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# Determination of the Odor Threshold Concentrations of Chlorobrominated Anisoles in Water

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Trihalophenols, which are drinking water disinfection byproducts (DBPs) formed by chlorination or chloramination practices, can be biomethylated into trihalogenated anisoles. These latter compounds have traditionally been suspected of causing odor episodes in drinking water around the world. The odor threshold concentration (OTC) of mixed chlorobrominated anisoles, which were previously synthesized, was determined by flavor profile analysis (FPA) performed by an experienced panel trained to identify odors and tastes in water. The odor threshold amount (OTA) was evaluated by using a gas chromatograph equipped with olfactometry (GC-O) and electron capture detectors (ECD). FPA results for mixed chlorobromoanisoles gave a theoretical OTCs range from 2 to 30 ng/L, the 2,6-diBr-3Cl-anisole being the most odorous compound. Rubber is the general descriptor described by panelists for these compounds, although earthy and musty are the following most cited descriptors.

**KEYWORDS: Chlorobrominated anisoles; odor; odor threshold concentration; gas chromatography coupled with olfactometry; flavor profile analysis; panel; treated water**

## INTRODUCTION

Tastes and odors in drinking water are among the major causes of complaints by consumers, and determining their origin and causes is one of the priority objectives of water companies. Earthy-musty is probably the most common descriptor associated to taste and odor events. Several different algae metabolites associated with these odor episodes and cyanobacteria metabolism byproducts such as geosmin and 2-methylisoborneol (MIB), with odor thresholds in water at the low ng/L level, are the two organic compounds that account for the majority of solved cases of odor events (1−3). Geosmin and MIB are efficiently removed in drinking water treatment plants (WTPs) by ozonation and/or powdered or granular activated carbon filtration (4). Their concentrations in treated water are usually lower than their odor threshold concentrations (OTCs). On the other hand, several organic compounds, such as iodinated halomethanes (5, 6), aldehydes (7), 2,6-dibromophenol (8), etc., have been identified as the origin of odor episodes and are byproducts that originate in the treatment process in WTPs and present OTC values at low µg/L. Recently, much attention has been paid to the presence of halogenated anisoles which can be formed by biomethylation of the corresponding halophenols which are initially formed as drinking water DBPs following chlorination

or chloramination practices (4, 9). They can impart an earthy-musty odor to water at low ng/L concentration levels. Odor threshold values ranging from 0.7 pg/L (10) to 7 ng/L (11) for 2,3,6-trichloroanisole and from 30 pg/L (10) to 50 ng/L (12) for 2,4,6-trichloroanisole have been reported. The taste and odor potential of bromo- and iodophenols and their corresponding anisoles have also been reported (13). Despite the discrepancies found in the literature, it is clear that very low levels of haloanisoles can cause taste and odor events in treated water. In addition, brominated anisoles can also be found in bromide-rich waters; for example, 2,4,6-tribromoanisole with an estimated odor threshold of 30 pg/L has been related with odor problems in treated waters (14). Very few data of chlorobromoanisoles are available in the literature (15), but several compounds have been determined at the low ng/L range in treated water (16). So, mixed chlorobromoanisoles are potential candidates in producing odor events in drinking waters containing low to medium bromide levels.

For the study of odor events, water companies employ the two-of-five test (17), the attribute rating test (18), or the flavor profile analysis (FPA) (19, 20), among others. FPA was primarily developed for the food and perfume industries (21−23) and was adapted for the water industry. The FPA method is now widely used as a sensory technique in studying tastes and odors in water, but the huge differences among the OTCs of different compounds in water limit the usefulness of the panel,

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which assesses the overall taste and odor characteristics of the sample. To avoid these limitations, FPA information can be complemented with that obtained by using gas chromatography-olfactometry (GC-O).

This technique combines the odor evaluation of the individual GC peaks eluting from a chromatographic column at an olfactory detector outlet and the chemical identification of the odorous compounds by GC-MS. Applications of the GC-O technique in solving taste and odor episodes in water have been reported (*24−27*).

The aim of this research was to determine the OTC values of mixed halogenated anisoles as well as their descriptors, using FPA and GC-O coupled with an ECD detector as the main techniques. The results obtained can be used by water companies to identify the cause of odor events in treated waters or to help in unresolved cases.

## MATERIALS AND METHODS

**Glassware.** All glassware has been washed with soapy water and rinsed with tap water, Milli-Q water, and finally with reagent-grade acetone. It was then air-dried and baked at 180 °C during 12 h. Aluminum foil was used to prevent the presence of dust or other contaminants.

**Reagents.** Mixed trihaloanisoles (2,4-dibromo-6-chloro-, 2,4-dichloro-6-bromo-, 2,6-dichloro-4-bromo-, 2,6-dibromo-4-chloro-, 2,3-dichloro-6-bromo-, 2,3-dibromo-6-chloro-, 2,5-dichloro-6-bromo-, 2,5-dibromo-6-chloro-, 2,6-dichloro-3-bromo-, 2,6-dibromo-3-chloroanisole-, and 2,3,6-tribromoanisoles) were synthesized with purities higher than 90% as described elsewhere (*16*). The compounds 2,4,6-tribromo-, 2,4,6-trichloroanisole, and pentachloroanisole were purchased from Sigma-Aldrich, whereas 2,3,6-trichloroanisole was acquired from Ultra Scientific (North Kingstown). Other reagents such as methanol purge-and-trap grade and acetone were from Sigma-Aldrich and Merck (Germany), respectively. Ultrapure water was from a Milli-Q water purification system (Millipore).

**Standard Solutions.** *FPA.* Individual mixed trihaloanisoles were dissolved in reagent water at 1 *μ*g/mL each. The solutions were usually prepared the day before testing and were kept at room temperature 1 h before the assay. Several dilutions were prepared immediately before odor evaluation.

*GC-O.* Two different standards of various haloanisoles in acetone at 100 ng/mL were prepared. The first contained 2,4,6-triCl-, 2,4-diCl-6Br-, 2,6-diBr-4Cl-, 2,4,6-triBr-anisoles, and pentachloroanisole, whereas the second included 2,3,6-triCl-, 2,5-diCl-6Br-, 2,6-diBr-3Cl-, 2,6-diCl-3Br-anisoles, and pentabromoanisole. These mixtures showed good chromatographic profiles and a complete separation of all compounds, thus preventing interferences of odors in the chromatographic run.

**Flavor Profile Analysis (FPA).** Odor evaluations were performed by FPA according to the method described in the literature (*19, 20*). Only one compound per session and week was sniffed by panelists. Encoded water samples (200 mL) contained in Erlenmeyer flasks (500 mL) with ground-glass stoppers and heated to 45 ± 1 °C for 15 min in a water bath were presented to the panelists (males and females from 22 to 30 years). The trained panel consisted at least of six persons (from a group of 18) per session. Odor was assessed by swirling the contents, removing the stopper, and immediately applying the nose to the mouth of the flask. Sessions were performed in a specially conditioned room (22 °C) kept free from interfering odors.

For each compound, the panel evaluated the response of water samples from the most diluted to the most concentrated and some blanks between them. Each solution presented to panelists was smelled, and the average of the intensity given by the panelists was recorded. The panelists had to describe the odor and to evaluate the response of odor intensity versus the concentration of each halogenated anisole, indicating its intensity (*I*) on a scale ranging from 0 (no odor) to 12 (very strong), with intermediate levels of 1 (beginning of perception), 2 (very faint), 4 (faint), and 8 (moderate). The average data obtained from panelists for each concentration except *I* = 0 values were represented as a logarithmic Weber−Fechner curve, which relates odor intensity to the logarithm of the compound concentration. The lowest concentration and average intensity recorded was that at which at least half of the panelists perceived the odor. From the equation obtained for each compound, the odor threshold concentration (OTC) was defined as the value of the concentration corresponding to an intensity average of *I* = 1.

**Gas Chromatography Coupled with Olfactometry (GC-O) Analysis.** The commercial olfactometric detector used (SGE; Australia) consists of a glass nose cone in which the steam of the GC is mixed with a humidified air steam (installed to prevent the mucous membrane from rapid drying), allowing the identification of individual components as they elute from the chromatographic column. Five assessors were selected from among people working in the laboratory without any panel experience and no special sensitivity to detect tastes and odors. They were asked to smell the effluent from the column and to give a verbal description of each odor and assess its intensity. Assessments of odor intensity were classified in three grades, with 1 (beginning of perception), 2 (medium), and 3 (strong). As with the FPA method, the intensity average results were correlated to the log of the amount (ng) of each haloanisole injected into the chromatographic column and smelled at the olfactometric detector. From the equation obtained for each compound, the odor threshold amount (OTA) was defined as the value of the amount sniffed by panelists corresponding to an intensity average of *I* = 1 and further confirmed by at least 60% of the assessors.

GC-O analyses have been performed on a Fisons Instrument GC-8000 (Manchester; U.K) gas chromatograph equipped with an ECD detector. A DB-5, 30 m × 0.25 mm i.d. × 0.25 *μ*m film thickness (J & W Scientific; CA) chromatographic column was used. It was connected to a small T-shaped piece of glass and diverted to two capillary columns with the same characteristics of the chromatographic column (20 cm each, DB-5), leading to the electron capture detector (ECD) and the olfactometric detector. The carrier gas was helium at 70 kPa, and 100 kPa of $N_2$ and air for ECD and olfactometric port makeup were used. The flow ratio between the effluents from the DB-5 columns to the ECD and the olfactometric detectors was approximately 1:1. The GC temperature program was from 30 °C (1 min) to 115 °C at a rate of 10 °C/min, from 115 to 200 °C at a rate of 5 °C/min, and finally up to 300 °C at 15 °C/min. It was then held for 7 min.

## RESULTS AND DISCUSSION

**Flavor Profile Analysis.** The FPA plot represented as a Weber−Fechner curve includes between five and seven points for each compound. The data obtained were adjusted to a linear regression curve with $r^2$ values ranging from 0.913 to 0.989. **Figure 1A** shows as an example the Weber−Fechner plots for 2,5-diCl-6-Br- and 2,6-diBr-3-Cl-anisoles. From the equation of each halogenated compound, the OTC values were obtained, and they are listed in **Table 1**. These values for trihaloanisoles ranged from 30 ng/L for the least odorous compound (2,4,6-triCl-anisole) to 2 ng/L for the most odorous one (2,6-diBr-3Cl-anisole). Some problems arose in the evaluation of odor intensity in water at high concentration values (i.e., 1 *μ*g/L) for all tested compounds. The Weber−Fechner curve linearity was lost at these concentrations probably due to nasal saturation. The results obtained at 45 °C in two different weekly sessions were slightly erratic for some compounds, that is, 2,6-diCl-3Br-anisole, but no problems were observed for the same compounds when solutions were smelled at room temperature. **Table 1** displays all OTC values obtained at 45 °C except for 2,6-diCl-3-Br-anisole, which is given at 25 °C.

The results showed that 2,4,6-triCl-anisole is less odorous (OTC = 30 ng/L) than 2,3,6-triCl-anisole (OTC = 5 ng/L); but for the brominated analogues, both OTC values are practically the same, 12 ng/L and 11 ng/L for 2,4,6-triBr-anisole and 2,3,6-triBr-anisole, respectively. For pentahalogenated compounds tested, OTC values were higher than those of trihaloanisoles. However, one would expect a higher OTC value for pentabro-
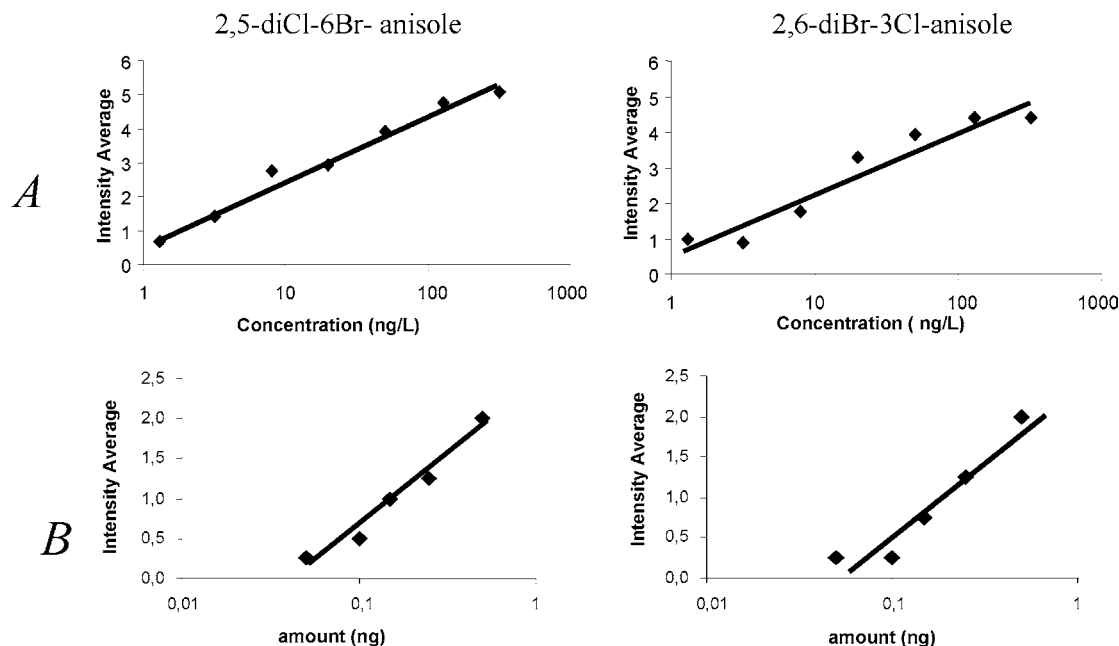
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**Figure 1.** Weber–Fechner plots of individual 2,5-diCl-6-Br-anisole and 2,6-diBr-3Cl-anisole obtained by FPA analysis (**A**) and by GC-O (**B**).

**Table 1.** Flavor Profile Analysis (FPA) and GC-Olfactometry (GC-O) of Mixed Haloanisoles

| compound | purity[a] (%) | equation W–F plot (OTC) | $r^2$ (OTC) | equation W–F plot (OTA) | $r^2$ (OTA) | OTC[b] (ng L$^{-1}$) | OTA[c] (ng) | main descriptor by FPA |
|---|---|---|---|---|---|---|---|---|
| 2,4,6-triCl | 99 | $y = 2.323 \log conc + 4.524$ | 0.989 | $y = 2.371 \log conc + 9.999$ | 0.951 | 30 | 0.16 | fruity, musty, earthy |
| 2,4,6-triBr | 98 | $y = 1.782 \log conc + 4.393$ | 0.931 | $y = 2.547 \log conc + 10.78$ | 0.982 | 12 | 0.14 | rubber, earthy |
| 2,3,6-triCl | 99 | $y = 1.899 \log conc + 5.364$ | 0.947 | $y = 2.068 \log conc + 8.872$ | 0.992 | 5 | 0.16 | earthy, sweety |
| 2,3,6-triBr | 90 | $y = 1.591 \log conc + 4.453$ | 0.954 | nd | nd | 11 | nd | rubber, sweety |
| 2,4-diCl-6-Br | 99 | nd | nd | $y = 1.275 \log conc + 5.623$ | 0.982 | nd | 0.24 | nd |
| 2,6-diCl-4-Br | 98 | $y = 1.034 \log conc + 3.563$ | 0.934 | nd | nd | 4 | nd | leather, rubber |
| 2,6-diBr-4-Cl | 90 | $y = 2.433 \log conc + 7.517$ | 0.960 | $y = 1.832 \log conc + 7.565$ | 0.995 | 2 | 0.18 | rubber, plastic |
| 2,3-diBr-6-Cl | 85 | $y = 2.489 \log conc + 5.673$ | 0.925 | nd | nd | 14 | nd | rubber, fruity |
| 2,3-diCl-6-Br | 90 | $y = 2.445 \log conc + 6.627$ | 0.957 | nd | nd | 5 | nd | rubber, fruity |
| 2,5-diBr-6-Cl | 80 | $y = 2.129 \log conc + 6.330$ | 0.969 | nd | nd | 6 | nd | rubber, musty |
| 2,5-diCl-6-Br | 98 | $y = 1.883 \log conc + 6.263$ | 0.934 | $y = 1.766 \log conc + 7.725$ | 0.965 | 2 | 0.16 | rubber, plastic |
| 2,6-diCl-3-Br | 95 | $y = 1.047 \log(x) + 3.221^c$ | $0.932^c$ | $y = 2.258 \log conc + 9.676$ | 0.910 | $3^c$ | 0.14 | rubber, cardboard |
| 2,6-diBr-3-Cl | 99 | $y = 1.674 \log conc + 5.499$ | 0.919 | $y = 1.858 \log conc + 7.974$ | 0.950 | 2 | 0.18 | rubber, musty |
| pentaCl | 98 | $y = 3.051 \log conc + 2.215$ | 0.979 | $y = 2.173 \log conc + 8.96$ | 0.975 | 240 | 0.16 | rubber, earthy |
| pentaBr | 98 | $y = 1.422 \log conc + 2.950$ | 0.913 | $y = 2.373 \log conc + 10.13$ | 0.914 | 43 | 0.14 | rubber, earthy |

[a] Determined by $^1$H NMR and GC/FID; confirmed by GC/MS. [b] Obtained by Weber–Fechner curve at 45 °C. [c] Obtained by Weber–Fechner curve at room temperature. [d] Main descriptor along the range studied. n.d.: not determined.

moanisole, which has a lower Henry constant ($K_H$) value, than for pentachloroanisole. Similar behavior was also previously observed for the iodinated halomethanes (*5*), of which iodoform, although the least volatile compound, is the most odorous. OTCs decreased with the number of iodine and bromine atoms in the molecule. For trihaloanisoles, this does not generally apply, and, as a general trend, no dependence of OTC versus number of chlorine−bromine atoms and/or structure, 2,4,6-/2,3,6-, was observed for tested compounds.

The OTC value evaluated by our panelists for 2,3,6-triCl-anisole was similar to the value previously reported by Guadagni et al. (*11*). For 2,4,6-triCl-anisole and 2,4,6-triBr-anisoles, our results were higher than the value reported for 2,4,6-triCl-anisole by Curtis (*10*) and U.K. Water Ind. Res. Ltd. (*13*). It should be mentioned that some of the compounds studied were detected by at least one panelist at levels below ng/L. These levels were the lowest experimental concentration levels detected to date for an organic compound in water by our flavor panel.

Rubber as the main descriptor and earthy and musty as the second choices were the predominant descriptors identified by

the panelists for these compounds in the range studied. Sweety, fruity, and plastic were also identified as other minority descriptors.

**Gas Chromatography Coupled with Olfactometry Analysis (GC-O).** Two standard solutions of mixed halogenated anisoles were injected repeatedly into the GC equipped with ECD and olfactory detectors to evaluate which is the minimal amount of odor that can be perceived. Plots, which relate odor intensity (1−3 scale) smelled at the olfactometric detector to the logarithm of the injected amount (*μ*g), were established giving $r^2$ values ranging from 0.910 to 0.995 (**Table 1** and **Figure 1B**, bottom). The OTA values ranged from 0.24 ng (2,4-diCl-6Br-anisole) to 0.14 ng (pentabromoanisole). **Figure 2** shows as an example the ECD chromatographic profile of five haloanisoles (0.15 ng each). The combination of the olfactometric detector and ECD detector provides enough sensitivity to identify haloanisoles even under their odor threshold values.

The results obtained for trihaloanisoles using the GC-O method did not follow the same trend as those previously obtained by the FPA method. The different sensitivity in odor
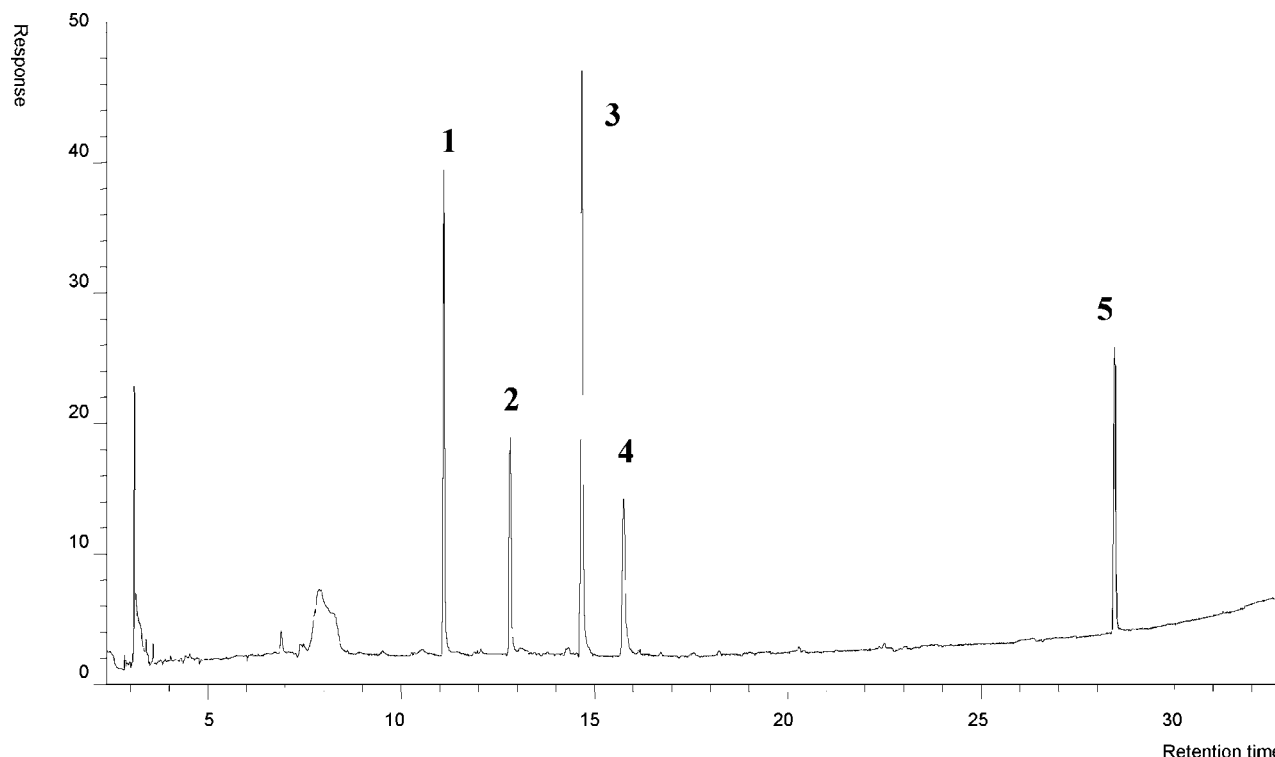
**Figure 2.** GC-ECD chromatogram of a standard mixture of halogenated anisoles used for olfactometry detection near their threshold odor (0.15 ng, each). Compounds: **1**, 2,3,6-triCl-anisole; **2**, 2,5-diCl-6-Br-anisole; **3**, 2,6-diBr-3-Cl-anisole; **4**, 2,6-diCl-3-Br-anisole; **5**, pentabromoanisole.

response of haloanisoles obtained with both methods can probably be explained by the different experimental conditions used. In FPA, the equilibrium water−air must be considered, whereas the compounds in the gas phase eluted from the chromatographic column are directly smelled in the GC-O method. The narrow ranges of OTA obtained suggest that the position and nature of the halogen have little effect on odor potency in the studied compounds.

Musty and earthy were the common descriptors indicated by assessors at amounts near the odor thresholds (OTA), whereas rubber was the main descriptor at higher amounts.

The similarity of descriptors and OTC values in all of the compounds tested makes it difficult to identify unequivocally the compound responsible for a determined event. It is probably the combination of all chlorobrominated anisoles present that gives the overall odor of the water sample.

**Conclusions.** The OTCs of mixed halogenated anisoles in water have been established in at low ng/L level, indicating that these compounds are among the most odorous compounds that can be found in drinking water. OTC and/or OTA values obtained by FPA and GC-O analysis of tested compounds showed no trend in the number of chlorine−bromine atoms and/ or 2,4,6-/2,3,6- structure. Moreover, no consistency was found in the calculated OTC and OTA values for a given compound by FPA and GC-O methods. This suggests a different sensitivity in odor signal for direct odor (GC-O) and the OTC determined in water (FPA). Nevertheless, the combination of a GC instrument equipped with ECD and olfactometric detectors enables odorous halogenated compounds to be detected at ultratrace levels, and halogenated anisoles, under their odor threshold. Rubber and earthy-musty are the main descriptors associated with chlorobromoanisoles in water by FPA panelists. The latter was the first choice at values in water near their OTC, whereas the former was the most cited descriptor at higher concentration. As low levels of these compounds are usually found in water, it seems reasonable to consider earthy-musty

as the most probable descriptor for those events in which chlorobromoanisoles at low ng/L levels are involved.

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