Evidence for extensive anaerobic dechlorination and transformation of the pesticide chlordecone (C\textsubscript{10}Cl\textsubscript{10}O) by indigenous microbes in microcosms from Guadeloupe soil

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SUPPLEMENTAL METHOD DETAILS (SMD)

1. Field Sampling and Microcosm Setup

A) Collection of Field Samples
All agricultural soil samples were collected from 0-20 cm depth. In 2010, soil, water and sludge samples were collected separately in polypropylene sampling bottles, while in 2018, a mix of soil and water was placed into 1-liter glass sampling jars that were filled to the top and sealed. All samples were shipped from Guadeloupe to University of Toronto for analysis where they were stored at 4°C until use.

B) Recipe for Artificial Groundwater Used in Microcosm Setup
From Middeldorp et al. (1998).

For 1 liter of water, the following compounds were added:

| Chemical        | Concentration | Molecular weight | Amount required |
|-----------------|---------------|------------------|-----------------|
|                 | (mM)          | (g/mol)          | (mg)            |
| NH₄Cl           | 1             | 53.49            | 53.5            |
| MgCl₂ (6 H₂O)   | 0.05          | 203.31           | 10.2            |
| MnCl₂ (4 H₂O)   | 0.02          | 197.9            | 4.0             |
| NaCl            | 0.12          | 58.44            | 7.0             |
| CaCl₂           | 6             | 147.02           | 882.1           |
| NaH₂PO₄         | 0.45          | 141.96           | 63.9            |
| KH₂PO₄          | 0.15          | 136.09           | 20.4            |
| Na₂SO₄          | 1             | 142              | 142.0           |
| NaHCO₃          | 1             | 86.01            | 86.0            |

The solution was made with distilled deionized water. It was autoclaved and then purged with N₂/CO₂ (80% /20%) for 1 hour. After, the artificial groundwater was moved into the glovebox. In the glovebox 10 mL of 100x sterile vitamins solution was added (recipe in Edwards and Grbić-Galić, 1994). The pH was measured and adjusted to pH 7.
2. Microcosm Sampling and Analysis

A) GC-FID Sample Preparation and Analysis
Microcosms were sampled using glass syringes and a 1 ml sample was added to a 10 ml headspace autosampler vials (Agilent) containing 5 ml of acidified deionized water (pH 2). The vials were then sealed with Teflon-coated septa and aluminum crimp caps (Chromatographic Specialties) for automated headspace injection onto the CG. Methane, ethene, ethane, and chlorinated ethene measurements were carried out as headspace analysis using an Agilent 7890A gas chromatograph (GC) equipped with a flame ionization detector (FID), a G1888 headspace autosampler and a J&W GS-Q column (30 m x 0.53 mm) (Agilent, Santa Clara, CA, USA). Helium was the carrier gas (11 ml/min) and the oven program was as follows: Hold at 35°C for 1.5 min, increase to 100°C at the rate of 15°C/min, then increase to 185°C at the rate of 5°C/min, hold 10 min, then increase to 200°C at the rate of 20°C/min, hold 10 min (total runtime 43.6 min). The GC had a packed inlet and a 3 ml sample loop. Headspace operating temperatures for oven, loop and transfer line were 70, 80 and 90°C respectively, while vial equilibration time, pressurization time, loop fill time, loop equilibration time and injection time were 40, 0, 0.2, 0 and 3 min respectively. During equilibration in oven, the samples were shaken at low speed. Data from the GC was integrated using ChemStation (Agilent). Calibration standards were prepared in the concentration range 0.2 to 2 mg/l (liq) for methane and ethene, and 1 to 20 mg/l for chlorinated ethenes.

B) IC Sample Preparation and Analysis
To measure chloride, nitrite, nitrate, sulfate, phosphate and acetate concentrations, samples (1 ml) were filtered through 0.2 μm nylon filters (Fisher Scientific). Analysis were performed using a Dionex ICS 2100 ion chromatograph with a Dionex IonPac AS18 analytical column (4x250 mm) and an ASRS 500 suppressor (Thermo Fisher Scientific). The samples were run isocratically at 23 mM KOH, 57 mA current and with a flow rate of 1 ml/min. Data from the IC was integrated using Chromelion (Thermo Fisher Scientific). Standards were prepared in the range 0.005 to 0.5 mM.

C) pH Measurements
One ml liquid samples were taken for “in-syringe” pH analysis using an Oakton pH spear (Oakton Instruments, Vernon Hills, USA). If needed, pH was adjusted with Na-bicarbonate or HCl to pH 7.
### D) LC-MS Sample Preparation and Analysis

**Summary of sample preparation methods and LC-MS methods on the different analysis dates:**

| Analysis date | Sample prep method | Scan Range | Instrument          | Eluent                                      | Flow rate | Column Temp | Gradient                                                                 |
|---------------|--------------------|------------|---------------------|---------------------------------------------|-----------|-------------|---------------------------------------------------------------------------|
| 02-Oct-13     | Method 1           | 140-520    | Thermo Exactive LCMS| Water (A)/Acetonitrile (B) gradient         | 0.2 ml/min| Ambient     | 50%B start, increase to 100%B over 25min, decrease to 50% B over 5min    |
| 27-Aug-14     | Method 1           | 130-550    | Thermo Exactive LCMS| Water (A)/Acetonitrile (B) gradient         | 0.2 ml/min| Ambient     | 50%B start, increase to 100%B over 25min, decrease to 50% B over 5min    |
| 24-Nov-16     | Method 2 *         | 120-1800   | Thermo Q-Exactive LCMS| Water (A)/Methanol (B) gradient            | 0.2 ml/min| 40°C        | 50%B start, increase to 100%B over 15min, hold for 9min, decrease to 50%B over 1min, hold for 5min |
| 02-Mar-17     | Method 3           | 150-750    | Thermo Q-Exactive LCMS| Water (A)/Methanol (B) gradient            | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |
| 15-Feb-18     | Method 3           | 150-750    | Thermo Q-Exactive LCMS| Water (A)/Methanol (B) gradient            | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |
| 06-Apr-18     | Method 4           | 150-750    | Thermo Q-Exactive LCMS| Water (A)/Methanol (B) gradient            | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |
| 09-Apr-18     | Method 4           | 150-750    | Thermo Q-Exactive LCMS| Water (A)/methanol (B) gradient w/5 mM ammonium acetate | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |
| 29-Jun-18     | Method 3, 4 and 5  | 150-750    | Thermo Q-Exactive LCMS| Water (A)/methanol (B) gradient w/5 mM ammonium acetate | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |
| 25-Sep-18     | Method 3, 4 and 6  | 150-750    | Thermo Q-Exactive LCMS| Water (A)/methanol (B) gradient w/5 mM ammonium acetate | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |
| 07-Mar-19     | Method 4           | 150-750    | Thermo Q-Exactive LCMS| Water (A)/methanol (B) gradient w/5 mM ammonium acetate | 0.3 ml/min| 40°C        | 50%B start, increase to 100%B over 8min, hold for 4min, decrease to 50%B over 1min, hold for 5min |

* For this run, samples were contained in plastic well plates with slit rubber mats. Compounds may have sorbed to the plastic, and standards had evaporated (slit mats do not seal well). For all other runs, samples were contained in glass vials with Teflon lined septa.
Details of the different sample preparation methods

Sample preparation method 1 (water phase with small amount of soil, liq/liq extraction):
1) Let soil settle in bottles overnight
2) Sample 2 mL liquid from microcosms
3) Extract with 15/85 % acetone/hexane, 2 cycles of extraction, each w/ 5mL of acetone/hexane
4) Filter the solvent phase through hydrophobic filter into glass vial
5) Evaporate filtrate to dryness and re-dissolve in 2mL of MeOH

Sample preparation method 2 (water phase):
1) Let soil settle in bottles overnight
2) Sample 1 mL liquid from microcosms
3) Filter sample into new glass vial through 0.2 μm PTFE syringe filter

Sample preparation method 3 (water phase):
1) Let soil settle in bottles overnight
2) Sample 0.75mL liquid from microcosms, avoiding getting any soil into the sample, and transfer to glass vial
3) Centrifuge @ 3000rpm for 5min
4) Transfer 0.5mL clear liquid from centrifuged samples into glass vials with 0.5mL MeOH, mix
5) Filter sample into new glass vial through 0.2 μm PTFE syringe filter

Sample preparation method 4 (samples with soil):
1) Shake bottle and sample 1mL slurry from microcosm
2) Add to glass vial containing 1mL MeOH, vortex, shake gently for 10min and let sit for about 30 min (or overnight)
3) Centrifuge @ 3000rpm for 5min
4) Filter sample into new glass vial through 0.2 μm PTFE syringe filter

Sample preparation method 5 (extraction of field samples, 5 ml slurry):
1) Shake bottle and sample 5mL slurry from sampling jar into a 15 ml glass centrifuge tube
2) Add 5 ml of a 15% acetone and 85% hexane mixture to the centrifuge tube
3) Shake gently for 10 min, let sample sit for 1 hour, shake for 1 min
4) Centrifuge @ 3000 rpm for 5min
5) Transfer the solvent phase into new glass vial
6) Filter sample into new glass vial through 0.2 μm PTFE syringe filter
7) Evaporate to dryness
8) Re-dissolve in 1 ml methanol

Sample preparation method 6 (extraction of field samples, 20 ml slurry):
1) Shake bottle and sample 5mL slurry from sampling jar into a 15 ml glass centrifuge tube (prepare 4 tubes with 5 ml sample for each field sample)
2) Add 5 ml of a 15% acetone and 85% hexane mixture to the centrifuge tube
3) Shake gently for 10min and let sample sit for 30 min
4) Centrifuge @ 3000 rpm for 5min
5) Transfer the solvent phase into new glass vial (combine solvent from the 4 tubes into one vial)
6) Repeat steps 2 to 5 one more time
7) Evaporate to dryness
8) Re-dissolve in 0.5 ml MeOH
9) Filter sample into new glass vial using 0.2 μm PTFE syringe filter
E) DNA Extraction, Amplicon Sequencing and Quantitative Polymerase Chain Reaction (qPCR) Analysis

Samples for DNA extraction and subsequent microbial community analysis were taken at different time points from the 6 transfers (GT5, GT20, GT33, GT4, GT15, GT3) and one TCE amended control (GT2). Slurry samples (1 mL) were collected, centrifuged at 10 000 rpm for 20 min, and cell pellets were frozen at -80°C for future DNA extraction. DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to manufacturer’s protocol with some modifications (see below). DNA concentrations were verified by a NanoDrop1000 Spectrophotometer (Thermo Fisher Scientific) and Qubit Fluorometric Quantitation (Thermo Fisher Scientific), and the extracts were stored at -80°C.

Microbial community composition of the samples was assessed by small subunit (SSU) rRNA gene fragment sequencing and Quantitative Polymerase Chain Reaction (qPCR) analysis. Samples from earlier sampling times (after 29 and 39 months) were sequenced using Pyrotag 454 sequencing and samples from later timepoints (76 and 79 months) were sequenced using Illumina MiSeq sequencing.

**Pyrotag 454 sequencing**

Samples were sequenced at Genome Quebec Innovation Centre using the Roche GS FLX Titanium technology (Roche Diagnostics Corporation, Indianapolis, IN). Extracted DNA samples were amplified by PCR using the universal primer set, 926f and 1392r (926f: 5’-AAACTYAAKGAATTGACGG-3’; 1392r: 5’-ACGGCGGTGTCAGTC-3’), targeting the V6-V8 variable region of the 16SrRNA gene from bacteria and archaea, as well as the 18S rRNA gene in eukarya (Engelbrektson et al. 2010). One to four independent 100 µL PCR amplification reactions were preformed per sample. Each PCR reaction was set up in sterile Ultra-Pure H2O containing 50uL of PCR mix (Thermo Fisher Scientific, Waltham, MA), 2 µL of each primer (forward and reverse, each from 10 µM stock solutions), 10 µL of a 3% BSA solution, and 4 µL of DNA extract. PCR reactions were run on a MJ Research PTC-200 Peltier Thermal Cycler (Bio-Rad Laboratories, Hercules, CA) with the following thermocycling program: 95 °C, 3 min; 25 cycles of 95 °C 30 s, 54 °C 45 s, 72 °C 90 s; 72 °C 10 min; final hold at 4 °C (modified from Ramos-Padron et al. 2011). The forward and reverse primers included adaptors, and the reverse primer also included 10bp multiplex identifiers (MID) for distinguishing multiple samples pooled within one sequencing region. The PCR products were verified on a 2% agarose gel and replicates were combined and purified using GeneJETTM PCR Purification Kit (Fermentas, Burlington, ON), according to the manufacturer’s instructions. The concentrations of PCR products were determined using a NanoDrop ND-1000 Spectrophotometer at a wavelength of 260 nm. The concentrations and qualities of the final PCR products were also evaluated by running them on 2% agarose gels and comparing band intensities to those from a serial dilution of ladders with known DNA concentrations. The purified PCR products were sent to Genome Quebec Innovation Centre, where they were checked for quality again, pooled and subject to unidirectional sequencing (i.e. Lib-1 chemistry) of the 16S gene libraries, using the Roche GS FLX Titanium technology (Roche Diagnostics Corporation, Indianapolis, IN).

**Illumina sequencing**

DNA extracts were sent to Genome Quebec Innovation Centre where they were sequenced using the Illumina MiSeq platform (Illumina Inc., San Diego, USA). Modified versions of the universal SSU primer set 926f and 1392r (926f-modified: 5’-
AAACTYAAAKGAATWGRCGG-3’; 1392r-modified: 5’-ACGGCGGTGWTGC-3’), targeting the V6–V8 variable region of the 16SrRNA gene from bacteria and archaea as well as the 18S rRNA gene in eukarya, were used (Ferris et al. 1996, and Engelbrektson et al. 2010).

Processing of amplicon sequencing
Sequence data from both sequencing technologies was processed in QIIME2 v2019.10 (Bolyen, E., et al. 2019). Primers were removed using the cutadapt plug-in QIIME2. Amplicon sequence variants (ASVs) were identified using the QIIME2 DADA2 plugin with the following settings: forward reads were trimmed to 260nt and reverse reads to 220, maximum expected errors both for forward and reverse reads were set to 3. Taxonomy was assigned to the ASVs using the feature-classifier plugin and machine-learning-based classification (the classify-sklearn option) using a SILVA v1.32 classifier trained on the region amplified by the primers used here. The 454 sequence data was demultiplexed and primers were removed in Geneious v10. The resulting reads were processed with DADA2 V1.1 in R with sequences trimmed to 400 nt, maximum errors set to 2 and homopolymer_gap_penalty= -1, band_size=32 to generate ASV (Callahan, B. J., et al. 2016). Taxonomy was assigned to the ASVs using the ‘assignTaxonomy’ function in the DADA2 package with the SILVA v1.32 database.

The ASVs obtained from both sequencing chemistries were imported into Geneious v.10 and aligned using MAFFT v7.450. Positions containing > 99% gaps were removed from the alignment, and 68bp at the 5’-end and 12bp at the 3’-end of the alignment were trimmed off, resulting in a 413bp alignment of 7966 ASVs. The alignment was used to produce a phylogenetic tree using FastTree v. 2.1.1.11. The ASV tables from both datasets were imported to Phyloseq v. 1.26 (McMurdie, P. J. and S. Holmes 2013) in R together with the phylogenetic tree. The ASVs were combined into operational taxonomic units (OTUs) using the tip-glom function in phyloseq with h=0.03. This function agglomerates all tips of the tree separated by a distance smaller than h into one taxon or OTU. This gave 4935 combined OTUs. The dataset was subsampled without replacement to an equal number of 4000 reads pr sample and a bar chart of the 100 top OTUs was drawn using the Fantaxic R-package (https://rdrr.io/github/gmteunisse/Fantaxtic/).

qPCR analysis
The abundance of total bacterial 16S rRNA genes in the microcosms was estimated by qPCR using a CFX96TM real-time PCR detection system, with a C1000 thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). DNA extracts were analyzed using the general bacterial 16S rRNA primers 055f (5’-ATGGCTGTCAGCT-3’) and 1392r (5’-ACGGGCGGTGTAC-3’) (Ferris et al. 1996). The 20 μl qPCR reactions were prepared in a PCR cabinet (ESCO Technologies, Gatboro, PA) and were made up by 10 μl of SsoFastTM EvaGreen® SuperMix (Bio-Rad Laboratories Inc., USA), 0.5 μl of each forward and reverse primers (10μM stock, making final concentration of 250 nM for both primers), 7 μl of UV treated UltraPure Distilled water (Invitrogen, Grand Island, NY, USA), and 2 μl of DNA extract diluted 10x. The qPCR cycle was as follows: 98°C for 2 min, 40 cycles of 98°C for 5 seconds and 55°C for 10 seconds, followed by an increase from 65°C to 95°C at 0.5°C increments over 10 seconds. Calibration curves for qPCR were prepared from serial dilutions of target-containing plasmids between 10^1 and 10^8 gene copies/ml. The general bacteria gene copy number detection limit was 1.1 E05 copies per ml.
Modifications to the DNeasy PowerSoil Kit manufacturer’s protocol

Link to Dneasy PowerSoil kit Protocol:

https://www.qiagen.com/ca/resources/resourcedetail?id=91cf8513-a8ec-4f45-921e-8938c3a5490c&lang=en

Modifications to protocol:
Step 1: Cell pellet was added to the tube (instead of soil sample)
Step 9: All of the supernatant was transferred to the collection tube (not only 600 μl)
Step 12: All of the supernatant was transferred to the collection tube (not only 750 μl)
Step 13: Keep the ratio of sample to C4 solution at 750:1200 (sample:C4)
Step 19: Added 50 μl H2O instead of 100 μl C6

References for supplemental method details

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852-7.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581-3.

Edwards EA, Grbic-Galic D. Anaerobic Degradation of Toluene and O-Xylene by a Methanogenic Consortium. Applied and Environmental Microbiology. 1994;60(1):313-22.

Engelbrektson A, Kunin V, Wrighton KC, Zvenigorodsky N, Chen F, Ochman H, et al. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. The ISME journal. 2010;4(5):642-7.

Ferris MJ, Muyzer G, Ward DM. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. Appl Environ Microbiol. 1996;62(2):340-6.

McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8(4):e61217.

Middeldorp PJM, van Aalst MA, Rijnaarts HHM, Stams FJM, de Kreuk HF, Schraa G, et al. Stimulation of reductive dechlorination for in situ bioremediation of a soil contaminated with chlorinated ethenes. Water Sci Technol. 1998;37(8):105-10.

Ramos-Padron E, Bordenave S, Lin S, Bhaskar IM, Dong X, Sensen CW, et al. Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. Environmental science & technology. 2011;45(2):439-46.
Figure S1: Microcosm transfers and their origins. Field samples FV 1, 2 and 3 were collected on a riverbank near a former banana field, and samples Bl inside an active agricultural banana production area in Guadeloupe.
Figure S2: History of microcosms. Cumulative chlordecone added (a), cumulative donor added (b), and cumulative methane produced (c) in active CLD amended (GT20, GT5, GT33 and GT4) and active CLD+TCE amended bottles (GT15, GT3) over the course of the study. See Tables S4 and S5 for raw data and details of amendments and analyses.
Figure S3: Methane production in active CLD amended microcosm during the first 1.5 years of monitoring. Data is shown for one of triplicates established for each soil type (soil type ID in brackets), but other replicates showed similar results.
Figure S4: TCE and its dechlorinated metabolites in a CLD+TCE amended microcosm during the first 2 years of monitoring (microcosm G15). The two other triplicates (G13 and G14) behaved similarly. TCE continued degrading in G15 and after transferring in GT15, but GT3 which was a transfer from G14 and G15, never degraded TCE (Figure S2 and Table S5).
Figure S5: LC/MS scan analysis of chlordecone in standard run on June 29th 2018
(instrument method and standard preparation method is described in main text and in Supplemental Method Details 2) (a) m/z 502.6874 EIC (extracted ion chromatogram), (b) m/z 548.6929 EIC, (c) m/z 562.7078 EIC, (d) m/z 516.7032 EIC, (e) full scan mass spectra at 9.9 min, (f) full scan mass spectra at 10.54 min

Abbreviations:
NL: Normalized Level (average spectrum displayed normalized to the average base peak)
RT: Retention Time
AA: Peak area (automatically detected area)
AH: Peak height (automatically detected height)
m/z: An ion’s mass-to-charge ratio
Figure S6 A: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolite monohydrochlordecone (MHCLD) m/z 468.7267 (A9a, loss of 1 Cl), data from sample GT20 June 29th 2018. The mass tracked in this study is the hydrate form of MHCLD.
Figure S6 B: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolite dihydrochlordecone (DHCLD) m/z 434.7661 (A8a, loss of 2 Cl), data from sample GT20 June 29\textsuperscript{th} 2018. The mass tracked in this study is the hydrate form of DHCLD.
Figure S6 C: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolite trihydrochloridecone (THCLD) m/z 400.8042 (A7a, loss of 3 Cl), data from sample GT3 March 7th 2019. The mass tracked in this study is the hydrate form of THCLD.
Figure S6 D: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolite B5a m/z 284.8616 (pentachloroindene, loss of 5 Cl), data from sample GT20 June 29th 2018.
Figure S6 E: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolite B4a m/z 250.9006 (tetrachloroindene, loss of 6 Cl), data from sample GT20 June 29th 2018.
Figure S6 F: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolite B3a m/z 216.9382 (trichloroindene, loss of 7 Cl), data from sample G19 March 7th 2019.
Figure S6 G: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolites C4a-b m/z 294.8899 (carboxylated tetrachloroindene, loss of 6 Cl), data from sample GT20 June 29th 2018.
Figure S6 H: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolites C3a-e m/z 260.9287(carboxylated trichloroindene, loss of 7 Cl), data from sample GT20 June 29th 2018.
Figure S6 I: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolites C2a-d m/z 226.9677 (carboxylated dichlorindene, loss of 8 Cl), data from sample GT20 June 29th 2018.
Figure S6 J: Extracted Ion Chromatogram, observed mass spectrum and theoretical mass spectrum for metabolites C1a-b m/z 193.0064 (carboxylated chloroindene, loss of 9 Cl), data from sample GT20 June 29th 2018.