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EFFECT OF XENOBIOTIC ESTROGENS AND STRUCTURALLY RELATED COMPOUNDS ON 2-HYDROXYLATION OF ESTRADIOL AND ON OTHER MONOOXYGENASE ACTIVITIES IN RAT LIVER

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Abstract—Previous study demonstrated that the administration for several days of 1-(*o*-chlorphenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (*o,p'*DDT) (estrogenic DDT derivative) or of tamoxifen (antiestrogen), but not of 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*DDE) (nonestrogen), to ovariectomized female rats dramatically diminished the induction of uterine ornithine decarboxylase (ODC) by subsequently administered estradiol [W. H. Bulger and D. Kupfer, *Archs Biochem. Biophys.* **182**, 138 (1977)]. The present investigation examines whether the inhibition of ODC induction by *o,p'*DDT and tamoxifen may have been due to enhanced hydroxylation of estradiol by the hepatic monooxygenase system. Additionally, the effects of other estrogenic and nonestrogenic xenobiotics on the major route of estradiol metabolism (2-hydroxylation) were examined. Treatment of ovariectomized (ovex) rats with *o,p'*DDT or *p,p'*DDE caused induction of hepatic estradiol-2-hydroxylation and increased demethylase activities of several substrates. Administration of Kepone (estrogenic) and Mirex (nonestrogenic), both inducers of hepatic monooxygenase, also increased 2-hydroxylation of estradiol. For comparative purposes, the effects on estradiol-2-hydroxylation of administration of classical estrogens (estradiol and diethylstilbestrol) and antiestrogen (tamoxifen) and inducers of monooxygenase activity (phenobarbital and 3-methylcholanthrene) were also studied. Treatment of ovariectomized and adrenalectomized (ovex/adx) or intact female rats with estradiol or ovex/adx animals with diethylstilbestrol had no effect on estradiol-2-hydroxylation. Similarly, tamoxifen did not alter the rate of estradiol-2-hydroxylation. The treatment of ovex/adx rats with 3-methylcholanthrene did not affect the rate of estradiol-2-hydroxylation. By contrast, ovex/adx female or intact male rats treated with phenobarbital exhibited induction of estradiol-2-hydroxylase activity. In the above studies only 2-hydroxyestradiol was found; there was no evidence for the formation of primary metabolites hydroxylated at other sites on estradiol. The current findings exclude the possibility that the previously observed inhibition of estradiol-mediated induction of ODC by pretreatment with *o,p'*DDT or tamoxifen (see article cited above) was due to enhanced hydroxylation of estradiol by liver monooxygenases. Also, it was concluded that there is no correlation between the ability to induce hepatic microsomal estradiol-2-hydroxylase activity and estrogenic (or antiestrogenic) properties of a given compound.

In a previous study, we observed that prior administration of 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (*o,p'*DDT) (estrogenic) or of tamoxifen (antiestrogenic) diminished the induction of uterine ornithine decarboxylase (ODC) by subsequently administered estradiol, suggesting that *o,p'*DDT could potentially be antiestrogenic [1]. However, it has been difficult to distinguish between classical antiestrogenic activity involving interfering interactions at the receptor site, such as that demonstrated by tamoxifen [2], and enhanced metabolism of estradiol by inducers of monooxygenase activity. Hydroxylation at the C-2 of estradiol is a major pathway of estrogen metabolism in rat and man [3–7]. The liver contains the highest levels of 2-hydroxylation activity in the rat [8] and this activity, which appears to be cytochrome P-450 mediated [9–11], is primarily in the microsomal fraction [9, 12, 13].

This study was undertaken to examine the possibility that *o,p'*DDT and tamoxifen may exert "antiestrogenic" activity by stimulating the hepatic hydroxylation of estradiol. Additionally, we extended these observations to other classes of xenobiotic estrogens and inducers of monooxygenases.

MATERIALS AND METHODS

Animals. Sprague–Dawley–CD rats (90–100 g) were obtained from the Charles River Breeding Laboratories (Wilmington, MA) and were maintained on Charles River Rat Formula and water *ad lib*. Bilateral ovariectomy (ovex) or adrenalectomy and ovariectomy (ovex/adx) was carried out under ether anesthesia. Animals were used 8–10 days after surgery. Male animals were employed without surgical treatment. Liver microsomes were prepared as previously described [14]. Microsomes used in cytochrome P-450 and P-448 determinations were resuspended in aqueous 1.15% KCl and centrifuged for a second time at 105,000 *g*.

Materials. Diethylstilbestrol, estradiol-3-methyl

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ether (3-MeO-E_2), EDTA-2Na, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, NADPH and activated charcoal untreated powder were purchased from the Sigma Chemical Co. (St. Louis, MO). The *o,p'*DDT and 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*DDE) were from the Aldrich Chemical Co. (Milwaukee, WI). Estradiol-17β was acquired from Steraloids Inc. (Wilton, NH). Kepone and Mirex were purchased from Chemical Service, Inc. (West Chester, PA). Phenobarbital Na was from Mallinckrodt, Inc. (St. Louis, MO). 3-Methylcholanthrene was purchased from the Eastman Kodak Co. (Rochester, NY). Ethylmorphine·HCl was acquired from Merck & Co. (Rahway, NJ). Tris–HCl and grade C dextran were from Schwarz/Mann (Orangeburg, NY). Insta-Gel was from the Packard Instrument Co. (Downers Grove, IL). Benzphetamine·HCl and tamoxifen citrate were gifts from the Upjohn Co. (Kalamazoo, MI) and ICI United States (Wilmington, DE) respectively. [2-^{3}H]Estradiol was provided by Dr. K. I. H. Williams and was repurified as previously described [15].

2-Hydroxylation of estradiol. A previously described method was employed [15]. Incubation was at 37° in a volume of 1 ml containing the following: sodium phosphate buffer (0.1 M, pH 7.4), 0.4 ml; $MgCl_2$ (0.1 M), 0.1 ml; microsomal suspension, 0.1 ml (100 μg protein in 1.15% KCl); 0.1 ml NADPH-generating system in buffer (glucose-6-phosphate, 4.5 μmoles; NADPH, 0.36 μmole; glucose-6-phosphate dehydrogenase, 1 I.U.); 0.1 ml of [2-^{3}H]estradiol (0.1 μCi) in buffer; 5 μl of estradiol (100 nmoles) in ethanol; EDTA, 1 μmole; and H_2O, 0.2 ml. Reaction was initiated with the NADPH-generating system and was terminated 20 min later with 1 ml of ice-cold $CaCl_2$ (0.016 M) and by placing the vials in an ice bath. Zero-time controls were kept on ice throughout. Samples were transferred into "culture" tubes (12 × 75 mm) containing dextran-coated charcoal (DCC) pellets, and contents were vortexed and subsequently shaken for 15 min at 0°. The DCC (charcoal 1%, dextran 0.05% in 10 mM Tris–HCl at pH 8) pellets were prepared by centrifugation (800 *g* for 10 min) of a 1-ml suspension of DCC and by discarding the supernatant fraction. Tubes containing sample and DCC were centrifuged (5000 *g* for 10 min), a 1-ml aliquot of supernatant fraction was placed in a scintillation vial containing 5 ml Insta-Gel plus 0.5 ml H_2O and radioactive content (dpm) was determined. Values were corrected for radioactivity remaining in zero-time control samples. The calculation of data was as previously described [15]. Assay conditions were found to be linear with respect to time and protein content. To ascertain that in each case we assessed the formation of 2-hydroxyestradiol, we subjected a representative pooled sample to high pressure liquid chromatography (HPLC). However, when samples were to be subjected to HPLC analysis, ascorbic acid (33 μmoles) was added to the above incubation mixture to protect catechol estrogens from further presumably nonenzymatic oxidation. After the $CaCl_2$ step, these samples were acidified (pH 1–2 with 1 N HCl), 200 mg NaCl was added, and samples were extracted three times with 2 ml anhydrous ether. Ether phases from each sample were pooled and washed with 1 ml of 20% aqueous NaCl; the ether phase was evaporated, at room temperature, with a stream of N_2 and the residue was stored at −20° under argon, until HPLC analysis. HPLC analysis was as previously described [15] employing a Whatman C_8 column (reversed phase) and an eluting solvent of 55% methanol/45% H_2O (containing 1% acetic acid) run at 2 ml/min. Compounds were monitored at 280 nm. Analysis as determined by co-injection on HPLC with authentic 4-hydroxyestradiol revealed no evidence for the presence of 4-hydroxyestradiol in extracts from the incubations (in the presence of ascorbic acid) of microsomes representing each treatment group employed in this study. However, the possibility of the formation of some polar metabolite(s) representing the hydroxylation of 2-hydroxyestadiol at C-4 or at other sites has not been ruled out.

Demethylation activity. Benzphetamine·HCl (2 μmoles), ethylmorphine·HCl (16 μmoles), or estradiol-3-methyl ether (2 μmoles) was incubated at 37° in an incubation mixture containing Tris–HCl buffer (0.1 M, pH 7.5, at 37°), 0.9 ml; $MgCl_2$ (0.1 M), 0.1 ml; NADPH-generating system (glucose-6-phosphate, 9 μmoles; NADP, 1 μmole; glucose-6-phosphate dehydrogenase, 2 I.U.), 0.1 ml; microsomal suspension (1 mg protein in 1.15% aqueous KCl), 0.1 ml; and H_2O to make a final volume of 2 ml. The estradiol-3-methyl ether was added in 20 μl acetone. Incubation was started by adding the NADPH-generating system and was for 20 min with benzphetamine and ethylmorphine and for 30 min with estradiol-3-methyl ether. Reaction was stopped by adding 0.6 ml of 20% $ZnSO_4$ followed by 0.6 ml of saturated $Ba(OH)_2$. After centrifugation (10 min), 2 ml of the supernatant fraction was analyzed for formaldehyde by adding 1 ml of the Nash reagent [16].

Benzpyrene hydroxylation. [^{14}C]Benzo[*a*]pyrene was incubated with a microsomal suspension (0.1 mg protein in 1.15% KCl) in a final volume of 1 ml for 10 min at 37°; reaction was started by addition of NADPH, and the phenolic products were quantitated radiometrically as previously described [17].

Cytochrome P-450 measurement. Cytochrome P-450 was determined by carbon monoxide difference spectrum of dithionite reduced microsomes using $E_{450\ nm} = 91\ mM^{-1}cm^{-1}$ [18].

Protein determination. The method of Lowry *et al.* [19], employing the modifications of Stauffer [20] and bovine serum albumin as a reference protein, was used.

Radioactivity determination. A Packard 460 CD Liquid Scintillation System was employed. Quench correction was by the channel ratio method.

RESULTS AND DISCUSSION

Previous studies with a variety of compounds demonstrated a strong correlation between degree of induction of uterine ODC and uterotropic activity [21–23], suggesting that induction of uterine ODC represents a *bona fide* marker for estrogenic activity. Hence, the observation, that prior administration of *o,p'*DDT or tamoxifen, but not of *p,p'*DDE,

Table 1. Effect of *o,p'*DDT and *p,p'*DDE on various monooxygenase activities in liver microsomes from ovariectomized female rats*

		Estradiol 2-hydroxylation†	3-MeO-E_2 *O*-demethylation‡	Benzphetamine *N*-demethylation‡	Ethylmorphine *N*-demethylation‡
Experiment 1	Control (5)	1.5 ± 0.1	1.6 ± 0.1	5.7 ± 0.4	4.0 ± 0.2
	*o,p'*DDT (5)	3.3 ± 0.2§	2.7 ± 0.2‖	18.3 ± 1.7§	10.4 ± 1.0§
	*p,p'*DDE (5)	3.4 ± 0.5¶	1.8 ± 0.1	21.8 ± 0.6§	14.8 ± 1.3§
Experiment 2	Control (5)	0.8 ± 0.2	1.1 ± 0.1	3.4 ± 0.1	2.7 ± 0.0
	*p,p'*DDE (5)	1.7 ± 0.1‖	1.5 ± 0.1¶	16.3 ± 0.5§	9.6 ± 0.5§

* Rats were injected i.p. one time each day for 2 days with 100 mg/kg *o,p'*DDT or *p,p'*DDE in 0.2 ml corn oil. Control rats were given 0.2 ml corn oil i.p. Microsomes were prepared 48 hr after the last injection. 3-MeO-E_2 = estradiol-3-methyl ether. | Values = $\bar{X}$ ± S.E. The number of incubations (N) is given in parentheses. | Statistical analysis was by Student's *t*-test.

† Expressed in nmoles 2-hydroxyestradiol formed per mg protein per min.

‡ Expressed in nmoles formaldehyde formed per mg protein per min.

§ $P \leq 0.001$, treated vs control.

‖ $P \leq 0.005$, treated vs control.

¶ $P \leq 0.025$, treated vs control.

inhibited induction of ODC by subsequently administered estradiol, was taken to suggest that *o,p'*DDT may exhibit both estrogenic and antiestrogenic properties. Such "antiestrogenic" activity may have been elicited by merely enhancing the metabolism of administered estradiol. To investigate this possibility we employed a regimen of *o,p'*DDT and *p,p'*DDE treatment that was identical to the one used in the above study [1]. These structurally similar chlorinated hydrocarbons have been shown to be inducers of various monooxygenase activities [23–25], but only *o,p'*DDT diminishes the estradiol-mediated induction of rat uterine ODC [1]. However, the results of the current study demonstrate that both *o,p'*DDT and *p,p'*DDE treatment increased 2-hydroxylation of estradiol in rat liver microsomes (Table 1). If the effect, reported earlier, of *o,p'*DDT on the response of the rat uterus to subsequently administered estradiol was mediated through an enhanced hydroxylation of estradiol, then it would have been expected that both *o,p'*DDT and *p,p'*DDE would exhibit similar apparent antiestrogenic action. This is clearly not the case [1]. In that earlier study, it was also demonstrated that treatment with tamoxifen (an antiestrogen) diminished the induction of uterine ornithine decarboxylase by subsequently administered estradiol [1]. For this reason it was also of interest to investigate the effect of tamoxifen on hepatic microsomal estradiol-2-hydroxylation, employing a regimen identical to the previous study. The results depicted in Table 2 demonstrate that tamoxifen treatment had no effect on the rate of microsomal estradiol-2-hydroxylation. This indicates that the antagonism by tamoxifen, observed in the previous study [1], was not due to enhanced monooxygenase activity. These findings are in agreement with those of other workers who observed that tamoxifen treatment had no effect on aryl hydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase activities in hepatic microsomes from female rats [26].

In another aspect of this investigation, a different class of xenobiotics (Kepone and Mirex) was studied. Kepone and Mirex are structurally similar and both compounds are inducers of hepatic monooxygenase activity [27, 28]. However, whereas Kepone is estrogenic [29–32], Mirex is not [29]. Treatment of ovariectomized rats with Kepone or Mirex resulted in the elevation of hepatic microsomal estradiol-2-hydroxylase activity (Table 3, experiments 1 and 2).

Table 2. Effect of tamoxifen citrate on various monooxygenase activities in liver microsomes from ovariectomized female rats*

		Estradiol 2-hydroxylation†	3-MeO-E_2 *O*-demethylation‡	Benzphetamine *N*-demethylation‡	Ethylmorphine *N*-demethylation‡
Experiment 1	Control (4)	0.8 ± 0.0	1.1 ± 0.1	3.5 ± 0.1	2.8 ± 0.0
	Tamoxifen (4)	0.9 ± 0.1	1.2 ± 0.1	4.0 ± 0.7	3.0 ± 0.5
Experiment 2	Control (5)	1.1 ± 0.1	0.9 ± 0.0	3.2 ± 0.5	2.1 ± 0.1
	Tamoxifen (5)	1.1 ± 0.1	1.2 ± 0.1§	3.9 ± 0.4	2.7 ± 0.2‖

* Rats were injected i.p. one time each day for 4 days with 1 mg/kg tamoxifen citrate in 0.2 ml H_2O. Control rats were given 0.2 ml H_2O i.p. Microsomes were prepared 24 hr after the last injection. 3-MeO-E_2 = estradiol-3-methyl ether. Values = $\bar{X}$ ± S.E. The number of incubations (N) is given in parentheses. Statistical analysis was by Student's *t*-test.

† Expressed in nmoles 2-hydroxyestradiol formed per mg protein per min.

‡ Expressed in nmoles formaldehyde formed per mg protein per min.

§ $P \leq 0.025$, treated vs control.

‖ $P \leq 0.050$, treated vs control.

Table 3. Effect of Kepone and Mirex on various monooxygenase activities in liver microsomes from ovariectomized (ovex) or ovariectomized-adrenalectomized (ovex/adx) female rats*

			Estradiol 2-hydroxylation†	3-MeO-E_2 *O*-demethylation‡	Benzphetamine *N*-demethylation‡	Ethylmorphine *N*-demethylation‡
Experiment 1	ovex	Control (5)	1.7 ± 0.1	1.0 ± 0.1	4.4 ± 0.2	3.6 ± 0.1
	ovex	Kepone (5)	5.2 ± 0.4§	2.0 ± 0.2‖	10.9 ± 1.0§	11.0 ± 1.4§
	ovex	Mirex (5)	3.7 ± 0.4‖	1.9 ± 0.1§	12.2 ± 0.5§	9.6 ± 0.6‖
Experiment 2	ovex	Control (6)	1.0 ± 0.1	ND¶	ND	ND
	ovex	Kepone (6)	2.9 ± 0.4§	ND	ND	ND
	ovex	Mirex (6)	1.9 ± 0.2‖	ND	ND	ND
Experiment 3	ovex/adx	Control (5)	0.9 ± 0.1	1.1 ± 0.1	4.5 ± 0.4	3.2 ± 0.1
	ovex/adx	Kepone (6)	1.8 ± 0.1§	1.9 ± 0.2**	10.0 ± 1.1‖	7.4 ± 0.9‖
	ovex/adx	Mirex (5)	2.2 ± 0.3§	2.5 ± 0.2§	13.9 ± 1.0§	10.6 ± 1.0§

* Rats were injected i.p. (one injection) with 40 mg/kg of Kepone or Mirex in 0.2 ml corn oil. Control rats were given 0.2 ml corn oil i.p. Microsomes were prepared 48 hr after the last injection. 3-MeO-E_2 = estradiol-3-methyl ether. Values = $\bar{X}$ ± S.E. The number of incubations (N) is given in parentheses. Statistical analysis was by Student's *t*-test.

† Expressed in nmoles 2-hydroxyestradiol formed per mg protein per min.

‡ Expressed in nmoles formaldehyde formed per mg protein per min.

§ $P \leq 0.001$, treated vs control.

‖ $P \leq 0.005$, treated vs control.

¶ Not determined.

** $P \leq 0.010$, treated vs control.

Table 4. Effect of estradiol and diethylstilbestrol (DES) on various monooxygenase activities in rat liver microsomes from intact and ovariectomized-adrenalectomized (ovex/adx) female rats*

			Estradiol 2-hydroxylation†	3-MeO-E_2 *O*-demethylation‡	Benzphetamine *N*-demethylation‡	Ethylmorphine *N*-demethylation‡
Experiment 1	Intact	Control (5)	0.8 ± 0.0	1.1 ± 0.0	3.3 ± 0.1	2.5 ± 0.1
	Intact	Estradiol (5)	0.9 ± 0.1	1.1 ± 0.0	3.5 ± 0.1	2.6 ± 0.1
Experiment 2	Ovex/adx	Control (6)	0.9 ± 0.0	ND§	ND	ND
	Ovex/adx	Estradiol (6)	0.9 ± 0.1	ND	ND	ND
Experiment 3	Ovex/adx	Control (6)	1.1 ± 0.1	1.1 ± 0.1	4.0 ± 0.3	2.8 ± 0.2
	Ovex/adx	DES (6)	1.1 ± 0.1	1.0 ± 0.1	3.8 ± 0.3	2.5 ± 0.2

* Rats were injected i.p. one time each day for 4 days with 100 μg/kg of estradiol or DES in 0.2 ml corn oil. Control rats were given 0.2 ml corn oil. Microsomes were prepared 24 hr after the last injection. 3-MeO-E_2 = estradiol-3-methyl ether. Values = $\bar{X}$ ± S.E. The number of incubations (N) is given in parentheses. Statistical analysis was by Student's *t*-test. All values with respect to control were not significantly different ($P > 0.05$).

† Expressed in nmoles 2-hydroxyestradiol formed per mg protein per min.

‡ Expressed in nmoles formaldehyde formed per mg protein per min.

§ Not determined.

Similar results were obtained when the experiment was repeated with ovex/adx animals (Table 3, experiment 3), indicating that the phenomenon was not caused by an ovarian or adrenal substance(s). These findings (Table 3) and the results with *o,p'*DDT and *p,p'*DDE (Table 1) indicate that the capacity to induce hepatic microsomal estradiol-2-hydroxylase activity is related to the ability of these compounds to induce monooxygenase in general and is independent of their estrogenic activity. The results depicted in Table 4 also support this conclusion. Treatment with estradiol had no effect on estradiol-2-hydroxylation by liver microsomes from intact or ovex/adx rats (Table 4, experiments 1 and 2). Similarly, treatment with diethylstilbestrol did not alter estradiol-2-hydroxylase activity (Table 4, experiment 3). Also, these compounds did not alter the *O*-demethylase or *N*-demethylase activities (Table 4, experiments 1 and 3). By contrast, other investigators [33] using a 10-fold higher dose (1 mg/kg) of steroidal estrogens have demonstrated, with female rats, moderate induction of hepatic microsomal aryl hydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase activities as well as increases in microsomal cytochrome P-450, indicating that extremely high doses of estrogens could elevate monooxygenase activity.

Since DDT is thought to be a phenobarbital-type inducer of monooxygenases [23], we examined the ability of phenobarbital to induce the 2-hydroxylation of estradiol. Additionally, for comparison purposes, the effect of a different type of inducer (3-methylcholanthrene) on estradiol-2-hydroxylation was also investigated. Treatment of ovex/adx female rats with 3-methylcholanthrene (3-MC) had no effect on the 2-hydroxylation of estradiol (Table 5). As anticipated, benzpyrene hydroxylation and cytochrome P-448 levels were elevated by this treatment (Table 5, experiment 1). Lack of induction of estradiol-2-hydroxylation by 3-MC has been reported previously for male rats [34]. By contrast, treatment of ovex/adx female rats with phenobarbital elevated hepatic microsomal estradiol-2-hydroxylation. This increase was also paralleled by increases in microsomal *O*-demethylase and *N*-demethylase activities and cytochrome P-450 levels (Table 5, experiment 2).

The results with phenobarbital were surprising, since previous studies demonstrated that phenobarbital treatment of male rats had no effect on hepatic microsomal estradiol-2-hydroxylation [13, 34]. However, even in male rats we observed that phenobarbital stimulated the 2-hydroxylation of estradiol (Table 5).* The discrepancy between our data (Table 5) and the earlier reports [13, 34] may reside in the contrast between the respective methodologies for the quantification of 2-hydroxyestradiol. Those studies may have underestimated 2-hydroxyestradiol in the phenobarbital-treated animals, because of

Table 5. Effect of 3-methylcholanthrene (3-MC) and phenobarbital (Pb) on various monooxygenase activities in rat liver microsomes from intact male and ovariectomized-adrenalectomized female rats*

			Estradiol 2-hydroxylation†	Benzpyrene hydroxylation‡	3-MeO-E_2 *O*-demethylation§	Benzphetamine *N*-demethylation§	Ethylmorphine *N*-demethylation§	P-450 (P-448)‖
Expt. 1	Female	Control (6)	0.5 ± 0.0	0.06 ± 0.01	ND¶	ND	ND	0.41 ± 0.02
	Female	3-MC (6)	0.6 ± 0.1	1.32 ± 0.17**	ND	ND	ND	1.06 ± 0.04**
Expt. 2	Female	Control (6)	0.36 ± 0.03	ND	1.4 ± 0.2	3.8 ± 0.5	5.2 ± 0.8	0.46 ± 0.01
	Female	Pb (5)	1.72 ± 0.12**	ND	2.6 ± 0.1**	15.6 ± 1.2**	20.6 ± 1.1**	0.99 ± 0.04**
Expt. 3	Male	Control (6)	1.62 ± 0.15	ND	ND	9.3 ± 1.5	ND	ND
	Male	Pb (6)	3.06 ± 0.17**	ND	ND	33.6 ± 1.2**	ND	ND
Expt. 4	Male	Control (6)	1.47 ± 0.10	ND	ND	10.8 ± 0.4	ND	ND
	Male	Pb (6)	2.41 ± 0.17**	ND	ND	22.7 ± 1.5**	ND	ND

* 3-Methylcholanthrene-treated rats were injected (25 mg/kg in 0.4 ml corn oil) i.p. one time each day for 4 days; microsomes were prepared 48 hr after the last injection. Phenobarbital-treated rats were injected (37.5 mg/kg in 0.2 ml H_2O) i.p. two times each day for 4 days; microsomes were prepared 12 hr after the last injection. Control rats were given 0.4 ml corn oil or 0.2 ml H_2O. 3-MeO-E_2 = estradiol-3-methyl ether. Values = $\bar{X}$ ± S.E. The number of incubations (N) is given in parentheses. Statistical analysis was by Student's *t*-test.

† Expressed in nmoles 2-hydroxyestradiol formed per mg protein per min.

‡ Expressed in nmoles phenolic products formed per mg protein per min.

§ Expressed in nmoles formaldehyde formed per mg protein per min.

‖ Expressed in nmoles cytochrome P-450 (P-448) per mg protein.

¶ Not determined.

** $P \leq 0.001$, treated vs control.

* The observations by Shiverick [35] reported in a brief communication (abstract) support our findings with respect to phenobarbital, *o,p'*DDT and tamoxifen treatment of female rats. However, in that study only the formation of catechol estrogen was determined without demonstration of the site of estradiol hydroxylation.

metabolism of 2-hydroxyestradiol into derivatives which were not quantitated by the assays employed. By contrast, our assay determines 2-hydroxylation of estradiol (release of 2-^{3}H) independently of the subsequent metabolism of 2-hydroxyestradiol and thus measures all 2-hydroxyestradiol formed. However, whether these apparent discrepancies are due to other factors as well remains to be established.

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REFERENCES

1. W. H. Bulger and D. Kupfer, *Archs Biochem. Biophys.* **182**, 138 (1977).
2. V. C. Jordan, G. Prestwich, C. J. Dix and E. R. Clark, in *Pharmacological Modulation of Steroid Action* (Eds. E. Genazzani, F. Di Carlo and W. I. P. Mainwaring), pp. 81–98. Raven Press, New York (1980).
3. J. Fishman, R. J. Cox and T. F. Gallagher, *Archs Biochem. Biophys.* **90**, 318 (1960).
4. J. Fishman, *J. clin. Endocr. Metab.* **23**, 207 (1963).
5. W. B. Keith and K. I. H. Williams, *Biochim. biophys. Acta* **210**, 328 (1970).
6. K. I. H. Williams, *Steroids* **15**, 105 (1970).
7. A. Bartke, R. E. Steele, J. G. Williams and K. I. H. Williams, *Steroids* **18**, 303 (1971).
8. A. R. Hoffman, S. M. Paul and J. Axelrod, *Biochem. Pharmac.* **29**, 83 (1980).
9. S. M. Paul, J. Axelrod and E. J. Diliberto, Jr. *Endocrinology* **101**, 1604 (1977).
10. S. M. Paul and J. Axelrod, in *Pharmacological Modulation of Steroid Action* (Eds. E. Genazzani, F. Di Carlo and W. I. P. Mainwaring), pp. 1–10. Raven Press, New York (1980).
11. H. A. Sasame, M. M. Ames and S. D. Nelson, *Biochem. biophys. Res. Commun.* **78**, 919 (1977).
12. S. M. Paul and J. Axelrod, *Life Sci.* **21**, 493 (1977).
13. R. L. Barbieri, J. A. Canick and K. J. Ryan, *Steroids* **32**, 529 (1978).
14. S. H. Burstein and D. Kupfer, *Ann. N.Y. Acad. Sci.* **191**, 61 (1971).
15. D. Kupfer, G. K. Miranda and W. H. Bulger, *Analyt. Biochem.* **116**, 27 (1981).
16. T. Nash, *Biochem. J.* **55**, 416 (1953).
17. J. E. Brown and D. Kupfer, *Chem. Biol. Interact.* **10**, 57 (1975).
18. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. C. E. Stauffer, *Analyt. Biochem.* **69**, 646 (1975).
21. A. M. Kay, I. Icekson and H. R. Lindner, *Biochim. biophys. Acta* **252**, 150 (1971).
22. S. Cohen, B. W. O'Malley and M. Stastny, *Science* **170**, 336 (1970).
23. D. Kupfer and W. H. Bulger, in *Estrogens in the Environment* (Ed. J. A. McLachlan), pp. 239–63. Elsevier-North Holland, New York (1980).
24. D. Kupfer, *CRC Crit. Rev. Toxic.* **4**, 83 (1975).
25. L. G. Hart and J. R. Fouts, *Proc. Soc. exp. Biol. Med.* **114**, 388 (1963).
26. W. A. Al-Turk, S. J. Stohs and E. B. Roche, *Drug Metab. Dispos.* **9**, 327 (1981).
27. D. L. Fabacher and E. Hodgson, *Toxic. appl. Pharmac.* **38**, 71 (1976).
28. L. S. Kaminsky, L. J. Piper, D. N. McMartin and M. J. Fasco, *Toxic. appl. Pharmac.* **43**, 327 (1978).
29. B. Hammond, B. S. Katzenellenbogen, N. Krauthammer and J. McConnell, *Proc. natn. Acad. Sci. U.S.A.* **76**, 6641 (1979).
30. V. P. Eroschenko and W. O. Wilson, *Toxic. appl. Pharmac.* **31**, 491 (1975).
31. R. D. Palmiter and E. R. Mulvihill, *Science* **201**, 356 (1978).
32. W. H. Bulger, R. M. Muccitelli and D. Kupfer, *Molec. Pharmac.* **15**, 515 (1979).
33. W. A. Al-Turk, S. J. Stohs and E. B. Roche, *Drug Metab. Dispos.* **8**, 143 (1980).
34. M. Numazawa, N. Soeda, Y. Kiyono and T. Nambara, *J. Steroid Biochem.* **10**, 227 (1979).
35. K. T. Shiverick, *Endocrinology* (Suppl.) **106**, 255 (Abstr. 721) (1980).