Project 1f - Rena PAH and Metals Chemistry

1: GC-MS Fingerprint Comparison of Rena Oil and Some October-November 2011 and January – February 2012 Oiled Sand and Tarball Samples

Professor Alistair Wilkins

Introduction

The selected ion mode (SIM) gas chromatography-mass spectrometry (GC-MS) fingerprints of three Rena tank oil samples and a series of oiled sand and tarball samples collected from coastal sites between October-November 2011 and January-February 2012 were determined.

Subsamples of bulk tarball samples or tarball fragments recovered from oiled sand samples were extracted by soaking with dichloromethane at room temperature. Rena tank oil samples were diluted with dichloromethane prior to GC-MS analysis.

Samples

The following samples were examined:

1) Rena Tank 3 oil, shared NIWA, Hill Laboratories and Waikato University sample supplied by the BOPRC (October 2011)
2) Rena Tank 5 oil, shared NIWAR, Hill Laboratories and Waikato University sample supplied by the BOPRC (October 2011)
3) Rena tank oil sample supplied via the Biological Sciences Department, The University of Waikato, Hamilton, New Zealand
4) TMMR000013, Mount Maunganui, beach tarball, 10-10-2011
5) TMMR000010, Pāpāmoa Beach, Taylors Road, 19-10-2011
6) TMMR000012, Pāpāmoa Beach, Taylors Reserve, 25-10-2011
7) TMMR000014, Pāpāmoa Beach, Taylors Reserve, tarball, 27-10-2011
8) TMMR000017, Mauao, 100 m SE of North Rock, oiled sand, 23-1-2012
9) TMMR000023, Mauao, opposite North Rock, oiled sand, 23-1-2012
10) TMMR000025, Tuapiro Road, oiled sand, 23-1-2012
11) TMMR000018, Matakana Island, Ocean Beach, sector 11, oiled sand, 1-2-2012
12) TMMR000020, Kulim Park, oiled sand, 8-2-2012
13) TMMR000024, Leisure Island, Causeway, oiled sand, 8-2-2012
14) TMMR000019, Maketū Estuary, Entrance, oiled sand, 10-2-2012.
GC-MS analyses

Two SIM GC-MS analyses were performed on each sample:

(i) A multiple ion suite, 50 to 340°C, for hydrocarbons and selected dicyclic to hexacyclic polyaromatic hydrocarbons (PAHs) and alkylated analogues. The following ions were included in the multiple ion suite:

- $m/z$ 57 for straight and branched chain hydrocarbons (alkanes)
- $m/z$ 128, 142, 156, 170 and 184 for naphthalene and alkylated naphthalenes
- $m/z$ 166, 180, 194 and 208 fluorene and alkylated analogues
- $m/z$ 178, 192, 206 and 220 for phenanthrene, anthracene and alkylated analogues
- $m/z$ 202, 216, 230 and 244 for fluoranthrene, pyrene and alkylated analogues
- $m/z$ 228, 242, 256, and 270 for benzo[a]anthracene, chrysene and alkylated analogues
- $m/z$ 252, 266, 280 and 294 for pentacyclic PAHs and alkylated analogues
- $m/z$ 276, 290, 304 and 318 for hexacyclic PAHs and alkylated analogues
- $m/z$ 184, 198, 234 and 248 for dibenzothiophene, tribenzothiophene and methyl analogues

(ii) $m/z$ 191 ion, 150 to 340°C, for hopanoid petroleum biomarker compounds

The identification of parent (non-alkylated) PAHs were established by comparison with the retention times and the total ion chromatogram (TIC) mode mass spectral fragmentation patterns determined for authentic specimens of 16 EPA priority PAHs purchased from Sigma Aldrich.

GC-MS fingerprint profiles for 8 groups of compounds are presented in Appendices 1.1 to 1.8:

- Appendix 1.1 Hydrocarbons
- Appendix 1.2 Naphthalenes
- Appendix 1.3 Phenanthrenes
- Appendix 1.4 Pyrenes
- Appendix 1.5 Benzo[a]anthracenes and chrysenes
- Appendix 1.6 Pentacyclic and hexacyclic PAHs
- Appendix 1.7 Dibenzothiophenes and tribenzothiophenes
- Appendix 1.8 Hopanoids

**TMMR02528 and TMMR02529 results reported by Hill Laboratories**

Two oiled sand samples collected from Ōmanu (TMMR02528) and Pāpāmoa Beach, Harrisons Cut (TMMR02529) on 2-10-2011 were submitted to Hill Laboratories for independent confirmation of peak identifications and determination of the level of the 16 EPA priority PAHs in the oiled sand samples. The ratio of sand to tarball material in the samples was not determined.
Hill Laboratories methodology quantitatively determines the levels of 16 EPA priority PAHs in subsamples of the mixed oil-sand material whereas the selected ion mode GC-MS fingerprinting technique qualitatively demonstrates the presence of PAHs and alkylated analogues of parent PAHs.

Figure 1 Ion mode GC-MS fingerprinting technique in oiled sand samples collected from Ōmanu (TMMR02528) and Pāpāmoa Beach, Harrisons Cut (TMMR02529) on 2-10-2011.

Hill Laboratories methodology quantitatively determines the levels of 16 EPA priority PAHs in subsamples of the mixed oil-sand material whereas the selected ion mode GC-MS fingerprinting technique qualitatively demonstrates the presence of PAHs and alkylated analogues of parent PAHs.

Tarball characteristics

The dominant extractable/soluble components of tarballs derived from bunker oils are straight and branched chain hydrocarbons (alkanes), polycyclic aromatic hydrocarbons (PAHs) and alkylated analogues of PAHs. Low levels of petroleum biomarker compounds such as C_{27}-C_{35} hopanoids are also detectable in bunker oil derived tarballs. The origin and significance of hopanoid biomarker compounds is reviewed in Appendix 1.8.

The extent to which evaporable lower molecular weight hydrocarbons (alkanes) and dicyclic naphthalenes and to a lesser extent also tricyclic PAHs such as anthracenes, phenanthrenes and dibenzothiophenes are found in tarballs is influenced by the weathering and degradative regimes the source bunker oil and weathered tarballs have been subjected to over time.
Tetracyclic (e.g. pyrenes, benz[a]anthracenes and chrysenes), pentacyclic and hexacyclic PAHs and hopanoid biomarker compounds are resistant to loss by weathering.

**Hydrocarbon Profiles**

The three *Rena* tank oil samples afforded SIM GC-MS *m/z* 57 \((C_6H_5^+\)) ion profiles which were dominated by straight and branched chain hydrocarbons with possessing 9-20 carbons atoms together with lesser levels of higher chain length hydrocarbons possessing 21 to 40+ carbon atoms SIM GC-MS hydrocarbon \((m/z\ 57\) fingerprints of the 18 examined tarball and oiled sand samples varied greatly (see Appendix 1.1). The October 2011 Pāpāmoa tarball samples afforded \(m/z\ 57\) profiles which were indicative of the loss of lower molecular weight hydrocarbons (up to \(C_{15}\)) primarily by evaporation to the atmosphere whereas some of the January-February 2012 and August-October 2012 tarball/oiled sand samples were hydrocarbon depleted to a much greater extent than that which can be ascribed to evaporative loss to the atmosphere alone.

**PAH, Dibenzothiophene and Tribenzothiophene Profiles**

Significant variability was apparent in the extent to which lower molecular weight PAHs such as naphthalene, methyl-naphthalenes and dimethylnaphthalenes and to a lesser extent phenanthrene and dibenzothiophene were retained in the examined tarball and oiled sand samples.

There was a remarkable consistency in the fingerprint patterns and ratio of peaks observed for parent and alkylated phenanthrenes, alkylated dibenzothiophenes, tetracyclic, pentacyclic, and hexacyclic PAHs such as fluoranthene, pyrene, chrysene, benzo[a]anthracene, di- and tritribenzoethiophenes and \(C_{27}-C_{35}\) hopanoid petroleum biomarker compounds.
Project 1f - Rena PAH and Metals Chemistry

2: SIM GC-MS Comparison of a Rena Tank Oil, an October 2011 Tarball and Four October 2012 Tarball and Oiled Sand Samples

Professor Alistair Wilkins

Introduction

Qualitative total ion chromatogram (TIC) and selected ion mode (SIM) gas chromatography-mass spectrometry (GC-MS) analyses were performed on the following samples:

1) *Rena* tank oil sample supplied via the Biological Sciences Department, University of Waikato, Hamilton, New Zealand

2) TMMR00014 Pāpāmoa Beach, Taylors Reserve, tarball 27-10-2011

3) TMMR02528 Ōmanu, oiled sand, 2-10-2012

4) TMMR02529 Pāpāmoa Beach, Harrisons Cut, oiled sand, 2-10-2012

5) TMMR00970 Waiairiki Reserve Beach surface oil patty, 26-10-2012

6) TMMR00971 Pāpāmoa Beach, Concord Ave, oily sediment (100 mm), 26-10-2012

The objective of the investigation was to determine if the four October 2012, tarball and oiled sand samples were likely to have originated from aged (weathered) *Rena*-like bunker oil, or from other source(s).

It was anticipated that if the 2-10-2012 and 26-10-2012 tarballs or oiled sand samples were derived from sourced *Rena* bunker oil(s) they would be hydrocarbon (especially C₆-C₁₆ hydrocarbon), naphthalene and alkylated naphthalene depleted) compared to a *Rena* bunker oil sample and the Pāpāmoa Beach, Taylors Reserve, tarball sample collected on 27-10-2011.
Results

Tarball, oil patty and sediment samples were extracted by soaking with dichloromethane at room temperature. The *Rena* tank oil sample was diluted with dichloromethane prior to GC-MS analysis.

Three GC-MS analyses were performed:

(i) TIC, 50 to 340°C for all detectable compounds

(ii) 36 ion suite, 50 to 340°C, for hydrocarbons, PAHs and alkylated PAHs

(iii) \( m/z \) 191 ion, 150 to 340°C, for hopanoid petroleum biomarker compounds

Selected ion mode (SIM) GC-MS ion profiles determined for a *Rena* tank oil sample, a 27-10-2011 Pāpāmoa Beach tarball sample and the four 2-10-2012 and 26-10-2012 tarball and oiled sand samples are given in Appendices 2.1 to 2.8:

Appendix 2.1  \( m/z \) 57 ion for hydrocarbons (alkanes)

Appendix 2.2  35 ion profile for PAHs and alkylated PAHs

Appendix 2.3  \( m/z \) 128, 142, 156 and 170 for naphthalene and mono-, di- and trialkylated naphthalene’s

Appendix 2.4  \( m/z \) 178, 192, 206 and 220 for phenanthrene and mono-, di- and trialkylated phenanthrenes

Appendix 2.5  \( m/z \) 202, 216 and 230 for pyrene and mono- and dialkylated pyrenes

Appendix 2.6  \( m/z \) 228, 242 and 256 for benz[a]anthracene, chrysene and mono- and dialkylated benz[a]anthracenes and chrysenes

Appendix 2.7  \( m/z \) 184, 198, 234 and 248 for dibenzo(thiophene, methyl dibenzo-thiophene, tribenzo(thiophene and methyl tribenzo-thiophene

Appendix 2.8  \( m/z \) 191 ion for hopanoid petroleum biomarker compounds.

The appended SIM GC-MS profiles can be compared with those presented in Report 1 for October-November 2011 coastal tarball and *Rena* tank oil samples.

Brief comments are included below some of the profiles.
Conclusions

The levels and ratios of hopanoids and tricyclic, tetracyclic and higher PAHs, as revealed by the SIM GC-MS fingerprint analyses, are consistent with the proposal that the 2-10-2012 and 26-10-2012 tarball and oiled sand samples are derived from weathered *Rena*-like bunker oil.

The extent to which hydrocarbons (alkanes) are depleted in the 2-10-2012 TMMR02528 and TMMR02529 samples and the 26-10-2012 TMMR00970 and TMMR00971 samples is greater than that which can be ascribed to loss by evaporation to the atmosphere alone. It is not known if tidal washing and/or biodegradation may have contributed to hydrocarbon depletion.

The array of dibenzo thiophene, methyl dibenzo thiophene, tribenzo thiophene and methyl tribenzo thiophene peaks found in the four October 2012 tarball and oiled sand samples is comparable to those found in the *Rena* tank oil sample, the October 2011 Pāpāmoa Beach tarball sample and other previously analysed October-November 2011 and January-February 2012 coastal tarball samples (see Chemistry Report Project f-1).
Project 1f - Rena PAH and Metals Chemistry  
3: SIM GC-MS Characterization of Polyaromatic Hydrocarbons (PAHs) in May-August 2012 Coastal Tuatua  

Professor Alistair Wilkins

Introduction

Freeze dried portions of tuatua samples collected from coastal sites May to August 2012 were ground to a fine powder and submitted to Hill Laboratories for determination of the levels of 16 EPA priority polyaromatic hydrocarbons (PAHs). Results are given in Appendix 3.1.

After the completion of Hill's analyses vials were returned to the University where selected samples were analysed using a 19 ion SIM GC-MS method to profile tricyclic, tetracyclic and pentacyclic PAHs and alkylated PAHs that were expected to be persistent in biota.

Discussion

The Bay of Plenty Regional Council (BOPRC) has periodically reported the levels of 16 EPA priority PAHs in coastal biota, including tuatua collected from Ocean Beach Road, Pāpāmoa and Taylors Reserve, Ōmanu.

18/10/2011 Tuatua PAH levels reported by Hill Laboratories

Elevated levels of PAHs were found by Hill Laboratories in extracts of two 18/10/2011 Ocean Beach Road and Taylors Reserve tuatua samples (124.8 and 98.2 µg/kg respectively) known to have been exposed to Rena sourced bunker oil (Table 3.1).
<table>
<thead>
<tr>
<th>MWt</th>
<th>Compound</th>
<th>11/45831 mg/kg</th>
<th>11/45834 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>Naphthalene</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>152</td>
<td>Acenaphthylene</td>
<td>0.0007</td>
<td>0</td>
</tr>
<tr>
<td>154</td>
<td>Acenaphthene</td>
<td>0.0022</td>
<td>0</td>
</tr>
<tr>
<td>166</td>
<td>Fluorene</td>
<td>0.0041</td>
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<td>178</td>
<td>Phenanthrene</td>
<td>0.0183</td>
<td>0.0084</td>
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<tr>
<td>178</td>
<td>Anthracene</td>
<td>0.0017</td>
<td>0.0007</td>
</tr>
<tr>
<td>202</td>
<td>Fluoranthene</td>
<td>0.0065</td>
<td>0.0055</td>
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<tr>
<td>202</td>
<td>Pyrene</td>
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<td>0.028</td>
</tr>
<tr>
<td>228</td>
<td>Benzo[a]anthracene</td>
<td>0.016</td>
<td>0.0144</td>
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<tr>
<td>228</td>
<td>Chrysene</td>
<td>0.0194</td>
<td>0.0184</td>
</tr>
<tr>
<td>252</td>
<td>Benzo[k]fluoranthene</td>
<td>0.0011</td>
<td>0.0008</td>
</tr>
<tr>
<td>252</td>
<td>Benzo[b]fluoranthene*</td>
<td>0.0068</td>
<td>0.0064</td>
</tr>
<tr>
<td>252</td>
<td>Benzo[a]pyrene (BaP)</td>
<td>0.0082</td>
<td>0.0082</td>
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<tr>
<td>276</td>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>0.0014</td>
<td>0.0012</td>
</tr>
<tr>
<td>278</td>
<td>Dibenzo[a,h]anthracene</td>
<td>0.0017</td>
<td>0.0014</td>
</tr>
<tr>
<td>276</td>
<td>Benzo[g,h,i]perylene</td>
<td>0.0041</td>
<td>0.0042</td>
</tr>
</tbody>
</table>

* includes benzo[j]fluoranthene

Total (mg/kg)  
11/45831: 0.1242  
11/45834: 0.0982  
Total (µg/kg)  
11/45831: 124.2  
11/45834: 98.2

1 Results are reported on a wet weight (as received) basis. They cannot be directly compared with the freeze dried results reported in Appendix 1 for May-August tuatua extracts.
Significant levels of higher molecular weight PAHs (202-278 Daltons; pyrene, chrysene, dibenzo[a,h]anthracene, etc.) are generally found in biota extracts exposed to lightly weathered bunker oils during an impact period. Lower molecular weight PAHs (naphthalene to fluorene: 128 to 166 Daltons), although more prevalent in bunker oils than higher molecular weight PAHs, are typically not major constituents of biota extracts.

It is apparent from subsequent BOPRC results that PAH levels in Pāpāmoa and Ōmanu tuatua have declined to near pre-Rena baseline levels (Figure 3.3). Notwithstanding this, it was of interest to determine if residual PAHs possessed Rena-like PAH and alkylated PAH fingerprints.

![Contaminant levels nearing the pre-Rena baseline](image)

**Figure 3** PAH levels in tuatua from Pāpāmoa and Ōmanu sites. Source: Bay of Plenty Regional Council (BOPRC), Contaminant monitoring update #4 (12 June 2012). URL: [http://www.boprc.govt.nz/sustainable-communities/Rena-environmental-monitoring/](http://www.boprc.govt.nz/sustainable-communities/Rena-environmental-monitoring/)

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May–August 2012 tuatua samples

The objective of the SIM GC-MS fingerprint analyses was to ascertain the extent to which it might be still be possible to identify residual PAH and alkylated PAH contributions attributable to exposure to bunker oil of the type used by the *Rena* in extracts of tuatua collected from Bay of Plenty sites that were impacted by oil lost from the *Rena*.

The sites from which samples were collected are given in Appendix 3.1. Site locations and impact period PAH levels (wet weight basis) as previously reported by the BOPRC are shown in Figure 3.4.

Freeze dried extracts were used in this investigation. Advantages of this approach are superior long-term comparability of results due to the elimination of variability attributable to the differing water content of analysed material and superior detection limits on a mg/kg dry weight basis.

Freeze dried material typically has a weight that is 2-3 times or more lower than that of wet weight of biota material.

![Environmental Sampling Sites for *Rena* Oil Spill - Kaimoana](image)

Figure 4 Coastal sampling site locations. Source: Bay of Plenty Regional Council (BOPRC). [URL](http://www.boprc.govt.nz/sustainable-communities/Rena-environmental-monitoring/Rena-kaimoana-sampling-results-pah-totals/)

The highest levels total PAHs reported by Hill Laboratories for the freeze dried May–August 2012 tuatua extracts were in the range 56-43 µg/kg for four Pāpāmoa samples and a Leisure Island sample. Levels in the range 37-35 µg/kg were found in another two Pāpāmoa samples and a Waihi (Bowentown) sample (see Appendix 1). All other freeze dried tuatua samples were found to contain < 30 µg/kg of total PAHs.

Results determined for freeze dried extracts of tuatua gathered from Waihau Bay and Whangaparaoa Bay revealed the presence of low but detectable levels of a selection of total PAHs in the range 6 to17 µg/kg. Although these sites are > 100 km from the Astrolabe Reef they are documented as being impacted by debris, containers and oil after the grounding incident.
Seven of the sixteen PAHs listed in Table 3.1 (and in Tables included in Appendix 3.1), namely benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene and indeno(1,2,3-cd)pyrene are believed to be probable human carcinogens (see http://en.wikipedia.org/wiki/Polycyclic_aromatic_hydrocarbon and refs sited therein).

The carcinogenic, mutagenic and teratogenic properties of these and other PAHs have been summarised by Luch (2005). Material Safety Data sheets (MSDS) for these compounds can be retrieved from a variety of web sources including Sigma-Aldrich: URL http://www.sigmaaldrich.com/safety-center.html

The highest level (29.2 µg/kg) of the seven health risk PAHs was found in the 21-6-2012 TMMR00460 Pāpāmoa Domain tuatua extract. Levels in the range 15-23 µg/kg were found in four other Pāpāmoa Domain tuatua extracts (TMMR00455, TMMR00456, TMMR00447 and TMMR00459) and a Leisure Island tuatua extract (TMMR0081) (see Appendix 1).

SIM GC-MS profiling of the Leisure Island samples

The SIM GC-MS profiles determined for parent and alkylated PAHs present in freeze dried TMMR00079 and TMMR00081 Leisure Island tuatua extracts and a Rena tank oil sample are compared in Appendix 2.

Phenanthrene and alkylated phenanthrene (Appendix 3.2, Figure 1), fluoranthene, pyrene, alkylated fluoranthene and alkylated pyrene (Figure 2), benz[a]anthracene, chrysene, alkylated benz[a]anthracene and alkylated chrysene (Figure 3), tribenzoephenone, alkylated tribenzoephenone (Figure 4) and pentacyclic PAH and alkylated pentacyclic PAH (Figure 5) profiles are included in Appendix 2.

The fingerprint profiles of the TMMR00079 and TMMR00081 Leisure Island tuatua extracts which Hill Laboratories reported levels of PAHs of 23.1 µg/kg level and 46.2 µg/kg respectively are consistent with the proposal that exposure to bunker oil of the type used on the Rena has contributed to the PAH and alkylated PAH levels detected in these extracts.

Phenanthrene and alkylated phenanthrene profiles

The SIM mode phenanthrene and alkylated phenanthrene profiles of 30 of the tuatua extracts and three replicate Rena tank oil profiles are presented in Appendix 3 (Figures 5a-f).

The sensitivity of the SIM GC-MS procedure was such that it was possible to demonstrate the presence of phenanthrene in one of the Leisure Island extracts (TMMR00079) and five of the Ōmanu extracts (TMMR00464, TMMR00466, TMMR00467, TMMR00468 and TMMR00469) at a level below the detection limit of Hill Laboratories PAH method.

Based on relative signal intensities (vertical scale signal count levels) the level of alkylated phenanthrenes was in all cases less than that of phenanthrene.

In all cases the phenanthrene and alkylated phenanthrene profiles presented in Appendix 3 are consistent with the proposal that exposure to bunker oil of the type used on the Rena has contributed to the residual phenanthrene and alkylated phenanthrene levels detected in the freeze dried extracts.
Project 1f - Rena PAH and Metals Chemistry
4: Polyaromatic Hydrocarbons (PAHs) in May–August 2012 Coastal Kina

Professor Alistair Wilkins

Introduction

Kina portions were freeze dried and ground to a fine powder and submitted to Hill Laboratories for determination of the levels of 16 EPA priority polyaromatic hydrocarbons (PAHs). Results are given in Appendix 4.1.

After the completion of Hill's analyses vials were returned to the University where selected samples were analysed using a 19 ion SIM GC-MS method to profile groups of PAHs and alkylated PAHs that were expected to be persistent in biota.

The sensitivity of the freeze dried PAH analyses utilised in recovery project investigations is greater than that of the wet weight methodology used to obtain results reported elsewhere.

Discussion of Results

The objective of the SIM GC-MS fingerprint analyses was to ascertain the extent to which it might be possible to identify PAH and alkylated PAH contributions attributable to bunker oil of the type used by the Rena.

Significant levels of tricyclic and tetracyclic PAHs such as phenanthrene, pyrene and chrysene (178, 202 and 228 Daltons) are often present in extract of biota that have been exposed to bunker oils. Lower molecular weight PAHs (naphthalene to fluorene: 128 to 166 Daltons), although more prevalent in bunker oils than higher molecular weight PAHs, are typically not major constituents of biota extracts.

May-August 2012 Kina Samples

The highest level of total PAHs reported by Hill Laboratories for the freeze dried May-August 2012 kina extracts was 73.2 µg/kg in the guts of a 10-8-2012 Plate Island (Motunau, site 4 deep) kina sample.

Levels of PAHs (primarily phenanthrene, fluoranthene and pyrene) in the range 51-25 µg/kg were found in the freeze dried guts or gonads (or both) of ten of the sixteen kina samples (guts and gonad extracts) that were analysed by Hill Laboratories (see Appendix 1).

Low but detectable levels of PAHs in the range 5.5 to 35.9 µg/kg were found in the freeze dried extracts of the guts and gonads of kina gathered from Waihau Bay (30/8/2012) and Whangaparaoa Bay (29/8/2012). Although these sites are remote (> 100 km) from the Astroabe Reef they were impacted by debris, containers and oil after the grounding incident. The BOPRC has reported that these sites were lightly or very lightly impacted by Rena oil (see Figure 1) and that only very low, near baseline, levels of PAHs were detectable in on a wet weight basis in kaimoana from these sites (URL: http://www.boprc.govt.nz/sustainable-communities/Rena-environmental-monitoring/Rena-kaimoana-sampling-results-pah-totals/).

SIM GC-MS profiles of five Mōtītī Island and a Whangaparaoa Island Bay kina sample
The SIM mode GC-MS fingerprint profiles determined for phenanthrene and alkylated phenanthrenes present in a *Rena* tank oil sample and the freeze dried gut extracts of five Mōtūti Island and a Whangaparaoa Island Bay kina sample are compared in Appendix 4.2.

![Map of Waiahu and Whangaparaoa Bay](image)

**Figure 1** Waiahu and Whangaparaoa Bay kaimoana sampling sites and impact period PAH levels (wet weight basis) as reported by the BOPRC.

*Rena* tank oils are characterized by four major and a fifth minor alkylated phenanthrene peak (m/z 192 ion responses) in the 15.6-16.2 minute region of the profiles. This pattern is replicated in the biota profiles.

A more complex series of dialkylated phenanthrene peaks occur in the 17.5-18.2 minute region of the *Rena* tank oil and the six biota m/z 206 ion (dialkylated phenanthrene) profiles.

The phenanthrene and alkylated phenanthrene fingerprint profiles determined for the five Mōtūti Island and a Whangaparaoa Bay kina gut extracts are consistent with the proposal that exposure to bunker oil of the type used on the *Rena* has contributed to the phenanthrene and alkylated phenanthrenes detected in these extracts.

**Health Risk PAHs**

Seven of the sixteen PAHs listed in in Tables included in Appendix 4.1, namely benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene and indeno(1,2,3,c,d)pyrene have been reported by the US Environmental Protection Agency (EPA) to be probable human carcinogens.

The carcinogenic, mutagenic and teratogenic properties of these and other PAHs have been summarised by Luch (2005).

The highest level (5.0 µg/kg) of the seven health risk PAHs was found in the guts of a Plate Island (Motunau, site 4 deep) kina sample (see Appendix 4.1). No EPA health risk PAHs were found in the majority of the gut or gonad extracts.
Project 1f - Rena PAH and Metals Chemistry
5: Polyaromatic Hydrocarbons (PAHs) in Air Dried August and November 2012 Otaiti Reef sediments
Professor Alistair Wilkins

Introduction

Sediments collected from nine Otaiti Reef sites (shallow and more deeply settled material; total 15 samples) on the 18th to 23rd August 2012 (Astrolabe 1-9) and from sixteen sites 200-1000 m distant from the reef (GS1-16 sites) on the 1st November 2012 were air dried and submitted to Hill Laboratories for determination of the levels of 16 EPA priority polyaromatic hydrocarbons (PAHs).

Sample sites and the location of the Rena wreckage are shown in Figures 1 and 2.

Results are given in Appendix 5.1 (Tables 1 and 2).

Figure 1 Astrolabe Reef sites (18th to 23rd August 2012).
18th-23rd August 2012 Astrolabe Reef sediment samples

The highest level of total PAHs determined by Hill Laboratories for the fifteen 18th-23rd August 2012 sediment samples was 27.788 mg/kg (27788 g/kg) in the site 2 deep reef sediment sample (Figure 3). This site is adjacent to the *Rena* wreckage.

Figure 3 PAHs (mg/kg) detected in the Astrolabe site 2 deep sediment sample.

The profile of PAHs observed for this sample and other Otaiiti reef sediment samples are consistent with them being derived from bunker oil(s) of the type used on the *Rena* and are comparable with those determined for the impact period (October-November 2011) and aged (February-August 2012) coastal tarball samples (see Reports 1 and 2).
A characteristic feature of bunker oil derived tarballs and aged sediment samples is that the loss of the majority of the lower molecular weight PAHs such as naphthalene, acenaphthylene, acenaphthene and fluorene is observed while higher molecular weight tetracyclic, pentacyclic and tetracyclic PAHs such as fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene as largely retained. On the other hand phenanthrene levels in ocean floor sediments are typically greater than those found in coastal tarballs (see Reports 1 and 2) due to the oil from which ocean floor sediments are derived not being exposed to long-term atmospheric evaporative effects.

![Figure 4 PAHs (mg/kg) detected in the Astrolabe site 4 shallow sediment sample.](image1)

![Figure 5 PAHs (mg/kg) detected in the Astrolabe site 5 deep sediment sample.](image2)

![Figure 6 PAHs (mg/kg) detected in the Astrolabe site 6 shallow sediment sample.](image3)

![Figure 7 PAHs (mg/kg) detected in the Astrolabe site 4 deep sediment sample.](image4)

Total PAH levels of 12.817 mg/kg (1.2817 g/kg), 11.323 mg/kg (1.1323 g/kg), 5.149 mg/kg (0.5149 g/kg) and 3.188 mg/kg (0.3188 g/kg) where found in the site 4 shallow, site 5 deep, site 6 shallow and site 4 deep reef sediments samples respectively (Figures 4 to 7).

As the levels of total PAHs decrease there is a tendency for % contributions of higher PAHs to increase as is evident for the site 4 deep reef sediment (see Figure 7).
Total PAH levels of 2.399 mg/kg, 1.921 mg/kg, 1.697 mg/kg and 1.136 mg/kg (2399 µg/kg, 1921µg/kg, 1.697 µg/kg and 1136 µg/kg respectively) where found in the site 6 deep, site 7 deep, site 7 shallow and site 8 shallow reef samples respectively (Figures 8 to 11).

Significant variability was apparent in the % contributions of di- and tricyclic PAHs, tetracyclic PAHs and pentacyclic PAHs (See Appendix 5.1).

With the exception only of the site 1 deep sediment sample the fluoranthene/pyrene ratio was > 1.0, whereas it was generally in the range 0.3-0.8 in freeze dried coastal kina gut extracts and < 0.33 in Rena tank oil samples recovered shortly after the grounding.

Seven of the sixteen PAHs listed in Appendix 5.1 (Tables 1 and 2), namely benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene and indeno(1,2,3,c,d)pyrene, have been reported by the US Environmental Protection Agency (EPA) to be probable human carcinogens.

The carcinogenic, mutagenic and tetragenic properties of these and other PAHs have been summarized by Luch (2005).

The highest levels of the seven EPA health risk PAHs (5588 µg/kg, 4675 µg/kg and 4334 µg/kg) were found in the site 2 deep, site 4 shallow and site 5 deep reef sediment samples respectively.
Levels of 1540 µg/kg, 1380 µg/kg, 1110 µg/kg, 809 µg/kg and 737 µg/kg were found in the site 4 deep, site 6 shallow, site 6 deep, site 7 shallow and site 7 deep reef sediment samples.

The levels of the seven EPA health risk PAHs detected in the other 18th-23rd August 2012 sediment samples varied from 11-317 µg/kg.

**Alkylated PAH contributions**

Alkylated PAHs of the type identified in *Rena* oil samples (see Reports 1 and 2) were present in all of the 18th-23rd August 2012 samples. Representative SIM-GCMS profiles for alkylated phenanthrene, fluoranthene, pyrene, chrysene and benzo[a]anthracenes, as detected in the Astrolabe 2 Deep (TMMR02630) and Astrolabe 4 Shallow (TMMR02617) sediment samples are included in Appendix 5.2.

**1st November 2012 outer reef sediment samples**

The highest level of total PAHs reported by Hill Laboratories for the twelve 1st November 2012 outer reef sediment samples extracts was 0.72 mg/kg (720 µg/kg) in the GS13 sample (Figure 12). Levels of 0.212 mg/kg, 0.112 mg/kg and 0.099 mg/kg (212 µg/kg, 112 µg/kg and 99 µg/kg respectively) were found in the GS14, GS6 and GS7 grab samples respectively. No PAHs were detected in the GS1, GS3, GS9 and GS15 samples.

Caution should be exercised when comparing the reported levels of compounds in sediment samples since the method of collection and biases of the collector can influence which portions of a typically non-homogenous ocean floor or reef sediment zone are gathered and presented for analysis.

![Astrolabe GS13 Grab sample](image1)

![Astrolabe GS14 Grab sample](image2)
The levels of the seven EPA health risk PAHs in the twelve 1st November outer reef sediment samples which had detectable levels of PAHs ranged from 2 to 232 μg/kg in the GS13 sample.
**Introduction**

 Portions of kina (guts and gonads), paua, crayfish, and four fish species collected from nine Astrolabe reef sites on the 18th to 23rd August 2013 were freeze dried and ground to a fine powder and submitted to Hill Laboratories for determination of the levels of 16 EPA priority polycyclic hydrocarbons (PAHs). Results are given in Appendix 6.1. (Table 1; Reef sites 1-9 are as marked in Figure 1).

 Caution should be exercised when comparing freeze dried PAH levels with those determined on a wet weight basis. Typically freeze dried levels are 2-3 times or more greater than those determined on a wet weight basis.

**Kina PAH levels**

 The highest level of total PAHs reported by Hill Laboratories for the freeze dried kina extracts was 24.191 mg/kg (24191 µg/kg) in the guts of the site 3 shallow kina sample (Figure 6.1). A much lower level of total PAHs (306 µg/kg) was detected in the gonads of this sample.

![Figure 1 PAHs (mg/kg) detected in the freeze dried gut extract of an Astrolabe Reef, site 3 shallow, kina sample](image)

 Levels of total PAHs in the range 0.914 to 0.416 mg/kg (914 to 416 µg/kg) were detected in the freeze dried gut extracts the site 7 shallow, site 1 shallow and site 2 shallow reef sites (see Figures 2, 3 and 4). As was the case for the site 3 shallow sample much lower total PAH levels in the range 0.04 to 0.179 mg/kg (40 to 179 µg/kg) were present in the gonads of these samples (40 to 179 µg/kg).

 All total PAH level of 0.188 mg/kg (188 µg/kg) was found in the freeze dried gut extracts of the site 4 shallow sample (Figure 5). Levels in the range 0.109-0.151 mg/kg (109-151 µg/kg) were found in extracts of the freeze dried 7 deep, 6 shallow and 6 deep samples.

 Less than 0.02 mg/kg (20 µg/kg) of total PAHs were detected in the freeze dried gut extracts of the 4 deep, 5 shallow, 5 deep, 8 shallow, 9 shallow and 9 deep kina samples.
Significant variability was apparent in the % contributions of di- and tricyclic PAHs, tetracyclic PAHs and pentacyclic PAHs (See Appendix 6.1).

Figure 2 PAHs (mg/kg) detected in the freeze dried gut extract of an Astrolabe Reef, site 7 shallow, kina sample.

Figure 3 PAHs (mg/kg) detected in the freeze dried gut extract of an Astrolabe Reef, site 1 shallow, kina sample.

Figure 4 PAHs (mg/kg) detected in the freeze dried gut extract of an Astrolabe Reef, site 2 shallow, kina sample.

Figure 5 PAHs (mg/kg) detected in the freeze dried gut extract of an Astrolabe Reef, site 4 deep, kina sample.

In most cases the fluoranthrene/pyrene ratio was > 1.0, whereas it generally in the range 0.3-0.8 in freeze dried coastal kina gut extracts and < 0.33 in *Rena* tank oil samples recovered shortly after grounding of the *Rena*.

Seven of the sixteen PAHs listed in Table 1, and in Tables included in Appendix 1, namely benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene and indeno(1,2,3-cd)pyrene have been reported by the US Environmental Protection Agency (EPA) to be probable human carcinogens.

The carcinogenic, mutagenic and teratogenic properties of these and other PAHs have been summarised by Luch (2005).

The highest level (11490 µg/kg) of the seven EPA health risk PAHs was found in the freeze dried gut extracts of the site 3 shallow kina sample (see Appendix 6.1). The levels of the seven EPA health risk PAHs detected in freeze dried kina gut extracts varied from 0-383 µg/kg.
A selection of the reef kina extracts were examined in SIM mode using a 36 ion suite which fingerprinted the presence of di-, tri- and tetracyclic PAHs and alkylated analogues of parent PAHs in the extracts. A diagnostic aspect of the SIM GC-MS profiles of *Rena* tank oils and tarballs derived from them is the presence four major methyl phenanthrene peaks in the ratio ca 1:1:1:1 with a fifth minor peak interspersed between the second and third eluting peak (see Reports 1-4). This pattern of methyl phenanthrene peaks was detected in the majority of reef kina extracts.

Illustrative m/z 178 (phenanthrene, 18.7 minutes) and 192 ion methyl phenanthrene profiles (5 peaks in the region 20.4 to 22.2 minutes) determined for four of the kina extracts are presented in Figures 6-8.

![Figure 6](image6.png)  
**Figure 6** TMRR02532 site 2 shallow kina (gonads) extract: m/z 178 and 192 ion profiles.

![Figure 7](image7.png)  
**Figure 7** TMRR02533 site 2 shallow kina (guts) extract: m/z 178 and 192 ion profiles.

![Figure 8](image8.png)  
**Figure 8** TMRR02566 Site 6 shallow kina (gonads) extract: m/z 178 and 192 ion profiles.

![Figure 9](image9.png)  
**Figure 9** TMRR02541 site 3 shallow kina (guts) extract: m/z 178 and 192 ion profiles.

**Paua, Crayfish and Fish Species**

Total PAH levels in the range 0.0095 - 0.057 mg/kg (9.5 – 57.1 µg/kg) were detected in four freeze dried Paua extracts (see Table 1, Appendix 1). Total PAH levels in the range 0.0008 – 0.0265 mg/kg (0.08 – 26.5 µg/kg) were detected in a site 7 deep crayfish (0.0126 mg/kg), a site 9 deep dwarf scorpion fish (0.0115 mg/kg), a site 8 shallow leather jacket (0.0363 mg/kg), a site 4 deep red moki (0.0008 mg/kg) and a site 1 sea perch extract (0.0265 mg/kg).
A much higher level of total PAHs (0.265 mg/kg; 26.5 µg/kg) was detected in a site 3 shallow blue cod muscle/liver sample. The array and level of PAHs (see Appendix 6.1, Table 1) found in the muscle/liver extract are consistent with their source being a bunker oil of the type used on the *Rena*.

SIM GCMS analyses demonstrated the presence in the four paua extracts and the blue cod muscle/liver extract of phenanthrene and five methyl phenanthrenes in ratios indicative of their origin from bunker oils of the type used on the *Rena*.

The m/z 178 (phenanthrene, 18.7 minutes) and 192 ion methyl phenanthrene profiles (5 peaks in the region 20.4 to 22.2 minutes) determined for the four paua extracts and the blue cod extract are presented in Figures 10-14. Some non-PAH peaks were also detected in the extracts. The signal to noise of the m/z 192 profiles determined for the crayfish, dwarf scorpion fish, leather jacket, red moki and sea perch extracts were such that presence of a series of methyl phenanthrenes in these extracts could not be established.
Figure 12 TMMR02562 site 6 shallow paua extract: m/z 178 and 192 ion profiles.

Figure 13 TMMR02549 site 4 shallow paua extract: m/z 178 and 192 ion profiles.

Figure 14 TMMR02586 site 3 shallow blue cod muscle/liver extract: m/z 178 and 192 ion profiles.
Project 1f - Rena PAH and Metals Chemistry
7: Identification of some Organic Compounds other than PAHs and Hydrocarbons in Otaiiti Reef Sediments

Professor Alistair Wilkins

Introduction

In order to ascertain whether or not GC-MS detectable organic compounds other than hydrocarbons and PAHs were present in Otaiiti Reef sediment samples, hand selected subsamples of 13 of the 15 inner reef sediments and the 16 outer reef sediment sample for which PAH levels have previously been determined (see Report 5), were soaked in dichloromethane and compounds present in the soakings were investigated using total ion chromatogram (TIC) and selected ion mode (SIM) GC-MS methods.

The TIC profiles of four of the examined reef sediment samples are given in Appendix 1. These profiles are representative of the widely varying TIC profiles determined for the reef sediment samples. The TIC of a hull scrap sample is included in Appendix X.

The TIC profile of the TMMR02630 (inner reef site 2 deep) sediment sample was dominated by low to intermediate molecular weight hydrocarbon peaks (C10-C20), together with lower levels of an interspersed series of PAH and alkylated PAHs and peaks attributable to some other compounds. Hill Laboratories have reported a total level of 27.788 mg/kg (27788 µg/kg) of PAHs in the TMMR02630 sediment sample.

The TIC profiles determined for the TMMR02617 (inner reef site 4 shallow), TMMR02624 (inner reef site 8 shallow) and TMMR01125 (outer reef GS13 site) sediment samples were dominated by peaks attributable to compounds other than hydrocarbons or PAHs. Hill Laboratories have reported total PAH levels of 12.817, 1.136 and 0.72 mg/kg (12817, 1136 and 720 µg/kg) respectively in these sediment samples.

Four groups or series of the compounds, in addition to PAHs and hydrocarbons of the type present in bunker oils of the type used on the Rena were identified in the TIC profiles of some of the examined reef sediments namely:

(i) tributyltin chloride (TBT)
(ii) sulphur (S8)
(iii) a series of compounds believed to be dimeric and trimeric cumene (cumyl) and a dicumylphenol analogues.
(iv) a series of tricresylphosphate (TCP) isomers.

Representative mass spectra and library mass spectra of tributyltin chloride, sulphur (S8) four cumyl-type compounds and a tricresylphosphate isomer are included in Appendix 2.

A 14 ion SIM GC-MS method was developed to detect TBT, S8, TCP and cumyl type compounds in reef sediment samples. While it is likely sulphur (S8) is a natural background compound, that is not likely to be the case for TBT, TCP and cumyl-type compounds. TBT is a well-known constituent of anti-fouling marine paints. TBT’s environmental impacts are well documented. In recent years its use has been prohibited in many countries.

Little is known about the source(s) and environmental impact of dimeric and trimeric cumyl adducts. Possibly they may be paint sourced components since TIC GC-MS analysis of the TMMR0549 hull scrap sample afforded peaks attributable to series of dimeric and trimeric cumyl adducts (see Chemistry Appendix 4) including the those detected in reef sediment extracts.
Tricresylphosphate is known to be a component of some hydraulic and lubricating oils. Modern production techniques typically afford mixtures of o- and p-cresyl products.

Lesser levels of phenyl or methylated cresyl isomers are generally also present in commercial TCP products. SIM GC-MS profiling (see Appendix 5) verified the present of phenyl and methylated cresyl isomers in the TMMR026xx reef sediment extract.

The environmental and human health impacts of m-cresyl isomers are greater than those of o- or p-cresyl isomers. Mackerer et al (see Appendix 5) have reviewed the neurotoxic and other human health effects arising from the use of TCP in jet engine oils.

Detection of TBT, S8 and TCP in Astrolabe reef sediment samples

The SIM GC-MS m/z 269 [for tributyltin chloride (TBT)], m/z 256 [for sulphur (S8)] and m/z 368 [for tricresylphosphate isomers (TCP)] ion profiles determined for the August 2012 (inner) and November 2012 (outer) Astrolabe reef sediment samples are presented in Chemistry Appendix 2 (Figures 1-13) and Appendix 3 (Figures 1-16), respectively. The site locations from which these samples were collected are shown in Figures included in the Appendices.

TBT, S8 and TCP isomers were detected in all of the inner reef (August 2012) sediment samples examined in the investigation.

TCP was only detected in the GS6 and GS13 outer reef sediment samples. The level of total PAHs in these samples was 0.112 and 0.72 mg/kg (112 and 720 μg/kg) respectively.

Detection of cumyl analogues in Astrolabe reef sediment samples

The SIM GC-MS m/z 236 ion profiles for dimeric cumene (dicumyl adduct) compounds believed to be 1,1,3-trimethyl-3-phenylindane and 2,4-diphenyl-4-methyl-E-pent-2-ene (see Appendix 1) and m/z 330 ion profiles for 2,4-bis(dimethylbenzyl)phenol determined for the August 2012 (inner) and November 2012 (outer) Astrolabe reef sediments sample are presented in Appendix 2 (Figures 14-26) and Appendix 3 (Figures 17-32), respectively.

The dicumyl adduct compounds, believe to be 1,1,3-trimethyl-3-phenylindane, 2,4-diphenyl-4-methyl-E-pent-2-ene and 2,4-bis(dimethylbenzyl)phenol, were detected in all of the inner reef (August 2012) sediment samples examined in the investigation.

Readily detectable levels of the compounds believed to be 1,1,3-trimethyl-3-phenylindane, 2,4-diphenyl-4-methyl-E-but-2-ene and 2,4-bis(dimethylbenzyl)phenol were present in the outer reef TMMR01125 GS13 sediment sample. This sample is characterized by the highest level of PAHs (0.720 mg/kg) determined for an outer reef sediment sample (see Report 5).

Other outer reef sediment samples were either devoid of these compounds or in the case of the TMMR01101 GS6, TMMR01129 GS11, TMMR01127 GS12 and TMMR01123 GS14 sediment samples characterized by the presence of only low or trace levels of them.
Introduction

The detection of tributyltin chloride (TBT), dimeric and trimeric cumyl type adducts and tricresylphosphate (TCP) isomers in Otaititi Reef sediments (see Report 7) prompted an examination of a selection of reef biota extracts gathered from sites 1, 2, 6 and 7 (Figure 1) using total ion chromatogram (TIC) and selected ion mode (SIM) GC-MS methods. The levels of PAHs found in the 12 examined extracts, as reported by Hill Laboratories are listed in Table 1.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>site</th>
<th>date</th>
<th>description</th>
<th>Total PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMMR02533</td>
<td>2 shallow</td>
<td>18-08-2012</td>
<td>kina, guts</td>
<td>0.416 mg/kg</td>
</tr>
<tr>
<td>TMMR02532</td>
<td>2 shallow</td>
<td>18-08-2012</td>
<td>kina, gonads</td>
<td>0.179 mg/kg</td>
</tr>
<tr>
<td>TMMR02567</td>
<td>6 shallow</td>
<td>21-08-2012</td>
<td>kina, guts</td>
<td>0.124 mg/kg</td>
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<tr>
<td>TMMR02566</td>
<td>6 shallow</td>
<td>21-08-2012</td>
<td>kina, gonads</td>
<td>0.066 mg/kg</td>
</tr>
<tr>
<td>TMMR02589</td>
<td>1 deep</td>
<td>21-08-2012</td>
<td>kina guts</td>
<td>0.093 mg/kg</td>
</tr>
<tr>
<td>TMMR02552</td>
<td>4 shallow</td>
<td>21-08-2012</td>
<td>kina guts</td>
<td>0.188 mg/kg</td>
</tr>
<tr>
<td>TMMR02568</td>
<td>7 deep</td>
<td>21-08-2012</td>
<td>kina guts</td>
<td>0.109 mg/kg</td>
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<tr>
<td>TMRR02575</td>
<td>7 shallow</td>
<td>21-08-2012</td>
<td>kina guts</td>
<td>0.914 mg/kg</td>
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<tr>
<td>TMMR02534</td>
<td>2 shallow</td>
<td>18-08-2012</td>
<td>paua</td>
<td>0.026 mg/kg</td>
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<tr>
<td>TMMR02572</td>
<td>7 shallow</td>
<td>21-08-2012</td>
<td>paua</td>
<td>0.057 mg/kg</td>
</tr>
<tr>
<td>TMMR02586</td>
<td>3 shallow</td>
<td>18-08-2012</td>
<td>blue cod, muscle + liver</td>
<td>0.264 mg/kg</td>
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<tr>
<td>TMMR02597</td>
<td>1 deep</td>
<td>18-08-2012</td>
<td>sea perch</td>
<td>0.027 mg/kg</td>
</tr>
</tbody>
</table>
Figure 1 Otago Reef sampling sites. Biota were collected from sites 1, 2, 6 and 7.

GC-MS Analyses

Biota extracts, returned from Hill Laboratories after analyses for polyaromatic hydrocarbons (PAHs) were examined in TIC and SIM GS-MS mode. Detection (bolded) and confirmation ions monitored in the 15 ion SIM suite were:

- **m/z 267** and **269** for tributyltin chloride
- **m/z 236, 91, 119** and **143** for dimeric and trimeric cumyl adducts, including compounds believed to be **1,1,3-trimethyl-3-phenylindane** and **2,4-diphenyl-4-methyl- E-pent-2-ene**
- **m/z 330** and **315** for **2,4-bis(dimethylbenzyl)phenol**
- **m/z 212** and **197** for **4-(dimethylbenzyl)phenol**
- **m/z 448** and **433** for **2,4,6-tris(dimethylbenzyl)phenol**
- **m/z 368, 354** and **165** for tricresyl phosphate and phenyldicresylphosphate isomers

Tributyltin chloride

A trace level of a peak that may be attributable to tributyltin chloride was detected in some of the kina extracts however its identification has not been confirmed. It is recommended that biota TBT levels are determined for samples submitted to Hill Laboratories.
**Dimeric cumyl adducts**

Dimeric cumyl adducts, believed to be (in elution order) 1,1,3-trimethyl-3-phenylindane, 2,4-diphenyl-4-methyl-pent-1-ene and 2,4-diphenyl-4-methyl-E-pent-2-ene, together with 2,4-bis(dimethylbenzyl)phenol were detected in most of the examined biota samples.

The NIST library mass spectra of 1,1,3-trimethyl-3-phenylindane and 2,4-diphenyl-4-methyl-E-pent-2-ene are characterized by strong m/z 236 (M+) ions while only a weak M+ ion is seen in the mass spectrum of 2,4-diphenyl-4-methyl-pent-1-ene. Strong m/z 143 fragment ions appear in the mass spectra of 1,1,3-trimethyl-3-phenylindane and 2,4-diphenyl-4-methyl-E-pent-2-ene while a strong m/z 119 ion fragment ion appears in the mass spectrum of 2,4-diphenyl-4-methyl-pent-1-ene (see NIST library mass spectra included in Appendix 1).

These characteristics are apparent (Figure 2) in the m/z 234, 119 and 143 ion profiles determined for peaks observed in the TMMR02533 site 2 kina gut extract.

![Figure 2 m/z 234, 119 and 143 ion profiles observed for three compounds believed to be dimeric cumyl compounds(17.8, 19.0 and 19.8 min) detected in the TMMR02533 site 2 kina gut extract.](image)

**Dimethylbenzylphenol (= dicumylphenol) compounds**

2,4-bis(dimethylbenzyl)phenol was detected in 11 of the 12 examined biota extracts, including the site 2 TMMR02533 kina gut extract (Figure 3). This compound has hitherto been detected in extracts of inner Astrolabe reef samples, two outer reef sediment samples and a hull scrap sample (see Report 7).

Since 2,4,6-tris(dimethylbenzyl)phenol, the trisubstituted analogue of 2,4-bis(dimethyl-benzyl)phenol was detected in the TIC GC-MS profiles of some reef sediment samples, it was of interest to determine whether or not it was present in biota extracts. A trace a level of 2,4,6-tris(dimethylbenzyl)phenol was only detected in site 2 TMMR2533 kina gut extract (Figure 4).
Tricresylphosphate isomers

Strong m/z 386 (M+) and m/z 368 (M-H2O)+ ions are exhibited by cholesterol and epimerized cholesterol analogues. Care is required to avoid GC-MS acquisition conditions which lead to the partial overlap of the m/z 368 ion responses of TCP isomers and cholesterol isomers.

Tricresylphosphate (TCP) isomers were only detected in the site 2 TMMR02533 kina gut (Figure 5) and site 1 blue cod muscle/liver sample (see Appendix 1). The ratio of o,o,o-, o,o,p-, o,p,p- and p,p,p-TCP isomers in extracts of these samples was in accord with that calculated in Report 7, Appendix 5, for a 57.3:43.7 ratio of o- to p- isomer contributions, as also found for reef sediment samples.
Discussion of results

Brief comments concerning the identification of dimeric cumyl adducts and 2,4-bis(dimethylbenzyl)-phenol in the extracts of the 12 examined biota samples appear in Appendix 1.

Based on the intensity of ion responses observed for the analyzed biota samples, the greatest level of dimeric cumyl adducts and 2,4-bis(dimethylbenzyl)phenol were found in the site 2 kina gut and site 1 blue cod muscle/liver sample.

Site 2 is immediately adjacent to the submerged remains of the stern section of the Rena wreck. An elevated level of TBT (a well-known marine paint constituent) has hitherto been detected in a sediment sample which Beca collected from this site and submitted to Hill laboratories for analysis.

Results determined for the TMMR00549 hull scrap (see Report 7) give rise to the hypothesis that the source of the dimeric cumyl adducts and 2,4-bis(dimethylbenzyl)phenol may be paint flakes ingested by grazing biota.

Based on the limited information available from this investigation, and from reef sediment profiling (see Report 7), including the signal to noise ratio of peaks detected in these investigations, it appears that dimeric cumyl adducts and 2,4-bis(dimethylbenzyl)phenol are more prone to persist in biota than is the case for tricresylphosphate isomers which were only detected in low/trace levels in 2 of the 12 biota samples examined in this investigation. Further analyses of a greater range of biota samples is required to substantiate this hypothesis.
Introduction

A number of sediment samples were collected from Otaiti Reef in August and November 2012 and submitted for urgent chemical examination for metals contamination.

Visual examination of sediment samples collected from the Otaiti Reef showed signs of copper staining, and two samples (TMM 2614 & 2630) appeared to be composed substantially of copper.

As a result it was decided to attempt an analysis of surface adsorbed metals using a dilute nitric acid leach followed by icp-ms analysis. This showed (Figure 2) the elevated presence of both copper and zinc on many of the samples, although the samples with high zinc were not those with high copper.
Initial analyses of a selection of biota samples using tetra-methyl ammonium hydroxide (TMAH) extraction for a range of metallic elements.

Initial analyses of a selection of biota samples using tetra-methyl ammonium hydroxide (TMAH) extraction for Background levels Pre Rena are: 2285 mg/kg
Background levels Pre *Rena* are: 5 mg/kg.

Background levels Pre *Rena* are: 2 mg/kg.
Background levels Pre *Rena* are: 0.4 mg/kg.

Background levels Pre *Rena* are: 3 mg/kg.
Background levels Pre *Rena* are: 0.5 mg/kg.

Background levels Pre *Rena* are: 3 mg/kg.
Background levels Pre Rena are: 0 mg/kg.
Background levels Pre Rena are: 2 mg/kg.

Background levels Pre Rena are: 0.09 mg/kg.

Maximum permissible concentrations in marine fish (mg/kg, wet weight) for human consumption are:
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<th>Element</th>
<th>Concentration</th>
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<tr>
<td>Lead</td>
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</tr>
<tr>
<td>Cadmium</td>
<td>0.2</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.5</td>
</tr>
<tr>
<td>Copper</td>
<td>10</td>
</tr>
<tr>
<td>Zinc</td>
<td>100</td>
</tr>
<tr>
<td>Aluminium</td>
<td>10</td>
</tr>
</tbody>
</table>

*NB. Dry weight concentrations will typically be 10 times higher.*

**Rena HFO as a source of Heavy Metals**

Samples of Tank 3 and Tank 5 and oil droplets collected from Taylor Road Reserve were analysed 3 ways.

(a) Direct digestion with 3:1 nitric:hydrochloric acid (Reverse aqua regia)
(b) Ashing at 450 °C followed by reverse aqua regia digestion
(c) Ashing at 600 °C followed by reverse aqua regia digestion.

Taylor Road Reserve sample was separated from entrained sand by mixing with sodium chloride brine, heating to 60 °C, emulsifying and centrifuging. Both fractions were ashed at 600 °C followed by reverse aqua regia digestion.

![Graph](graph.png)

*Figure 3 Metals in various Rena Oil samples (from the ship’s tanks and sediments).*
Figure 4 Metals from oil samples at Anchor Road (droplets and mixed in sand).

Figure 5 Anchor Road Sand background metals levels (controls).
Background levels (Pre-Rena) from Taylor Road Beach sand. (Si and Hg, not determined).

<table>
<thead>
<tr>
<th>Metal</th>
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Conclusions

Of the material sampled, 3 samples would exceed international environmental health limits set for lead, 26 would exceed limits set for cadmium, mercury is probably not a problem, between 3 and 6 exceed the limits for copper, 2 exceed the limits for zinc and 18 exceed the limit for aluminium. Two kaimoana samples had very high concentrations of copper.

Bibliography