ABSTRACT

Earlier, we reported the formation of unidentified strong irritants in reaction mixtures of terpenes and ozone. The identified products included aldehydes, ketones and carboxylic acids. Here we report the effects of relative humidity and reaction time on irritant formation in our flow reaction system using limonene and isoprene. Upper airway irritation was measured in mice (via reduction of respiratory rate) using ca. 5 and 35% relative humidity and long and short reaction times. Maximum irritation was observed for low humidity/short time reaction mixtures for both terpenes and the combination of high humidity and long reaction time gave the least irritation.

In order to estimate the comparative irritation of limonene/ozone mixtures, we studied irritation effects at four ozone concentrations using low humidity/short time conditions holding other parameters constant. The mixture containing limonene/ozone at 46/0.5 ppm exhibited an irritation effect, which was the same magnitude as that of pure limonene.

INDEX TERMS

Indoor air chemistry, Ozone, Terpene, Mouse bioassay, Upper airway irritation

INTRODUCTION

We have suggested that terpene/ozone reaction products (α-pinene, R-(+)-limonene, ozone) include unidentified, potent airway irritants (Wolkoff et al., 1999, Clausen et al., 2001, Wilkins et al., 2001). Reaction time and relative humidity are the two of the most interesting reaction parameters with respect to the formation of highly irritating (and thus chemically reactive) products indoors. Formation of ozonides and peroxides during ozone reactions of terpenes has been reported (Griesbaum et al., 1998, Sauer et al., 1999, Hewitt and Kok, 1991). We investigated the effect of increased relative humidity and reaction time on irritant concentration using the mouse bioassay.

In order to estimate the irritation potency of the limonene/ozone reaction mixture relative to known irritants, ozone concentration was varied and the respiratory rate was monitored.

METHODS

Materials

O₃ was generated photochemically as described earlier (Wolkoff et al., 1999). The age of the reaction mixture was determined by the transport time through the flow tube reactor and exposure chamber. For the low humidity experiments, medicinal grade air was used to evaporate the terpene. Pure oxygen at ca. 0.5 l/min was irradiated and added to the terpene stream to give a total flow of ca. 17 l/min (Clausen et al., 2001). For high humidity experiments the medicinal air was bubbled through warm water (ca. 30° C) to give final

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mixtures with ca. 35±3% relative humidity. Humidity was measured with a Testo 650 temperature/humidity measuring instrument (Testo GmbH & Co., D-79849 Lenzkirch). The biological test method followed the ASTM method modified as previously described (Clausen et al., 2001). For limonene the reaction times were 16 and ca. 30 seconds, while for isoprene we utilized ca. 30 and 90 seconds because of the slower reaction rate.

Experimental procedure
Each experiment consisted of a 15 min pre-exposure period during which breathing parameters for the unexposed mice were recorded. The exposure period was 30 min, followed by a 15 min recovery period. Data for the pre-exposure period was not significantly different for different groups of mice. To facilitate comparison, differences in effects were expressed as relative decrease from baseline. Time dependence of the effects was studied by two-way analysis of variance and regression analysis, using Minitab statistical software, version 10 xtra (Minitab Inc.)

Control experiments were performed using pure laboratory air. The starting concentrations of the mixtures of terpenes and O₃ were for limonene; 48 ppm/ 4ppm and for isoprene; 500 ppm/ 4 ppm respectively. The high concentrations of terpenes were necessary to ensure the reaction of >90% of the ozone.

To estimate the irritation potency of the limonene/ozone reaction mixture , the ozone concentration was varied and the respiratory rate was monitored, using low relative humidity/short time reaction conditions to achieve the maximum effect.

Effects – sensory irritation
When a substance stimulates the trigeminal nerve endings, it may cause a painful sensation in humans. In mice, it causes a reflexively induced decrease in respiratory rate, which is caused by an elongation of the period from the end of the inspiration until the start of the expiration, called the time of break. The concentration of a substance which causes a 50% decrease in respiratory rate is called RD₅₀, while the concentration found by extrapolating the dose/response curve to 0 response is the RD₀.

RESULTS
Residual ozone concentrations for mixtures with 4 ppm as the starting concentration were 45 ppb and 140 ppb, respectively for limonene and isoprene. Thus we expect no contribution to the physiological effects here from ozone, since the NOEL is > 1 ppm.
No significant airway limitation or pulmonary irritation was observed. Reduction in respiratory rate is shown in figure 1 for all the conditions tested. Reduction in respiratory rate for the short time /low humidity conditions was statistically larger than that for long time/high humidity conditions for both terpenes. Although the differences were not statistically significant when comparing other pairs of reaction conditions, there appears to be trends for both terpenes indicating that either an increase in reaction time or relative humidity results in decreased irritation.
Using the short time/low humidity conditions, respiratory rate was studied using limonene/ozone mixtures at the concentrations; 46/4, 46/2, 46/1 and 46/0.5 ppm/ppm. The 48/0.5 ppm mixture exhibited an effect on the respiratory rate which had the same magnitude as that of pure limonene (Clausen et al., 2001) or air. The plot for respiratory rate reduction as a function of ozone utilized is shown in figure 2.

**DISCUSSION AND CONCLUSIONS**

The identified products from the limonene/ozone reaction included acetone, limonene oxides, 4-acetyl-1-methylcyclohexene, carvone, 3-isopropenyl-6-oxoheptanal, formaldehyde, acrolein, acetaldehyde, formic acid and acetic acid (Clausen et al., 2001). Acetone, methylvinyl ketone, methacrolein, formaldehyde, formic acid, acetic acid were identified in the isoprene/O$_3$ reaction mixture (Wilkins et al., 2001). The sums of the contributions of the irritant compounds identified could not explain the observed effects, however.

The small decrease in irritation observed when the reaction time was increased by a factor of 2-3 suggests that within the time scale of these experiments (~ 1 min), the irritant(s) formed are reasonably stable. Criegee intermediates cannot be excluded from consideration as direct contributors to irritation, taking their reaction rates with water vapor into consideration (Hatakeyama and Akimoto, 1994). The small reduction of irritation with the presence of a modest amount of water, compared to previous studies (Sauer et al., 1999, Hewitt and Kok, 1991), suggests that irritant(s) react slowly with water, relative to the reaction time scale. The NOEL the limonene/O$_3$ reaction mixtures is similar in magnitude to that for formaldehyde (0.3 ppm, Nielsen et al., 1999), indicating similar irritant potency. Detailed kinetic studies have shown that mixtures of irritants exhibit competitive agonism, and in the range 10-60% reduction of respiratory rate, effects are additive (Nielsen et al., 1988, Cassee et al., 1996).
CONCLUSIONS AND IMPLICATIONS
Low humidity and short reaction times will favor the concentration of irritants generated by the reactions of the terpenes, limonene and isoprene with ozone. Whether the results presented can be generalized to other reaction conditions remains to be clarified by additional experiments.
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REFERENCES