

Occurrence of Bacteria and Viruses on Elementary Classroom Surfaces and the Potential Role of Classroom Hygiene in the Spread of Infectious Diseases

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The presence of microorganisms on common classroom contact surfaces (fomites) was determined to identify the areas most likely to become contaminated. Six elementary classrooms were divided into control and intervention groups (cleaned daily with a quaternary ammonium wipe) and tested for heterotrophic bacteria. Three classrooms were also tested for norovirus and influenza A virus. Frequently used fomites were the most contaminated; water fountain toggles, pencil sharpeners, keyboards, and faucet handles were the most bacterially contaminated; desktops, faucet handles, and paper towel dispensers were the most contaminated with viruses. Influenza A virus was detected on up to 50% and norovirus on up to 22% of surfaces throughout the day. Children in the control classrooms were 2.32 times more likely to report absenteeism due to illness than children in the intervention classrooms and were absent longer (on average). Improved classroom hygiene may reduce the incidence of infection and thus student absenteeism.

Keywords: contamination; surfaces; environment; pathogens; schools; classroom hygiene; absenteeism

INTRODUCTION

Bacteria and viruses may survive on environmental surfaces and may subsequently be transferred to a person's hands on contact. Microbial survival on inanimate surfaces (fomites) depends on a variety of factors including the species, the relative humidity or moisture content, the temperature, the surface materials and properties, and, in the case of viruses, whether they are enveloped or nonenveloped. Nonenveloped viruses, such as norovirus, are fairly stable in the environment (Barker, Stevens, & Bloomfield, 2001). For instance, the nonenveloped human astroviruses have been found to persist for up to 60 days on dry

nonporous surfaces and for up to 90 days on dry porous surfaces (Abad et al., 2001). Enveloped

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viruses are less stable; nevertheless, the enveloped influenza A virus may survive for up to 48 hr on dry surfaces (Bean et al., 1982) and the enveloped SARS coronavirus has been found to survive on fomites for up to 96 hr (Duan et al., 2003).

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Bacteria tend to be found in higher numbers on porous surfaces and under moist conditions (Rusin, Maxwell, & Gerba, 2002). Transfer rates of microbes to hands are more efficient from hard, nonporous surfaces such as stainless steel (Rheinbaben, Schünermann, Gross, & Wolff, 2000; Rusin et al., 2002). A 40% transfer rate was observed for *Escherichia coli* from a nonporous laminate surface to fingers in one study (Scott & Bloomfield, 1990). Rusin et al. (2002) found bacterial transfer rates of 38.5% to 41.8% from the telephone and rates of 27.6% to 40.0% from a sink faucet handle to a person's hand with minimal contact times. In the study by Rheinbaben et al. (2000), the bacteriophage Φ X174 was transferred from an inoculated doorknob to 14 successive people by contact and then to an additional six successive persons through the shaking of hands.

The transfer of bacteria and viruses from fomites to hands and from hands to fomites has been demonstrated in numerous studies (Ansari, Sattar, Springthorpe, Wells, & Tostowaryk, 1988; Boyce, Potter-Bynoe, Chenevert, & King, 1997; Bures, Fishbain, Uyehara, Parker, & Berg, 2000; Manning, Archibald, Bell, Banerjee, & Jarvis, 2001; Rusin et al., 2002; Salvat, Toquin, Michel, & Colin, 1995). Nevertheless, the contribution of such microbial transfer to the spread of diseases is unclear. In a study by Reynolds, Watt, Boone, and Gerba (2005), 25 of 54 (46%) day care center surfaces tested positive for human biochemical markers (hemoglobin, urea, amylase) and 35 of 54 (65%) tested positive for protein levels greater than 200 μ g/ml, suggesting contamination by

blood, mucus, sweat, saliva, or urine. Rotavirus has been found on 16% to 30% of surfaces in day care centers (Butz, Fosarelli, Dick, Cusack, & Yolken, 1993; Keswick, Pickering, DuPont, & Woodward, 1983; Wilde, Pickering, Eiden, & Yolken, 1992). In a study by Gwaltney and Hendley (1982), over 50% of individuals became ill after handling coffee cup handles and other objects that had been contaminated with rhinovirus, strongly suggesting transmission via fomites. In another study, the detection of influenza virus on greater than 50% of household and day care center fomites correlated with the seasonal incidence of disease in the community (Boone & Gerba, 2005).

MATERIALS AND METHODS

Classroom Intervention With Disinfecting Wipes (DWs)

In the current study, DWs containing quaternary ammonium were used daily to clean surfaces in a set of intervention classrooms. In addition, student illness and absenteeism records were kept to determine the impact of DW use. This study was undertaken to determine the levels of heterotrophic bacteria and the presence of norovirus and influenza A virus on common nonporous classroom surfaces and was not meant to be an epidemiological study. Heterotrophic bacterial plate counts (HPCs) were included as a general indicator of microbiological quality of an environmental surface to identify the areas in the classroom most likely to become contaminated by microorganisms. Norovirus and influenza A virus were included as indicators of the potential for spread of gastrointestinal and respiratory viruses via classroom fomites.

Between January 26 and March 15, six elementary school classrooms (with a total of 148 students) from one school in the Seattle, Washington, area were included in an intervention study using quaternary ammonium DW¹ to determine whether the regular use of DWs (Clorox Company, Oakland, California) reduced bacterial levels. Teachers in each classroom collected information regarding the incidence of gastrointestinal and respiratory illnesses (self-reported by the students) leading to student absenteeism during this period. Following

TABLE 1. Test Surfaces Sampled for Heterotrophic Plate Count (HPC) Bacteria in Control and Intervention Classrooms

<i>Classroom Surface</i>	<i>Total Area Sampled (cm²)</i>	<i># Samples Per Room</i>
Student desktop	25	4
Student chair back	25	4
Computer keyboard	25	3
Computer mouse	208	3
Entrance doorknob	266	1
Exit doorknob	266	1
Sink faucet handle	210	1
Water fountain toggle	72	1
Soap dispenser lever	45	1
Sink countertop	25	1
Paper towel dispenser lever	35	1
Manual pencil sharpener handle	6.6	1

their return to the classrooms, the teachers interviewed the students regarding the reason for the absence to determine whether it was due to illness or for other reasons. The teachers were given a list of symptoms (e.g., coughing, sneezing, fever, headache, sinus problems, sore throat, vomiting, abdominal pain, diarrhea) to ask about to guide these interviews.

There were three control classrooms (A, B, and C) including Grades 5, 1, and 4, respectively. The children's age range was 6–11 years old across the grades. The control classrooms did not receive any DW intervention. Three classrooms (D, E, and F) including Grades 5, 1, and a mixture of 3 and 4, respectively, were included as intervention classrooms. Adult parent volunteers wiped all of the test surfaces (i.e., all of the surfaces listed in Table 1) with DW in the morning each weekday prior to the arrival of the students. The use of such DWs has been demonstrated to immediately reduce bacterial numbers on surfaces in previous experiments performed by our laboratory (Bright & Rusin, 2003). Although the teachers were aware of the intervention or the lack thereof, the students in the intervention classrooms were unaware of the DW being used in their classroom.

Surface Sampling and Enumeration of HPC Bacteria

A total of 12 different types of surfaces were sampled in each classroom (Table 1). Multiple desks, chair backs, computer keyboards, and computer mice were included. Samples were collected

in the afternoons on January 26, February 2, February 23, and March 8 in each classroom by swabbing surfaces with BBL™ CultureSwabs™ with Amies medium without charcoal (Becton Dickinson, Franklin Lakes, New Jersey). The samples were placed on ice for transport back to the laboratory. The bacterial numbers were enumerated via the spread plate method on R2A medium (Difco, Sparks, Maryland; American Public Health Association [APHA], 2005), and the number of HPC bacteria per square centimeter was then calculated for each surface.

Norovirus and Influenza A Virus Detection on Classroom Surfaces

Viral samples were collected on February 12 by swabbing surfaces with BBL™ CultureSwabs™ with Amies medium without charcoal (Becton Dickinson, Franklin Lakes, New Jersey) in the morning, at midday recess, and in the afternoon in the three control classrooms A, B, and C. The surfaces sampled were a subset of those previously sampled for bacteria including student desktops, sink faucet handles, paper towel dispenser handles, soap dispenser levers, water fountain toggles, and entrance doorknobs. Only the classroom surfaces expected to have higher bacterial loads were tested for viruses due to the additional substantial costs of performing reverse transcriptase polymerase chain reaction (RT-PCR) on virus samples. The purpose of this sampling was to determine whether norovirus and influenza A viral exposure was occurring in the classroom during the course of the intervention study.

RT-PCR for the Detection of Influenza A and Norovirus

RT-PCR was performed following the influenza virus procedure described by Boone and Gerba (2005) for both viruses. The primers used to amplify and identify influenza A virus were those described by Wright, Wilson, Novosad, Dimock, and Werber (1995) and Vabret et al. (2000). The primers used for norovirus were those described by Vinjé et al. (2003).

All PCR products were detected using 2% agarose gel electrophoresis. Positive samples were purified using a QIAquick PCR Purification Kit

TABLE 2. Summary of Heterotrophic Plate Count (HPC) Bacteria Recovered From Surfaces in Control (No Treatment) and Intervention Classrooms (Wiped With Quaternary Ammonium Disinfecting Wipe [DW])^a

<i>Fomite</i>	<i>Treatment</i>	<i>N</i> ^b	<i>Geometric Mean (per cm²)</i>	<i>Standard Deviation</i>	<i>p</i> ^c
Water fountain toggle	None	12	26.42	69.6	.089
	DW	12	7.22	63.3	
Manual pencil sharpener handle	None	12	5.14	4.7	.434
	DW	12	4.45	3.2	
Computer keyboard	None	36	2.39	9.7	.058
	DW	36	0.83	4.0	
Sink faucet handle	None	12	1.05	26.4	.971
	DW	12	0.86	17.0	
Paper towel dispenser lever	None	12	0.62	4.1	.143
	DW	12	2.19	176.6	
Sink countertop	None	12	0.61	2.8	.967
	DW	12	0.67	4.3	
Computer mouse	None	36	0.41	10.1	.518
	DW	36	0.14	9.1	
Student desktop	None	48	0.28	1.5	.799
	DW	48	0.26	1.9	
Student chair back	None	48	0.23	7.3	.258
	DW	48	0.19	0.3	
Soap dispenser lever	None	12	0.14	0.5	.119
	DW	12	0.46	13.0	
Exit doorknob	None	12	0.04	0.3	.584
	DW	12	0.04	0.1	
Entrance doorknob	None	12	0.02	0.1	.147
	DW	11	0.05	0.2	

a. Sample sites are ranked in descending order from the most contaminated surface to the least contaminated.

b. *N* = total number of samples taken.

c. *p* = bacterial counts from the control and intervention classrooms were compared by analysis of variance. This difference is considered significant if $p \leq .05$.

(Qiagen Inc., Valencia, California) and sequenced using a 377 ABI sequencer from Applied Biosystems (Roche Molecular Systems Inc. Branchburg, New Jersey). Sequencing is a commonly used method for the confirmation of RT-PCR-positive products to prevent false-positive results (Vinjé et al., 2003).

Statistical Analysis

One-way analysis of variance (ANOVA) tests were performed to determine whether the bacterial counts from the control and intervention classrooms were statistically different ($p \leq .05$).

Information related to child absenteeism, in addition to concurrent gastrointestinal or respiratory illness (self-reported by students to teachers), was recorded during the entire 7-week duration of the study for students in both the control and intervention classrooms. The odds ratio of

developing a respiratory or gastrointestinal illness leading to absenteeism was determined for the control classrooms relative to the intervention classrooms using Statcalc of EpiInfo version 6.0.

RESULTS

HPC Bacterial Contamination of Surfaces

The geometric means of the numbers of HPC bacteria found on each classroom surface are presented in Table 2. The geometric mean was used due to the presence of outlying data values. Counts were grouped by the sample site (e.g., desktop, keyboard) and by classroom type (i.e., control vs. intervention). The water fountain toggle and the manual pencil sharpener handle were determined to be the most contaminated surfaces (per square centimeter) and the classroom entrance and exit doorknobs were found to be the

TABLE 3. Occurrence of Influenza A (Infl A) Virus and Norovirus (Noro) on Surfaces in Three Control Classrooms^a

Fomite	Morning		Recess		Afternoon		Total	
	Infl A	Noro	Infl A	Noro	Infl A	Noro	Infl A	Noro
Student desktop	1/10	2/10	1/10	2/10	3/7	2/7	5/27	6/27
Sink faucet handle	1/3	0/3	1/2	1/2	2/2	0/2	4/7	1/7
Paper towel dispenser	1/2	0/2	0/1	1/1	1/1	0/1	2/4	1/4
Soap dispenser	0/1	0/1	0/1	0/1	ND	0/1	0/2	0/3
Water fountain toggle	0/3	1/3	0/2	0/2	0/2	0/2	0/7	1/7
Entrance doorknob	0/3	0/3	1/2	0/2	1/2	0/2	2/7	0/7
Total	3/22	3/22	3/18	4/18	7/14	2/15	13/54	9/55

NOTE: ND = not determined.

a. Results are presented as the ratio of the number of positive samples to the total number of samples collected.

least contaminated surfaces (Table 2). There were no statistically significant ($p \leq .05$) differences between the bacterial numbers found on the surfaces of control and intervention classrooms or between the three control classrooms or the three intervention classrooms (data not shown). There were also no significant differences between counts when classroom grade (e.g., first grade vs. fifth grade) was considered (data not shown).

Viral Occurrence

Influenza A virus was detected on surfaces in all three control classrooms. It was found on 24% (13 of 54) of all classroom surfaces tested (Table 3), including 13.6% of surfaces in the mornings, 16.7% at midday recess, and 50% in the afternoon. The virus was most commonly detected on student desktops (5 of 27), sink faucet handles (4 of 7), paper towel dispensers (2 of 4), and entrance doorknobs (2 of 7) but was not detected on the water fountain toggle or the soap dispenser in any of the classrooms.

Norovirus was detected on surfaces in only two of the three classrooms. The virus was found on 16.4% (9 of 55) of all classroom surfaces tested (Table 3) including 13.6% of surfaces in the morning, 22.2% at midday recess, and 13.3% of classroom surfaces in the afternoon. Norovirus was detected on student desktops (6 of 27), a paper towel dispenser (1 of 4), a sink faucet handle (1 of 7), and a water fountain toggle (1 of 7) but was not detected on the soap dispenser or the entrance doorknob in any of the classrooms.

TABLE 4. The Number of Students Absent During the Study Period (January 26 to March 15)

Week	Control Classrooms (Rooms With Absent Students)	Intervention Classrooms (Rooms With Absent Students)
1	5 (A, B, C)	4 (D, E)
2	7 (A, B, C)	3 (D)
3	6 (A, B, C)	0
4	0	0
5	4 (A, C)	4 (E, F)
6	11 (A, B, C)	2 (D)
7	6 (A, C)	5 (D, E, F)

During the 2 weeks prior to the viral sampling date, from three to six children had been absent from each of these control classrooms due to illness. All three classrooms had at least one student that had been absent as a result of illness as recently as February 11 (the day before viral sampling).

Summary of Illnesses

The numbers of students absent due to illness per week in the control and intervention classrooms are shown in Table 4. Some students were absent for at least 1 day in more than one time period (e.g., a student may have been absent during Week 1 and then again during Week 5). In Week 3, a total of six students from the three control classrooms were absent due to illness; no students were absent from any of the intervention classrooms. This was the only week in which students were absent due to illness from only the control but not the intervention classrooms.

TABLE 5. Student Symptoms (Respiratory or Gastrointestinal) and Absenteeism in Control and Intervention Classrooms During the Study Period

	Classroom	Student Grade Level	Total # Students	# Ill Students (% of Total)	Total Classroom Absenteeism (Days)	Median Days Absent Per Ill Student
Control	A	Fifth	25	9 (36.0)	22	2
	B	First	26	9 (34.6)	21	2
	C	Fourth	23	8 (34.8)	14	2
	Totals		74	26 (35.1 ^a)	57	2 ^b
Intervention (DW use)	D	Fifth	26	6 (23.1)	10	1.5
	E	First	24	5 (20.8)	7	1
	F	Third/fourth	24	3 (12.5)	5	1
	Totals		74	14 (18.9 ^a)	22	1 ^b

a. Average of the three classrooms.

b. Median of the three classrooms.

Otherwise, a total of at least two students were absent from the intervention classrooms and a total of at least four students were absent from the control classrooms in each week of the study, with the exception of Week 4 in which no students were absent from any of the classrooms included in the study.

Student illness summary data during the 7-week study period (from January 26 to March 15) are presented in Table 5. The intervention classrooms were treated with DWs during this entire period. In the control classrooms, 26 of 74 children (35.1%) became ill (with either gastrointestinal or respiratory symptoms) with a total of 57 days absent (mean = 2.2 days per ill student, median = 2.0 days per ill student); 14 of 74 children (18.9%) became ill in the intervention classrooms with a total of 22 days absent (mean = 1.6 days per ill student, median = 1.0 day per ill student).

During this intervention study, children in the control classrooms were 2.32 times more likely to become ill than the children in the intervention classrooms ($p = .026$, 95% CI = 1.03–5.28, odds ratio determined using EpiInfo version 6 Statcalc).

DISCUSSION

Based on the existing literature, all of the classroom surfaces examined in this study are likely to have high transfer rates to the hands of children through normal contact and use during the day because all were made of nonporous materials (Rheinbaben et al., 2000; Rusin et al., 2002) such as plastics, veneers/laminates, and stainless steel.

The water fountain toggle and the manual pencil sharpener handle are used by numerous students throughout the day and were found to be the two most bacterially contaminated classroom surfaces in this study. The sink faucet handle and the paper towel dispenser lever are likewise used by multiple students and were also among the most contaminated classroom surfaces. The computer keyboard was the third most contaminated fomite. Keyboards are frequently used by multiple students and experience an extended contact time with a person's hand during use. The student desktop is commonly used throughout the day and is touched quite frequently; however, most desks are used by only an individual student. The doorknob is not frequently touched during the average school day, as doors are often propped open. Frequently or heavily used fomites are most likely contaminated by such use and therefore carry higher heterotrophic bacterial loads than other lightly used fomites.

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Sink faucet handles (4 of 7 samples, 57%), paper towel dispenser levers (2 of 4 samples, 50%), and student desktops (5 of 27 samples, 18.5%) were the surfaces most often contaminated by influenza A virus. Norovirus was detected on these fomites as

well. As mentioned previously, these are among the most frequently and heavily used fomites in the classroom. Interestingly, the water fountain toggle, the most heavily contaminated surface by bacteria, tested negative for influenza A virus in all samples (0 of 7) and only 1 of 7 (14.3%) tested positive for norovirus. Influenza A virus was detected on 2 of 7 (28.6%) of the entrance doorknobs tested though norovirus was not detected on any doorknobs (0 of 7). There is therefore no clear correlation between bacterial and viral contamination of classroom environmental surfaces. The manual pencil sharpener handle and the computer keyboard were not tested for the presence of viruses.

Viruses such as norovirus that cause vomiting or diarrhea have the potential for contamination of the environment, especially as the result of poor personal hygiene. Evans et al. (1998) reported that 607 of 680 (89%) reported norovirus outbreaks were attributed to person-to-person transmission, including transmission due to poor hand hygiene and surface-to-surface transmission (Barker et al., 2001). In addition, outbreaks of norovirus infections in passengers on cruise ships during successive trips have strongly implicated environmental contamination in the transmission of the virus (Barker et al., 2001).

Although influenza A virus is not generally thought to be readily transmitted via fomites, other viruses such as the parainfluenza viruses, rhinoviruses, and respiratory syncytial virus irritate the respiratory epithelium and induce coughing and sneezing, thereby facilitating their transmission by not only the airborne route but also environmental contamination (Barker et al., 2001; Hall, Douglas, & Geiman, 1980). It is often difficult to test for the presence of specific respiratory viruses in schools unless there is an identified outbreak underway. In contrast, influenza A virus has a predictable seasonality (Monto, 2004) during which it can be targeted for detection as an indicator of viral contamination. The presence of influenza A virus on fomites during the flu season highlights the potential for other respiratory viruses to contaminate classroom surfaces, possibly contributing to their transfer from person to person.

Although there was presumably an immediate drop in the bacterial load on surfaces following the intervention (based on our previous work), there was no statistical difference between the bacterial contamination of surfaces in the control and intervention classrooms. This brings into question the

validity of testing for heterotrophic bacteria to model the risk of contracting an infectious disease. Nevertheless, the identification of sites within the classroom that typically have a higher bacterial load could be useful in identifying potential areas of concern for contamination by pathogens. Researchers may then target these areas for a more thorough study of the occurrence of specific bacterial pathogens in the classroom.

Although there were no statistically significant differences in the bacterial levels found on classroom surfaces in this study, there was a statistically significant difference in attendance between intervention and control classrooms. Classrooms undergoing daily intervention with DWs were found to have a statistically significant reduction in student absenteeism due to illness. In the current study, we tested for the presence of influenza A and norovirus to determine whether these viruses were occurring in the classroom during the time of the investigation; however, the testing for these viruses was not included as part of the intervention. It is possible that the intervention (using a quaternary ammonium compound [QAC] DW) was more effective at reducing the numbers of viral pathogens (particularly lipid viruses such as influenza A virus) present in the classroom than it was at reducing the numbers of bacterial pathogens. QACs have been demonstrated to be very effective against lipid viruses (Eleraky, Potgieter, & Kennedy, 2002) but not as effective against non-enveloped viruses (Doultree, Druce, Birch, Bowden, & Marshall, 1999; Eleraky et al., 2002). Nevertheless, some studies have suggested that some QACs may also be effective against nonlipid viruses such as feline calicivirus (commonly used as a surrogate for human norovirus; Jimenez & Chiang, 2006). More work is therefore needed to determine the role of viral contamination of classroom surfaces in the spread of infectious diseases.

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There are approximately 164 million lost school days each year in the United States among

students in kindergarten through 12th grade, with a yearly average of 4.5 days absent per student (Benson & Marano, 1998). Numerous handwashing studies have been conducted in day care centers and hospital pediatric wards in which a decrease in the incidence of infection has been observed (Barker et al., 2001). In an elementary school, children involved in an experimental handwashing program were absent 50.6% fewer times (277 vs. 140) than children that did not participate (Guinan, McGuckin, & Ali, 2002). Mandatory handwashing programs have also reduced the incidence of gastrointestinal illnesses and absenteeism in elementary school students (Kimel, 1996; Master, Longe, & Dickson, 1997). Comparable results have been observed with similar handwashing programs or the use of hand sanitizers in schools and day care centers (Butz, Larson, Fosarelli, & Yolken, 1990; Hammond, Ali, Fendler, Dolan, & Donovan, 2000). A few of these handwashing studies have also included some efforts at improving environmental hygiene (Barker et al., 2001). Despite this, very little attention has been paid to the effects of classroom environmental hygiene alone.

The results of the current study suggest that proper classroom environmental hygiene such as the use of DWs may also reduce the transfer of pathogenic bacteria and viruses between fomites and the hands of children and thereby reduce the spread of diseases. This preliminary work emphasizes the need for further epidemiological studies using more classrooms and greater numbers of students to determine the potentially important role of microbial contamination, particularly that by viruses, of classroom fomites in the transfer and spread of infectious diseases and how this relates to student absenteeism rates.

SCHOOL NURSING IMPLICATIONS

School nurses may use the information garnered by this study and previous handwashing efficacy studies to develop educational programs to increase student and teacher awareness regarding how pathogenic microorganisms are spread and to emphasize the importance of hygiene, both personal and environmental, in limiting the spread of infectious diseases in the classroom setting. School nurses should consider conducting educational sessions in the classroom to

demonstrate both effective hand washing techniques and effective disinfectant wipe usage. During these sessions, the occasions when one should wash their hands or wipe down environmental surfaces should be described in detail.

School nurses could also encourage the use of disinfectant hand wipes in a portion of the classrooms in their schools and gather long-term information on student absenteeism rates between those classrooms engaged in using such interventions and those that are not. Nurses could employ parent volunteers (as was done in the current study) and thus involve the parents in their children's health. Because school nurses often have limited resources and time, this information could enable them to direct their efforts to programs that will have the greatest effectiveness on reducing student absenteeism rates.

NOTE

1. Active ingredients: *n*-alkyl (C14, 60%; C16, 30%; C12, 5%; C18, 5%) dimethyl benzyl ammonium chloride and *n*-alkyl (C12, 68%; C14, 32%) dimethyl ethylbenzyl ammonium chloride[0].

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