TOXICOLOGY OF ATMOSPHERIC DEGRADATION PRODUCTS OF SELECTED HYDROCHLOROFLUOROCARBONS

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EXECUTIVE SUMMARY

The potential environmental degradation products of the hydrochlorofluorocarbons are trifluoroacetic acid (TFA), mixed fluorochloroacetic acids, and hydrofluoric acid. There is no available toxicologic data on mixed fluorochloroacetic acids. The additional fluoride burden, arising from environmental degradation of hydrochlorofluorocarbons, (1 to 3 ppb) in rainwater is trivial compared to levels in fluoridated drinking water (1 ppm), and would provide an insignificant risk to humans.

Overall there is sparse available toxicologic data on TFA. The acute lethality of TFA in mice suggests that it is only slightly toxic, and that its lethal effects at high doses are not dependent on its metabolism.

While a no adverse effect level has not been determined, 240 mg TFA/kg in rats produced no bone marrow or small intestinal effects, 25 mg TFA/kg produced no body weight gains, relative testis weight gains, or testicular histologic changes in rats, and 2000 mg/kg every second day for 14 days in mice produced no hepatic necrosis, or heart and kidney histological changes.

TFA at 2000 mg/kg in mice significantly decreased hepatic NADPH and reduced glutathione levels, but after 24-hr both levels returned to normal. Administration of 150 mg TFA/kg/day for 5-6 days to rats decreased the hepatic glycogen content by 24%, the percent liver/body weight by 43%, hepatic pyruvate kinase activity by 42%, and increased hepatic glycerol 1-phosphate oxidase activity by 125%. Thus the lowest dose at which effects have been reported is 150 mg/day for 5-6 days.

TFA is not mutagenic, but no carcinogenicity data is available. However, trichloroacetic acid is hepatocarcinogenic in mice, although it is also not mutagenic in the Ames assay. While no chronic toxicity data on TFA is available it appears likely that on the basis of its resistance to metabolism, rapid clearance, lack of mutagenic potential, and low acute toxicity TFA is unlikely to exhibit significant chronic toxic effects. For a more complete assessment of TFA toxicity chronic studies are required, as well as acute studies in species other than the mouse. The potential for TFA to act as a peroxisome proliferator should be investigated, to gain insight into its hepatocarcinogenic potential.

A German Senate Commission for the Evaluation of Health Hazards in the Work Environment has recommended that a blood TFA level of 2.5 μg/ml is risk-free. However, the assessment has no experimental or epidemiologic basis.

1. INTRODUCTION

Trifluoroacetic acid (TFA) is a liquid bp 72.4°C, mp 15.4°C, with a sharp biting odor. It has been proposed as the product of environmental degradation of the hydrochlorofluorocarbons HCFC-123, HCFC-124, HFC-134a, and HFC-125. Compounds HCFC-141b and HCFC-142b could yield mixed fluorochloroacetic acids, for which there is no available toxicologic data. The release of hydrochlorofluorocarbons into the environment could also give rise to HF, but the additional fluoride burden (1 to 3 ppb) in rainwater is trivial compared to levels in fluoridated drinking water (1 ppm), and would provide an insignificant risk to humans (Murray, 1986; World Health Organization, 1984; US EPA, 1986). Thus, in this paper only the toxicologic data on TFA is reviewed to assess the potential risks of environmental exposure.

Pharmacokinetics

There is little or no available data on the absorption, disposition, and elimination of TFA. In healthy human volunteers the half-life for renal excretion of TFA administered intravenously is 16 hr (Holaday and Cummah, 1976). In patients receiving halothane anesthesia the resultant metabolically-formed TFA had a half-life in the blood of 52-60 hr (Dallmeier and Henschler, 1977).

For rats (200-260 g) administered 1.3 mmol TFA/kg/day (150 mg TFA/kg/day) for 5 days the plasma TFA levels (μmol/ml blood) were 0.7 and 0.75 after 1 day, 0.8 and 1.0 after 2 days, 1.1 and 1.25 after 3 days, 1.06 and 0.6 after 4 days, and 1.3 and 1.1 after 5 days. Each value represents a single estimation and results are for two different animals at each time point (Stier et al., 1972). The average TFA concentration in the livers of these animals was 1.1 μmol/g liver. These values represent approximately 10% of the administered dose of TFA in the liver and 10% in the plasma (Stier et al., 1972).

TFA is not metabolized to any significant extent by rats (Fraser and Kaminsky, 1988). In humans TFA is not metabolized and is quantitatively excreted in urine (D.A. Holaday and R. Cummah, personal communication reported in Fiserova-Bergerova, 1977). Fluoro substituents, when constituents of a trifluoromethyl group, are metabolically stable relative to monofluoro substituents. Thus TFA is not metabolically defluorinated, in contrast to fluoroacetic acid, which has been demonstrated to be defluorinated by the rat hepatic microsomal system (Smith et al., 1977; Kostyniak et al., 1978).

Acute Toxicity

The majority of studies on TFA toxicity involve acute administration. Several LD50 values have been reported. For male Swiss-Webster, albino mice administered sodium trifluoroacetate intraperitoneally, values of > 400 mg/kg (> 2.9 mmol/kg) (Rosenberg, 1971), > 2000 mg/kg (> 14.7 mmol/kg) (Airaksinen and Tammisto, 1968), > 5000 mg/kg (> 37 mmol/kg) (Blake et al., 1969), and > 2000 mg/kg (> 14.7 mmol/kg) (Airaksinen et al., 1970) were obtained. TFA itself produced death in two of five mice treated intraperitoneally at 150 mg/kg (1.1 mmol/kg), probably as a consequence of its acidity (Blake et al., 1969). The LD_{50} of sodium trifluoroacetate when administered intravenously to mice was 1,200 mg/kg (10.5 mmol/kg) (Airaksinen and Tammisto, 1968). Preadministration of phenobarbital (40 mg/kg/day) for three days, or L-cysteine, isoniazid, ethanol, 4-iodopyrazole, or allopurinol administration 10 min before and 3 hr after sodium trifluoroacetate administration did not affect its LD_{50}, suggesting that the acetate was not being metabolized to toxic products (Airaksinen et al., 1970). Acute lethalities of the ord-

er reported for TFA, categorize it as a "slightly toxic" substance (Klaasen et al., 1986). Other toxic endpoints, primarily involving effects on metabolic activity, have also been investigated. Sodium trifluoroacetate in saline was administered by a single injection intraperitoneally to Swiss, albino, male mice (17-20 g) (Rosenberg, 1971). By 12-hr after administration of 2000 mg/kg (14.7 mmol/kg) the concentration of the coenzyme NADPH in the liver was statistically significantly decreased from 0.44 to 0.25 μmol/g. The decreased levels returned to normal by 24-hr after administration (Rosenberg, 1971). Reduced glutathione levels in the livers were also statistically significantly decreased from 4.33 to 3.94 μmol/100 mg at 12-hr after administration and by 24-hr these levels had also returned to normal (Rosenberg, 1971).

Male Wistar rats (200-260 g) were administered TFA in the drinking water for 5-6 days, equivalent to a dose of 150 mg TFA/kg body weight/day (1.3 mmol TFA/kg body weight/day) (Stier et al., 1972). In TFA-treated rat liver relative to untreated rat liver, the soluble protein increased by 6%, the glycogen content decreased by 24%, the neutral fat increased by 10% and the percent liver weight to body weight by 43%. None of these effects were evaluated for statistical significance. A number of enzyme activities in liver of TFA-treated animals was altered relative to control rat livers: pyruvate kinase decreased by 42%, phosphoglycerate kinase decreased by 10%, glycerol 1-phosphate oxidase increased by 125%, glycerol-phosphate dehydrogenase decreased by 4%, malic enzyme increased by 4%, glucose 6-phosphate dehydrogenase decreased by 17%, glyceraldehyde 3-phosphate dehydrogenase decreased by 4%, enolase decreased by 7%, malate dehydrogenase decreased by 10%, isocitrate dehydrogenase decreased by 10% and NADPH-oxidase increased by 7%. Again differences were not evaluated for statistical significance and it is doubtful whether any of the activities, with the possible exception of pyruvate kinase and glycerol 1-phosphate oxidase, were significantly affected.

In several studies TFA has been administered to animals, but without producing the certain specific effects which were sought. However, there has been no systematic attempt made to determine a no adverse effect level for TFA. TFA was neutralized in water and administered orally in a single dose to ten-week-old male Alpk/AP strain rats (Lloyd et al., 1988; Lloyd et al., 1986). At doses of 10 or 25 mg/kg (0.09 or 0.22 mmol/kg) body weight gains and relative testis weight were not affected, and no histological changes were noted in the testes relative to untreated controls (Lloyd et al., 1988; Lloyd et al., 1986). Equivalent doses of 2,2,2-trifluoroacetaldehyde and 2,2,2-trifluoroethanol significantly reduced body weight gain and relative testis weight, and produced histologically-detectable testicular damage.

When TFA (240 mg/kg, 2.1 mmol/kg) was administered intravenously to male Wistar rats no bone marrow or small intestinal toxicity was detected (Fraser and Kaminsky, 1988). The metabolic precursors of TFA, 2,2,2-trifluoroethanol and 2,2,2-trifluoroacetaldehyde, at equimolar doses produced significant decreases in intestinal dry weight and leukocyte counts (Fraser and Kaminsky, 1988).

Swiss male mice (17-20 g) were injected intraperitoneally with sodium trifluoroacetate at 1000 mg/kg (7.4 mmol/kg) and killed 24 hr later, at 2000 mg/kg (14.7 mmol/kg) and killed at 12 or 24 hr later, at 2000 mg/kg (14.7 mmol/kg) every second day for 14 days and killed on the 14th day (Rosenberg and Wahlstrom, 1971). There were no TFA-induced histological changes in hearts or kidneys of any of the treated mice. At the lowest dose of sodium trifluoroacetate (1000 mg/kg) histological changes in the liver were noted including a cloudy swelling of the hepatocytes with a slight fat accumulation. At the higher dose (2000 mg/kg) vacuolization of the hepatocytes was detected at all time periods (Rosenberg and Wahlstrom, 1971). However, even after multiple doses no hepatic necrosis was detected.

BIOLOGICAL AND HEALTH EFFECTS

Mutagenicity

TFA has been tested for mutagenicity in the Ames bacterial assay using two histidine-dependent strains of Salmonella typhimurium, TA98 and TA100 (Baden et al., 1976). TFA (150 mg/plate, 1.32 mmol/plate) was incubated in an agar-overlay assay at 37°C for 2 days without a microsomal activating system. No indication of mutagenicity was obtained under conditions where a positive control (N-methyl-N'-nitro-N-nitrosoguanidine) was highly mutagenic. This lack of mutagenicity of TFA was confirmed under similar conditions (Waskell, 1978). In a third study sodium trifluoroacetate was not mutagenic with Salmonella typhimurium TA98, TA100, and TA1535 at all concentrations used up to 35.7 mg/ml (0.26 mmol/ml), in the presence or absence of polychlorinated biphenyl (Aroclor 1254)-induced rat liver or testes post mitochondrial supernatants (Blake et al., 1981). The concentration of TFA in these assays was the maximum non-toxic concentration for the bacteria.

It is important to note that while trichloroacetic acid has also been found to be nonmutagenic in the Ames assay (Rapson et al., 1980; Waskell, 1978), it is a hepatocarcinogen in mice (Herren-Freund, 1987). Trichloroacetic acid has been demonstrated to produce peroxisome proliferation in mice (DeAngelo et al., 1986), which has been proposed as one of the mechanisms of hepatocarcinogenesis (Reddy et al., 1980), although it is questionable whether this mechanism applies in humans (Elcombe et al., 1985). Based on these reports the potential of TFA to act as a peroxisome proliferator should be investigated to gain insight into its potential as a hepatocarcinogen.

In Vitro Toxicology

TFA at 4 mM (450 mg/l) reduced the binding of the drugs warfarin and phenytoin to human serum albumin to 56% and 85% of controls, respectively. TFA at 10 mM (1140 mg/l) correspondingly reduced the binding to 44% and 77% (Dale, 1986). The results suggest that TFA generally affects the conformation of the albumin. The potential of TFA to produce metabolic disturbances was tested in vitro in cultured Morris rat hepatoma 7288C cells (Ishii and Corbascio, 1971). TFA (2.0 mM, 230 mg/l) did not affect uridine or thymidine uptake, while at 10 mM (1140 mg/l) leucine and acetate uptake by the cells was not affected. Thus at these concentrations DNA, RNA, protein and lipid synthesis by the cells was not affected.

When TFA was infused at 200 μmol/hr into 100 ml of perfusion medium for an isolated perfused rat liver the levels of lactate and pyruvate decreased after 10 min (Stier et al., 1972). TFA produced a higher uptake and turnover of lactate and pyruvate. This result is unusual in that fluorinated compounds usually inhibit rather than accelerate metabolic processes.

Antigenicity

Aqueous solutions of chicken serum globin (10-15 mg/ml) and TFA (1.2 M) were mixed in a ratio of 1 to 1.5 at 4°C for 15 min. The mixture was dialyzed against water and lyophilized to produce a complex of TFA and chicken serum globin, which was used to immunize rabbits (Rosenberg and Wahlstrom, 1973). Under these conditions TFA acted as a hapten and elicited antibodies. The clinical significance of this observation is unknown.

Analysis

Several methods are available for the analysis of TFA in biological material. In urine or serum TFA is quantitated by neutralizing with sodium hydroxide, esterifying with 2,2,2-trichloroethanol, and gas chromatographic analysis with a nickel-63 electron-capture detector (Witte et al., 1977). The detection limit is 1 μg TFA/ml body fluid. Another method uses isotachophoresis to quantitate urinary or blood TFA (Mario et al., 1980). In serum TFA has been methylated and the head space vapor phase analyzed by gas chromatography on Poropak Q (Fraser and Kaminsky, 1987).

2. CONCLUSIONS

Overall there is sparse available toxicologic data on TFA. The acute lethality of TFA in mice suggests that it is only slightly toxic, and that its lethal effects at high doses are not dependent on its metabolism.

While a no adverse effect level has not been determined, 240 mg TFA/kg in rats produced no bone marrow or small intestinal effects, 25 mg TFA/kg produced no body weight gains, relative testis weight gains, or testicular histologic changes in rats, and 2000 mg/kg every second day for 14 days in mice produced no hepatic necrosis, or heart and kidney histological changes.

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