4,4-DICHLORODIPHENYLETHYLENE (4.4-DDE) IS A 3-METHYLCHOLANTHRENE-TYPE INDUCER IN THE DEER MOUSE (*Peromyscus maniculatus)* BUT AN AROCLOR-LIKE INDUCER IN THE VOLE *(Microtus ochrogastor).*

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Introduction

Technical grade DDT, a mixture of 2,2-bis (4-chlorophenol)-1,1,1-trichloroethane (4,4'-DDT) and 2-(2-chlorphenol)-2-(4-chlorophenol)-1,1,1-trichloroethane (2,4'-DDT), was used worldwide as a pesticide from the 1940's to the early 1970's. The use of DDT in the USA was banned in 1972 following the decline of many passerine species and impaired reproduction in raptors due to eggshell thinning (1). It is still used in many third-world countries where preservation of human life outweighs environmental concerns. As originally formulated, technical DDT contained approximately 85% 4,4'-DDT and 15% 2,4'-DDT. In the environment, 4,4'-DDT is converted to 4,4'-DDE. Like DDT, 4,4'-DDE has been shown to biomagnify in animal tissues. Cattle feeding on grassland formally used as an orchard accumulated 4,4'-DDE from the grass into milk fat and adipose tissues (2). DDT and similar halogenated aromatic hydrocarbons have been found in milk and this may be a major route of excretion in lactating females (3,4,5). 4,4'-DDE residues can be found in appreciable levels in many human and animal tissues including ovarian follicular fluid (6) and breast adipose tissue (7).

The different congeners of DDE and DDT have different effects in tissues. The 2,4' congener of DDT binds to the estrogen receptor and elicits estrogen-like effects (8). Moreover, technical DDT and its metabolites exert uterotrophic effects in experimental animals (9). DDT is also reported to adversely affect the male reproductive system (10). 4,4'-DDE acts as an antiandrogen in the rat by competitive inhibition with testosterone and dihydrotestosterone binding to the androgen receptor (11). Early gestational exposure (preimplantational) to DDT results in decreased brain and body weights and embryo lethality but not deformations (12).

DDT/DDE concentrations in tissues depend on bioavailability and metabolism. Also, intestinal bacteria such as *Aerobacter aerogenes* have been shown to degrade DDT to DDE in the rat (13). DDT is also converted to DDE by DDT-dehydrochlorinase, a glutathione (GSH) dependent reaction (14). This conversion is observed in both mammalian and avian species (15). Increased activity of glutathione S-transferase correlates with DDT resistance in houseflies (14). The non-enzymatic conversion of DDT to DDE has also been reported.

DDT and DDE congeners induce liver microsomal enzymes including cytochrome P450s (CYP) and UDP-glucuronosyl transferases (UGT). However, the isozymes induced, particularly with DDE, appear to be species and strain dependent. Alternatively, the substrate specificity of the isozymes induced may vary between strain and species. DDT is reported to induce cytochrome P450B1 and P450B2 in mammalian and avian liver (14). However, the identity of the CYP enzymes induced by DDE is less clear. Studies by Haake et al (16) demonstrated that DDE induced benzo[a]pyrene 3-hydroxylase activity, an activity associated with CYP1A1 in the rat. CYP1A1 induction is mediated through both Ah receptor-mediated and hypoxia-mediated pathways and is found following exposure to xenobiotics such as TCDD and 3-methylcholanthrene. In contrast, Nim et al (17) report that 4,4'-DDE is a CYP2B inducer in the Fischer 344/NCr rat and show that both immunoreactive CYP2B protein and CYP2B mRNA are induced following exposure to 4,4'-DDE in the diet.

In this study, we show that 4,4-DDE exposure results in different patterns of CYP isozyme in the deer mouse and the vole. These findings are significant since both the deer mouse and the vole represent New World rodent species frequently used as sentinels for human and wildlife exposure. An increase in CYP1A1 activity would normally be interpreted as an indication of PAH or HAH exposure and not the result of DDE exposure thus resulting in an erroneous risk assessment. Moreover, increases in CYP1A1 and 1A2 activities effect gonadal steroid metabolism and result in increased bioactivation of some pro-carcinogens whereas increases in CYP2B are linked to increased clearance of many drugs.

Material and Methods

Animal Care and Treatment-Deer mice (Peromyscus maniculatus), 28 to 35 days old, and voles (Microtus ochrogastor) were obtained from a breeding colony in house. Mice and voles were acclimated for 7 days before dosing and (housed 5 per cage) were maintained on hardwood shavings, fed and watered ad libitum and kept on a 12 hour light –dark cycle. Mice or voles were dosed by gavage with 0, 0.1, 0.3, 1.0, 3.0 or 10 mg/kg 4,4'-DDE dissolved in corn oil such that the animals received a dose volume of 5 ml/kg body weight. Each animal received two doses of compound 24 hours apart. Animals were euthanized by CO2 asphyxiation 48 hours after the last dose of compound. The uterus and liver were removed, weighed, and placed into a tube containing ice-cold homogenization buffer (Tris 10 mM; sucrose 0.4%; EDTA 0.1 mM).

Cytochrome P450 Activity-Alkylresorufin-O-dealkylase activity was measured in hepatic microsomes by a modification of the method reported by Pohl and Fouts (18). Microsome preparation was performed using a modification of the technique described by Denomme (19). The microsomes were stored at –80°C until assayed. The fluorometer settings were 540 nm excitation and 590 nm emission, each with a 10 nm slit width. The actual protein content of each sample was confirmed by using a modification of the Bradford (20) method in a Molecular Devices Thermomax microplate reader at a wavelength of 595 nm. The data was reported as picomoles of resorufin formed per minute per mg protein (pmol/min/mg protein), or alternatively, as percent of control EROD activity.

Cytochrome P450 Immunoprecipitation and Western Blotting-Microsomes were solubilized in sodium dodecyl sulfate (SDS) buffer, mixed with loading buffer, and loaded onto a 4-12% gradient SDS polyacrylamide gel (SDS-PAGE) at 5 µg/well protein. The proteins were transferred from the gel to nitrocellulose membrane using a NOVEX electroblot module. The membrane was exposed to the primary antibody for 1 hour then rinsed 4 times with PBS containing 1% Tween. The membrane was then exposed to the secondary antibody for 1 hour and rinsed 4 times with PBS-Tween. The blot was developed using the Amersham ECL kit using the manufacturer's directions and exposed to x-ray film for 5 minutes. The intensity of the bands was quantitated using a 32-bit scanner and NIH Image software. Immunoprecipitation studies were performed by adding antibody to the microsomes and measuring EROD or BROD activity as described above. These assays were run in duplicate with non-specific antibody used as a control.
S**tatistics and Data Handling**-Differences in responses among doses were determined by 2-way ANOVA for unbalanced data or the General Linear Model if data groups passed the Shapiro-Wilk normality and the Levene's variance test. Level of significance was set at $p \leq 0.05$.

Results and Discussion
The following table (TABLE 1) compares the effects of 4,4'-DDE exposure to voles and deer mice upon alkylresorufin-O-dealkylase activity. Note that both the pattern and magnitude of induction are different between these species. In particular, the deer mouse appears much more sensitive to 4,4'-DDE in terms of the magnitude of EROD and MROD induction. In contrast, the vole seems insensitive to the doses of DDE used. Significant induction was only observed at the highest doses. Note DeMo equals deer mouse.

TABLE 1 DDE-Induced Alkylresorufin-O-Dealkylase Activities in Vole and Deer Mouse

Dose, mg/kg	Vole BROD	Vole EROD	Vole MROD	Vole PROD	DeMo BROD	DeMo EROD	DeMo MROD	DeMo PROD
0	50	215	100	50	50	80	400	10
1	75	225	120	65	90	750	1100	15
3	110	265	185	80	110	1400	1200	12
10	90	330	280	95	125	3050	1350	8

The following table (TABLE 2) describes the alteration in specific alkylresorufin-O-dealkylase activities with increasing amounts of antibody to rat CYP1A1. Control is goat serum in lieu of antibody. Note that antibody treatment substantially reduces activity at low concentrations except for vole BROD suggesting that CYP1A1 is largely responsible for deer mouse EROD and BROD activities as well as vole EROD, MROD and PROD activities.

TABLE 2 Inhibition of Alkylresorufin-O-Dealkylase Activities by Antibody to Rat CYP1A1

Antibody	Control	DeMo EROD	DeMo BROD	Vole EROD	Vole BROD	Vole MROD	Vole PROD
0	100	100	100	100	100	100	100
0.5	100	85	50	90	95	100	100
1	100	65	45	100	90	85	90
3	100	45	20	55	85	65	60
5	100	42	12	40	80	40	50
7.5	100	40	11	30	80	39	40
10	100	38	10	20	80	36	40
15	100	35	10	23	82	37	40

The following table (TABLE 3) describes the alteration in specific alkylresorufin-O-dealkylase activities with increasing amounts of antibody to rat CYP2B. Again, control is goat serum in lieu of antibody. Note that CYP2B antibody treatment produces a concentration dependent decrease in vole BROD and PROD activities but does not affect deer mouse EROD or PROD activities nor vole EROD or MROD activities. There appears to be some overlap for substrate affinity between vole CYP1A1 and CYP2B for pentoxyresorufin. This study demonstrates that one cannot assume there is species conservation among the cytochromes P450 for all substrates and points out the difficulty in cross-species comparison without confirmatory data.

TABLE 3 Inhibition of Alklyresorufin-O-Dealkylase Activities by Antibody to Rat CYP2B

Antibody	Control	DeMo EROD	DeMo BROD	Vole EROD	Vole BROD	Vole MROD	Vole PROD
0	100	100	100	100	100	100	100
0.5	100	96	117	110	90	110	100
1	100	82	98	105	80	95	70
3	100	92	96	105	40	100	50
5	100	82	91	100	10	96	20
7.5	100	100	98	90	8	105	10
10	100	97	93	110	5	91	20
15	100	77	97	95	8	95	15

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