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Soluble, semivolatile phenol and nitrogen compounds in milk-processing wastewaters

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ABSTRACT

Potable water is an essential and major input in processing our food supplies, and the continued growth in food manufacturing is placing increased pressure on this limited resource. Recycling and reuse of factory wastewater can lessen potable water use but requires a detailed understanding of wastewater properties. This study uses solid-phase extraction techniques with gas chromatography-mass spectrometry analysis to investigate trace-level semivolatile organic species in various waste and reference waters associated with the Burra Foods milk-processing plant located in Southeastern Australia. Our focus was on contaminants containing phenolic and heterocyclic nitrogen functional groups, which, because of their toxicity and persistence, may limit options for water recycling and reuse. Effluent from the wastewater treatment plant of the factory showed both the highest soluble carbon burden (47 mg/kg) and concentrations of target compounds. The target species found in these effluents included methyl phenol (13 mg/kg), hydroxy indole (9.8 mg/kg), synthetic tolyltriazoles (5.1 mg/kg) and alkyl phenol ethoxylates (0.2 mg/kg). Given the environmental stability of the tolyltriazoles, they may act as chemical markers where these effluents are used for purposes such as irrigation. Milk evaporator condensate waters, in contrast to the effluent, contained very few target species, with only low levels of pyrrolidine and piperidine derivatives such as ethylglutarimide (450 μg/L) detected. Although there were fewer target microcontaminants overall in the potable and creek reference waters, these samples had characteristic profiles. The potable water analysis revealed hydroxy cineole (2.1 μg/L) and the creek analysis revealed dichlorohydroxyacetophenone (0.3 μg/L), which were not detected in other waters. The compounds found in the wastewaters are likely to have been derived from milk or synthetic chemicals used in factory operations. The presence of nitrogen compounds in all the different milk-processing waters suggest their likely source was milk, probably milk phosphoproteins subjected to thermal, chemical, or microbial degradation. Our benign results for the condensates suggest it may be possible to substitute condensate for potable water with minimal pretreatment, both within the plant and in other applications, such as irrigation of recreation turf.

Key words: gas chromatography-mass spectrometry, milk processing, recycled water, wastewater

INTRODUCTION

Australia is one of the world's driest continents (Garnaut, 2008), with average rainfalls varying between 0 mm in the Simpson desert to 3,200 mm in coastal areas (Bureau of Meteorology, 2008). Recent drought conditions in much of the country (National Climate Centre, 2008), combined with climate change, are likely to decrease rainfall over much of temperate Australia (Australian Government Department of Climate Change, 2008). This places even greater pressure on the already limited water resources of the country, especially in provincial locations.

The impact on regional potable water supplies in Australia from food-related production is significant. Agricultural industries in Australia account for approximately 65% (2004 to 2005) of total water usage (Commonwealth of Australia, 2008). The dairy industry is the third largest rural industry in Australia, supplying 11% of the world dairy trade and directly employing approximately 40,000 people (Dairy Australia, 2008). The approximately 1.7 million cows in Australia produce more than 9,200 ML of milk annually, which is processed into a range of value-added products, including whole milk, milk powder, butter, and cheese (Dairy Australia, 2008). These processing facilities consume on average 386 ML of potable water annually and produce 452 ML per annum of wastewater (Allinson and Dyer, 2007). The variability in water use between processing facilities would suggest that improved in-factory water

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Table 1. Characteristics of wastewater from dairy plants

Item	pH	Biochemical oxygen demand, g/m^3	Fat, %	N, g/m^3	P, g/m^3	Electrical conductivity, μS/cm
Cheese/milk powder,[1] effluent	10.6	1,500	—	0.01	35	2,600
Cheese/evaporated milk,[2] clean stream	—	12	—	—	—	880
Cheese/evaporated milk,[2] dirty stream	8 to 12	700 to 1,700	0.005 to 0.03	50 to 70	10	2,600
Cheese[3]	6.9	2,800	0.1	150	42	3,500
Whey[4]	4.6	35,000	0.08	1,400	640	—

[1]D. Kleinert, Murray Goulburn Cooperative Co. Ltd., Rochester, Victoria, Australia; personal communication.
[2]K. Wild, Burra Foods Pty. Ltd., Korumburra, Victoria, Australia; personal communication.
[3]R. Knight, Murray Goulburn Cooperative Co. Ltd., Leitchville, Victoria, Australia; personal communication.
[4]Barnett et al. (1994).

use efficiency may be one way to lessen the demand on local potable water supplies.

Burra Foods Pty. Ltd. (38°25′37″ S, 145°49′16″ E; Figure 1) produces customized fresh and frozen dairy ingredients for the food manufacturing sector, with more than 60% going to export markets. Using milk from farms in the Gippsland region of Southeastern Australia, their Korumburra factory processes more than 10,000 kilotonnes of milk solids annually. Over the last 3 yr, Burra Foods has reduced potable water use from 28 kL per tonne of milk solids to 13 kL per tonne of milk solids, a water savings of more than 150 ML a year. These savings have been achieved in part by the separation of evaporator condensate and other clean water streams from the milk waste stream. This has provided opportunities to substitute potable water with wastewater, both within the factory and for irrigation applications in the wider community.

Dairy factory wastewaters commonly contain milk, by-products of the processing operations, cleaning products, and various additives that may be used in the factory. Bovine milk typically contains water (87%), fat (4%), protein (3.5%), lactose (4.7%), and other minerals and inorganic compounds (0.8%; Bylund, 1995). The fat content ranges from 3 to 5%, with a major component being triacylglycerol (98%). Other fat components include phospholipids (0.5 to 1% of fat) and sterols (0.2 to 0.5% of fat; Jensen et al., 1991). Depending on the diet of the cows, milk can also contain other trace organic compounds, such as plant-derived terpenes.

Figure 1. Location of the Burra Foods milk-processing factory in Korumburra, Victoria, Australia.

The composition of dairy factory wastewaters depends on the types of products produced at the factory, the chemicals used in operations, and whether wastewater streams within the factory are segregated. Typical characteristics of individual milk-processing wastewaters are presented in Table 1. The data demonstrate that condensate collected from the evaporation of milk or whey is relatively clean, although it may contain volatile organic components.

The reuse and recycling of particular process waters, both within the factory and to the local area (i.e., irrigation of recreation reserves), requires an assessment of their likely impact. In this study, we examine major input, process, and effluent waters from the Burra Foods processing factory and water from a nearby creek. The objective was to identify and compare the concentrations of dissolved organic semivolatile micropollutants. In particular, this study focuses on compounds that exhibit environmental persistence, environmental toxicity, or both, such as those with aromatic and heterocyclic rings. These include the more toxic and persistent phenols (Davì and Gnudi, 1999; Schüssler and Nitschke, 1999) and heterocyclic nitrogen and amine compounds (Sacher et al., 1997; Schüssler and Nitschke, 1999; Weiss and Reemtsma, 2005), which contrasts with the typical milk carbohydrates and lipids generally reported in milk-processing waters (Barnett et al., 1994).

MATERIALS AND METHODS

The origins of the waters used in this study and their relationships to the processing that occurs at Burra

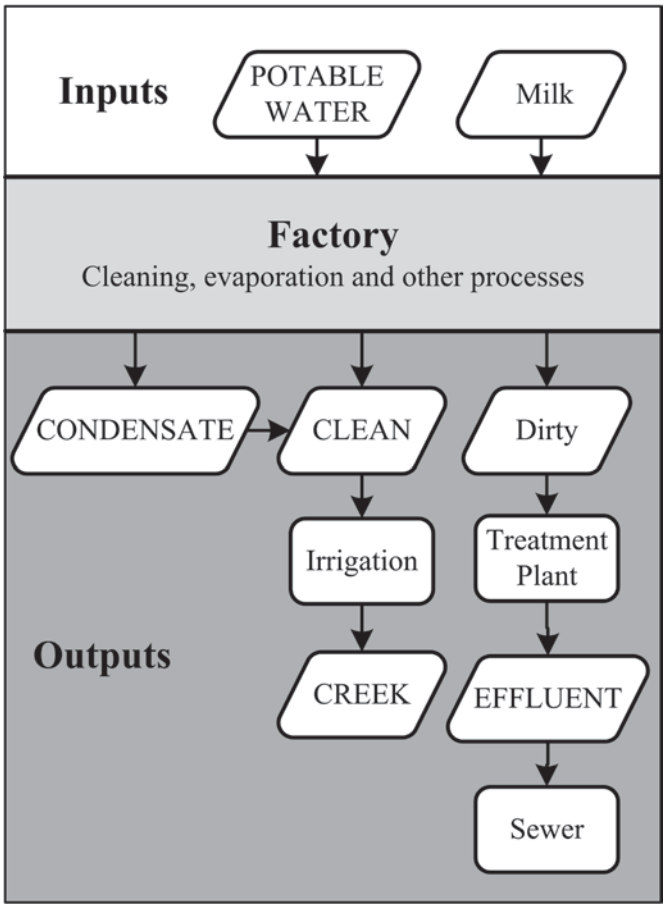

Figure 2. Schematic of water flows at the Burra Foods milk-processing factory Korumburra, Victoria, Australia, and sampling points. Sample points are indicated by capital letters.

Foods Pty. Ltd. are illustrated in Figure 2. Factory wastewaters included condensate from the dryers, a combined clean water stream, which was composed largely of condensate, and effluent from bioreactors, in which process waters with a higher solids content, such as the initial clean-in-place washes, are combined and aerobically digested. Potable water was sampled at the factory inlet, and creek water was sampled from Foster's Creek slightly downstream from the factory. Several samples of each water type were collected over a 3-wk period during April 2008. The samples represent the typical average composition for each location sampled a minimum of 3 times, covering different production regimens at the factory.

Water samples (40 to 80 L) were collected in 20-L polypropylene containers via sampling ports at the plant or directly from the creek and were stored at 4°C. All materials (e.g., hosing and valves) in contact with the samples were prerinsed with 1% Extran MA03 (Merck, Kilsyth, Australia), 10% hydrochloric acid (analytical reagent grade, Ajax Chemicals, Taren Point, Australia), deionized water, and finally excess sample before use.

Water samples were analyzed for TS, pH, electrical conductivity, dissolved reactive phosphorus (**DRP**), total dissolved phosphorus (**TDP**), total dissolved nitrogen (**TDN**), NO_2/NO_3, and ammonia using standard methods (Eaton et al., 2005). Total solids were measured on an RC210D analytical balance (Sartorius Mechatronics Australia Pty. Ltd., Oakleigh, Australia). All pH measurements were conducted using a HI9321 pH meter (Hanna Instruments Pty. Ltd., Keysborough, Australia). Electrical conductivity was measured using a Model 900C conductivity meter (TPS Pty. Ltd., Brisbane, Australia). Analysis of DRP, TDP, TDN, NO_2/NO_3, and ammonia were conducted using a Lachat Quickchem Series 8000 flow injection analyzer (DKSH Australia Pty. Ltd., Hallam, Australia). The filtered samples were tested for dissolved organic carbon (**DOC**) with a Shimadzu TOC-V [Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Sydney, Australia] using a catalytic combustion tube-infrared analyzer.

Extractions were completed within 1 wk of sample collection. The waters were prefiltered with Schleicher and Schuell GF6 glass fiber filter paper (PerkinElmer, Rowville, Australia) that had been muffled to 450°C to remove the bulk of undissolved material. The filtrate was passed via a siphon arrangement onto preconditioned [10 mL of methylene chloride and then 10 mL of methanol (HPLC grade, Merck, Kilsyth, Australia)] 6-mL Bond Elut 1-g polar **PPL** (a styrene divinyl benzene type solid adsorbent phase with a nominal pore size of 150 Å) solid-phase extraction (**SPE**) cartridges (Varian Inc., Mulgrave, Australia) using a Vac Elut 20-extraction manifold (Varian Inc.)

The SPE cartridges were used because they have been found to be more effective than C_{18} coated silica to isolate additional polar and nitrogen-rich species compared with those amenable to established C_{18}-based methods (Dittmar et al., 2008). The use of PPL SPE cartridges has become more widely accepted, with US Environmental Protection Agency method 528 having established a PPL SPE extraction technique for phenols in potable waters (Munch, 2000). The cartridges were top-loaded with a plug of ChemTube-Hydromatix diatomaceous earth (Varian Inc.) to assist residual solids management and were replaced once the flow rate was reduced to less than 0.1 mL/min. No more than 5 L was processed on any individual cartridge.

The loaded SPE cartridges were dried for 30 min on the extraction manifold by drawing air through them. Internal standards (d_6-phenol, tribromophenol and d_{14}-chrysene, Accustandard Inc., New Haven, CT) were added and each cartridge was eluted with 5 mL of acetone. Aliquots of the acetone extract from each water type

covering different sampling dates were combined and the composites were dried with anhydrous sodium sulfate, followed by solvent exchange into dichloromethane or hexane as required. The extract was split and these splits were analyzed either directly or after solid-phase cleanup (by GC-MS after derivatization).

The solid-phase cleanup procedure involved sequential elution from neutral alumina using hexane and 1:9 (vol/vol) methanol:dichloromethane to preseparate neutral and phthalate ester components from the more polar target species. This was due to the inevitable contribution from neutral and phthalate compounds associated with the plastic sample traps, tubing, resin bleed, and workup and so were removed before analysis to reduce interferences and simplify the chromatograms. Methylation of samples using diazomethane dissolved in diethyl ether was conducted to protect the GC column and improve the chromatography in regions where the free acids eluted. Methylated products were then split and a fraction was reacted with bis(trimethylsilyl)-trifluoroacetamide plus 1% trimethylchlorosilane to prepare their trimethylsilyl ethers. The preparation of trimethylsilyl ethers was performed to assist in the structural elucidation of mass spectra caused by their selective targeting of active hydrogen atoms in hydroxy and amino functional groups.

The GC-MS instrument (CP8400 GC and Saturn 2200 ITMS; Varian Inc., Middelburg, the Netherlands) was equipped with an 8400 autosampler and a 1079 split-splitless injector operated at 290°C. The split vent was closed during injection, opened to 1:80 after 0.20 min, and reduced to 1:15 after 1.0 min. A Varian FactorFour capillary column, VF-5ms, 30 m × 0.25 mm i.d. and 0.25-μm film thickness, was used to effect separation using helium carrier gas pressure programmed to a constant flow (1 mL/min.). The column oven was programmed to hold at 75°C for 2 min, increase to 320°C at 8°C/min, and hold for a further 14 min. The transfer line to the mass spectrometer was heated to 170°C and the trap was operated at 150°C. In MS mode, the scan range was 35 to 450 amu with 0.61 s/scan.

Where certified reference materials were unavailable, tentative identities were assigned to compounds based on their retention time and mass spectral data. Mass spectra were compared with the National Institute of Standards and Technology-Environmental Protection Agency-National Institutes of Health 2005 library (Gaithersburg, MD), with all computer spectral matches (minimum $R^2 = 85\%$) checked manually. Peak structural assignments were further validated by comparing their retention times and mass spectra with trimethylsilylated and nontrimethylsilylated samples.

Samples were also analyzed in GC-MS-MS mode optimized for phenols in the EPA 8270 Phenols Mix (Supelco, Bellefonte, PA). The GC parameters and MS temperatures were as described above. Nonresonant collision-induced dissociation waveforms were optimized and used when possible. Resonant collision-induced dissociation waveforms were required for reproducible fragmentation of 8 EPA SW-846 standard phenols (Accustandard Inc.) and the internal standard 1,3,5-bromo-2-methoxybenzene. Storage voltages were increased to maximize the resolution of the parent fragment without ejecting important daughter ions. Scan ranges included daughter and parent fragments but were minimized to reduce the necessary scan time (0.44 to 0.59 s/scan). Defaults were used for all other parameters.

RESULTS AND DISCUSSION

The properties of the Burra Foods samples are presented in Table 2 and are consistent with the characteristics of milk-processing wastewaters presented previously. This suggests that the internal waste- and water-handling protocols of the factory are consistent with industry norms. There was a general increase in constituent concentrations from potable waters through the factory process waters to the final effluent. Potable water fed to the factory showed the lowest DOC, at 0.5 mg/L, followed by condensate (2.1 mg/L) and clean water (5.4 mg/L). Given that condensate makes the largest volumetric contribution to the clean water supply, this presumably reflects a higher carbon burden in the other clean water components. In accord with the very high organic loads treated through the aerobic bioreactors (primary flush waters, etc.), the effluent discharged from the treatment plant of the factory showed a 10-fold increase in DOC over the clean process water (47 mg/L). The reference natural water, in our case represented by the creek, showed a relatively high DOC of 9.3 mg/L, suggesting a probable contribution from degrading plant material during the sampling period (April 2008).

The TS results indicated the relative clean state of the water used and processed by Burra Foods, with all but the effluent water being well within the recommended drinking water range of 500 mg/L of total dissolved solids (Eaton et al., 2005), and was comparable with the potable water sample, at 274 mg/L of TS. All the water samples were slightly alkaline. The electrical conductivity of the waters correlated well with their TS values ($R^2 = 0.996$), implying the majority of the dissolved species were in an ionic form.

The DRP was highest (2.2 mg/L) in the effluent, with the clean water also a having an environmentally significant concentration, at 0.3 mg/L (Environment Protection Authority, 1996). The TDP followed the same trend as the reactive phosphorus, which is consis-

Table 2. Standard chemical properties for water samples collected during April 2008 from the Burra Foods milk-processing plant

	Chemical property[1]								
Sample	DOC, mg/L	TS, mg/L	pH	EC, μS/cm	DRP, mg P/L	TDP, mg P/L	TDN, mg of nitrogen/L	NO_2/NO_3, mg of nitrogen/L	Ammonia, mg of nitrogen/L
Potable	0.5	274	7.97	430	<0.02	<0.02	0.64	<0.02	<0.02
Condensate	2.1	49	8.33	8	<0.02	<0.02	0.64	0.18	0.46
Clean	5.4	284	9.40	450	0.34	0.437	5.89	1.53	0.03
Creek	9.3	612	8.59	924	0.17	0.235	1.59	0.77	0.11
Effluent	47.2	1,310	8.44	1,876	2.18	3.233	25.55	0.23	15.46

[1]DOC = dissolved organic carbon; EC = electrical conductivity; DRP = dissolved reactive phosphorus; TDP = total dissolved phosphorus; TDN = total dissolved nitrogen. TS <0.45 μm.

tent with soluble phosphorus being added to the clean water in the condensate component.

The nitrogen concentrations were also highest in the effluent sample, with microbial treatment under aerobic conditions being insufficient to decompose and then completely eliminate the more intractable sources of nitrogen, such as some proteins. The R^2 correlating TDP with TDN was very high, at 0.99, and implies that the same source (i.e., CN phosphoprotein) was responsible for both (Rodwell, 2006). The clean water showed a higher nitrogen concentration than the condensate, again suggesting that the noncondensate waters added to the clean water were providing a disproportionally greater proportion of the pollutant load. The residual TDN and TDP concentrations (5.9 and 0.44 mg/L, respectively) support the view that condensate may incorporate liquid microdroplets carried over into the vapor stream during drying. The contribution of aerosols to the condensate is dependent on evaporator operating conditions, and elevated nitrogen and potassium levels are markers for less volatile milk products (Jensen et al., 1991). However, the overall low concentrations of nitrogen found in the condensate (which were comparable to nitrogen concentrations of the potable water sample) suggest that, from a nutrient standpoint, condensate could substitute for potable water more broadly within the plant.

The ammonia concentrations suggest the bulk of the effluent nitrogen was present in the ammonia form, in contrast to the condensate and clean waters, in which other species dominated. This may indicate that the digesters were not optimized and nitrification (conversion of ammonia to nitrate), an aerobic process, was slower than denitrification (conversion of nitrate to nitrous oxides or nitrogen gas), an anaerobic process.

Selected GC-MS analytical results are presented in Table 3 and Figures 3 and 4. The SPE cartridges were used to extract organic compounds and to process the large volumes of water required to assess sub-part per million (mg/kg) components (Fontanals et al., 2005). The disc form of this sorbent trap technology has been used previously to investigate trace levels of steroids (Kelly, 2000) and antimicrobials (Agüera et al., 2003) in analytically complex wastes and environmental waters. For example, an efficient methodology has been developed based on SPE cartridges for the study of dissolved OM in seawater (Dittmar et al., 2008). Because the samples examined in this paper were expected to contain complex compounds of microbial origin and to have varied ion concentrations, it was expected that the SPE cartridges would be similarly effective in this application.

The separation afforded by GC when combined with the detection and identification capabilities inherent in MS provided a means of analyzing the complex mixture of volatile species isolated from the SPE process (Fontanals et al., 2005). A stacked GC-MS chromatogram for polar compounds isolated from the 5 water samples is presented in Figure 3. The chromatograms revealed that the SPE cartridge acetone extracts were varied in their composition, and peak areas were consistent with the DOC burden of the parent waters. Figure 4 presents a selected ion screen from the total ion chromatograms presented in Figure 3. The ions chosen are characteristic for aromatic and nitrogen compounds and simplify the overall chromatograms. Comparison of Figure 4 with Figure 3 illustrates, by their similarity in key peak ratios and negligible extraneous peaks, that the target species were major contributors to the samples collected, rather than being a small subset of a larger library of compounds.

In addition to the target peaks, the traces in Figures 3 and 4 revealed that complex unresolved OM was present in the creek and, to a lesser extent, the potable sample via the broad elevated baseline disturbance in its chromatogram. This riverine material is either semivolatile or low-volatile high molecular weight material and produced the broad, unresolved envelope of peaks during GC injection via its thermal degradation. The lower concentration of this material in the potable water presumably reflects the treatment afforded water in this region. This includes sedimentation and flocculation to remove such humic materials that may cause turbidity (South Gippsland Water, 2008). That detectable

Table 3. Peak identification list for methylated polar fractions from GC-MS analysis

Retention time, min	Tentative identification	Indicative concentration,[1] ng/L					
		Blank	Clean	Condensate	Creek	Effluent	Potable
6.29	4-Methylphenol	—	1.1	—	—	13,000	—
8.00	Pyrrolidine, 1-acetyl	—	—	—	0.32	360	—
9.20	3-Ethyl-4-methyl-1*H*-pyrrole-2,5-dione	—	22	19	3.6	32	31
9.65	4-Ethyl-2,6-piperidinedione [3-ethylglutarimide]	—	3.9	450	—	140	2.6
10.20	4-Methoxybenzeneacetonitrile	—	—	—	—	640	—
10.62	Dimethylbenzoxazole	—	—	—	—	460	—
11.50	1-(4-Methylethenyl)phenyl-ethanone [isopropenylacetophenone]	—	—	3.3	—	3.8	—
12.06	3,5-Dichloro-2-hydroxyacetophenone	—	—	3.2	1.6	—	—
12.27	*exo*-2-Hydroxycineole	—	—	—	—	—	2.1
12.36	Dichloro-2-hydroxyacetophenone isomer	—	—	—	0.32	—	—
12.51	2-(2-Methyl-propenyl)-cyclohexanone	—	1.1	—	—	—	—
12.78	Dihydro-methyl-2*H*-indol-2-one isomer	—	—	—	0.35	180	—
12.81	2,3-Dihydro-3,3-dimethyl-1*H*-inden-1-ol	—	—	—	1.6	150	—
13.49	5-Hydroxyindole [1*H*-indol-5-ol]	—	—	—	—	9,800	—
13.84	*N*-(2-phenylethyl)acetamide,	—	—	—	—	1,400	—
14.05	1,3-Dihydro-1-methyl-2*H*-indol-2-one	—	—	—	—	150	—
14.25	4-Tolyltriazole	—	—	—	—	4,400	—
14.28	2-Bromo-4-(1,1-dimethylethyl)phenol	—	11	—	—	—	—
14.80	1*H*-Indole-1-carboxaldehyde, 2,3-dihydro	—	—	—	—	23	—
14.88	1,3-Dihydro-2*H*-indol-2-one	—	—	—	—	2,700	—
14.97	5-Tolyltriazole	—	—	—	—	730	—
18.25	1-Methyl-2-acetylindole	—	—	—	—	13	—
18.61	1,4-Dione,hexahydro-3-(2-methylpropyl)-pyrrolo[1,2-a]pyrazine	—	—	—	—	54	—
19.14	Brominated aromatic amine compound, MW = 320	—	16	—	—	210	—
19.90	1-Benzylphthalazine	—	—	—	—	560	—
20.88	Dihydro-methoxy-methyl-1*H*-1,5-benzodiazepin-2-one	—	18	—	—	54	—
22.35	4-*tert*-Octylphenol diethoxylate	—	—	—	—	86	—
22.63	Phenol, 4,4′-(1-methylethylidene)bis- [Bisphenol A]	—	—	—	—	240	—
25.43	4-*tert*-Octylphenol triethoxylate	—	—	—	—	170	—

[1]The compound identification in this table was based on National Institute of Standards and Technology mass spectral match quality ($R^2 > 0.85$) and GC retention time shifts pre- and postsilylation derivatization.

concentrations of this material were found by GC-MS analysis may be due to the drought affecting the region at the time of sampling.

The bulk of the compounds presented in Table 3 were either degradation products from carbohydrates and proteins or relatively intractable synthetic chemical compounds used in the operation and maintenance of the factory. As expected, the highest compound concentrations were detected in the waters with the highest DOC, namely, the effluent and clean composite process water streams. The condensate was practically devoid of target species.

Given the toxicity of phenolic compounds, an additional specific search for the most common phenolic environmental pollutants was conducted. Investigation by the more sensitive GC-MS-MS technique for the presence of standard phenolic US Environmental Protection Agency (2007) priority pollutants revealed detectable levels (>1 μg/kg) of only phenol and methyl phenol. Interestingly, the effluent sample recorded the highest concentration of any target species, with 4-methyl phenol (13 mg/kg). 4-Methyl phenol is associated with anaerobic biodegradation of tyrosine and tryptophan (Schüssler and Nitschke, 1999) and supports the suggestion that conditions within the anaerobic digester may not have been optimal. Nitro- and halogenated phenols on the US Environmental Protection Agency priority pollutant list were undetected and are not considered germane to the waters associated with operations of the Burra Foods factory.

The water samples contained water-soluble volatile acids and fatty acids along with polymer-degradation products and phthalates. For example, 3,5-dichloro-2-hydroxyacetophenone and its isomer were found in both the condensate and the creek samples but could be attributed to the plastic stabilizer present in the environment or within the sampling containers. These sorts of compounds were considered either artifacts, ubiquitous, nontoxic, or readily degraded in the environment and were not investigated further.

The only compound found in all samples was 3-ethyl-4-methyl-1*H*-pyrrole-2,5-dione. This analog of the common aroma compound maleimide is also a ubiquitous natural product found in many different plant species, and its discovery is not surprising (Shimoda et al., 1995).

Another class of compounds found in the samples could be linked to protein degradation. An isomer of 2*H*-indol-2-one along with 2,3-dihydro-3,3-dimethyl-1*H*-inden-1-ol were discovered in both creek and effluent

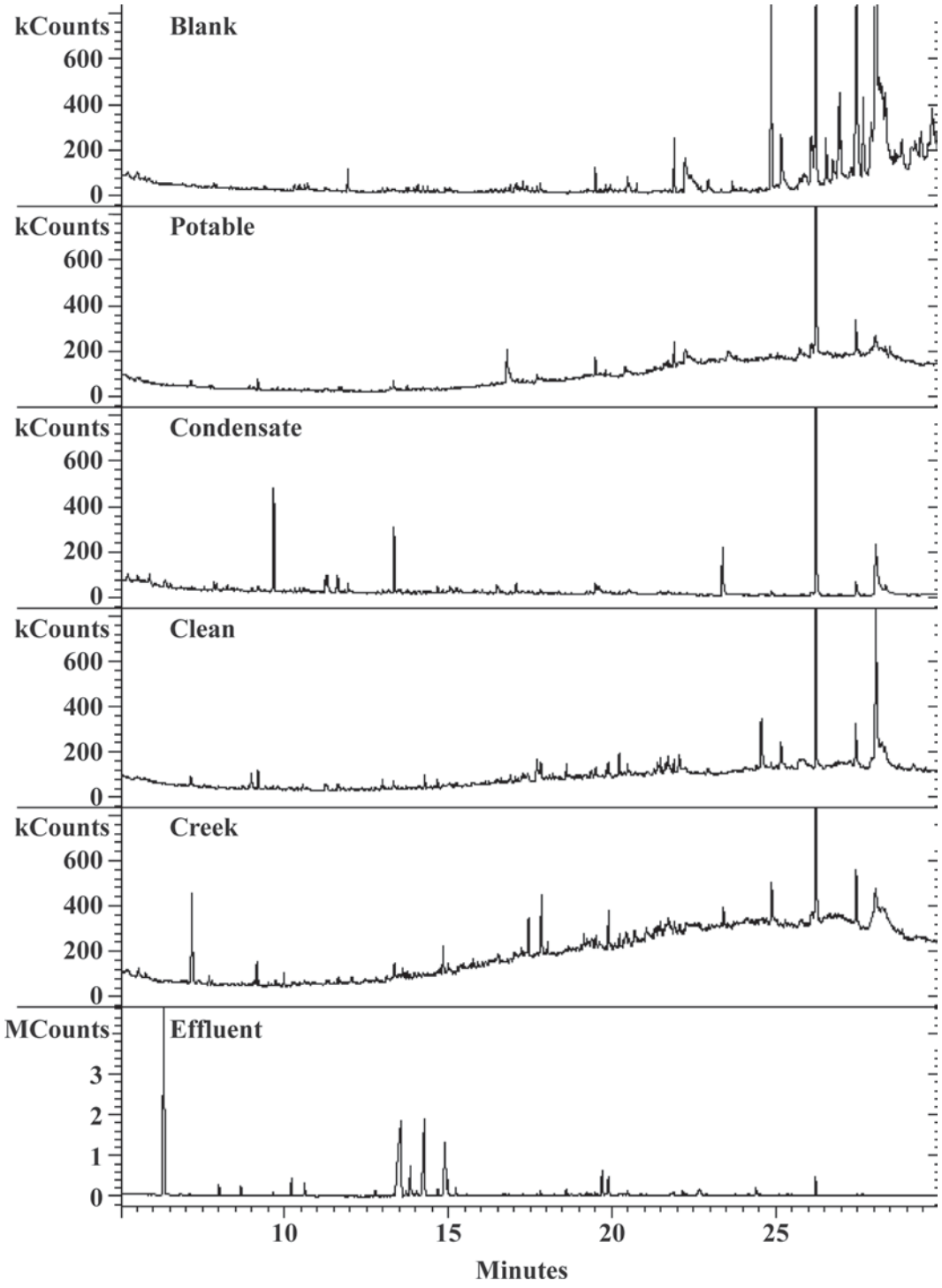

Figure 3. Stacked GC-MS total ion chromatograms of the methylated acetone extracts from solid-phase microextraction cartridges.

samples and are likely to be breakdown products of the essential AA tryptophan (Cox and King, 1943). Other tryptophan degradation products found solely in the effluent included 5-hydroxyindole (9.8 mg/kg), 1,3-dihydro-2*H*-indol-2-one (2.7 mg/kg), and 2,3-dihydro-1*H*-indole-1-carboxaldehyde (23 μg/kg; Rodwell, 2006).

Natural compounds not specifically related to milk were also found, including isoprenoids; the *exo*-2-hydroxycineole found in the potable water sample has been associated with methyl phenol in landfill leachate caused by anaerobic processes (Li et al., 2007). More likely, considering the ubiquitous eucalyptus trees

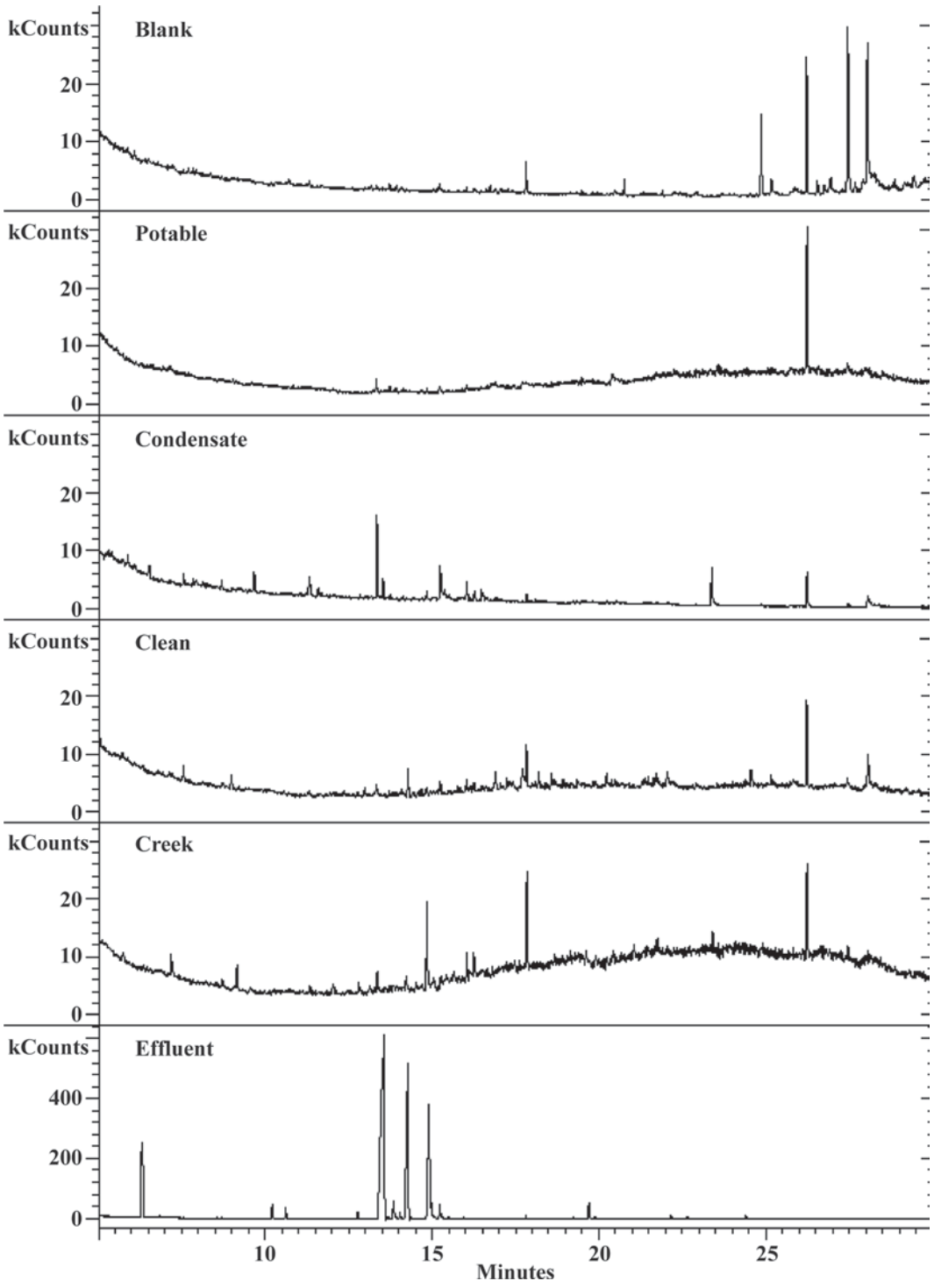

Figure 4. Stack of GC-MS select combined characteristic aromatic and nitrogen-containing compound ions (m/z 77 + 91 + 80 + 86 + 133) chromatograms of the methylated acetone extracts from solid-phase microextraction cartridges.

found in Australia, *exo*-2-hydroxycineole was derived as a bacterial metabolite from eucalyptol, an essential oil found in this plant species (Miyazawa et al., 2001).

The compound 4-ethyl-2,6-piperidinedione (or 3-ethylglutarimide) was found in clean, condensate, and effluent waters. This was the major contributor to the condensate, at 450 μg/kg, and may be present because of carryover from other parts of the processing of the plant. The small molecular weight and extensive hydrogen-bonding characteristics of this molecule could allow

for uptake with water to the condensate stream and explain its relatively high concentration compared with the other streams. A wide range of piperidine derivatives are pharmaceutically active but are not necessarily toxic. Those of the glutarimide class are also protein metabolites or degradation products of milk and have been found in the oxidation of whey products (Ferretti and Flanagan, 1971). The root structure glutarimide is central to a range of plant pesticides, both natural and synthesized (Kondo et al., 2000). The variance in concentrations between condensate and effluent process streams (140 μg/kg) may be due to further bacterial breakdown of the glutarimide moiety, as has been reported elsewhere (Volpp and Dounchis, 1971).

In addition to those compounds derived from milk, the wastewaters contained synthetic chemicals. Of interest was the presence of a benzodiazepine, a widely and well-used class of psychoactive drugs, which was found in both the clean and effluent supply. The one discovered, dihydro-methoxy-methyl-1*H*-1,5-benzodiazepin-2-one is not a commonly known variant (Horton et al., 2003). Although its has been used as an antianxiety agent in veterinary applications (Rehm and Schatzmann, 1984), the species identified was an unusual isomer for this class of compounds, which could mean that it may be a previously unreported metabolite of another benzodiazepine.

Artificial chemical additives may end up in factory wastewaters either deliberately, as in flush agents, or surreptitiously, via leaks. Three main classes of these compounds that were detected in the samples were bisphenols, triazoles, and ethoxylates. The factory additives used for cleaning have a direct contribution to wastewaters, whereas those used in processes such as temperature and corrosion control (triazoles and glycols) may also appear in specific wastewaters. However, the effluent water was the only sample to report these compounds, confirming that the factory water-handling procedures were successful in preventing the mixing of low organic burden streams with the primary rinse and machinery waters. Samples such as the condensate should not contain the target additives, given its feed source and the strict clean-in-place protocols in operation at the factory, unless the contamination occurred downstream from the evaporators. Bisphenol A was detected (240 μg/kg) in the effluent and is suspected of being hazardous to humans (vom Saal and Hughes, 2005) as an endocrine disruptor (Wetherill et al., 2007). Its origin may be associated with antioxidant dosage or as a monomer from the degradation of epoxy resins and protective polymer coatings in the factory.

Other additives detected in the effluent were the tolyltriazoles (Weiss and Reemtsma, 2005). These are widely used corrosion inhibitors with limited biological activity or toxicity. The various tolyltriazoles isomers (Richardson, 2006) have markedly different degradation behavior. The 2 tolyltriazole compounds found in this study were 2 isomeric forms, 4- and 5-tolyltriazole. Both were significant contributors to the target compounds, with the more stable 4-tolyltriazole isomer being more abundant, at 4.4 mg/kg. These anthropogenic compounds are typically used to control corrosion in cooling-water systems treated with oxidizing biocides. Tolyltriazoles are resistant to microbial degradation and are increasingly being reported in natural waters (Richardson, 2006). These intractable properties and their significant contribution to the effluent discharge water suggest that tolyltriazoles could be used to monitor leakage from sites where effluents are applied to land, assuming, of course, that the isomers found in the effluent are not ubiquitous in the environment. Also detected in the effluent were ethoxylated octyl phenols (di- and triethoxylates); these polyethylene glycol derivatives are used in coolants and as nonionic detergents, enabling protein solubilization and phase partitioning (Mazzola et al., 2008).

CONCLUSIONS

This study, which used PPL SPE trap technology coupled with GC-MS analyses to target the more polar semivolatile organic nitrogen and oxygen compounds, has shown that Burra Foods process waters are variable in their concentrations of these chemicals. The origin of most of these water-soluble species was most likely the result of either milk protein decomposition or the chemical additives used to clean and maintain the plant. The separation of the water streams, at first analysis, revealed that the most toxic of the chemicals found had been safely removed to the effluent stream. The effluent analysis also revealed the highest levels of these soluble phenolic and heterocyclic nitrogen compounds, in accordance with the incomplete conversion of the high-protein milk wastes sent to the digesters. Tolyltriazoles that are used within factory processes have potential utility as markers for effluent input.

Other process streams, such as the composite clean water, were significantly lower in phenolic- and nitrogen-containing compounds, with its main component, the condensate, identified as a high-quality water that merits further investigation for use as a potable water substitute in other parts of the factory. The condensate, excepting the relatively innocuous ethylglutarimide, contained only a few low-concentration soluble, semivolatile phenol, and nitrogen compounds and the data presented suggest that with minimal treatment, the condensate could also be used to supplement natural creek water.

The initial investigation into the different wastewaters at Burra Foods is consistent with condensate being a benign water source that could be substituted for potable water without significant pretreatment. Further analysis of how milk-processing plants may recycle their wastewaters and other recyclable wastes is continuing.

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