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The nephrotoxicity and hepatotoxicity of 1,1,2,2-tetrafluoroethyl-L-cysteine in the rat

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Abstract Recent studies have shown that tetrafluoroethylene is a renal and hepatic carcinogen in the rat. In this study, we have examined the ability of a single i.p. dose of 1,1,2,2-tetrafluoroethyl-L-cysteine (TFEC), a major metabolite of tetrafluoroethylene, to produce hepatic and renal injury in male and female rats. We have also examined the effect of blocking the renal organic anion transport system with probenecid and of inhibiting the activity of cysteine conjugate β-lyase with aminooxyacetic acid on the extent of renal injury produced by TFEC. Doses of ≥12.5 mg/kg TFEC produced renal tubular necrosis to the pars recta of the proximal tubules within 24 h in both male and female rats. This was associated with an increased kidney to body weight ratio and plasma urea at doses of ≥25 mg/kg. No consistent evidence of liver injury was seen at doses up to 50 mg/kg TFEC in rats of either sex, although occasional vacuolation of hepatocytes and a small dose-related increase in liver to body weight ratio was observed. Prior treatment of female rats with probenecid completely prevented the renal injury produced by either 25 or 50 mg/kg TFEC as judged by plasma urea and histopathology. However, prior treatment of female rats with aminooxyacetic acid afforded no protection against the nephrotoxicity produced by either TFEC or the cysteine conjugate of hexachloro-1,3-butadiene. Thus no major sex difference in nephrotoxicity in the rat was seen with TFEC, while accumulation of TFEC, or its N-acetyl derived metabolite, into renal proximal tubular cells via a probenecid sensitive transport system appears to be a key event in the mechanism of nephrotoxicity. The lack of protection observed with the cysteine conjugate β-lyase inhibitor, aminooxyacetic acid, may reflect the inability to completely inhibit the mitochondrial form of this enzyme and thereby prevent the formation of the reactive metabolite. Our acute studies provide no insight concerning the liver carcinogenicity of tetrafluoroethylene.

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Introduction

Fluoroalkenes are a group of commercially important monomers used in the polymer industry, which are nephrotoxic to experimental animals. Recently tetrafluoroethylene (TFE) has been reported to produce an increased incidence of renal and hepatic tumours in both sexes of F344/N rats in a 2 year carcinogenicity study (NTP 1995). In this study, rats were exposed by inhalation to TFE for 6 h/day, 5 days/week at a concentration of 0, 156, 312 or 625 ppm for males and 0, 312, 625 or 1250 ppm for females over 103 weeks. At the higher doses in both sexes there was an increased incidence of renal tubular degeneration and hyperplasia, and the incidence of renal adenoma and carcinoma for males was 3, 5, 9, 13 and for females was 0, 3, 3, 10 with 50 animals/dose per sex. The incidence of hepatocellular adenoma and carcinoma in this study was for males 4, 7, 15, 5 and females 0, 7, 12, 8 with 50 animals/dose per sex.

Overall this study shows that male rats are slightly more sensitive to the chronic toxicity and carcinogenicity of TFE than female rats. Earlier studies reported that TFE caused diuresis and an increased urinary excretion of glucose and F^- ion in rats exposed to 3500 ppm for 30 min (Dilley et al. 1974). Subsequent studies by Odum and Green (1984) reported that TFE was metabolized in the rat by conjugation with hepatic glutathione and that further processing led to the formation of the cysteine conjugate (1,1,2,2-tetrafluoroethyl-L-cysteine, TFEC). No evidence was found of oxidative metabolism, via cytochrome P450, as reported with tetrachloroethylene. TFEC was shown to be a substrate for the renal enzyme cysteine conjugate β-lyase, which leads to the formation of stoichiometric amounts of pyruvate, ammonia and a reactive species which was thought to be responsible for the

nephrotoxicity. A similar mechanism of toxicity to the kidney has been proposed for a number of chlorinated and fluorinated ethylenes and for the by-product hexachloro-1,3-butadiene (for recent reviews see Dekant and Vamvakas 1993, Commandeur et al. (1995).

Only limited information is available on the toxicity of the glutathione derived metabolites of TFE in rats; a single oral dose of 100 mg TFEC/kg caused extensive nephrotoxicity within 24 h with no indication of hepatotoxicity as judged by clinical chemical markers (Odum and Green 1984). Moreover Commandeur et al. (1988) reported that the mercapturic acid of TFE was nephrotoxic, but not hepatotoxic, at dose of ≥50 μmol/kg (13 mg/kg) i.p. TFEC also caused nephrotoxicity in calves following a single intravenous injection of ≥10 mg/kg, where it produced a marked azotaemia and necrosis to the pars recta of the proximal tubules (Lock et al. 1996). The cysteine conjugate and mercapturic acid of TFE are toxic to isolated rat proximal tubular cells. Prior treatment with aminooxyacetic acid, an inhibitor of cysteine conjugate β-lyase, afforded some protection against the cytotoxicity as did probenecid, a competitive inhibitor of the renal organic anion transport system (Boogaard et al. 1989).

The aims of this study were (1) to examine the dose-response relationship for the production of nephrotoxicity or hepatotoxicity with TFEC in both male and female rats as a structurally related chemical, pentachloro-1,3-butadienyl-L-cysteine, which produces nephrotoxicity by a similar mechanism and shows a marked sex difference in response, with female rats being much more sensitive than male rats (Hook et al. 1983; Ishmael and Lock 1986). (2) To investigate whether administration of probenecid, a competitive inhibitor of the renal organic anion transport system to rats, will afford protection against the nephrotoxicity and (3) whether administration of aminooxyacetic acid, an inhibitor of cysteine conjugate β-lyase will also provide protection against the nephrotoxicity.

Materials and methods

Chemicals

S-(1,1,2,2-tetrafluoroethyl)-L-cysteine and N-acetyl-S-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine (PCBD-NAC) were synthesized as described by Odum and Green (1984) and Nash et al. (1984), respectively, and had a purity >99%. Probenecid and aminooxyacetic acid were purchased from Sigma Chemical Company (Poole, Dorset, UK). A stock solution of probenecid (0.5 M) was prepared in isotonic saline by making the solution alkaline and then adjusting the pH to 7.4. All remaining chemicals were of the highest purity commercially available.

Animals and treatment

Female and male Alderley Park (Alpk/AP) albino rats of 180–210 g body wt. were used for all of the studies. The rats were obtained from the Animal Breeding Unit at Alderley Park and fed pelleted PCD diet supplied by Special Diet Services Ltd. (Stepfield, Witham, Essex, UK). All animals were housed in stainless-steel, wire bottomed cages, at an environmental temperature of 20 ± 2 °C with a relative humidity of 45 ± 7%, and submitted to a light cycle from 06.00 h to 18.00 h. Animals were fasted overnight prior to dosing and for the entire post-dosing period but given free access to water.

For the initial studies to examine for any sex difference, TFEC was given by i.p. injection as a solution in isotonic saline at 5 ml/kg for doses of 3.125–12.5 mg/kg and as a suspension in corn oil at 5 ml/kg for the 25 and 50 mg/kg doses. For the studies with probenecid or aminooxyacetic acid, TFEC was given as a solution in isotonic saline at 10 ml/kg or 5 ml/kg i.p. respectively. PCBD-NAC was administered by i.p. injection in polyethylene glycol 400 (PEG 400) at 2 ml/kg body weight. Probenecid was administered i.p. at 0.5 mmol/kg in isotonic saline at 1 ml/kg, 0.5 h prior to TFEC administration and 7 h after TFEC administration. Aminooxyacetic acid was given at 50 mg/kg i.p. in isotonic saline at 1 ml/kg, 30 min before 12.5 mg/kg PCBD-NAC and 3 h before and again 30 min after 25 mg/kg PCBD-NAC or 1 h before TFEC. Control animals where appropriate received isotonic saline. PEG 400 or corn oil at the appropriate dose volume.

Detection of renal damage

Twenty four hours after TFEC or PCBD-NAC administration, the animals were killed by inhalation of an overdose of halothane vapour. Blood was collected from the heart into a syringe containing heparin and centrifuged to separate the plasma. Liver and kidneys were removed, blotted dry and weighed. Portions of the liver and kidneys (which included the cortex, medulla and papilla) were fixed in Formol saline (10%); paraffin sections (5 μm in thickness) were prepared and stained with haematoxylin and eosin for histopathological examination. Slides were examined without the pathologist having prior knowledge of the various treatment groups. The extent of renal tubular necrosis was scored as described in the legend to Table 1. The concentration of plasma urea was determined by the method of Marsh et al. (1965) and the activities of the plasma enzymes alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were determined by commercially available methods (Boehringer Corp. Mannheim, Germany) in conjunction with a Vitatron analyser.

Measurement of cysteine conjugate β-lyase activity

Fresh rat renal cortex was homogenized with 2 vol of ice-cold 0.32 M sucrose using an Ultraturrax homogenizer and centrifuged at 105 000 g at 4 °C for 60 min. The cytosol fraction was removed and stored at −70 °C prior to assay for enzymatic activity. The protein content was determined by the method of Lowry et al. (1951). Cysteine conjugate β-lyase activity with TFEC as substrate was measured at several substrate concentrations in the presence and absence of aminooxyacetic acid by monitoring the release of pyruvic acid as described by Stevens and Jakoby (1983).

Statistics

Differences between control and treated groups were analysed using ANOVA followed by Student's *t*-test with Bonferroni adjustments for multiple comparisons.

Results

Nephrotoxicity of TFEC to male and female rats

Administration of TFEC to male rats at a dose of 25 mg/kg or 50 mg/kg produced a small but statistically

Table 1 Effect of TFEC on liver and kidney to body weight ratio, plasma urea and renal pathology in the male rat. (*TFEC* 1,1,2,2-tetrafluoroethyl-L-cysteine)

Treatment[a]	Liver/body weight × 100	Kidney/body weight × 100	Plasma urea (mg %)	Extent of renal necrosis[b]				
				0	1+	2+	3+	4+
Control	3.24 ± 0.05 (4)[c]	0.74 ± 0.02	39 ± 4	4				
3.125 mg/kg	3.49 ± 0.06 (3)	0.76 ± 0.02	44 ± 7	3				
6.25 mg/kg	3.66 ± 0.05 (3)	0.77 ± 0.01	48 ± 5	2	1			
12.5 mg/kg	3.62 ± 0.08 (3)	0.84 ± 0.01	55 ± 4			2	1	
25 mg/kg	3.68 ± 0.09* (4)	0.94 ± 0.03**	170 ± 40**				2	2
50 mg/kg	3.93 ± 0.10* (3)	1.00 ± 0.03**	193 ± 13**				1	2

[a] Male rats were fasted overnight prior to dosing with TFEC. For 25 and 50 mg/kg the chemical was given as a suspension in corn oil at 5 ml/kg i.p., while for the other doses it was given as a solution in isotonic saline i.p. at 5 ml/kg body weight

[b] The extent of renal necrosis was scored as follows: 1+, minimal, involving isolated straight proximal tubules; 2+, moderate, involving several straight proximal tubules; 3+, extensive, seen as a distinct band of damage in the outer stripe of the outer medulla with occasional tubular casts; 4+, severe, a more diffuse band involving the outer medulla and the inner cortex with many tubular casts

[c] Values are mean ± SEM with the number of animals in parentheses

* Significantly different from control, $P < 0.05$ by ANOVA

** Significantly different from control, $P < 0.01$ by ANOVA

significant increase in liver to body weight ratio within 24 h of dosing (Table 1), without affecting the activities of plasma alanine aminotransferase, aspartate aminotransferase or alkaline phosphatase (data not shown). These doses also produced a statistically significant increase in kidney to body weight ratio and elevation in plasma urea (Table 1), while at the lower dose of 12.5 mg/kg there was a trend towards an increase in both these parameters which was not statistically significant. Histological examination of the liver showed normal morphology at all doses examined, while in the kidney TFEC at 25 mg/kg and 50 mg/kg produced a distinct band of necrosis in the outer stripe of the outer medulla, which extended into the medullary rays with the presence of tubular casts (Fig. 1B, Table 1). At a lower dose of 12.5 mg/kg TFEC there was a moderate renal tubular necrosis involving the straight portion of the proximal tubules in two rats and more extensive necrosis in another rat, while at 6.25 mg/kg only one rat out of three had a minimal lesion in the straight portion of the proximal tubules (Table 1).

A similar dose-response relationship was seen in female rats administered TFEC. Liver to body weight ratio was not statistically increased at any dose level, although there was a trend towards an increase at 50 mg/kg (Table 2). No increases were seen in the activities of plasma alanine aminotransferase, aspartate aminotransferase or alkaline phosphatase (data not shown). Histopathological examination showed that the liver was essentially normal, although one animal at 12.5 mg/kg showed minimal vacuolation of hepatocytes and another at 50 mg/kg had a small area of focal necrosis. Doses of ≥12.5 mg/kg TFEC produced an increase in kidney to body weight ratio and elevation in plasma urea. Although not all of the increases were statistically significant (Table 2), these increases were related to renal injury where extensive necrosis to the straight portion of the proximal tubule was seen. At 6.25 mg/kg TFEC no renal tubular necrosis was observed (Table 2). Nephrocalcinosis was evident in the kidneys of all female rats and was unrelated to TFEC treatment.

Effect of probenecid or aminooxyacetic acid on TFEC or PCBD-NAC mediated nephrotoxicity

The nephrotoxicity of TFEC was examined in rats treated with either aminooxyacetic acid, an inhibitor of β-lyase (Elfarra et al. 1986) or probenecid, an organic anion transport inhibitor (Weiner 1990). In addition, the effect of aminooxyacetic acid on the nephrotoxicity produced by PCBD-NAC was examined. Prior treatment of female rats with probenecid at a dose, which had previously been shown to protect against hexachloro-1,3-butadiene-induced nephrotoxicity (Lock and Ishmael 1985), afforded complete protection against the nephrotoxic effects of both 25 mg/kg and 50 mg/kg TFEC (Fig. 1C, Table 3). Treatment with TFEC alone produced the expected increase in kidney to body weight ratio and azotaemia, which was associated with morphological evidence of necrosis to the straight portion of the proximal tubules (Table 3). Prior treatment with probenecid completely prevented the TFEC-induced renal tubular necrosis (Table 3), although at the higher dose of TFEC there was an increase in both kidney to body weight ratio and plasma urea (Table 3). Treatment with probenecid alone produced a small but statistically significant increase in liver to body weight ratio and in plasma alanine aminotransferase activity (Table 3), which was also seen in those groups given the combination treatment.

Histological examination of the liver showed evidence of minimal fibrinous peritonitis, with an occasional focus of inflammatory cell infiltrate following probenecid alone and TFEC plus probenecid. One out of the four rats given TFEC alone at 25 mg/kg showed minimal hepatocyte vacuolation, while three out of the five rats

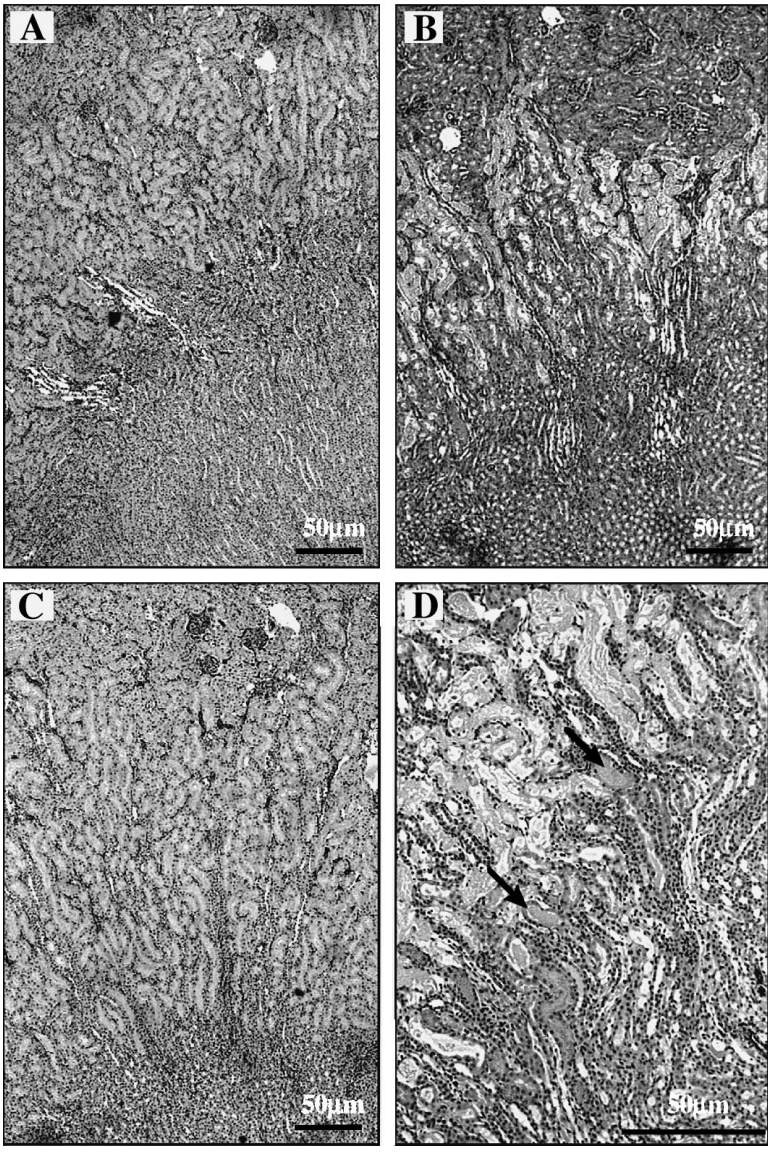

Fig. 1 Sections of female rat kidney 24 h after i.p. administration of **A** isotonic saline at 1 ml/kg showing no evidence of necrosis; **B** TFEC at 20 mg/kg showing a distinct band of necrosis in the outer stripe of the outer medulla (score 3+; see legend to Table 1); **C** TFEC at 50 mg/kg and probenecid 0.5 mmol/kg 0.5 h before and 7 h after TFEC, showing no evidence of necrosis; **D** TFEC at 20 mg/kg and aminooxyacetic acid 50 mg/kg 1 h before TFEC showing severe necrosis (score 4+; see legend to Table 1) with many tubular casts as indicated by the *arrows*. Haematoxylin and eosin staining (*TFEC* 1,1,2,2-tetrafluoroethyl-L-cysteine)

Table 2 Effect of TFEC on liver and kidney to body weight ratio, plasma urea and renal pathology in the female rat

Treatment[a]	Liver/body weight × 100	Kidney/body weight × 100	Plasma urea (mg %)	Extent of renal necrosis[b]				
				0	1+	2+	3+	4+
Control	3.21 ± 0.16 (4)[c]	0.72 ± 0.02	36 ± 2	4				
3.125 mg/kg	3.17 ± 0.05 (4)	0.73 ± 0.02	38 ± 2	4				
6.25 mg/kg	3.37 ± 0.09 (3)	0.77 ± 0.03	36 ± 3	3				
12.5 mg/kg	3.31 ± 0.10 (3)	0.81 ± 0.01	88 ± 7			1	2	
25 mg/kg	3.34 ± 0.14 (3)	0.88 ± 0.02*	97 ± 9				3	
50 mg/kg	3.84 ± 0.17 (3)	0.91 ± 0.02**	166 ± 26**					3

[a] Female rats were dosed with TFEC, i.p. as described in Table 1
[b] The extent of renal necrosis was scored as described in Table 1
[c] Values are mean ± SEM with the number of animals in parentheses
* Significantly different from control, $P < 0.05$ by ANOVA
** Significantly different from control, $P < 0.01$ by ANOVA

given 50 mg/kg TFEC also showed minimal hepatocyte vacuolation, while another animal showed a small focus of necrotic hepatocytes.

Aminooxyacetic acid is a known inhibitor of pyridoxal phosphate dependent enzymes and a good inhibitor of cysteine conjugate β-lyase (Elfarra et al. 1986; Commander et al. 1995). We have confirmed these findings, by showing that aminooxyacetic acid will inhibit the ability of rat renal cytosol to metabolize TFEC to pyruvate. The kinetics of pyruvate formation in the

Table 3 Effect of prior treatment with probenecid on TFEC-induced hepatic and renal function in the female rat. (*ALT* Alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase)

Treatment[a]	Liver/body weight × 100	ALT (U/1)	AST (U/1)	ALP (U/1)	Kidney/body weight × 100	Plasma urea (mg%)	Extent of renal necrosis[b]			
							0	1^+	2^+	3^+	4^+
Control	3.36 ± 0.09 (9)[c]	30 ± 3	159 ± 16	126 ± 10	0.76 ± 0.02	37 ± 2	9				
TFEC 25 mg/kg	3.47 ± 0.12 (5)	21 ± 1	49 ± 4**	138 ± 16	0.90 ± 0.03**	99 ± 5***				5	
TFEC 50 mg/kg	3.92 ± 0.11 (5)	24 ± 2	86 ± 5**	132 ± 8	0.90 ± 0.02***	200 ± 26***				2	3
Probenecid alone	3.71 ± 0.05** (11)	50 ± 3***	155 ± 9	149 ± 13	0.79 ± 0.02	43 ± 3	11				
Probenecid + TFEC 25 mg/kg	4.07 ± 0.08** (4)	57 ± 2	191 ± 28	169 ± 10	0.83 ± 0.02	44 ± 2	4				
Probenecid + TFEC 50 mg/kg	4.31 ± 0.05*** (4)	64 ± 5*	181 ± 22	161 ± 14	0.93 ± 0.02**	76 ± 5***	4				

[a] Female rats were dosed with probenecid at 0.5 mmol/kg, i.p., 30 min before and 7 h after TFEC

[b] The extent of renal necrosis was scored as described in Table 1

[c] Values are mean ± SEM with the number of animals in parentheses

* Significantly different from appropriate control, $P < 0.05$ by ANOVA

** Significantly different from appropriate control, $P < 0.01$ by ANOVA

*** Significantly different from appropriate control, $P < 0.001$ by ANOVA

Fig. 2 Kinetic analysis of the inhibition by aminooxyacetic acid of rat renal cysteine conjugate β-lyase with TFEC as substrate. For details see the Materials and methods. Linear regression analysis yielded the best fit and gave a correlation coefficient of $r = 0.999$ for the control (■) and $r = 0.997$ for 10 μM aminooxyacetic acid (□)

presence of TFEC alone obeyed Michaelis-Menten kinetics, the K_m and V_{max} being 5 mM and 30 nmol/min per mg protein respectively. Aminooxyacetic acid is a potent competitive inhibitor with a K_i of 3 μM (Fig. 2). Based on this finding and the findings of others (Elfarra et al. 1986; Commandeur et al. 1987) who have used aminooxyacetic acid in vivo, we dosed rats with 50 mg/kg i.p. aminooxyacetic acid, 1 h prior to dosing with TFEC at 20 mg/kg or PCBD-NAC at 6.25 mg/kg. This treatment did not protect the rats against the nephrotoxicity as judged by the increased kidney to body weight ratio, elevation in plasma urea or extent of renal tubular necrosis (Fig. 1D, Table 4).

In a further study we gave aminooxyacetic acid at 50 mg/kg, 3 h prior to dosing with PCBD-NAC at 12.5 mg/kg followed by a booster dose of aminooxyacetic acid at 25 mg/kg, 15 min after the PCBD-NAC. Again this did not afford any protection against the nephrotoxicity, based on kidney to body weight ratio, increase in plasma urea and renal pathology; in fact in both experiments the aminooxyacetic acid tended to increase the extent of renal damage (Table 4).

Discussion

The toxicity of the cysteine S-conjugate of tetrafluoroethylene to both male and female rats has been studied at doses ranging from 3.125 mg/kg to 50 mg/kg. A dose of ≥12.5 mg/kg TFEC produced nephrotoxicity with minimal changes in the liver. These finding are in agreement with an earlier study conducted in male rats at a single dose level of 100 mg/kg (Odum and Green 1984) and with studies by Commandeur et al. (1988) with the mercapturic acid of TFE, which produced nephrotoxicity with little evidence of hepatotoxicity.

Table 4 Effect of prior treatment with aminooxyacetic acid (AOAA) on TFEC-and PCBD-NAC-induced renal function in the female rat. (*PCBD-NAC* N-Acetyl-S-(1,2,3,4,4-pentachloro-1,3-butadienyl)-L cysteine, *ND* not determined)

Treatment[a]	ALT (U/1)	AST (U/1)	ALP (U/1)	Kidney/body weight × 100	Plasma urea (mg%)	Extent of renal necrosis[b]			
						0	1^+	2^+	3^+	4^+
Control	38 ± 3[c](3)	145 ± 8	149 ± 6	0.82 ± 0.05	34 ± 2	3				
PCBD-NAC 6.25 mg/kg	39 ± 9(7)	117 ± 21	159 ± 24	0.86 ± 0.05	88 ± 28	3		2	1	1
PCBD-NAC 12.5 mg/kg	39 ± 6(6)	138 ± 30	201 ± 17*	0.93 ± 0.03	160 ± 24**			1	3	2
TFEC 20 mg/kg	ND(4)	ND	ND	0.83 ± 0.02	118 ± 6***				4	
AOAA alone	17 ± 2(8)	92 ± 15	ND	0.75 ± 0.01	55 ± 3	8				
AOAA + PCBD-NAC 6.25 mg/kg	23 ± 3(5)	98 ± 13	ND	0.85 ± 0.02***	149 ± 30**			1	3	1
AOAA + PCBD-NAC 12.5 mg/kg	24 ± 5(3)	62 ± 7	ND	1.04 ± 0.04***	270 ± 8***					3
AOAA + TFEC 20 mg/kg	ND(5)	ND	ND	0.80 ± 0.02*	134 ± 12***				5	

[a] Female rats were dosed with aminooxyacetic acid at 50 mg/kg, i.p., at the times stated in the Results followed by either TFEC or PCBD-NAC

[b] The extent of renal necrosis was scored as described in Table 1

[c] Values are mean ± SEM with the number of animals in parenthesis

* Significantly different from appropriate control, $P < 0.05$ by ANOVA

** Significantly different from appropriate control, $P < 0.01$ by ANOVA

*** Significantly different from appropriate control, $P < 0.001$ by ANOVA

Fig. 3 Comparison of plasma urea concentration in male and female rats 24 h after a single i.p. dose of either TFEC or S-(1,1,2,3,4,4-pentachloro-1,3-butadienyl)-L-cysteine, (PCBC). The PCBC data were taken from Ishmael and Lock (1986). PCBC female rats (■), PCBC male rats (□), TFEC female rats (●), TFEC male rats (○). *Statistically significantly different from male rats given PCBC, $P < 0.001$

TFEC produced a dose-dependent renal tubular necrosis with selective injury to the pars recta of the proximal tubule, similar to that produced by hexachloro-1,3-butadiene and its glutathione derived conjugates (Lock and Ishmael 1979; Ishmael and Lock 1986). No sex difference in nephrotoxicity was seen, which contrasts with that for the cysteine conjugate of hexachloro-1,3-butadiene, where the female is more sensitive than the male (Fig. 3).

The basis for the sex difference with hexachloro-1,3-butadiene is not currently understood, but it is seen with the parent compound, glutathione, cysteine and mercapturic acid conjugates (Ishmael and Lock 1986). The sex difference is unlikely to be related to a difference in the renal enzyme cysteine conjugate β-lyase, based on the present work with TFEC. However, there could be a related sex difference in hepatic or renal rates of deacetylation or re-acetylation of the cysteine conjugate of hexachloro-1,3-butadiene, which has been shown to be an important factor in determining the renal toxicity of a series of 1,1-difluorinated ethylenes (Commandeur et al. 1988, 1991), or another mechanism still to be elucidated.

The pathway of metabolism of TFEC to form a reactive intermediate was established by Commandeur et al. (1989) following the earlier studies of Odum and Green (1984), TFEC undergoes metabolism via cysteine conjugate β-lyase to form difluorothionoacyl fluoride, which is thought to be responsible for the cellular toxicity in vivo. Formation of this reactive metabolite by the enzyme β-lyase located in the mitochondria leads to inhibition of mitochondrial respiration and hence ATP synthesis (Hayden and Stevens 1990; Groves et al. 1993) and inhibition of the mitochondrial enzyme lipoyl dehydrogenase (Lock and Schnellmann 1990). More

recently, the presence of N-(difluorothionoacetyl) lysine adducts have been detected in renal proteins following administration of TFEC (Harris et al. 1992; Hayden et al. 1991a; Chen et al. 1992; Bruschi et al. 1993). These adducts are also formed in vitro with rat liver and kidney subcellular fractions (Commandeur et al. 1989; Hayden et al. 1991b). The reactive intermediate formed via β-lyase metabolism of TFEC does not appear to react with DNA as TFEC was shown to be non-mutagenic in the Ames *Salmonella* assay with, or without, renal S9 fraction (Green and Odum 1985). It is therefore surprising that TFE was carcinogenic to the kidney in a recent bioassay (NTP 1995), suggesting that the tumours may have arisen by a non-genotoxic mechanism, perhaps related to the persistent proximal tubular degeneration and regeneration produced by continuous exposure to TFE over a 2 year period.

TFEC following administration is likely to undergo metabolism in the liver to form the mercapturic acid (Commandeur et al. 1991). It can then be delivered to the kidney and concentrate in proximal renal tubular cells, via the organic anion transport system located on the basolateral membrane, similar to that established for hexachloro-1,3-butadiene (Lock and Ishmael 1985; Lock et al. 1986). Our studies have shown that prior treatment of rats with probenecid, a competitive substrate for the renal organic anion transport system, afforded complete protection against the nephrotoxicity produced by TFEC. Thus sufficient TFEC must accumulate in renal cells and then undergo de-acetylation (Commandeur et al. 1991) to afford the cysteine conjugate, which is the substrate for β-lyase, to produce the reactive acylating metabolite responsible for the toxicity. These findings are in agreement with studies in isolated renal proximal tubular cells where probenecid afforded partial protection against the mercapturate of TFEC but not against TFEC itself (Boogaard et al. 1989). Our attempts to inhibit cysteine conjugate β-lyase in vivo by administration of aminooxyacetic acid to rats followed by administration of either TFEC or PCBD-NAC failed to prevent the toxicity as judged by the range of criteria used, with renal pathology being the primary criterion. These findings are in general agreement with those of others who found aminooxyacetic acid afforded only limited protection against the nephrotoxicity produced by S-(1,2-dichlorovinyl)-L-cysteine (Elfarra et al. 1986) and 1,1-dichloro-2,2-difluoroethylene (Commandeur et al. 1987). These authors used the appearance of glucose in urine as the primary indicator of nephrotoxicity, but did not report histological findings in the kidneys.

No consistent evidence of hepatotoxicity was seen following TFEC. Occasional vacuolation of hepatocytes was seen and there was a small dose-related increase in liver to body ratio. This effect may be similar to that reported for hexachloro-1,3-butadiene where hydropic changes were seen in rat liver, with no evidence of necrosis (Lock et al. 1982). In conclusion, these studies have confirmed that the kidney is the major target organ for toxicity following a single dose of TFEC to male or female rats. There was only limited evidence of liver toxicity. No sex difference was seen in the renal toxicity, with a dose of ≥12.5 mg/kg producing selective necrosis to the pars recta of the proximal tubules. Probenecid totally protected the rats against the nephrotoxicity, suggesting that accumulation into proximal tubular cells via the organic anion transport system is a prerequisite for the toxicity. Attempts to inhibit cysteine conjugate β-lyase to a sufficient extent in vivo, with aminooxyacetic acid, failed to provide any protection against the nephrotoxicity.

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