Bull Environ Contam Toxicol (2010) 85:160–164
DOI 10.1007/s00128-010-0053-6

Identification of Alkyl-methoxypyrazines as the Malodorous Compounds in Water Supplies from Northwest Spain

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Received: 25 February 2010 / Accepted: 14 June 2010 / Published online: 6 July 2010

Abstract A study of organic compounds which caused several odor problems in water supplies from NW Spain was carried out. Sour dish-cloths, potato bin and musty were the main descriptors associated with these events. Closed loop stripping analysis combined with sensory-GC and GC/MS detection was used. The compound producing the initial odor event was 3,5-dimethyl-2-methoxypyrazine, identified for the first time as the compound responsible for odor incidents in finished water. Concentration levels were up to 300 ng/L in the effluent from an activated sludge wastewater treatment plant (WWTP) and 3 ng/L in finished water. 3-isobutyl-2-methoxypyrazine and other alkyl methoxypyrazines were the compounds responsible for the odor events that occurred several months later. Concentration levels in the mg/L range were measured in the activated sludge of a WWTP.

Keywords 3,5-Dimethyl-2-methoxypyrazine · 3-i-Propyl-2-methoxypyrazine · 3-i-Butyl-2-methoxypyrazine · Odor events · Water

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The presence of bad taste or smell in drinking water is usually perceived by consumers as unsafe and the major cause of complaints. The European Directive includes the taste and odor parameter in water intended for human consumption. Therefore, a rapid response to identify the compound(s) responsible of the event and to remove it in the treatment process is of paramount importance to guarantee safe and aesthetic water.

Both natural and anthropogenic compounds are the sources of pollutants producing taste and odors in water. Geosmin, 2-methyl-isoborneol (MIB) and haloanisoles are by the far the most commonly reported compounds of biological origin causing musty and earthy off-flavors in water at ng/L levels (Malleviale et al. 1987; Suffet et al. 1995). Industrial and sewage effluents, leachates from landfills, pipe joint lubricants, etc. have been frequently described as the source of taste and odors coming from anthropogenic compounds.

Closed loop stripping analysis (CLSA), a preconcentration technique developed by Grob and Zürcher (1976) is the analytical tool most frequently reported in the literature used to identify the compound or compounds responsible for taste and odor episodes (Boleda et al. 2007). Other extraction techniques such as solid-phase micro-extraction (SPME) (Diaz et al. 2004) or stir-bar sorptive analysis (SBSE) (Benanou et al. 2003) have also been gaining more relevance in odor and taste analysis. All these techniques can be combined with GC/MS detection to identify compounds at ng/L levels or lower. Additionally, sensory-GC has been also used to discriminate compounds with characteristic and/or intense odors from others in complex chromatograms by sniffing at an olfactory port and thus helping to identify the compound(s) responsible for an event (Ventura et al. 1997).

From September 2008 until March 2009, several odor incidents have been noticed in wastewater, river and treated water in several small towns located in the region of Galicia (NW-Spain). The most common descriptors for treated water were “sour dish-cloths”, potato bin and musty. Therefore, the aim of the present study was to identify which compound or compounds were responsible for the objectionable odors of the river water and treated water. The methodology used was CLSA combined with GC/MS and sensory-GC. Origin, range of concentrations in wastewater, surface and finished water and the procedures carried out to avoid more odor events are also reported.

Materials and Methods

Figure 1 shows the geographical location of the odor incidents. Barces River supplies raw water to the drinking water treatment plant (DWTP) located there. This river is also the final recipient of effluents of the municipal solid waste treatment plant (MSW) of Galicia, the effluents of which are treated in two WWTPs in series. The former has an activated sludge treatment and the latter a physico-chemical treatment. The final effluent is discharged into the Rego da Iña creek and ends in Porto Pereiro River, a tributary of the Barces River. Samples from wastewater treatment plant (WWTP), river and treated water were collected in amber glass bottles (1 L), transported and stored at 4°C until their analysis within 48 h. Sodium thiosulfate was added to destroy residual chlorine in treated water samples.

A 3,5-dimethyl-2-methoxypyrazine standard was provided by a generous gift of the Australian Wine Research Institute (Dr. M. A. Sefton and Dr. D. L. Capone). 3-isopropyl- and 3-isobutyl-2-methoxypyrazines were from Sigma–Aldrich Chemie (Steinheim, Germany). For CLSA analyses, 1-chloroalkanes (C_5, C_6, C_{12}, C_{16}) were purchased from Fluka (Büchs, Switzerland) and the 1-chloroalkanes (C_8, C_{10}, C_{14} and C_{18}) were from Sigma–Aldrich. Other reagents used were carbon disulfide *for spectroscopy* from Merck (Darmstadt, Germany) and methanol *purge and trap grade* from Sigma–Aldrich. Ultrapure water was from Milli-Q® water purification system (Millipore Corp., Beldford, MA).

Analyses were carried out in a commercial CLSA apparatus (Brechbüler, Switzerland). Water samples (1 L or diluted with Milli-Q) were spiked with 1-chloroalkanes (C_5, C_6, C_{10}, C_{12}, C_{16} and C_{18}) to give a final concentration of 800 ng/L for each compound. We used 5 mg-activated carbon filters. The samples were stripped for 1 h. Temperatures of 45 and 55°C were used for water-bath and carbon filter, respectively. After stripping, the carbon filters were spiked with C_8 and C_{14} 1-chloroalkanes at the same concentrations as the spiked water samples in order to control the filter elution. The filters were then extracted with 40 µL of CS_2 (20 µL + 20 µL).

Quantitation was performed by the internal standard method. For each compound, the response is calculated relative to 1-chloro-C_6 at two calibration levels. The calibration analyses were obtained by analyzing Milli-Q water samples spiked with 1-chloro-C_6 and different spiked solutions of 2-methoxy-3,5-dimethylpyrazine (0.25 and 1 µg/L). The unknown concentrations in the sample are then obtained from the mean response factors using the

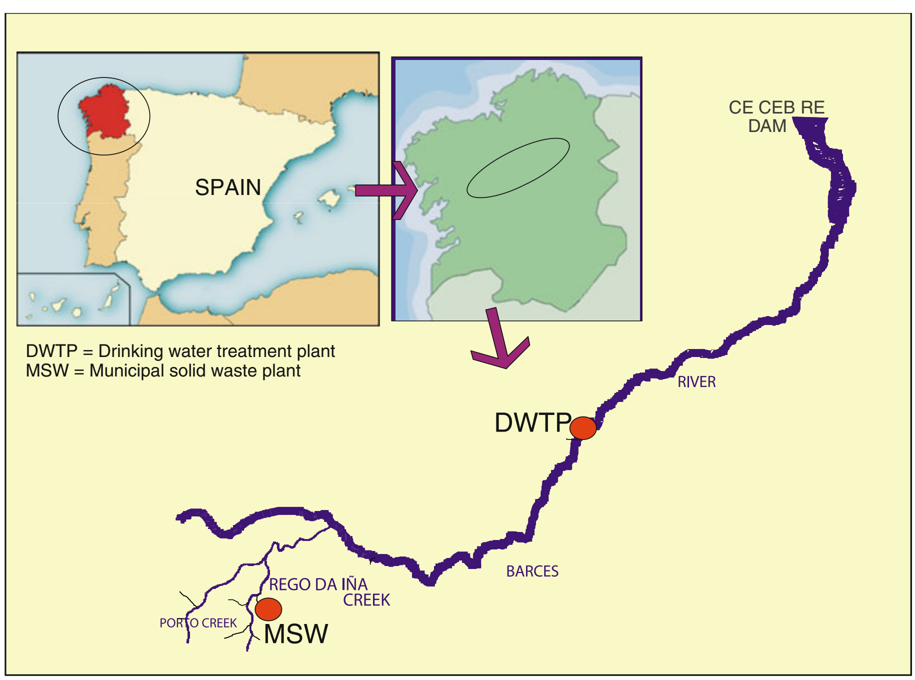

Fig. 1 Geographic location of the studied area (NW Spain)

formula $C_z = A_z\ C_i/R_zA_i$ where C_z; A_z and C_i; A_i are the concentration and peak areas of the unknown compound and the internal standard, respectively and R_z is the response factor for the target compound.

Analyses of CLSA extracts were carried out on a Trace GC2000 (Thermo Instruments, USA) mass spectrometer. Injections (1 µL of CS_2 extracts) were made cold "on column" into a 50 m × 320 µm i.d. (0.2 µm film thickness) CP-Sil 19-CB column (Varian Inc, USA). A deactivated precolumn (2 m) was used. The GC temperature program was 30°C (5 min) to 285°C (10 min) at a rate of 3°C/min. Helium was used as the carrier gas (60 kPa). The mass spectrometer was operated in electron impact mode (70 eV). The source temperature was kept at 300°C. Mass spectra were acquired by scanning from 35 to 450 Da.

Results and Discussion

In September 2008 a very unpleasant smell appeared in the finished water of several towns in Galicia (NW Spain), coming from the raw water of Barces River. The odor was characterized as humid, earthy, "sour dish-cloths" and potato bin. Since earlier events in a nearby area were related to the presence of geosmin, samples from both Barces River raw water and treated water were analyzed to detect the presence of this compound and 2-methyl-isoborneol, 2,3,4- and 2,3,6-trichloroanisoles, 3-isobutyl-2-methoxypyrazine and 3-isopropyl-2-methoxypyrazine which are usually described as compounds producing these kinds of odors. None of these target compounds were present in the samples analyzed. In parallel to these tests, a full analysis of water following the European Directive 98/83/EC which regulates the quality of water for human consumption was performed. No compounds which could be considered a basis for preventing the distribution of water were identified.

As the odor persisted, a set of new samples was analyzed by CLSA, GC/MS and sensory-GC. Finished water leaving the drinking water treatment plant (DWTP) showed the presence of 3,5-dimethyl-2-methoxypyrazine. This compound was responsible for the odor of the sample and it is mainly described in the literature as causing a fungal must taint in wine corks (Simpson et al. 2004), in roasted coffee (Czerny et al. 2000) and the obnoxious odors produced in old machine cutting-fluid emulsions because of microbial activity (Mottram et al. 1984; Yasuhara et al. 1986). However, this is the first time that is reported as the compound responsible for odor incidents in finished water. The odor threshold for 3,5-dimethyl-2-methoxy pyrazine in water has been determined as low as 0.4 ng/L (Czerny et al. 2000). Quantification of samples was made possible by a gift of an authentic standard from the Australian Wine Research Institute and was established as 3 ng/L, that is 7.5 times more odorous than its threshold in water. Previously, two different chromatographic columns (DB-1701 and DB-5) were tested to ensure its unequivocal identification in order to avoid misidentification between 3,5-dimethyl-2-methoxypyrazine and 3,6-dimethyl-2-methoxypyrazine, two isomers which have nearly identical spectra and very similar retention times on GC (Simpson et al. 2004).

Once the pyrazine was identified as the compound responsible for the bad smell of the treated water, additional samples were taken to find its origin. The same compound 3,5-dimethyl-2-methoxypyrazine was also identified in the Rego da Iña creek at concentration levels of 840 ng/L. The main descriptor associated with these odorous samples was sour dish-cloths.

These results led us to focus our attention on the effluents of the MSW plant. Table 1 displays the concentration levels of the different alkyl-methoxypyrazines during the September-December 2008 incidents. Thus, 3,5-dimethyl-2-methoxypyrazine was identified in further odor events in the activated sludge basin, in the effluent of the activated sludge process and the effluent of the physico-chemical treatment of the MSW plant. Concentration levels of 2010 ng/L (November 2008), 300 ng/L (December 2008)

Table 1 Concentration levels in ng/L (ppt) of alkyl-methoxypyrazines during September–December 2008 odor events

Compound	Activated sludge MSW		Physico chemical MSW		Rego da Iña		DWTP	
	Influent	Effluent	Effluent		Creek		Treated	
	11/21/08	12/23/08	10/21/08	05/19/09	09/24/08	05/19/09	09/24/08	05/19/09
3,5-Dimethyl-2-methoxypyrazine	2010	300	500	–	840	–	3	–
Ethylmethylpyrazines	1000	ND	ND	–	–	–	–	–
3-i-Propyl-2-methoxypyrazine	–	–	–	–	–	–	–	–
3-i-Butyl-2-methoxypyrazine	–	–	–	–	–	–	–	–
Sum of Hydrocarbons	0.2×10^6	ND	ND	3.2×10^6	0.1×10^6	1.7×10^6	–	–

ND not determined, – means < LOQ

Table 2 Concentration levels in μg/L (ppb) of alkyl-methoxypyrazines during February–March 2009 odor events

Compound	Activated sludge MSW				Rego da Iña		DWTP
	Influent		Effluent		Creek		Treated
	02/13/09	03/24/09	02/13/09	03/24/09	02/13/09	03/24/09	03/24/09
3,5-Dimethyl-2-methoxypyrazine	–	0.005	–	–	–	–	–
3-i-Propyl-2-methoxypyrazine	–	2615	–	5470	–	–	–
3-i-Butyl-2-methoxypyrazine	–	12940	–	21915	–	–	–
Unidentif. alkylmethoxypyrazines	–	1815	–	4525	–	–	–

and 500 ng/L (October 2008) were measured in these samples, respectively. The influent also contained several unidentified substituted ethyl methylpyrazines with an estimated total concentration of different isomers around 1,000 ng/L but they were not present in the effluent. Other sampling points (not shown) such as storm drains and a leachate from the landfill did not show the presence of the target pyrazines.

Cutting-fluid emulsions have been suggested (Mottram et al. 1984; Yasuhara et al. 1986) as one of the sources of 3,5-dimethyl-2-methoxypyrazine and this was investigated. Hydrocarbons were present in the activated sludge basin (0.2 mg/L), in the effluents of the MSW (not quantified) and in the creek (0.1 mg/L) when odor events occurred. However, several months later (May 2009), the effluent of the physico-chemical treatment plant and the Rego da Iña creek presented elevated levels of hydrocarbons (3.2 mg/L and 1.7 mg/L, respectively) but pyrazines were absent. Therefore, no apparent correlation between the presence of hydrocarbons from cutting-oil emulsions and pyrazines could be established.

MSW appeared to be the likely origin of the odor event. The following actions were carried out: The polluted Rego da Iña creek was diverted to prevent pollution of the Barces River. This avoided contamination of raw waters used for drinking water production. In the activated sludge treatment plant, water was emptied and some sludge was partially replaced with fresh sludge from another facility.

However, once this clean-up was completed, new odor events were noticed a few months later (March 2009). In this new event, the main identified compounds in the influent and the activated sludge basin of the WWTP were 3-i-propyl-2-methoxypyrazine; 3-i-butyl-2-methoxypyrazine and several unidentified alkyl-methoxypyrazines at concentration levels of several mg/L (see Table 2). These extremely high concentrations precluded the determination of other minority compounds such as 3,5-dimethyl-2-methoxy pyrazine that could be only identified at the influent of the activated sludge at a very low trace levels (5 ng/L). The main descriptor associated with these odorous samples was earthy-musty and potato bin. No more samples were analyzed because the extremely objectionable odor made it easy to determine the origin of the incident. As the Rego da Iña creek was still diverted, no pollution of the river occurred and the DWTP was not affected.

To solve this new problem, water from the activated sludge treatment plant was emptied followed by addition of calcium hydroxide to the sludge, followed by complete removal. A fresh sludge from another urban WWTP was added. The measure appears to be effective as no odor problems have reappeared and no pyrazines have been found in the periodical analysis performed since then.

The present study has showed that odor events in water supplies from NW Spain are related to the presence of different alkyl-methoxypyrazines. The compound causing the odor described as sour dish-cloths was 3,5-dimethyl-2-methoxypyrazine with a concentration level of 3 ng/L in finished water. It is the first report of 3,5-dimethyl-2-methoxypyrazine as the malodorous compound in water supplies. The subsequent odor events were linked to the presence of the more common 3-i-propyl- and 3-i-butyl-2-methoxypyrazines which were present at extremely high concentration levels (mg/L) in the activated sludge of a MSW plant. The preventive measures carried out after the first odor incident, which consisted of the diversion of the creek carrying the effluents of the MSW plant, prevented the pollution of the raw surface water and the DWTP.

References

Benanou D, Acobas F, de Roubin MR, David F, Sandra P (2003) Analysis of off-flavours in the aquatic environment by stir bar sorptive extraction-thermal desorption-capillary GC/MS/olfactometry. Anal Bioanal Chem 376:69–77

Boleda MR, Diaz A, Martí I, Martin-Alonso J, Matia L, Romero J, Ventura F (2007) A review of taste and odour events in Barcelona's drinking water area (1990–2004). Water Sci Technol 55:217–221

Czerny M, Grosch W (2000) Potent odorants of raw Arabica coffee: their changes during roasting. J Agric Food Chem 48:868–872

Diaz A, Ventura F, Galceran MT (2004) Identification of 2, 3-butanedione (diacetyl) as the compound causing odor events in treated water at trace levels. J Chromatogr A 1034:175–182
Grob K, Zürcher F (1976) Stripping of trace organic substances from water: equipment and procedure. J Chromatogr 117:285–294
Mallevialle J, Suffet IH (eds) (1987) Identification and Treatment of Tastes and Odors in Drinking Water. AWWA Research Foundation and Lyonnaise des Eaux. Denver (CO)
Mottram DS, Patterson RLS, Warrilow E (1984) 2,6-dimethyl-3-methoxypyrazine: a microbiologically-produced compound with an obnoxious musty odour. Chem Ind 448-449
Simpson RF, Capone DL, Sefton MD (2004) Isolation and identification of 2-methoy-3, 5-dimethylpyrazine, a potent musty compound from wine corks. J Agric Food Chem 52:5425–5430
Suffet IH, Mallevialle J, Kawczynski E (eds) (1995) Advances in Taste and Odor Treatment and Control. AWWA Research Foundation and Lyonnaise des Eaux. Denver (CO)
Ventura F, Romero J, Parès J (1997) Identification of dicyclopentadiene and its derivatives as compounds causing odor in groundwater supplies. Environ Sci Technol 31:2368–2374
Yasuhara A, Yamanaka Y, Ogawa T (1986) Volatile compounds in machine cutting-fluid emulsion. Agric Biol Chem 50:1765–1770