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FORMATION AND TOXICITY OF ANESTHETIC DEGRADATION PRODUCTS

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■ **Abstract** Toxic degradation products are formed from a range of old and modern anesthetic agents. The common element in the formation of degradation products is the reaction of the anesthetic agent with the bases in the carbon dioxide absorbents in the anesthesia circuit. This reaction results in the conversion of trichloroethylene to dichloroacetylene, halothane to 2-bromo-2-chloro-1,1-difluoroethylene, sevoflurane to 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A), and desflurane, isoflurane, and enflurane to carbon monoxide. Dichloroacetylene, 2-bromo-2-chloro-1,1-difluoroethylene, and Compound A form glutathione *S*-conjugates that undergo hydrolysis to cysteine *S*-conjugates and bioactivation of the cysteine *S*-conjugates by renal cysteine conjugate β-lyase to give nephrotoxic metabolites. The elucidation of the mechanisms of formation and bioactivation of degradation products has allowed for the safe use of anesthetics that may undergo degradation in the anesthesia circuit.

INTRODUCTION

The hazards associated with human exposure to degradation products of volatile anesthetics have long been recognized. The effects of light, air, and heat on chloroform to produce phosgene, along with other degradation products, are well known. The interaction of volatile anesthetic agents within the anesthetic circuit itself was also a concern. The most notable interaction was that of trichloroethylene with soda lime to produce dichloroacetylene. This highly toxic compound produced considerable morbidity and, perhaps, mortality, and it is discussed in this review because of its historical importance. As these problems were identified and corrected, concern shifted to the toxicity associated with the metabolism of inhaled anesthetics. Fluoride-induced nephropathy associated with methoxyflurane and the halothane-associated hepatoxicity were of concern to anesthesiologists. Recently, however, there has been renewed interest in the formation and toxicity of degradation products of volatile anesthetics, particularly the formation of

Compound A from sevoflurane and the formation of carbon monoxide from anesthetics with -CHF_2 groups, i.e., desflurane, enflurane, and isoflurane.

This review addresses the formation, fate, and animal and human toxicity of dichloroacetylene (from trichloroethylene); 2-bromo-2-chloro-1,1-difluoroethylene (from halothane); 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene or Compound A (from sevoflurane); and carbon monoxide (from desflurane, isoflurane, and enflurane). All of these degradation products have known or suspected toxic potential for humans.

DICHLOROACETYLENE FORMATION FROM TRICHLOROETHYLENE

Introduction

The successful human use of trichloroethylene as a general anesthetic and analgesic was first reported by Striker et al. in 1935 (1). Trichloroethylene was introduced as an alternative to other inhaled anesthetics, such as diethyl ether and cyclopropane, because it possessed several advantages, including nonflammability, maintenance of cardiovascular stability, and general lack of postoperative side effects. It remained popular in some countries as a general anesthetic and analgesic well into the 1970s and is still used today in some parts of the world. Soon after its introduction, however, there appeared reports that its use was occasionally associated with cranial nerve neuropathies, particularly of the trigeminal nerve (2, 3). Subsequently, the formation of dichloroacetylene was implicated in the observed toxicity of trichloroethylene (4, 5).

Formation and Fate of Dichloroacetylene

Dichloroacetylene **2** is formed by the base-catalyzed elimination of HCl from trichloroethylene **1** (Figure 1) (6); this reaction is dependent on temperature and on the base-content of the soda lime (5). Dichloroacetylene is highly unstable and decomposes to give phosgene (the most abundant degradation product) and several other compounds (7), but their formation has not been implicated in dichloroacetylene-induced cranial nerve damage. Moreover, dichloroacetylene is

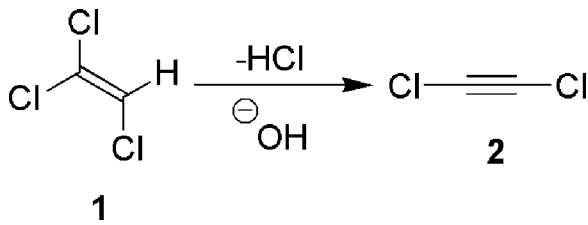

Figure 1 Base-catalyzed conversion of trichloroethylene **1** to dichloroacetylene **2**.

stabilized by high concentrations of trichloroethylene, which may limit its decomposition during anesthesia.

The metabolic fate of dichloroacetylene has been investigated in experimental animals. In rats exposed by inhalation to [^{14}C]dichloroacetylene (40 ppm, 1 h), *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine **7** (Figure 2) (61.8%), 2,2-dichloroethanol (12.2%), 2,2-dichloroethyl glucuronide (4.5%), dichloroacetic acid (8.9%), chloroacetic acid (4.7%), and oxalic acid (8.3%) were excreted in the urine over 96 h (8). The finding that mercapturate **7** is the major metabolite of dichloroacetylene indicates that glutathione-dependent metabolism is the major pathway of metabolism.

The biotransformation and bioactivation of a range of nephrotoxic and cytotoxic haloalkenes is dependent on glutathione *S*-conjugate formation and activation of cysteine *S*-conjugates by cysteine conjugate β-lyase. This pathway includes glutathione transferase-catalyzed glutathione *S*-conjugate formation, hydrolysis of the conjugates by γ-glutamyltransferase and dipeptidases to give the corresponding cysteine *S*-conjugates, active uptake of the cysteine *S*-conjugates by the kidney, and bioactivation by cytosolic and mitochondrial β-lyases. Reviews about the β-lyase pathway have appeared (9, 10).

Dichloroacetylene **2** undergoes bioactivation by the β-lyase pathway (Figure 2). The reaction of dichloroacetylene **2** with glutathione is catalyzed by rat hepatic and renal glutathione *S*-transferases to give *S*-(1,2-dichlorovinyl)glutathione **3** (11). The bioactivation mechanism of *S*-(1,2-dichlorovinyl)glutathione **3** has been elucidated (12): *S*-(1,2-dichlorovinyl)glutathione **3** is hydrolyzed by γ-glutamyltransferase and dipeptidases to give *S*-(1,2-dichlorovinyl)-L-cysteine **4**, which undergoes bioactivation by renal cysteine conjugate β-lyase or detoxication by *N*-acetylation to give *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine **7**, which is the major urinary metabolite. *S*-(1,2-Dichlorovinyl)-L-cysteine **4** undergoes a β-lyase-catalyzed β-elimination reaction to give 1,2-dichloroethenethiolate **5**, pyruvate, and ammonia. Thiolate **5** may lose chloride to give chlorothioketene **6** or may tautomerize to give chlorothionoacetyl chloride **8**. Both thioketene **6** and thionoacyl chloride **8** may contribute to the toxicity of *S*-(1,2-dichlorovinyl)-L-cysteine **4**, but the finding that thioketene **6** is highly unstable in aqueous environments favors a role for thionoacyl chloride **8** (13). The formation of 1,2-dichloroethenethiolate **5** and chlorothioketene **6** has been demonstrated by Fourier-transform ion cyclotron resonance mass spectrometry (14).

Toxicity

The toxicity of dichloroacetylene and its glutathione and cysteines *S*-conjugates has been investigated in experimental animals and in a range of in vitro systems. The high reactivity of dichloroacetylene has prevented investigation of its cytotoxicity.

ANIMAL TOXICITY AND IN VITRO STUDIES Dichloroacetylene is nephrotoxic, nephrocarcinogenic, neurotoxic, and hepatotoxic in laboratory animals, but nephrotoxicity is the prominent feature of dichloroacetylene-induced toxicity (15–18).

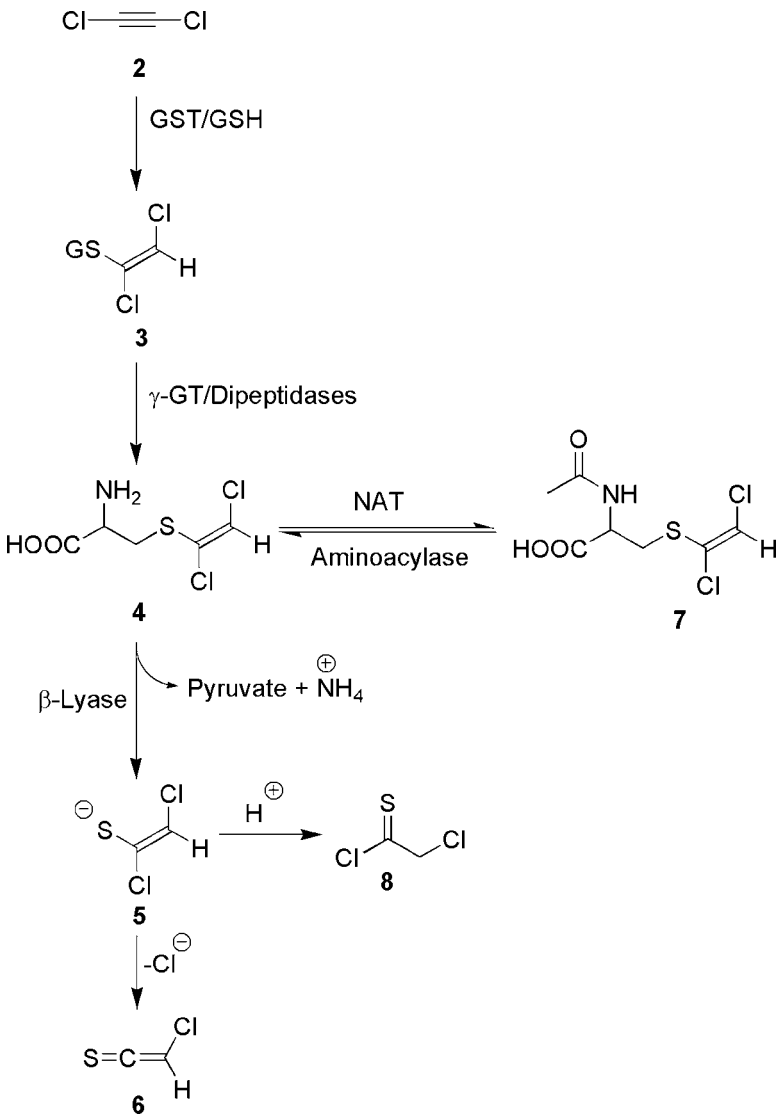

Figure 2 Glutathione *S*-transferase- and cysteine conjugate β-lyase-dependent bioactivation of dichloroacetylene **2**. **3**, *S*-(1,2-dichlorovinyl)glutathione; **4**, *S*-(1,2-dichlorovinyl)-L-cysteine; **5**, 1,2-dichloroethenethiolate; **6**, chlorothioketene; **7**, *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine; **8**, chlorothionoacetyl chloride. GSH, glutathione; GST, glutathione *S*-transferase; γ-GT, γ-glutamyltransferase; NAT, *N*-acetyltransferase.

Rabbits exposed to dichloroacetylene show extensive tubular and focal necrosis in the collecting tubules and accompanying clinical-chemical indices of renal damage, including marked increases in blood urea nitrogen concentrations. As indicated above, the nephrotoxicity of dichloroacetylene is associated with β-lyase-dependent bioactivation (11). Dichloroacetylene is a potent nephrocarcinogen in rats and mice: Cystadenomas and adenocarcinomas of the proximal tubules were observed in all animals exposed to dichloroacetylene (18). The hepatotoxicity of dichloroacetylene is characterized by fatty degeneration of parenchymal cells, but only transient elevations in serum transaminases are observed (17).

The neurotoxicity of dichloroacetylene in rabbits is characterized by morphological changes in the sensory and motor trigeminal nuclei and in the facial and oculomotor nerves and by functional neurological deficits that are manifested as decreased thermal sensitivity (16). In mice, damage to the Purkinje layer of the cerebellum is also observed (15). The mechanism of the neurotoxicity of dichloroacetylene has not been elucidated. Both *S*-(1,2-dichlorovinyl)glutathione **3** and *S*-(1,2-dichlorovinyl)-L-cysteine **4** are efficiently taken up by the brain, and β-lyase activity is present in the brain, indicating a possible role for the β-lyase pathway in dichloroacetylene-induced neurotoxicity in rodents (19, 20).

The cytotoxicity of dichloroacetylene itself has apparently not been reported. The dichloroacetylene-derived conjugates *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine are, however, cytotoxic in isolated rat renal proximal tubular cells (21). The cytotoxicity of *S*-(1,2-dichlorovinyl)glutathione is blocked by the γ-glutamyltransferase inhibitor acivicin and by the dipeptidase inhibitors 1,10-phenanthroline and phenylalanylglycine, indicating that hydrolysis of the glutathione *S*-conjugate to the cysteine *S*-conjugate is required for toxicity. The β-lyase inhibitor (aminooxy)acetic acid blocks the cytotoxicity of both the glutathione and cysteine *S*-conjugates. *S*-Conjugate-induced mitochondrial dysfunction plays an important role in *S*-(1,2-dichlorovinyl)-L-cysteine-induced cytotoxicity (22). Similarly, *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine are cytotoxic in pig kidney-derived cultured LLC-PK1 cells, and their cytotoxicity is blocked by (aminooxy)acetic acid (23).

Pure dichloroacetylene is mutagenic in *Salmonella typhimurium* strain TA100 but not in strain TA98 (24). The glutathione and cysteine *S*-conjugates of dichloroacetylene are also mutagenic in the Ames test with *S. typhimurium* strain TA2638 (25). The β-lyase inhibitor (aminooxy)acetic acid blocks the mutagenicity of both *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2-dichlorovinyl)glutathione, indicating a role for β-lyase in *S*-conjugate-induced mutagenicity. γ-Glutamyltransferase, which catalyzes the hydrolysis of glutathione *S*-conjugates to cysteine *S*-conjugates, is present in extracts of *S. typhimurium* and converts the glutathione *S*-conjugates to cysteine *S*-conjugates, which undergo β-lyase-dependent bioactivation. Finally, *S*-(1,2-dichlorovinyl)-α-methyl-DL-cysteine, which cannot undergo bioactivation by the pyridoxal phosphate-dependent β-lyase, is not mutagenic. *S*-(1,2-Dichlorovinyl)-L-cysteine induces unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts and expression of *c-fos* and *c-myc* in LLC-PK1 cells (26, 27).

HUMAN TOXICITY Damage to cranial nerves is a distinctive feature of dichloroacetylene poisoning in man and is often associated with symptoms such as skin irritation, headache, nausea, dizziness, and confusion (28). This unusual pattern of symptoms was described in one of the early reports of toxicity after trichloroethylene anesthesia (4, 29). Patients given trichloroethylene through soda-lime-containing circuits showed neurological symptoms that ranged from mild trigeminal anesthesia to general encephalitis and death. The most striking feature in all patients was trigeminal neuropathy, but many patients also showed involvement of other cranial nerves. Although the investigators did not establish the exact cause of the toxicity, they believed that the most likely cause was dichloroacetylene formed by a chemical reaction of trichloroethylene with soda lime. Accordingly, they recommended that soda lime not be used during anesthesia with trichloroethylene. Detailed studies of the conditions required to produce dichloroacetylene from trichloroethylene in soda lime revealed that not only were the temperature and base content of the soda lime important, but also its degree of hydration: Only dry soda lime produced significant amounts of dichloroacetylene (5).

Interestingly, nephrotoxicity, which is a prominent feature of dichloroacetylene-induced toxicity in rodents, was apparently not observed in human subjects anesthetized with trichloroethylene. Although the evidence strongly indicates that dichloroacetylene undergoes β-lyase-dependent bioactivation in rodents, the failure to observe nephrotoxicity in human subjects may be attributed to the low β-lyase activities present in human kidney tissue (30–32). The possible role of β-lyase-dependent bioactivation in the observed neurotoxicity of dichloroacetylene merits further investigation.

Serious toxicity after trichloroethylene anesthesia ceased to be a problem once the cause was identified. Anesthesia circuits that lacked carbon dioxide absorbents were used to deliver trichloroethylene. Additionally, the base content of absorbents was reduced and their formulations were changed to minimize the temperature increase during use so that if they were used in error during trichloroethylene anesthesia, risks would be minimized. Despite the apparent safety of modern absorbents, they have been implicated in the production of all of the other degradation products of currently used anesthetics described in this review.

2-BROMO-2-CHLORO-1,1-DIFLUOROETHYLENE FROM HALOTHANE

Introduction

Halothane was introduced into clinical anesthesia practice in 1956 (33) and soon became the most commonly used volatile anesthetic because of its lack of flammability and its desirable anesthetic properties. By the early 1960s, however, reports appeared that its use was occasionally associated with a type of fulminant hepatitis. Although rare, approximately 1 case in 35,000 administrations, concern about this

so-called halothane-associated hepatitis eventually led to its decline in popularity, especially after the introduction of isoflurane. Nevertheless, halothane is still used in many parts of the world and finds limited clinical use in the United States, most notably for pediatric anesthesia. Halothane-associated hepatitis is now believed to be associated with its cytochrome P450-dependent metabolism to trifluoroacetyl chloride, which trifluoroacetylates lysine residues in liver proteins to give neoantigens that result in a drug-induced allergic hepatitis (34, 35).

2-Bromo-2-chloro-1,1-difluoroethylene, which has not been implicated in the pathogenesis of halothane-associated hepatitis, was identified as a minor (<0.005% w/w) impurity in halothane (36–38). Later studies also showed that 2-bromo-2-chloro-1,1-difluoroethylene is present in the breath of human subjects anesthetized with halothane (39). *S*-(2-Bromo-2-chloro-1,1-difluoroethyl)-*N*-acetyl-L-cysteine had previously been identified as a urinary metabolite of halothane (40), and its formation from 2-bromo-2-chloro-1,1-difluoroethylene is discussed below.

Formation and Fate of 2-Bromo-2-chloro-1,1-difluoroethylene

2-Bromo-2-chloro-1,1-difluoroethylene **10** is formed by the base-catalyzed elimination of HF from halothane **9** in the presence of soda lime (Figure 3) (36, 39). Inhaled 2-bromo-2-chloro-1,1-difluoroethylene presumably undergoes rapid metabolism in the body because it is not detectable in the expired gases of patients within minutes of being disconnected from the anesthetic circuit (39).

2-Bromo-2-chloro-1,1-difluoroethylene reacts readily and nonenzymatically with sulfur nucleophiles, such as glutathione and cysteine (41). The pseudo first-order rate constants for the reaction of 2-bromo-2-chloro-1,1-difluoroethylene with cysteine and glutathione are $1.7 \pm 0.4 \times 10^{-4}$ sec^{-1} and 1.77 ± 0.20 to $2.02 \pm 0.22 \times 10^{-4}$ sec^{-1}, respectively. The reaction of 2-bromo-2-chloro-1,1-difluoroethylene with sulfhydryl groups is about 50 times faster than its rate of hydrolysis.

The glutathione *S*-transferase-dependent metabolism of 2-bromo-2-chloro-1,1-difluoroethylene **10** has been described. *S*-(2-Bromo-2-chloro-1,1-difluoroethyl)-*N*-acetyl-L-cysteine **17** (Figure 4) is present in the urine of human subjects

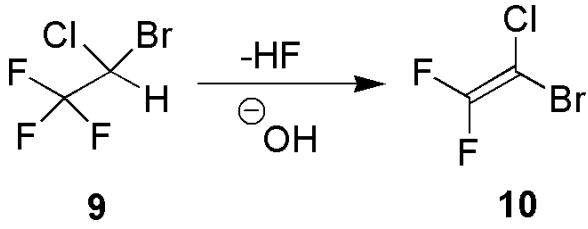

Figure 3 Base-catalyzed conversion of halothane **9** to 2-bromo-2-chloro-1,1-difluoroethylene **10**.

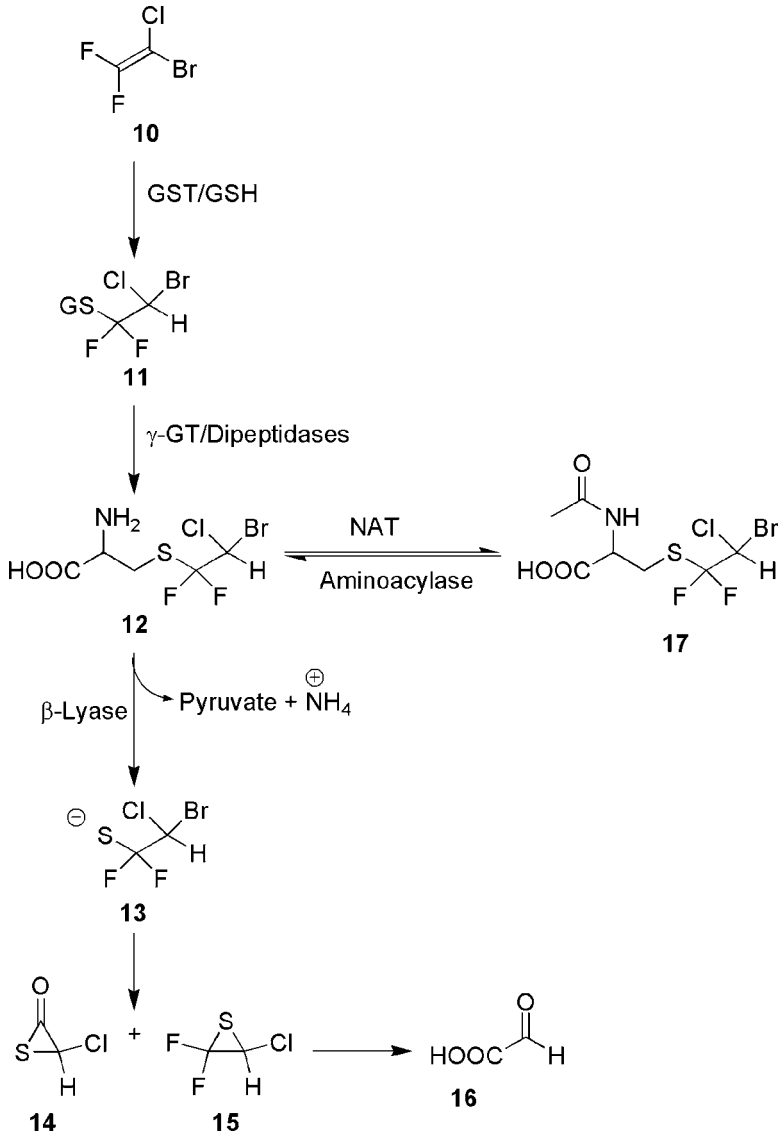

Figure 4 Glutathione *S*-transferase- and cysteine conjugate β-lyase-dependent bioactivation of 2-bromo-2-chloro-1,1-difluoroethylene **10**. **11**, *S*-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione; **12**, *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine; **13**, 2-bromo-2-chloro-1,1-difluoroethanethiolate; **14**, 2-chloro-α-thiolactone; **15**, 2,2-difluoro-3-chlorothiirane; **16**, glyoxylic acid; **17**, *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-*N*-acetyl-L-cysteine. GST, glutathione *S*-transferase; GSH, glutathione; γ-GT, γ-glutamyltransferase; NAT, *N*-acetyltransferase.

anesthetized with halothane (40, 42). Its formation can be rationalized by the addition of glutathione to 2-bromo-2-chloro-1,1-difluoroethylene **10** to give *S*-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione **11**, which may undergo γ-glutamyltransferase- and dipeptidase-catalyzed hydrolysis to give (2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine **12** (Figure 4). *N*-Acetylation would give the observed mecapturate *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-*N*-acetyl-L-cysteine **17**.

Cysteine *S*-conjugate **12** undergoes β-lyase-dependent bioactivation: Glyoxylic acid **16** was identified as a terminal metabolite of *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine **12** (43). This was an unexpected finding, because other bromine-lacking cysteine *S*-conjugates afford dihaloacetic acids as terminal products. Detailed mechanistic studies showed that 2-chloro-α-thiolactone **14** may be an intermediate in the bioactivation of *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine (Figure 4). Subsequent experiments also showed, however, that 3-chloro-2,2-difluorothiirane **15** is also formed as an intermediate in the β-lyase-catalyzed bioactivation of *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine **12** (44) (Figure 4). Hydrolysis of thiirane **15** would give glyoxylic acid **16**. Subsequent computational chemistry studies indicated that a role for 2-chloro-α-thiolactone **14** is unlikely and that 3-bromo-2,2-difluorothiirane **14** may be more important (45).

The finding that 3-chloro-2,2-difluorothiirane **15** is formed in the biotransformation of cysteine *S*-conjugate **12** marked the first demonstration of 2,2,3-trihalothiirane formation, although 2,2,3-trihalothiiranes had earlier been suggested, but not identified, as possible intermediates in the bioactivation of cysteine *S*-conjugates (46, 47). 2,2,3-Trihalothiirane formation from cysteine *S*-conjugates was later confirmed by Commandeur et al. (48).

Toxicity

The toxicity of 2-bromo-2-chloro-1,1-difluoroethylene and its glutathione and cysteines *S*-conjugates has been investigated in experimental animals and in vitro systems.

ANIMAL TOXICITY AND IN VITRO STUDIES 2-Bromo-2-chloro-1,1-difluoroethylene is nephrotoxic in mice: Its LC_{50} is approximately 0.025% (v/v) (36). Animals exposed to 2-bromo-2-chloro-1,1-difluoroethylene show kidney damage characterized by intense renal tubular degeneration. To determine whether the formation of 2-bromo-2-chloro-1,1-difluoroethylene in the anesthetic circuit might lead to kidney damage, monkeys were anesthetized with halothane, but no abnormalities were found on postmortem examination. In dogs anesthetized with halothane, concentrations of 0.00005 to 0.001% (v/v) 2-bromo-2-chloro-1,1-difluoroethylene are found in the reservoir bag, but no macroscopic or microscopic changes are observed on postmortem examination.

Detailed studies on the mechanism of 2-bromo-2-chloro-1,1-difluoroethylene-induced kidney damage have been conducted and were designed to test the hypothesis that 2-bromo-2-chloro-1,1-difluoroethylene **10** undergoes glutathione

S-transferase- and cysteine conjugate β-lyase-dependent bioactivation. *S*-(2-Bromo-2-chloro-1,1-difluoroethyl)glutathione **11** and *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine **12**, the glutathione and cysteine conjugates of 2-bromo-2-chloro-1,1-difluoroethylene **10**, are nephrotoxic in Fischer 344 rats (49). The nephrotoxicity of the *S*-conjugates is characterized by diuresis and increases in urine glucose and protein concentrations, in blood urea nitrogen concentrations, in kidney/body weight percentages, and in serum glutamate-pyruvate transaminase activities. Morphological examination of the kidneys of rats given either *S*-conjugate showed severe damage to the proximal tubules. Hepatic lesions were seen in some rats given the highest concentration studied (500 μmol/kg).

Both *S*-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione and *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine are cytotoxic in cultured LLC-PK1 cells (49). The cytotoxicity of *S*-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione is blocked by the γ-glutamyltransferase inhibitor acivicin, and the cytotoxicity of both *S*-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione and *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine is inhibited by the β-lyase inhibitor (aminooxy)acetic acid. Also, *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-DL-α-methylcysteine, which cannot undergo β-lyase-catalyzed bioactivation, is not cytotoxic. These data demonstrate that the observed nephrotoxicity of 2-bromo-2-chloro-1,1-difluoroethylene is attributable to the formation and bioactivation of *S*-(2-bromo-2-chloro-1,1-difluoroethyl)glutathione and *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine by the β-lyase pathway.

The mutagenicity of 2-bromo-2-chloro-1,1-difluoroethylene has also been investigated. In studies with the Ames *Salmonella* auxotroph reversion test, 2-bromo-2-chloro-1,1-difluoroethylene induced both base-substitution and frameshift mutations in *S. typhimurium* strains TA92, TA98, and TA100 (50). With a transformable strain of *Bacillus subtilis*, the induction of Spo^- mutants was observed. These experiments were conducted in the absence of hepatic microsomal fractions and indicate that 2-bromo-2-chloro-1,1-difluoroethylene may be a direct-acting mutagen.

Further studies showed that 2-bromo-2-chloro-1,1-difluoroethylene is not mutagenic in the Ames test conducted in liquid culture in the absence or presence of a microsomal activating system (51). When cells growing in enriched media were used, 2-bromo-2-chloro-1,1-difluoroethylene induced an increase in revertants, and the addition of S-9 fractions decreased the number of revertants. 2-Bromo-2-chloro-1,1-difluoroethylene purified by preparative gas chromatography is not mutagenic in the Ames test, whereas an unpurified commercial preparation is mutagenic (52).

The mercapturate *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-*N*-acetyl-L-cysteine **17** is not mutagenic in the Ames test with *S. typhimurium* strains TA1535 and TA100 in the absence or presence of S-9 fractions, whereas mercapturate **17** inhibited growth of the recombination repair-deficient strain M45 in the *B. subtilis* "*rec*" assay (53).

Other studies showed that *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine **12** is mutagenic in the Ames test with *S. typhimurium* strain TA2638 (54). The

mutagenicity of *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-L-cysteine is inhibited by (aminooxy)acetic acid, indicating a role for β-lyase, which is present in *S. typhimurium* (55); furthermore, *S*-(2-bromo-2-chloro-1,1-difluoroethyl)-DL-α-methylcysteine, which is not a β-lyase substrate, is not mutagenic. The finding that mercapturate **17** is not mutagenic (see above) may indicate that *S. typhimurium* lacks aminoacylase activity that catalyzes the hydrolysis of the mercapturates to the cysteine *S*-conjugates.

HUMAN TOXICITY The formation of 2-bromo-2-chloro-1,1-difluoroethylene under clinical conditions was established by Sharp et al. (39). 2-Bromo-2-chloro-1,1-difluoroethylene was detectable in patients connected to a rebreathing circuit containing soda lime, but not in patients connected to a nonrebreathing Bain circuit. If a semiclosed system was used to deliver 1% halothane at a 5 liter min^{-1} total flow, the concentration of 2-bromo-2-chloro-1,1-difluoroethylene was less than 1 ppm, whereas it rose to approximately 5 ppm if a completely closed system was used to deliver 1% halothane at a 0.5 liter min^{-1} total flow. 2-Bromo-2-chloro-1,1-difluoroethylene disappeared from the patient's breath within minutes after disconnection from the anesthetic circuit, indicating that it may be rapidly metabolized.

The study by Sharp et al. was not designed to determine the toxicity of 2-bromo-2-chloro-1,1-difluoroethylene in man (39). Even so, the authors suggested that the presence of up to 5 ppm 2-bromo-2-chloro-1,1-difluoroethylene was a cause for concern. Although this is much less than the LC_{50} of 250 ppm in mice, they pointed out that the lethal concentration and the concentrations that produce nonlethal tissue damage in man are unknown.

Additional studies on the toxicity of 2-bromo-2-chloro-1,1-difluoroethylene in man have apparently not been reported and, thus, any implication that toxic concentrations may be achieved during halothane anesthesia remains speculative. Indeed, other than the rare cases of massive liver damage seen postoperatively, halothane is a remarkably nontoxic drug and, in particular, does not cause nephrotoxicity, a characteristic feature of 2-bromo-2-chloro-1,1-difluoroethylene toxicity in animals.

2-(FLUOROMETHOXY)-1,1,3,3,3-PENTAFLUORO-1-PROPENE (COMPOUND A) FORMATION FROM SEVOFLURANE [FLUOROMETHYL 1-(TRIFLUOROMETHYL)-2,2,2-TRIFLUOROETHYL ETHER]

Introduction

Sevoflurane is a fluorinated volatile anesthetic agent that is approved for use in over 40 countries, including the United States. Its low blood-gas partition coefficient allows rapid induction and awakening (56). In addition, sevoflurane is nonirritating to the airways and is, therefore, useful for inhaled induction. Although sevoflurane underwent clinical trials in the United States in the 1970s and

was considered an excellent anesthetic, two concerns about its potential toxicity have been raised: First, sevoflurane undergoes metabolism to inorganic fluoride, which has the potential to induce nephrotoxicity. Second, sevoflurane undergoes Baralyme®- and soda lime-dependent degradation to the fluoroalkene Compound A, which is nephrotoxic in rats.

Formation and Fate of Compound A from Sevoflurane

Sevoflurane **18** undergoes a base-catalyzed dehydrofluorination reaction in the anesthetic circuit to form Compound A **19** (Figure 5). The degradation of sevoflurane to Compound A is catalyzed by the bases (NaOH, KOH) present in soda lime and Baralyme® (57–59). Compound A and 1-(methoxy)-2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropane (Compound B) are the major degradation products of sevoflurane that are formed by reaction of sevoflurane with soda lime (60). The concentrations of Compound A found in anesthesia circuits are usually less than 20 ppm, although higher concentrations have been reported (60–66). Compound A concentrations are higher when Baralyme® rather than soda lime is used and when dry rather than wet absorbents are used (61, 64, 65). Compound A formation is also greater at low (0.5 to 1 liter) fresh gas flows than at higher (2 to 6 liters) fresh gas flows, perhaps because of the higher canister temperatures generated at low flow rates (64, 67). 1-Methoxy-2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropane (Compound B) is also formed in anesthesia circuits, but its concentration is considered to be too low to be of toxicological concern: No toxicity was observed in rats exposed for 3 h to 2400 ppm Compound B (60, 62). 1-Methoxy-2-(fluoromethoxy)-1,1,3,3-tetrafluoro-2-propene (Compound C) and (*E*)- and (*Z*)-1-methoxy-2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propene (Compounds D and E) are also formed as degradation products of sevoflurane (59, 68), but no information about their fate or toxicity is apparently available.

The biotransformation of Compound A has been studied in human hepatic microsomal fractions (69). Human cytochrome P450 2E1 catalyzes the defluorination of Compound A, although significant NADPH-independent defluorination of Compound A is also observed. The enzymatic defluorination of Compound A was significantly inhibited by sevoflurane, which is also a substrate for cytochrome P450 2E1 (70).

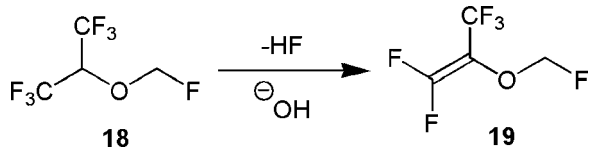

Figure 5 Base-catalyzed conversion of sevoflurane **18** to 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A) **19**.

Compound A also undergoes glutathione-dependent metabolism. In rats given Compound A intraperitoneally, diastereomeric *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione **20** and (*E*)- and (*Z*)-*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]glutathione **21** (Figure 6) are excreted in the bile, and the corresponding mercapturates, diastereomeric *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **26** and (*E*)- and (*Z*)-*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine **27** (Figure 7), respectively, are excreted in the urine (71, 72). Moreover, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid **24**, the expected product of the β-lyase-catalyzed metabolism of both cysteine *S*-conjugates **22** and **23**, is excreted in the urine of rats given Compound A **19** (73). These findings show that Compound A is metabolized by the β-lyase pathway. Cysteine *S*-conjugates **22** and **23** undergo β-lyase-catalyzed biotransformation in rat, human, and nonhuman primate renal cytosol and mitochondria, and β-lyase activity is lower in human kidney tissue than in rat or nonhuman primate kidney tissue (32). Also, cysteine *S*-conjugate **22** is biotransformed by rat renal cytosol to pyruvate, fluoride, and 2-fluoromethoxy-3,3,3-trifluoropropanoic acid **24**, which undergoes degradation to 3,3,3-trifluorolactic acid **25** (Figure 6) (74). In addition, although cysteine *S*-conjugate **23** is a substrate for β-lyase, it also undergoes a rapid ($t_{1/2} \approx 5$ min) intramolecular cyclization reaction to give the thiazole 2-[1-(fluoromethoxy)-2,2,2-trifluoroethyl]-4,5-dihydro-1,3-thiazole-4-carboxylic acid, which, because it lacks a free amino group, cannot serve as a β-lyase substrate (74). Hence, these data show that Compound A undergoes β-lyase-dependent metabolism.

Studies designed to quantify relative metabolite excretion (mercapturates **26** and **27** versus 2-fluoromethoxy-3,3,3-trifluoropropanoic acid **24**) in rats given Compound A showed that the formation and excretion of 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid **24** is greater than the formation and excretion of mercapturates **26** and **27**, again demonstrating the predominance of the β-lyase pathway in the metabolism of Compound A (75).

Compound A–derived mercapturates **26** and **27** (Figure 7) undergo little metabolism in rats (76). When [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine and [*acetyl*-$^2H_3$]*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine were given to rats, approximately 15% and 5%, respectively, were excreted as the unlabeled compounds, indicating minimal hydrolysis and acetylation of the released cysteine *S*-conjugates. The observed hydrolysis of Compound A–derived mercapturates is catalyzed by human and rat kidney cytosol and by acylases I and III. Mercapturate **26**, but not mercapturate **27**, was mildly nephrotoxic in rats, indicating hydrolysis and bioactivation by the β-lyase pathway.

The metabolism of Compound A formed from sevoflurane in the anesthetic circuit of human subjects anesthetized with sevoflurane has also been studied (77). The human subjects were anesthetized with sevoflurane (1.25 minimum alveolar concentration, 3%, 2 liter min^{-1}, 8 h), and urine was collected for 72 h after anesthesia. Analysis of the urine samples by ^{19}F NMR spectroscopy and GC-MS showed

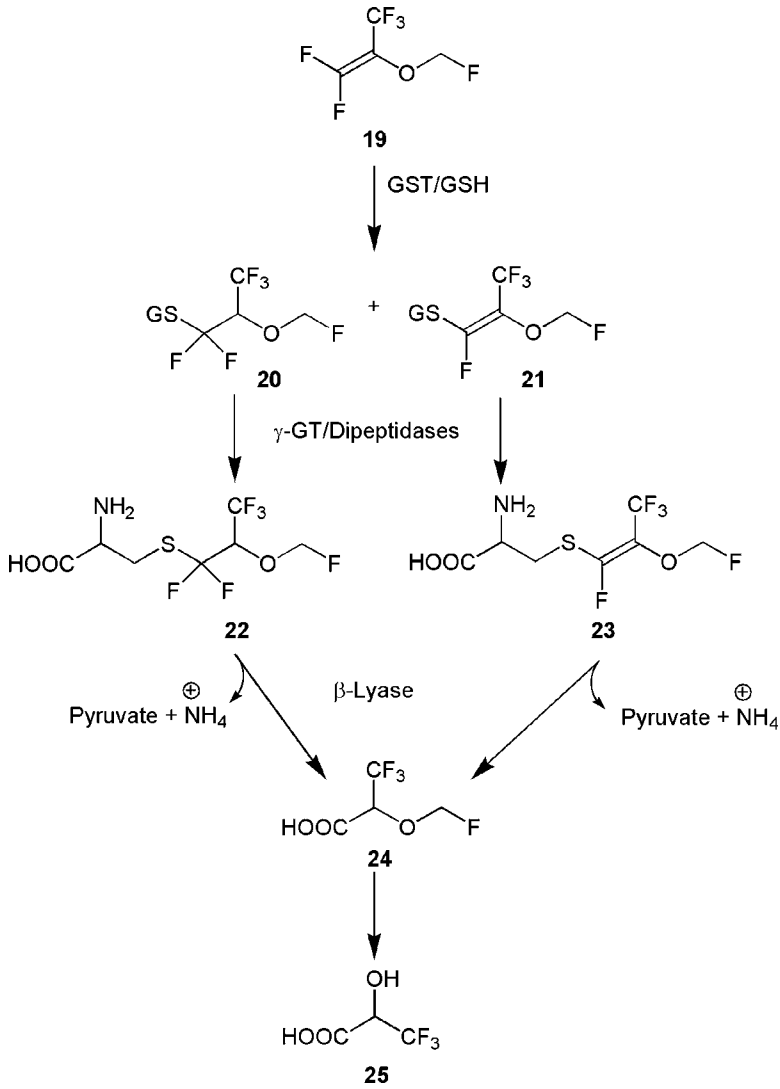

Figure 6 Glutathione *S*-transferase- and cysteine conjugate β-lyase-dependent bioactivation of 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A) **19**. **20**, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione; **21**, *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]glutathione; **22**, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine; **23**, *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine; **24**, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid; **25**, trifluorolactic acid. GST, glutathione *S*-transferase; GSH, glutathione; γ-GT, γ-glutamyltransferase; β-lyase, cysteine conjugate β-lyase.

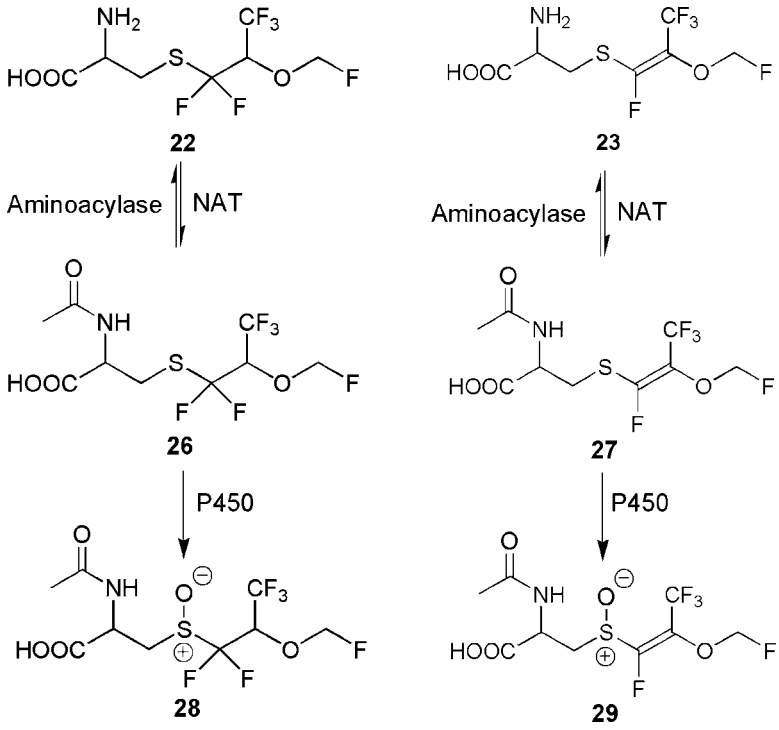

Figure 7 *N*-Acetylation and sulfoxidation of *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine **22** and *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine **23**. **26**, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine; **27**, *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine; **28**, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine sulfoxide; **29**, *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine. NAT, *N*-acetyltransferase; P450, cytochrome P450.

the presence of the Compound A metabolites *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **26**, (*E*)- and (*Z*)-*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine **27**, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid **24**, 3,3,3-trifluorolactic acid **25**, and inorganic fluoride, indicating metabolism of Compound A by the β-lyase pathway. Similar results were found in human subjects anesthetized with sevoflurane under conditions designed to maximize Compound A formation (75). The inspired Compound A concentrations were 29 $\pm$ 14 ppm (range 10–67 ppm). Mercapturates **26** and **27** were identified along with 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid **24**.

In vitro studies on the *N*-acetylation, *N*-deacetylation, and β-lyase-catalyzed biotransformation of Compound A–derived cysteine *S*-conjugates and

mercapturates by human kidney microsomes and cytosol showed significant interindividual variability (78). In human kidney cytosol, the rates of *N*-acetylation of cysteine *S*-conjugates **22** and **23** were 0.024 ± 0.01 (range 0.008–0.045) nmol mercapturate mg^{-1} min^{-1} and 0.024 ± 0.02 (range 0.001–0.07) nmol mercapturate mg^{-1} min^{-1}, respectively. Similar results were obtained in human kidney microsomes: The rates of *N*-acetylation of cysteine *S*-conjugates **22** and **23** were 0.025 ± 0.02 (range 0.005–0.055) nmol mercapturate mg^{-1} min^{-1} and 0.030 ± 0.02 (range 0.001–0.06) nmol mercapturate mg^{-1} min^{-1}, respectively. The β-lyase-catalyzed biotransformation of cysteine *S*-conjugates **22** and **23** amounted to 0.051 ± 0.04 (range 0.004–0.14) nmol pyruvate mg^{-1} min^{-1} and 0.26 ± 0.08 (range 0.10–0.40) nmol pyruvate mg^{-1} min^{-1}, respectively. The rates of hydrolysis of the mercapturates **26** and **27** were 1.25 ± 0.57 (range 0.8–2.5) nmol mg^{-1} min^{-1} and 0.17 ± 0.10 (range 0.05–0.37) nmol mg^{-1} min^{-1}, respectively. These data show that rates of β-lyase-catalyzed bioactivation of Compound A–derived cysteine *S*-conjugates in human kidney tissue were greater than the rates of *N*-acetylation of the cysteine *S*-conjugates and that the rates of *N*-deacetylation of Compound A–derived mercapturates were greater than the rates of *N*-acetylation of Compound A–derived cysteine *S*-conjugates. Hence, rates of bioactivation (β-lyase and *N*-deacetylation) of cysteine *S*-conjugates of Compound A exceed rates of detoxication (*N*-acetylation) in human kidney tissue.

Recent studies also show that Compound A–derived cysteine *S*-conjugates and mercapturates undergo biotransformation to the corresponding sulfoxides (79). The sulfoxidation of (*Z*)-*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine **26** and *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **27** to give sulfoxides **28** and **29**, respectively (Figure 7), is catalyzed by rat liver microsomal fractions; little sulfoxidation is observed in renal microsomal fractions. In contrast to the mercapturates, *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine **22** underwent nonenzymatic sulfoxidation. Although both cytochromes P450 and flavin-containing monoxygenases catalyze sulfoxidation reactions, P450 3A isoforms are the major enzymes responsible for the sulfoxidation of Compound A–derived mercapturates. Finally, the sulfoxidation of mercapturates **26** and **27** is significantly greater in rat than in human liver microsomes.

Toxicity

The toxicity of Compound A and its glutathione and cysteine *S*-conjugates has been investigated in experimental animals and in vitro systems.

ANIMAL TOXICITY AND IN VITRO STUDIES Compound A is nephrotoxic in Wistar rats exposed by inhalation for 1 h to 700 to 1400 ppm Compound A or for 3 h to 110 to 460 ppm Compound A (60). The LC_{50}s for 1-h exposures of male and female rats are 1090 ppm and 1050, respectively, and, for 3-h exposures, 420 ppm and 400 ppm, respectively. The toxicity of Compound A is characterized by renal tubular

necrosis, increased urine glucose and protein concentrations, and increased blood urea nitrogen concentrations. Lung congestion and hyperemia are also observed, but hepatotoxicity was not reported.

A more detailed investigation of the toxicity of Compound A reported a LC_{50} of 331 ppm for a 3-h exposure in Wistar rats (80). Compound A–induced nephrotoxicity is characterized by corticomedullary necrosis; significant evidence of corticomedullary necrosis was not seen in 10 rats exposed to 50 ppm Compound A, but was seen in rats exposed to concentrations of Compound A greater than 100 ppm. Liver and brain injury, but not lung injury, were observed in some animals. The effect of exposure time on Compound A–induced toxicity has also been studied (81). The LC_{50}s in Wistar rats exposed to Compound A for 6 or 12 h were 203 or 127 ppm, respectively. As in other studies, the nephrotoxicity is characterized by corticomedullary necrosis. Proliferating cell nuclear antigen, which is an indicator of cell proliferation, increased with increasing exposure concentration. Lung injury was seen only at near-lethal concentrations of Compound A. Additional studies on Compound A–induced toxicity showed a threshold for nephrotoxicity, as measured by histopathological examination, of 150 to 200 ppm for a 1-h exposure (82).

The toxicity of Compound A was studied in Sprague-Dawley rats exposed by nose-only inhalation to 0, 30, 61, 114, or 202 ppm Compound A (83). Increases in blood urea nitrogen and creatinine concentrations are observed in male and female rats exposed to 202 ppm Compound A, and renal tubular necrosis is observed in rats exposed to 114 or 202 ppm Compound A.

The mechanism of Compound A–induced nephrotoxicity has not been fully resolved, but most evidence implicates the β-lyase pathway. A range of 1, 1-difluoroalkenes undergo β-lyase-dependent bioactivation, including 2-bromo-2-chloro-1,1-difluoroethylene (49, 54), bromotrifluoroethylene (54), chlorotrifluoroethylene (84), 1,1-dichloro-2,2-difluoroethylene (54), hexafluoropropene (85), and tetrafluoroethylene (86).

Evidence for a role for the β-lyase pathway in Compound A–induced nephrotoxicity has been presented: (Aminooxy)acetic acid, a β-lyase inhibitor (12), partially blocks Compound A–induced nephrotoxicity and reduces the excretion of 2-fluoromethoxy-3,3,3-trifluoropropanoic acid **24** in rats given Compound A (71, 73). Also, the Compound A–derived cysteine *S*-conjugates *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine **22** and *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine **23** are substrates for rat, human, and nonhuman primate renal β-lyase (32). Finally, the Compound A–derived glutathione *S*-conjugates *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]glutathione **20** and *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]glutathione **21** and cysteine *S*-conjugate *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine **22** and *S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-L-cysteine **23** are nephrotoxic in rats in vivo, and their nephrotoxicity is partially blocked by (aminooxy) acetic acid (87).

Martin et al. purported to show that the β-lyase pathway is not involved in the mechanism of Compound A–induced nephrotoxicity (88, 89). These workers

reported that the β-lyase inhibitor (aminooxy)acetic acid and the γ-glutamyltransferase inhibitor acivicin either failed to block or increased Compound A–induced nephrotoxicity. The interpretation of these studies is limited, however, by the experimental design: In one study (88), only one exposure concentration (150 ppm) and one exposure time (3 h) were used. In a second study (89), the concentrations of Compound A used (600 and 800 ppm for 1 h) are greater than one half of the observed LC_{50} of Compound A in Wistar rats (60). Moreover, in both studies nephrotoxicity was assessed only by histopathological studies; no clinical chemical parameters were reported. Subsequent studies showed that (aminooxy)acetic acid and acivicin also potentiate the nephrotoxicity of Compound A–derived glutathione and cysteine *S*-conjugates (90). The observation that acivicin potentiates the toxicity of Compound A has been confirmed by others (91, 92). The mechanism by which acivicin may increase the nephrotoxicity of Compound A is not apparent. Acivicin also increases the nephrotoxicity of hexachlorobutadiene in rats (93), but blocks hexachlorobutadiene-induced nephrotoxicity in mice (94); hexachlorobutadiene undergoes β-lyase-dependent bioactivation in rats (95). Lantum et al. (96) tested the hypothesis that acivicin may reduce renal glutathione concentrations and, thereby, render the kidney susceptible to injury. It was found, however, that acivicin significantly increases renal glutathione concentrations. Finally, probenecid blocks the nephrotoxicity of Compound A, perhaps by preventing the renal uptake of glutathione and cysteine *S*-conjugates of Compound A (91, 92). Additional studies are needed to clarify fully the mechanism of Compound A–induced nephrotoxicity in rats and, particularly, the effects of acivicin. Nevertheless the weight of the evidence supports a role for the β-lyase pathway in the nephrotoxicity of Compound A in rats.

The cytotoxicity of Compound A and several of its metabolites has been studied in human-kidney-derived HD-2 cells (97). Compound A was cytotoxic only at concentrations greater than 0.9 mM, which is higher than would be achieved during the clinical use of sevoflurane. Glutathione *S*-conjugates **20** and **21** of Compound A were not cytotoxic in HK-2 cells. The Compound A–derived cysteine *S*-conjugates **22** and **23** were cytotoxic in HK-2 cells, but were much less cytotoxic than *S*-(1,2-dichlorovinyl)-L-cysteine **4**. The mercapturate (*Z*)-*S*-[2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine **27** was cytotoxic at the highest concentration tested (2.7 mM), but *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **26** was not cytotoxic.

The Compound A–derived sulfoxides **28** and **29** (Figure 7) are also cytotoxic in HK-2 cells (97); indeed, these sulfoxides are more cytotoxic than the corresponding cysteine *S*-conjugates and mercapturic acids. Several sulfoxides of haloalkene-derived cysteine *S*-conjugates or mercapturates are nephrotoxic in vivo or cytotoxic in vitro, or both, including *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide (98), *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine sulfoxide (99), *S*-(1,2,3,4,4-pentachlorobutadienyl)-*N*-acetyl-L-cysteine sulfoxide (100), *S*-(trichlorovinyl)-*N*-acetyl-L-cysteine sulfoxide (99), and (*cis*-3-chloro-2-propenyl)-*N*-acetyl-L-cysteine and (*trans*-3-chloro-2-propenyl)-*N*-acetyl-L-cysteine (101). These findings

raise the question of whether sulfoxidation of cysteine *S*-conjugates or mercapturates is also a bioactivation pathway in addition to the β-lyase pathway. As expected, the nephrotoxicity of *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide, *S*-(1,2-dichlorovinyl)-*N*-acetyl-L-cysteine sulfoxide, and *S*-(trichlorovinyl)-*N*-acetyl-L-cysteine sulfoxide is not blocked by the β-lyase inhibitor (aminooxy)acetic acid (98). Moreover, *S*-(1,2-dichlorovinyl)-L-cysteine sulfoxide is more cytotoxic in rat renal distal tubular cells than in proximal tubular cells, but *S*-(1,2-dichlorovinyl)-L-cysteine-induced nephrotoxicity is characterized by necrosis of renal proximal tubular cells rather than distal tubular cells (102). Finally, the α-methyl analogs of *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine are not nephrotoxic in rats (12, 87). These compounds cannot undergo β-lyase-catalyzed bioactivation but could presumably undergo sulfoxidation. Hence, studies on the sulfoxidation of α-methyl analogs of cysteine *S*-conjugates and the toxicity of the sulfoxides are needed to resolve this point.

Mutagenicity studies showed that Compound A does not induce reverse mutations, either in the absence or presence of a S-9 activating system, with *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 or with *Escherichia coli* strain WP2uvrA (60). Also, Compound A did not induce chromosome aberrations or increase the number of micronuclei in bone-marrow cells (60).

HUMAN TOXICITY The human toxicity of Compound A has been reviewed (103, 104). As discussed above, Compound A is produced when sevoflurane is delivered through absorbent-containing circuits. Concern has been expressed that sevoflurane-derived Compound A may place patients at increased risk for Compound A–induced kidney damage. First, the relatively low safety factor of Compound A concentrations produced in anesthetic circuits compared with concentrations that produce toxicity in rats is a potential concern. The safety factor is hard to assess but is estimated to be in the range of 2 to 8, which is far less than the safety factor of 10 to 100 that is commonly accepted by many toxicologists as providing an adequate margin of safety (105). Second, evidence of renal injury in human volunteers anesthetized with sevoflurane has been reported (106, 107). Other workers have, however, failed to find evidence of renal injury or have observed mild and transient evidence of renal injury in human subjects anesthetized with sevoflurane compared with reference anesthetics (see, for example, 62, 63, 108–113). Also, a retrospective evaluation of the effect of sevoflurane on renal function in adult surgical patients (1941 patients received sevoflurane and 1495 received control agents) found no evidence that sevoflurane administration is associated with renal injury (114).

There are well-documented scientific reasons why the nephrotoxicity of Compound A in man would be expected to be less than that in rats: If β-lyase-dependent bioactivation is the basis of the observed nephrotoxicity of Compound A, human kidney tissue has much lower β-lyase activities than does rat kidney (30–32, 115). Allometric scaling, which does not account for metabolic differences, indicates

that the human threshold for Compound A–induced nephrotoxicity would be approximately 9000 ppm h^{-1}, which is approximately 20 times the highest reported human exposure (113). To date, it is estimated that more than 120 million human subjects worldwide have been anesthetized with sevoflurane and no established cases of Compound A–associated renal damage have been reported (personal communication, R.D. Ostroff, Abbott Laboratories, Abbott Park, IL).

CARBON MONOXIDE FORMATION FROM DESFLURANE, ISOFLURANE, AND ENFLURANE

Introduction

Although the toxicity of carbon monoxide is well established, significant intraoperative exposure of patients to carbon monoxide has been thought to be unlikely. Thus, the reports by Moon et al. that patients anesthetized with isoflurane or enflurane had elevated blood carboxyhemoglobin (COHb) saturations were unexpected (116, 117). As discussed below, it was later found that the source of the carbon monoxide was a reaction between anesthetics bearing a -CHF_2 moiety, e.g., isoflurane and enflurane, and the carbon dioxide absorbent (118).

The toxicity and biological effects of carbon monoxide have been summarized, as has the issue of xenobiotic-derived carbon monoxide toxicity (119, 120). Because the carbon monoxide toxicity is well understood, this section focuses on the mechanism and conditions of the formation of carbon monoxide from anesthetic agents.

Formation and Fate of Carbon Monoxide

The observation of elevated COHb saturations in anesthetized patients led to an attempt to determine the causes and conditions under which carbon monoxide is produced in anesthesia circuits. Moon et al. showed increases in carbon monoxide concentrations if enflurane or isoflurane was allowed to stand in cartridges of soda lime (116). In most samples, the carbon monoxide concentrations were less than 20 ppm, which is well below the concentrations that had been observed under clinical conditions. In some (5%) samples, however, carbon monoxide concentrations greater than 100 ppm were found, and the concentrations exceeded 1000 ppm in a few of the samples.

Investigation of the degradation of several anesthetics in soda lime and Baralyme® showed that significant amounts of carbon monoxide were generated from desflurane, enflurane, and isoflurane, which contain the -CHF_2 moiety, whereas insignificant amounts were generated from halothane and sevoflurane, which lack the -CHF_2 group (121). Carbon monoxide production is dependent on the dryness and type of absorbent: Carbon monoxide formation increases as the absorbent water content decreases (122), and generation of carbon monoxide is greater with barium hydroxide lime than with soda lime (121). The use of absorbents that lack

strong bases, such as Amsorb®, prevents the formation of carbon monoxide (and the formation of Compound A from sevoflurane) (123).

The mechanisms by which desflurane and isoflurane are converted to carbon monoxide have been elucidated (124). Their studies, based on well-established carbene and elimination chemistry, describe a mechanism for the formation of carbon monoxide from anesthetics bearing a -CHF_2 moiety. Base-catalyzed abstraction of a proton from desflurane **30a** or isoflurane **30b** (Figure 8) affords the intermediate carbanions **31a,b**. Elimination of halide from the intermediate carbanions gives difluorocarbene **32** and trifluoroacetaldehyde **34**. Hydrolysis of carbene **32** affords carbon monoxide **33**. The hydrolysis of methylene carbenes to carbon monoxide is known (125). Difluorocarbene **32** was trapped by reaction with α-methylstyrene to give 1,1-difluoro2-methyl-2-phenylcyclopropane, thereby establishing its formation as an intermediate. Moreover, the oxygen atom in carbon monoxide is derived from water: When [^{18}O]H_2O was incorporated into barium hydroxide, [^{18}O]carbon monoxide was formed. Deuterium substitution in the -CHF_2 group of desflurane and isoflurane resulted in decreased carbon monoxide formation. Although mechanistic studies on the formation of carbon monoxide from enflurane have apparently not been reported, a pathway similar to that described for desflurane and isoflurane can be envisioned.

The biological fate of carbon monoxide is largely determined by its elimination via the respiratory tract, although a small fraction of the body burden of carbon monoxide is oxidized to carbon dioxide (126, 127).

Toxicity

The toxicity of carbon monoxide has been thoroughly investigated in animal studies and in cases of accidental or intentional human exposure; hence, only brief comments about human carbon monoxide toxicity are warranted.

HUMAN TOXICITY Moon et al. reported 28 cases of unexplained elevations of COHb saturations during anesthesia (116, 117). Eight cases had COHb saturations greater than 27%, and three cases had saturations of 30% or greater. To determine the source of the carbon monoxide, gas samples were collected from inside the soda lime canisters in idle anesthesia machines on 320 occasions. Carbon monoxide concentrations were less than 20 ppm in 271 of the samples, between 100 and 1000 ppm in 10, and greater than 1000 ppm in 6.

The clinical significance of exposure to carbon monoxide concentrations in this range and the physical properties and toxicity of carbon monoxide are well known. The Haldane equation describes quantitatively the competition between oxygen and carbon monoxide for the same ferrous heme binding sites on hemoglobin:

$$\frac{[Hb(CO)_4]}{[Hb(O_2)_4]} = 245\frac{[P_{co}]}{[Po_2]}.$$

The constant, 245 at pH 7.4, indicates that if $Pco = 1/245P_{O_2}$, then blood will be half-saturated with oxygen and half with carbon monoxide at equilibrium.

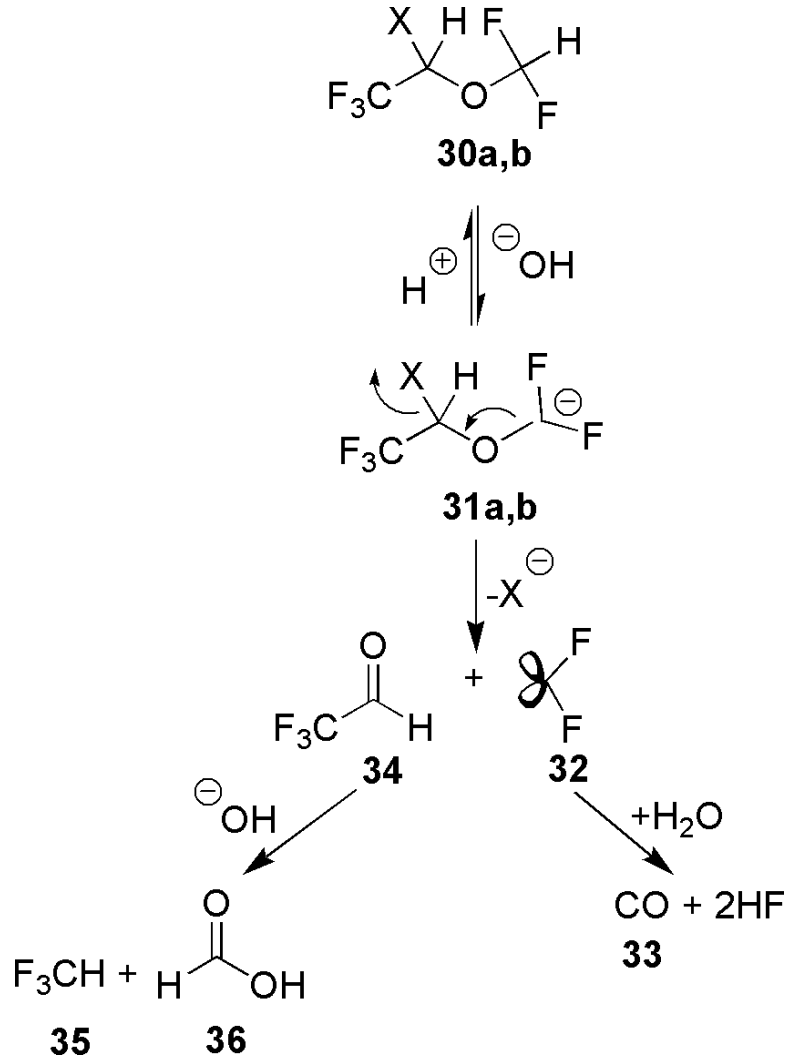

Figure 8 Base-catalyzed conversion of desflurane **30a** and isoflurane **30b** to carbon monoxide. **31a,b**, intermediate carbanions; **32**, difluorocarbene; **33**, carbon monoxide; **34**, trifluoroacetaldehyde; **35**, trifluoromethane; **36**, formic acid.

For a human breathing room air containing only approximately 0.085% (850 ppm) carbon monoxide at sea level, the COHb saturation will be 50% at equilibrium. This relationship accounts for the dangerous toxicity of low concentrations of carbon monoxide. The toxicological effects of carbon monoxide depend on a range of factors, including preexisting anemia and cardiovascular disease. In general, COHb

saturations between 30% and 50% result in tachycardia, hypoxic ECG changes, headache, weakness, nausea, dizziness, and failing vision. Saturations between 50% and 80% result in coma, convulsions, and death.

CONCLUSIONS

The degradation of anesthetic agents to toxic products has been associated with both old and modern agents. The common element that leads to the formation of degradation products is the bases in carbon dioxide absorbents in the anesthetic circuit. With some agents, e.g., sevoflurane, the degradation of an anesthetic to a toxic product was discovered during manufacture or early in its clinical use. With other agents, e.g., desflurane, isoflurane, and enflurane, however, the degradation products were discovered after many years of clinical use. The elucidation of the mechanisms of anesthetic degradation also provides a means for minimizing the formation of toxic degradation products by use of absorbents that lack strong bases, e.g., Amsorb®, rather than Baralyme® or soda lime.

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Annual Review of Pharmacology and Toxicology
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CONTENTS