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Species Differences in Responsiveness to 1,4-Bis[2-(3,5-dichloropyridyloxy)]-benzene, a Potent Phenobarbital-Like Inducer of Microsomal Monooxygenase Activity

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SUMMARY

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1,4-Bis[2-(3,5-dichloropyridyloxy)]-benzene (TCPOBOP), previously shown to be an extremely potent inducer of the phenobarbital pleiotropic response in mice, was surprisingly ineffective in the rat. Hepatic aminopyrine *N*-demethylase, benzphetamine *N*-demethylase, NADPH-cytochrome *c* reductase, microsomal epoxide hydrolase and ϕ aldehyde dehydrogenase activities, cytochrome P-450, and liver weight were all increased in the rat by sodium phenobarbital, but not by TCPOBOP at a dose of 10 mg/kg (equivalent to 50 times the mouse ED_{50}). At higher doses, TCPOBOP did produce an increase in hepatic benzphetamine *N*-demethylase activity, but this response plateaued at about 40% of the maximal response produced by phenobarbital. TCPOBOP appears to have diminished potency and intrinsic activity in the rat, but it does not antagonize the effect of phenobarbital (i.e., act as a partial agonist). In four strains of mice and Syrian golden hamsters, TCPOBOP (3 mg/kg) was a potent inducer of hepatic aminopyrine *N*-demethylase activity, but four strains of rats and guinea pigs failed to respond. This greatly diminished potency of TCPOBOP in the rat (ED_{50} 1.2×10^{-4} moles/kg) compared with the mouse ($ED_{50} = 4.9 \times 10^{-7}$ moles/kg) is not attributable to (a) a rapid metabolism or excretion, because in both species [^{3}H] TCPOBOP has a long half-life and is stored primarily as the parent compound; or (b) a toxic effect preventing the hepatic induction response. The species difference in sensitivity to TCPOBOP appears to be due to some evolutionary change affecting the mechanism of induction by TCPOBOP but not by phenobarbital.

INTRODUCTION

The induction of hepatic microsomal monooxygenase activity and other coordinately expressed enzymes by the administration of foreign chemicals has been extensively studied since the phenomenon was first described over 25 years ago (1). Most of the compounds which induce hepatic monooxygenase produce one of two patterns of response: phenobarbital is the prototype of one class and MC[2] is the prototype of the other group (2).[3] Phenobarbital increases liver weight, stimulates proliferation of the smooth endoplasmic reticulum, induces NADPH-cytochrome P-450 reductase and one or more species of cytochrome P-450 (7), and increases the associated monooxygenase activity toward a wide variety of substrates (2). In contrast, 3-methylcholanthrene produces a modest increase in the smooth endoplasmic reticulum, induces little or no reductase, and induces a distinct species of cytochrome P-450, with the associated monooxygenase activity increased toward a narrower spectrum of substrates. In addition, both drugs induce a number of nonmonooxygenase enzymes: phenobarbital induces UDP-glucuronosyltransferase (8), glutathione-*S*-transferase (9), ϕ-aldehyde dehydrogenase (10), and micro-

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[2] The abbreviations used are: MC, 3-methylcholanthrene; TCPOBOP, 1,4-bis[2(3,5-dichloropyridyloxy)]-benzene; SDS, sodium dodecyl sulfate.

[3] In addition to the two "classical" responses typified by phenobarbital and 3-methylcholanthrene, several other distinct patterns of hepatic enzyme induction produced by other compounds have been reported recently: pregnenolone 16α-carbonitrile (3), isosafrole (4), 2-(3)-*tert*-butyl-4-methoxyphenol (5), and ethanol (6).

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somal epoxide hydrolase activities (11); and MC induces UDP-glucuronsyltransferase (with substrate specificity distinct from that induced by phenobarbital) (8), DT-diaphorase (12), τ-aldehyde dehydrogenase (10), and choline kinase activities (13). Phenobarbital induces monooxygenase activity primarily in the liver and proximal small intestines, whereas MC affects many tissues. Foreign chemicals are usually classified as phenobarbital-like or MC-like on the basis of the hepatic microsomal monooxygenase activity they induce, and it is assumed, but infrequently documented, that these compounds faithfully evoke the entire pleiotropic response produced by the prototype compounds.

The mechanism of this coordinate enzyme induction produced by MC and MC-like compounds has been shown recently to involve the stereospecific high affinity binding of these compounds to a cytosol protein which mediates their nuclear uptake and presumably the ensuing response (14, 15). This advance is in large part attributable to the availability of a potent agonist, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which binds to the cytosol with a high affinity ($K_D = 2.7 \times 10^{-10}$ M) and which has a long biological half-life, permitting one to separate the process of enzyme induction from that of metabolism of the inducing compound.

The mechanism by which phenobarbital exerts its pleiotropic effect is unclear. The remarkable variety of compounds which appear to produce this response and the lack of any obvious structural similarity among these compounds make it difficult to postulate that all of them act by combining with a single specific recognition site. However, all of these compounds are very weak agonists and are usually administered at doses of 10^{-3}–10^{-4} moles/kg. The lack of a discernible structure-activity relationship among these compounds may be due to their minimal potency.

We have recently described a potent and long-acting phenobarbital-like agonist, TCPOBOP, which in B6D2F$_1$/J mice is 650 times as potent as phenobarbital in inducing hepatic aminopyrine *N*-demethylase activity, with an ED_{50} of 4.9×10^{-7} moles/kg (16). Maximally effective doses of phenobarbital or TCPOBOP, or both compounds administered in combination, induce hepatic aminopyrine *N*-demethylase activity, cytochrome P-450, and NADPH-cytochrome *c*-reductase to the same extent. The cytochrome(s) P-450 induced by both compounds is similar by carbon monoxide and ethyl isocyanide difference spectra and SDS-polyacrylamide gel electrophoresis of liver microsomes. Both compounds increase liver weight, proliferation of the hepatic smooth endoplasmic reticulum, and induction of hepatic microsomal epoxide hydrolase and cytosolic glutathione *S*-transferase activities in B6D2F$_1$/J mice. Both TCPOBOP and phenobarbital induce monooxygenase activity in the proximal intestines, but not in the skin. A single maximally effective dose of TCPOBOP (7.5×10^{-6} moles/kg) induces hepatic aminopyrine *N*-demethylase activity for over 20 weeks in B6D2F$_1$/J mice, reflecting the long biological half-life of the parent compound. In summary, TCPOBOP is a potent and long-acting inducer of the phenobarbital pleiotropic response in the mouse.

In the course of these investigations of TCPOBOP, we observed that a dose of the compound which produced maximal induction of hepatic aminopyrine *N*-demethylase activity in the mouse (7.5×10^{-6} moles/kg) was ineffective in the rat, and, even at 100 times this dose, TCPOBOP produced a submaximal induction of enzyme activity in rat liver. This diminished potency of TCPOBOP in the rat is not attributable to rapid metabolism or excretion of the compound or to the production of a hepatotoxic response which inhibits enzyme induction. In this report, we present these studies of effects of TCPOBOP on hepatic enzyme induction in the rat and other species.

MATERIALS AND METHODS

Materials. 4-Dimethylaminoantipyrine and *trans*-styrene oxide were purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wisc.). Cytochrome *c*, monosodium glucose 6-phosphate, glucose 6-phosphate dehydrogenase (from torula yeast), NADPH, NADP, NADH, and NAD were purchased from Sigma Chemical Company (St. Louis, Mo.). Propionaldehyde was obtained from Eastman Organic Chemicals (Rochester, N. Y.). LK5DF-silica gel plates were bought from Kontes Company (Vineland, N. J.). Protosol was purchased from New England Nuclear Corporation (Boston, Mass.), and RIA-Solvo II scintillation cocktail was purchased from Research Products International Corporation (Elk Grove Village, Ill.). [7-^{3}H] *Trans*-styrene oxide (30 mCi/mmole) was bought from Amersham Searle Corporation (Arlington Heights, Ill.). L-Benzphetamine hydrochloride and L-norbenzphetamine were gifts of The Upjohn Company (Kalamazoo, Mich.).

TCPOBOP and [2-^{3}H]TCPOBOP were synthesized as reported previously (16). *N*-[*methyl*-3H]Benzphetamine was synthesized from norbenzphetamine and [^{3}H]HCHO by Dr. Andrew Kende (University of Rochester, Rochester, N. Y.) and had a specific activity of 10.7 mCi/mmole.

Animals. Sprague-Dawley and Fischer 344 rats and golden Syrian hamsters were obtained from Gibco Animal Resources Laboratories (Madison, Wisc.). Wistar and Long-Evans rats were purchased from the Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Long-Evans rats were also purchased from Simonsen Laboratories, Inc. (Gilroy, Calif.). Guinea pigs were obtained from the Bio-Lab Corporation (St. Paul, Minn.), and 1-week-old chickens were purchased from Sunnyside Farms (Oregon, Wisc.). All of the above animals were products of outbred colonies. Hybrid B6D2F$_1$/J mice and other inbred strains were obtained from the Jackson Laboratory (Bar Harbor, Maine). All of the animals except the chicks were housed in hanging wire cages, permitted unlimited access to laboratory chow (Wayne Mouse Breeder Blox, Allied Mills, Chicago, Ill.) and water, and kept on a 12-hr light/12-hr dark cycle.

Sodium phenobarbital was dissolved in 0.9% sodium chloride solution and injected i.p. daily for 3 days. TCPOBOP was dissolved in corn oil and injected i.p., usually as a single dose. In the experiment on the pharmacokinetics of [^{3}H]TCPOBOP (Fig. 3), the compound was dissolved in *p*-dioxane (0.4 ml/kg) to promote more rapid

absorption. The age and sex of the animals and the dosage regimen for each experiment are given in the legends to the tables or figures.

The inducibility of rat hepatic ϕ-aldehyde dehydrogenase activity by phenobarbital is polymorphic in commercial outbred rat colonies. The trait is determined by two alleles at one locus (17), with the following three phenotypes being distinguishable: poorly inducible (rr), moderately inducible (Rr), and highly inducible (RR). We used Long-Evans rats from the Simonsen Laboratories, which have a high incidence of RR animals in the colony (17), and administered sodium phenobarbital, 100 mg/kg, daily for 3 days. Under ethyl ether anesthesia, the rats were partially hepatectomized and aldehyde dehydrogenase activity was determined in the cytosol of the liver tissue excised. Male and female rats with the highest induced enzyme activity (RR) were selected and bred to give progeny with a uniform phenotype (highly inducible) which were then used in the experiment in Table 3.

Cell fractionation. Liver cell fractions were prepared as previously described (16).

Enzyme assays. Aminopyrine *N*-demethylase and benzphetamine *N*-demethylase activities were measured in the 10,000 × *g* supernatant fraction of liver essentially by the method of Cochin and Axelrod (18), in which the formaldehyde product is converted to a chromagen by the Hantzch reaction (19). Assay conditions were as previously described (16). These monooxygenase activities are expressed as moles of product formed per minute per milligram of tissue (wet weight).

Benzphetamine *N*-demethylase activity was determined in hepatic microsomes (Fig. 4) using a radiometric assay similar to the assay for aminopyrine *N*-demethylase activity (20). The assay was performed in 0.5 ml, containing benzphetamine, approximately 1×10^5 dpm, in 0.5 μmole of substrate.

NADPH-cytochrome *c* reductase activity was measured in liver microsomes by the method of Masters *et al.* (21).

Microsomal epoxide hydrolase activity was assayed by using [7-^{3}H]*trans*-styrene oxide as a substrate by modifications of the method of Oesch *et al.* (11) as recently described (22). In this experiment, the microsomes were stored at −70° until assayed within 7 days.

Aldehyde dehydrogenase activity was assayed in the 100,000 × *g* supernatant fraction of liver, which was stored at −70° in isotonic salt solution containing 30% glycerol and 1 mM dithiothreitol. The assay was performed as described by Deitrich *et al.* (17), using a 1-ml total volume that contained 30 μmoles of pyrophosphate, pH 9.6, 33 nmoles of pyrazole, 1 nmole of NAD, and 3.3 μmoles of propionaldehyde. The propionaldehyde was distilled under nitrogen and stored at −20° until used.

Cytochrome P-450 was measured in liver microsomes by the method of Omura and Sato (23) on a Cary 118C recording spectrophotometer.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of hepatic microsomes was performed in 0.75-mm thick slab gels using an upper stacking gel of 3% and a lower gel of 7.5% total acrylamide (30:8) by the method of Laemmli (24), and the gels were stained with Coomassie blue (25).

Quantification, extraction, and identification of tissue radioactivity. Following the administration of [^{3}H]TCPOBOP to Sprague-Dawley rats and B6D2F$_1$/J mice, the animals were killed at various intervals; samples of inguinal fat, liver, and other tissues were solubilized in Protosol, and the radioactivity was quantified as previously reported. Counting efficiency ranged from 15% for blood to 40% for fat. The data in Fig. 2B for mice has been previously reported (16) and is included for comparison.

Pooled samples of adipose tissue taken from rats 10 and 30 days after administration of [^{3}H]TCPOBOP were saponified, and the radioactivity was extracted into hexane and chromatographed on silica gel thin-layer plates as described previously (16).

Proteins were determined by the method of Lowry *et al.* (26), using bovine serum albumin as a standard.

RESULTS

TCPOBOP is a potent and long-acting phenobarbital-like inducer of hepatic microsomal monooxygenase activity in B6D2F$_1$/J mice (16). TCPOBOP and phenobarbital induce hepatic aminopyrine *N*-demethylase activity to the same maximal extent in the mouse, with parallel log-dose curves, but TCPOBOP is approximately 650 times more potent, with an ED$_{50}$ of 4.9×10^{-7} moles/kg (~200 μg/kg).

Comparison of the effects of these two compounds in the rat gives surprisingly different results (Table 1). Sodium phenobarbital is approximately equipotent in the rat and mouse, and maximally effective regimen produces hepatomegaly and a 6-fold increase in hepatic aminopyrine *N*-demethylase activity in the rat. TCPOBOP, at 3 mg/kg/day, produces little liver enlargement and only a 25% increase in enzyme activity; even at a dose of 30 mg/kg/day, more than 450 times the ED$_{50}$ dose in the mouse, TCPOBOP produces only a doubling of the enzyme activity in the rat. A more detailed comparison of the effects of these compounds is seen in Table 2. Sodium phenobarbital (100 mg/kg/day) produces a significant increase in liver weight, substantial increases in aminopyrine *N*-demethylase, benzphetamine *N*-de-

TABLE 1

The effect of phenobarbital and TCPOBOP on aminopyrine N-Demethylase activity in rats

Sprague-Dawley rats weighing approximately 50 g were injected i.p. for 3 days with corn oil (2.5 ml/kg), TCPOBOP dissolved in corn oil, or sodium phenobarbital in saline (10 ml/kg). The animals were killed 24 hr after the last dose and their livers were assayed for enzyme activity. Each value is the mean ± standard error for five animals.

Treatment	Dose	Liver/body wt. × 100	Aminopyrine *N*-demethylase activity
	mg/kg/day × 3 days		*nmoles HCHO/mg wet wt./min*
Control	—	4.15 ± 0.03	0.148 ± 0.009
Phenobarbital	100	5.83 ± 0.20	0.937 ± 0.054[a]
TCPOBOP	3	4.62 ± 0.07	0.185 ± 0.014[a]
TCPOBOP	10	4.53 ± 0.18	0.309 ± 0.028[a]
TCPOBOP	30	4.77 ± 0.09	0.304 ± 0.063[a]

[a] Significantly different from control group at $p < 0.01$, using a one-tailed Student's *t*-test.

TABLE 2

The effect of phenobarbital and TCPOBOP on hepatic microsomal monooxygenase activities in Sprague-Dawley rats

Female Sprague-Dawley rats, approximately 120 g in weight, were divided into four treatment groups: (a) control animals received isotonic sodium chloride (10 ml/kg) daily for 3 days; (b) sodium phenobarbital dissolved in isotonic sodium chloride (100 mg/kg/day for 3 days); (c) TCPOBOP, 10 mg/kg dissolved in corn oil administered as a single dose on Day 1; and (d) combined administration of sodium phenobarbital (100 mg/kg/daily for 3 days) and TCPOBOP (10 mg/kg). Three days after the initial injection, the animals were killed and the enzyme activities were determined. Each value represents the mean ± standard error of determinations on four animals.

	Liver/body wt. × 100	Aminopyrine *N*-demethylase activity	Benzphetamine *N*-demethylase activity	Cytochrome P-450	NADPH-cytochrome *c* reductase activity
		nmoles HCHO/mg wet wt./min		*nmoles/mg protein*	*nmoles/mg protein/min*
Control	4.81 ± 0.14	0.136 ± 0.011	0.071 ± 0.008	0.415 ± 0.025	13.8 ± 0.5
Phenobarbital	6.35 ± 0.16	0.849 ± 0.075	0.870 ± 0.091	1.088 ± 0.051	29.6 ± 0.8
TCPOBOP	4.86 ± 0.07	0.131 ± 0.007	0.100 ± 0.003	0.456 ± 0.026	15.2 ± 0.3
Phenobarbital and TCPOBOP	6.32 ± 0.19	0.769 ± 0.082	0.774 ± 0.080	1.031 ± 0.076	31.4 ± 1.8

methylase, and NADPH-cytochrome *c* reductase activities, and a 2.5-fold increase in cytochrome P-450. TCPOBOP (10 mg/kg) evokes no substantial increase in these parameters. The effects produced by coadministration of phenobarbital and TCPOBOP do not differ from those produced by phenobarbital alone.

A comparison of the liver microsomal protein pattern from mouse and rat after treatment with phenobarbital and TCPOBOP by SDS-polyacrylamide gel electrophoresis is seen in Fig. 1. In the mouse, the compounds administered alone or in combination produce a similar increase in proteins in the region of 49,000–56,000 mol wt, a pattern distinct from that produced by pregnenolone-16α carbonitrile or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In the rat, phenobarbital produces an observable increase in protein bands, but TCPOBOP (30 mg/kg) does not.

A highly inducible and discriminating measure of the phenobarbital response in the rat is obtainable through the induction of hepatic φ-aldehyde dehydrogenase activity. By using Long-Evans rats selected and bred for the highly inducible phenotype (see Methods) we examined the effect of phenobarbital and TCPOBOP on this enzyme activity. As seen in Table 3, phenobarbital produces an 11-fold increase in φ-aldehyde dehydrogenase activity, TCPOBOP (10 mg/kg) evokes no change, and the coadministration of both compounds produces a response comparable with that of phenobarbital. We also measured microsomal epoxide hydrolase activity in these animals. Phenobarbital or the coadministration of phenobarbital and TCPOBOP produced a 2-fold increase in epoxide hydrolase activity, but TCPOBOP alone produced no change in activity.

To account for this disparity between the exceptional potency of TCPOBOP in mice and its minimal effectiveness in rat, we explored a number of possibilities.

1. *Pharmacokinetics.* The greatly reduced potency of TCPOBOP in the rat might arise from its rapid metabolism and/or excretion by this species. To test this possibility, we administered to Sprague-Dawley rats and B6D2F$_1$/J mice a single i.p. dose of [^{3}H]TCPOBOP (3 mg/kg, 5.34×10^5 dpm/μg) and analyzed the radioactivity in various tissues at subsequent times. As seen in Fig. 2, in both species accumulation of radioactivity was greatest in the adipose tissue; the concentration in the liver was approximately one order of magnitude lower, and lower still in the kidney, lung, brain, and blood (data not shown). The radioactivity stored in the fat of both species declined slowly over a 30-day period. Adipose tissue samples obtained from rats and mice 10 and 30 days after administration of [^{3}H]TCPOBOP were hydrolyzed in alkali and then extracted into hexane. Of the total adipose tissue radioactivity, the fraction extracted into hexane in the mouse was 88.0 ± 2.3% and in the rat it was 79.2 ± 2.4%. Virtually all of the radioactivity extracted from both species migrated with the same R_F as [^{3}H]TCPOBOP in thin-layer chromatography in two solvent systems. We conclude that, in both species, TCPOBOP has a long biological half-life and is stored primarily in the fat as the parent compound. We cannot account for the marked difference in biological potency of TCPOBOP in the rat and mouse by any difference in the pharmacokinetics of this compound in these species.

2. *Toxicity.* It is conceivable that TCPOBOP produces some toxic effect in the rat which interferes with the coordinate pattern of enzyme induction. We have ob-

TABLE 3

The induction of hepatic cytosol aldehyde dehydrogenase and microsomal epoxide hydrolase activities in rats by phenobarbital and TCPOBOP

Immature Long-Evans rats, weighing approximately 100 g, bred from parents of the phenotype highly inducible for φ-aldehyde dehydrogenase (RR) were divided into four treatments groups: (a) control animals received isotonic sodium chloride, 10 ml/kg/day for 3 days; (b) sodium phenobarbital in saline, 100 mg/kg/day for 3 days; (c) TCPOBOP, 10 mg/kg, in corn oil administered as a single dose; and (d) the combined administration of phenobarbital and TCPOBOP. The animals were sacrificed 3 days after the initial injection and the hepatic enzyme activities assayed as described under Methods. Each value is the mean ± standard error of determinations on four animals (three female and one male animal in each group).

	Aldehyde dehydrogenase activity	Epoxide hydrolase activity
	nmoles/mg protein/min	*nmoles/mg protein/min*
Control	33.6 ± 2.4	35.1 ± 2.0
Phenobarbital	385 ± 17.8	78.4 ± 4.8
TCPOBOP	34.3 ± 2.4	32.5 ± 4.3
Phenobarbital plus TCPOBOP	361 ± 12.7	74.4 ± 6.5

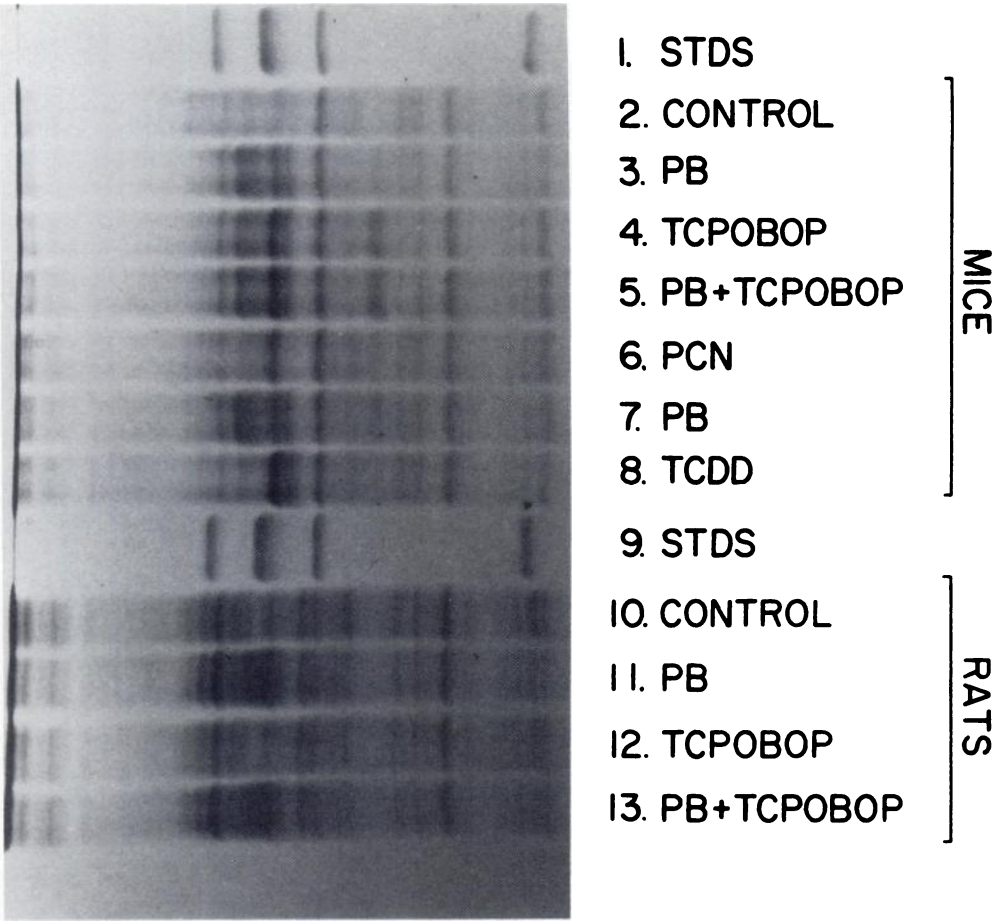

FIG. 1. *Gel electrophoresis patterns of hepatic microsomal proteins from rats and mice pretreated with phenobarbital, TCPOBOP, or other inducers*

Hepatic microsomal protein from B6D2F$_1$/J mice (15 μg) or Sprague-Dawley rats (25 μg) were electrophoresed in SDS-polyacrylamide slab gel (7.5% total acrylamide, 9.5 cm long) and stained as described under Methods. *Lanes 1* and *9*, protein standards (from *left* to *right*): fumarase, 49,000; glutamate dehydrogenase, 53,000; catalase, 58,000; and phosphorylase A, 80,000; *Lanes* 2–8 were microsomal proteins from mice which received the following treatments: *2*, control; *3*, phenobarbital (*PB*), 100 mg/kg daily for 3 days; *4*, TCPOBOP, 3 mg/kg; *5*, combined PB and TCPOBOP treatments; *6*, pregnenolone 16α-carbonitrile (*PCN*), 25 mg/kg daily for 3 days; *7*, PB; and *8*, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*TCDD*), 96.6 μg/kg; *Lanes* 10–13 were microsomal proteins from rats which received the following treatments: *10*, control; *11*, PB, the same regimen as used for mice; *12*, TCPOBOP, 30 mg/kg; and *13*, combined PB and TCPOBOP treatments.

served no gross pathology in treated animals, nor any alterations in routine histological examination of the liver. Furthermore, the extent of induction of hepatic microsomal monooxygenase activity produced by phenobarbital was diminished neither by the simultaneous nor by prior administration of TCPOBOP (30 mg/kg). There is no evidence that TCPOBOP produces any toxic action on rat liver which inhibits enzyme induction.

3. *Action as an agonist with diminished potency and/or diminished intrinsic activity.* In B6D2F$_1$/J mice, TCPOBOP is a potent inducer of the phenobarbital pleiotropic response, i.e., it induces the same battery of enzyme activities to the same maximal extent as does phenobarbital. This suggests that both compounds share a common mechanism of action and may compete for the same hypothetical receptor. In the rat, we have observed that TCPOBOP is not completely inactive (Table 1), but at high doses (150 or 450 times the ED_{50} dose in the mouse), TCPOBOP evokes a modest increase in hepatic aminopyrine N-demethylase activity equivalent to 15–20% of the maximal response produced by phenobarbital. If we assume that TCPOBOP and phenobarbital act on a common receptor, several possibilities might account for the diminished activity of TCPOBOP in the rat: (a) TCPOBOP acts as a partial agonist, with a potency comparable with that observed in the mouse, i.e., the compound binds to the receptor with a high affinity, but has a reduced intrinsic activity and evokes a submaximal response even at an infinite dose; (b) TCPOBOP is a full agonist but with a diminished potency (receptor affinity), i.e., TCPOBOP is capable of producing induction comparable with that produced by phenobarbital, but only at very high doses; or (c) TCPOBOP is a partial agonist with diminished potency in the rat.

Consider the first hypothesis, that in the rat TCPOBOP acts as a partial agonist with an affinity for the putative receptor similar to that in the mouse. A corollary is that TCPOBOP should alter the sensitivity of the rat to phenobarbital, i.e., shift the dose-response curve to the right. As seen in Fig. 3, the log dose-response curve for the induction of hepatic aminopyrine *N*-demethylase activity is not altered by the administration of TCPOBOP (10 mg/kg, a dose which is maximally inducing in the mouse but noninducing in the rat). Since TCPOBOP

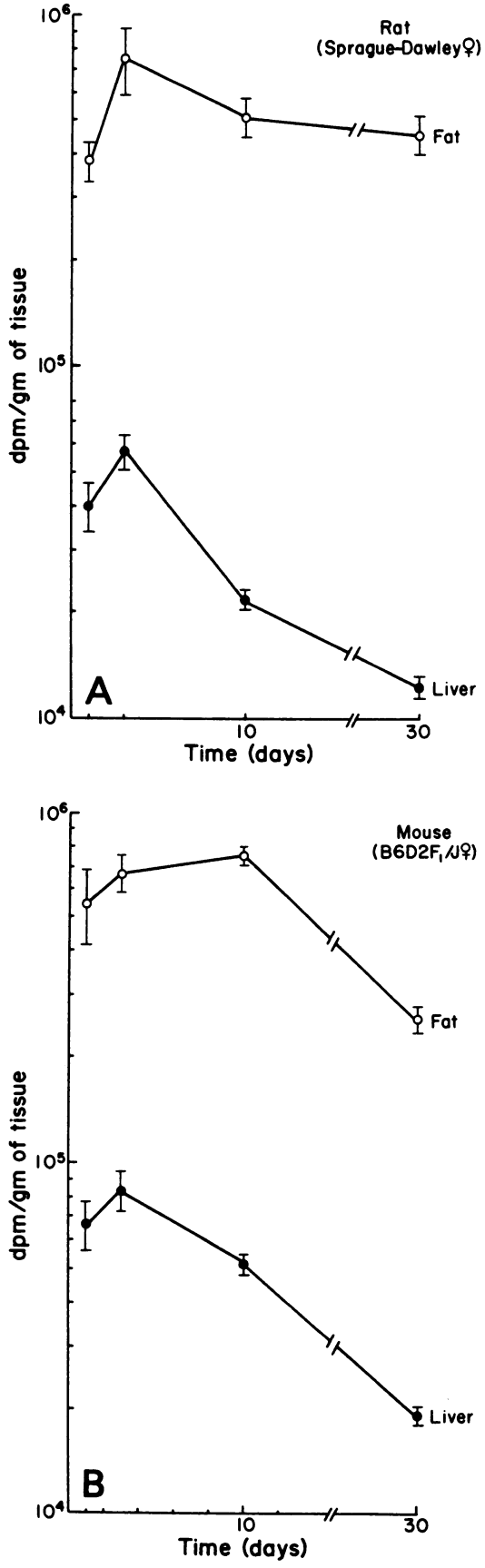

FIG. 2. *Pharmacokinetics of [^{3}H]TCPOBOP in the rat and mouse*
Female Sprague-Dawley rats, 7 weeks old and weighing approximately 180 g, and B6D2F$_1$/J female mice, 9 weeks old, were administered a single i.p. injection of 7.5 × 10^{-6} moles/kg of [^{3}H]TCPOBOP (2.15 × 10^7 dpm/μmole) in *p*-dioxane (0.4 ml/kg). At subsequent time points, the animals were killed and the radioactivity in liver and fat was quantified as described under Materials and Methods. A, Sprague-Dawley rats; B, B6D2F$_1$/J mice. The data on mice have been presented previously (16) and are included for comparison. Each *point* represents the mean ± standard error of determinations on five or six animals.

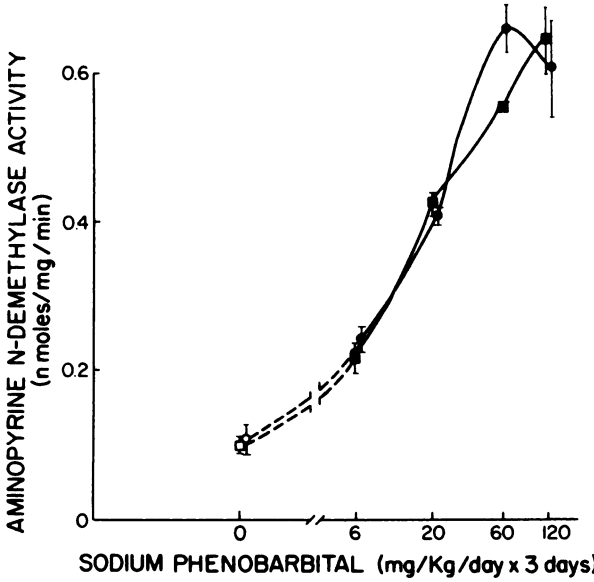

FIG. 3. *Log dose-response relationship for the induction of hepatic aminopyrine N-demethylase activity in rats by phenobarbital with or without the coadministration of TCPOBOP*
Immature female Sprague-Dawley rats were divided into two treatment groups. One group received varying doses of sodium phenobarbital dissolved in isotonic sodium chloride administered daily for three days (■) or the vehicle alone (□). A second group received the same treatment plus the administration of a single dose of TCPOBOP, 10 mg/kg dissolved in corn oil on Day 1 (●), or just corn oil and saline injection (○). Twenty-four hours after the last injection, the animals were killed and hepatic enzyme activity was assayed. Each *point* represents the mean ± standard error of determinations on four or five animals, except the rats which received the largest dose of phenobarbital where $n = 3$.

does not act as a competitive antagonist at this dose, it must have a lower affinity for the putative receptor in the rat than in the mouse.

To explore the hypotheses that TCPOBOP acts as either a full or a partial agonist, but with diminished affinity for the receptor in the rat, we performed the experiment seen in Fig. 4. At very high doses of TCPOBOP, the hepatic concentrations of the compound inhibit monooxygenase activity measured *in vitro*. To eliminate this effect, we measured benzphetamine *N*-demethylase activity in hepatic microsomes which were washed with bovine serum albumin, a procedure shown to remove TCPOBOP.[4]

Rats were treated with varying doses of TCPOBOP, administered alone or jointly with a maximally effective regimen of phenobarbital. As seen in Fig. 4, phenobarbital alone produced a 13.3-fold increase in enzyme activity. TCPOBOP at high doses produced an increase in benzphetamine *N*-demethylase activity. At 100 mg/kg (equivalent to 500 times the mouse ED$_{50}$), TCPOBOP increased the enzyme activity to 42% of the maximal response

[4] A. Poland and E. Glover, unpublished observation.

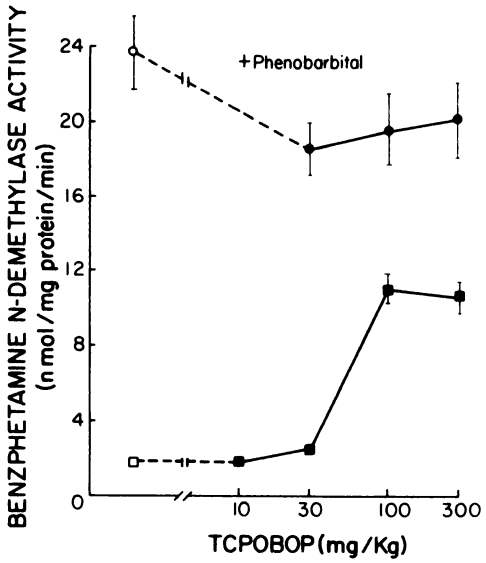

FIG. 4. *Interaction between phenobarbital and TCPOBOP on the induction of hepatic microsomal benzphetamine N-demethylase activity in the rat*

Female Sprague-Dawley rats, weighing 50–60 g received one of the following treatments: (a) corn oil, 15 ml/kg (□); (b) a single administration of varying doses of TCPOBOP dissolved in corn oil (■); (c) sodium phenobarbital (100 mg/kg) dissolved in saline, administered daily for 3 days (○); or (d) the combined administration of sodium phenobarbital (100 mg/kg) daily for 3 days, plus a single injection of a varying dose of TCPOBOP (●). Three days after the initial injection, the animals were killed and hepatic microsomes were prepared. The microsomal pellets were resuspended in 10 mM EDTA in a 1.15% potassium chloride solution, resedimented, and stored in 0.25 M sucrose at −70° until the enzyme was assayed. The microsomes were then washed twice with 10 ml of 1.15% potassium chloride containing bovine serum albumin (10 mg/ml) and once with 10 ml of 0.15 M potassium phosphate buffer, pH 7.4, by resuspension and centrifugation. This procedure was found to remove 95% of the [^{3}H]TCPOBOP associated with liver microsomes following *in vivo* administration of the compound. Benzphetamine *N*-demethylase was assayed in the washed microsomes as described under Materials and Methods. Each value represents the mean ± standard error of determinations on four animals.

evoked by phenobarbital, and at 300 mg/kg TCPOBOP produced no greater increase. This plateauing of the response produced by TCPOBOP suggests that the compound might indeed act as a partial agonist. If this is the case, then at these high doses TCPOBOP should saturate the putative receptor and diminish the effectiveness of the full agonist, phenobarbital. However, in rats which received 100 and 300 mg/kg TCPOBOP plus phenobarbital, the enzyme activity was not significantly different from that in rats which received only phenobarbital. In this experiment, we were unable to demonstrate any antagonism between TCPOBOP and phenobarbital, nor could we produce a maximal response with TCPOBOP comparable with the effect of phenobarbital.

Our observations have been based on the comparison of one strain of mice, B6D2F$_1$/J, and one strain of rats, Sprague-Dawley. We compared the capacity of TCPOBOP and phenobarbital to induce hepatic aminopyrine *N*-demethylase activity in several strains of mice and rats, as well as guinea pigs, hamsters, and chickens. Each strain was divided into three treatment groups: (a) control, (b) phenobarbital, and (c) TCPOBOP. As seen in Table 4, in four strains of mice TCPOBOP produced an 8- to 11-fold increase in hepatic aminopyrine *N*-demethylase activity, comparable with the response produced by phenobarbital. In contrast, the four strains of rats responded to phenobarbital with approximately a 6-fold induction of enzyme activity, but showed little response to TCPOBOP (3 mg/kg). Similarly, in the guinea pig, enzyme activity is induced by phenobarbital, but not appreciably by TCPOBOP. The hamster and chicken were responsive to both compounds, although the chicken was less responsive to TCPOBOP than phenobarbital, on the basis of the single experiment performed.

TABLE 4

The effect of phenobarbital and TCPOBOP on aminopyrine N-demethylase activity in various species

B6D2F$_1$/J, DBA/2J, and RF/J adult female mice, C3H/HeJ adult male mice, Sprague-Dawley, Fischer, Wistar, and Long-Evans rats (all weanling females, 40–75 g), Syrian Golden Hamsters (females, 50–75 g), and guinea pigs (males, 160 g) were administered a single i.p. injection of corn oil (7.5 ml/kg), TCPOBOP (3 mg/kg in corn oil), or sodium phenobarbital (100 mg/kg in saline) daily for 3 days. Chickens (1 week of age) of both sexes were similarly treated except the dose of phenobarbital on successive days was 40, 60, and 80 mg/kg. Hepatic aminopyrine *N*-demethylase activity was assayed under standard conditions for all animals, except that, for chickens, the incubation period was 15 min and, for guinea pigs, the pH optimum was 7.8. Each value is the mean ± standard error of determinations on three or four animals.

	Aminopyrine *N*-demethylase activity *nmol HCHO/mg wet wt./min*			TCPOBOP/ Control
	Control	Phenobarbital	TCPOBOP	
Mice				
B6D2F$_1$/J	0.235 ± 0.007[a]	2.38 ± 0.10	2.70 ± 0.07	11.5
DBA/2J	0.435 ± 0.028	—	3.58 ± 0.11	8.2
C3H/HeJ	0.433 ± 0.038	—	4.08 ± 0.27	9.4
RF/J	0.526 ± 0.044	—	4.23 ± 0.37	8.0
Rats				
Sprague Dawley	0.143 ± 0.012	1.05 ± 0.04	0.196 ± 0.301	1.4
Fischer 344	0.170 ± 0.004	0.985 ± 0.033	0.157 ± 0.015	0.9
Wistar	0.159 ± 0.023	1.18 ± 0.04	10.142 ± 0.014	0.9
Long Evans	0.160 ± 0.010	1.06 ± 0.05	0.198 ± 0.017	1.2
Guinea Pig	0.362 ± 0.049	0.933[b]	0.390 ± 0.062	1.1
Hamster, Syrian Golden	0.586 ± 0.077	1.32 ± 0.03	1.32 ± 0.05	2.3
Chicken	0.061 ± 0.004	0.206 ± .018	0.128 ± 0.004	2.1

[a] $n = 5$.
[b] $n = 2$.

DISCUSSION

In a previous report, we found TCPOBOP to be an extremely potent phenobarbital-like inducer in the mouse, inducing the same battery of enzymes to approximately the same maximal extent as phenobarbital, and differing primarily in its greater potency and longer duration of action. In contrast, in the present study, we found that TCPOBOP is a weak and rather ineffective inducer in the rat (with greatly reduced potency, and possibly reduced intrinsic activity). At a dose of 10 mg/kg (equivalent to 50 times the mouse ED$_{50}$), TCPOBOP produced no significant increase in the rat in hepatic aminopyrine *N*-demethylase, benzphetamine *N*-demethylase, NADPH-cytochrome *c* reductase, microsomal epoxide hydrolase, or ø-aldehyde dehydrogenase activities, and no increase in hepatic cytochrome P-450 content

or liver weight. Phenobarbital increased all of these parameters. Even at the highest doses of TCPOBOP tested in the rat, 300 mg/kg (equivalent to 1500 × the mouse ED_{50}), the compound induced hepatic microsomal benzphetamine *N*-demethylase activity to only 40% of the maximal response observed with phenobarbital. The potencies of phenobarbital to induce hepatic microsomal monooxygenase activity in the mouse [$ED_{50} = 1.07 \times 10^{-4}$ moles/kg × 3 days (16)] and the rat [$ED_{50} = 0.67 \times 10^{-4}$ moles/kg/day × 3 days (Fig. 3)] are very similar. Although TCPOBOP is a potent inducer in the mouse ($ED_{50} = 4.9 \times 10^{-7}$ moles/kg), it is far less effective in the rat ($ED_{50} = 1.2 \times 10^{-4}$ moles/kg).[5] This greatly diminished potency of TCPOBOP in the rat is not attributable to more rapid metabolism and/or excretion of the compound by this species, or to the production of a hepatic toxicity which prevents the induction response. While all of the results cited above contrast the effects of TCPOBOP in a single strain of mice, B6D2F_1/J, and rats, Sprague-Dawley, the conclusions appear to be more general. A dose of TCPOBOP (7.5×10^{-6} moles/kg) which induced hepatic aminopyrine *N*-demethylase activity in four strains of mice and Syrian golden hamsters failed to increase enzyme activity appreciably in four strains of rats or in guinea pigs.

We cannot provide a precise explanation for the species difference in responsiveness to TCPOBOP. However, it is informative to consider these results with respect to several possible models for the mechanism of this pleiotropic response.

Perhaps the simplest model involves postulation that phenobarbital and like-acting compounds evoke the pleiotropic response by binding a single common receptor, a pool of saturable high-affinity sites. A major objection to this model is the absence of any apparent structure-activity relationship among the diverse chemicals which produce a phenobarbital-like response. Since nearly all of these compounds have low potency (administered at 10^{-4}–10^{-3} moles/kg), we may fail to discern the chemical similarity among them which is essential for binding to the common putative receptor. Alternatively, one might envision several distinct receptors which bind different subclasses of compounds, but which evoke the same pleiotropic response. Finally, we may postulate that these diverse chemicals do *not* bind to specific, saturable sites, but act by means of some common physical properties, analogous to the mechanism by which anesthetics produce general anesthesia or the mechanism by which osmotic diuretics produce diuresis.

If TCPOBOP and phenobarbital do not act on a specific receptor but exert their effects by some common physical properties, it is difficult to explain some change occurring between animal species which alters the effect of one compound and not the other. Our data present a strong argument against a nonreceptor model for this induction response.

If both compounds act on a common receptor, presumably in the divergent evolution of the mouse and rat, some change occurred in the receptor which does not affect the potency of a weak agonist such as phenobarbital but which greatly alters the potency (and presumably the binding affinity) of TCPOBOP. If both compounds compete for a common receptor, then TCPOBOP should act as either a full or a partial agonist in the rat; i.e., at some large dose, TCPOBOP either should produce the same maximal effect as phenobarbital or antagonize the action of phenobarbital. Our failure to observe either of these effects might reflect the limitations of our experiment; alternatively, this might suggest another model: TCPOBOP and phenobarbital could bind to separate, independent receptors, and both of these receptors could evoke the same induction response. In this case, an evolutionary change in the receptor which binds TCPOBOP would explain the species difference in sensitivity to TCPOBOP without affecting phenobarbital potency and account for our failure in the rat to demonstrate that TCPOBOP was either a full agonist like phenobarbital or that it could antagonize phenobarbital.

TCPOBOP, the most potent phenobarbital-like inducer presently known in the mouse, is a surprisingly weak agonist in the rat. This difference in species sensitivity to TCPOBOP is not attributable to a difference in metabolism and/or excretion of the compound, but appears to reside in its mechanism of action. Among the numerous foreign chemicals which act as phenobarbital-like inducers, we are unaware of another compound for which a comparable difference in species sensitivity has been observed. The difference in species responsiveness to TCPOBOP is a strong argument against the nonreceptor model of this pleiotropic response. At present, we cannot provide compelling evidence to distinguish between a model in which phenobarbital and TCPOBOP act on a single common receptor site and one that postulates that compounds act at separate recognition sites.

[5] This estimate of potency of TCPOBOP in the rat is based on the data in Fig. 4 and on the assumption that the maximal response observed is the maximal effect TCPOBOP is capable of producing (intrinsic activity = 0.4). We have measured the potency of TCPOBOP to induce hepatic benzphetamine *N*-demethylase activity in the rat; in the mouse the ED_{50} for this response is 3.4×10^{-7} moles/kg (unpublished data), which is very similar to that for the induction of hepatic aminopyrine *N*-demethylase activity ($ED_{50} = 4.9 \times 10^{-7}$ moles/kg).

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