected before the release of irrigation water. The population density of living *T. granosa* observed from the sampling events was very low, ranging from 0.0 to 0.161/m^2. The lowest population density of living *T. granosa* was observed after the release of irrigation water. These results showed that *T. granosa* in the cultivation plots were significantly affected when the irrigation water was released from the paddy plantation to the coastal area.

3.2. In situ water parameters

The in situ water parameters (salinity, turbidity, pH, and DO) from



(a)



(b)

Fig. 3. Chromatogram of Diuron standard at 1.00 ppm concentration (a), and the chromatogram of the sample (b).

each sampling point during the sampling events are shown in Fig. 4. Sampling points P1–P4 were within the channel and paddy field, while S5 and S6 were in coastal area. Therefore, we separately discuss water parameter results for freshwater and marine water. The pH levels of the sampling events were significantly different with a p-value < 0.05. A low reading (pH 4–7) was found in the third sampling event, which occurred after irrigation water was released, compared to the readings from the first (pH 6–8) and second (pH 7.5–9.5) sampling events, which refer to the phases before and during the discharge of irrigation water. The pH readings of sampling points were significantly different with a p-value < 0.05. The turbidity and DO levels had fluctuating trends between sampling events, and both parameters were significantly different between sampling points ($p < 0.05$). The turbidity of sampling points P4 (125 NTU) and P2 (B) (207 NTU) was high in the second sampling event which took place during the release of irrigation water. This might be because the water contained more mud and sediment due to the high stream of water towards the water gate, and the mixing effect observed during the sampling works. Low water salinity at P1 – P4 indicates the source of water at the water body is the Sungai Tengi, which flows through irrigation channels and domestic drainage systems.

In situ water parameters at the *T. granosa* sampling plots of S5 and

Table 2
Mortality rate and the population density of blood cockle, *Tegillarca granosa*, collected in cultivation plots at the coastal area of Sekinchan.

Sampling event	Sampling Site	Range Shell Length (cm)	Total number of individuals	Number of individuals		Mortality (%)	Density of alive individuals (/m^2)
				Alive	Dead		
1st sampling; Before irrigation water release	S5	0.2–1.4	211	201	10	4.74	0.161
	S6	0.3–1.4	93	84	9	9.68	0.067
2nd sampling; During irrigation water release	S5	0.2–1.4	156	146	10	6.41	0.117
	S6	0.3–1.4	37	34	3	8.11	0.027
3rd sampling; After irrigation water release	S5	0.2–1.4	60	37	23	38.33	0.030
	S6	0.3–1.4	0	0	0	0	0

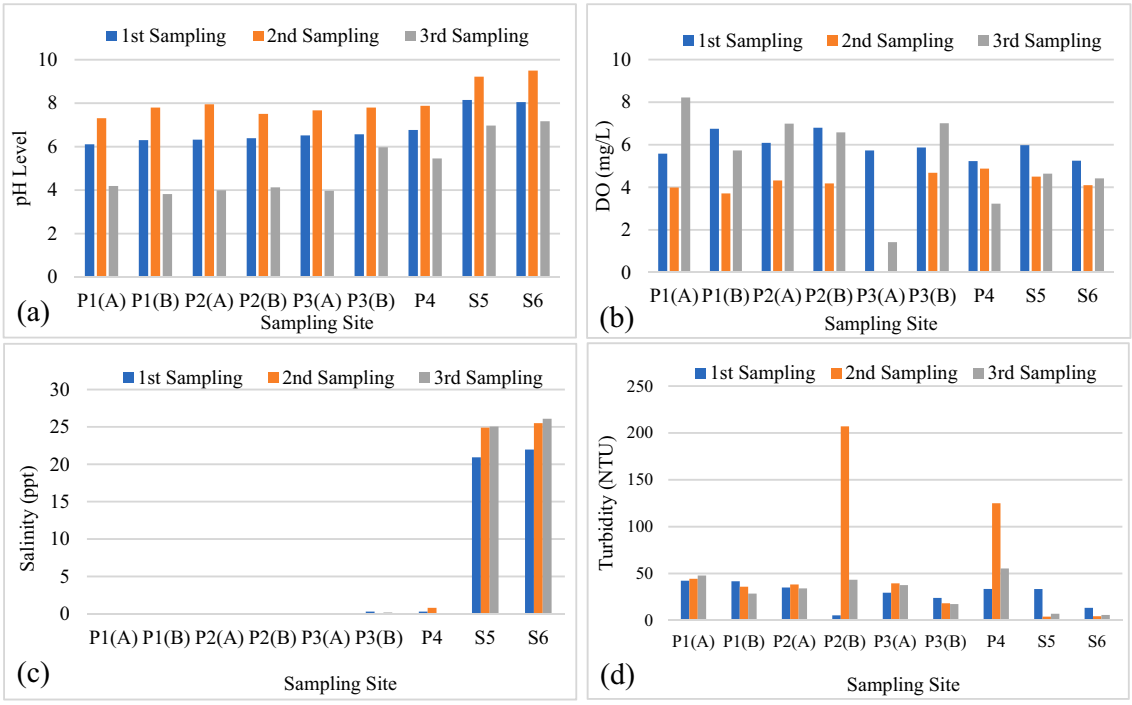


Fig. 4. Level of water parameter a) pH, b) DO (mg/L), c) salinity (ppt), and d) turbidity (NTU) recorded from Sekinchan, Selangor.

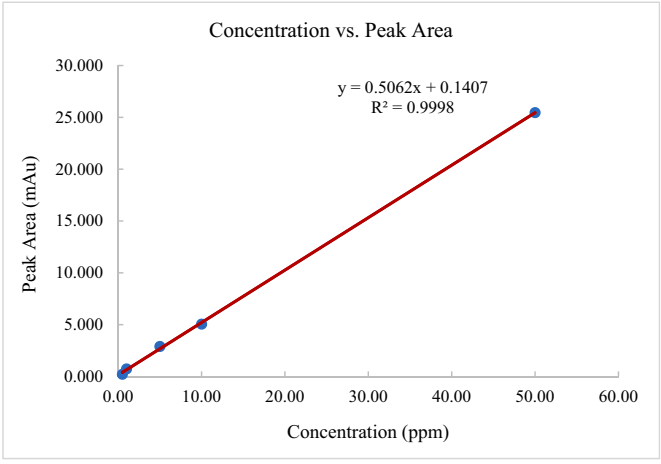


Fig. 5. Calibration curve of Diuron standards using HPLC analysis.

S6 showed the pH of the seawater was higher than that suitable for the habitat of *T. granosa*, ranging from pH 7.1 to pH 8.5 (Oon, 1980). The lowest pH at S5 and S6 was pH 7, recorded at the third sampling event, which refers to the phase after the release of irrigation water (Fig. 4). pH 8 was recorded at the first sampling event, which was before the release of irrigation water and pH 9.2 and pH 9.5 were recorded at the second sampling, which was during the release of irrigation water (Fig. 4). As shown in Fig. 4, the DO readings were all below 7 mg/L and below the suitable DO level for the habitat of *T. granosa* (Jalal et al., 2009). The salinity and turbidity at S5 and S6 were found to be at an acceptable level for *T. granosa*. The optimal salinity for the habitat of *T. granosa* is in the range of 25–30 ppt (Tiensongrusmee and Pontjoprawiro, 1988). In general, the findings suggested the pH and DO levels observed in the *T. granosa* sampling plots were not suitable for *T. granosa*.

3.3. Diuron calibration curve

A calibration curve for Diuron using a HPLC-UV instrument was derived based on the calibration standards ranging from 0.5 to 50.0 ppm. A linear relationship between the concentrations versus the peak area was observed for Diuron standards using HPLC-UV analysis. A linear regression coefficient, R^2 = 0.9998, was obtained from the equation derived from the calibration curve of the standards, y = 0.5062x + 0.1407, where the y-axis is the peak area of the Diuron absorbance from the HPLC analysis, and the x-axis is the concentration of Diuron injected into the instrument, in ppm (Fig. 5). This indicates that the response of the HPLC-UV detector to Diuron residues was good, with a linearity factor of $R^2 > 0.999$.

Table 3
The concentrations of Diuron in water samples before, during, and after the release of irrigation water at water channel (A), and paddy field (B). Concentration in ppb.

Sampling point	First sampling event; before irrigation water release	Second sampling event; during irrigation water release	Third sampling event; after irrigation water release
P1(A)	ND	2113	2480
P1(B)	ND	ND	3910
P2(A)	1589	ND	ND.
P2(B)	ND	ND	2761
P3(A)	ND	2177	ND
P3(B)	ND	ND	ND
P4(A)	1322	ND	3583
S5	ND	ND	2456
S6	ND	ND	ND

Remark: ND; not detected.

3.3.1. Diuron concentration in water samples

Diuron residues in water samples are presented in Table 3. Diuron was present at two different sites in the first and second sampling events, and at six of nine sampling points at the third sampling event, which is the phase after the irrigation water was released from the paddy field (Table 3). The concentration of Diuron was found to increase proportionally with the sampling events. Diuron was recorded at 1322 ppb and 1589 ppb, respectively, in the samples from sampling points P4(A) and P2(A) collected at the first sampling event. Much higher Diuron concentrations were recorded in the samples from P1(A) and P3(A) during the second sampling event, with concentrations of 2113 ppb and 2177 ppb, respectively. A significant increment in the number of sites showing the presence of Diuron in the water samples was observed in the third sampling event, with concentrations ranging from not detected (ND) to 3910 ppb, as shown in Table 3. Diuron concentration was only detected at one of the *T. granosa* cultivation plot (S6) in samples from the third sampling event, with values as high as 2456 ppb, even though the site was in the coastal area. This indicates that Diuron becomes more concentrated during the release of irrigation water from the paddy field and transported to the coastal area. The environmental conditions worsen after the irrigation water is discharged from the paddy field.

Herbicide was applied by farmers during the paddy maturing phase. This was before the irrigation water of the plantation was discharged from the plantation. Results indicated that the highest contamination of Diuron occurred in the phase after the irrigation water was discharged from the paddy field, and higher concentrations were recorded in the paddy field than in the water channel. This was expected because, after the release of irrigation water, the remaining herbicide in the paddy field became concentrated due to the low volume of water remaining in the field. It is shown that when the volume of water is reduced, the contaminants can be more concentrated (Moorthy, 2007). Moreover, the half-life of Diuron in the water was relatively long. It was reported by Sakugawa et al. (2010) that the half-life of Diuron in water was 9–38 days when the water samples were exposed to natural sunlight. As observed from the sampling results, a low concentration of Diuron in the water samples from the channel and coastal area before and during the release of irrigation water was expected because the channel was still receiving water from the main source of water supply of Sungai Tengi, thus diluting the contaminant present in the irrigation water.

In comparison to results of previous studies, it was found that the highest Diuron concentration recorded in Sekinchan was also found among the high value for both water in river water and in the marine water as shown in Table 4. The highest Diuron level in river water was recorded as high as 6742 ppb in the Southampton Water, United Kingdom (Thomas et al., 2001) while the highest level in marine water was found as high as 3050 ppb in western Japan (Okamura et al., 2003). The high values in the current study were expected due to the cumulative recent introduction of Diuron at the paddy field that is the point source. Since study of Diuron application in paddy plantations is limited, the most likely explanation relates to the optimal Diuron level applied to control weeds in other types of plantation. A study by Konlan et al. (2016) recommended an optimal Diuron concentration of 1.0% solution (2 kg ha^{-1} in 200 L of water) for early post-emergence weed management in cowpea and maize in the forest zone of Ghana and places of similar agro-ecologies (Konlan et al., 2016). The recommended value in Konlan et al. (2016) is equivalent to 1,000,000 ppm of Diuron per hectare and application in paddy plantations in Malaysia is thought to be at similar levels, based on the high level of Diuron in the water sample at Sekinchan, Selangor recorded after the release of the irrigation water.

3.3.2. Diuron concentration in sediment samples

The concentrations of Diuron residue in sediment samples collected from the study area are shown in Table 5. Diuron was not detected in the sediment from most sampling points because concentrations were below the limit of detection of the instrument, which was 0.5 ppm. The low concentration of Diuron observed in sediment in the study could be caused by various factors. One of the potential reasons is that Diuron has a low tendency to be absorbed into soil and sediment (Moncada, 2004), and persists in the water column rather than in sediment or soil. With a high solubility in water of 36,400 ppb at 25 °C, Diuron is readily dissolved and dispersed in the water column (Moncada, 2004). Therefore, in paddy fields, although the herbicide is mostly transported into the sediment, it has a higher tendency to return to the water column, rather than accumulating in the surface of the sediment. Diuron has high mobility in sediment; as suggested by Alva and Singh (1990), the amount of adsorbed Diuron can be influenced by the proportion of organic matter in the sediment. The authors found that a large quantity of the herbicide leached into sediment, resulting in increased contaminated water, because Diuron can more easily leach into deeper soil due to the small amount of organic matter in the sediment. Furthermore, when the herbicide was absorbed onto the sediment, its persistence in water increased (Ying and Williams, 2000).

In addition, it is been reported that the half-life of Diuron in sediment is in the range of 22 to 49 days (Ramli et al., 2012), and that Diuron in sediment can degrade more quickly due to biological degradation. According to Salvestrini et al. (2002), abiotic degradation of the herbicide in water is caused by hydrolysis, which is an irreversible reaction yielding the derived compound, 3,4-DCA, as the only product. Giacomazzi and Cochet (2004) noted that, in the environment, the organic and inorganic matter of soils dissolved in aqueous phase can catalyze the chemical degradation of Diuron. According to Ellis and Camper (1982), in biotic degradation, Diuron can degrade more efficiently due to mixed microbial populations typically found in the environment. As a result, the biotic and abiotic degradation of Diuron in sediment could lead to the accumulation of 3,4-DCA, which is a highly toxic derivative (Giacomazzi and Cochet, 2004). To summarize, the high solubility and mobility of Diuron make it easier for the herbicide in sediment to be transported back to the aqueous state. In addition, biodegradation and other processes promote the degradation of Diuron in sediment.

Other factors leading to low levels of Diuron in sediment samples could be related to the spray method used to apply the herbicide (Plimmer, 1992). The loss and degradation of herbicide can occur during and after spraying through the decomposition process, evaporation into the air, or leaching into the soil of the crop. Diuron can thus be absorbed by the microbes in the sediment or adsorbed on soil particles (van der Werf, 1996).

The concentration of Diuron in sediment was compared to other studies as shown in Table 6. The Diuron concentrations in the sediment at Sekinchan were, as expected, lower than those of some of the reported studies in Table 6. The highest recorded Diuron concentration

Table 4
The mean concentrations (ppb) of Diuron found in water in this study with other reported studies.

Location and country		Range concentration (ppb)	Reference
River	River Crouch, United Kingdom	5–305	River
	Southampton Water, United Kingdom	< 1–6742	Thomas et al. (2001)
	River Hamble, United Kingdom	10.5–305.2	Boxall et al. (2000)
	River Orwell, United Kingdom	21.9–768	Boxall et al. (2000)
	Sekinchan water channel, Selangor, Malaysia	ND – 3910	This study
Coastal	Sutton Harbour, United Kingdom	1–334	Coastal
	Coastal waters in western Japan	ND – 3050	Okamura et al. (2003)
	Maizuru Bay, Japan	10–257	Eguchi et al. (2010)
	Port of Osaka, Japan	0.8–267	Harino et al. (2004)
	Port Klang, Selangor, Malaysia	ND – 530	Hanapiah et al. (2017)
	Johor, Malaysia	1–285	Ali et al. (2014)
	Kemaman, Terengganu, Malaysia	ND – 42	Ali et al. (2014)
	Sekinchan coastal waters, Selangor, Malaysia	ND – 2460	This study

Remark: ND; not detected.

Table 5
The concentrations of Diuron in sediment samples before, during, and after the release of irrigation water at water channel (A), and paddy field (B). Concentration in ppb.

Sampling point	First sampling event; before irrigation water release	Second sampling event; during irrigation water release	Third sampling event; after irrigation water release
P1(A)	ND	ND	ND
P1(B)	ND	ND	ND
P2(A)	ND	ND	ND
P2(B)	ND	ND	ND
P3(A)	ND	ND	ND
P3(B)	ND	ND	ND
P4	ND	ND	ND
S5	ND	ND	ND
S6	ND	ND	ND

Remark: ND; not detected.

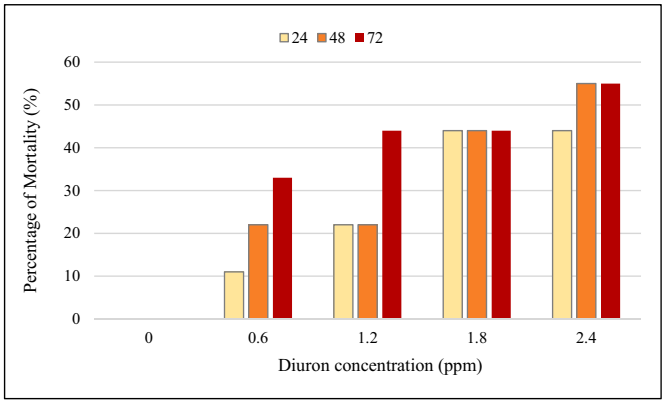


Fig. 6. Mortality of blood cockles after exposed to four different Diuron concentration (0, 0.6,1.2,1.8, 2.4 ppm) at 24, 48 and 72-h.

was found in a sample from Osaka Port, Japan (1350 ppb), followed by Indonesia (740 ppb) and the River Orwell, United Kingdom (395 ppb), as reported in Harino et al. (2005), Harino et al. (2012) and Boxall et al. (2000). Available value recorded in this study found much greater than the level recorded in Port Klang as reported by Hanapiah et al. (2017).

3.4. Acute toxicity test of Diuron on Tegillarca granosa

Mortality results of *T. granosa* after exposure to four different Diuron concentrations (0.6, 1.2, 1.8, and 2.4 ppm) at 24-, 48-, and 72-h are shown in Fig. 6. The range of mortality percentages was within 10% and the maximum was 55% recorded between 0.6 and 2.4 ppm. No mortality was observed in control tanks (0 ppm) throughout the 72-h observation. The mortality percentage proportionally increased by duration of exposure among *T. granosa* in the 0.6 ppm test tank and by increments of the tested Diuron concentration (Fig. 6). A large fluctuation of mortality percentage was recorded among samples exposed to a concentration of 1.2 ppm: mortality was stable at 22% under 24-h and 48-h, before a sharp rise to 55% after 72-h exposure (Fig. 6). Throughout the observation, a largely stable mortality trend at 55% was found in samples exposed to a concentration of 1.8 ppm. Fig. 6 also shows that the highest mortality was recorded in samples exposed to 2.4 ppm after 48-h exposure and the value was constant after 72-h exposure. The median lethal concentration (LC_{50}) of Diuron in adult *T. granosa* after 72-h was 1.84 ppm (Fig. 7). Since limited study of Diuron toxicity has been conducted for *T. granosa* and other bivalves, the findings in this study are not comparable with those related to the same species or other bivalves.

3.5. Diuron contamination as the possible explanation of mass Tegillarca granosa mortality

The increase of *T. granosa* mortality rate occurred over the time of

Table 6
The concentrations notation of Diuron found in sediment in this study with other reported studies.

Location and country	Range concentration (ppb)	Reference
Indonesia	0.04–740	Harino et al. (2012)
Port of Osaka, Japan	< 0.64–1350	Harino et al. (2005)
Osaka Bay, Japan	15–84	Balakrishnan et al. (2012)
Fukuyama, Japan	12–90	Balakrishnan et al. (2012)
River Hamble, United Kingdom	< 12–13	Boxall et al. (2000)
River Orwell, United Kingdom	< 12–395	Boxall et al. (2000)
Southampton Water, United Kingdom	0.4–6.2	Thomas et al. (2000, 2002)
Port Klang, Selangor, Malaysia	< 0.04–4.8	Hanapiah et al. (2017)
Sekinchan, Selangor, Malaysia	ND	This study

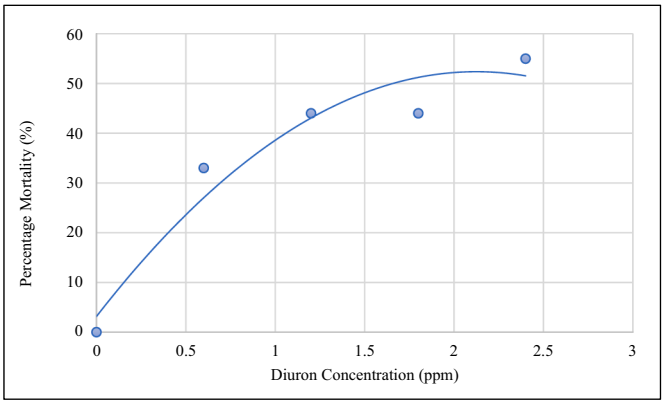


Fig. 7. Median lethal concentration of Diuron exposure on blood cockle *T. granosa* at 72-h exposure based on Probit analysis.

the sampling events and increasing trends were similarly observed for Diuron concentration in water samples between the sampling events. These results are shown in Tables 2 and 3. It is of note that the number of *T. granosa* in the first sampling was significantly higher than that in the third sampling. During the third sampling event (after the release of irrigation water from the paddy field), the presence of agricultural waste in the surrounding area was observed, which was related to runoff from the paddy field that drifted into the channel and coastal area, thus further contaminating the coastal waters of the *T. granosa* cultivation area. Although the LC_{50} level of Diuron in cockles was identified as 1.84 ppm, which is a considerably higher concentration compared to the background level within the study area, this compound nonetheless has a significant impact on cockle cultivation activities within the study site. The inconsistency of the findings of the toxicity study of the Diuron level obtained from field screening is expected due to the differences in *T. granosa* size. Samples in the study area ranged in size from 0.2 to 1.4 cm (Table 2), whereas samples used for the toxicity study were larger, ranging from 2 to 3 cm in length as previously mentioned. Due to these size differences, it is suspected that *T. granosa* samples have different susceptibility to environmental stressors. The samples used in the toxicity study can be considered to have reached the juvenile stage and thus survived stressors in their original habitat and, as a result, have metabolisms that can be better expected to regulate higher concentrations of Diuron. In contrast, the samples collected from Sekinchan were considered to be spats with a high sensitivity to lower concentrations of Diuron. During their early life stages, marine invertebrates are vulnerable to environmental stress, resulting in higher mortality, with low mortality associated with maturity (Byrne, 2011).

In addition, due to the chemical properties of the herbicide, Diuron is highly soluble in the water column rather than in sediment or soil (Moncada, 2004), making it easier to be absorbed by the cells of the organisms. The effects of the herbicide may lead to direct toxic actions in tissues or cells, impacting biological mechanisms, including the immune system, of target organisms such as marine bivalves (Cajaraville et al., 1996; Coles et al., 1994). This could further reduce the survival rates of *T. granosa* by increasing their susceptibility to a wide range of infectious diseases, due to being weakened by the presence of the pollutant (Gagnaire et al., 2007). Previous studies have shown that Diuron could potentially have various effects on marine bivalves, such as on the immune parameters of *Crassostrea gigas* at the molecular, biochemical, and cellular levels (Gagnaire et al., 2007; Bouilly et al., 2007; Tanguy et al., 2005).

Furthermore, *T. granosa* mostly feed on microorganisms from the surrounding detritus and benthic microalgae (Broom, 1985). A study by Ma et al. (2002) proved that Diuron is highly toxic to certain microalgae by reducing their chlorophyll-a levels. Hence, high concentrations of Diuron in water might affect the survival of microalgae, thus causing a chain reaction in the ecosystem food chain, disturbing the feeding activity of *T. granosa*, and increasing the *T. granosa* mortality rate.

In conclusion, this study suggested that Diuron is applied during paddy cultivation activities in the study area and has a potentially negative effect on *T. granosa* farming activities that receive the discharge of irrigation water from paddy fields. Furthermore, unsuitable pH and DO readings in the *T. granosa* sampling plots are also linked to the paddy plantations. This research provided new insights into the mortality rate of *T. granosa* in the study area.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Zurfatiha Zulkarnain and **Nurul Zatul Amira Anuar**: involved in planning, conceptualization, methodology, performed the analysis and drafted the original manuscript, **Ferdaus Mohamat-Yusuff**: planning, conceptualization, methodology, supervision, performed the analysis and drafted the original manuscript. **Amirul Azuan Md Joni** and **Syaizwan Zahmir Zulkifli**: reviewing and editing the manuscript; **Ahmad Ismail, Faradiella Mohd Kusin**, **Khairul Nizam Mohamed**, **Zufarzaana Zulkeflee** and **Zulfa Hanan Asha'ari**: method validation, project administration and supervision; **Aziz Arshad**: aided in funding acquisition.

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