Persistence of Volatile Compounds in the Breath after Their Consumption in Aqueous Solutions

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The persistence of volatile compounds in the breath was monitored after their consumption in aqueous solutions. Factors studied were variation in volatile release patterns between panelists, effect of adding hydroxy propyl methyl cellulose (HPMC), and differences among compounds. For any given compound, the extent of volatile persistence was broadly similar for all panelists. Adding HPMC at concentrations in excess of c^* did not substantially affect persistence. The largest differences in persistence were observed when compounds were compared (>20-fold). The differences were modeled using a quantitative structure property relationship approach, based on the persistence data from 41 compounds. Major components of the model were terms that described the hydrophobicity and vapor pressure of a molecule. The model was validated with a test set, which showed that there was a significant correlation between persistence predicted by the model and the actual values observed.

Keywords: *QSPR; APCI; API; HPMC; aroma release; viscosity*

INTRODUCTION

One of the key components of the eating (or drinking) experience is the aftertaste, which will be influenced by differences in volatile persistence in the breath. Factors that may have a direct effect on persistence include human physiology and the nature of the food matrix itself. Thickening agents have been reported to affect aroma release at concentrations $>c^*$, the point at which coil overlap and entanglement occurs (Baines and Morris, 1987). Roberts et al. (1996), using a dynamic headspace system (designed to simulate in-mouth conditions), observed a decrease in the concentration of aroma compounds as viscosity increased, the decrease being greatest for the more volatile compounds. These results suggest that increasing viscosity reduces the diffusion of aroma compounds, which become depleted at the matrix surface, decreasing their gas phase concentration. Consequently, we might expect to see a decrease in the persistence of volatile compounds in the breath following ingestion, as viscosity increases.

Direct measurement of the breath volatile concentration during eating has also been used to study volatile persistence. Increasing fat content increased volatile persistence in both biscuit and yogurt systems (Brauss et al., 1999a,b**)**, presumably by providing a reservoir for volatile release. Increasing the viscosity could have a similar effect in vivo, reducing the rate of clearance of the bolus from the throat and thus increasing volatile persistence.

In addition to the effects of human physiology or the food matrix, persistence may be affected by the physical characteristics of the compounds themselves. Aroma compounds are chemically diverse, covering a wide range of molecular characteristics and making it difficult to predict their behavior. It has, however, been possible to produce empirical models, using the quantitative structure property relationship approach (QSPR), which describes the behavior of volatile compounds in some perfumery and food systems (Labows et al., 1997; Friel et al., 2000; Linforth et al., 2000). These models are based on fundamental factors that are common to all molecules (size, shape, polarity, etc.) and can be used not only to describe them but also to predict their behavior.

This paper investigates the effect of viscosity and variation between individual panelists on volatile persistence in breath after consumption of aqueous volatile solutions. These effects are compared with any structural or physicochemical differences associated with the compounds themselves.

MATERIALS AND METHODS

Solution Preparation. HPMC (Methocel, Dow, Schwalbach Germany) solutions were prepared by dispersing HPMC in water at 80 °C and allowing the solutions to cool to 4 °C (with constant stirring). Aqueous solutions of each volatile (100 mg/kg) were prepared with vigorous shaking using an SF1 flask shaker (Stuart Scientific, Redhill, U.K.) for 1 h. These were then diluted (1:9, v/v) with water or HPMC solutions to produce a final volatile concentration of 10 ppm in 0, 0.1, or 1% HPMC. Following dilution, all solutions were mixed overnight on an SRT2 roller bed (Stuart Scientific) at room temperature. All solutions contained 2% sucrose to increase their palatability.

Solution Sampling Protocol. Panelists (three males and three females, aged between 22 and 40 years of age) were instructed to inhale, place either 3, 7, or 15 mL of the test solution in their mouths, swallow, and then exhale and inhale normally while their breath was sampled into the MS Nose (Micromass, Manchester, U.K.). Panelists were given water to cleanse their mouths between samples. The breath of the panelists was analyzed to check for detectable traces of compounds persisting on the breath prior to the consumption of samples; samples were consumed only if their breath did

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10.1021/jf000488n CCC: $19.00
Published on Web 11/01/2000

not contain detectable amounts of the volatile present in the sample.

Mass Spectrometry. Breath was sampled into the MS Nose at a flow rate of 30 mL/min. The mass spectrometer was used in selected ion mode, monitoring two ions at a time with a dwell time of 11 ms and a corona discharge of 4 kV. The cone voltage was 18 V for all compounds except dimethylpyrazine, for which it was set to 26 V. The ions monitored were the protonated molecular ion (MH^+) with the exception of linalool and α-terpineol, which dehydrated to form the (MH^+) $- H_2O$ ion. Compounds were added singularly to each solution, and the mass spectrometer was used in selected ion mode to monitor the major ion associated with each compound. A signal was observed only when a solution containing the test compound was present; no signals were observed for control samples (water with no added volatiles), nor was there interference from compounds naturally present in the breath.

Calculation of Persistence Values. For many of the least persistent compounds the breath volatile concentration fell so rapidly that it was not possible to use data over a series of exhalations to determine persistence. Data for the breath volatile concentration for the first and second exhalations after the solutions had been swallowed was available for all compounds (except methyl furan, which showed no persistence). The ratio of the concentration of aroma compounds in the first and second exhalations provided a simple consistent measure of persistence, sufficient to characterize the differences between compounds.

The strength of persistence was calculated by expressing the peak height for the volatile in the second exhalation after swallowing as a percentage of the first. Compounds that were persistent would have values approaching (or possibly even exceeding) 100%, whereas the nonpersistent compounds would produce much lower values (i.e., tending toward zero).

QSPR Modeling. Physicochemical parameters describing the compounds were calculated using the chemical modeling program CaChe 3.1 (Oxford Molecular, Oxford, U.K.). Physicochemical parameters for the QSPR model were then selected using partial least squares (PLS) regression (Guideline + 7.2, Camo, Trondheim, Norway), followed by multiple linear regression (MLR) using Design Expert 5.0 (Statease, Minneapolis, MN). PLS revealed the parameters that explained most of the variation in the data set (highest regression coefficients); these were analyzed further using MLR for statistical significance. Parameters that were statistically significant ($P < 0.05$) were used to generate the final model.

RESULTS AND DISCUSSION

Persistence of Individual Compounds. Volatile persistence in the breath during eating has been reported only for systems in which other matrix components are present (lipids, hydrocolloids, protein, etc.). To study volatile persistence with minimal matrix interactions, anethole and *p*-cymene were consumed as a dilute aqueous solution while their breath volatile concentration was monitored. This clearly demonstrated that anethole was far more persistent than *p*-cymene (Figure 1), such that anethole could be observed in exhalations 0.5 min after the solution was swallowed.

Two main effects were observed: first, for persistent compounds the concentration of volatiles in the breath declined more slowly over successive breaths (as expected) and, second, there were differences in the shape of the peak produced by the first exhalation after swallowing. Nonpersistent compounds showed a sharp peak at the start of the exhalation followed by a small shoulder at the base of the peak. Persistent compounds, however, had much larger shoulders relative to the maximum volatile intensity. These differences are probably caused by the way in which compounds are transported from the throat, through the upper airways, and out through the nostrils. Consequently, there may be substantial differences among the breath profiles of individuals.

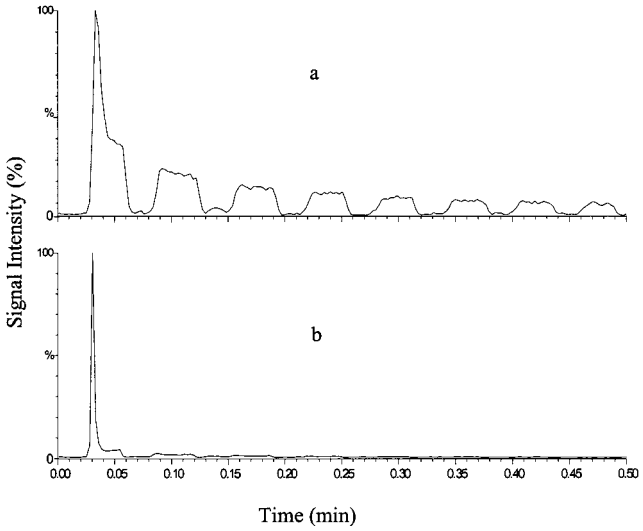

Figure 1. Breath-by-breath profiles for the consumption of an aqueous solution containing anethole (a) and *p*-cymene (b). The solution was consumed at 0 min, and the signal intensity for each compound was followed for 0.5 min thereafter. The maximum signal intensity has been normalized to 100% for each compound.

Table 1. Average Persistence of Six Compounds Consumed in 3 mL Aliquots of Aqueous Solutions by Six Panelists[a]

	panelist							
compound	1	2	3	4	5	6	av[b]	SD[c]
anethole	12	31	19	23	28	24	23	7
benzaldehyde	11	9	16	20	22	13	15	5
dimethylpyrazine	64	62	45	64	60	69	61	8
ethyl butyrate	5	2	9	6	11	6	6	3
linalool	32	46	29	32	43	42	37	7
α-terpineol	62	65	55	69	57	56	61	6

[a] Each value is the average of two replicates. [b] Overall mean value for each compound. [c] Corresponding standard deviation.

Differences in Persistence among Individuals. Dilute solutions of six volatiles were consumed by six panelists to determine the interpanelist differences in the strength of persistence. There were substantial differences in the persistence of the six compounds (Table 1), such that dimethylpyrazine and α-terpineol were 10 times more persistent than ethyl butyrate. These differences were much larger than those observed among panelists. The greatest variation among panelists was observed for ethyl butyrate. This compound was the least persistent compound studied and produced a sharp peak at the start of the first exhalation (similar to *p*-cymene, Figure 1). This rapid change in volatile concentration was difficult to monitor, even with the mass spectrometer set to its fastest rate of data acquisition (dwell time = 11 ms) and may depend more on experimental variation than genuine differences among individuals.

Effect of HPMC on Persistence. One of the other factors that may affect volatile persistence is the food matrix itself. To investigate the extent to which viscosity could affect persistence, two further solutions containing HPMC at concentrations above (1%) and below (0.1%) c^* ($c^* = 0.57\%$) were also consumed by the panel. The persistence values observed for these two solutions

Table 2. Average Persistence of Six Compounds Consumed in 3 mL Aliquots of 0.1 or 1% HPMC Solutions by Six Panelists[a]

	panelist							
compound	1	2	3	4	5	6	av	SD
0.1% HPMC								
anethole	24	32	20	22	29	22	25	5
benzaldehyde	7	9	7	12	20	18	12	6
dimethylpyrazine	59	73	45	59	63	64	60	9
ethyl butyrate	2	1	6	10	10	5	6	4
linalool	22	41	32	37	62	34	38	14
α-terpineol	48	59	57	57	69	64	59	7
1% HPMC								
anethole	13	32	29	25	28	20	24	7
benzaldehyde	8	13	16	10	23	15	14	5
dimethylpyrazine	81	69	58	62	83	75	71	10
ethyl butyrate	5	3	4	9	10	9	6	3
linalool	25	72	54	42	59	43	49	16
α-terpineol	50	65	47	60	74	61	60	10

[a] Each value is the average of two replicates.

(Table 2) were virtually identical to those observed for the aqueous solution in the absence of HPMC (Table 1). Dimethylpyrazine was the only compound that showed a statistically significant increase in persistence with the addition of 1% HPMC ($P < 0.05$, paired sample t test); however, the magnitude of the increase was only 18%.

One possible explanation for the absence of an effect of the HPMC was that the aliquots of solution were too small (3 mL) and were diluted by saliva on ingestion, resulting in solutions with an HPMC concentration $<c^*$. To explore this possibility, three of the initial six compounds were consumed in larger quantities by two panelists. HPMC did not significantly affect volatile persistence (Figure 2) even when consumed as 15 mL aliquots. Furthermore, this experiment also demonstrated that volatile persistence was not affected by the volume of sample consumed. The only observable effect was a decrease in the standard deviation for the 15 mL samples compared to the 3 mL samples; consequently, in all further experiments the panelists consumed 15 mL aliquots.

The experiment investigating the effect of sample volume also included dimethylpyrazine, to confirm whether the differences observed in the first experiment were genuine. No significant differences were observed in the persistence of this compound, and the most logical conclusion is that the previous results were the product of chance rather than true differences in persistence.

A comparison of Tables 1 and 2 shows that there were some consistent differences among panelists. Panelists 5 and 6 both showed greater average volatile persistence than panelist 1 ($P < 0.01$, paired sample t test), who had the lowest average persistence values. However, even in the most extreme case (a comparison of panelist 1 with panelist 5), the average increase in persistence was only 40%.

Modeling the Differences in Persistence. The greatest differences in persistence were observed among compounds, rather than among individuals, or as a result of the addition of HPMC. The differences among compounds will depend on their physical characteristics and, with sufficient data, can be modeled using the empirical QSPR approach. Panelist 6 produced persistence values close to the average on each occasion and was selected for the consumption of a wider range of volatile compounds in aqueous solution to generate the data required for modeling. A total of 41 compounds were consumed (to produce a total of 53 persistence values with replication); these showed considerable differences in the strength of persistence (Table 3), comparable to the range of values observed for the original set of six test compounds (Table 1). The most persistent compound was 3-ethyl-2-methylpyrazine, with a volatile concentration in the second exhalation after swallowing equal to 90% of that observed for the first exhalation. In contrast, methyl furan showed the least persistence and was not detected in the second exhalation after swallowing.

The initial model (eq 1) had just four physicochemical parameters, Log P, an estimate of the octanol−water partition coefficient, Log ρ_L, an estimate of the vapor pressure (Liang and Gallagher, 1998), carbonyl group count, and ether linkage count. The model had a good

$$\text{persistence} = 117 - 23 \times \text{Log } P - 15 \times \text{ether linkage} - 23 \times \text{Log } \rho_L - 9.1 \times \text{carbonyl group} - 15 \times \text{Log } P^2 - 3.2 \times \text{Log } \rho_L{}^2 + 3.6 \times \text{Log } P^3 \quad (1)$$

overall regression coefficient ($R^2 = 0.88$) for the correlation between persistence values predicted by the model and the actual values observed (Figure 3). The predictive correlation coefficient ($rCV^2 = 0.84$) was only slightly lower than R^2, suggesting that the model would have reasonable predictive powers. The calculations that generate values for Log P and Log ρ_L include factors based on structural fragments of the molecule, some of which are related to oxygen-containing functional groups. The carbonyl count and the ether linkage count were minor components of the model and could be considered as correction factors for the Log P and Log ρ_L calculations. The outlier, with a negative predicted persistence in Figure 3 (ethyl methyl furan), was poorly described by the model, despite the fact that similar molecules such as methyl furan were adequately described by the model (predicted value for methyl furan = 4.0; observed = 0.0).

Evaluation of the Model. To test the model, 10 compounds were removed from the model to produce a test set (the internal test set). This resulted in the deletion of 12 values in total, due to the removal of all replicates of any one test compound. The regression coefficients were then recalculated for the smaller data set, resulting in eq 2. This equation was very similar to

$$\text{persistence} = 115 - 21 \times \text{Log } P - 16 \times \text{ether linkage} - 23 \times \text{Log } \rho_L - 10 \times \text{carbonyl group} - 15 \times \text{Log } P^2 - 2.6 \times \text{Log } \rho_L{}^2 + 3.6 \times \text{Log } P^3 \quad (2)$$

eq 1, indicating the stability of the model. In addition, the values for R^2 and rCV^2 were comparable to those of the full model (0.92 and 0.88, respectively), indicating that the new model had predictive powers similar to those of the original one.

Another way of testing the predictive power of the model was to use a further series of compounds that had not been analyzed previously. These were consumed and their persistence in the breath was determined, forming an additional test set (the external test set). Equation 2 was used to predict the amount of persis-

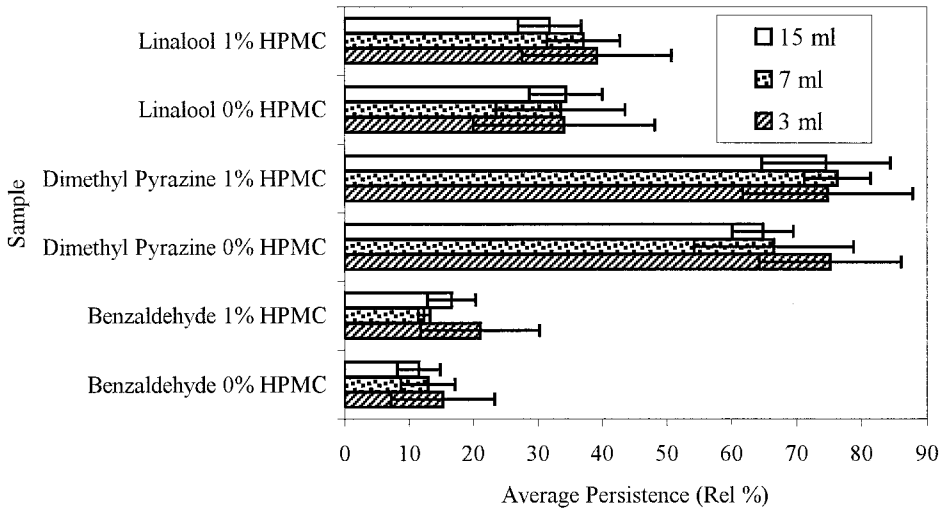

Figure 2. Average persistence values for volatiles in solutions of 0 or 1% HPMC consumed in 3, 7, or 15 mL aliquots. Each result is the average of four replicates ± the standard deviation.

Table 3. Average Persistence (%P) of Compounds in the Breath[a]

compound	%P	compound	%P
3-ethyl-2-methylpyrazine	90	anethole	19
dimethylpyrazine	85	menthone	16
ethyl lactate	81	hexenal	14
dimethylpyrazine	75	hexanone	14
α-terpineol	70	menthyl acetate	11
2,3-diethylpyrazine	69	ethyl decanoate	11
pyrazine	68	1,4-cineole	11
diethyl succinate	67	ethyl methyl furan	10
pyrazine	66	nonanone	9
ethanol	66	decan-2-one	9
pyrazine	65	methyl acetate	7
guaiacol	65	hexanal	6
dimethylpyrazine	65	menthyl acetate	6
carvone	61	methyl acetate	6
ethanol	60	decanal	6
ethanol	58	isoamyl acetate	6
linalool	54	menthofuran	5
anethole	38	limonene	5
propan-2-ol	36	butanone	5
α-damascenone	35	ethyl hexanoate	4
furfuryl acetate	34	isoamylbutyrate	4
ethyl undecanoate	31	menthofuran	3
octanol	25	ethyl butyrate	3
ethyl undecanoate	25	*p*-cymene	1
menthol	23	octanone	1
nonanone	22	methyl furan	0
benzaldehyde	20		

[a] Fifteen milliliter aliquots of solution were consumed by one panelist. Each value is the mean of two replicates. Where more than one value is shown for a compound, these are the results of consuming aqueous solutions of these compounds on separate occasions.

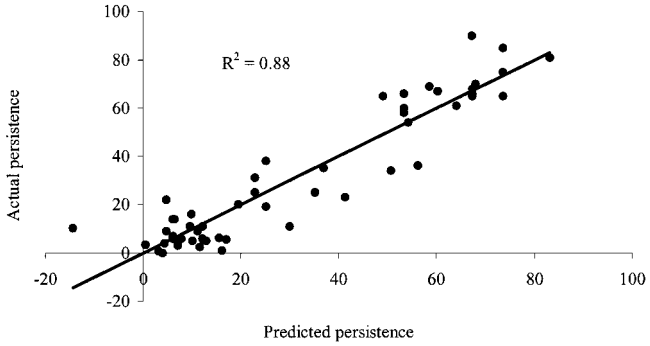

Figure 3. Comparison of the persistence predicted using eq 1 with the actual values observed experimentally.

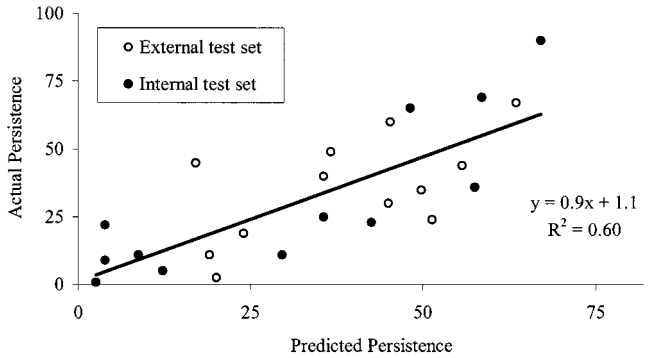

Figure 4. Validation of the persistence model using internal and external test compounds.

tence for the test sets, and these values were then compared with the observed persistence values (Figure 4). Both the internal and external data sets showed a significant correlation between the predicted and actual values ($P < 0.05$ and $P < 0.001$, respectively), with an overall regression coefficient of 0.6 for the entire data set.

Final Model. On the basis of the results of the model evaluation, the physicochemical parameters selected did appear to adequately predict the strength of volatile persistence in the breath after their consumption in an aqueous solution. Therefore, it was possible to move onto the final stage of model development, in which all data values (65 in total including the external test set) were used to estimate the regression coefficients. The resulting eq 3 included all of the parameters previously found

$$\text{persistence} = 114 - 24 \times \text{Log } P - 16 \times \text{ether linkage} - 23 \times \text{Log } \rho_L - 8.0 \times \text{carbonyl group} - 14 \times \text{Log } P^2 - 2.6 \times \text{Log } \rho_L{}^2 + 3.3 \times \text{Log } P^3 \quad (3)$$

to correlate significantly with the variation in the data set ($P < 0.05$). In addition, the values for the regression coefficients for the parameters were similar to those of eqs 1 and 2 ($R^2 = 0.83$, $rCV^2 = 0.78$), reflecting the stability of the model. Ethyl methyl furan, which was an outlier in the initial model, was still an outlier in this final model (Figure 5). Additional physicochemical

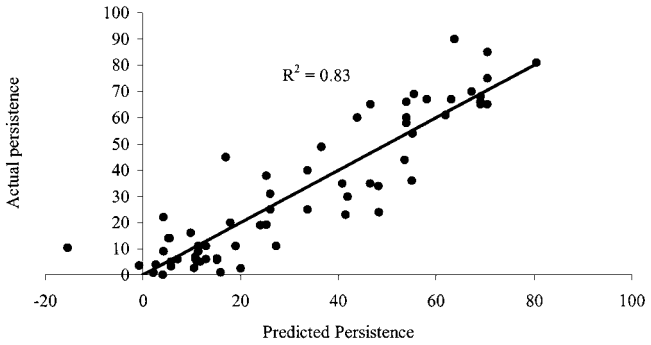

Figure 5. Final model (eq 3) showing the correlation between predicted and actual persistence values.

parameters might allow a more accurate prediction of the behavior of this compound; however, that would require a much larger data set.

The polarity and vapor pressure of a molecule are important parameters correlating with the persistence of aroma compounds, indicating the mechanisms involved. After swallowing, the surface of the throat will have been coated with volatiles in solution and there will be compounds in the gas phase above it. During the subsequent exhalation, the gas phase component will be expelled, resulting in the initial breath volatile concentration. Compounds that are more water soluble (low Log *P*) or that have low vapor pressure (low Log ρ_L) would be most likely to repartition into the mucous layers of the upper airways, whereas the more nonpolar (high Log P) compounds with higher volatility (high Log ρ_L) would partition into the mucous the least. The latter would be the least persistent compounds, effectively passing through the upper airway as a plug of gas (observed half-peak width = 20−30 ms) with little reservoir for their replenishment. The former compounds would have a greater reservoir in the throat and the nasal mucosa, resulting in greater persistence over successive breaths and the altered peak shape of the first exhalation.

This of course raises an additional question: is the intensity of retronasal aroma perception dependent on the height (maximum intensity) or the area (intensity integrated over the entire breath) of volatile delivered to the olfactory epithelium?

Conclusions. The persistence of aroma compounds in the breath can vary substantially, even when the compounds are consumed in the simplest of matrices, an aqueous solution. The variation observed among compounds can be modeled using the QSPR approach. Two of the main physicochemical parameters in the model were Log *P* and Log ρ_L, which describe the hydrophobicity/hydrophilicity and vapor pressure of the molecules, respectively. These parameters were found to be important in the modeling of the differences in the intensity and timing of volatile release from gelatin/sucrose gels (Linforth et al., 2000). It appears to be likely that these two parameters are key factors describing the behavior of aroma compounds in vivo.

ACKNOWLEDGMENT

R.S.T.L. thanks Firmenich for funding my postdoctoral research fellowship.

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Received for review April 19, 2000. Revised manuscript received August 21, 2000. Accepted August 24, 2000.

JF000488N