Persistence in Alterations in the Ontogeny of Cerebral and Hepatic Cytochrome P450s following Prenatal Exposure to Low Doses of Lindane

Ashu Johri,* Alok Dhawan,* Ram Lakhan Singh,+ and Devendra Parmar*†

*Developmental Toxicology Division, Industrial Toxicology Research Centre, Lucknow 226 001, Uttar Pradesh, India; and †Department of Biochemistry, Dr R. M. L. Awadh University, Faizabad 224 001, Uttar Pradesh, India

Received August 16, 2007; accepted October 28, 2007

Oral administration of low doses (0.0625, 0.125, or 0.25 mg/kg body weight, po, corresponding to 1/1400th, 1/700th, or 1/350th of LD₅₀, respectively) of lindane, an organochlorine insecticide, to pregnant dams from gestation day 5–21 was found to produce dose-dependent alterations in the ontogenic profile of xenobiotic-metabolizing cytochrome P450s (CYPs) in the brain and liver of offspring. The increase in the cerebral and hepatic mRNA expression of CYP1A1, 1A2, 2B1, 2B2, and 2E1 was also found to be associated with an increase in the catalytic activity of these CYP isoenzymes in the brain and liver of the offspring at different stages during postnatal development. Interestingly, though the levels of CYPs were severalfold lower in brain when compared to the liver, almost equal magnitude of induction in these CYPs in brain have suggested that like in the liver, brain CYPs are responsive to the transplacental induction by environmental chemicals and that the increase is transcriptionally regulated. Moreover, due to its lipophilic nature, lindane may partition in mother’s milk leading to further exposure of the offspring during the critical period of neurodevelopment which may explain the increase in CYP mRNA expression and associated catalytic activity especially during the early postnatal period. Interestingly, the increase in mRNA expression of these CYP isoforms was found to persist up to adulthood, suggesting that the low doses of lindane administered to the dams might program the brain and liver of the offspring to persistently express the xenobiotic-metabolizing CYP isoforms. As CYP-dependent metabolism of lindane is involved in its neurobehavioral toxicity, the potential of lindane to imprint the expression of cerebral and hepatic CYPs may help in identifying the role of these enzymes in the developmental neurotoxicity of the pesticide.

Key Words: γ-hexachlorocyclohexane (lindane); cytochrome P450; expression; gestation; rat.

Lindane (γ-hexachlorocyclohexane), an organochlorine pesticide, has been used extensively worldwide in agriculture and public health. The residues of lindane have been found to accumulate in the environment and reported to gain entry into human body through the food chain (Gunderson, 1988). Marked neurological and hepatotoxic effects have been observed in experimental animals following acute and chronic exposure to lindane (Smith, 1991; Videla et al., 1991). Neurotoxicity of lindane has been shown to involve different manifestations of hyperactivity, and at high doses, convulsions have been reported (Martinez and Martinez-Conde, 1995; Tussel et al., 1987). Although the mechanism of action of lindane is yet to be clearly established, studies suggest that γ-aminobutyric acid (GABA) receptor-ionophore complex is the primary site of action for lindane (Abalis et al., 1985).

Metabolic studies have shown that lindane is biotransformed by hepatic cytochrome P450 (CYP) monooxygenase enzymes to form various metabolites (Agency for Toxic Substance and Disease Registry ATSDR, 1998; Liu and Morgan, 1986). Chadwick et al. (1981) have demonstrated that pretreatment of rats with inducers of hepatic enzymes significantly influenced the metabolism and excretion of lindane and its metabolites by altering specific metabolic pathways. Studies from our laboratory have demonstrated that lindane produces an increase in the expression of hepatic and brain CYPs in adult rats, and this increase was further found to correlate with its neurobehavioral toxicity (Parmar et al., 2003). No significant increase in the incidence of convulsions following pretreatment of 3-methylcholanthrene (MC), an inducer of CYP1A1/1A2 isoenzymes, while a significant increase in the lindane-induced convulsions in phenobarbital (PB), an archetypal inducer of CYP2B1/2B2 isoenzymes or ethanol, inducer of CYP2E1-catalyzed reactions, pretreated rats led us to suggest that the induction of different CYP isoenzymes, especially in brain, have the potential to intensify or protect against the toxicity of lindane by modulating their concentrations at the target sites.

Though the metabolism of lindane and its effects on CYPs are relatively well characterized in the adults, not much is known about its effects in the developing animals. Studies have demonstrated that the developing organisms may be more sensitive to the adverse effects of environmental toxicants to which they may be exposed in utero (Barr et al., 2005;
Faustman et al., 2000). Human and animal studies have suggested a link between lindane exposure during pregnancy and fetal effects. Lower doses of lindane, which do not produce any effects in the adults, were found to induce behavioral, neurochemical, metabolic, and even structural changes in developing rats (Rivera et al., 1998; Serrano et al., 1990). An association has been observed between intrauterine growth retardation and elevated maternal blood concentrations of lindane (Siddiqui et al., 2003). As CYP-dependent metabolism of lindane is involved in its neurobehavioral toxicity, the present study attempted to investigate if prenatal exposure to lindane has the potential to imprint the expression of cerebral and hepatic CYPs, which may also help in identifying the role of these enzymes in the developmental neurotoxicity of the pesticide.

### MATERIALS AND METHODS

#### Chemicals

- Lindane-technical grade, phenylmethylsulfonyl fluoride (PMSF), NADPH, 7-ethoxycoumarin (EC), 7-pentoxyresorufin (PR), N-nitrosodimethylamine (NDMA), resorufin, bovine serum albumin, DC, diethyl pyrocarbonate, Tween-20, and dithiothreitol (DTT) were procured from Sigma-Aldrich (St Louis, MO).
- Trizol reagent was obtained from Life Technologies (Carlsbad, CA, USA).
- dNTP mix, M-MuLV reverse transcriptase, and DNA polymerase were procured from MBI Fermentas (Hanover, MD, USA).
- All other chemicals used were of the highest purity commercially available and procured either from BDH, New Delhi (a subsidiary of E. Merck, Mumbai, India) or SISCO Research Laboratories Pvt Ltd (Mumbai, India). PB sodium salt was a gift from Biodeal Laboratories (Pune, India).

#### Animals and Treatment

- Adult male (~12 weeks old) and female (~10 weeks old) albino Wistar rats of proven fertility were obtained from the Animal House facility of Industrial Toxicology Research Centre, Lucknow. All the animals were maintained on a commercial pellet diet and water ad libitum in a temperature-controlled room with a 12-h light/dark cycle and cared for in accordance to the policy laid down by Animal Care Committee of Industrial Toxicology Research Centre. The animal experimentation was approved by the Ethics Committee of the Centre.
- A total of 132 female rats were allowed to mate with 44 adult males (3:1). On day 0 of pregnancy (confirmed by a positive vaginal smear), the pregnant rats (128 numbers) were randomly divided into 11 batches of four groups each. Animals in group 1, 2, and 3 received po 0.0625, 0.125, or 0.25 mg/kg body weight (bw) of lindane corresponding to 1/1400th, 1/700th, or 1/350th of LD50, respectively, from gestation day 5–21 once per day. Animals in group 4 served as control and received corn oil in an identical manner. On the day of parturition, the average litter size was adjusted to eight per dam in all the groups with equal number of males and females as far as possible. Each group contained 32 dams with 128 male offspring. The male offspring were sacrificed on postnatal day (pnd) 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, and 90. Liver and brain were immediately removed, snap frozen, and processed for isolation of microsomes (pnd 10, 20, 30, 40, 50, 60, and 90) or total RNA. For isolation of microsomes for the assay of enzyme activities, two brain or liver samples were pooled from the offspring sacrificed at pnd 10, 20, 30, 40, 50, 60, and 90. For isolation of RNA, one brain and liver samples from the offspring at pnd 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, and 90 were processed.

#### Preparation of Microsomes

Brain and liver tissues were homogenized in four volumes of ice-cold 0.25M potassium phosphate buffer, pH 7.25, containing 0.15M KCl, 0.25mM PMSF, 0.01M EDTA, and 0.1mM DTT. Subcellular fractionation of the brain and liver homogenate was performed as described earlier (Parmar et al., 1998). In brief, the postmitochondrial supernatant obtained after centrifugation of the homogenate at 14,000 × g for 20 min was again centrifuged at 105,000 × g for 60 min to separate the microsomes. The pellets were resuspended in microsome dilution buffer containing 0.1M potassium phosphate buffer, pH 7.25, 20% (vol/vol) glycerol, 0.25mM PMSF, 0.01M EDTA, and 0.1mM DTT and stored at −80°C until further analysis. Protein content of the samples was estimated by the method of Lowry et al. (1951).

#### RNA Isolation

Total RNA was extracted from brain and liver using Trizol Reagent (Life Technologies) according to the manufacturer’s protocol. The protocol utilizing Trizol reagent, a monophasic solution of phenol and guanidium isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi (1987).

#### Enzyme Assays

All enzyme reactions have earlier been shown to be linear with time, substrate, and enzyme concentration (Parmar et al., 1998).

**7-Ethoxycoumarin-O-deethylase and 7-pentoxyresorufin-O-dealkylase assay.** The activities of 7-ethoxycoumarin-O-deethylase (EROD) and 7-pentoxyresorufin-O-dealkylase (PROD) were determined in brain and liver microsomes by the method described by Parmar et al. (1998). In brief, the reaction mixture in 1.25 ml contained 50mM Tris buffer, pH 7.5, 25mM MgCl2, 0.050mM ER or PR, and suitable amounts of microsomal enzyme. The reaction was initiated by the addition of 0.50mM NAPDH and was incubated at 37°C for 30 min for brain and for 10 min for liver. Reaction was terminated by adding 2.0 ml of methanol, and the mixtures were centrifuged at 2000 × g for 10 min. Levels of resorufin in the supernatant were measured using a Perkin Elmer LS 55 Luminescence Spectrometer at excitation wavelength of 550 nm and emission wavelength of 585 nm.

**N-nitrosodimethylamine demethylase assay.** N-nitrosodimethylamine demethylase (NDMA-d) activity was assayed by a slight modification of the method of Castonguay et al. (1991). The assay mixture contained a suitable amount of protein, 70mM Tris-HCl, pH 7.4, 10.0mM semicarbazide, 14.0mM MgCl2, 215.0mM KCl, 1.000mM NAPDH, and 4mM NDMA in final volume of 1.0 ml. The reaction mixture was incubated at 37°C for 30 min for brain and for 10 min for liver. The reaction was stopped by the addition of 1.5 ml of 12.5% trichloroacetic acid. After centrifugation at 2000 × g for 15 min, 2.0 ml of the supernatant was mixed with 1.0 ml of Nash reagent containing 6M ammonium acetate, 60mM acetyl acetone, and 0.15M acetic acid. The tubes were then incubated at 70°C for 20 min, and HCHO formed was measured at 415 nm.

#### Semiquantitative Reverse Transcription-PCR Analysis

cDNA was synthesized from total RNA isolated from brain or liver of control or lindane-exposed offspring by the methodology followed by Johri et al. (2006, 2007). Prior to the amplification of CYPs, normalization was carried out with glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the housekeeping gene. The primers used were those that have been earlier described (Hodgson et al., 1993; Omiecinski et al., 1999; Schilter and Omiecinski, 1993; Soh et al., 1996). The PCR mixture for CYP1A1, 1A2, 2B1, 2B2, and 2E1 in 50 ml contained 1× PCR buffer, 0.2mM dNTP mix, 0.3μM of each CYP1A1, 2B1, 2B2, or 2E1 primers or 0.4μM of each CYP1A2 primers, 2 μl cDNA, and 1.5 U Taq DNA polymerase from MBI Fermentas. MgCl2 at the final concentration of 3.0mM was used for CYP1A1 and 2B2, while 1.5mM was used for CYP2B1 and 2E1 and 1.0mM for CYP1A2.

To ensure that PCR reactions were within the linear range, PCR reactions were initially standardized using different concentrations of cDNA and PCR cycles. Liver cDNA samples corresponding to 3.1, 6.2, 12.5, 25, and 50 ng of total RNA and brain samples corresponding to 9.3, 18.7, 37.5, 75, and 150 ng of total RNA were amplified in 50-μl reaction volume as described in our earlier study (Johri et al., 2007). Optimization of liver and brain cDNA
concentrations using 30 PCR cycles revealed that the correlation coefficient values of the cDNA dilution curves were higher than 0.98 ($R^2 > 0.98$) for all the CYPs. Likewise, optimization of the PCR cycles to be used for subsequent PCR amplifications revealed that PCR reactions were linear from 27 to 40 cycles for all the CYPs. Accordingly, 30 cycles for CYP1A1, 1A2, 2B1, 2B2, and 2E1 were used for all the PCR reactions.

The final PCR conditions used were initial denaturation at 94°C for 3 min, then 30 cycles of denaturation at 93°C for 45 s; annealing at 58.2°C for CYP1A1, 55°C for CYP2B1, 53°C for CYP1A2, or 60°C for CYP2B2 for 1 min; and extension at 72°C for 1.5 min. One cycle of final extension at 72°C for 10 min was also used. For CYP2E1, nested PCR was carried out similarly using initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing of primers at 50°C for 30 s, extension at 72°C for 5 min, and final extension at 72°C for 5 min. A 2-μl aliquot from first PCR reactions were reamplified using nested primers for CYP2E1 following the same conditions. PCR products (194 bp for GAPDH, 341 bp for CYP1A1, 793 bp for CYP1A2, 380 bp for CYP2B1, 163 bp for CYP2B2, and 750 bp for CYP2E1) were analyzed by agarose gel electrophoresis using VERSA DOC Imaging System Model 1000 (Bio-Rad, Hercules, CA, USA). The densitometry was performed using Quantity One Quantitation software of Bio-Rad, and the ratio between the mean optic densities of PCR products of various CYP isoforms and GAPDH from three numbers of samples pooled from different animals were used to calculate the statistical significance.

**Statistical Analysis**

All values are presented as mean ± SEM. Main effects of treatment, dose, and time on each of the enzymatic assays (NDMA-d, EROD, and PROD) and mRNA expression of individual CYP isoforms were ascertained using the three-way ANOVA considering treatment, dose, and time as dependent variables and each parameter separately as dependent variable. Prior to the ANOVA, normality of assumptions of data and homogeneity of variance between the groups was ascertained. *Post hoc* analysis for comparing the two groups was done by calculating the least significant differences at 5% level of significance using residual mean squares for calculating the $t$ values.

**RESULTS**

Reverse transcriptase (RT)–PCR analysis with primers specific for rat liver GAPDH resulted in the formation of PCR products of expected band size of 194 bp in the RNA isolated from the liver or brain of control rats or rats prenatally exposed to lindane. PCR amplification with primers specific for rat liver GAPDH resulted in the formation of products of almost equal intensity in the cDNA formed from reverse transcription of RNA extracted from liver or brain isolated from offspring born to control or lindane-treated dams (data not shown).

**mRNA Expression of CYP1A Isoforms**

RT-PCR analysis of the RNA extracted from liver or brain isolated from the offspring born to control or lindane-treated dams revealed that PCR products of correct size (341 bp for CYP1A1, 793 bp for CYP1A2) were formed with CYP1A1 and 1A2 primers, respectively. As evident from the Figures 1 and 2 and densitometric analysis of the PCR products, prenatal

![FIG. 1](https://academic.oup.com/toxsci/article-abstract/101/2/331/1640504) Effect of prenatal exposure of lindane on brain and liver CYP1A1 mRNA expression in offspring. (A) Representative ethidium bromide–stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder, lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offspring, respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from MC-pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from control and exposed offspring. All the values represent mean ± SEM of the ratio between the optic densities, mean optic density of CYP isoforms, and GAPDH from three different animals for each time point ($n = 3$). *$t < 0.05$ when compared with controls.
exposure to 0.0625, 0.125, or 0.25 mg/kg bw of lindane was found to produce a dose-dependent increase in the mRNA expression of both CYP1A1 and 1A2 in brain at the higher doses of 0.125 or 0.25 mg/kg. Densitometric analysis further revealed severalfold higher increase in CYP1A2 mRNA expression as compared to CYP1A1 expression at birth in the brain of offspring exposed prenatally to the relatively higher doses of lindane (0.125 or 0.25 mg/kg bw). Though a decline was observed in the induced levels of CYP1A1 and 1A2 mRNA expression with increasing postnatal age, the increase in the mRNA expression was found to persist up to adulthood (Figs. 1 and 2). Significant increase was observed even up to pnd 90 (for CYP1A1) or pnd 60 (for CYP1A2) in the mRNA expression of CYP1A1 and 1A2 isoforms in brain of the offspring exposed prenatally to the relatively higher doses of lindane (0.125 or 0.25 mg/kg bw). As evident from Figures 1 and 2, the induction pattern followed a similar trend as in brain, with the increase in CYP1A1 mRNA expression remaining significant even up to pnd 90 and that in CYP1A2 mRNA expression up to pnd 60 or 70 in the liver of offspring prenatally exposed to 0.125 or 0.25 mg/kg of lindane. Furthermore, a greater increase was observed in the CYP1A1 and 1A2 mRNA expression in liver as compared to brain at different postnatal ages (Figs. 1 and 2). As seen in the brain, prenatal exposure to the lowest dose (0.0625 mg/kg) of lindane did not produce any significant change in the hepatic mRNA levels of CYP1A1 or CYP1A2 at birth or during postnatal development (Figs. 1 and 2).

mRNA Expression of CYP2B Isoforms

RT-PCR analysis of the RNA extracted from liver or brain isolated from the offspring born to control or lindane-treated dams revealed that PCR products of correct size (380 bp for CYP2B1 and 163 bp for CYP2B2) were formed with CYP2B1 and 2B2 primers. Prenatal exposure to the low doses of lindane was found to produce a dose-dependent increase in the mRNA expression of CYP2B1 and 2B2 isoforms in brain of offspring at different pnds during development (Figs. 3 and 4).
Densitometric analysis revealed that though the expression of CYP2B1 and 2B2 isoforms was severalfold higher as compared to control at birth, maximum fold increase in CYP2B1 mRNA expression was observed at pnd 10 and in CYP2B2 mRNA expression at pnd 20 in the brain of offspring exposed to the relatively higher doses of lindane (0.125 or 0.25 mg/kg). The levels of CYP2B1 and 2B2 then showed a decline though the increase persisted up to pnd 90 in the brain of the offspring (Figs. 3 and 4). Interestingly, in contrast to that observed with the expression of CYP1A isoforms, a significant increase in CYP2B1 mRNA expression was also observed in brain of the offspring at weaning exposed to the lowest dose (0.0625 mg/kg) of lindane (Figs. 3 and 4).

Similar to that observed with brain, prenatal exposure to the different doses of lindane was found to produce a dose-dependent increase in the postnatal development of CYP2B1 and 2B2 isoforms in the liver of the exposed offspring. As evident from Figures 3 and 4, severalfold increase in the CYP2B1 and 2B2 mRNA expression was observed at birth in liver of the offspring exposed to the relatively higher doses (0.125 or 0.25 mg/kg) of lindane. Densitometric analysis revealed that the increase in CYP2B1 and 2B2 mRNA levels continued up to pnd 30; thereafter, the levels of CYP2B1 and 2B2 mRNA showed a decline, though the increase was found to be significant even up to pnd 90 at the relatively higher doses (0.125 or 0.25 mg/kg) of lindane (Figs. 3 and 4).

**mRNA Expression of CYP2E1**

RT-PCR analysis of the RNA isolated from brain of the offspring born to lindane-treated dams with rat liver CYP2E1 primers revealed that prenatal exposure to the different doses of lindane produces dose-dependent increase in the ontogeny of CYP2E1 isoform (Fig. 5). Densitometric analysis revealed that the increase in the mRNA expression of CYP2E1 isoform was statistically significant at relatively higher doses of 0.125 or 0.25 mg/kg in brain of the exposed offspring. It was further found that maximum increase in the mRNA expression of CYP2E1 occurs at pnd 0. A decline was thereafter observed in the induced levels of CYP2E1 mRNA expression with increasing postnatal age. However, the increase in the mRNA expression was found to persist up to adulthood (Fig. 5). Significant increase even up to pnd 60 was observed in the mRNA expression of CYP2E1 in brain of the offspring prenatally exposed to relatively higher doses (0.125 or 0.25 mg/kg) of lindane. No significant change was, however, observed in the CYP2E1 mRNA expression in the brain of offspring exposed to the lowest dose of lindane (Fig. 5).

As observed with brain, prenatal exposure to the different doses of lindane was found to modify the postnatal development of CYP2E1 in the liver of the exposed offspring. Similar to that observed in brain, a dose-dependent increase was observed in the ontogeny of CYP2E1 in the liver of the

---

**FIG. 3.** Effect of prenatal exposure of lindane on brain and liver CYP2B1 mRNA expression in offspring. (A) Representative ethidium bromide–stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder and lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offspring, respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from PB-pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from control and exposed offspring. All the values represent mean ± SEM of the ratio between the optic densities, mean optic density of CYP isoforms, and GAPDH from three different animals for each time point (n = 3). *t < 0.05 when compared with controls.
offspring prenatally exposed to lindane, especially at the relatively higher doses of lindane (Fig. 5). As evident from Figure 5, maximum increase in CYP2E1 mRNA levels was observed at pnd 0. Densitometric analysis further revealed that the induction pattern followed a similar trend as seen in brain, with the increase in CYP2E1 mRNA expression remaining significantly increased even up to pnd 90. Significant increase in the mRNA expression of CYP2E1 was also observed in the liver of offspring exposed prenatally to the lowest dose of lindane (0.0625 mg/kg) at birth and pnd 5. Furthermore, as evident from Figure 5, a greater increase was observed in CYP2E1 mRNA expression in liver as compared to brain at different postnatal ages.

Enzymatic Analysis

CYP1A-dependent EROD activity. Oral administration of different doses (0.0625, 0.125, or 0.25 mg/kg bw) of lindane to the pregnant rats produced a dose-dependent increase in the CYP1A-dependent EROD activity in the brain microsomes isolated from the offspring (Fig. 6). The increase was found to persist up to pnd 60, with the effect being significant up to pnd 40 at relatively higher doses (0.125 or 0.25 mg/kg bw) and up to pnd 50 at the highest dose of 0.25 mg/kg bw (Fig. 6A). Similar dose-dependent increase in the EROD activity was found in the liver microsomes isolated from exposed offspring.

The increase observed in the hepatic EROD activity was found to decline with increasing age. Though the hepatic enzyme activity remained increased even up to pnd 90 in the exposed offspring, this increase was found to be significant only at the highest dose of lindane (Fig. 6B).

CYP2B-dependent PROD activity. Oral administration of different doses of lindane (0.0625, 0.125, or 0.25 mg/kg bw) was found to produce a dose-dependent increase in the cerebral PROD activity of the offspring born to dams exposed to lindane (Fig. 7). Significant increase was observed in the activity of PROD in brain microsomes isolated from the offspring prenatally exposed to relatively higher doses of lindane (0.125 or 0.25 mg/kg). This significant increase in PROD activity was found to persist up to pnd 30 in the brain microsomes isolated from offspring exposed to relatively higher doses of lindane (0.125 or 0.25 mg/kg bw). At the highest dose of 0.25 mg/kg bw, the increase was found to be significant even up to pnd 40 (Fig. 7A).

As seen with brain, PROD activity in liver was found to increase dose dependently in the offspring prenatally exposed to lindane. However, in contrast to the brain, the effect was found to persist up to pnd 90 in the microsomes prepared from liver of the offspring prenatally exposed to relatively higher doses of lindane. The increase in the hepatic PROD activity was further found to be statistically significant up to pnd 50 in the offspring exposed to relatively higher doses of 0.125 or 0.25 mg/kg bw.
while at the highest dose of 0.25 mg/kg bw, the increase was found to be significant even up to pnd 60 (Fig. 7B).

**CYP2E-dependent NDMA-d activity.** As seen with CYP2E1 mRNA expression, oral administration of different doses (0.0625, 0.125, or 0.25 mg/kg bw) of lindane to the pregnant rats produced a dose-dependent increase in the CYP2E1-dependent NDMA-d activity in brain microsomes isolated from exposed offspring (Fig. 8A). However, the increase in the NDMA-d activity was found to be statistically significant up to pnd 30 in the brain microsomes isolated from offspring exposed to relatively higher doses of lindane (0.125 or 0.25 mg/kg bw), while at the highest dose of 0.25 mg/kg, this significant increase persisted up to pnd 40 (Fig. 8A). Similar to brain, the dose-dependent increase in NDMA-d was found to be significant up to pnd 40 in the liver microsomes isolated from the offspring born to dams exposed to relatively higher doses of lindane (0.125 or 0.25 mg/kg bw), while at the highest dose (0.25 mg/kg), the increase in the hepatic NDMA-d activity persisted up to pnd 50 (Fig. 8B).

**DISCUSSION**

The present study demonstrating an increase in the expression and catalytic activity of cerebral and hepatic CYPs during postnatal development following prenatal exposure to low doses of lindane assumes significance in view of the earlier reports that CYP-mediated metabolism of the pesticide is involved in its neurobehavioral toxicity (Chadwick et al., 1981; Parmar et al., 2003). It has been earlier shown that lindane, a mixed type of inducer of CYPs, induces its own metabolism by increasing the expression and activity of various CYP

**FIG. 5.** Effect of prenatal exposure of lindane on brain and liver CYP2E1 mRNA expression in offspring. (A) Representative ethidium bromide–stained agarose gels. Lane 1 contains 1.0 kb plus DNA ladder and lane 2 contains RT-PCR product without RNA. Lanes 3–13 contain 5 μl of RT-PCR product of RNA isolated from brain or liver of 0-, 5-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, and 90-day-old rat offspring, respectively. Lane 14 contains 5 μl of RT-PCR product of RNA isolated from PB-pretreated animals. (B) Densitometric analysis of the RT-PCR products of RNA isolated from control and exposed offspring. All the values represent mean ± SEM of the ratio between the optic densities, mean optic density of CYP isoforms, and GAPDH from three different animals for each time point (n = 3). *t < 0.05 when compared with controls.

**FIG. 6.** Effect of prenatal exposure of lindane on EROD activity in brain and liver of offspring. All the values represent mean ± SEM of six samples pooled from different animals for each time point (n = 6). *t < 0.05 when compared to control.
when compared to control. Isoenzymes may have an impact on the accumulation and resulting toxicity of lindane (Agency for Toxic Substance and Disease Registry, ATSDR, 1998; Chadwick et al., 1981; Liu and Morgan, 1986), significant increase in the mRNA expression and associated catalytic activity of xenobiotic-metabolizing CYPs at birth and later indicates the responsiveness and sensitivity of fetal and neonatal CYPs to lindane reaching the liver and brain.

Our data indicating alterations in the ontogeny of cerebral and hepatic CYPs following prenatal exposure of lindane may have neurotoxicological consequences as these CYPs are closely associated with the neurotransmission process. Recent studies have indicated that ligands of GABA and peripheral benzodiazepine receptor induce CYP2B proteins at the transcriptional level and that this induction is mediated through the nuclear receptor–binding sites, NR1 and NR2 of PB-responsive unit, suggesting a common induction mechanism (Roberge et al., 2004). Studies have also shown that CYPs are involved in the dopaminergic neurotransmission, known to be altered by lindane (Llorens et al., 1991; Nissbrandt et al., 2001; Rivera et al., 1998; Sunol et al., 1988). Likewise, adrenoceptor-dependent signaling pathways, which modulate the inducibility of CYP1A1 and 1A2 genes by the Ah receptor ligands, could be involved in lindane-induced alterations in CYPs (Konstandi et al., 2005). Alterations in the cerebral and hepatic CYPs by lindane may not only modify the toxification or detoxification of the insecticide but also possibly modulate the affinity of lindane toward these receptors. The alterations in the ontogeny of cerebral and hepatic CYPs, particularly CYP2E1, involved in the generation of free radicals, are of significance in view of the earlier studies demonstrating oxidative stress to be one of the mechanisms involved in lindane toxicity (Azzalis et al., 1995; Videla et al., 1991). Considering the highly lipophilic nature of lindane and high levels of polyunsaturated fatty acids and low levels of antioxidant enzymes in brain (Somani et al., 1996), severalfold increase in the cerebral and hepatic CYPs particularly CYP2E1 at birth and during postnatal development in prenatally exposed offspring have suggested their involvement in the sensitivity of brain to oxidative stress and its possible role in lindane-induced developmental neurotoxicity.

Furthermore, the alterations in the ontogeny of the cerebral and hepatic CYP isoenzymes during postnatal development even after exposure to low doses of lindane in utero could also be possibly related with the endocrine-disrupting activity of lindane. Both, estrogenic as well as antiestrogenic and antiandrogenic properties of lindane have been reported in rats (Aarif and Huisingh, 2001; Dalsenter et al., 1997b; Maranghi et al., 2007). A number of studies have suggested alterations in
hypothalamic-pituitary-gonadal, hypothalamic-pituitary-thyroid, and hypothalamic-pituitary-adrenal axis following prenatal exposure to lindane (Dalsenter et al., 1997a; Maranghi et al., 2007). The imprinted overinduction of hepatic CYP isoenzymes reported after PB exposure, associated with an abnormality in the circulating growth and sex hormone profiles in the neonatal rats (Agrawal and Shapiro, 1996), may also help in explaining the persistence in the alterations of the cerebral and hepatic CYP isoenzymes even up to adulthood following prenatal exposure to low doses of lindane. As these hormones are known to be altered by lindane, the alterations in the ontogeny and, furthermore, persistence in the alterations of cerebral and hepatic CYPs could probably result from lindane-induced disruption of endocrine homeostasis in the offspring following prenatal exposure.

In conclusion, results of the present study have provided evidence that placental transfer of lindane possibly occurs following gestational exposure of low doses of lindane which leads to alterations in the ontogeny of the cerebral and hepatic CYPs. These alterations in the ontogeny and persistence in the increase in the mRNA expression of CYPs up to adulthood is of immense significance as CYP induction has been shown to be involved in lindane-induced neurobehavioral toxicity. Furthermore, the close association of CYPs with physiological functions of the brain, such as neurotransmission, steroid synthesis, and inactivation etc., has indicated that alterations in postnatal development of CYPs in brain and liver following prenatal exposure of lindane may possibly influence internal regulatory and metabolic pathways in the brain which may help in explaining the developmental neurotoxicity of lindane.

ACKNOWLEDGMENTS

The authors are grateful to the Director, Industrial Toxicology Research Centre, Lucknow, for his keen interest and support in carrying out the study. A.J. is thankful to Indian Council of Medical Research, New Delhi, for providing Senior Research Fellowship. The authors are grateful to Mr Neeraj Mathur, Scientist, Industrial Toxicology Research Centre (ITRC), for carrying out statistical analysis. The technical assistance of Mr B. S. Pandey and Mr Rajesh Misra is also gratefully acknowledged. ITRC Communication Number 2567. Senior Research Fellowship of Indian Council of Medical Research, New Delhi vide 3/1/2/11/Env/04-NCD-I.

REFERENCES


PERSISTENCE IN ALTERATIONS IN THE ONTOGENY OF CEREBRAL AND HEPATIC CYTOCHROME P450s 339

Downloaded from https://academic.oup.com/toxsci/article-abstract/101/2/331/1640504 by guest on 31 January 2019


