Reactions of Halogenated Hydroperoxides and Peroxyl and Alkoxyl Radicals from Isoflurane in Aqueous Solution

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Model systems, based on aqueous solutions containing isoflurane ($CHF_2OCHClCF_3$) as an example, have been studied in the presence and absence of methionine (MetS) to evaluate reactive fates of halogenated hydroperoxides and peroxyl and alkoxyl radicals. Primary peroxyl radicals, $CHF_2OCH(OO^{\bullet})CF_3$, generated upon 1-e-reduction of isoflurane react quantitatively with MetS via an overall two-electron oxidation mechanism to the corresponding sulfoxide (MetSO). This reaction is accompanied by the formation of oxyl radicals $CHF_2OCH(O^{\bullet})CF_3$ that quantitatively rearrange by a 1,2-hydrogen shift to $CHF_2OC^{\bullet}(OH)CF_3$. According to quantum-chemical calculations, this reaction is exothermic ($\Delta H = -5.1$ kcal/mol) in contrast to other potentially possible pathways. These rearranged $CHF_2OC^{\bullet}(OH)CF_3$ radicals react further via either of two pathways: (i) direct addition of oxygen or (ii) deprotonation followed by fluoride elimination resulting in $CHF_2OC(O)CF_2^{\bullet}$. Route i yields the corresponding $CHF_2OC(OO^{\bullet})(OH)CF_3$ peroxyl radicals, which eliminate $H^+/O_2^{\bullet-}$. The resulting ester, $CHF_2OC(O)CF_3$, hydrolyzes further, accounting for the formation of HF, trifluoroacetic acid, and formic acid with a contribution of 45% and 80% in air- and oxygen-saturated solutions, respectively. A competitive pathway (ii) involves the reactions of the secondary peroxyl radicals, $CHF_2OC(O)CF_2OO^{\bullet}$. The two more stable of the three above mentioned peroxyl radicals can be distinguished through their reaction with MetS. Although the primary $CHF_2OCH(OO^{\bullet})CF_3$ oxidizes MetS to MetSO in a 2-e step, the majority of the secondarily formed $CHF_2OC(O)CF_2OO^{\bullet}$ reacts with MetS via a 1-e transfer mechanism, yielding $CHF_2OC(O)CF_2OO^-$, which eventually suffers a total breakup into $CHF_2O^- + CO_2 + CF_2O$. Quantum-chemical calculations show that this reaction is highly exothermic ($\Delta H = -81$ kcal/mol). In air-saturated solution this pathway accounts for about 35% of the overall isoflurane degradation. Minor products (10% each), namely, oxalic acid and carbon monoxide originate from oxyl radicals, $CHF_2OC(O)CF_2O^{\bullet}$ and $CHF_2OCH(O^{\bullet})CF_3$. An isoflurane-derived hydroperoxide $CHF_2OCH(OOH)CF_3$ in high yield was generated in radiolysis of air-saturated solutions containing isoflurane and formate either via a H-atom abstraction from formate by the isoflurane-derived peroxyl radicals or by their cross-termination reaction with superoxide $O_2^{\bullet-}$. $CHF_2OCH(OOH)CF_3$, is an unstable intermediate whose multistep hydrolysis is giving $H_2O_2 + 2HF + HC(O)OH + CF_3CH(OH)_2$. In the absence of MetS, about 55% of $CHF_2OCH(OO^{\bullet})CF_3$ undergo termination via the Russell mechanism and 27% are involved in cross-termination with superoxide ($O_2^{\bullet-}$) and peroxyl radicals derived from *t*-BuOH (used to scavenge $^{\bullet}OH$ radicals). The remaining 18% of the primary peroxyl radicals undergo termination via formation of alkoxyl radicals, $CHF_2OCH(O^{\bullet})CF_3$.

Introduction

The chemical and biomedical interest in isoflurane ($CHF_2OCHClCF_3$) as a target for redox and free radical attack relative to the, e.g., much more studied halothane ($CF_3CHClBr$) lies in its molecular structure. It contains an ether bridge and also carries a CHF_2 group, which, in contrast to the CF_3 group, is less stable toward oxidative attack. Both structural parameters render isoflurane-derived radicals much more labile species with respect to bond cleavage and hydrolysis. In addition to these structural aspects, two further reasons can be forwarded as justification for the study presented and discussed in this paper. For one, isoflurane belongs to the family of halogenated anaesthetics that are still widely used for the maintenance of anaesthesia of outpatients.[1−3] Isoflurane induces and maintains general anesthesia by depression of central nervous functions and resultant loss of consciousness. Despite its low rate of biotransformation (approximately 0.2% in humans) some metabolites, e.g., trifluoroacetic acid (TFAA), are thought to induce rare, although serious side effects such as hepatotoxicity.[4] Other serious side effects known are tachycardia, respiratory depression, nausea, and disturbance of liver function. All these medical effects and clinical conditions can be related, at least in part, to its redox and radical chemistry.

Furthermore, the degradation of isoflurane has an important environmental aspect. Together with halothane and other chlorofluoro hydrocarbons, isoflurane has been used for many

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years for a variety of purposes. It is estimated that the current emissions of halothane and isoflurane are 1500 and 750 tons/yr, respectively.[5] Atmospheric oxidation processes of halothane[6] and isoflurane[7] result, for example, in the formation of $CF_3C(O)Cl$, of which approximately 60% undergo hydrolysis to give trifluoroacetic acid with a global deposition rate of 800 tons/year. TFAA has been detected at low levels in surface water, rain, and tropospheric air samples.[8,9] The bulk of existing data suggests that TFAA is a chemically almost undegradable, long lasting environmental sink in fluorocarbon chemistry.[10]

All redox and radical reactions of organic material in an oxygen-containing, i.e., also natural, environment inherently invoke the formation of peroxyl radicals. If the latter carry halogen functionalities, their chemistry is particularly interesting and complex. Halogenated peroxyl radicals are not only resonably good one-electron oxidants, but with certain compounds, such as organic sulfides or inorganic iodide, they also readily engage in multi-electron oxidation mechanisms[11−13] affecting both product yields and the identity of the products. For this reason we have conducted experiments in systems containing methionine. This amino acid serves, first of all, the function of an organic sulfide. But, in addition, it establishes direct relevance to the above-mentioned biological (medical) implication and, furthermore, is an easy-to-handle, not smelling organic sulfide.

One of the important product radicals generated in the multistep oxidation by halogenated peroxyl radicals are alkoxyl radicals. Recently, we have investigated this in detail for the free-radical degradation of halothane,[14] employing MetS as a selective agent for quantitative transformation of its halogenated peroxyl radicals into corresponding alkoxyl radicals. The aim of this present work has, therefore, been to investigate the key intermediates and final products of isoflurane degradation to provide the chemical basis for a better understanding of its toxic side effects.

It is well-known that halogenated peroxyl and alkoxyl radicals and hydroperoxides are the key intermediates in the redox-mediated degradation of halogenated organic compounds. In many cases it is, however, very difficult to distinguish between the final stable products derived from $RHalOO^{\bullet}$, $RHalO^{\bullet}$, or RHalOOH, as they are often the same. Therefore, the more general purpose of our study is to enable this distinction. Our approach is based on model systems that allow selective generation of halogenated hydroperoxides or oxyl radicals and the study of their reactive fates irrespective of their precursor.

Experimental Section

All chemicals were laboratory reagent grade and used without further purification. Isoflurane was received from Maybridge Chemical Co. Ltd. TFAA, 2-methylpropan-2-ol, *d*,*l*-methionine (MetS), and *d*,*l*-methionine sulfoxide (MetSO) were supplied by Aldrich. Oxalic acid, HCOONa, NaCl, NaF, and Br_2 were received from Merck. Trifluoroacetaldehyde hydrate, $CF_3CH(OH)_2$ (TFAAld), was obtained from Maybridge Chemical Co. Ltd. 2-Methylpropan-2-ol was distilled before using.

Solutions were always prepared freshly from Millipore-filtered water ($R > 18$ MΩ). The desirable pHs of the solutions were adjusted by addition of $HClO_4$ or NaOH.

The γ-radiolysis was carried out in the field of a ^{60}Co γ-source. Total adsorbed doses, as determined by Fricke dosimetry,[15] were in the range 50−300 Gy. The pulse radiolysis experiments were carried out with a 12 MeV linear electron accelerator (50 ns electron pulses, Institut für Oberflächenmodifizierung e. V., Leipzig, Germany).[16]

TABLE 1: Reductive Degradation of Isoflurane in 10 mM Air-Saturated Aqueous Solution Containing 5 vol % *t*-BuOH in the Absence or Presence of 1 mM MetS[a]

product	without MetS (system I)	with MetS (system II)
HCl	2.0	2.0
HF	4.6	7.3
$CF_3C(O)OH$ (TFAA)	0.7	0.9
CF_3CHO (TFAAld)[b]	1.1	0
CO_2	0.3	1.6
HC(O)OH	2.0	2.0
HO(O)CC(O)OH	0.03	0.2
CO	0.03	0.2
MetSO		2.4
total fluorine	10.0 (100%)	10.0 (100%)
total carbon	6.0 (100%)	6.0 (100%)

[a] Products and their yields (G values). Dose rate = 0.35 Gy s^{-1}. [b] As hydrate.

Ionic products (including CO_2, which was analyzed in the form of HCO_3^- ions) were measured by high-performance ion chromatography (IC) employing a Dionex 2010i machine. TFAAld was analyzed in the form of TFAA by means of IC after oxidation with excess NaOBr at pH 12.3, reflux for 2 h at 85 °C, and cooling to room temperature. Analysis of CO was made by gas chromatography. The yields of MetSO were determined by HPLC (Inertsil column, 250 × 4.6 mm, 5 μ, ODS II, H_2O/CH_3OH = 95/5 vol % as eluent at 1 dm^3 min^{-1}, UV detection at 210 nm). Hydroperoxides were determined using Allen's reagent.[17] Separation of the (hydro)peroxides by HPLC was on a 25 cm reversed phase column using water as eluent. The (hydro)peroxides were detected by postcolumn reaction with molybdate-activated iodide.

The quantum-chemical calculations were carried out using the Gaussian 03 package.[18] For the systems under study, geometries were optimized by applying the density functional theory (DFT) approach with the B3LYP hybrid functional.[19,20] Stationary points were characterized by frequency calculations. For geometry optimizations, the standard 6-31+G(d,p) basis sets[21] were used. To investigate the influence of a solvent on the molecular structure of the radicals, geometry optimizations were carried out using the self-consistent reaction field (SCRF) polarized continuum model (PCM).[22,23]

Radiation chemical yields are given in units of 10^{-7} mol J^{-1} absorbed energy and denoted as G, i.e., in the following the factor of "10^{-7}" and the dimension "mol J^{-1}" will not explicitly be written for reason of simplification. The G have been determined by the commonly used standard procedure, i.e., from the initial slopes of yield−dose plots, covering typically 4−5 measurements. Error limits of all radiation based data are estimated to 10% at most, unless specifically noted.

All experiments were done at room temperature.

Results and Discussion

Two different air-saturated, aqueous systems containing 0.01 mM isoflurane and 5% (v/v) *t*-BuOH were investigated with respect to final products and yields obtained upon γ-irradiation: one in the absence of MetS (system I), the other in the presence of 0.001 mM MetS (system II). The results are listed in Table 1.

As in the case of halothane, $CF_3CHClBr$,[14] there is strong evidence that the mechanisms of isoflurane degradation in the absence and in the presence of MetS are completely different. For example, trifluoroacetaldehyde (TFAAld) is one of the major products in the system without MetS, but it is completely absent in the MetS-containing system. Carbon dioxide yields, on the

other hand, considerably increase in the presence of the organic sulfide (from $G = 0.3$ to 1.6). The same is true for the yields of the minor products CO and oxalate, which are increased by a factor of 7 in the presence of MetS. Fluoride yields also increase significantly, namely, by more than 50%. Comparing the isoflurane system with the halothane system, it is noted that, despite of some qualitative similarities, the respective quantitative aspects reveal significant differences, clearly reflecting the different molecular composition of the two halogenated compounds.

The radiation chemical basis for the reactions occurring in our systems are the primary radicals from the radiolysis of the solvent and the transients generated from their reactions with the isoflurane in the oxygenated, *t*-butanol-containing solutions.

Radiolysis of water results in the formation of three highly reactive radical species: hydrated electrons (e_{aq}^-), hydrogen atoms ($H^\bullet$), and hydroxyl radicals ($^\bullet OH$)[24] as described in

$$H_2O \xrightarrow{\gamma\text{-irradiation}} e_{aq}^-, H^\bullet, {}^\bullet OH, \text{other species} \qquad (1)$$

The radiation chemical yields of the primary species from water radiolysis, G, range from 0.6 for $H^\bullet$ to 2.8 for e_{aq}^- and $^\bullet OH$, respectively.

The reaction of isoflurane with hydrated electrons leads, in oxygenated solution, to the peroxyl radicals $CHF_2OCH(OO^\bullet)CF_3$. Mechanistically, their formation involves a dissociative electron capture and subsequent, fast oxygen addition to the thus generated C-centered radical (reactions 2a,b).

$$CHF_2OCHClCF_3 + e_{aq}^-, O_2 \rightarrow Cl^- + CHF_2OCH(OO^\bullet)CF_3 \qquad (2a,b)$$

The $^\bullet OH$ radicals will not directly react with isoflurane but, under the experimental conditions, will quantitatively be scavenged by *t*-BuOH, leading to the corresponding alkylperoxyl radicals (reactions 3a,b; 95% yield).[25]

$$(CH_3)_3COH + {}^\bullet OH, O_2 \rightarrow H_2O + {}^\bullet OOCH_2(CH_3)_2COH \qquad (3a,b)$$

The comparatively small yield of hydrogen atoms may undergo a variety of reactions but, under our experimental conditions, will be mostly scavenged by oxygen giving hydroperoxyl radicals (87%, $k_4 = 1.1 \times 10^{10}$ M^{-1} s^{-1}).[26]

$$H^\bullet + O_2 \rightarrow HO_2^\bullet / H^+ + O_2^{\bullet -} \qquad (pK_a = 4.8) \qquad (4)$$

The rest will react with MetS (10%, $G \approx 0.06$) and *t*-BuOH (3%, $G \approx 0.02$). Other possible reaction like H or Cl abstraction from isoflurane should be negligible, based on the available rate constants for structurally similar compounds.[26]

Both $HO_2^\bullet$ and $^\bullet OOCH_2(CH_3)_2COH$ radicals are unreactive toward MetS under the prevalent experimental conditions and, therefore, need not to be considered.

Fate of Halogenated Hydroperoxide Derived from Isoflurane. A molecular transient of mechanistic significance in our systems turned out to be the hydroperoxide, $CHF_2OCH(OOH)CF_3$. It is well-known that halogenated hydroperoxides (denoted in general terms as RHalOOH) are the products of 1-e reduction of $RHalOO^\bullet$ by appropriate electron donors, and numerous rate constants have been measured for such reactions.[26] However, it was impossible to evaluate reactive fates of RHalOOH, except for their immediate further reduction by the same electron donors. Therefore, we have created an alternative model system that avoids this complication, and in which RHalOOH is exclusively generated via an H-atom abstraction by $RHalOO^\bullet$ and/or cross-termination of the latter with $O_2^{\bullet -}$.

$CHF_2OCH(OOH)CF_3$, specifically, has been generated by γ-radiolysis of a system consisting of 10 mM isoflurane and 10 mM HCO_2Na in an air-saturated solution, starting at natural pH. The identifyable products were HCl, HF, H_2O_2, HC(O)OH, and $CF_3CH(OH)_2$ (TFAAld), formed at a stoichiometric ratio of 1:2:1:1:1.

Most interestingly, and other than in systems I and II, no trifluoroacetic acid $CF_3C(O)OH$ (TFAA) is formed. This shows that the hydroperoxide-precursor peroxyl radicals $CHF_2OCH(OO^\bullet)CF_3$ do not undergo self-termination reaction because this would lead to TFAA via either the Russell mechanism or the corresponding alkoxyl radicals $CHF_2OCH(O^\bullet)CF_3$ (see below). Thus, the only reactive pathways leading to hydroperoxide would be cross-termination of the peroxyl radical with superoxide $O_2^{\bullet -}$ (reaction 5) and possibly also hydrogen abstraction abstraction from formate (reaction 6). Sources for the superoxide are possible direct scavenging of e_{aq}^- and $H^\bullet$ by O_2, and formation through the sequence $^\bullet OH + HC(O)O^- \rightarrow CO_2^{\bullet -} + H_2O$, followed by $CO_2^{\bullet -} + O_2 \rightarrow O_2^{\bullet -} + CO_2$.

The hydroperoxide $CHF_2OCH(OOH)CF_3$ is an unstable intermediate, indicated by the fact that only H_2O_2 could be detected immediately after γ-irradiation, both by UV spectroscopy and via HPLC with postcolumn reaction, using activated Allen's reagent.[17] The experimentally observed products and product ratios $G(F^-)/G(Cl^-) = 2{:}1$ and $G(F^-)/G(\text{TFAAld}) = 2{:}1$ can be explained by a multistep hydrolysis of the hydroperoxide, leading to two HF, one HC(O)OH, and one $CF_3CH(OH)_2$ per each HCl equivalent (reactions 7−10). At present, there are no explicit kinetic data available for the hydrolysis reactions 7 and 8. Reaction 9 should be fast, in analogy to HCl elimination from CCl_3OH ($k > 8 \times 10^5$ s^{-1}).[27] The rate of hydrolysis of formyl fluoride HC(O)F could be of the same order as that for formyl chloride ($k \sim 6 \times 10^2$ s^{-1}).[28] It is noted, though, that HC(O)Cl predominantly fragments into HCl and CO (94%),[28] whereas HC(O)F does not fragment but only hydrolyzes.

$$CHF_2OCH(OO^\bullet)CF_3 + O_2^{\bullet -} + H^+ \rightarrow CHF_2OCH(OOH)CF_3 + O_2 \qquad (5)$$

$$CHF_2OCH(OO^\bullet)CF_3 + HC(O)O^- \rightarrow CHF_2OCH(OOH)CF_3 + CO_2^{\bullet -} \qquad (6)$$

$$\downarrow H_2O$$

$$CHF_2OCH(OH)CF_3 + H_2O_2 \qquad (7)$$

$$\downarrow H_2O$$

$$CHF_2OH + CF_3CH(OH)_2 \qquad (8)$$

$$\downarrow$$

$$HC(O)F + HF \qquad (9)$$

$$\downarrow H_2O$$

$$HC(O)OH + HF \qquad (10)$$

In conclusion, the mechanism outlined in reactions 5−10 explains all products observed in this special system without any involvement of transient oxyl radicals.

Reaction of Peroxyl Radicals Derived from Isoflurane with MetS. In the absence of formate as solute, practically no $O_2^{\bullet -}$ is available through the above reaction of $CO_2^{\bullet -}$ with oxygen. Also, any generation of $O_2^{\bullet -}$ through its elimination from the isoflurane-derived peroxyl radicals is negligible because of the fast and efficient scavenging of the latter by MetS. Consequently, no hydroperoxide is formed through this route. The

specific formate-free solution studied by radiolysis consisted of 10 mM isoflurane, 10 mM MetS and 20 vol % *t*-BuOH in an air-saturated aqueous solution at pH 6. The lack of TFAAld among the products means that also its precursor, i.e., the primary isoflurane hydroperoxide, is not formed in this system. This excludes specifically any contribution by a one-electron oxidation of methionine through the primary peroxyl radical, as hypothetically formulated in

$$CHF_2OCH(OO^\bullet)CF_3 + MetS + H^+ \not\rightarrow CHF_2OCH(OOH)CF_3 + MetS^{\bullet+} \quad (11)$$

This is corroborated by pulse radiolysis, which shows no detectable formation of any of the well-known follow-up transients of the sulfur-centered radical cation as there are, depending on pH and MetS concentration, the three-electron-bonded, strongly absorbing *intra*molecularly S∴N or *inter*molecularly S∴S bonded radical cations, respectively.

The obtained result does not completely exclude the possible occurrence of reaction 11 but, at least, sets the value of its rate constant below 1×10^6 M^{-1} s^{-1}. The latter was obtained for the analogous reaction of the peroxyl radicals from halothane, $CF_3CHClOO^\bullet$,[29] which should be of higher reactivity compared to $CHF_2OCH(OO^\bullet)CF_3$ due to the higher electron-withdrawing effect of the chlorine substituent compared to the CHF_2O group.

Hence, in analogy to the halothane system, primary peroxyl radicals derived from isoflurane are concluded to react with MetS via an overall 2-e oxidation mechanism, leading to MetSO and corresponding oxyl radicals:

$$CHF_2OCH(OO^\bullet)CF_3 + MetS \rightarrow CHF_2OCH(O^\bullet)CF_3\ (\mathbf{A}^\bullet) + MetSO \quad (12)$$

This reaction will also not be very fast, as it is in the case for peroxyl radicals with several halogen atoms at the peroxyl-carrying carbon.[11,12,30] But it is faster than, for example, most of the bimolecular termination processes of the peroxyl radical, e.g., the Russell mechanism, because that would result in (not detected) TFAAld.

In conclusion, all the molecular products generated in the above system besides MetSO must originate from reactions of the oxyl radical $CHF_2OCH(O^\bullet)CF_3$ (abbreviated in the following as $\mathbf{A}^\bullet$).

Reactions of Oxyl Radicals Derived from Isoflurane. Alkoxyl radicals, in general, are known as one-electron oxidants,[31] more powerful than the corresponding peroxyl radicals. For example, a reduction potential of $E^\circ = 2.3 \pm 0.3$ V has been calculated for the reaction $CCl_3O^\bullet + e^- \rightarrow CCl_3O^-$ whereas an E° of only 1.15 ± 0.16 V has been reported for $CCl_3OO^\bullet + e^- \rightarrow CCl_3OO^-$.[31] Much lower reduction potentials pertain, however, to oxyl radicals with less halogen substitution at the oxyl-carrying carbon. This should, accordingly, also apply to the isoflurane-derived one. Reaction 13, for example, clearly does not occur because the direct isoflurane product from 1-e oxidation of MetS, i.e., $CHF_2OCH(OH)CF_3$ would eventually hydrolyze to TFAAld (which has not been found).

$$\mathbf{A}^\bullet + MetS + H^+ \rightarrow CHF_2OCH(OH)CF_3 + MetS^{\bullet+} \quad (13)$$

Alternatively, oxyl radicals $\mathbf{A}^\bullet$ could undergo two possible β-fragmentations. Reaction 14 can be ruled out for two reasons: TFAAld was not detected and, according to our quantum chemical calculations, this step is highly endothermic ($\Delta H = +20.9$ kcal/mol). The other possibility, namely cleavage of a trifluoromethyl radical (reaction 15) cannot be proven or disproven on the basis of final products, all of which may originate through different routes as well. It can be eliminated though on the basis of mechanistic considerations and also because it is endothermic ($\Delta H = +4.8$ kcal/mol) (although much less than reaction 14). A most probable process, on the other hand, is an exothermic 1,2-H shift (reaction 16, $\Delta H = -5.1$ kcal/mol). This well-established reaction of alkoxyl radicals typically proceeds with rate constants in the 10^5-10^6 s^{-1} range.[32−37]

$$\mathbf{A}^\bullet \rightarrow CHF_2O^\bullet + CF_3CHO \qquad \Delta H = +20.9 \text{ kcal/mol} \quad (14)$$

$$\mathbf{A}^\bullet \rightarrow {}^\bullet CF_3 + CHF_2OCHO \qquad \Delta H = +4.8 \text{ kcal/mol} \quad (15)$$

$$\mathbf{A}^\bullet \rightarrow CHF_2OC^\bullet(OH)CF_3 \qquad \Delta H = -5.1 \text{ kcal/mol} \quad (16)$$

As will be further illustrated, all the final products from isoflurane can, indeed, be explained on the basis of reaction 16 and further transformations of the rearranged primary oxyl radical.

Follow-Up Reactions of $CHF_2OC^\bullet(OH)CF_3$. ***Route A:*** Trifluoroacetic acid (TFAA) is one of the characteristic products in both our systems I and II. Its formation in the MetS-containing solution can be explained by route A, involving oxygen addition to the rearranged oxyl radical with subsequent fast $HO_2^\bullet$ elimination from peroxyl radicals (reactions 17 and 18). Trifluoroacetic acid ester (TFAE) formed in this sequence undergoes hydrolysis giving TFAA and difluoromethanol (reaction 19) with the latter ending up as 2 HF and formic acid (reactions 9 and 10). Based on the yield of TFAA ($G = 0.9$) and the overall degradation yield of isoflurane (equal to the chloride yield, $G = 2.0$), the contribution of this pathway amounts to 45%. The results obtained from the γ-radiolysis of the same system but in oxygen-saturated solution, could be taken as an additional proof for the occurrence of route A. The selectivity of TFAA formation is now increasing up to 80%, based on the yields of TFAA and HCl ($G = 0.8$ and 1.0, respectively). Other possible routes are not leading to TFAA as it will be shown below.

$$CHF_2OC^\bullet(OH)CF_3 + O_2 \rightarrow CHF_2OC(OO^\bullet)(OH)CF_3 \quad (17)$$

$$CHF_2OC(OO^\bullet)(OH)CF_3 \rightarrow HO_2^\bullet + CHF_2OC(OH)CF_3\ (TFAE) \quad (18)$$

$$\downarrow H_2O$$

$$CHF_2OH + CF_3C(O)OH \quad (19)$$

Overall Stoichiometry of Route A:

$$HCl + 2HF + CF_3C(O)OH + HC(O)OH + MetSO$$

Route A predicts fluoride and TFAA formation at a ratio of 2:1. As can be realized from Table 1, the experimentally found overall ratio is, however, much higher ($G(HF)/G(TFAA) = 8$). Also, carbon dioxide, carbon monoxide, and oxalic acid are not yet accounted for. To explain the formation of these products, starting from $CHF_2OC^\bullet(OH)CF_3$, we assume this radical to exist in an acid−base equilibrium (eq 20) and to suffer β-elimination of fluoride from the anionic form, via reaction 21.

$$CHF_2OC^\bullet(OH)CF_3 \leftrightarrow CHF_2OC^\bullet(O^-)CF_3 + H^+ \quad (20)$$

This kind of fluoride elimination has been established in the case of halothane for the radical $CF_3C^{\bullet}(O^-)OH$,[14] and characterized as an exothermic reaction (according to our quantum-chemical calculation $\Delta H = -4.8$ kcal/mol). The same holds for our present radical for which fluoride elimination from its anionic form $CHF_2OC^{\bullet}(O^-)CF_3$ is even more exothermic ($\Delta H = -8.7$ kcal/mol). HF elimination from the neutral radical $CHF_2OC^{\bullet}(OH)CF_3$, on the other hand, is endothermic ($\Delta H = +7.8$ kcal/mol). Consequently, the pK_a of the hydroxyl group becomes a parameter of interest. A p$K_a = 6$ has been determined for the similar radical $CF_3C^{\bullet}(OH)H$.[38] Our radical, $CHF_2OC^{\bullet}(OH)CF_3$, should possess an even lower pK_a due to the stronger electron-withdrawing effect of the CHF_2O-group compared to that of H. Its pK_a may, in fact, be close to 1.7 reported for the radical $(CF_3)_2C^{\bullet}OH$.[39]

As indicated already by the dependence of the TFAA yield as function of oxygen concentration, the fluoride elimination is in competition with reaction 17. Reaction 21 leads to the generation of a secondary halogenated alkyl radical, which in the presence of oxygen will be transformed into the corresponding peroxyl radical via reaction 22.

$$CHF_2OC^{\bullet}(O^-)CF_3 \rightarrow F^- + CHF_2OC(O)CF_2^{\bullet} \quad (21)$$

$$\downarrow O_2$$

$$CHF_2OC(O)CF_2OO^{\bullet} \quad (22)$$

Follow-Up Reactions of $CHF_2OC(O)CF_2OO^{\bullet}$. This peroxyl radical may enter into two follow-up mechanisms, route B1 or B2, the initial processes being a 1-e or 2-e oxidation of MetS (reaction 23 and 26, respectively), giving two key intermediates: the halogenated hydroperoxide anion or an oxyl radical.

The fluorine substituents at the peroxyl-carbon render $CHF_2OC(O)CF_2OO^{\bullet}$ a much better oxidant than the primary isoflurane peroxyl radical, $CHF_2OCH(OO^{\bullet})CF_3$. A 1-e oxidation of MetS is, therefore, a feasible process, as has, in fact, been found to occur quite efficently (although not quantitatively) with, e.g., $CCl_3OO^{\bullet}$ as oxidant.[29] Further transformations of $CHF_2OC(O)CF_2OO^{\bullet}$ via route B1 are suggested to involve a complete breakdown of the carbon sceleton as depicted by reaction 24, which resembles an already described analogous process, namely, $^-O(O)CCF_2OO^- \rightarrow CO_3^{2-} + COF_2$.[40]

Route B1:

$$CHF_2OC(O)CF_2OO^{\bullet} + MetS \rightarrow CHF_2OC(O)CF_2OO^- + MetS^{\bullet+} \quad (23)$$

$$\downarrow$$

$$CHF_2O^- + CO_2 + CF_2O \quad (24)$$

$$\downarrow \qquad \downarrow H_2O$$

$$F^- + HC(O)F \qquad 2\,H^+/F^- + CO_2 \quad (25a,b)$$

Overall Stoichiometry of Route B1:

$$HCl + 5HF + 2CO_2 + HC(O)OH + MetSO + MetS^{\bullet+}$$

Reaction 24 can be viewed as a nucleophilic attack of the hydroperoxide anion onto the partially positive charged carbon atom in β-position. The resulting decomposition of the peroxyl anion leads to carbon dioxide, carbonyl difluoride, and the anion of difluoromethanol.

Further hydrolysis (reactions 25a,b and 10) is giving another equivalent of carbon dioxide and formic acid together with a high fluoride yield. Here, it is important to point out that reaction 24 has a strong driving force, because it is characterized by very high exothermicity ($\Delta H = -81.0$ kcal/mol) because of the formation of two carbonyl compounds (CO_2 and COF_2). The contribution of route B1 will be estimated later because none of the products is specific for this route and, consequently, requires quantitative knowledge of the contributions by the other routes.

The 2-e oxidation (reaction 26) competes with the 1-e oxidation and is the starting process of route B2. It provides plausible pathways for the formation of oxalic acid and carbon monoxide, which are minor but specific products. The key intermediate in this B2 mechanism is the oxyl radical $CHF_2OC(O)CF_2O^{\bullet}$. It is a powerful oxidant and reacts with MetS (reaction 27) under formation of the $MetS^{\bullet+}$ radical cation and the halogenated oxyl anion. Under release of fluoride (reaction 28) and hydrolysis (reaction 29) the latter turns into the oxalic acid ester $CHF_2OC(O)C(O)OH$. This molecular intermediate, in turn, further degrades into various products (reactions 30a and 30b). CHF_2OH, as an unambiguous ester hydrolysis product, will eventually end up as formic acid and fluoride (reactions 9 and 10). The remainder of the transient molecule may, however, enter two possible pathways, yielding either oxalic acid as a direct ester hydrolysis product (reaction 30a) or CO and CO_2 as a result of simple fragmentation (reaction 30b). Both pathways appear reasonable because they are reminiscent of well-known degradation processes in the chemistry of halogenated organic molecules. For example, HC(O)Cl predominantly fragments into CO + HCl, and HC(O)F predominantly hydrolyzes to HC(O)OH. An alternative mechanism yielding CO, based on β-cleavage of the above oxyl radical under formation of CF_2O and CHF_2OCHO, and CO elimination from the latter aldehyde is discarded because this process is endothermic ($\Delta H = +5.1$ kcal/mol).

Route B-2:

$$CHF_2OC(O)CF_2OO^{\bullet} + MetS \rightarrow MetSO + CHF_2OC(O)CF_2O^{\bullet} \quad (26)$$

$$CHF_2OC(O)CF_2O^{\bullet} + MetS \rightarrow MetS^{\bullet+} + CHF_2OC(O)CF_2O^- \quad (27)$$

$$\downarrow$$

$$CHF_2OC(O)C(O)F + F^- \quad (28)$$

$$\downarrow H_2O$$

$$CHF_2OC(O)C(O)OH + HF \quad (29)$$

$$CHF_2OC(O)C(O)OH + H_2O \rightarrow CHF_2OH + HO(O)CC(O)OH \quad (30a)$$

$$\rightarrow CHF_2OH + CO + CO_2 \quad (30b)$$

Overall Stoichiometry of Route B-2:

$$HCl + 5HF + [C(O)OH]_2 + CO + HC(O)OH + 2MetSO + MetS^{\bullet+}$$

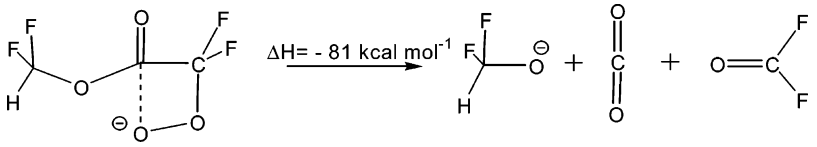

TABLE 2: Comparison of the Calculated and Experimental Yields (*G* Values) of Products in System II (with MetS).

product	route A 45%	route B1 35%	route B2 20%	total calcd	total exptl
HCl	0.9	0.7	0.4	2.0	2.0
HF	1.8	3.5	2.0	7.3	7.3
$CF_3C(O)OH$	0.9	0	0	0.9	0.9
CF_3CHO	0	0	0	0	0
CO_2	0	1.4	0.2	1.6	1.6
HC(O)OH	0.9	0.7	0.4	2.0	1.8
HO(O)CC(O)OH	0	0	0.2	0.2	0.2
CO	0	0	0.2	0.2	0.2
MetSO	0.9	0.7	0.8	2.4	2.4
total fluorine	4.5	3.5	2.0	10.0	10.0
total carbon	2.7	2.1	1.2	6.0	6.0

Both oxalic acid and CO are exclusive markers for route B2. Each of them is formed at a yield of $G = 0.2$, accounting for 10% each of the overall mechanism. Their combined yield of $G = 0.4$, accordingly makes up for a total contribution of 20% by route B2.

Routes A and B2 together account for 65% of the overall isoflurane degradation; hence route B1 should be responsible for the remaining 35%. To check this against the material balance, the individual product yields via the various routes were calculated on the basis of their above evaluated contributions and compared to the experimental values. The results are presented in Table 2.

As can be seen, the calculated product yields arising from isoflurane are in excellent agreement with the experimental ones. So, the proposed mechanism is adequately describing the formation and yields of all products.

Because all the products are coming from the isomerized alkoxyl radicals $CHF_2OC^{\bullet}(OH)CF_3$ ($G = 0.9$) or its further product $CHF_2OC(O)CF_2^{\bullet}$ ($G = 1.1$) the rate constant for the 1.2-H-shift (reaction 16) can be estimated to $k_{16} = 4.6 \times 10^5$ s^{-1} under the reasonable assumption of $k_{18} = 2 \times 10^9$ M^{-1} s^{-1} applying for the majority reactions of alkyl radicals with oxygen.[41]

Concerning the material balance discussed above, one may ask the question to what extent the $MetS^{\bullet+}$ radical cations (generated in reactions 11, 13, 23, and 27) may directly contribute to the various, above listed products. The only possible candidate would be CO_2, which is efficiently generated in the $^{\bullet}OH$-induced oxidation of methionine.[42] In our peroxyl-induced system this does, however, not take place because in any direct 1-e-oxidation, the amino group of the methionine must be deprotonated for this mechanism.[29] The protonation/deprotonation of the N-terminal amino group is, of course, an equilibrium process and, therefore, a small fraction of the N-terminus is deprotonated, even at physiological pH. Theoretically, sulfur-radical cations may thus form and, via rate-determining deprotonation of the amino group, convert into CO_2. This process could even lead to the decay of the $(S\therefore S)^+$ species under pulse radiolysis conditions. The lacking CO_2 yields can, however, satisfactorily be explained by a competitive process: although the pK_a of the amino group is ca. 10.5 and may not change very dramatically through oxidation of the sulfur (separated via three aliphatic C-centers from the amino group), the pK_a of the α-methyl/methylene groups at the sulfur radical cation are ca. −2 and −6, as calculated by Rauk et al.[43] Hence, it appears that deprotonation of the CH_2/CH_3 groups are much more feasible, yielding additional carbon-centered radicals. Consequently, decarboxylation becomes lower, even of negligible importance, once the sulfur radical cation is formed around neutral pH. The reason this pH condition does not apply to the $^{\bullet}OH$-induced process is, in this case, the initial $^{\bullet}OH$ adduct to the sulfur atom instantaneously removes a proton from the $-NH_3^+$ group and, therefore, decarboxylation takes place also in neutral and even slightly acid solutions.[43] A quantitative material balance including the direct fate of methionine, such as time-resolved detection of $MetS^{\bullet+}$ and any degradation products thereof, could not be conducted for reasons of too short lifetimes, unspecificity of detection parameters, and unsuitable reaction conditions.

Fate of Peroxyl Radicals Derived from Isoflurane in the Absence of MetS. As can be realized upon inspection of Table 1, the yields of the various degradation products are very different whether or not MetS was present. As in the MetS-containing solution the chloride yield identifies the yield of initial one-electron reduction of isoflurane and the resulting primary peroxyl radical yield, $CHF_2OCH(OO^{\bullet})CF_3$. Mechanistically, it can be stated already at this point that in the MetS-free system none of the 2-e-oxidation reactions of the halogenated peroxyl radicals can take place nor any of the follow-up reactions initiated by this process. All observed products can arise, therefore, only from the commonly known standard peroxyl chemistry. We further like to point out that all listed yields are significant from the analytical point of view, i.e., even the very small yields of CO and $(COOH)_2$ are real and must not be considered impurities.

Our discussion will start with the carbon monoxide and oxalic acid yields. In the MetS-containing system, they originate exclusively from hydrolytic degradation of $CHF_2OC(O)C(O)F$ which, in turn, was formed via the secondary oxyl radical $CHF_2OC(O)CF_2O^{\bullet}$ as a precursor. The mechanistic question, accordingly, is, How can these molecular and radical species be formed without the MetS-based processes?

Clearly, any mechanism involving reduction of a possibly formed secondary oxyl radical $CHF_2OC(O)CF_2O^{\bullet}$ (as in reaction 26) and its subsequent reactions (eqs 27−29) must be discarded because of the lack of a suitable reductant. The only way to generate $CHF_2OC(O)C(O)F$ through any of the possible peroxyl−peroxyl reactions is a Russell-type cross termination between the secondary isoflurane and the *t*-butanol-derived peroxyl radicals, as formulated in the reaction sequence 31 and 32.

$$CHF_2OC(O)CF_2OO^{\bullet} + HOC(CH_3)_2CH_2OO^{\bullet} \rightarrow HOC(CH_3)_2C(O)H + O_2 + CHF_2OC(O)CF_2OH \quad (31)$$

$$CHF_2OC(O)CF_2OH \xrightarrow{-HF} CHF_2OC(O)C(O)F \quad (32)$$

CO may theoretically also arise from an oxyl radical $CHF_2OC(O)CF_2O^{\bullet}$, generated in a bimolecular reaction of two $CHF_2OC(O)CF_2OO^{\bullet}$ radicals. As will be shown in the Appendix, this possibility can, however, be discarded on thermodynamic grounds. The fact that CO and $(COOH)_2$ are formed at equal yields, as in the route B2 case, also speaks against this alternative pathway.

The combined yields of CO and $(COOH)_2$, derived upon hydrolysis of $CHF_2OC(O)C(O)F$ (reactions 30a,b), amount to $G = 0.06$, or about 15% of those obtained in the system with MetS. Reaction 31 thus accounts only for a small part of all peroxyl radical termination processes. The main reason for this is that there is only a low yield of the secondary peroxyl radical $CHF_2OC(O)CF_2OO^{\bullet}$ to begin with. In the MetS-free systems the necessary precursor of the latter, namely, the primary oxyl radical, $CHF_2OCH(O^{\bullet})CF_3$ can only be formed through the termination process, reaction 33. This reaction is, however, in

$$2CHF_2OCH(OO^\bullet)CF_3 \rightarrow 2CHF_2OCH(O^\bullet)CF_3 + O_2 \quad (33)$$

competition with the presumably much more efficient Russell mechanism, eq 34.[44] A further possible route could be the so-called concerted termination ("Bennett reaction"),[45] eq 35. For reviews on peroxyl radical reactions see refs 46 and 47. Both

$$2CHF_2OCH(OO^\bullet)CF_3 \rightarrow CHF_2OC(O)CF_3\ (TFAE) + O_2 + CHF_2OCH(OH)CF_3 \quad (34)$$

$$2CHF_2OCH(OO^\bullet)CF_3 \rightarrow 2CHF_2OC(O)CF_3 + H_2O_2 \quad (35)$$

these mechanisms predict an ultimate ratio of G(fluoride)/G(chloride) = 2:1. The experimentally found ratios are 3.65:1 and 2.3:1 for the systems with and without MetS, respectively. In the MetS-containing system all products from isoflurane are exclusively derived from the primary halogenated oxyl radicals, the yield of which is identical with the primary reduction yield, i.e., 100%. The experimentally observed ratio G(fluoride)/G(chloride) = 2.3:1 in the MetS-free system can thus be explained if just 18% of the primary isoflurane radicals $CHF_2OC^\bullet HCF_3$ enter into those oxyl radical routes that yield a F^-/Cl^- ratio of 3.65:1 and the other 82% enter any of the 2:1 ratio yielding mechanisms.

On this basis, the yields of other products from the oxyl radicals routes are calculated by multiplying their yields obtained in the presence of MetS by the factor 0.18 (see Table 3).

Concerning reaction 35, so far no evidence has been obtained for any significant contribution of the concerted termination mechanism by halogenated peroxyl radicals in aqueous solutions. If this holds true for isoflurane as well, then the majority of TFAA in the MetS-free system should originate from the Russell-type termination. The yield of TFAA from this termination pathway will be identical to that of the aldehyde TFAAld, i.e., G = 0.55. Subtracting this from the overall observed G(TFAA) = 0.7 leaves G = 0.15 for the TFAA formation through the oxyl routes (which do not result in TFAAld). The contribution of Russell termination in the overall isoflurane degradation thus amounts to 55% (G = 1.1 from the overall G = 2.0). This together with the above-mentioned 18% contribution through the oxyl radical route leaves 27% or G = 0.55 for still further reactions of the primary peroxyl radicals. The only ones to be envisaged are cross-terminations with either peroxyl radicals from *t*-butanol and superoxide, as formulated in reactions 36 and 5, respectively.

$$CHF_2OCH(OO^\bullet)CF_3 + HOC(CH_3)_2CH_2OO^\bullet \rightarrow HOC(CH_3)_2C(O)H + O_2 + CHF_2OCH(OH)CF_3 \quad (36)$$

As to be seen from Table 3, the calculated values based on all these figures are in very good agreement with the experimentally observed yields, confirming the proposed mechanism of isoflurane degradation in the absence of MetS.

Conclusions

The new approach employed in this work allowed for the first time, via radiation chemical studies, a detailed investigation of the reactive fates of halogenated hydroperoxides and alkoxyl radicals, independent of each other and their precursor peroxyl radicals. On the basis of detailed product studies and supporting quantum-chemical calculations, mechanisms of the degradation of isoflurane in different systems have been evaluated. The results of this study suggest that the toxicological side effects of isoflurane may be associated with the formation of the highly reactive halogenated peroxyl and oxyl radicals ($CHF_2OCH(OO^\bullet)CF_3$/$CHF_2OCH(O^\bullet)CF_3$, and $CHF_2OC(O)CF_2OO^\bullet$/$CHF_2OC(O)CF_2O^\bullet$) as well as of toxic molecular products such as HF, CF_2O, and $CF_3C(O)OH$.

TABLE 3: Comparison of the Calculated and Experimental Yields (G Values) of Products in System I (without MetS).

product	route via $RO^\bullet$	route via Russell's termination	routes via cross-termination	calcd yield	exptl yield
HCl	0.35	1.1	0.55	2.0	2.0
HF	1.3	2.2	1.1	4.6	4.6
$CF_3C(O)OH$	0.15	0.55	0	0.7	0.7
CF_3CHO	0	0.55	0.55	1.15	1.1
CO_2	0.3	0	0	0.3	0.3
HC(O)OH	0.35	1.1	0.55	2.0	2.0
HO(O)CC(O)OH	0.03	0	0	0.03	0.03
CO	0.03	0	0	0.03	0.03
total fluorine	1.75	5.5	2.75	10.0	10.0
total Carbon	1.05	3.3	1.75	6.1	6.0

Appendix

In competition to reaction 26 (1-e oxidation of MetS) radical $CHF_2OC(O)CF_2O^\bullet$ may possibly suffer C−C bond rupture giving rise to carbon difluoride and an acyl type radical $CHF_2OC^\bullet{=}O$:

$$CHF_2OC(O)CF_2O^\bullet \rightarrow CHF_2OC^\bullet{=}O + COF_2 \quad (37)$$

The latter may undergo four different pathways (reactions 38-41a) with an overall stoichiometry of HCl + 5HF + 2CO_2 + CO_2/CO + 3MetSO.

$$CHF_2OC^\bullet{=}O \rightarrow CHF_2O^\bullet + CO \quad (38)$$

$$CHF_2OC^\bullet{=}O \rightarrow CHF_2^\bullet + CO_2 \quad (39)$$

$$CHF_2OC^\bullet{=}O + H_2O \rightarrow CHF_2OC^\bullet(OH)_2 \quad (40)$$

To distinguish between reactions 38−40, we performed

$$CHF_2OC^\bullet{=}O + O_2 \rightarrow CHF_2OC(O)OO^\bullet \xrightarrow{MetS} CHF_2OC(O)O^\bullet + MetSO \quad (41a,b)$$

$$\downarrow$$

$$CHF_2O^\bullet + CO_2 \quad (42)$$

$$\downarrow$$

$$F_2C^\bullet(OH) \quad (43)$$

$$\downarrow O_2$$

$$HO_2^\bullet + COF_2 \quad (44)$$

quantum calculations. Reaction 38 can, accordingly, be neglected because of its high endothermicity (ΔH = 19.8 kcal/mol). Reactions 39 and 40 are both exothermic (ΔH = −43.5 and −0.1 kcal/mol, respectively). However, both should be strongly suppressed in the oxygenated solution because of reaction 41a.

Assuming that $CHF_2OC(O)OO^\bullet$ will oxidize MetS via the 2-e mechanism under formation of acyloxyl radicals $CHF_2OC(O)O^\bullet$, the most likely reaction of the latter would be decarboxylation (reactions 42). Further reactions of $CHF_2O^\bullet$ will be 1,2-H shift with subsequent oxygen addition and $HO_2^\bullet$ elimination, finally leading to another equivalent of carbon dioxide (see reactions 43 and 44 followed by reaction 24b). However, assigning G = 0.7 for this route (as an alternative to routes

B1/B2), a complete material balance could not be obtained anymore. Calculated yields for CO_2 and MetSO would be significantly higher as the experimentally observed (3.0 vs 1.6 and 3.7 vs 2.3, respectively) and those for formic acid would be considerably lower (1.3 vs 2.0). This eliminates these reactions from consideration.

Any other alternatives with the same stoichiometry as for routes B1/B2 (with $HCl:CO_2:MetSO = 1:2:1$) are also not apparent. For this reason, reactions 41−44 can be neglected.

In summary, the formation of $CHF_2OC^{\bullet}{=}O$ species (reaction 37) must be discarded, and hence, routes B1/B2 are, at least indirectly, supported.

We have analyzed also an alternative to the route A possibility for TFAA formation via β-cleavage from rearranged primary oxyl radicals:

$$CHF_2OC^{\bullet}(OH)CF_3 \rightarrow CHF_2^{\bullet} + CF_3C(O)OH \qquad (45)$$

However, such a reaction contradicts the experimentally observed significant increase of TFAA yield in the oxygen-saturated system compared to the air-saturated one.

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