Formation of Strong Airway Irritants in Mixtures of Isoprene/Ozone and Isoprene/Ozone/Nitrogen Dioxide

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We evaluated the airway irritation of isoprene, isoprene/ozone, and isoprene/ozone/nitrogen dioxide mixtures using a mouse bioassay, from which we calculated sensory irritation, bronchial constriction, and pulmonary irritation. We observed significant sensory irritation (approximately 50% reduction of mean respiratory rate) by dynamically exposing the mice, over 30 min, to mixtures of isoprene and O_3 or isoprene, O_3, and NO_2. The starting concentrations were approximately 4 ppm O_3 and 500 ppm isoprene (+ approximately 4 ppm NO_2). The reaction mixtures after approximately 30 sec contained < 0.2 ppm O_3. Addition of the effects of the residual reactants and the identified stable irritant products (formaldehyde, formic acid, acetic acid, methacrolein, and methylvinyl ketone) could explain only partially the observed sensory irritation. This suggests that one or more strong airway irritants were formed. It is thus possible that oxidation reactions of common unsaturated compounds may be relevant for indoor air quality.
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It has been proposed that reactions between unsaturated volatile organic compounds (VOCs) and oxidants (e.g., terpenes and ozone) may produce chemically reactive products that irritate the eye and airway (*1*). Some of the known reaction products are aldehydes, carboxylic acids, and hydroperoxides, which may cause irritation at concentrations relevant for indoor air (*1,2*). Some epidemiologic studies of airway irritation symptoms are consistent with the hypothesis that ozone in combination with unsaturated VOCs (e.g., from human activities or building furnishings) contribute to nasal resistance and eye irritation (*3*). However, until the recent report of the formation of irritants from the reactions of O_3 and (+)-α-pinene (*4*) and O_3 and limonene (*5*), the only experimental evidence that supports this hypothesis was the observations of indoor air oxidation reactions reported by Weschler and Shields (*6,7*).

Our objective was, using a mouse bioassay, to provide experimental evidence for the formation of irritating substances in mixtures of O_3 and isoprene, a common plant and microbial metabolite (*8,9*) and one of the major organic constituents of air exhaled by humans (*10,11*). This assay analyzes the respiratory pattern of mice exposed to airborne chemicals (e.g., VOCs). When the upper airway is exposed to irritants, the respiratory rate is reduced because stimulation of the nasal trigeminal nerves reflexively induces a break in breathing after inhalation. When pulmonary irritants are present, the vagal nerves are stimulated, which often creates a pause in breathing before inhalation and thus also a reduction of the respiratory rate. These effects are concentration-dependent over a wide range of concentrations and they are distinguished by analysis of the respiratory parameters (*12*). They are usually expressed as percent of baseline or percent decrease from baseline. Thus the threshold concentration for reduction of the respiratory rate (RD_0), which can be estimated from the dose–response relationship, corresponds to the no-effect level (NOEL). The RD_{50} used here is the concentration of a substance required to cause 50% decrease in respiratory rate.

The atmospheric chemistry of isoprene with ozone and nitrate radicals has been investigated extensively (*13–18*). The ozone reaction is reported to give methacrolein, methyl vinyl ketone, hydroxy hydroperoxides, the two isomeric monoepoxides, 3-methylfuran, propene, and many secondary oxidation products of these, depending on the reaction conditions (*13–15*). The reported reaction products of isoprene with NO_3• consist primarily of nitro or hydroxy aldehydes formed by 1,4 addition processes (*16–18*).

Experimental Details

Chemicals. Isoprene (> 98%, cat. no. 59250) was obtained from Fluka (Fluka Chemie AG, Copenhagen, Denmark) and contained approximately 1% C_{10} impurities determined by gas chromatography–mass spectrometry (GC-MS) analysis. Methylvinyl ketone, methacrolein, 2-methyl-2-vinyloxirane, and 3-methyl-2(5H)-furanone were supplied by Aldrich Chemicals (www.sigma-aldrich.com). We used O_2 [99.999%, N_2 < 5 ppm (product no. 500158, Hydrogas Denmark, Glostrup, Denmark)] to generate O_3 to avoid contamination with nitrogen oxides. Nitrogen containing 150 ppm nitrogen dioxide was diluted to approximately 4 ppm in the O_3/NO_2 reaction.

Methods. We generated O_3 photochemically (*19*) with a mercury lamp in a thermostated lamp housing controlled by a high-performance variable power supply, as described earlier (*4*). We transferred O_3 in pure O_2 at 0.5 L/min through a steel tube [internal diameter (i.d.) = ~ 2 mm] into an approximately 13 m Teflon reaction flow tube (i.d. = 2.2 cm) and diluted to an airflow of approximately 18 L/min from the VOC generator or directly with similar dilution without the VOC generator (O_3 exposure) to the mouse exposure chamber, a cylindric glass vessel (vol 2.3 L), mounted vertically with coplanar mouse ports at 90° angles. The Teflon reaction flow tube was connected directly to the isoprene vapor generator, Pitt No. 1 (*20*), which was fed by an ice-cooled syringe pump. The isoprene concentration was monitored by infrared spectroscopy (Miran 1A; Foxboro Co., Foxboro, MA, USA). The steel tube from the O_3 generator protruded through the wall of the Teflon reaction flow tube so the outlet was directed upstream. O_3 concentrations were monitored by a Photometric O_3 Analyzer (Model 400; API, Inc., San Diego, CA, USA), interfaced to a personal computer. The sampling cycle was 8 sec. The analyzer was calibrated with an internal O_3 source at six concentrations. We measured the O_3 concentration loss through the Teflon reaction flow tube at less than 1%. Variation of O_3 concentrations in the mouse chamber was ± 2% over 1 hr. The age of the reaction mixture, determined by the transport time through the flow tube and exposure chamber, was approximately 30 sec, and 96% of the O_3 was consumed.

We analyzed the aldehydes (C_1–C_6) by sampling on DNPH-coated silica gel and then by DAD/HPLC (*21*) while we collected acids (C_1 and C_2) on Na_2CO_3-coated

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Chromosorb PAW (Dansk Miljoe Center A/S, Galten, Denmark) and analyzed them by gas chromatography-flame ionization detection (GC-FID) of their methyl esters (*22*). VOCs (methylvinyl ketone, methacrolein, and unidentified products) were concentrated on Tenax TA (Supelco International, Bellefonte, PA, USA) with a syringe (100 mL) and analyzed immediately by GC-MS after thermal desorption (*4*). We took all air samples in duplicate, in the same plane as the mouse ports and as close as possible to the breathing zones of the mice. Variation in the monitored reactant concentrations were < 5%.

Experimental protocol. The biologic test method followed the American Society for Testing and Materials method (*23*), further developed and computerized by Boylstein et al. (*24,25*) and Vijayaraghavan et al. (*26,27*). We recorded plethysmograph data by a dataloger and analyzed each curve with a computer to calculate the parameters. Experiments were performed between 0900 and 1700 hr.

Effects

Sensory irritation. When a substance stimulates the trigeminal nerve endings, it may cause a painful sensation in humans (*12*). In mice, it causes a reflexively induced decrease in respiratory rate (*f*), 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 (TB).

Airflow limitation. This effect is caused by bronchoconstriction, edema, or accumulation of mucous in the conducting airways. This increases the time of expiration (TE) and decreases expiratory flow rate. The parameter used to characterize airflow limitation is the expiratory flow rate at half of the tidal volume (VT), which is abbreviated VD. When VT changes, VD is expected to change as well. Thus, one adjusts for changes in VT by plotting the VD/VT ratio versus the exposure concentration.

Pulmonary irritation. Stimulation of the vagal nerves at the alveolar level may produce two types of respiratory effects. One is rapid shallow breathing, in which *f* is increased and VT is decreased. The other is an increase in time from the end of the expiration to the initiation of the following inspiration, called the time of pause (TP). This effect can be recognized and quantified by either the effect on TP or the decrease in *f*. The concentration that causes a 50% decrease in respiratory rate is called RD_{50}, and the concentration found by extrapolating the dose–response curve to 0 response is the RD_0.

Because we observed only sensory irritation, we report only changes in respiratory rate.

Experiments. Each experiment consisted of a 15-min preexposure period during which we recorded breathing parameters for the unexposed mice. The exposure period was 30 min, followed by a 15-min recovery period. These parameters were uniformly applied to dose–response experiments for mixtures, pure substances, and air blanks. The chamber conditions were 23 ± 2°C and 10 ± 5% relative humidity. All experiments with O_3 were performed at 22% O_2 content. The total airflow through the exposure chamber was approximately 17–18 L min^{-1} for all experiments, and the air was introduced in a uniform manner. In each experiment, a naive group of 4 male BALB/c mice (M&B A/S, Ry, Denmark), maintained under standard conditions, was exposed, head only, in separate body plethysmographs. We compared the mean effect for 12 mice for the period between the 11th and 20th minute to values obtained during the pre-exposure period, to determine the exposure effects. Data for the preexposure period were not significantly different for different groups of mice.

To facilitate comparison, differences in effects were expressed as percent of baseline or relative decrease from baseline. Time dependence was studied by two-way analysis of variance and regression analysis, using Minitab statistical software (Minitab 13 for Windows; Minitab Inc., State College, PA, USA). *p*-Values < 0.05 were considered statistically significant.

Dose–response curves were established for isoprene, methacrolein (*28*), O_3 and formaldehyde (*29*); we used data for methylvinyl ketone, allylglycidyl ether, NO_2, formic acid, acetic acid, acetone, saturated aldehydes, and 3-methylfuran to calculate/estimate the response for these substances in the reaction mixtures. We estimated the total irritation attributable to identified components. Detailed kinetic studies have shown that mixtures of irritants exhibit competitive agonism, and that in 10–60% reduction of breathing frequency, effects are hypoadditive (*30,31*). We performed control experiments using laboratory air. The starting concentrations of the mixture of isoprene and O_3 (and isoprene, O_3, and NO_2) were approximately 500 ppm and 3.7 ppm (~ 500 ppm, 3.7 ppm, and 3.9 ppm) respectively. The high concentration of isoprene was necessary to ensure the reaction of > 90% of the ozone so the concentration was close to the NOEL. The irritation effect of methylvinyl ketone was calculated from literature data (*32*).

Results and Discussion

Breathing parameter response during exposure to isoprene. We observed no significant effects on breathing parameters during exposure to isoprene at concentrations ≤ 15×10^3 ppm. No animals died during the exposure or recovery periods.

Reaction of isoprene with O_3. About 0.2 ppm ozone (~ 4% of the original concentration) and approximately 500 ppm isoprene were unreacted and approximately 0.3 ppm formaldehyde, 1.4 ppm methacrolein, 0.6 ppm methylvinyl ketone, 0.3 ppm acetone, 0.7 ppm formic acid, and 0.4 ppm acetic acid were formed in the approximately 30-sec-old reaction mixture (Figure 1). The concentrations of some of the irritants (especially formaldehyde, methylvinyl ketone, and methacrolein) may be slightly underestimated because of reactions with O_3 during sampling on Tenax TA. The linear saturated aldehydes with more than two carbon atoms, identified in the DNPH analysis, were probably artifacts from contaminants in the flow system. In addition to the identified

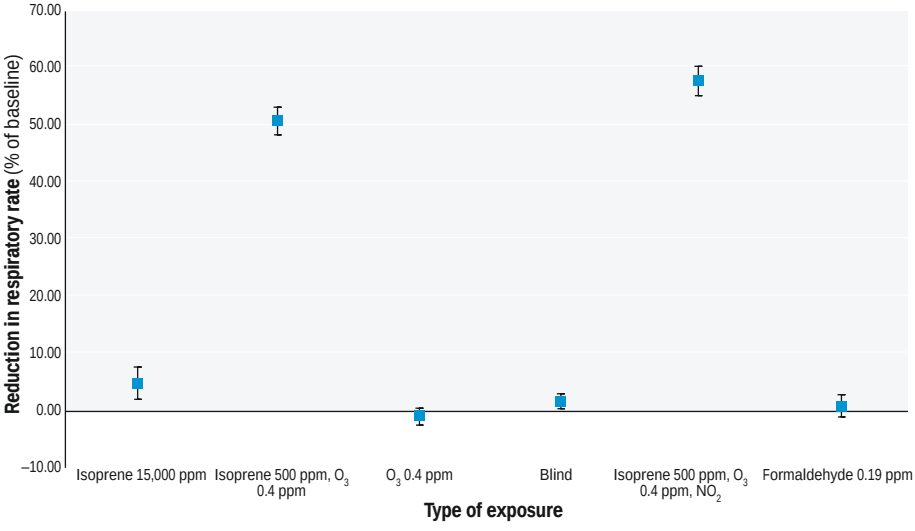

Figure 1. Average reduction in respiratory rate caused by exposure to isoprene/oxidant mixtures and controls with 95% confidence limits (11th to 20th min of exposure).

products, there were two major products of unknown structure in the carbonyl analyses and several small unidentified peaks in the GC-MS analysis. We observed only traces of Tenax TA artifacts (< 0.1 ppm), undoubtedly because of the small sample volume. Small amounts (~ 50 ppb) of C_{10} oxidation products were detected from the approximately 1% $C_{10}H_{16}$ impurities in both reactions. We disregarded these because of their low levels.

Assuming that isoprene reacted with one mole of ozone (thus neglecting secondary reactions, e.g., hydroxyl radicals), approximately 2.3 ppm of products (C_3 or larger) could be accounted for from 3.7 ppm ozone.

The bioassay results include only effects on the respiratory rate (Figure 1), because sensory irritation was the dominating effect of the individual substances and the mixture determined by analysis of the respiratory parameters. The concentrations of all substances identified in the reaction mixture, except for methacrolein, were below or equal to established NOEL or estimated irritation threshold levels (RD_0). Thus we expected their contributions to be minor, as reflected in their rather low calculated effect levels (Table 1). Although O_3 at a higher concentration may increase the respiratory rate ("rapid shallow breathing") (*29*), 0.2 ppm is far below its NOEL and thus we disregarded its contribution. The reaction mixture (isoprene/ozone) caused a mean reduction of 52% in the respiratory rate. This was significantly different from the effects of the laboratory air exposure ($p < 0.001$, *t*-test) as well as from exposures to residual O_3, isoprene, and formaldehyde at the concentrations measured.

Reaction of isoprene with O_3/NO_2. About 0.2 ppm ozone (~ 4%) and 500 ppm isoprene were unreacted and about 0.5 ppm formaldehyde, 1.4 ppm methacrolein, 1.2 ppm methylvinyl ketone, 0.1 ppm acetone, 0.9 ppm formic acid, 0.5 ppm acetic acid, and 0.5 ppm isoprene epoxides were present in the approximately 30-sec-old reaction mixture. About 0.2 ppm of 3-methylfuran and 0.1 ppm each of 3-methyl-2(5H)-furanone and the tentatively identified substances methylvinyl ketone epoxide and 4-methyl-2(5H)-furanone were also detected by GC-MS. Because of the rather low concentrations of the three tentatively identified products, they are not included in Table 1. We do not anticipate them to be especially irritating substances. About 3.2 ppm of products (C_3 or larger) could be accounted for from 3.7 ppm O_3, assuming 1:1 stoichiometry. Besides the identified products, there were two products of unknown structure in the carbonyl analyses and several small unidentified peaks in the GC-MS analysis.

We expected only formaldehyde, methylvinyl ketone, and methylacrolein to contribute significantly to irritation of the reaction mixture, reflected by their calculated effect levels and the sum of these (see Table 1). The sum of the effects should be an overestimation of the actual contributions because these effects can be hypoadditive. We disregarded the effects of 0.2 ppm O_3 and about 20 ppb NO_2. We observed a mean reduction in the respiratory rate of 58% for the mixture isoprene/O_3/NO_2 (Figure 1). For convenience, we used the RD_{50} values to evaluate the biologic effects, instead of the actual irritation (52 and 58%, respectively).

Laboratory and field studies have shown that O_3 reacts with unsaturated indoor VOCs to form formaldehyde as well as other products (*1*,*2*). Our objective here was to find evidence for the formation of other potent airway irritants by mixing O_3 and O_3/NO_2 with isoprene, a major volatile compound in exhaled air. We chose the initial concentrations of isoprene, O_3, and NO_2 on the basis of the reaction rates and their concentration–response relationships, so the residual concentrations in the reaction mixture were close to but below their NOELs in the bioassay.

Recent studies of the reaction of isoprene and O_3 reported products identified by FTIR, including formaldehyde, methylacrolein, methyl vinyl ketone, formic acid, carbon monoxide, and carbon dioxide, in addition to minor amounts of hydroperoxymethyl formate, formic acid anhydride, methanol, and ketene (*15*,*33*). Furthermore, the authors (*15*,*33*) also emphasized that the amounts of the main products, methacrolein and methyl vinyl ketone, reported depended greatly on reaction conditions, especially relative humidity (in our experiments < 10%). The amounts of methacrolein and methyl vinyl ketone reported here agree essentially with those reported earlier, in which ozone was slowly added to a reaction chamber filled with isoprene (*13*). With the isoprene/O_3 ratios used here, conditions for further reaction with hydroxyl radical were expected to be optimal. However, the amount of 3-methylfuran, an isoprene/OH• reaction product (*34*), was < 1% of the O_3 concentration, which may be in agreement with a OH• yield of 0.27 and a 3-methylfuran yield of about 0.04 from OH• and isoprene (*35*). Another factor to consider is the difference in product stabilities under different reaction conditions (different modes of OH• production). The reaction products identified here depend, of course, on the methods used for isolation/identification

Table 1. Calculated reduction in breathing rate caused by reactants and products from reaction 1 (isoprene/ozone) and reaction 2 (isoprene/ozone/NO_2) including sensory irritation properties (NOEL or RD_0 and RD_{50}).

Substance	Concentration (ppm)	NOEL[a] (ppm)	RD_{50} (ppm)	Calculated effect[b] (% reduction)
Isoprene	~ 500	~11,000	~ 57,200	
Formaldehyde	0.3	0.3 (*29*)	3.1	
Formic acid	0.7	10 (RD_0) (*45*)	480	
Acetic acid	0.4	13 (RD_0) (*45*)	310	
Acetone	0.3[c]	7,500	23,500	
Methylvinyl ketone	0.6	0.8 (*32*)	5.2	
Methacrolein	1.4	1.3 (RD_0) (*28*)	10.4	2
C_2–C_6 linear saturated aldehydes	~ 0.3	100 (RD_0)[d]		
Ozone	0.14	>1 (*29*)		
Sum of calculated effects				2%
Isoprene	~ 500	~11,000	~ 57,200	
Formaldehyde	0.5	0.3 (*29*)	3.1	11
Formic acid	0.9	10 (RD_0) (*45*)	480	
Acetic acid	0.5	13 (RD_0) (*45*)	310	
Acetone	0.1[c]	7,500	23,500	
3-Methylfuran	0.2[c]	—[e]		
Methylvinyl ketone	1.2	0.8	5.2	16
Methacrolein	1.4	1.3	10.4	2
Isoprene epoxides	0.5	1.4[f]	5.7	
C_2–C_6 linear saturated aldehydes	~ 0.3	100 (RD_0)[d]		
Ozone	0.17	>1 (*29*)		
NO_2	< 0.02	—[g]		
Sum of calculated effects				29%

[a]The NOEL of the sensory irritation effect in mice, if not available, is set equal to the threshold concentration (RD_0), obtained from the curvilinear relationship of the percent decrease in respiratory rate versus the logarithm of the exposure concentration (ppm), taken from the cited literature. The similarity between NOEL and RD_0 for formaldehyde may justify the use of RD_0 as NOEL. [b]Calculated from dose–response data. [c]Determined in toluene equivalents [d]The C_2–C_6 linear saturated aldehydes have their RD_0 values for Swiss-Webster mice > 100–400 ppm (*46*). The lowest value (> 100 ppm) is used here. [e]Increased eye blink (slight irritation but no other symptom) was observed in humans at 1 ppm (*47*). [f]Value for allylglycidyl ether was used (*48*). [g]Odor detection threshold in humans, 0.19 ppm (*49*).

(Tenax adsorption, thermal desorption/MS, carbonyl derivatization/HPLC); thus they do not reflect accurately the composition of the reaction mixtures because some of the products are undoubtedly thermally unstable (e.g., peroxides, dicarbonyl compounds, nitrates). Calculation of irritation on the basis of the products identified is thus, by nature, insufficient, requiring additional experiments using milder separation/analysis conditions.

The reaction of isoprene with O_3 and NO_2 was conceived as a model for realistic conditions, when both oxidants are present. Because the rate of the reaction of O_3 with NO_2 is somewhat larger than that of O_3 with isoprene (3.2 compared to 1.43 $cm^3 \times 10^{-17}$/molecule/sec), we anticipated that the product composition would reflect both the reaction with O_3 and NO_3• (*13,36*). Because both of these O_3 reactions are relatively slow (~ 1/10 that of O_3 and limonene, for example), it was necessary to use a high isoprene concentration, approximately 100 × $[O_3]$, to assure the complete reaction of both of the pulmonary irritants, O_3 and NO_2, in the flow system used. The overall effects of these reaction conditions appear to be that most of the isoprene reacted with O_3 and that the isoprene/NO_3• reaction played a relatively minor role as reflected by the composition of the reaction products. About 20% of the products (isoprene epoxides + furanones, based on the O_3 consumed) may have been derived from the reaction of isoprene and NO_3• (*16,18,37*). It is highly likely that unstable products such as hydroperoxy-nitrates or nitrate-aldehydes decompose during thermal desorption (*38*). All of the products identified in the two reactions have been described previously except the two furanone derivatives. The unsubstituted furanone [2(3H)-furanone] has been reported from the reaction of furan with NO_3• (*39*). It is possible that the furanones identified in this work are the result of oxidation of 3-methylfuran or that they are formed by rearrangements of products, in which the terminal carbon atoms of isoprene are oxidized, followed by ring closure. Alternatively, they may be artifacts caused by thermal degradation of labile open chain derivatives during thermal desorption. Formation of epoxides was reported in reactions of O_3 with α-pinene (*40*) and limonene (*5*) under similar conditions, so identification of the three epoxides here, isoprene monoepoxides (two isomers) and methylvinyl ketone epoxide was consistent with earlier work.

Further examination of reaction intermediates and their decomposition, involving cold isolation procedures and kinetic ultraviolet-visible spectroscopy and Fourier transform infrared spectroscopy will probably provide more detailed product identification and mechanistic characterization of these reactions under the special flow reaction conditions used.

The identified VOCs expected to contribute most to the sensory irritation effects of the mixtures are formaldehyde, methacrolein, and methylvinyl ketone. Because the effects of airway irritants are assumed to be additive or hypoadditive (*1*), the substances measured cannot account for the observed effect (Table 1). That the calculated effects for the two reaction mixtures were considerably less than those observed suggests that (potent) unidentified irritant(s) were formed in the reaction mixtures. Possibly, unstable reaction product(s) such as hydroperoxides or, in the O_3/NO_2 reaction, peroxynitrates, nitrohydroperoxides, or nitroaldehydes, by analogy to the reaction of butadiene (*39*), are responsible for the unexplained upper airway irritation. The formation of peroxyacetyl nitrate has been reported in model mixtures of α-pinene, O_3, and NO_2 (*41*).

An intriguing question, which is partly the basis for the investigation, is whether these oxidative processes contribute significantly to human airway irritation during periods of elevated indoor O_3 (and/or NO_2) concentrations and/or in crowded buildings with low air exchange rates (elevated isoprene and other reactive olefin concentrations). The concentration of isoprene in the lung is low (25–200 ppb) and somewhat lower in an occupied room (~ 20 ppb) (*42*). At these low concentrations, the oxidation processes are much slower, but it is possible that during extended exposure they could contribute to the reported airway irritation. Aldehydes have been identified in bronchoaveolar lavage of rats exposed to 0.5–10 ppm ozone undoubtedly from the oxidation of unsaturated fatty acids (*43*). These authors suggested that oxidation intermediates (ozonides, hydroxy-hydroperoxides) might be involved in the inflammatory process.

It is also interesting that blood levels of isoprene in humans have been reported to be 1–5 mg/m^3 whereas the other mammals investigated had blood levels of < 70 $\mu g/m^3$ (*44*), suggesting that some aspects of terpene biosynthesis in humans are unique.

The upper airway irritation observed in mice exposed to isoprene/oxidant mixtures could not be explained by the concentrations of residual reactants and reaction products identified by some conventional sampling/analytic procedures. It is likely that strongly irritating, unstable products are responsible for the unexplained airway irritation. The practical and rather ironic implication of these observations may be that humans themselves produce one of the compounds, which may contribute to upper airway irritation indoors.

References and Notes

1. Wolkoff P, Clausen PA, Jensen B, Nielsen GD, Wilkins CK. Are we measuring the relevant indoor pollutants? Indoor Air 7:92–106 (1997).
2. Weschler CJ, Shields HC. Potential reactions among indoor pollutants. Atmos Environ 31:3487–3495 (1997).
3. Höppe P, Praml G, Rabe G, Lindner J, Fruhmann G, Kessel R. Environmental ozone filed study on pulmonary and subjective responses of assumed risk groups. Environ Res 71:109–121 (1995).
4. Wolkoff P, Clausen PA, Wilkins CK, Hougaard KS, Nielsen GD. Formation of strong airway irritants in a model mixture of (+)-α-pinene/ozone. Atmos Environ 33:693–698 (1999).
5. Clausen PA, Wilkins CK, Wolkoff P, Nielsen GD. Chemical and biological evaluation of a reaction mixture of R-(+)-limonene/ozone: formation of strong airway irritants. Environ Int 26:511–522 (2001).
6. Weschler CJ, Shields HC. Production of the hydroxyl radical in indoor air. Environ Sci Technol 30:3250–3258 (1996).
7. Weschler CJ, Shields HC. The influence of ventilation on reactions among indoor pollutants: modeling and experimental observations. Indoor Air 10:92–100 (2000).
8. Loreto F. Emission of isoprenoids by plants: their role in atmospheric chemistry, response to the environment, and biochemical pathways. J Environ Pathol Toxicol Oncol 16:119–124 (1997).
9. Kuzma J, Nemecek-Marshall M, Pollock WH, Fall R. Bacteria produce the volatile hydrocarbon isoprene. Curr Microbiol 30:97–103 (1995).
10. Stone BG, Besse TW, Duane WC, Evans CD, DeMaster EG. Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. Lipids 28:705–708 (1993).
11. Fenske JD, Paulson SE. Human breath emissions of VOCs. J Air Waste Manag Assoc 49:594–598 (1999).
12. Alarie Y. Sensory irritation by airborne chemicals. Crit Rev Toxicol 2:299–363 (1973).
13. Paulson SE, Flagan RC, Seinfeld JH. Atmospheric photooxidation of isoprene part II: the ozone-isoprene reaction. Int J Chem Kinet 24:103–125 (1992).
14. Hakola H, Arey J, Aschmann SM, Atkinson R. Product formation from the gas-phase reactions of OH radicals and O_3 with a series of monoterpenes. J Atmos Chem 18:75–102 (1994).
15. Sauer F, Schäfer C, Neeb P, Horie O, Moortgat GK. Formation of hydrogen peroxide in the ozonolysis of isoprene and simple alkenes under humid conditions. Atmos Environ 33:229–241 (1999).
16. Skov H, Hjorth J, Lohse C, Jensen NR, Restelli G. Products and mechanisms of the reactions of the nitrate radical (NO_3) with isoprene, 1,3-butadiene and 2,3-dimethyl-1,3-butadiene in air. Atmos Environ 26A:2771–2783 (1992).
17. Ellermann T, Nielsen OJ, Skov H. Absolute rate constants for the reaction of NO_3 radicals with a series of dienes at 295 K. Chem Phys Lett 200:224–229 (1992).
18. Kwok ESC, Aschmann SM, Arey J, Atkinson R. Product formation from the reaction of the NO_3 radical with isoprene and rate constants for the reactions of methacrolein and methyl vinyl ketone with the NO_3 radical. Int J Chem Kinet 28:925–934 (1996).
19. Dohan JM, Masschelein WJ. The photochemical generation of ozone: present state-of-the-art. Ozone Sci Eng 9: 315–334 (1987).
20. Wong KL, Alarie Y. A method for repeated evaluation of pulmonary performance in unanestesthetized, unrestrained guinea pigs and its application to detect effects of sulfuric acid mist inhalation. Toxicol Appl Pharmacol 63:72–90 (1982).
21. Fung K, Grosjean D. Determination of nanogram amounts of carbonyls as 2,4-dinitrophenylhydrazones by high-performance liquid chromatography. Anal Chem 53:168–171 (1998).
22. Williams KE, Esposito GG, Rinehart DS. Sampling tubes for the collection of selected acid vapors in air. Am Ind Hyg Assoc J 42:476–478 (1981).
23. ASTM. Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals. Philadelphia:American Society for Testing and Materials, 1984;1–16.

24. Boylstein LA, Anderson SJ, Thomson RD, Alarie Y. Characterization of the effect of airborne mixture of chemicals on the respiratory tract and smoothing polynominal spline analysis of the data. Arch Toxicol 69:579–589 (1995).
25. Boylstein LA, Luo J, Stock MF, Alarie Y. An attempt to define a just detectable effect for airborne chemicals on the respiratory tract in mice. Arch Toxicol 70:567–578 (1996).
26. Vijayaraghavan R, Schaper M, Thompson R, Stock MF, Boylstein LA, Luo JE, Alarie Y. Computer assisted recognition and quantitation of the effects of airborne chemicals acting at different areas of the respiratory tract in mice. Arch Toxicol 68:490–499 (1994).
27. Vijayaraghavan R, Schaper M, Thompson R, Stock MF, Alarie Y. Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. Arch Toxicol 67:478–490 (1993).
28. Larsen ST, Nielsen GD. Effects of methacrolein on the respiratory tract of mice. Toxicol Lett 114:197–202 (2000).
29. Nielsen GD, Hougaard KS, Larsen ST, Hammer M, Wolkoff P, Clausen PA, Wilkins CK, Alarie Y. Acute airway effects of formaldehyde and ozone in BALB/c mice. Hum Exp Toxicol 18:400–409 (1999).
30. Nielsen GD, Kristiansen U, Hansen L, Alarie Y. Irritation of the upper airways from mixtures of cumene and *n*-propanol. Arch Toxicol 62:209–215 (1988).
31. Cassee FR, Arts JHE, Groton JPG, Feron VJ. Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70:329–337 (1996).
32. Müller J, Greef G. Recherche de relations entre toxicite de molecules d'interet industriel et proprietes physicochemiques: test d'irritation des voies aeriennes superieures applique a quatre families chemiques. Food Chem Toxicol 22:661–664 (1984).
33. Neeb P, Sauer F, Horie O, Moortgat GK. Formation of hydroxymethyl hydroperoxide and formic acid in alkene ozonolysis in the presence of water vapour. Atmos Environ 31:1417–1423 (1997).
34. Atkinson R, Aschmann SM, Tuazon EC, Arey J, Zielinska B. Formation of 3-methylfuran from the gas-phase reaction of OH radicals with isoprene and the rate constant for its reaction with the OH radical. Int J Chem Kinet 21:593–604 (1989).
35. Atkinson R, Aschmann SM, Arey J, Shorees B. Formation of OH radicals in the gas phase reactions of O_3 with a series of terpenes. J Geophys Res 97:6065–6073 (1992).
36. Finlayson-Pitts BJ, Pitts JN Jr. Atmospheric Chemistry: Fundamentals and Experimental Techniques. New York:John Wiley & Sons, 1986;528.
37. Tuazon EC, Atkinson R. A product study of the gas-phase reaction of isoprene with the OH radical in the presence of NO_x. Int J Chem Kinet 22:1221–1236 (1990).
38. Tuazon EC, Alvarado A, Aschmann SM, Atkinson R, Arey J. Products ot the gas-phase reactions of 1,3-butadiene with OH and NO_3 radicals. Environ Sci Technol 33:3586–3595 (1999).
39. Berndt T, Böge O, Rolle W. Products of the gas phase reactions of NO_3 radicals with furan and tetramethylfuran. Environ Sci Technol 31:1157–1162 (1997).
40. Wolkoff P, Clausen P, Wilkins K, Nielsen GD. Unpublished data.
41. Gårdfeldt K, Langer S. Formation of peroxyacetylnitrate (PAN) in indoor environment [Abstract]. Environ Sci Pollut Res 5[3], 170. 1998.
42. Cailleux A, Turcant A, Premel-Cabic A, Allain P. Volatile organic compounds in indoor air and in expired air as markers of activities. Chromatographia 37:57–59 (1993).
43. Pryor WA, Bermudez E, Cueto R, Squadrito GL. Detection of aldehydes in bronchoalveolar lavage of rats exposed to ozone. Fundam Appl Toxicol 34:148–156 (1996).
44. Cailleux A, Cogny M, Allain P. Blood isoprene concentrations in humans and in some animal species. Anal Biochem 210:268–276 (1993).
45. Nielsen GD, Hansen LF, Hammer M. Eye and Airway Irritation Effects of Organic Acids. Copenhagen:Working Environment Fund, 1996.
46. Steinhagen WH, Barrow CS. Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharmacol 72:495–503 (1984).
47. Wålinder R, Ernstgård L, Gullstrand E, Johansson G, Norbäck D, Venge P, Wieslander G. Acute effects of experimental exposure to four volatile compounds associated with water-damaged buildings and microbial growth. In: Proceedings of Indoor Air '99, 8–13 August 1999, Vol.2. Edinburgh, Scotland:Watford:Building Research Establishment Ltd, 1999;606–610.
48. Gagnaire F, Zissu D, Bonnet P, De Ceaurriz JC. Nasal and pulmonary toxicity of allyl glycidyl ether in mice. Toxicol Lett 39:139–145 (1987).
49. Devos M. Nitrogen dioxide. In: Standardized Human Olfactory Thresholds (Devos M, Patte F, Rouault J, Laffort P, van Gemert LJ, eds). New York:Oxford University Press, 1990;124.