Heterogeneous oxidation of squalene film by ozone under various indoor conditions

**Abstract** The effects of indoor conditions (ozone concentration, temperature, relative humidity (RH), and the presence of NOx) on heterogeneous squalene oxidation were studied with Attenuated Total Reflectance–Fourier Transform Infrared spectroscopy. The heterogeneous kinetics of squalene-ozone reaction revealed a pseudo-first-order reaction rate constant of $1.22 \times 10^{-5}$/s at $[O_3] = 40$ ppb. Oxidation kinetics were insensitive to temperature over the range of $24-58 \pm 2^\circ C$ as well as to RH and presence of NOx. Products, however, were affected by the environmental parameters. As temperature was increased, fewer surface products and more low molecular weight gaseous products were observed. Lower air exchange rates also enhanced gas phase reactions, allowing for formation of secondary gas phase products. As RH increased, there was a shift in product distribution from ketones to aldehydes, and the presence of NOx during squalene ozonolysis resulted in the formation of nitrated oxidation products. Identified surface products included 6-methyl-5-hepten-2-one, geranyl acetone, and long chain ketones and aldehydes, while gas phase products included formaldehyde, acetone, 4-oxopentanal (4-OPA), glyoxal, and pyruvic acid.

**Practical Implications**
Heterogeneous oxidation of squalene resulted in surface products including long chain aldehydes and ketones, and gas phase products including formaldehyde, a known human carcinogen (IARC 2006), and bicarbonyl compounds like: 4-oxopentanal (4-OPA), glyoxal, and pyruvic acid that are characterized as asthma triggers and sensitizers (Anderson et al., 2007; Jarvis et al., 2005). In addition, ozonolysis experiments in the presence of NOx showed the formation of nitrated surface oxidation products. Such nitrated products may have higher mutagenicity, carcinogenicity, or allergic potential than their nitrate free counterparts (Franze et al., 2005; Pitts, 1983). Kinetic studies determined that at moderate ozone levels of 40 ppb (Uhde and Salthammer, 2007), and an estimated skin surface density of $4 \times 10^{15}$ molecules/cm$^2$, surface reaction would lead to a minimum product formation flux of $4 \times 10^{10}$ molecules cm$^2$/s. As squalene is naturally occurring and continually produced by the human body, its concentration in the indoor environment cannot be controlled. However, this study highlights the importance of regulating air exchange rate, temperature, and ozone level in the indoor environment on the formation of potentially harmful or irritating squalene oxidation products.

**Introduction**
As people spend most of their time indoors, they may be more susceptible to health risk by exposure to indoor air pollution than outdoor. Because of high indoor pollutant emissions and high ratios of breathing to ventilation rates, indoor intake fractions are relatively high. Thus, human exposure for a typical pollutant released indoors is on the order of 1000 times more likely than outdoors (Smith, 1988). Zhu et al. (2005) observed that in a relatively stagnant environment, like an indoor environment, inhaled air comes from the lower body, being directed upward by metabolic heating. This implies that pollutants released closer to the human body are likely to have higher impact on indoor human exposure. Squalene is a major component of human skin sebum found on the skin surface as well as worn clothing. Although squalene is not known to have adverse health effects, it may participate in reactions with indoor oxidants such as O$_3$ and NO$_3$, yielding harmful secondary products. This has been supported by recent studies on ozone reactions with skin oils which resulted in volatile aldehydic and ketonic products (Coleman et al., 2008;
Ozone is known to initiate indoor chemistry. It has been observed that heterogeneous chemistry plays a significant part in oxidation (Fick et al., 2005; Wisthaler et al., 2005) and in some cases, the latter has faster reaction rates and higher product yields (Moise et al., 2008). Although, many researchers have studied indoor surface reactions, only a few gaseous and surface products have been identified, and the effect of environmental conditions on this chemistry remains not fully understood (Morrison, 2008). In the case of surface reactions, condensed phase products may cause human health concerns via dermal contact or hand to mouth transfer and gas phase products via inhalation.

Squalene is a non-volatile triterpene with high unsaturation, known to react quite rapidly with ozone (Wells et al., 2008). In fact, a few studies were conducted on squalene ozone oxidation and major findings included the formation of acetone, 6-methyl-5-hepten-2-one (6-MHO), 4-OPA, geranyl acetone (GA), and long-chain aldehydes (Fruekilde et al., 1998; Wells et al., 2008). Additionally, dark exposure of squalene sorbed on fabric to room air was found to generate polar functionalities like: O-H, C=O, and C-O (Park and Obendorf, 1994). Formation of such oxygen containing fragments and carbonyl functionalities, particularly dicarboxyls, have been characterized as asthma triggers and sensitzers (Anderson et al., 2007; Jarvis et al., 2005). Additional gas phase products from terpene oxidation include formaldehyde, a known human carcinogen (IARC 2006), and OH radicals that react quickly with most organic compounds found in air (Calogirou et al., 1999) forming secondary products. In addition to ozone reactions, nitration of some organic compounds from reactions with NOx have resulted in products with higher mutagenicity and carcinogenicity (Franze et al., 2005; Pitts, 1983; Tokiwara et al., 1994). Thus, squalene may react with indoor oxidants to form indoor air pollutants contributing to adverse health effects (Wells et al., 2008).

In the work presented here, squalene-ozone surface reaction rates and products were investigated. Although, the aforementioned studies identified some products, and squalene oxidation kinetics were reported under specific conditions, the effect of environmental factors were not considered. In this study, kinetics were measured in situ using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy, and the effects of ozone concentration (10^{11}-10^{15} molec/cm^3), relative humidity (RH) (< 10% and > 70%), temperature (24, 37, and 58°C), and presence of NOx during ozonolysis, were investigated. Condensed and gas phase products were identified and oxidation mechanisms postulated. The effects of temperature, humidity, ozone concentration and NOx on surface rate constants have not been reported previously.

**Methods**

Experimental

A detailed description of a similar experimental set-up and procedure is provided by Segal-Rosenheimer and Dubowsky (2007). In summary, the ATR-FTIR experimental system consisted of a ZnSe ATR crystal (7.5 x 0.8 cm^2, HATRPlus by Pike Technologies Inc., Madison, WI, USA), which was placed in a custom-built flow-through stainless steel chamber (volume 5.4 l) with a gas inlet pointed toward the ATR crystal. The ATR accessory was attached to a Bruker Tensor 27 FTIR with a deuterated triglycine sulfate detector. Gas phase carbonyl species were collected downstream on dinitrophenylhydrazine (DNPH) coated silica cartridges (P/N WAT037500; Waters Corp., Milford, MA, USA), following an inline KI ozone scrubber (P/N WAT054420; Waters Corp.) to avoid additional oxidation upon the cartridge.

In a typical experiment, ozone was generated by irradiating a dry flow of O2 and N2 with a 185 nm light (Jelight ozone generator, Model 600, Irvine, CA, USA). Its concentration was measured using a UV–VIS spectrophotometer (Spectronic® 601; Milton Roy Inc., Ivyland, PA, USA) for high concentrations (≥ 10^{15} molec/cm^3) or an ozone monitor (Bionics Instrument Co. TG-KBPII, Lakezur, IL, USA) for low concentrations (≤ 10^{13} molec/cm^3). Ozone concentration was controlled by varying the mixing ratios of O2/N2 and the exposure length to UV light. After recording a background spectrum of the clean ATR crystal, a thin squalene film was generated by placing 100 µl of 2 mm solution of squalene (Analytical standard; Aeros Organics, Yehud, Israel) in chloroform (Analytical reagent; Acros Organics, Yehud, Israel) directly on the ATR crystal, and letting the solvent evaporate. Complete removal of the solvent was confirmed by ATR spectra. The ATR flow-through chamber was then sealed and spectral measurements were started simultaneously with diverting the ozone flow to the ATR chamber. In a separate batch experiment, gas-phase species were detected in a small volume long path IR cell (Infrared analysis Inc., model 6.2 V, Anaheim, CA, USA). A high resolution FTIR (Bruker Vertex 70) with MCT (Mercury Cadmium Telluride) detector was used for gas-phase analysis.

Reaction rate constants were measured for ozone levels ranging between 10^{11}-10^{15} molec/cm^3 (30 ppb–60 ppm) at low RH (< 10%). The effects of three additional parameters were investigated: high RH (> 70%), temperatures (24, 37, and 58 ± 2°C), and NOx presence.
Humidification was obtained by bubbling dry nitrogen gas through DI water (18.2 MΩ water, Millipore) and adding it to the ozone carrying gas in the mixing cell. High RH experiments were performed at moderate ozone levels (30–60 ppb).

Temperature was controlled with a heating tape and thermocouple feedback attached to the exterior of the reactor. The interior reactor temperature was also monitored with a second thermocouple (AHLBORN Almemo®, type K, ZA9020-FS, Holzkirchen, Germany). For experimental simplicity, replicate experiments were conducted in parallel in small glass reactors that were submerged in water baths (MRC Inc., Holon, Israel) at different temperatures and at ozone levels 400–500 ± 20 ppb.

NOx experiments were performed under dry conditions (< 15% RH) at 28 ± 2°C. For simultaneous squalene exposure to NOx and ozone, two identical collapsible Teflon chambers were connected symmetrically via a T junction to the inlet of a home-made stainless steel reactor (volume 0.160 l) attached to the ATR. One chamber contained NOx mixture of [NO] = 1.5 ± 0.7 ppm, [NO2] = 4.8 ± 0.4 ppm, and [O3] < 50 ppb (prepared by titrating NO, Calgaz Calibration gases, with ozone). NO and NO2 concentrations in the chamber were measured by chemiluminescence NOx analyzer (API Teledyne, Model 200E, San Diego, CA, USA). The second chamber contained a mixture of N2/O2/O3 ([O3] = 12.3 ± 0.4 ppm). The stainless steel reactor was followed by a peristaltic pump (P/N 7553–75 Cole Parmer) withdrawing the gases via the reactor at a 1:1 ratio.

Chromatographic products analysis

In addition to spectroscopic analysis, the residue of the squalene film was analyzed by Gas Chromatography–Mass Spectroscopy (GC-MS). For surface product identification and quantification, aliquots of 200 μl of chloroform were deposited on the ATR crystal after oxidation, gently shaken, and pipetted off. This was repeated to a total volume of 1 ml. For identification, 10 μl samples were injected into the GC-MS (Varian CP-3800 GC with MS trap detector Varian Saturn 2000, run in EI mode). Injector temperature was 250°C, and analysis was performed using a capillary column (Varian FactorFour, DB-5 column; 30 m; 250 um I.D; film thickness 0.25 um) following a temperature gradient starting at 40°C (10 min hold) with MS filament off, followed by temperature ramping (10°C/min) up to 300°C with filament on. Ions were collected in the range of 40–500 m/z. For simplicity, GC-FID (Varian CP-3800 GC with CP-8410 autoinjector and CP-Sil 5CB, 15 m column) was used for routine quantification of squalene loss. In the latter, injector temperature was 250°C with split ratio of 10 and column temperature was varied between 130–240°C (30°C/min) and 240–290°C (10°C/min). Volatile aldehyde and ketone species were identified and quantified after derivatization upon DNPH cartridges, using High Performance-Liquid Chromatography (HPLC) with Photo-Diode Array (PDA) detection (Agilent 230 solvent delivery, 410 autosampler, 335 PDA detector) as described by WatersCorp (1994). In summary, the cartridges were backflushed with acetonitrile (ACN) (Mallinkrodt, HPLC grade) to a total volume of 2 ml. 20 μl of sample were injected onto a Hypersil GOLD column (Thermo Electron, Co.; 3 μm particle, 150 × 4.6 mm) using a bisolvent system (A and B) at 1.2 ml/min flow rate. The solvent gradient was varied from 100% of A (water/ACN/tetrahydrofuran: 60/30/10 v/v/v) to 100% B (water/ACN: 40/60 v/v) in 10 min followed by 20 min hold. Derivatized products were detected at 360 nm. DNPH standard solutions of formaldehyde, acetaldehyde, and acetone (Calibration standards; Supelco, Rehovot, Israel) were used for quantification, while DNPH standards of 6-MHO, glyoxal, and pyruvic acid (Analytical reagent; Acros Organics) were synthesized using the procedure described by WatersCorp (1994). Geranyl acetone was purchased from Sigma Aldrich (analytical grade 96%).

Results and discussion

Squalene-ozone FTIR spectra characterization

The spectrum of a fresh squalene thin-film can be viewed in Figure 1. The main absorption bands are those associated with the alkane, -CH3 and -CH2 groups. The methyl and methylene antisymmetric stretches are at 2967 and 2919 cm, respectively, while the -CH2 symmetric stretch is observed at 2853/cm (Lin-Vien et al., 1991). The -CH2 scissor (1445/cm) and -C(CH3) symmetric bends (1379/cm) are also noticeable. Bands associated with the trialkyl alkene functions are observed as -CH wag (835/cm) and weak -C=C stretches (1668/cm) (Chi and Obendorf, 1998).
and -CH stretches (3052–3020/cm) (Lin-Vien et al., 1991; Park and Obendorf, 1994).

The oxidation of squalene was monitored in real time using the ATR-FTIR spectrometer. As the reaction progressed, product build-up was indicated by increases in the carbonyl region (1775–1680/cm) and in the 'finger-print' region (1600–1000/cm). Loss of the alkyl stretches was observed by decreases in the 2965–2850/cm region, but absorbance bands of squalene and its products could not be quantitatively separated because of spectral overlap. The observed decrease in absorbance at this spectral region was because of volatile product formation and to a smaller extent, reaction at the double bond (i.e. loss of -C=CH). Differences in the extinction coefficients between squalene and its products as well as secondary radical chemistry are also possible.

Alternately, clear spectral differences in ozonolysis products were observed depending on the extent of reaction at the double bonds (see Figure 2). In all experiments several -C-O absorption bands were observed at 1300–1000/cm region. Absorbance and relative ratio between these bands differed depending on ozone concentration. In general, as ozone concentration increased there was a shift in products from aldehydes to a mixture of various ketones, aldehydes, and alcohols. At a very high ozone level ([O₃] = 22 ppm), a broad -OH absorption band could be observed with a maximum at 3400/cm indicative of alcohols, and a broad peak centered at 1717/cm representing aldehydic and ketonic carbonyl groups. At medium levels of ozone ([O₃] = 200 ppb), very little absorbance at the -OH band was observed, but the formation of a broad carbonyl was evident in addition to higher -C=O bands. At very low ozone levels ([O₃] = 50 ppb), very little -OH and weaker -C=O absorbance was observed, but a clear narrow carbonyl absorption band centered at maximum 1728/cm could be seen, indicative of an aldehyde functionality. These results suggest that an increase in ozone level from 50 to 200 ppb resulted in the formation of secondary oxidation products and possibly oligomers or secondary ozonides with -C-O functionalities. Additional increase to 22 ppm resulted in other secondary oxidation products containing more -OH groups, possibly because of secondary OH radical reactions. As OH radical formation resulting from terpene ozonolysis can reach 60–90% yields (Aschmann et al., 2002), subsequent secondary reaction products containing -C-O and -OH functionalities because of radical reactions would not be surprising (Finlayson-Pitts and Pitts, 2000).

Complementary identification of condensed phase ozonolysis products was performed by GC-MS. At 40–50 ppb ozone (in dry N₂), several compounds were identified (numbers in parenthesis refer to product number in Scheme 1): 6-MHO (4), 4,8,13,17,21-tetramethyl-octadeca-4,8,12,16,20-pentaene-al (TOP) (5), GA (6), and 5,9,13-trimethyl-tetradeca-4,8,12-triene-al (TTT) (7). Although 4,9,13,17-tetramethyl-octadeca-4,8,12,16-tetraeneal (TOT) (2) was not identified because of chromatographic overlap with squalene, this product as well as (4), and (7) were previously identified by Fruekilde et al. (1998). All the above compounds contain many of the absorption bands similar to squalene, including those representing -CH₂, -CH₃, and -C=CH₂ bonds, in addition to carbonyl frequencies as shown in Figure 2b. The IR spectra also indicated the formation of products containing -C-O functionalities, possibly secondary ozonides as a result of primary product rearrangement and secondary reactions. However, thermal liability of these compounds prevented identification under the present experimental conditions (Norgaard et al., 2006).

Gas phase product analysis of oxidized squalene film under batch conditions was performed in the long-path IR cell (containing 3 × 10¹⁸ molecules gaseous O₃ and 2 × 10¹⁷ molecules squalene on glass slides). The volatile products and acetone spectra can be viewed in Figure 3. Characteristic gas phase acetone bands include those at 1740, 1370, and 1230/cm (PNNL 2008), while characteristic 4-OPA bands include 2970, 2921, 2821, 2722, and 1740/cm (Fruekilde et al., 1998), allowing for identification by spectral comparison. Acetone (1) is a product of primary and secondary reactions, while 4-OPA (8) is a product of secondary reactions.

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**Fig. 2** Increasing absorption at three spectral regions associated with formation of surface oxidation products under various ozone concentrations. (a) Build up of -OH absorbance bands are prevalent at 22 ppm ozone, (b) carbonyl peak broadens and red-shifted as ozone concentration is increased, and (c) more C-O bonds are observed at 200 ppb ozone than 40 ppb or 22 ppm.
reactions exclusively (Fruekilde et al., 1998), as shown in Scheme 1.

Although, Scheme 1 indicates the formation of carboxylic acids, this analysis required additional derivatization methods that were not available at the time of experimentation.

Reaction mechanisms

Based on kinetics, products, and known ozone-terpene reaction mechanism, the squalene-ozone reaction mechanism is postulated in Scheme 1.

Reactions between ozone and unsaturated compounds are known to occur via addition of ozone across the double bond to form an unstable primary ozonide. In the case of squalene, six double bonds are available, with no apparent preference based on electron withdrawing groups, stability of cations, or steric hindrance (Nunes et al., 2005). The primary ozonide immediately cleaves into an aldehyde or ketone and a Criegee intermediate (Scheme 1, paths a–b, c–d, and e–f). The Criegee intermediate can stabilize or decompose via various pathways including dissociation, ester/isomerization, and H-migration/hydroperoxide.
The loss rate of squalene ozonolysis can be described by Equation (1). In excess ozone (i.e. present experimental conditions) the rate law can be written as pseudo-first-order with respect to squalene (Equation (2)), where: [sq]₀ is the surface concentration of squalene, [O₃]₀ is the surface concentration of ozone, k₁ is the pseudo-first-order surface rate constant, k is the second-order surface rate constant, and t is the time interval. Given that the rate loss of squalene is equal to the rate formation of products, the experimental data can be plotted and fit to a negative exponential growth model (Equation (3)), where [P]ᵢ and [P]ₜ is the product concentration at time t and time infinity, and kᵡ may be extracted.

\[
\frac{d[\text{sq}]}{dt} = -k[\text{O}_3][\text{sq}], \quad (1)
\]

\[
\frac{d[\text{sq}]}{dt} = -k_1[\text{sq}] \quad \text{where} \quad k_1 = k[\text{O}_3], \quad (2)
\]

\[
[P]_t = [P]_\infty (1 - \exp(-k_1t)). \quad (3)
\]

As absorbance is linearly proportional to concentration according to the Beer–Lambert law, the ratios between concentrations or absorbance will yield the same observed k₁. Thus absorbance values were used instead of concentrations, for simplicity.

At times close to zero, Equation (3) becomes linear (based on Taylor expansion of 1-exp⁻ᵃᵃ with a slope of k₁. In experiments with ozone levels ≤ 250 ppb no secondary oxidation reactions were observed and long experimental durations were required for reaching [P]ₜ. Thus, in these experiments, k₁ was extracted from the initial linear rate, to eliminate sources of error from changing experimental conditions during such durations.

Alternately, at high ozone concentrations (> 2 ppm), where a reduction in carbonyl absorbance during reaction indicated secondary reaction, spectral analysis was performed via the ‘inverse method’. Detailed description of this procedure is given in Segal-Rosenheimer and Dubowski (2008). In general, within the appropriate wavenumber range (3100–2700/cm, which did not show formation of secondary reaction products), the linear combination of initial and final spectra was calculated for each spectrum during reaction. The coefficients of final and initial spectra were plotted as a function of reaction time and the exponential fitting used to extract k₁.

For confirmation, both methods, integrated absorbance at the carbonyl band and inverse linear combination, were used at mid-ozone levels and the resulting k₁ values were equal (within experimental error).

Figure 5 (note the log-log scale) shows that the k₁ values at the various ozone concentrations fit well by Equation (4), suggesting a Langmuir-Hinshelwood type mechanism.
Where: $k_1$ (s) is the pseudo-first-order reaction rate constant, \( k \) (\( \text{cm}^2\text{s/molecules} \)) is the second order surface rate constant, \([S]\) (\( \text{molec/cm}^2 \)) is the total surface density of \( \text{O}_3 \) adsorption sites, \( K_{\text{O}_3} \) (\( \text{molec/cm}^3 \)) is the ratio between ozone adsorption and desorption rate coefficients, and \([\text{O}_3]_{\text{gas}}\) is the gaseous ozone concentration near the surface (\( \text{molecule/cm}^3 \)).

The parameters \( k[S] \) (the maximum first order surface rate constant in the presence of saturated ozone sites) and \( K_{\text{O}_3} \) obtained from the fit are 0.0048 ± 0.0007/s and \((2.6 \pm 0.73) \times 10^{-15} \text{molec/cm}^3\), respectively. This is in agreement with the accumulating evidence in the literature showing that many heterogeneous processes proceed via quick chemisorption of gaseous reactant (ozone) followed by slower surface reaction with sorbed reactant (squalene) (Ammann et al., 2003; Dubowski et al., 2004; Mmereki et al., 2004). As the deviation from linear correlation between the observed \( k_1 \) and \([\text{O}_3]_{\text{gas}}\) occurs only at very high ozone levels (\( > 5 \times 10^{15} \text{molec/cm}^3 \)), the possibility that under such extreme levels, additional mechanistic effects are resulting in lower rate constants rather than saturation of surface sites (i.e. L-H mechanism), cannot be ruled out.

Comparison to previous studies of surface oxidation is not straightforward as both substrate chemical (Kwamena et al., 2007) and physical properties (Kahan et al., 2006; Pöschl et al., 2001) affect the surface kinetics. However, \( k[S] \) and \( K_{\text{O}_3} \) for ozonolysis of thin film cypermethrin adsorbed on ZnSe were determined to be 0.0007 ± 0.0001/s and \((4.7 \pm 1.7) \times 10^{-16} \text{molec/cm}^3\), respectively (Segal-Rosenheimer and Dubowski, 2007). The complexity of the cypermethrin structure and the deactivation of its double bond because of the adjacent chlorines, are most likely the causes of the lower \( K_{\text{O}_3} \) and \( k[S] \) observed for its reaction on ZnSe. Recently, Wells et al. (2008) reported reaction probability of 4.5 \((\pm 1.4) \times 10^{-4}\) for ozone uptake by squalene film on glass. This is approximately 45 times higher than the reaction probability calculated based on the present obtained kinetics (following the procedure of Pöschl et al., 2001). The current research employed thicker squalene films relative to those used by Wells et al. (2008) (10–100 layers vs. sub-monolayer, respectively). However, previous studies on oleic acid (which like squalene is liquid under room temperature) have shown that inhomogeneous films with a multilayer island pattern are formed at both sub- and multilayer quantities (Kwamena et al., 2006; Rosen et al., 2008). If not all molecules were accessible for reaction in the current setting, it may have lead to an underestimation of the reaction probability. On the other hand, as Wells et al. (2008) extracted squalene oxidation kinetics by monitoring ozone concentrations at the reactor’s outlet, ozone loss via secondary gas phase reactions would lead them to overestimate surface reaction probability.

Reaction probabilities for ozone with sebum containing clothing (Coleman et al., 2008) and hair (Pandrangi and Morrison, 2008) were found to be on the order of \( 10^{-4} \) and \( 10^{-4} \) to \( 10^{-5} \), respectively. Differences are not surprising given the disparity in medium and morphology and considering that squalene is only one of many ozone reactive components of sebum.

Effect of RH

The effect of RH on kinetics was measured at moderate levels of ozone (40–50 ppb). The difference in oxidation kinetics at RH < 10% (\( N = 3 \)) and at RH > 80% (\( N = 6 \)) was not statistically significant (\( t \)-test at \( \alpha = 0.05 \)), although differences in products formation could be observed spectrally in the fingerprint region (see Figure 6). Comparable carbonyl frequencies were observed, but the absorbances associated with -C-O bonds under dry conditions were not present under

![Fig. 5](image-url)  
**Fig. 5** Plot of pseudo-first-order surface rate constants vs. gas phase ozone concentration for the reaction of squalene film with ozone. Error in ozone concentration reflect uncertainties in spectrophotometer readings and flow rates, while error in rate constants reflect statistical error of data fittings.

![Fig. 6](image-url)  
**Fig. 6** Comparison of oxidized squalene film under [\( \text{O}_3 \)] = 40 ppb, low and high RH (dashed and solid lines, respectively). Differences between dry and humid conditions observed in the fingerprint region, while similarities are observed in the carbonyl region.
high RH indicating a change in reaction pathway(s). Instead of following the hydroperoxide channel (Scheme 1), stabilized Criegee radical may be quenched by reaction with water vapor to form α-hydroxyhydroperoxides followed by decomposition to carbonyls plus hydroperoxide (Sauer et al., 1999; Winterhalter et al., 2000). However, no observed change in OH radical formation during gas phase alkene ozonolysis in 36–74% RH was observed by Atkinson and Arey (2003). This implies that certain aspects of the mechanism are still not yet fully understood. Surface product identification showed no change from dry conditions (products (4), (5), (6), (7), and (8) positively identified). However, qualitative comparison showed a product distribution shift from predominately low molecular weight ketones, to an even distribution of ketones and high molecular weight aldehydes when RH was high, similar to the increased aldehyde yield observed in gas phase ozonolysis of α-pinene under humid conditions (Warscheid and Hoffmann, 2001).

Effect of temperature

The temperature effect at 24°C, 37°C and 58°C (± 2°C) was observed on the flow-through ATR spectrometer set up at [O₃] = 400 ± 20 ppb and air exchange rate (AER) of 3/h, and for simplification, using three small reactors (v = 0.16 l each) set parallel and submerged in water baths at the specified temperatures. In the later, [O₃] = 500 ± 20 ppb and AER = 7.5 and 32.2/h were used. All temperature experiments were run under dry conditions. The same surface products were identified, although as temperature increased, desorption was enhanced and the amount of products retained on the surface decreased. Volatility of squalene itself was less sensitive to temperature changes in this range. Therefore, surface kinetics were compared through quantification of remaining squalene after equal reaction times (using extraction and GC analysis). Similar to Thornberry and Abbott (2004) and de Gouw and Lovejoy (1998) who observed weak or very low temperature dependence on ozone uptake coefficient on unsaturated organic liquids, no significant effect on surface kinetics could be observed within experimental error. Not surprisingly, at higher temperature, kinetics of gas phase secondary reactions were faster, resulting in a shift to lower molecular weight gaseous products. Significant amounts of formaldehyde (3), acetaldehyde, and acetone (1) were identified in the gas phase. Under the low AER conditions (3/h), the molar yields of formaldehyde, acetaldehyde, and acetone increased as temperature increased from 24°C to 58°C. At the higher AER conditions (32/h), the same gas phase products were observed, but in different distributions and lower yields, most likely because of the significant decrease in residence time for secondary reaction formation. This is in agreement with Weschler et al. (2007), who observed a higher ratio of volatile 4-OPA to 6-MHO at AER = 4.4/h vs. 8.8/h during ozone reaction with an occupied aircraft cabin. Yields for formaldehyde and acetaldehyde were similar for all temperatures, while acetone increased as temperature increased. In addition, trace levels of gaseous 6-MHO were detected but in levels that were too low for quantification. Interestingly, Coleman et al. (2008) reported much higher 6-MHO formation under experimental conditions of [O₃] = 120 ppb and AER = 23/h. The absence of 6-MHO in the current experiments may be because of the higher ozone levels resulting in quick secondary oxidation of 6-MHO into acetone and other lower molecular weight compounds. The increased acetone yield with increased temperature highlights the gas phase kinetics dependence, while the reduction in secondary gas phase product yields at higher AER reflects the effect of lower residence time. Additional identified products included glyoxal and pyruvic acid, low molecular weight bicarboxyls.

Effect of NOₓ

To obtain a general impression of the impact of NOₓ presence during ozonolysis, separate NOₓ studies were performed under extreme concentrations. As mentioned above, the gas mixture introduced to the ATR reactor was withdrawn from two Teflon chambers attached symmetrically. Separate experiments determined that the pump withdrawal was equal from both chambers. Initial conditions in the NOₓ chamber were as follows: [NO] = 1.5 ± 0.7 ppm, [NOₓ] = 4.8 ± 0.4 ppm, and [O₃] < 50 ppb, while the second chamber contained [O₃] = 12.3 ± 0.4 ppm. Given the flow rate, dilution, tube dimensions, and rate constants of equations (5–8), (Sander et al., 2003) the concentrations entering the reactor were determined as follows: [NO] = 50 ± 25 ppb, [NOₓ] = 2.5 ± 0.3 ppm, [O₃] = 6.2 ± 0.1 ppm, and [NO₃] = 1 ± 0.15 ppb (nitrate photolysis in the transfer line was calculated to be negligible).

\[
NO + O_3 \rightarrow NO_2 + O_2, \quad k = 1.9 \times 10^{-14} \text{cm}^3/\text{molec/s} \tag{5}
\]

\[
NO_2 + O_3 \rightarrow NO_3 + O_2, \quad k = 3.2 \times 10^{-17} \text{cm}^3/\text{molec/s} \tag{6}
\]

\[
NO_2 + NO_3 \rightarrow N_2O_5, \quad k = 1.2 \times 10^{-12} \text{cm}^3/\text{molec/s} \tag{7}
\]

\[
N_2O_5 \rightarrow NO_2 + NO_3, \quad k = 3.8 \times 10^2 \text{cm}^3/\text{molec/s}. \tag{8}
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The reactions with squalene film were monitored in real time. In addition to carbonyls, the products of
NOx reactions are expected to contain organonitrates, often found to have higher vapor pressures (Presto et al., 2005; Zhang et al., 2006). Therefore, absorbance bands associated with the alkane, -CH3 and -CH2 groups (3025–2810/cm), were selected to monitor kinetics instead of the carbonyl region. For comparison, ozone experiments in the absence of NOx were performed in this experimental set-up under similar experimental conditions ([O3] = 6.1 ± 0.7 ppm). There was no statistical difference (t-test, x = 0.05) found between the pseudo-first-order reaction rate of squalene with NOx/O3 and just O3 for the limited number of experiments performed in this setting (N = 4). However, differences in product formation were observed spectrally including the presence of organonitrates and organoperoxyxynitrates (Figure 7). The formation of absorption bands at 1630, 1280, and 867/cm are consistent with organonitrates (Allen et al., 1994; Hallquist et al., 1999), while the band at 1291/cm is consistent with organoperoxyxynitrates. The absence of the characteristic organoperoxyxynitrates 790/cm band may be because of a blue-shift into the 867/cm peak as observed by Hung et al. (2005).

**Conclusion**

The ubiquitous nature and high unsaturation of squalene makes it an important compound for indoor pollution studies. Therefore, surface ozonolysis reaction of squalene was investigated. Extracting kinetics at various levels of ozone under dry conditions at 24 ± 2°C showed that the reaction can be fitted to a Langmuir–Hinshelwood reaction mechanism, with a maximum pseudo-first-order surface rate constant (i.e. \( k_{[S]} \)) of 0.0048 ± 0.0007/s and \( K_{O_3} = (2.6 ± 0.73) \times 10^{-15} \) molec/cm^2. Surface kinetics were unaffected by an increase in RH (> 70%), however, a shift in product distribution from ketones to aldehydes was observed. Additionally, kinetics were insensitive to temperature in the range 24–58 (±2)°C. At moderate ozone levels of 40 ppb, a pseudo-first-order reaction rate of 1.22×10^{-5}/s was determined, describing a half life of 15.7 h. With respect to squalene, which is naturally and continually produced by the human body, a more appropriate description of its impact might be with regard to its concentration on the skin and subsequent product formation. Using a sebum thickness of 0.7 µm with a 5% w:w ratio squalene to sebum (Chi and Obendorf, 1998), and molecular geometry similar to anthracene (Kwamena et al., 2006), squalene density on the skin was calculated on the order of 4 × 10^{15} molecules/cm^2. At a moderate ozone level of 40 ppb, that would lead to a minimum product formation flux of 4 × 10^{10} molecules/cm^2/s, which is on the same order of magnitude as VOC emission rates observed from reaction between laundered and soiled clothing fabrics and 55–116 ppb ozone (Coleman et al., 2008).

Surface products identified included 6-MHO (4), TOP (5); geranyl acetone (6); and TTT (7); as well as unidentified long chain alcohols. All products were observed at 24°C under moderate ozone conditions with or without RH. However, as temperature increased, the products partitioning between surface and gas phase changed, with less products remaining on the surface consistent with their volatilization (e.g. 6-MHO was not detected at 37°C or 58°C, while TTT was).

Gaseous products included acetone (1), formaldehyde (3), acetaldehyde, glyoxal, and pyruvic acid at various yields and ratios depending on ozone level, temperature, and AER. At the higher ozone levels and at lower AER, higher yields of the low molecular weight products were detected. In addition, higher temperatures accelerated secondary gas-phase reactions, resulting in a higher concentration of low molecular weight secondary products. For comparison, Fruekilde et al. (1998) observed acetone, 6-MHO, geranyl acetone, and 4-OPA at significant concentrations in the gas phase. However, their experimental conditions included a very high AER (flow of 1 l/min in a flow tube of 0.5 cm ID) at lower ozone levels (45–100 ppb vs. 400–500 ppb). As increased ozone level, AER, and temperature have all been shown to enhance secondary oxidation products, it is difficult to predict exactly the oxidation products under normal indoor ozone levels of 40 ppb and AER of 0.6–2/h (USEPA 2005). However, it is likely that the low molecular weight carbonyls and bicarbonyls will be more prevalent.

This study highlights the potential of squalene as a significant contributor to indoor pollution via ozonolysis reactions. This is of particular concern because of the possible health implications associated with the identified squalene oxidation products. For instance, formaldehyde is a known human carcinogen (IARC 2006), and bicarbonyl compounds like 4-OPA, glyoxal, and pyruvic acid are characterized as asthmatics and sensitizers via induction of cellular damage (Anderson et al., 2007; Jarvis et al., 2005). In addition, NOx experiments showed the formation of nitrated surface oxidation products, which might have higher adverse
health potential than their nitrate free counterparts (Franze et al., 2005; Pitts, 1983). The higher volatility of the nitrated products seen by Presto et al. (2005) and others, make these secondary pollutants a greater concern because of the high abundance of squalene near the body.

Squalene is naturally occurring and continually produced by the human body, therefore its concentration in the indoor environment cannot be controlled. However, this study highlights the importance of AER, temperature and ozone level in the indoor environment on the formation of potentially harmful squalene oxidation products.

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