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# Differential Modulation of Hepatic Cytochrome P-450 Enzymes in Rat and Syrian Hamster by 4′-Trifluoromethyl-2,3,4,5-Tetrachlorobiphenyl

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**ABSTRACT: The effects of a single injection (40 mg/kg) of 4′-trifluoromethyl-2,3,4,5-tetrachlorobiphenyl (CF3) on hepatic cytochrome P-450 monooxygenases were assessed in rat and syrian hamster. The CF3 treatment significantly increased the total amount of cytochrome P-450 in both species. In rats, CF3 treatment caused marked increases in ethoxyresorufin *O*-deethylase (EROD), arylhydrocarbon hydroxylase (AHH), and testosterone 7$\alpha$-hydroxylase activities but significantly reduced the activities of benzphetamine *N*-demethylase (BzND), erythromycin *N*-demethylase (ErND), testosterone 6$\beta$, 16$\alpha$, and 16$\beta$-hydroxylases, and formation of androstenedione. Administration of CF3 to hamsters strongly induced the activities of EROD, AHH, BzND, testosterone 15$\alpha$, and 16$\alpha$-hydroxylases, and androstenedione production, whereas ErND, testosterone 6$\beta$, and 7$\alpha$-hydroxylases were decreased. Administration of CF3 to rats induced the CYP1A family proteins and CYP2A1, while CF3 reduced the level of CYP2B1, and, to a lesser extent, of CYP6$\beta$2. In hamsters, CF3 treatment significantly induced the CYP1A2, CYP2A1, CYP2A8, and CYP2B1 isozymes, whereas the CYP6$\beta$2 level was decreased. The ability of hepatic microsomes to activate aflatoxin B1 and benzo(*a*)pyrene was elevated by CF3 treatment in hamsters, while activation of aflatoxin B1 was decreased in microsomes from CF3-treated rats. These results showed differences in the CF3-induced pattern of rat and hamster cytochrome P-450 monooxygenases.**

KEY WORDS: **4′-Trifluoromethyl-2,3,4,5-tetrachlorobiphenyl (CF3), Cytochrome P-450, Rat, Hamster, Enzyme Induction.**

## INTRODUCTION

Cytochromes P-450 (CYP) catalyze the biotransformation of a wide variety of xenobiotics and endogenous substrates and can be distinguished on the basis of their structure, substrate specificity, or response to various types of compounds (1–3). Several isoforms and their specific activities are induced when animals are treated with naturally occurring or man-made chemicals. The use of such CYP inducers has been of advantage in the elucidation of the mechanisms of xenobiotics toxification and detoxification in different species (4,5). Comparative biotransformation and metabolite profile data are important elements in the choice of an animal model in toxicity studies.

Much knowledge about CYP has been obtained in rats, mice, and humans compared with other species. Recently, the hamster has become popular as an animal model in the studies of toxicity, notably in carcinogenicity studies. This animal shows higher basal drug metabolism activities and CYP content and is more efficient in activating certain carcinogens (6–8). It has been demon-

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strated that hamsters differ from rats in their response to CYP inducers (9,10).

Among the CYP inducers, polychlorobiphenyls (PCB) and their derivatives have been well studied. Commercial PCB mixtures are potent inducers of several isoforms of hepatic CYP in a number of species, including rat, mouse, hamster, monkey, and man (11–13). Commercial mixtures cause "mixed-type" induction and several isozymes of P-450 are induced. However, the availability of synthetically pure PCB congeners has allowed an assessment of the regulation of the different isozymes of CYP and other components of hepatic drug metabolism. Structure-activity relationships have been defined (14–17). Recently, a series of 4'-substituted-2,3,4,5-tetrachlorobiphenyls have been synthesized and tested as inducers of rat liver microsomal CYP (18). Among them, the trifluoromethyl-substituted derivative, named CF3, was shown to be an effective inducer of CYP1A family and CYP2B subfamily proteins, and more interestingly, the CYP2A subfamily proteins, known to be relatively resistant to induction in rats (19). In contrast, in hamsters, one member of the CYP2A subfamily (CYP2A8) was mainly induced by 3-methylcholanthrene treatment, constituting approximately 40% of the total amount of CYP (20).

The aim of this study was to compare the modulation by CF3 treatment of the biotransformation system in rats and hamsters, especially on the CYP2A subfamily, and on the other hand, to compare these effects of CF3 with our previous studies assessed on rats treated with prototypic polychlorobiphenyls (17,21,22). The results demonstrated important differences in the CYP enzymes between rats and hamsters.

## MATERIALS AND METHODS

### Chemicals

Aflatoxin B1 (AFB1), 7-ethoxyresorufin, benzphetamine, erythromycin, and testosterone were purchased from Sigma Chemicals (St. Louis, MO). NADPH, NADH, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase were obtained from Boehringer–Mannheim (Mannheim, Germany). Benzo(*a*)pyrene (B(*a*)P), 7-ethoxycoumarin, and other reagents were obtained from Wako Chemical (Osaka, Japan). 4'-trifluoromethyl-2,3,4,5-tetrachlorobiphenyl (CF3) was synthesized by the aryl aryl coupling (23) of *p*-trifluoromethylaniline (Aldrich Chemical Co., Milwaukee, WI) in 10-fold excess of 1,2,3,4-tetrachlorobenzene (Aldrich Chemical Co., Milwaukee, WI). The crude reaction product was purified by Florisil and alumina chromatography and by recrystallization from methanol. White crystalline product had a melting point of 64–65°C and was >99% pure, as determined by gas chromatographic analysis. Structure of the compound was confirmed by NMR and mass spectrometry. Polyclonal antibodies to CYP1A1 and CYP2B1 of rat were obtained from OXYgene (Dallas, TX). Other chemicals were of the highest grade commercially available.

### Animals Treatment and Hepatic Microsomes Preparation

Male Sprague Dawley rats (180 g) and male Syrian golden hamsters (80 g) were obtained from Nippon SLC (Hamamatsu, Japan) and were provided with drinking water ad libitum and fed a normal diet. The animals received a single intraperitoneal injection of CF3 dissolved in corn oil, at a dose of 40 mg/kg, whereas control animals received an equal volume of corn oil (2 mL/kg of body weight). The animals were killed by decapitation on the fourth day after injection, and the livers were perfused, quickly excised, and hepatic microsomal fractions were prepared as previously described (24). Liver microsomes were stored at −80°C until use required.

### Assays

Microsomal protein concentration was determined by the method of Lowry *et al.* with bovine serum albumin as standard (25). Total cytochrome P-450 content was assayed by the method of Omura and Sato (26). The 7-ethoxyresorufin *O*-deethylase (EROD) (27), ethoxycoumarin *O*-deethylase (ECOD) (28), and arylhydrocarbon hydroxylase (AHH) (29) activities were fluorimetrically determined, as previously described, with slight modifications. The *N*-demethylation of benzphetamine and erythromycin was assayed by the generation of formaldehyde according to the methods of Lu *et al.* (30) and Wrighton *et al.* (31), respectively. The testosterone hydroxylase activities were determined by the rate of formation of the corresponding products, as described by Yamazoe *et al.* (32).

### SDS PAGE Electrophoresis and Immunoblotting Analysis

Microsomal proteins were analyzed on 10%-acrylamide gels in the presence of SDS according to the method of Laemmli (33). The resolved pro-

teins were transferred onto nitrocellulose sheets and immunostained for the antibodies directed against rat CYP1A1, CYP2A1, CYP6β2, and CYP2B1 and antibodies directed against hamster CYP1A2 and CYP2A8 produced in our laboratory (24). PAB antirat CYP2A1 and CYP6β2 were kindly donated by Drs. K. Nagata and Y. Yamazoe (Department of Pharmacology, School of Medecine, Keio University, Tokyo). Isozyme protein bands were detected using the peroxidase-diaminobenzidine-tetrahydrochloride method (1).

### Mutagenicity Test

The mutagenicity test was carried out principally by a modification of the Ames test (34). The assay was performed using *Salmonella typhimurium* TA100 in the presence of different proportions of microsomal proteins and AFB1 (0.25 μg/plate dissolved in 50 μL of DMSO) or B(*a*)P (5 μg/plate dissolved in 50 μL of DMSO).

### Statistics

Enzymatic activities of control and treated groups were compared statistically using Student's *t*-test and were considered to be significantly different at $p < 0.05$.

## RESULTS

### Assays

Treatment with CF3 caused a marked increase in CYP content in rat and hamster by about twofold (Table 1). In rats, treatment with CF3 reduced by half the *N*-demethylation of benzphetamine and erythromycin, whereas the activities of ethoxycoumarin *O*-deethylase, arylhydrocarbon hydroxylase, and ethoxyresorufin *O*-deethylase were increased 7-, 8.5-, and 14-fold, respectively. In hamsters, the administration of CF3 caused a 50% reduction in erythromycin *N*-demethylase, whereas the activities of benzphetamine *N*-demethylase, arylhydrocarbon hydroxylase, ethoxycoumarin, and ethoxyresorufin, *O*-deethylase were significantly increased 1.6-, 3-, 4-, and 8-fold, respectively.

### Testosterone Hydroxylase Activities

The effects of CF3 on the testosterone hydroxylases in rat and hamster liver microsomes are shown in Table 2. Microsomes from CF3-treated rats exhibited an enhanced ability to oxidize testosterone at the 6α and 7α positions, by 4.8- and 6.5-fold, respectively. The activities of 6β-, and 16β-hydroxylase, and androstenedione production were decreased by half, whereas marked 87 and 90% reductions were observed for the 16α- and 2α-hydroxytestosterone formations, respectively. The oxidation of testosterone at other positions was not significantly changed (2β, 15α, and 15β C). In hamsters, CF3 treatment resulted in a weak increase in androstenedione and 16α-hydroxytestosterone production (about 1.5-fold), whereas a marked increase in 15α-hydroxytestosterone formation (16-fold) was observed. The oxidation of testosterone at the 2α and 7α positions was significantly decreased by 50%, whereas a marked reduction (76, 90, and 92%) of the 2β-, 6β-, and 15β-hydroxytestosterone production was observed. Oxidation at the 6β and 16β positions was not affected by CF3 treatment.

### Western Blotting Analysis

Immuboblots obtained with rat and hamster microsomal preparations using polyclonal antibodies (PAB) directed against different rat and hamster CYP are presented in Figure 1. When the antibodies to rat CYP1A1 were employed, CF3 treatment strongly induced the corresponding bands in rat, whereas no immunoreactive protein was detected in CF3-treated hamster (A). In contrast, CF3 treatment significantly increased the intensity of the protein band immunorelated to hamster CYP1A2 in both rat and hamster (B). This induction was higher in hamsters as compared to rats. Using the antibodies directed against rat CYP2A1, administration of CF3 to rat and hamster obviously increased the level of the immunoreactive proteins. However, a lesser induction was observed in hamsters (C). An intense band immunoreactive to antihamster CYP2A8 antibody was only recognized in the CF3-treated hamster microsomes (D). Using the antibodies directed against rat CYP2B1, CF3 treatment caused a decrease of the intensity of the corresponding bands in rats, whereas it increased the intensity of the immunoreactive proteins in hamsters. In microsomes from treated and untreated animals, two proteins, immunorelated with rat CYP6β2, were observed (F). Treatment with CF3 slightly decreased the intensity of the two bands in both rat and hamster microsomes.

### Mutagenicity Test

The effects of CF3 on the mutagenic activation of AFB1 and B(*a*)P by both rat and hamster micro-

**TABLE 1.** Effects of CF3 on P-450 Activities in Rat and Hamster Liver

| *Microsomal Parameters*[a] | *Sprague Dawley Rat* | | *Syrian Golden Hamster* | |
|---|---|---|---|---|
| | *Control* | *CF3* | *Control* | *CF3* |
| Total P-450 (nmol/mg) | 0.83 ± 0.065[b] | 1.66 ± 0.083*[c] | 0.89 ± 0.031 | 2.12 ± 0.058* |
| ECOD (nmol/min/mg) | 1.05 ± 0.043 | 7.60 ± 0.56* | 1.85 ± 0.039 | 7.50 ± 0.65* |
| EROD (nmol/min/mg) | 0.09 ± 0.005 | 1.27 ± 0.034* | 0.06 ± 0.004 | 0.45 ± 0.014* |
| AHH (nmol/min/mg) | 0.16 ± 0.011 | 1.35 ± 0.076* | 0.23 ± 0.009 | 0.69 ± 0.034* |
| BzND (nmol/min/mg) | 4.74 ± 0.36 | 2.64 ± 0.15* | 4.94 ± 0.022 | 7.78 ± 0.21* |
| ErND (nmol/min/mg) | 1.89 ± 0.083 | 0.88 ± 0.053* | 5.76 ± 0.17 | 2.62 ± 0.10* |

[a]ECOD, ethoxycoumarin *O*-deethylase; EROD, ethoxyresorufin *O*-deethylase; AHH, arylhydrocarbon hydroxylase; BzND, benzphetamine *N*-demethylase; ErND, erythromycin *N*-demethylase.
[b]Values are expressed per milligram of microsomal protein and as a mean ± SD for five animals.
[c]Significance of the differences vs. controls at $*p < 0.01$.

**TABLE 2.** Effects of CF3 on Testosterone Hydroxylases in Rat and Hamster Liver

| *Testosterone Metabolites*[a] | *Testosterone Metabolism (pmol/min/mg)* | | | |
|---|---|---|---|---|
| | *Sprague Dawley Rat* | | *Syrian Golden Hamster* | |
| | *Control* | *CF3* | *Control* | *CF3* |
| 2α OHT | 395 ± 53[b] | 43 ± 9*[c] | 41 ± 2 | 22 ± 2* |
| 2β OHT | 144 ± 9 | 98 ± 16 | 1773 ± 200 | 425 ± 33* |
| 6α OHT | 35 ± 4 | 170 ± 9* | 96 ± 15 | 90 ± 33 |
| 6β OHT | 1580 ± 157 | 810 ± 150* | 4578 ± 508 | 490 ± 10* |
| 7α OHT | 636 ± 73 | 4100 ± 200* | 3975 ± 650 | 2157 ± 52* |
| 15α OHT | 35 ± 9 | 50 ± 20 | 160 ± 25 | 2634 ± 215* |
| 15β OHT | 41 ± 6 | 44 ± 14 | 1128 ± 126 | 86 ± 13* |
| 16α OHT | 561 ± 63 | 73 ± 13* | 20 ± 2 | 34 ± 4* |
| 16β OHT | 94 ± 19 | 56 ± 16* | 270 ± 30 | 250 ± 20 |
| Androstenedione | 1084 ± 190 | 538 ± 62* | 604 ± 136 | 856 ± 26* |

[a]The abbreviations denote the hydroxylated testosterone metabolites.
[b]Values are expressed per milligram of microsomal protein and as a mean ± SD for five animals.
[c]Significance of the differences vs. controls at $*p < 0.05$.

somes are presented in Figure 2. For AFB1 activation, the slope of the CYP-induced mutants decreased with liver fractions from the different groups in the following order: CF3-treated hamster, untreated hamster, untreated rat, and CF3-treated rat. For B(*a*)P activation, although CF3 treatment markedly increased the activation of B(*a*)P by hepatic microsomes, no species differences were observed. It is to be noted that hamster hepatic microsomes were more potent in activating both AFB1 and B(*a*)P than were rat microsomes.

## DISCUSSION

CF3 treatment caused a marked modification of the catalytic activities and isozymes pattern of the CYP monooxygenases in both rat and hamster liver.

First, in rats, CF3 caused a marked increase in the level of proteins immunoreactive with anti-CYP1A1 and CYP1A2 antibodies and in the activities of ethoxyresorufin *O*-deethylase (EROD) and arylhydrocarbon hydroxylase (AHH). Using anti-hamster CYP1A2 to analyze the microsomal rat CYP1A2 level, the induction of CYP1A2 was weaker than the CYP1A1 one. In rats, the activities of EROD and AHH are related to the CYP1A1 and to a lesser extent to the CYP1A2 (35,36). In a previous study, using a higher dose of CF3, Parkinson *et al.* reported an induction by as much as 65-fold of the CYP1A1 (18). The increase of the AHH activity by CF3 is in agreement with our previous

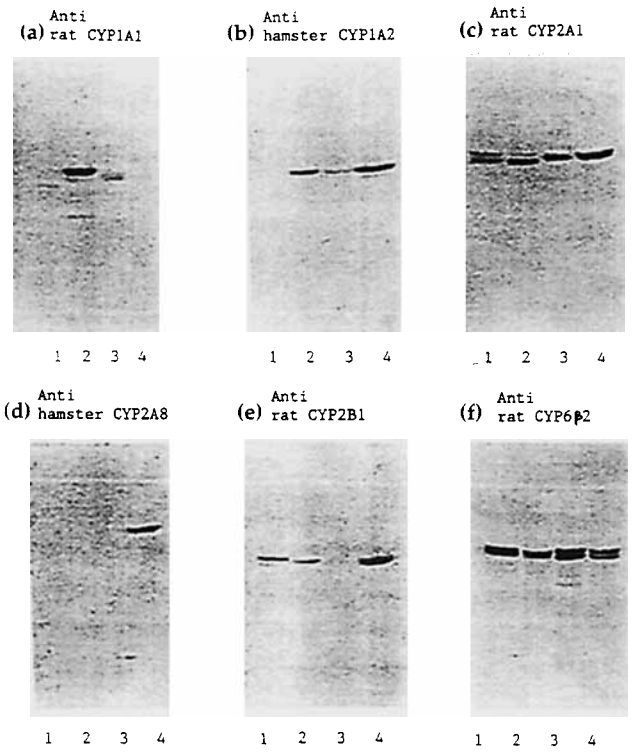


**FIGURE 1.** Western blot analysis of hepatic microsomes from rat (control, lane 1, and CF3-treated, lane 2) and hamster (control, lane 3, and CF3-treated, lane 4). The antibodies employed were (A) antirat CYP1A1, (B) antihamster CYP1A2, (C) antirat CYP2A1, (D) antihamster CYP2A8, (E) antirat CYP2B1, and (F) antirat CYP6β2. Microsomal proteins (7.5 μg) were applied on the 10% acrylamide gel and analyzed as described in the Materials and Methods section.

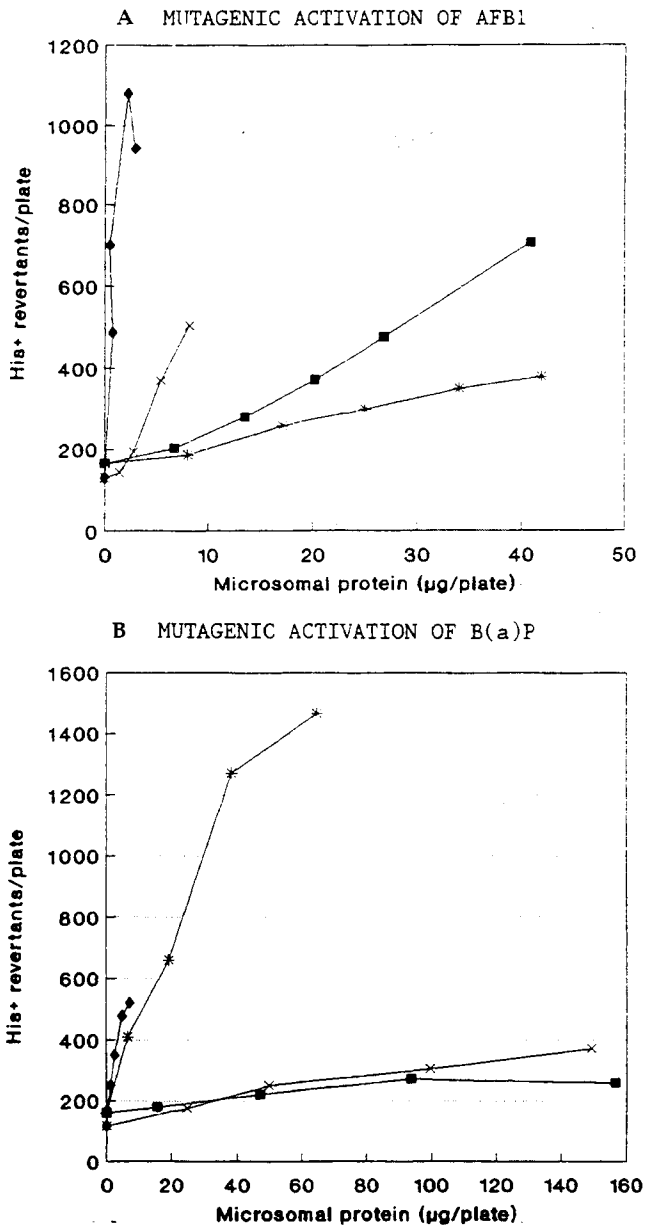


**FIGURE 2.** Effects of CF3 on the mutagenic activation of (A) AFB1 and (B) B(*a*)P by rat and hamster hepatic microsomes (—■— control rat, —✱— CF3-treated rat, —×— control hamster, —◆— CF3 treated hamster). The assays were performed as described in the Materials and Methods section with AFB1 (0.25 μg/plate) or B(*a*)P (5 μg/plate) dissolved in 50 μL of DMSO.

studies on the effects of single treatment of rats with the prototypic 3,3′,4,4′-tetrachlorobiphenyl (21,22). In hamsters, CF3 caused a great induction of the proteins immunorelated to CYP1A2. We were unable to demonstrate the presence of hepatic CYP1A1 in either untreated and CF3 treated-hamster. Recent studies reported similar results in 3-methylcholanthrene (3MC) and β-napthoflavone (BNF)-treated hamsters (37,38). Moreover, despite a clear increase in AHH activity, a stronger induction of EROD was observed in CF3-treated hamsters. Recent studies have led to the conclusion that hamster liver AHH activity was catalyzed by other enzymes than rat liver AHH (39). On the other hand, it has been found that catalytic activity of hamster CYP1A2 does not include EROD (39,40). In both species, the microsomal activation of benzo(*a*)pyrene (B(*a*)P) was elevated in the CF3-treated animals. These results agree well with the western blotting and enzyme activities data. Indeed, in rats, activation of B(*a*)P in mutagenic metabolites is essentially mediated by the cytochrome CYP1A1 and CYP1A2 isozymes (41). In hamsters, the CYP1A family readily catalyzes activation of polycyclic aromatic hydrocarbons, e.g., B(*a*)P (42).

Second, in rats, CF3 treatment significantly increased both the CYP2A1 level and the rate of oxidation of testosterone at the 7α position. In contrast, in hamsters, CF3 treatment strongly induced CYP2A8 level and 15α-hydroxytestosterone pro-

duction, while a significant decrease in testosterone hydroxylation at the 7α position was observed. In rats, CYP2A1 is specifically involved in the formation of 7α-hydroxytestosterone (43–45). In hamsters, the testosterone 15α hydroxylation is mediated principally by CYP2A8 (unpublished data). CYP2A8 is the major enzyme induced in 3MC treated hamsters, constituting about 40% of the total liver CYP, and is mainly responsible for the microsomal activation of AFB1 (20,46,47). Thus, the increase of CYP2A8 and its related activity after CF3 treatment is consistent with the marked induction of the microsomal activation of AFB1. In rats, CF3 treatment failed to induce CYP2A8, not expressed in untreated rats, which agrees well with the studies suggesting that this isozyme is specific to the hamster (48). Moreover, it appears that CF3 is the most effective inducing agent of rat CYP2A1 protein, whereas 3MC, PB, or Aroclor 1254 caused only a modest increase in CYP2A1, and its related activity (18,45,49). In microsomes from both control and CF3-treated hamsters, we detected proteins that cross-reacted with antirat CYP2A1. In which proportion this CYP2A1-like catalyzes the 7α hydroxylation of testosterone remains to be clarified.

Third, in both rat and hamster, ErND activity and 6β-hydroxylation of testosterone were significantly decreased by CF3 treatment. In rat, these activities are principally mediated by the CYP3A1 (43,50). In recent study, Wood *et al.* (51) observed a strong decrease in the formation of 6β-hydroxytestosterone by the treatment of rat with 3MC and Aroclor 1254. Furthermore, western blot using antirat CYP6β2 revealed a significant decrease in the level of the immunostained proteins in CF3-treated hamsters. In contrast, in CF3-treated rats, despite the marked decrease in 6β-hydroxytestosterone production, western blot indicated a slight decrease in CYP6β2. CYP6β2 is a novel form of CYP that catalyzes the testosterone 6β-hydroxylation, and it is reported to belong to the CYP3A subfamily (52). The decrease in the ability of microsomes from CF3-treated rats agrees well with the decrease of the CYP3A protein level. Indeed, in control rats, a member of the CYP3A subfamily is responsible for activating $AFB_1$ to mutagenic compounds (53).

Fourth, in rats, CF3 treatment failed to induce CYP2B1 proteins, while in hamsters, it caused a significant increase in the proteins that cross-reacted with antirat CYP2B1. Parkinson *et al.* (18), using higher dose of CF3, observed a 24-fold induction of CYP2B1 isozyme in rat hepatic microsomes. Similar strong induction was observed by the treatment of rat with PCBs and related compounds. However, a decrease of CYP2B1 level, at an already low level in untreated rats, has been reported under administration of 3MC and other polyaromatic hydrocarbon (PAH) to rats (35). In rats, after CF3 treatment, the activities of benzphetamine *N*-demethylase (BzND) and testosterone 16β-hydroxylase were decreased. This is consistent with the decrease in CYP3A1, an isozyme that is responsible, in untreated rat, for the *N*-demethylation of benzphetamine and 16β hydroxylation of testosterone (54). On the other hand, the decrease of the activity of BzND agrees well with the decrease in the production of 2α-hydroxytestosterone; both of them are related to CYP2C11 (55). In contrast, in hamsters, CF3 treatment increased the BzND and testosterone 16β-hydroxylase activities. In hamsters, the 16β-hydroxytestosterone production is far less specific to CYP2B subfamily proteins than in rat (56). Nevertheless, hamster proteins similar to rabbit P-450 LM2 (rat CYP2B1) have catalytic activity toward benzphetamine (57).

Our results revealed that CF3 treatment showed similar effects, as did treatment with 3MC and related compounds in rat and hamster. The modulation of CYP isozymes differs between rat and hamster. The interspecies variations should be elucidated by studying catalytic activities of isolated CYP enzymes and the mechanisms by which isozymes are induced in each species.

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