

**TEMPERATURE EFFECTS
ON
DRINKING WATER ODOR PERCEPTION**

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ABSTRACT

Thirteen volunteer panelists were trained according to *Standard Method 2170*, flavor profile analysis (FPA). Following training these panelists underwent triangle test screening to determine whether or not they could detect the odorants used in this study. Following triangle testing, panelists underwent directional difference testing to determine if temperature affected odor perception when presented with two water samples. Following directional difference testing, panelists used FPA and evaluated water samples that contained odorants at either 25 °C or 45 °C. Samples containing geosmin cooled to 5 °C were also evaluated.

Sensory analyses experiments indicate that odor intensity is a function of both aqueous concentration and water temperature for geosmin, MIB, nonadienal, n-hexanal, free chlorine, and 1-butanol. The higher water temperature resulted in an increase in odor intensity for some, but not all, concentrations of geosmin, 2-methylisoborneol, *trans*-2, *cis*-6-nonadienal, n-hexanal, free chlorine, and 1-butanol. Additionally, above 400 ng/L of geosmin, 400 ng/L of MIB, and 100 ng/L the odor intensity was equal to or less than the odor intensity at 600, 600, and 200 ng/L, respectively. Henry's Law should predict that an increase in concentration would increase the amount of odorant the panelist comes into contact with; however, results demonstrated that at specific aqueous odorant concentrations odor perception did not follow Henry's Law. Odor response to drinking water containing isobutanol was affected by concentration but not water temperature.

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AUTHOR'S PREFACE

This work is presented in the Virginia Tech manuscript format. While the first chapter is a review of the literature pertaining to drinking water taste-and-odors, each additional chapter is a separate manuscript formatted for journal submission. Chapters II and III document in detail the affect of temperature upon drinking water odor perception. This research was prepared for and supported by the American Water Works Association Research Foundation (AWWARF), while and the Edna Bailey Sussman Foundation provided additional support. Chapter II, entitled Water Temperature as it Affects the Perception of Drinking Water Odorants has been published in the *Proceedings of the American Water Works Association Water Quality Technology Conference: Nashville, Tennessee* (2001).

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CHAPTER I

LITERATURE REVIEW

SIGNIFICANCE OF DRINKING WATER ODOR

The results of more than 100 publications related to drinking water odor are described in this literature review. These publications point out that odor is a common problem in drinking water and affects consumer perception. Drinking water odor problems have been identified globally in countries such as Australia, Canada, France, Japan, South Africa, and the United States of America (Kikuchi et al., 1973; Slater and Blok, 1983; Krasner et al., 1985; Bartels and Suffet, 1987; Ito et al., 1988; Wnorowski and Scott, 1992; Bruchet et al., 1992). Burlingame and Dahme (1989) cited many sources, which demonstrated that this problem has been reported in the United States of America as far back as the 1880s.

Utility customers may receive drinking water with very different characteristics from the water leaving the treatment plant. Disinfectant residuals, disinfection byproducts, and microbial byproduct concentrations above the odor threshold level (as low as nanogram per liter) may all be present when water arrives at consumer taps. Several common drinking water odors that have been identified are chlorinous, earthy, musty, cucumber, and grassy (Sigworth, 1957; Gallup Poll, 1973; Burlingame et al., 1987; Dietrich et al., 1991; Suffet et al., 1999).

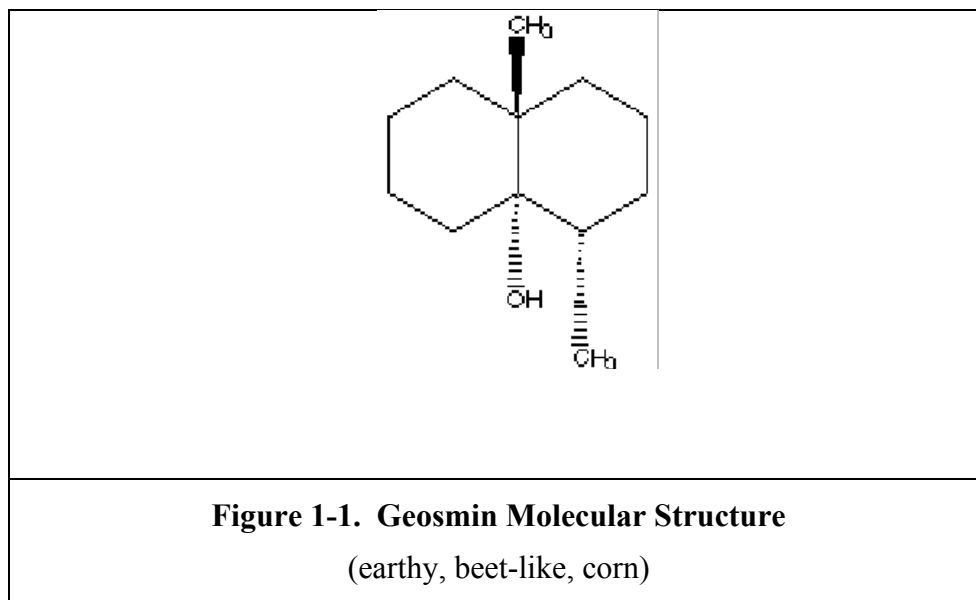
Generally, when individuals are unhappy with water odor they complain to the water utility. Few to many complaints can be logged depending on the severity or durations of the taste-and-odor episodes. These complaints can be infrequent, such as a total of 35 complaints logged at a rural Virginia water authority (1995-2001) or number more than 1000 at a Massachusetts water authority during a 7-month period (Sung, 1990).

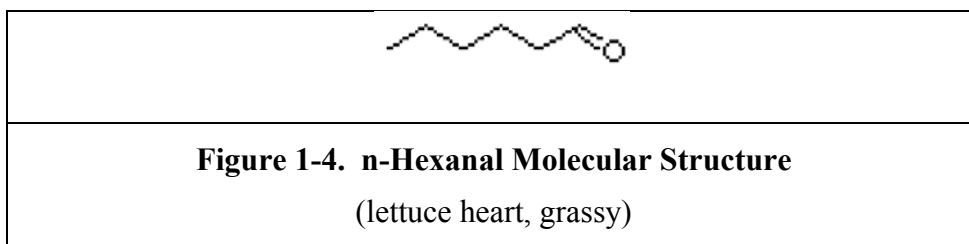
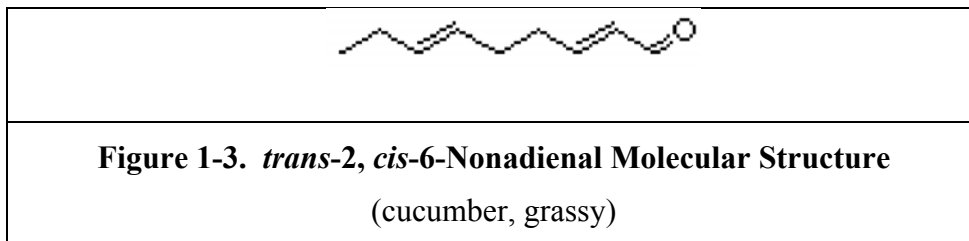
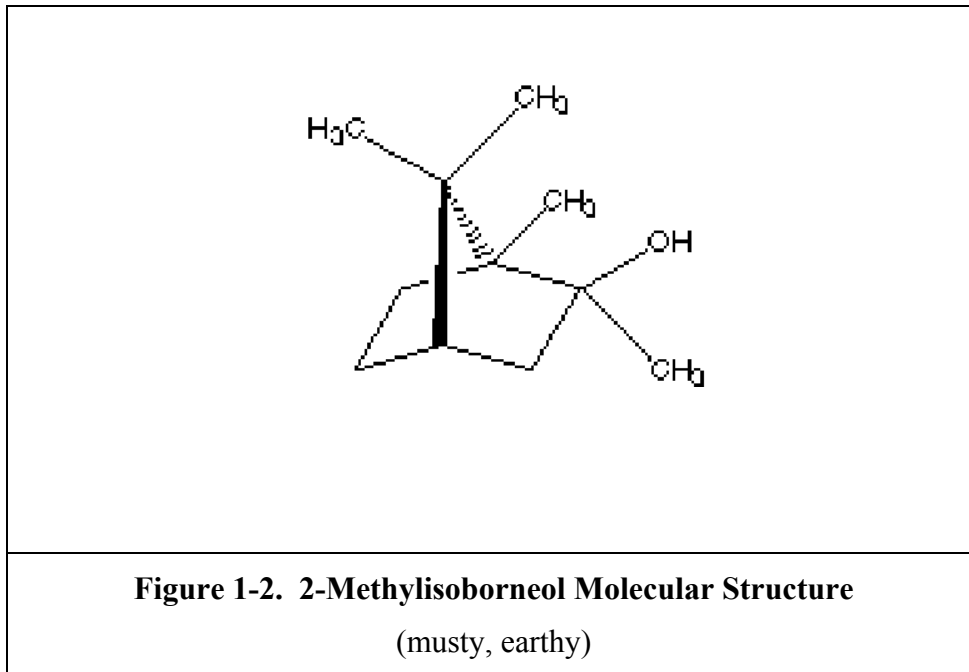
Utilities are challenged to consistently provide aesthetically pleasing water because the public is constantly evaluating these qualities. As a result, when drinking water odor episodes occur many local and national media report them (Mayer, 1994; Wood, 1995; Cohn, 1996; Cohn, 1997; Hack, 2000). Several media reports indicated that the odorous drinking water caused consumers to be unhappy, concerned, and even outraged.

COMMON DRINKING WATER ODORANTS

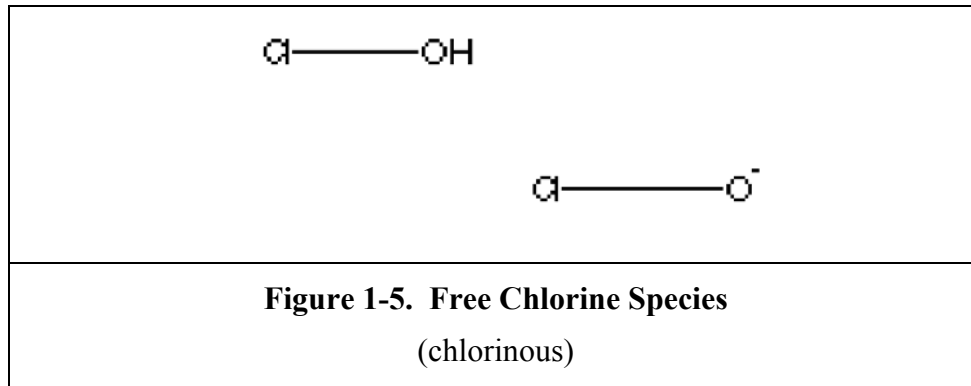
The water industry has identified numerous chemicals that cause drinking water odor. Some odors that have been identified by consumers are medicinal, earthy, grassy, septic, chlorinous, swimming pool, tobacco-like, and rotten egg. In this study seven odorants were used: geosmin, MIB, nonadienal, n-hexanal, free chlorine, isobutanol, and 1-butanol.

Four metabolites produced by actinomycetes, algae, or cyanobacteria and that have been identified in drinking water are geosmin, 2-methylisoborneol (MIB), *trans*-2, *cis*-6-nonadienal (nonadienal), and n-hexanal (Gerber and Lechevalier, 1965; Safferman et al., 1967; Medsker et al., 1968; Izaguirre et al., 1982; Burlingame et al., 1986; Negoro et al., 1988; Hayes and Burch, 1989; Wnorowski and Scott, 1992; AWWARF, 1993; Rashash, 1994; Dietrich et al., 1995; Rashash et al., 1997; Suffet et al., 1999; Young et al., 1999). The molecular structures and common odor descriptors are provided in Figures 1-1-4 (Tinoco et al., 1978; Mallevalle and Suffet, 1987; Burlingame et al., 1991; AWWARF, 1993; Rashash, 1994; Jones et al., 1995; Krasner, 1995; Rashash et al., 1997; Suffet et al., 1999; Burlingame et al., 2000; Sigma-Aldrich, 2001).

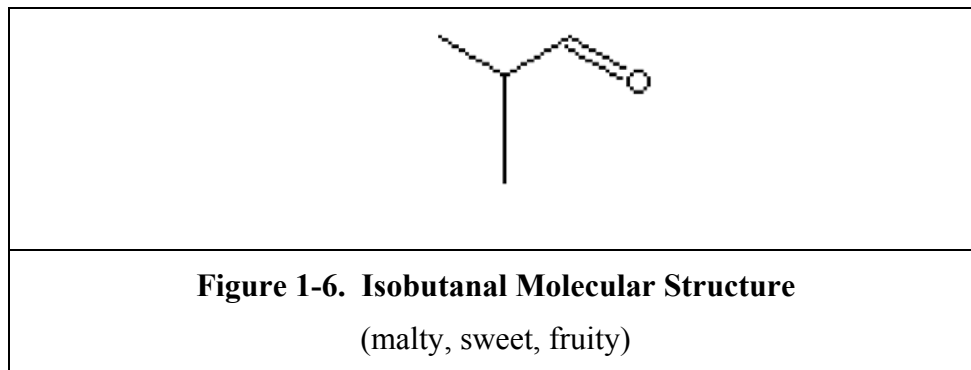




Free chlorine, Cl_2 , HOCl and OCl^- , is the most widely applied disinfectant in the United States of America, and has been associated with a chlorinous odor (Figure 1-5). Its chlorinous odor has the ability to mask earthy and musty odors present. This is one reason why when chlorine levels lower, earthy and musty odors can be more readily detected (Worley, 2000). Additionally, chlorinous odors are one of the most common drinking water complaints (Burlingame et al., 1987; Burlingame and Dahme, 1989; Suffet et al., 1996).



Isobutanal, which has a malty, sweet fruit odor, has been identified as a byproduct of ozonation and chlorination (Figure 1-6). The food industry has also identified isobutanal as being derived from the breakdown of the amino acid valine (Amoore et al., 1976). Moreover, isobutanal was identified at a concentration of 54 ug/L in Canadian drinking water (Hrudey et al., 1988; Froese et al., 1999; Suffet et al., 1999).



While 1-butanol is not a drinking water odorant, it has been studied in air pollution, chemical engineering, and sensory science (Baker, 1962; Laing, 1985; Stevens et al., 1989; Burlingame et al., 1991; Olsson et al., 1994; APHA, 1998; Cometto-Muniz et al., 1999). The molecular structure and common odor descriptors for 1-butanol are provided in Figure 1-7 (Verschueren, 1983; Mallevalle and Suffet, 1987). A few researchers have referred to 1-butanol being used as an odor calibration standard (Baker, 1962; Burlingame et al. 1991).



Figure 1-7. 1-Butanol Molecular Structure
(sweet, rancid)

ODOR DETECTION

Odorant Transport. Odorant molecules must first leave the water and become airborne before they can be detected. This process is called volatilization, and depends upon water solubility, vapor pressure, and the Henry's Law constant of the compound. Henry's Law defines the relationship where equilibrium is reached between the gaseous concentration and the liquid concentration. Volatilization occurs until equilibrium is reached between these two phases mediated by a Henry's Law constant.

Every compound has a Henry's Law constant, m (dimensionless) or K_h (pressure-mass per volume), that is specific to a temperature and pressure. Two equations are presented which illustrate Henry's Law, equations 1 and 2, respectively.

$$y_{\text{air}} = m * C_{\text{water}} \quad \text{Equation 1}$$

$$P_v = K_h * C_{\text{water}} \quad \text{Equation 2}$$

In equation 1, y_{air} is defined as the concentration in the gas phase (mass per volume) and C_{water} is defined as the aqueous concentration (mass per volume) (Metcalf and Eddy, 1991). In equation 2, P_v is defined as the vapor pressure and C_{water} is defined as the water solubility of the compound (mass per volume). Both equation 1 and 2 were used in this study to determine Henry's Law constants and these constants were then compared to data found in the literature. Water solubility, vapor pressure, and other physical and chemical properties for the seven compounds used in this study are provided in Table 1-1. Henry's Law constants were found in the literature for geosmin, MIB, isobutanol, and 1-butanol. Conversely, Henry's Law constants were calculated using measured water solubility and vapor pressure data found in the literature to check the variability of measured data (Table 1-2).

Table 1-1. Physical and Chemical Properties

Odorant	MW (g/mol)	Density at 20 °C (g/mL)	Water Solubility (mg/L)	Vapor Pressure (atm)	$\Delta H^{\circ}_{\text{soln}}$ (kJ/mole) ¹	Ref.
1-Butanol	74.12	0.810	74,000 (25 °C)	8.80×10^{-3}	59.86	a, d, g, i, k
Free Chlorine	70.91	Not Found	7,290 (20 °C)	Not Found	Not Found	d, j
Geosmin	182.31	0.949	150.2 (20 °C)	5.49×10^{-5}	Not Found	e, b
n-Hexanal	100.00	0.815	5,640 (30 °C)	0.10×10^2	54.01	d, f, h, k, c
Isobutanal	72.10	0.785	91,000 (20 °C)	2.24×10^{-1}	Not Found	f, g, i
2-Methylisoborneol	154.25	0.929	194.5 (20 °C)	7.26×10^{-5}	Not Found	e, b
<i>trans</i> -2, <i>cis</i> -6- Nonadienal	138.21	0.868	Not Found	Not Found	Not Found	g

- a. Nirmalakhandan, 2001.
- b. Pirbazari et al., 1992.
- c. Young et al., 1999.
- d. Syracuse Research Center: Chemfate, 2001.
- e. Lalezary et al., 1984.
- f. Amoore et al., 1976.
- g. Weaste and Astle, 1979.
- h. Aldrich Chemical, 2000.
- i. Verschueren, 1983.
- j. AWWA, 1999.
- k. Sanders, 1999.

¹ H stands for enthalpy of dilute solution.

Table 1-2. Henry's Law Constants

Odorant ²	Temp. (°C)	m Reported	m Calculated	Ref.
1-Butanol	25	0.0003	0.0004	a, b, d, e, f
Free Chlorine	Not Found	Not Found	Insufficient data	
Geosmin	20	0.0023	0.0028	d
n-Hexanal	Not Found	0.0109	Insufficient data	b
Isobutanal	20	0.0080	0.0074	a, d
2-Methylisoborneol	20	0.0027	0.0024	d
<i>trans</i> -2, <i>cis</i> -6- Nonadienal	20	0.0045	Insufficient data	c

- a. Nirmalakhandan, 2001.
- b. Syracuse Research Center: Chemfate, 2001.
- c. Estimated by Zander et al., 1999.
- d. Larzeny et al., 1984.
- e. Brennan et al., 1998.
- f. Altschuh et al., 1999.

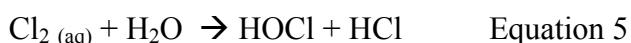
Henry's Law constants at different temperatures can be calculated using the van't Hoff equation, Equation 3. Variables in the van't Hoff equation were defined as follows: m_1 is the Henry's Law constant at T_1 , m_2 is the Henry's Law constant at T_2 , R (8.315 joule/mole-°K) is the universal gas constant, T_1 (°K) is the temperature at m_1 , T_2 (°K) is the temperature at m_2 , and $\Delta H^\circ_{\text{soln}}$ (joule/mole) is the standard molar enthalpy of a dilute solution containing that compound.

² m is defined as a dimensionless Henry's Law constant, whereas K_h is defined with units atm-m³/mole.

$$\ln (m_1 / m_2) = (\Delta H^{\circ}_{\text{soln}} / R) * [(1/ T_2) - (1/ T_1)] \quad \text{Equation 3}$$

Before an odor can be perceived, the gaseous concentration must be greater than an individual's odor threshold concentration (OTC). The OTC is defined as the minimum concentration required for an individual to detect an odorant and varies from person to person (Lawless and Heymann, 1998). Typical OTCs for the seven compounds used in this study are shown in Table 1-3.

Free chlorine was the only chemical used in this study where speciation was dependant on pH. Four reactions describe the chlorine species in water as illustrated in equations 4-7.



This study was most concerned with the dissociation of hypochlorous acid (HOCl), which yields a proton and a hypochlorite ion (Equation 7). The dissociation of hypochlorous acid can be expressed using an equilibrium constant K. The equilibrium constant for this reaction is presented in equation 8 (AWWA, 1999).

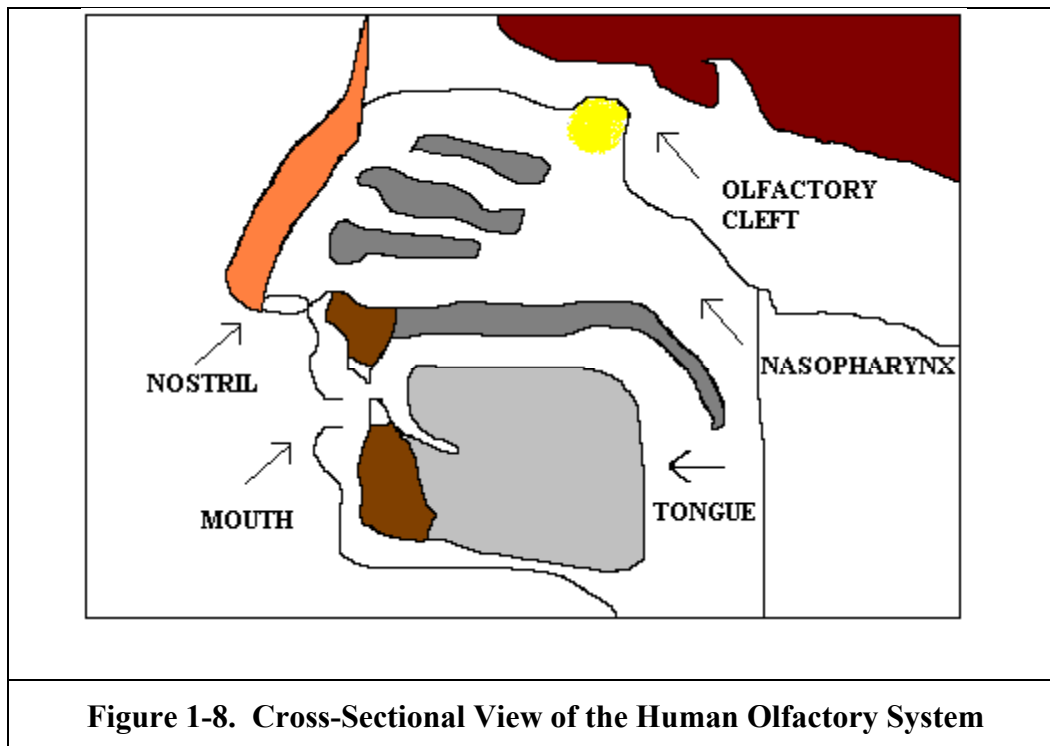
$$K = [\text{H}^+] [\text{OCl}^-] / [\text{HOCl}] = 2.898 \times 10^{-8} \text{ moles/ L at } 25 \text{ } ^\circ\text{C} \quad \text{Equation 8}$$

Sense of Smell. The sense of smell, commonly referred to as olfaction, is extremely complex. Drinking water odor is a product of the nervous system created by the transfer of electrical signals to the brain once odorants reach the olfactory receptors (Hudson, 2000). Additionally, the flow rate and duration of odorant contact has been found to influence perception (Schneider et al., 1966). Odorants arrive at the olfactory receptors by either entering the nasal cavity through the nostrils or by traveling up passages from the nasopharynx to the olfactory cleft (Figure 1-8). The olfactory cleft is located at the top of the nose, which contains the olfactory epithelium. This olfactory epithelium is a sheet of cells containing the olfactory receptors.

Table 1-3. Aqueous Odor Threshold Concentrations

Odorant	Aqueous Odor Threshold Concentration, ug/L	Ref.
1-Butanol	0.002 – 0.005	a, b, c, d
Free Chlorine	280 – 360 pH dependent	e
Geosmin	0.006 - 0.01	f, g, h
n-Hexanal	0.06 – 1.9	a
Isobutanal	0.15 - 2.0	i, j, k
2-Methylisoborneol	0.002 - 0.02	a, h, g
<i>trans</i> -2, <i>cis</i> -6-Nonadienal	0.002 - 0.013	h, l, m, n

- a. Burlingame et al., 1991.
- b. Baker, 1962.
- c. Lillard and Powers, 1975.
- d. Stahl, 1973.
- e. Krasner and Barrett, 1984.
- f. Persson, 1980.
- g. Krasner et al., 1983.
- h. Rashash et al., 1997.
- i. Hrudehy et al., 1998.
- j. Guadagni et al., 1963.
- k. Guadagni et al., 1972.
- l. Burlingame et al., 1992.
- m. Whitfield and Last, 1991.
- n. Hayes and Burch, 1989.



Some consumers experience a lesser sense of smell, reduction in smell, or loss of smell entirely. Anosmia is the condition in which the sense of smell is reduced or lost entirely. This can be diagnosed at birth (congenital anosmia) or caused by Alzheimer's disease, a traumatic head injury, a cold, or infection (Wuensch, 2001). More specifically, individuals can have different degrees of anosmia. Total anosmia is the inability of an individual to detect any odorants; partial anosmia is the inability to detect some odorants, while specific anosmia is specific to one or a limited number of odorants. Adaptation is the condition in which odor intensity is decreased as a result of continued exposure to the same or different odorant, self- and cross-adaptation, respectively (Berglund and Engen, 1993; Dalton and Wysocki, 1996). Moreover, an individual's ability to detect odorants declines with age (Kaneda et al., 2000).

ADDITIONAL INFLUENCES ON ODOR PERCEPTION

Concentration Effects. The aqueous concentration has been found to influence the descriptor of nonadienal and camphor.

A 2-5 ng/L nonadienal solution has been described as having a cucumber odor, while at 10-20 ng/L the grassy odor is found (AWWARF, 1993; Rashash, 1994; Rashash et al., 1997; Burlingame et al., 2000). Furthermore, Burlingame et al. (1992) reported waxy, oily, and rancid descriptors for nonadienal solutions.

Conova (2001) reported that Dr. Linda Buck of the Howard Hughes Medical Institute at Harvard Medical School found that low camphor concentrations exhibited a urine odor, while higher camphor concentrations had a forest smelling descriptor. An explanation was that increases in concentration caused more receptors to become involved in information processing. Theimer et al. (1999) has explained that the odor descriptor change occurs as a result of additional types of molecule-receptor interaction.

Molecular Structure. Molecular structure and spatial orientation affects the odor quality, intensity, and threshold. Leitereg et al. (1971) and Haring et al. (1972) both reported that isomers sometimes vary in odor quality and thresholds. As described by Theimer et al. (1977), many theories also exist which propose that the “molecules’ conformation at the moment of contact to the receptor” may influence the descriptor, intensity and threshold. Theimer et al. (1977) cited many theories indicate that molecules require a certain shape to enter receptors (Amoore, 1952, 1962; Davies 1953; Theimer and Davies 1967). Jacob (2001) reported that six-carbon benzene ring side chain locations influenced odor descriptors. Other research demonstrated that larger rings (14 to 19 carbon atoms) were rearranged considerably without altering the odor (Jacob, 2001). Additionally, branched chain aldehydes had much lower odor thresholds than straight chain compounds (Guadagni et al., 1963).

Multi-Odorant Mixtures. The number of odorants present and the complexity of the mixture have been found to influence a person’s ability to detect more than one odor (Berglund and Olsson, 1993; Olsson, 1994). The odor intensity of a mixture has been found to be less than, equal to, or greater than the sum of the responses to the individual components (Olsson, 1994; Cometto-Muniz et al., 1999). Additionally, Worley et al. (2001) identified that the chlorinous odor can mask earthy and musty odors present.

Research also indicates that humans can only identify up to four odorants in multi-component mixtures (Livermore and Laing, 1998).

The results of Livermore and Laing (1998) indicated that when more than four odors were present in a mixture, only a small number of the subjects identified more than four of the components. All samples used during this study were single component solutions, one odorant per solution.

SENSORY EVALUATION METHODS FOR DRINKING WATER

Sensory evaluation methods used in drinking water research have been adapted from the food and beverage industry (Carnicross et al., 1950; Mularski, 1968; Mielgaard et al., 1999). Of these methods, triangle testing is used to determine whether a person can detect a specific odorant. During triangle testing, an individual is presented with three coded samples, two samples identical, one different (i.e. odorous, odorous, odor free), and chooses the sample that they believe is different (Mielgaard et al., 1999). Mielgaard et al. (1999) has provided statistical calculations, which can be used to determine a panelist's ability to detect the different sample.

Directional difference testing sometimes referred to as paired comparison testing, is used to determine which way a particular sensory characteristic differs between two water samples (Mielgaard et al., 1999). During directional difference testing, two coded samples are presented to people. First, individuals smell the samples from left to right and then choose the sample they believe has a stronger characteristic. Depending on the test instructions, "no difference" verdicts may be permitted. Again, tables are provided in Mielgaard et al. (1999) to determine the direction of the sensory response.

Currently, the threshold odor test (*Standard Method 2150B*) and flavor profile analysis (*Standard Method 2170B*) are the most popular drinking water odor sensory analysis methods (APHA, 1998). The threshold odor test, commonly referred to as TON, is used to determine an overall odor intensity of the sample. During TON, "the sample is diluted with odor-free water until the definitely least perceptible odor is achieved," and analysis typically occurs at 60 °C; however, 40 °C is also used (APHA, 1998).

Flavor profile analysis (FPA) requires a minimum of four trained panelists to assign intensity ratings to each taste-and-odor attribute identified in the water sample. These panelists rate each taste-and-odor attribute using a 7-point category scale (i.e. 0 to 12, even numbers) and then come to a consensus.

Standard Methods for the Examination of Water and Wastewater recommends FPA on odor be conducted at 25 °C or 45 °C; however, some utilities have conducted FPA at 20 °C (Krasner et al., 1985; Seligman et al., 1992). One utility performs FPA at 45 °C because they have found that the higher water temperature allows their panelists to be more sensitive (Burlingame et al., 1991). FPA has accepted taste reference standards (sour, salty, bitter, sweet); though, odor reference standards do not exist. Some researchers have integrated odor standards into their research such as geosmin, MIB, and n-hexanal (Burlingame et al., 1991; Bruchet et al., 1992; Cotsaris et al., 1995).

TEMPERATURE EFFECTS ON ODOR PERCEPTION

The temperature of source water can fluctuate seasonally and depends upon the type of source. For example, groundwaters are nearly constant temperatures, while surface water temperature depends strongly on climate (Viessman and Hammer, 1993). In Philadelphia Pennsylvania, the summer time water temperature of rivers and drinking water is about 25 °C, whereas the wintertime temperature can be 10-15 °C depending on the winter conditions (Burlingame, 2001). Additionally, a southwest Virginia water authority has seen raw water temperatures approaching 1-2 °C in the winter months, and as high as 20 °C during the summer (Knocke, 2000). Similarly the water supply of the City of Appleton, Wisconsin typically exceeds temperatures above 18 °C during late summer (Young et al., 1999). Once drinking water leaves the water utility consumers can come into contact with drinking water that has passed through a hot water heater or has been held in conveyance pipes for an extended period of time. In consumer's homes, water is typically used from below ambient temperatures to as high as 65 °C.

Food industry research indicates that product temperature influences consumer perception (Sizer and Harris, 1985; Wallengren and Moller, 1986; Baron and Penfield, 1996). In one study, Sizer and Harris (1985) evaluated serving temperature on perceived sensory heat and pungency. Their results indicated that the rate at which an individual felt a burn caused by inhalation of the chemical caspacin was dependent upon sample serving temperature.

Results from a study on common industrial chemicals demonstrated that odor threshold values for 1-butanol at 20 °C, 40 °C, and 60 °C decreased as water temperature increased (Alexander et al., 1982). Alexander et al. (1982) also observed that as water temperature increased from 20°C to 40°C to 60°C, the observed mean OTCs decreased from 0.8 mg/L, 0.3 mg/L, and 0.27 mg/L, respectively. They concluded that as water temperature increased less 1-butanol was required to arrive at the OTC.

Drinking water industry research indicated that sample serving temperature influenced how much consumers liked their drinking water (Pangborn and Bertolero, 1972). Pangborn and Bertolero (1972) found that temperature affected taste intensities, and also concluded that chilling it increased consumer palatability and acceptance of drinking water.

Water temperature affected the perception of dichloramine solutions at 25 °C and 40 °C (Bartels et al., 1986). The Weber-Fechner plot revealed that dichloramine odor intensities were greater at 45 °C than at 25 °C. Similarly, Burlingame et al. (1991) conducted a study to determine the affect of testing temperature on odor reference standards. Resembling Bartels et al. (1986), Burlingame et al. (1991) concluded that elevating the presentation temperature affected the perceived odor intensity, and odor intensities at 45 °C were higher than at 25 °C.

REPORTED FPA ODOR INTENSITY DATA

FPA odor intensity values were found in taste-and-odor literature for geosmin, MIB, nonadienal, n-hexanal, and free chlorine (Table 1-4). During this literature search, consistent and accepted standards for an odorant concentration and its corresponding FPA odor intensity were not found. Although, data found in the literature were used to approximate FPA 4-6 odor intensities and check the consistency of the results of this study.

Table 1-4. FPA Odor Intensities at 45 °C

Odorant	FPA Odor Intensity	Concentration (ug/L)	References
Free Chlorine	4.0	1000, 1800	d
	6.0	3000	d
		pH dependant	
Geosmin	4.0	0.015 – 0.100	a, b, c
	8.0	0.056 – 0.920	c
n-Hexanal	4.0	0.0026 – 0.0062	c
	6.0	0.019 – 0.068	c
	8.0	0.140 – 0.750	c
2-Methylisoborneol	4.0	0.005 – 0.100	a, b, c
	8.0	0.042 – 0.230	c
<i>trans</i> -2, <i>cis</i> -6-Nonadienal	3.5, 4.0	0.050, 0.300	a, b

- a. Rashash, 1994.
- b. Rashash et al., 1997.
- c. Burlingame et al., 1991.
- d. Krasner and Barrett, 1984.

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CHAPTER II

WATER TEMPERATURE AS IT AFFECTS THE PERCEPTION OF DRINKING WATER ODORANTS

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ABSTRACT

Panelists were trained in flavor profile analysis (FPA) and evaluated water samples that contained odorants at either 25 °C or 45 °C. The higher water temperature resulted in an increase in odor intensity for some, but not all, concentrations of geosmin, 2-methylisoborneol, *trans*-2, *cis*-6-nonadienal, n-hexanal, free chlorine, and 1-butanol. The odor response of isobutanol was not affected by temperature changes.

INTRODUCTION

Understanding the effects of water temperature on odor response will aide utilities in their efforts to minimize, control, and understand drinking water odor problems and complaints. In consumer's homes, water is typically used from below ambient temperatures to as high as 65 °C. Many drinking water utilities and researchers conduct drinking water odor testing at temperatures from ambient to 60 °C¹. Although the literature suggests that water temperature influences odor response, there are few quantitative data reported^{2,3,4}. Data reported for the food and beverage industries indicated that sample serving temperature affected consumer perception⁵⁻⁸.

Six odorants commonly identified in drinking water and one common air pollutant were investigated in this research. Table 2-1 describes the odor characteristics of these compounds.

Geosmin, 2-methylisoborneol (MIB), *trans*-2, *cis*-6-nonadienal (nonadienal), and n-hexanal are drinking water-associated odorants that are produced by actinomycetes, cyanobacteria or algae and occur in both water supplies and tap waters⁹⁻¹⁹. These compounds are very difficult to remove by conventional water treatment processes and sometimes occur at concentrations above the odor threshold in consumer's tap water.

Free chlorine, which includes hypochlorous acid (HOCl) and hypochlorite ion (OCl⁻), is the most widely applied disinfectant in the United States. It is the drinking water compound most associated with a chlorinous odor; the majority of the odor complaints in American drinking water concern chlorine^{20,21,22}. Chlorine has the ability to mask earthy and musty odors when present²³, which may be one of the reasons why chlorinous odors are among the most common drinking water complaints.

Isobutanal has been identified as a byproduct from ozonation and chlorination; it is formed by oxidation of the amino acid valine^{18,24,25}. Isobutanal was identified at a concentration of 54 ug/L in Canadian drinking water²⁴. Although 1-butanol is not a drinking water odorant, it has been studied in air pollution, chemical engineering, and sensory science^{1,3,26-30}. More specifically, 1-butanol has been researched as a standard for sensory analysis^{3,30}.

The objective of this research was to evaluate the effect of temperature (25 °C and 45 °C) on odor intensity for geosmin, 2-methylisoborneol, nonadienal, n-hexanal, isobutanal, free chlorine, and 1-butanol. Odor intensity was measured by flavor profile analysis (FPA) and quantitative description analysis (QDA).

MATERIALS AND METHODS

Reagents. The following chemicals were used in the project: 1-butanol (CAS 71-36-3; Sigma-Aldrich Chemical, Milwaukee, WI), geosmin (CAS 16423-19-1; Sigma, St. Louis, MO), n-hexanal (CAS 66-25-1; Sigma, St. Louis, MO), isobutanal (CAS 78-84-2; Sigma-Aldrich Chemical), 2-methylisoborneol (CAS 2371-42-8; Supelco, Bellefonte, PA), calcium carbonate A.C.S. grade (CAS 1317-65-3; Fisher Scientific) *trans*-2, *cis*-6-nonadienal (CAS 557-48-1; Aldrich Chemical), citric acid (CAS 77-92-9; Sigma-Aldrich Chemical), and quinine monohydrochloride dihydrate (CAS 6119-47-7; Sigma-Aldrich Chemical).

Consumer grade sucrose (sugar) and sodium chloride (table salt) were also used in these experiments. Reagent water was obtained from a Nanopure ultrapure water system (Barnstead/Thermolyne, model #D4744, Dubuque, IA).

Glassware. All glassware was initially soaked in 10% nitric acid solution for 48 hours. Before each use, glassware was washed with Sparkleen[®] odor-free detergent (Fisher Scientific), rinsed 10 times with tap water, and rinsed 3 times with reagent water. If any odors remained, this process was repeated. All 500 mL wide mouth Erlenmeyer flasks were stored with approximately 200 mL reagent water to prevent the formation of chalky odors. Immediately before the Erlenmeyer flasks were used, the glassware was rinsed again (10 times tap water, 3 times reagent water).

Free Chlorine Solutions. Free chlorine solutions were prepared with Clorox[®] bleach in reagent water containing 50 mg/L calcium carbonate. The pH was adjusted to 7.5 with 10% hydrochloric acid solution. The concentration of free chlorine was measured using a free chlorine Hach Kit (Hach Company, Loveland, CO).

Panelists. The Human Subject Committee at Virginia Tech approved the study protocol. All the subjects were either students or staff at Virginia Tech that ranged from 20 to 45 years and consisted of 6 males and 7 females. Subjects were not provided any monetary compensation for their participation. Prior to training and testing, panelists were advised not to wear perfumes, hand lotions, scented soaps, or similar odorous products, and were also asked to refrain from eating, drinking, and smoking at least 30 minutes before testing. Panelists underwent initial odor training using the *Sense of Smell Kit* (Carolina Biological Supply Company, Burlington, NC), which consisted of identifying a variety of odorants. Panelists were trained according to the AWWA manual on Flavor Profile Analysis (FPA) that uses a 7-point ordinal rating scale¹⁴. The subjects were then screened for their ability to detect the specific odorants using triangle testing and samples with an FPA odor intensity ranging between 4 to 6 FPA units³⁸. Only panelists who could correctly identify the odorant at the 95% confidence level were allowed to participate in temperature effect testing. Based on this testing 9-13 persons were able to participate during odor testing of each compound.

Sensory Test Sessions. All samples presented to panelists were coded with 3-digit random numbers³⁸. All experiments were performed under minimal background odors.

Odor evaluations were conducted with 200 mL odorant solution in 500 mL wide-mouth, glass Erlenmeyer flasks. Concentrations of odorant solutions were selected such that the lowest concentration yielded a FPA rating of about 4 and that the higher concentrations yielded FPA ratings of approximately 6-10. Temperature was controlled by either warming a sample solution inside a water bath (45 ± 1.5 °C) or allowing the sample to remain on the tabletop at room temperature (25 ± 1.5 °C). Quantitative Data Analysis (QDA) or modified FPA rating involved analyzing 3 to 5 samples using a 7-point category scale, which ranged in even values from 0 to 12^{14, 39}. Odor rating was conducted in 500 mL wide-mouth Erlenmeyer flasks containing 200 mL odorant solution.

Before panelists began rating unknown samples, they were instructed to: (1) calibrate their taste scales with a FPA sour solution of 4 and 8, (2) sniff a geosmin FPA 4 reference standard (30 ng/L, 45 °C, Erlenmeyer flasks) to calibrate their odor intensity scale, and (3) use a minimum 90 second break time between each sniff to avoid adaptation to the odorant. Individual ratings were recorded for each panelist. For each test, Erlenmeyer flasks containing odor free reagent water and a geosmin FPA 4 reference standard were provided in a 45 °C water bath. Mean odor intensity values and standard errors were calculated from the individual rating data.

Statistical Analysis. A NCSS 97 statistical package was used to perform two-way Analyses of Variances on data from all compounds except chlorine, which required a 1-way ANOVA. An alpha value of 0.05 was selected as the significance level for all data interpretation.

RESULTS

Odor intensities of water samples that contained geosmin, MIB, nonadienal, hexanal, free chlorine, butanol, and isobutanol were determined at 25 °C and 45 °C (Figures 2-1 through 2-7). For all odorants, except isobutanol, odor intensity increased as temperature increase for at least one concentration that was tested.

For geosmin, the differences in earthy odor intensity between 25 °C and 45 °C for all concentrations tested were between 1.0 - 2.5 FPA units (Figure 2-1).

A two-way ANOVA demonstrated that temperature had a significant effect on odor response ($p < 0.01$) while concentration had no significant effect on odor response ($p \cong 0.1$). This may be due to the variability in the odor intensity ratings among panelists and the fact that the odor intensity of the 600 ng/L geosmin was rated equal to or less than the odor intensity of the 400 ng/L samples. The interaction term, temperature*concentrations, was also not significant ($p = 0.73$).

Similar results to geosmin were observed for 2-MIB (Figure 2-2), in which the ratings of the musty odor between 25 °C and 45 °C varied by 1.5 – 2.0 FPA units. A two-way ANOVA test indicated that both temperature ($p < 0.01$) and concentration ($p = 0.02$) had a significant affect on odor response with increasing temperature and increasing concentration resulting in increased odor intensity. The interaction term temperature*concentrations was not significant ($p = 0.85$). The concentration term was significant even though the graph indicates that the odor intensity of the 600 ng/L 2-MIB was less than that of the 400 ng/L samples.

Results for the determination of the cucumber odor for nonadienal are presented in Figure 2-3. The odor intensity difference between nonadienal solutions at 25 °C and 45 °C are between 0 – 1.0 FPA unit, which is less than the difference determined for geosmin or 2-MIB. Temperature had a greater affect on the odor response of nonadienal at 50 ng/L than at the concentrations of 100 or 200 ng/L where there was little odor intensity difference with increased temperature. A two-way ANOVA test indicated that temperature ($p = 0.19$), concentration ($p = 0.06$), and the interaction term temperature*concentration ($p = 0.62$) was not significant for nonadienal.

Data for n-hexanal are presented in Figure 2-4 and indicate that at a concentration of 50 ng/L, the odor intensity of a solution at 45 °C was much greater than that of a solution at 25 °C; this trend was reversed at a concentration of 800 ng/L. Statistical analysis indicated that temperature ($p = 0.89$) and concentration ($p = 0.56$) were not significant, but that the interaction term between temperature and concentration ($p = 0.02$) was significant. The data for chlorine are presented in Figure 2-5. Only one concentration of chlorine was tested, and for 1.88 mg/L Cl_2 , the temperature significantly ($p = 0.04$) affected odor perception. The chlorine odor was more intense at 45 °C than at 25 °C.

The sensory analysis data for 1-butanol are presented in Figure 2-6. Like geosmin and 2-MIB, increasing temperature and increasing concentration resulted in an increased odor intensity rating for this compound at the levels tested; although, the 2-way ANOVA test indicated that the differences were not significant for temperature ($p = 0.09$), concentration ($p = 0.08$), or the interaction term ($p = 0.66$) in spite of the 2.0 FPA unit difference between the intensity ratings at each temperature.

Data for two concentrations of isobutanol are presented in Figure 2-7. The statistical analysis indicated that the effect of concentration was significant ($p = 0.02$), while the effect of temperature ($p = 0.12$) and the interaction term ($p = 0.86$) were not significant. Thus, increasing the concentration resulted in an increased odor perception for isobutanol, but increasing the temperature had no effect.

Table 2-2 presents a summary of the statistical analyses by individual compound. The reader is reminded that these results apply only to these experiments. If different concentrations were studied, the results could be very different because the general trend of increased odor with increased concentration and increased temperature was not consistent across the conditions tested.

DISCUSSION

Panelists stated that using the geosmin FPA 4 reference standard (30 ng/L, 45 °C, Erlenmeyer flasks) helped calibrate their sense of smell to an odor intensity value of four. The authors conclude that the use of a geosmin FPA 4 odor reference standard did not negatively influence a panelist's ability to judge FPA odor intensity because geosmin, MIB, nonadienal, hexanal, and free chlorine FPA odor intensity data were consistent with other FPA odor intensity data obtained from the literature^{3,15,17,31}. Odor reference standards should be evaluated and developed to allow panelists a benchmark from which they can judge other odorous water samples.

The magnitude of the effect of 25 °C and 45 °C water temperatures on odor intensity rating by panelists was specific to each odorant.

In general, for solutions of geosmin, 2-MIB, nonadienal, n-hexanal, 1-butanol and chlorine with an FPA rating of 4 (moderate), an increase in odor intensity was determined for the warmer solutions (45 °C) compared to the solutions at 25 °C. No difference was detected for an isobutanol solution at a concentration equivalent to an FPA 4 rating. At concentrations that produced odor intensities greater than FPA 4, variable results were observed with some odorants always producing increased odor intensities at 45 °C compared to 25 °C (geosmin, 2-MIB, chlorine), while for the other odorants (1-butanol, nonadienal, n-hexanal isobutanol) the effect of temperature was variable as the concentration increased above that equal to FPA 4.

Clearly, temperature and concentration affected the odor intensity rating for compounds in drinking water, but as indicated by the ANOVA results (Table 2-2), the effect was not significant or consistent for all compounds. The effect of temperature on odor involves both water solubility and vapor pressure of the individual compound. These can be complimentary or competing affects depending on the physical/chemical properties, the relationship of temperature, and the Henry's law constant for that compound. Consequently, the results of this study should not be directly applied to other compounds, temperatures, or concentrations for which data were not presented.

In this research, the data indicated that the higher water temperature enhanced the odor intensities for solutions that contained geosmin, 2-MIB, nonadienal, n-hexanal, chlorine, or 1-butanol. The results of this study agreed with those of previous researchers. The odor intensities of the dichloramine drinking water samples warmed to 45 °C were greater than in samples warmed to 25 °C for the concentrations tested². Burlingame et al. (1991) concluded that conducting FPA rating tests at 45 °C enabled panelists to better detect drinking water odorants.

Geosmin and MIB FPA rating data indicated that after about a concentration of 600 ng/L geosmin or 2-MIB, panelists could not detect an increase in odor intensity with an increase in concentration; at higher concentrations, the Weber-Fechner relationship was no longer applicable³⁹. This phenomenon was also noticed for nonadienal and n-hexanal, where for the higher concentrations tested, an increase in concentration did not result in an increase in responsiveness by panelists. A likely explanation is that too many odorant molecules stimulated the olfactory system and no increase in response could be recorded.

A similar explanation could apply when an increase in temperature produced an increase in response at a lower concentration but not at a higher concentration.

For all odorant concentrations tested, odor intensities for water that contained geosmin, 2-MIB, or free chlorine were increased at 45 °C compared to solutions at 25 °C. This is an important finding because geosmin, MIB, and free chlorine are three of the most common drinking water odorants. Consumers can more readily detect the odors of these three compounds in warm water than in cold water. This suggests that utilities should conduct sensory analyses at temperatures greater than ambient, and that testing a consumer's warm or hot water may be more representative of an odor problem than tests on their cold water. More research is required to determine the effect of water temperature on drinking water odor response for other compounds, concentrations, and temperature, as well as multiple odorant mixtures.

CONCLUSIONS

- Use of a geosmin FPA 4 odor reference standard (30 ng/L, 45 °C, Erlenmeyer flasks) to calibrate the panelists was well received by the panelists and assisted in producing consistent odor ratings.
- Increasing the water temperature from 25 °C to 45 °C resulted in a higher odor intensity rating for all concentrations of geosmin, 2-MIB, and chlorine that were tested.
- Increasing the water temperature from 25 °C to 45 °C resulted in an increased odor intensity rating for some, but not all, concentrations of *trans*-2, *cis*-6-nonadienal and n-hexanal. The odor intensity of isobutanal solutions did not change with a change in temperature.
- Changing the temperature will affect both water solubility and vapor pressure of an individual compound. These can be complimentary or competing effects depending on the physical/chemical properties, and the relationship of temperature and the Henry's law constant for that compound. Consequently, the results of this study should not be directly applied to other compounds, temperatures, or concentrations for which data were not presented.

- For many drinking odorants at moderate concentrations, conducting odor analysis at 45 °C allowed panelists to perceive greater odor intensities compared to analysis of the same concentration at 25 °C. Thus, consumers generally perceive more intense odor in warm water than cold water for moderate concentrations (FPA rating of about 4).
- At some level, increasing the concentration will not result in an increase in odor intensity. For geosmin and 2-MIB, the leveling of the response occurred between 400 - 600 ng/L; for *trans*-2, *cis*-6-nonadienal this occurred between a concentration of 100 and 200 ng/L.

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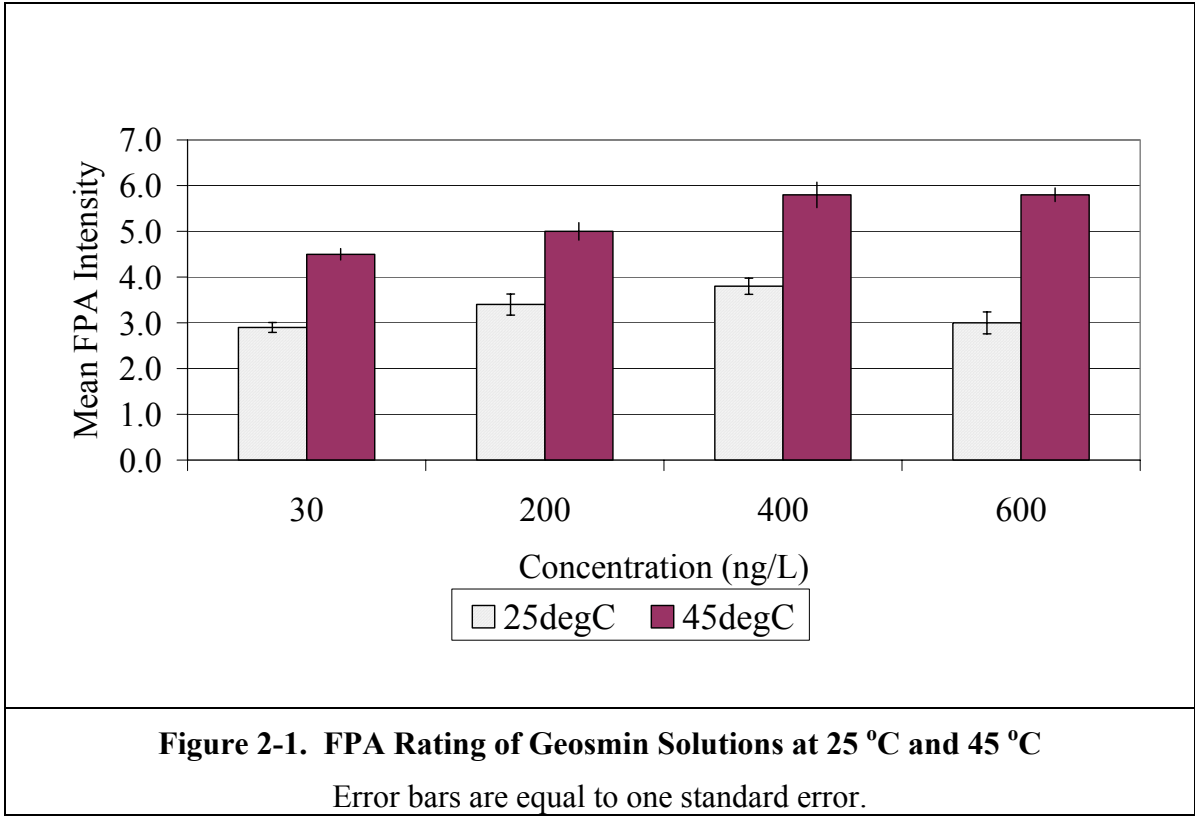
Table 2-1. Aqueous Odor Threshold Concentrations

Odorant	Common Odor Descriptor	Aqueous Odor Threshold Concentration, ug/L	References
1-Butanol	rancid	0.002 – 0.005	3, 30, 40, 41
Chlorine	chlorinous	280 – 360 pH dependent	31
Geosmin	earthy	0.006 - 0.01	17, 32, 33
n-Hexanal	lettuce heart	0.06 – 1.9	3
Isobutanol	malty, fruity	0.15 - 2.0	24, 34, 35
2-Methylisoborneol	musty	0.002 - 0.02	3, 17, 33
<i>trans</i> -2, <i>cis</i> -6-Nonadienal	cucumber	0.002 - 0.013	12, 17, 36, 37

Table 2-2. Summary of Results from ANOVA Test for Significance of Temperature and Concentration on Odor Response.

Odorant	Significance of Term in ANOVA		
	Temperature	Concentration	Temperature*Concentration
Geosmin	Significant	No	No
2-MIB	Significant	Significant	No
Nonadienal	No	No	No
n-Hexanal	No	No	Significant
1-Butanol	No	No	No
Chlorine ¹	Significant	----	----
Isobutanol	No	Significant	No

1. Only one chlorine concentration was tested.



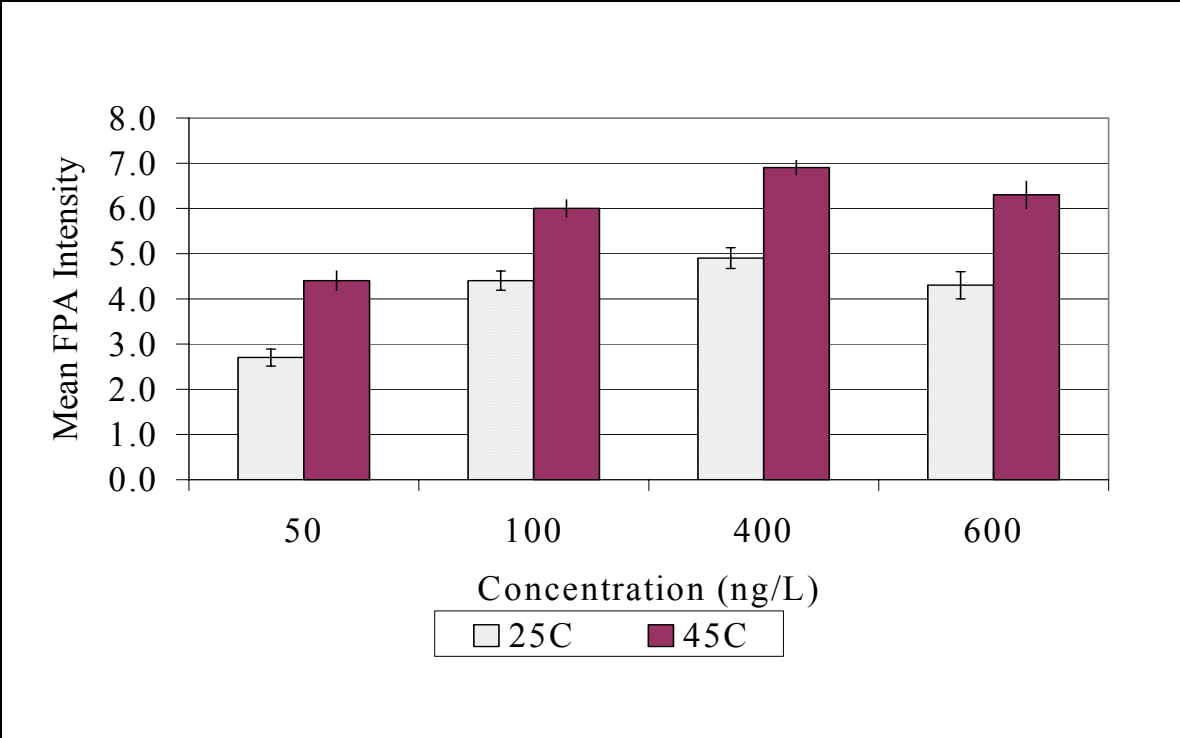


Figure 2-2. FPA Rating of MIB Solutions at 25 °C and 45 °C

Error bars are equal to one standard error.

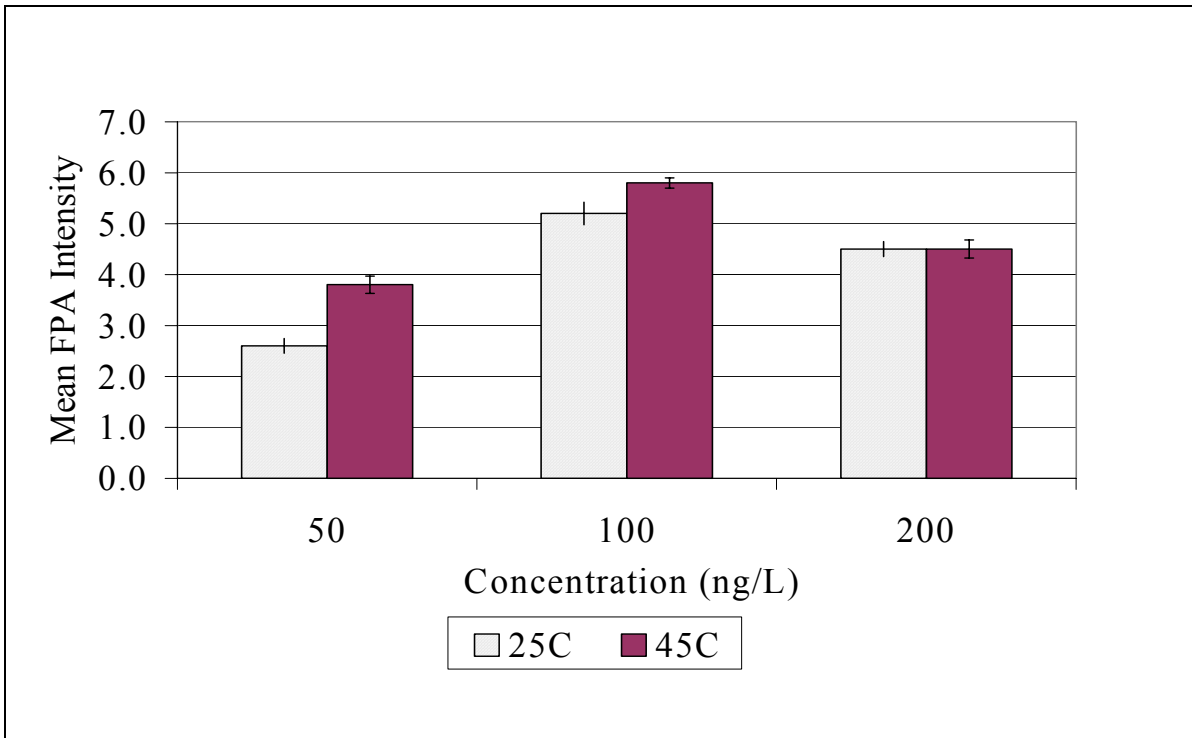


Figure 2-3. FPA Rating of *trans*-2, *cis*-6-Nonadienal Solutions at 25 °C and 45 °C

Error bars are equal to one standard error.

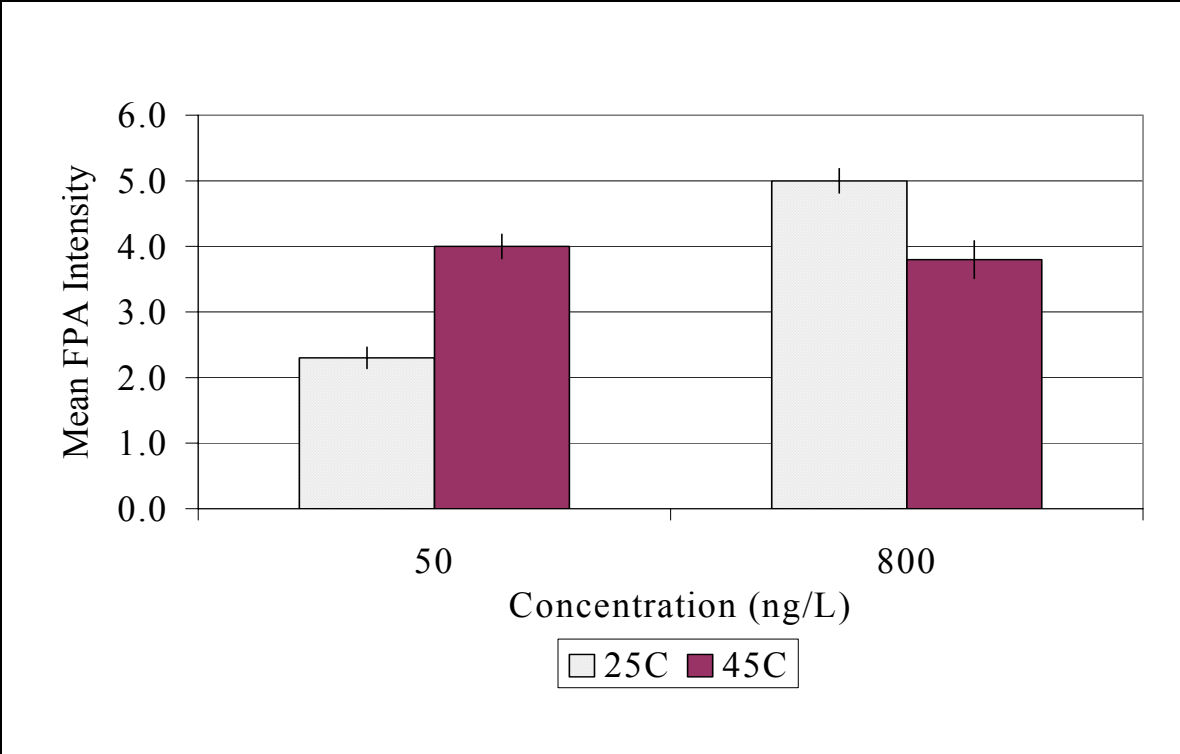


Figure 2-4. FPA Rating of n-Hexanal Solutions at 25 °C and 45 °C

Error bars are equal to one standard error.

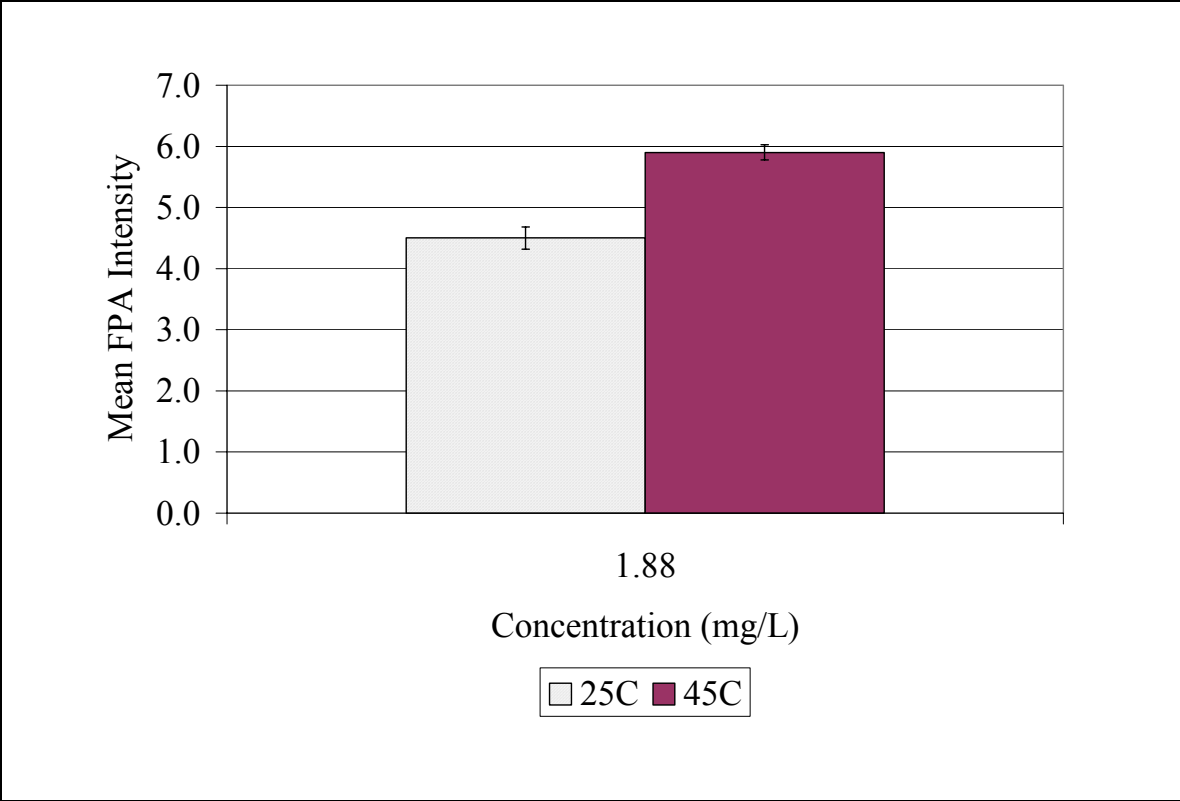


Figure 2-5. FPA Rating of Free Chlorine Solutions at 25 °C and 45 °C
Error bars are equal to one standard error.

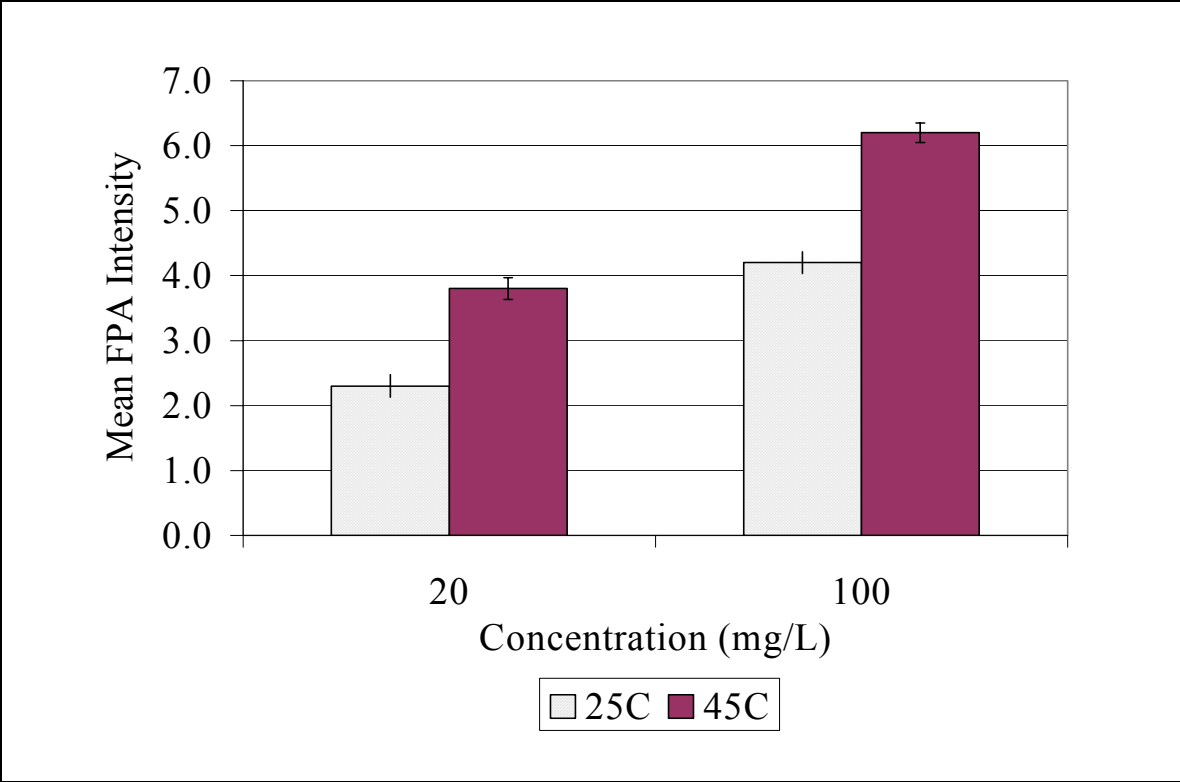


Figure 2-6. FPA Rating of 1-Butanol Solutions at 25 °C and 45 °C

Error bars are equal to one standard error.

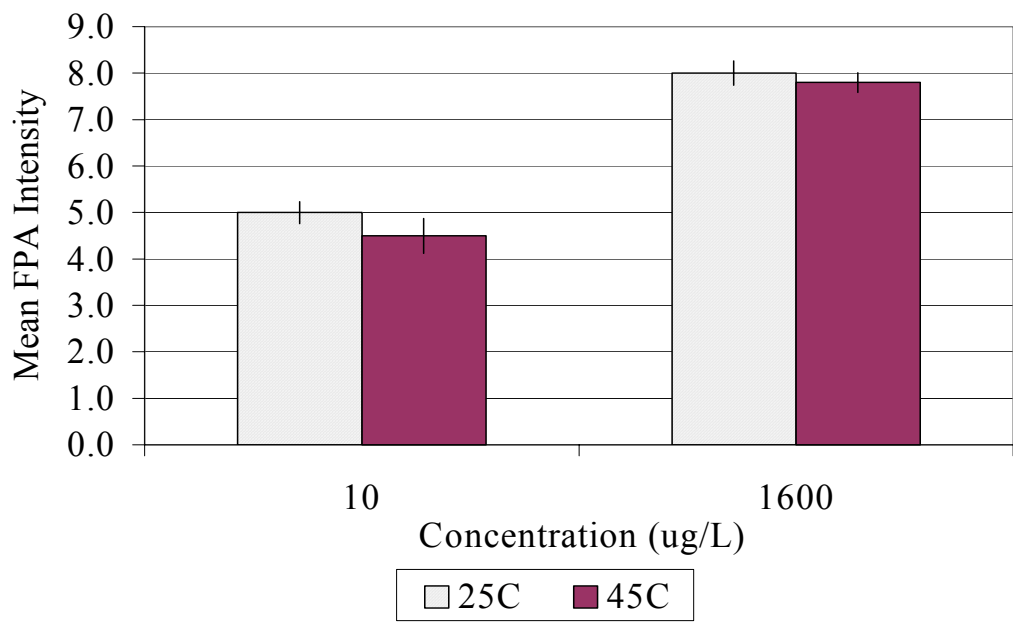


Figure 2-7. FPA Rating of Isobutanal Solutions at 25 °C and 45 °C

Error bars are equal to one standard error.

CHAPTER III

RELATIONSHIP BETWEEN ODOR INTENSITY, CONCENTRATION, AND TEMPERATURE FOR DRINKING WATER ODORANTS

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ABSTRACT

Sensory analyses experiments indicated that, for the concentrations and temperatures tested, odor intensity was a function of both aqueous concentration and water temperature for geosmin, MIB, nonadienal, n-hexanal, free chlorine, and 1-butanol. Odor response to drinking water containing isobutanol was affected by concentration but not water temperature. Treatment strategy concerns and sensory analysis recommendations are provided.

Key words: Odor, drinking water, temperature, geosmin, MIB, nonadienal, chlorine, isobutanol, butanol, FPA.

INTRODUCTION

Odor is a common drinking water problem that occurs throughout the world in countries that include Australia, Canada, France, Japan, South Africa, and the United States of America (Kikuchi et al., 1973; Krasner et al., 1985; Bartels and Suffet, 1987; Ito et al., 1988; Bruchet et al., 1992; Wnorowski and Scott, 1992; Hack, 2000). Problematic drinking water odors were reported in the United States as early as the 1880s as cited by Burlingame and Dahme (1989). Common drinking water odors have been described as chlorinous, earthy, musty, and cucumber (Sigworth, 1957; Gallup Poll, 1973; Burlingame et al., 1987; Dietrich et al., 1991; Suffet et al., 1999; Burlingame and Tanjucto, 2001).

The olfactory system -or sense of smell- is extremely complex. Odorants arrive at the olfactory receptors by either entering the nasal cavity through the nostrils or by traveling up passages from the nasopharynx to the olfactory cleft.

Odorants stimulate the nervous system and cause transfer of electrical signals from the olfactory receptors to the brain (Hudson, 2000). One of the significant issues is that perception is not only influenced by odorant contact with receptors but also the flow rate through the nose and the duration of odorant contact (Schneider et al. 1966).

Henry's Law predicts the amount of a drinking water odorant that enters the gas phase and can possibly be inhaled. Henry's Law is based on a thermodynamic equilibrium that relates gas phase and aqueous phase concentrations. Before an odor can be perceived, the gaseous concentration must be greater than an individual's odor threshold concentration (OTC). The OTC is defined as the minimum concentration required for an individual to detect an odorant; OTC varies from person to person (Lawless and Heymann, 1998). Typical OTCs for the seven compounds used in this study are shown in Table 3-1.

While anosmia is an abnormality in which the ability to smell specific or all odors is lost, adaptation is a normal response that involves a loss in sensitivity due to continued exposure to an odorant (Berglund and Engen, 1993; Gagnon et al., 1994; Dalton and Wysocki, 1996). Self-adaptation occurs when odor intensity decreases following continued exposure to the same odorant. Cross-adaptation occurs as a result of exposure to one odorant, which decreases the odor intensity of another odorant (Berglund and Engen, 1993; Dalton and Wysocki, 1996). This reduction in smell can allow individuals to become accustomed to an odor and be unable to detect it (Ekman et al., 1967).

Microbial byproducts, disinfectants, and disinfection byproducts are common drinking water odorants. Geosmin, MIB (2-methylisoborneol), *trans*-2, *cis*-6-nonadienal (nonadienal), and n-hexanal have been identified in numerous water supplies and tap waters. The common odor descriptors are: geosmin (earthy, beet-like), MIB (musty, earthy), nonadienal (cucumber, grassy), and n-hexanal (lettuce heart, grassy) (Safferman et al., 1967; Tinoco et al., 1978; Izaguirre et al., 1982; Slater and Blok, 1983; Burlingame et al., 1986; Mallevialle and Suffet, 1987; Hayes and Burch, 1989; Wnorowski and Scott, 1992; Burlingame et al., 1991; AWWARF, 1993; Rashash, 1994; Dietrich et al., 1995; Jones and Korth, 1995; Krasner, 1995; Rashash et al., 1997; Suffet et al., 1999; Young et al., 1999; Burlingame and Waer, 2000; Sigma-Aldrich, 2001).

Table 3-1. Aqueous Odor Threshold Concentrations and Reported Aqueous Concentrations for FPA Values

Odorant	Aqueous Odor Threshold Concentration (ug/L)	Concentration yielding a FPA Intensity of 4 at 45 °C (ug/L)	Concentration yielding a FPA Intensity of 8 at 45 °C (ug/L)	References
1-Butanol	0.002 – 0.005	---	---	c
Free Chlorine	280 – 360 pH dependent	1000, 1800	--	c
Geosmin	0.006 – 0.01	0.015-0.100	0.056 – 0.920	a, d, h
n-Hexanal	0.06 – 1.9	0.0026- 0.0062	0.140 – 0.750	a, g
Isobutanal	0.15 - 2.0	--	--	e, f
2-Methylisoborneol	0.002 - 0.02	0.005-0.100	0.042 – 0.230	a, d, h
<i>trans</i> -2, <i>cis</i> -6-Nonadienal	0.002 - 0.013	0.050, 0.300	--	d, g

- a. Burlingame et al., 1991.
- b. Lillard and Powers, 1975.
- c. Krasner and Barrett, 1984.
- d. Rashash et al., 1997.
- e. Guadagni et al., 1963.
- f. Guadagni et al., 1972.
- g. Cotsaris et al., 1995.
- h. Larzeny et al., 1991.

These compounds are metabolites excreted by actinomycetes, cyanobacteria, or algae, and are very difficult to remove by conventional water treatment processes.

The most widely applied disinfectant in the United States is free chlorine, which includes Cl_2 , HOCl and OCl^- . Its chlorinous odor has the unique ability to mask earthy and musty odors present, and this is one of the reasons earthy and musty odors are better detected when chlorine levels are lower (Worley et al., 2002). The chlorinous odor is one of the most common drinking water complaints (Burlingame et al., 1987; Burlingame and Dahme, 1989; Suffet et al., 1996).

Isobutanol has been identified as a byproduct from ozonation and chlorination (Hrudey et al., 1988; Froese et al., 1999; Suffet et al., 1999). Its common odor descriptors are malty, sweet, and fruity (Hellmann and Small, 1973; Amoore et al., 1976; Mallevalle and Suffet, 1987). Even though 1-butanol is not a drinking water odorant; it has been studied in air pollution, chemical engineering and sensory science (Laing, 1985; Stevens et al., 1989; Burlingame et al., 1991; Olsson, 1994; APHA, 1998; Cometto-Muniz et al., 1999). 1-Butanol has also been studied as an air-quality standard for sensory analysis and has been described as having a rancid odor (Baker, 1962). Quantitative sensory analysis data for aqueous samples were not found in the literature for either isobutanol or 1-butanol.

Various methods have been developed to provide qualitative descriptions and quantitative measurements of odor intensity. One of the most popular drinking water odor sensory analysis methods is flavor profile analysis, Standard Method 2170 (APHA, 1998). During flavor profile analysis (FPA), a minimum of four trained panelists use a 7-point category scale to assign an intensity rating to individual drinking water taste-and-odor attributes. The recommended FPA odor analysis temperature is 25 °C or 45 °C. The flavor profile analysis method does not require use of odor standards during FPA rating, however, some researchers have integrated geosmin, MIB, and n-hexanal odor references into their research (Burlingame et al., 1991; Bruchet et al., 1992; Cotsaris et al., 1995; APHA, 1998). The basis of this integration is to provide panelists a predetermined odor intensity rating from which to judge other odorous water samples.

Although consumers use drinking water at temperatures that vary from cold to hot, few researchers have quantitatively analyzed how water temperature affects odor response.

Two previous studies reported that dichloramine odor intensities were greater at 40 °C than at 25 °C, and the odor intensities for geosmin, MIB, and n-hexanal solutions were greater at 45 °C were greater than at 25 °C (Bartels et al., 1986; Burlingame et al., 1991). Additionally, Pangborn and Bertolero (1972) found that water temperature influenced how subjects liked their drinking water. These researchers also concluded that chilling drinking water increased consumer palatability and acceptance.

Food industry research has indicated that sample temperature influences consumer thresholds and responses (Sizer and Harris, 1985; Wallengren and Moller, 1986; Baron and Penfield, 1996). Sizer and Harris (1985) determined that serving temperature affected the rate at which an individual felt the chemical burn from capsaicin, a common food additive.

Alexander et al. (1982) conducted a study whereby odor thresholds were measured for 3 industrial chemicals dissolved in water at 20 °C, 40 °C, and 60 °C (Table 3-2). Results demonstrated that as temperature increased, a lower concentration of the chemical was required to reach the odor threshold concentrations for these chemicals.

Table 3-2. Mean Odor Threshold Concentrations of 1-Butanol, Diethanolamine, and Propylene glycol methyl ether at 20 °C, 40 °C, and 60 °C

Chemical	Mean Odor Threshold Concentrations, ug/L ¹		
	20 °C	40 °C	60 °C
1-Butanol	800	300	270
Diethanolamine	4,000,000	160,000	160,000
Propylene glycol methyl ether	16,000	7,400	4,000

1. Adapted from Alexander et al. (1982)

The objectives of this research were to determine whether temperature and/or concentration affected odor response for geosmin, 2-methylisoborneol, *trans*-2, *cis*-6-nonadienal, n-hexanal, free chlorine, isobutanal, and 1-butanol solutions. Specific objectives were: 1) determine if odorant solutions at 25 °C had the same odor intensities as solutions at 45 °C; 2) determine the FPA rating of odorant solutions at different concentrations and temperatures; 3) determine differences in FPA ratings of geosmin solutions at 5 °C, 25 °C, and 45 °C.

MATERIALS AND METHODS

Reagents. The following high-purity chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, MO): 1-butanol (CAS 71-36-3), geosmin (CAS 16423-19-1), n-hexanal (CAS 66-25-1), isobutanol (CAS 78-84-2), *trans*-2, *cis*-6-nonadienal (CAS 557-48-1), citric acid (CAS 77-92-9), and quinine monohydrochloride dihydrate (CAS 6119-47-7). Also used in this research were 2-methylisoborneol (CAS 2371-42-8; Supelco, Bellefonte, PA) and calcium carbonate A.C.S. grade (CAS 1317-65-3; Fisher Scientific). Consumer grade sucrose (sugar) and sodium chloride (table salt) were also used in these experiments. Reagent water was obtained from a Nanopure[®] ultrapure water system (Barnstead/Thermolyne, model #D4744, Dubuque, IA).

Glassware and Plastic ware. A 10% nitric acid solution was used to soak all of the glassware for 48 hours. Sparkleen[®] odor-free detergent (Fisher Scientific) was used to wash all glassware. Before all experiments, glassware was rinsed 10 times with tap water, and rinsed 3 times with reagent water. This process was repeated until no odors remained. Two hundred milliliters of reagent water was added to each 500 mL wide mouth Erlenmeyer flask to prevent the formation of chalky odors. Sixteen ounce DART[®] K-resin cups and lids were used as received from the manufacturer without further treatment (Dart Container Corporation, Mason, MI).

Free Chlorine Solutions. Clorox[®] bleach was used to prepare free chlorine solutions. These solutions consisted of 50 mg/L calcium carbonate reagent water. Ten percent hydrochloric acid solution was used to adjust solution pH to 7.5. The free chlorine concentration was measured using a free chlorine Hach Kit (Hach Company, Loveland, CO).

Panelists. The Human Subjects Committee at Virginia Tech approved the study protocol. All subjects were ages 20 to 45, either students or faculty and consisted of 6 males and 7 females. Prior to training and testing, panelists were advised not to wear perfumes, hand lotions, scented soaps, or similar odorous products. These panelists were also asked to refrain from eating, drinking, and smoking at least 30 minutes before testing. Panelists first underwent an introductory session by which they used the *Sense of Smell Kit* (Carolina Biological Supply Company, Burlington NC) to become familiar with their sense of smell.

Following this training, panelists were trained in flavor profile analysis (FPA) according to the AWWA manual (AWWARF, 1993).

The subjects were then screened for their ability to detect specific odorants using triangle testing. This method is explained in further detail by Meilgaard et al. (1999) and Whelton and Dietrich (2001). Only persons who were shown to be able to detect a specific odorant were allowed to participate in data generation for that odorant. Based on this testing, 12 individuals were used for geosmin, 9 for MIB, 13 for nonadienal, 10 for n-hexanal, 10 for isobutanol, and 13 for 1-butanol.

Sensory Test Sessions. All experiments were performed under negligible background odors. Directional difference testing involved the presentation of two 200 mL water samples (A and B) in 16 ounce DART®-brand, translucent, plastic cups. DART®-brand cups were previously shown to be odor-free and to perform similar to Erlenmeyer flasks (Worley, 2000). Prior to testing, the 16 ounce plastic cups were placed inside clean ceramic coffee mugs to prevent the panelist from detecting the temperature of the water sample. During testing, panelists were presented with the cup (inside the mug), but were not allowed to touch it. The test administrator opened and closed the samples for all panelists. Panelists were asked to determine which sample had greater odor intensity. In this experiment the concentrations of geosmin, MIB, and nonadienal were equal. Also, the water temperature of sample A was (25 ± 1.5 °C), while the water temperature of sample B (45 ± 1.5 °C).

Flavor profile analysis was conducted using 200 mL odorant solutions in 500 mL wide-mouth Erlenmeyer glass flasks. Concentrations of odorant solutions were selected such that the lowest concentration yielded a moderate FPA rating of about 4 and that the higher concentrations yielded FPA ratings of approximately 6-10. Samples were either warmed inside a water bath (45 ± 1.5 °C) or allowed to remain on the tabletop at room temperature (25 ± 1.5 °C). No more than 6 samples were evaluated in a particular sensory session.

During each FPA session, panelists were provided with a weak (FPA 4) and a moderate (FPA 8) sour standard to calibrate their sense of taste. To calibrate their sense of smell, panelists were provided a 30 ng/L geosmin solution (FPA 4) in an Erlenmeyer flask at 45 °C. After calibrating with both the taste and the odor standards, panelists evaluated individual water samples.

Panelists assigned a FPA rating value to a water sample, ranging from 0 to 12, even numbers (AWWARF, 1993; Lawless and Heymann, 1998). According to *Standard Methods*, the group of panelists must come to a consensus FPA value for each water sample. However, similar to Quantitative Data Analysis (QDA) (Lawless and Heymann, 1998), a mean and standard deviation was calculated for each water sample that the panelists rated.

Computational and Statistical Analyses. A 2-tailed t-test was applied to directional difference data for geosmin, MIB, and nonadienal responses with an alpha value of 0.10 (Zar, 1999). For FPA data, 1-tailed t-tests were performed with an alpha value of 0.05 (Zar, 1999). Trend line slopes for geosmin and MIB Weber-Fechner plots were compared to determine whether or not they were different at an alpha value of 0.05 (Zar, 1999).

RESULTS

Odor Intensities of Solutions at 25 °C and 45 °C. Directional difference tests were used to determine which of two samples had the more intense odor. Samples varied in odorant concentration and temperature. During the first series of experiments, single odorant solutions of geosmin, MIB, and nonadienal concentrations were held constant ($C_1 = C_2$) at concentrations designed to result in a moderate odor intensity of FPA 4. The solution temperatures were maintained at either 25 °C or 45 °C. Statistical analyses indicated that the panelists perceived the odor intensity of the 45 °C solution as greater than that of the 25 °C solution (Table 3-3). Experiment replications demonstrated that the panel was consistent in their response.

FPA Rating of Geosmin and MIB at 25 °C and 45 °C. For geosmin solutions of equal concentration, the FPA ratings of earthy odor intensity were greater at 45 °C than at 25 °C (Table 3-4). The odor intensity difference between the 25 °C and 45 °C geosmin solutions ranged from 1.6 to 2.8 FPA units with the odor intensity difference increasing as the concentration increased. Weber-Fechner lines for these data are presented in Figure 3-1. A test of the 25 °C and 45 °C regression lines determined that the slopes were not statistically different and the lines were parallel. The data for the 600 ng/L geosmin solution were not included in the regression analyses because the QDA intensities were equal to or less than those for the 400 ng/L solutions. This suggested that the panelist olfactory system was saturated.

**Table 3-3. Directional Difference Experiments with Single Odorant Concentrations
Constant and Temperature Varied at either 25 °C or 45 °C**

Odorant and Concentration	Trial Number	Number of Panelists Choosing 45 °C Solution as More Intense	Significant at 90% confidence ¹
Geosmin (30 ng/L)			
	1	10/12	YES
	2	11/12	YES
	3	10/12	YES
	4	10/11	YES
	5	10/11	YES
	6	10/11	YES
	7	12/12	YES
	8	10/11	YES
MIB (30 ng/L)			
	1	7/8	YES
	2	7/8	YES
Nonadienal (40 ng/L)			
	1	11/13	YES
	2	11/13	YES

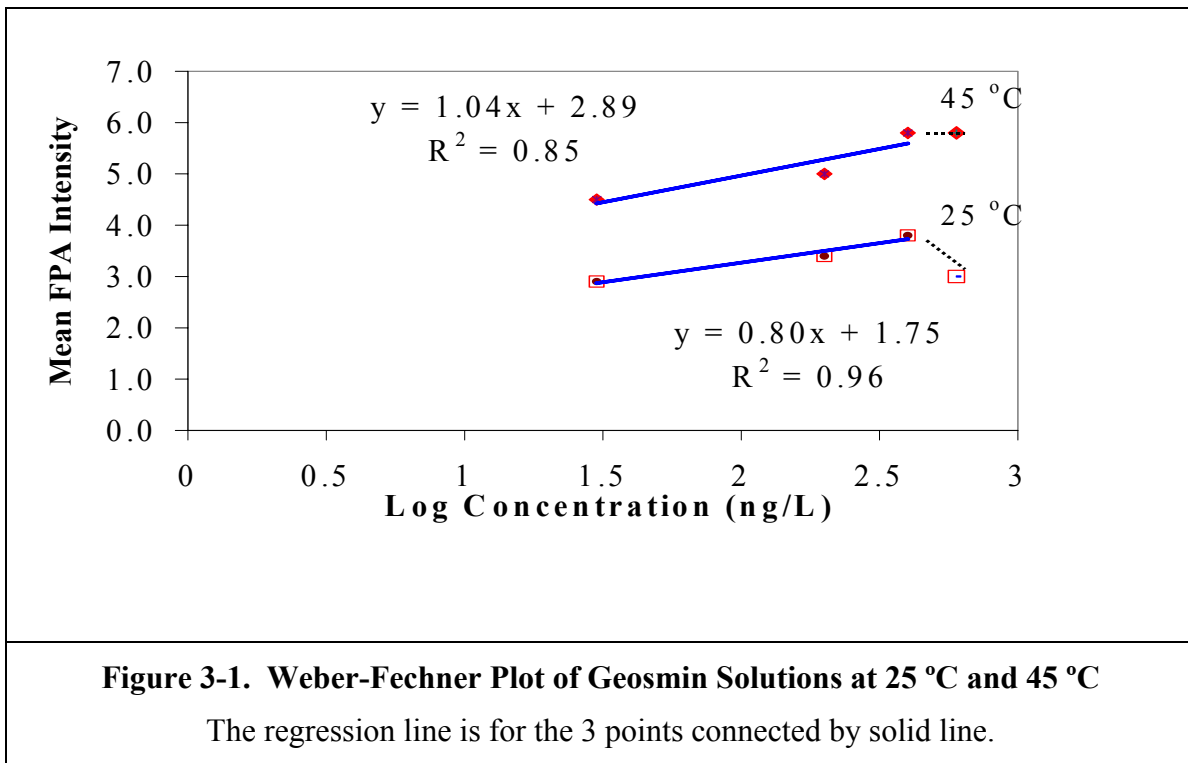
1. 2-tailed t-test

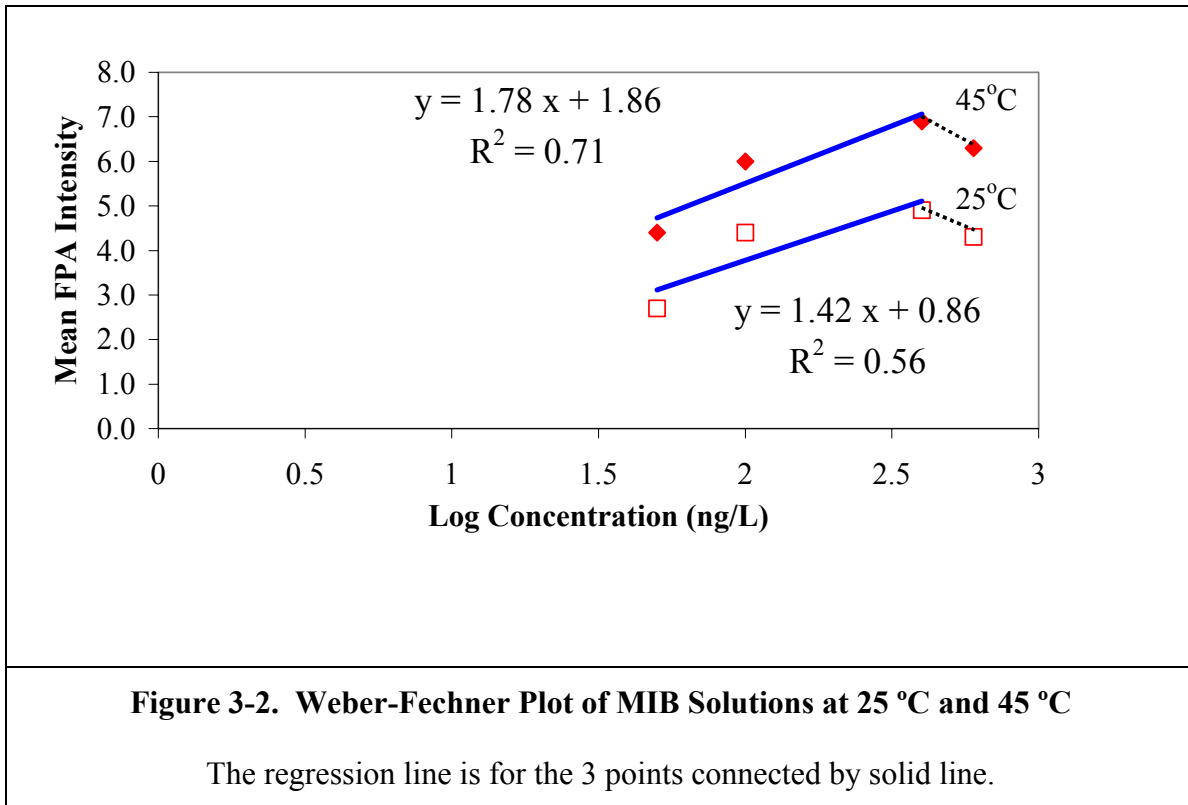
All musty odor intensities of MIB solutions were statistically greater at 45 °C than at 25 °C (Table 3-4). Results of a statistical analysis of the 25 °C and 45 °C trend line slopes concluded that the slopes were not different from one another and the lines were parallel (Figure 3-2). Odor intensity differences for the solutions at both temperatures varied from 1.6 to 2.0 FPA units.

Table 3-4. FPA Rating of Geosmin and MIB Solutions at 25 °C and 45 °C

Odorant	Concentration (ng/L)	Odor Intensity ¹ of Solution at 25 °C	Odor Intensity ¹ of Solution at 45 °C	Difference in Odor Intensity (45 °C - 25 °C)	Significant at 95% Confidence ²
Geosmin	30	2.9	4.5	1.6	YES
	200	3.4	5.0	1.6	YES
	400	3.8	5.8	2.0	YES
	600	3.0	5.8	2.8	YES
MIB	50	2.7	4.4	1.7	YES
	100	4.4	6.0	1.6	YES
	400	4.9	6.9	2.0	YES
	600	4.3	6.3	2.0	YES

1. The value for the odor intensity is the mean of the FPA ratings by individual panelists; n = 9-12; Standard error values were between 0.1 and 0.3 for all means.
2. One tailed t-test.





FPA Rating of Nonadienal, n-Hexanal, and Isobutanal at 25 °C and 45 °C.

Cucumber odor intensity data are reported in Table 3-5. A difference was found for only the 50 ng/L nonadienal solutions; a 1-tailed t-test demonstrated that the odor intensities of the 100 ng/L and 200 ng/L solutions at 45 °C were not greater than at 25 °C (Table 3-5). As concentration increased, the odor intensity difference decreased from 1.2 to 0 FPA units between nonadienal solutions (Figure 3-3).

Similar to the nonadienal solution at 50 ng/L, a 1-tailed t-test indicated that the lettuce heart odor intensity of the 50 ng/L n-hexanal solution was greater at 45 °C than at 25 °C. The 45 °C 800 ng/L solution of n-hexanal did not have greater odor intensity than the 25 °C solution (Table 3-5). FPA odor intensity differences were 1.7 FPA units at 50 ng/L and -1.2 FPA units at 800 ng/L.

A Weber-Fechner plot of isobutanol at 45 °C is provided in Figure 3-4. FPA data and 1-tailed t-test results indicated that the malty odor intensities of solutions at 45 °C were not greater than solutions at 25 °C (Table 3-5). The differences observed were –0.5 FPA units at 10 ug/L concentration and –0.2 FPA units at 1600 ug/L concentration.

FPA Rating of Chlorine and 1-Butanol at 25 °C and 45 °C. Free chlorine and 1-butanol FPA results are provided in Table 3-6. As the 1-tailed t-test results indicated, all free chlorine and 1-butanol solutions at 45 °C had greater odor intensities than solutions prepared at 25 °C. The chlorinous odor intensity difference between the 25 °C and 45 °C solutions of free chlorine (1.88 mg/L, pH 7.5) was 1.4 FPA units. The rancid odor intensity differences of 1-butanol were 1.5 and 2.0 FPA units in the 20 and 100 mg/L solutions. A Weber-Fechner plot at 45 °C is shown for 1-butanol in Figure 3-5.

FPA Rating of Geosmin at 5 °C. When panelists rated two 30 ng/L geosmin solutions, one cooled to 5 °C and the other warmed to 45 °C, the mean FPA ratings were 2.0 and 4.5 FPA units, with standard errors of 0.2 and 0.1 FPA units, for the 5 °C and 45 °C samples, respectively. A 1-tailed t-test indicated that the odor intensity of the 30 ng/L solution at 45 °C was greater than at 5 °C ($p = 0.007$).

When panelists compared 30 ng/L geosmin samples at 5 °C and 25 °C, statistical analyses demonstrated that the odor intensity of the 30 ng/L geosmin sample at 25 °C was not greater than the odor intensity at 5 °C ($p=0.134$). Further testing and statistical analyses determined that when panelists were presented again with two 30 ng/L geosmin solutions, one at 5 °C and one at 25 °C, but were told that the 30 ng/L geosmin reference standard had an FPA =6 rating, the odor intensity of the 25 °C solution was not greater than the 5 °C solution ($p = 0.216$) (Table 3-7). Thus, although scaling affected the absolute intensity, it did not affect the relative odor intensity.

**Table 3-5. FPA Rating of Nonadienal, n-Hexanal, and Isobutanal Solutions
at 25 °C and 45 °C**

Odorant	Concentration (ng/L)	Odor Intensity ¹ of Solution at 25 °C	Odor Intensity ¹ of Solution at 45 °C	Difference in Odor Intensity (45 °C - 25 °C)	Significant at 95% Confidence ²
Nonadienal	50	2.6	3.8	1.2	YES
	100	5.2	5.8	0.6	NO
	200	4.5	4.5	0.0	NO
n-Hexanal	50	2.3	4.0	1.7	YES
	800	5.0	3.8	-1.2	NO
Isobutanal	10	5.0	4.5	-0.5	NO
	1600	8.0	7.8	-0.2	NO

1. The value for the odor intensity is the mean of the FPA ratings by individual panelists, n = 9-13; Standard error values were between 0.1 and 0.3 for all means.
2. One tailed t-test.

Table 3-6. FPA Rating of Free Chlorine and 1-Butanol Solutions at 25 °C and 45 °C

Odorant	Concentration (ng/L)	Odor Intensity ¹ of Solution at 25 °C	Odor Intensity ¹ of Solution at 45 °C	Difference in Odor Intensity (45 °C - 25 °C)	Significant at 95% Confidence ²
Chlorine	1.88	4.5	5.9	1.4	YES
1-Butanol	20	2.3	3.8	1.5	YES
	100	4.2	6.2	2.0	YES

1. The value for the odor intensity is the mean of the FPA ratings by individual panelists; n =12-13; Standard error values were between 0.1 and 0.3 for all means.
2. One tailed t-test.

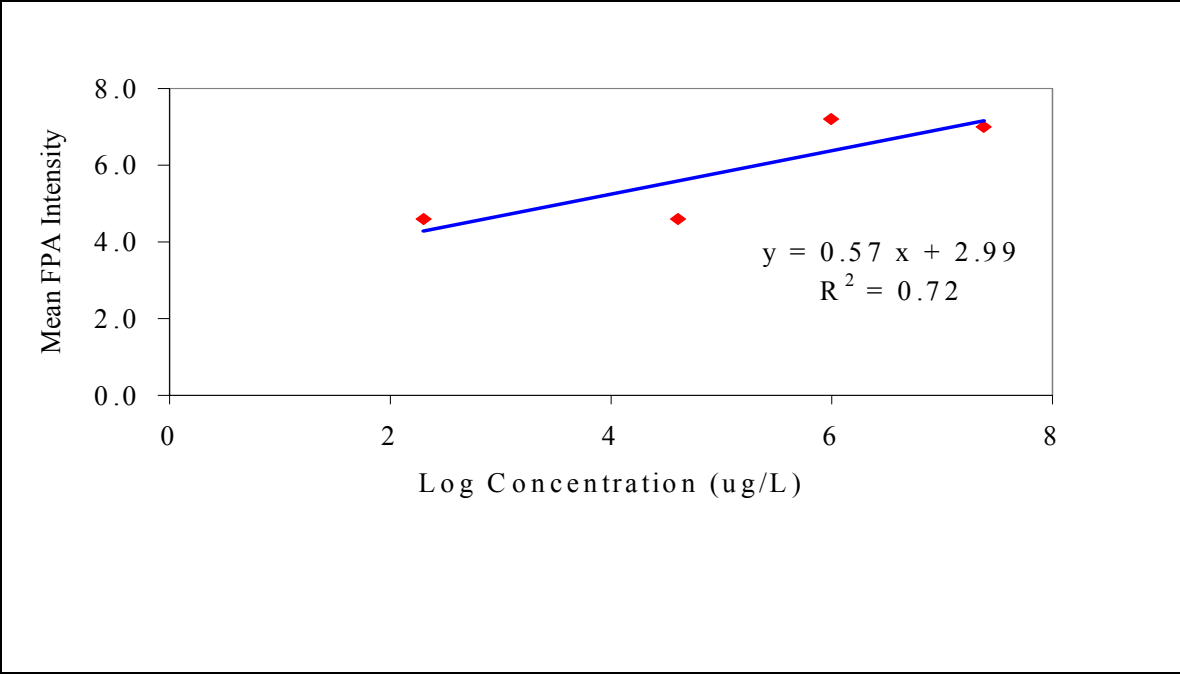


Figure 3-4. Isobutanol Weber-Fechner Plot of Solutions at 45 °C

n = 9

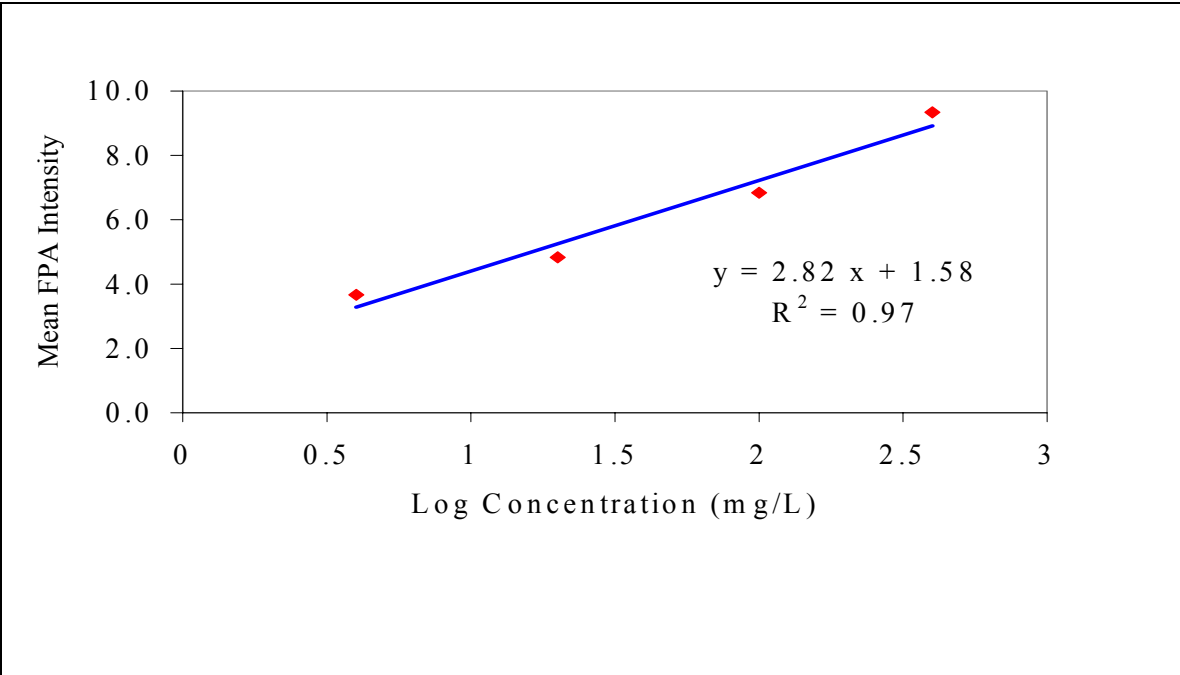


Figure 3-5. 1-Butanol Weber-Fechner Plot of Solutions at 45 °C

n = 9

Table 3-7. Geosmin FPA Rating at 5 °C and 25 °C using either a FPA 4 or FPA 6 Odor Reference Standard

Aqueous Concentration ng/L	Odor Intensity			
	Rating with a FPA 4 Reference Standard ¹		Rating with a FPA 6 Reference Standard ¹	
	Solution at 5 °C ²	Solution at 25 °C ²	Solution at 5 °C ²	Solution at 25 °C ²
30	2.0	2.9	2.5	3.5
100	----	----	2.4	3.1

1. Geosmin reference standard: 30 ng/L geosmin warmed to 45 °C.
2. Standard errors values were between 0.1 to 0.2 FPA units for all values.

DISCUSSION

Relation to the Literature. Concentrations of geosmin, MIB, nonadienal, n-hexanal, free chlorine, and isobutanol used in this research were selected to yield ratings of FPA 4-8 and were typical of concentrations found in drinking water supplies and tap waters (except for 1-butanol). Moreover, FPA ratings of geosmin, MIB, nonadienal, n-hexanal, and free chlorine from this research were consistent with FPA data found in the drinking water literature (Krasner and Barrett, 1984; Burlingame et al., 1991; Rashash, 1997; Rashash et al., 1997). This research found that as the aqueous concentration of 1-butanol was increased and the temperature of the 1-butanol solution was increased, then the perceived odor intensity increased. This is similar to previous sensory research with vapor-phase 1-butanol (Alexander et al., 1982).

Similar to the food and beverage industry research on product temperature, these results support that drinking water temperature affect odor response by consumers (Pangborn and Bertolero, 1972; Sizer and Harris, 1985; Bartels et al., 1986; Wallengren and Moller, 1986; Burlingame et al., 1991; Baron and Penfield, 1996). This is an important finding to the water industry because responsiveness to an odorant, odor strength, and magnitude of the differences perceived can be concentration and temperature dependent. Pangborn and Bertolero (1972), reported that drinking cold water was preferred by consumers.

The results of this study demonstrated that many drinking-water odorants were more difficult to detect in cold water than warm water.

Temperature Affects at 25 °C and 45 °C. The effect of water temperature on odor response was not significant or consistent for all compounds or all concentrations tested. The human senses could only perceive this increased vapor concentration for selected aqueous concentrations. For example, the odor intensities of MIB at 400 ng/L were greater than the odor intensities at 600 ng/L.

An increase in odor intensity was observed for higher temperature solutions of geosmin, MIB, nonadienal, n-hexanal, free chlorine, and 1-butanol when the concentration was equivalent to that producing an odor intensity of FPA 4 at 45 °C. This discovery is very important because geosmin, MIB, and free chlorine are three of the most common drinking water odorants and consumers will notice these odors more readily in warm water.

Indications of panelist adaptation and sensory overload were found for analysis of geosmin for concentrations at or above 400 ng/L and MIB for concentrations at or above 400 ng/L (Table 3-4), nonadienal for concentrations at or above 100 ng/L (Table 3-5), and n-hexanal for concentrations at or above 50 ng/L (Table 3-5). Sensory overload is the olfactory system's inability to process the amount of information because too many odorant molecules are present. This finding is important from a water treatment standpoint because consumers may be unable to detect odor differences at higher concentrations or hotter water temperatures for certain odorants. For example, isobutanol odor response was unaffected by temperature at 10 and 1600 ug/L at 25 °C and 45 °C.

Temperature affected odor response to geosmin and MIB solutions similarly. As concentration increased, both earthy and musty odor intensities at 25 °C increased at the same slope as 45 °C solutions (Figures 3-1 and 3-2).

Temperature Affects at 5 °C. Odor responsiveness to water containing geosmin demonstrated that a greater odor intensity difference was found between 5 °C and 45 °C solutions than between 25 °C and 45 °C or 5 °C and 25 °C solutions. An implication of this is that when consumers compare odorous drinking water with greater temperature differences (5 °C and 45 °C vs. 25 °C and 45 °C) they are more likely to find a greater odor intensity difference.

Water Treatment Strategy. Depending on the odorant present and its water concentration, lowering the odorant concentration may or may not have a positive affect on odor intensity. When odorant concentrations exist that encourage adaptation, removing odorants below those concentrations may promote conditions that are conducive to consumers detecting differences between waters, i.e. nonadienal and n-hexanal. Also, by removing geosmin and MIB concentrations from 600 ng/L to 400 ng/L consumers may detect an increase in odor intensity. These product changes could result in increased complaint volume and consumer dissatisfaction.

CONCLUSIONS

- For odorant solutions prepared at an FPA rating of 4, water temperature affected the perception of solutions containing geosmin, MIB, nonadienal, n-hexanal, free chlorine, and 1-butanol.
- Panelists will more effectively detect solutions containing geosmin, MIB, nonadienal, free chlorine, and 1-butanol at 45 °C than at 25 °C.
- Adaptation was noticed for water containing between 400 to 600 ng/L geosmin, 400 to 600 ng/L, 2-MIB, 100 and 200 ng/L nonadienal, 50 and 800 ng/L n-hexanal, and 10 and 1600 ug/L isobutanol.
- The comparison of waters containing geosmin at 30 or 100 ng/L concentrations with a greater temperature difference may elicit a greater odor intensity difference than comparing waters with a lower temperature difference.
- FPA data for geosmin at 5 °C and 25 °C imply that similar to an odor threshold, a threshold for difference detection may exist. Thus, a concentration difference may be required for the olfactory system to be able to perceive a difference between two solutions.

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VITA

Andrew James Whelton was born July 26, 1978 in Lowell, Massachusetts to Richard and Mary Whelton of Arlington and Revere, Massachusetts. Andrew has one brother, Ryan who is 1.5 years younger of age and one sister, Laura, who is 6 years younger.

Prior to attending Virginia Tech in 1996, Andrew worked at MBC Freight Consultants Inc. (Chelsea, MA). Over the course of 3 summers, he coordinated trucking logistics and domestic freight deliveries, while managing a warehouse workforce of approximately 10 employees. During this time Andrew worked along side his brother Ryan, and cousins Ken Wilhelm and David Staub.

While at Virginia Tech, Andrew worked for the Massachusetts Department of Environmental Protection (Worcester, MA) where he was trained as a multi-media environmental inspector. Additionally, he obtained his HAZWOP/ER certification in the fall of 1998. From 1999-2000 Andrew worked with Dr. Andrea Dietrich and other researchers on a small drinking water system grant provided by the Virginia Department of Health. This work was published in the *Journal of American Water Works Association* in 2000. Following graduation with a Bachelors of Science degree in Civil Engineering in spring of 2000, Andrew worked at Olver Inc. (Blacksburg, VA) where he primarily evaluated existing and alternative disinfection strategies at a water authority located in southwest Virginia.

Andrew entered Virginia Tech's Environmental Engineering program to pursue a Masters of Science degree in Fall 2000. During graduate study, Andrew aided Dr. Andrea Dietrich in training water utilities in Indiana, New Jersey, and Virginia on new methods developed for drinking water odor detection. In November, 2001 Andrew accompanied seven other researchers to the American Water Works Association Water Quality Technology Conference in Nashville, Tennessee and co-hosted a seminar entitled "New Practical Methods for Drinking Water Odor Detection: Beyond Flavor Profile Analysis (FPA) and Threshold Odor Number (TON)." In August 2001, the Graduate Student Association awarded Andrew the title of Graduate Student of the Month for his contributions to graduate-level research. Following graduation in December 2001, Andrew will be working with the U.S Army Center for Health Promotion and Preventive Medicine as an independent contractor in Aberdeen Proving Ground, Maryland.