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# Selective Induction of Cytochrome P450e by Kepone (Chlordecone) in Primary Cultures of Adult Rat Hepatocytes

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## SUMMARY

Cytochromes P450b and P450e (IIB1 and IIB2, respectively) are two remarkably similar microsomal hemoproteins whose inductions in rat liver are generally believed to be coordinately controlled by such xenobiotics as phenobarbital. To critically examine this assumption, we used a new system of primary cultures of adult rat hepatocytes on Matrigel to evaluate whether organochlorine pesticides, as "phenobarbital-like" agents, directly induce these cytochromes in parallel in the liver parenchymal cell. For 14 of the pesticides we tested, as well as for phenobarbital, P450b and P450e mRNAs, measured on Northern blots, rose in concert as much as 58- and 6-fold, respectively, over the amounts in incubated control cultures. Kepone (chlordecone) treatment of the cultures increased P450e mRNA in a dose-dependent manner that disclosed a 10-fold greater potency, compared with cultures exposed to phenobarbital. Kepone also resembled phenobarbital in these experiments, in that there were dose-dependent increases in the amounts of hepatocellular P450p, P450pcn2, $P450_{PB-1}$, P450f, and NADPH-cytochrome P450 oxidoreductase mRNAs. However, in the same kepone-treated cells, P450b mRNA or P450b immunoreactive protein was induced only slightly, if at all. In contrast, additions to the medium of mirex, a structural analog of kepone, effectively induced both P450b and P450e mRNAs and their proteins. Selective induction of P450e by kepone in the hepatocyte cultures, the first pharmacologic dissociation of the induction of P450b and P450e mRNAs and proteins, was not apparent in kepone-treated rats, where both P450b and P450e mRNAs were increased to equivalent extents. We conclude that the P450b and P450e genes may be expressed independently by process(es), possibly involving extrahepatic factors, that can be defined with the present hepatocyte culture system.

The P450 gene superfamily of microsomal hemoproteins, which are prominent in the liver, includes at least nine distinct mammalian gene families, each of which is further subdivided into one to eight subfamilies, whose respective constituents share at least 59% amino acid sequence similarity (1). Four of the P450 gene families, designated I to IV (1), contain members that are markedly induced in liver by xenobiotics. For example, P450c[1] and P450d (gene subfamily IA) are induced in rat by polycyclic aromatic hydrocarbons (1, 2). In contrast, P450b and P450e (subfamily IIB) are forms induced by PB and the "PB-like" compounds, which include certain organochlorine pesticides, polychlorinated biphenyls, P450 inhibitors, and imidazole antimycotic drugs (1, 3–7). P450p (IIIA1) is inducible by glucocorticoids, PB, and the PB-like compounds (1, 6–9). $P450_{LA\omega}$ (IVA1) is induced by peroxisome proliferators but not by polycyclic aromatic hydrocarbons, PB, or glucocorticoids (1, 10). In addition to P450b and P450e, PB and the PB-like compounds also increase "constitutive" members of the IIC gene subfamily (i.e., P450f and $P450_{PB-1}$) (11, 12) and several non-P450 enzymes, including P450 OR (13, 14).

The PB-inducible genes P450b and P450e encode remarkably similar proteins that share 97% amino acid sequence identity (15–17). With few exceptions, P450b and P450e catalyze the same oxidations of steroid hormones and xenobiotics, although P450b displays higher activity toward most known substrates (4, 5, 18–21). In addition to their structural relatedness, P450b and P450e share many of the same regulatory characteristics. In contrast to P450c and P450d, which are often coinduced but not coordinately controlled (22–25), most of the available evidence suggests that P450b and P450e are induced in parallel in rat liver by PB and PB-like compounds (26–28). Such observations, together with preliminary analysis of the structures of these genes (29), have suggested that P450b and P450e utilize a common induction pathway.

Recent studies have shown that P450b and P450e are affected by extrahepatic hormonal factors. For example, the levels of

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[1]By the nomenclature recommended by Nebert *et al.* (1), P450c, P450d, P450b, P450e, P450p, P450pcn2, P450f, and $P450_{PB-1}$ correspond to P450s IA1, IA2, IIB1, IIB2, IIIA1, IIIA2, IIC7, and IIC6, respectively.

**ABBREVIATIONS:** P450, cytochrome P450; PB, phenobarbital; P450 OR, NADPH-cytochrome P450 oxidoreductase, *o*, *p*-DDD, 1-1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane.

P450b and P450e protein in rat liver rise after hypophysectomy and are lowered by administration of growth hormone (30). In light of these data, past assumptions about the effects of PB and PB-like inducers on the hepatic P450s in whole animals must be reconsidered to include the possibility of indirect actions of xenobiotics on hormonal homeostasis. It has not been possible to test this idea, because most systems for culture of liver cells require a complex serum-containing medium and fail to express such liver-specific functions as induction of P450b/e. However, we recently developed a new system of primary monolayers of rat hepatocytes cultured on Matrigel, a reconstituted basement membrane, that permits PB induction of P450b and P450e mRNAs and proteins (31). Moreover, addition of physiologic amounts of growth hormone to the hepatocyte cultures blocks PB induction of P450b/e, as is seen *in vivo* (32). With the availability of specific probes to P450b and P450e mRNAs, we now have the opportunity to examine the direct effects, on hepatic P450b and P450e gene expression, of PB-like compounds, focusing on the organochlorine pesticides. We report here that one of these organochlorine pesticides, kepone (chlordecone), alone was found to be a highly selective inducer of P450e mRNA and protein.

## Experimental Procedures

**Materials.** Adult male Sprague-Dawley rats (Dominion Laboratories, Dublin, VA), weighing 175–200 g, were maintained in wire-bottomed cages, with free access to animal chow and water, for 2 weeks before use. Collagenase type I was purchased from Cooper Biochemical Co. (Malvern, PA). Sodium PB was obtained from J. T. Baker, Inc. (Phillipsburg, NJ). Organochlorine pesticides (reported purities generally >99%) were obtained from the following sources: aldrin, γ-chlordane, γ-chlordene, dieldrin, heptachlor, and *trans*-nonachlor were from Velsicol Corp. (Chicago, IL); α-chlordane, *o,p*-DDD, endosulfan, endrin, heptachlor epoxide, β-, γ-, and δ-hexachlorocyclohexanes, kepone, and oxychlordane were from the United States Environmental Protection Agency (Research Triangle Park, NC); and mirex was from Supelco, Inc. (Bellefonte, PA). Kepone alcohol was prepared as previously described (33). The specific oligonucleotides to P450b and P450e described by Omiecinski *et al.* (28) were synthesized in our laboratory, using a Biosearch DNA synthesizer, and purified by high performance liquid chromatography. The oligonucleotides to P450p and P450pcn2 described by Gonzalez *et al.* (34) were synthesized by Dr. Bryan Johnson of the University of Alabama (Birmingham, AL). A cloned cDNA fragment to P450c (P210) (22) was provided by Dr. John Fagan (Mahareshi International University, Fairfield, IA), cDNAs to P450f and $P450_{PB\text{-}1}$ (35) were supplied by Dr. Frank Gonzalez (National Cancer Institute, Bethesda, MD), and a cDNA to P450 OR (pOR-7) (14) was the gift of Dr. Charles Kasper (University of Wisconsin, Madison, WI).

**Hepatocyte cultures and drug treatments.** Rat hepatocytes were isolated from adult male Sprague-Dawley rats (230–280 g) and plated onto 60-mm plastic dishes coated with 120–150 μl of Matrigel, as previously described (31). Cultures were maintained in a humidified incubator at 35°, under an atmosphere of 5% $CO_2$/95% air. The culture medium, a modification of Waymouth MB-752 containing insulin ($1.5 \times 10^{-7}$ M) as the only hormone, was renewed daily. In each experiment, cells were incubated for the first 3 days with medium only and were then treated with medium containing drug at the doses and durations indicated in the individual figure legends. Drugs were added to the cultures as concentrated stock solutions in water (PB) or dimethylsulfoxide (organochlorine pesticides; maximum, 0.3% of total volume).

**Animals and treatments.** Four groups of three male Sprague-Dawley rats (280–300 g) were treated with PB (in water; 80 mg/kg/day × 3 days, intraperitoneally), kepone (in corn oil; 20 mg/kg × one dose, orally), mirex (in corn oil; 20 mg/kg × one dose, orally), or corn oil (one dose, orally). Rats treated with corn oil, kepone, or mirex were killed 72 hr after dosing, whereas PB-treated rats were killed 24 hr after the last dose.

**Northern blot analyses.** Total RNA was isolated from the pooled cells of three culture dishes, as previously described (31). RNA samples (10 μg) were resolved on denaturing 1% agarose gels and either capillary transferred onto reinforced nitrocellulose filters (Optibind; Schleicher and Schull, Keene, NH) or electrophoretically transferred onto nylon filters (Nytran; Schleicher and Schull). Oligonucleotides were radiolabeled to greater than $5 \times 10^8$ cpm/μg, using a commercial 5′-labeling kit (BRL, Gaithersburg, MD) and [γ-$^{32}$P]ATP (4500 Ci/mmol; ICN Biomedicals, Inc., Costa Mesa, CA) as substrate. Prehybridization, hybridization, and washing conditions for the P450b, P450e, P450p, and P450pcn2 oligonucleotides have been previously described (36). The cDNA inserts were radiolabeled to greater than $10^8$ cpm/μg, using a nick translation kit (BRL) and [α-$^{32}$P]dCTP (3000 Ci/mmol; ICN Biomedicals) as substrate. Prehybridization and hybridization conditions for cDNAs have been described previously (6). Final washing conditions for the cDNAs were 0.1× standard saline citrate (20× standard saline citrate = 3 M sodium chloride, 0.3 M sodium citrate, pH 7.5), 0.1% sodium dodecyl sulfate, at 65° for 30 min. RNA bands were visualized by autoradiography and quantified by scanning densitometry.

**Western blot analyses.** Microsomes were prepared by differential centrifugation of the sonicates from eight pooled culture dishes, as previously described (31). Microsomal protein samples (50 μg) were resolved on sodium dodecyl sulfate-polyacrylamide (10%) gels and electrophoretically transferred to nitrocellulose filters. Blots were blocked for 1 hr at 37° with 3% bovine serum albumin and 10% calf serum in phosphate-buffered saline (137 mM sodium chloride, 2.68 mM potassium chloride, 8.10 mM sodium phosphate dibasic, 1.47 mM potassium phosphate monobasic, pH 7.4) and then developed successively with 1) goat polyclonal antibody recognizing both P450b and P450e (7), 2) rabbit anti-goat IgG, 3) peroxidase-goat antiperoxidase complex, and 4) 0.03% *N,N*-diaminobenzidine tetrahydrochloride and 0.006% hydrogen peroxide.

**Biochemical assays.** Microsomal protein concentrations were determined by the method of Schacterle and Pollack (37), using bovine serum albumin as the standard. Lactate dehydrogenase activities were determined in medium samples and cell homogenates essentially as described (38).

## Results

**Effects of organochlorine pesticides on P450b and P450e mRNAs in cultured rat hepatocytes.** Sixteen organochlorine pesticides representing four different chemical classes, i.e., 1) chlorinated ethanes (*o,p*-DDD), 2) cyclodienes (α-chlordane, γ-chlordane, γ-chlordene, oxychlordane, *trans*-nonachlor, heptachlor, heptachlor epoxide, aldrin, endrin, dieldrin, and endosulfan), 3) hexachlorocyclohexanes (β-, δ-, and γ-hexachlorocyclohexanes), and 4) kepone, were added to the medium (all at $10^{-5}$ M, except γ-chlordane, which was at $3 \times 10^{-6}$ M) of cultures of male rat hepatocytes that had been incubated for 3 days in standard medium. Northern blot analysis revealed that 14 of the pesticides produced parallel inductions of P450b and P450e mRNAs, ranging from about 40 to 100% of the response produced by PB (increases of as much as 58- and 6-fold over untreated control values for P450b and P450e mRNAs, respectively) (Fig. 1). Endosulfan treatment of the cells, at $10^{-5}$ M, gave essentially no induction of either P450b or P450e mRNA. Only one pesticide, kepone, produced a selective induction, increasing P450e mRNA 4.5-fold over the level in 120-hr untreated hepatocytes (75% of the response produced by PB in this experiment), whereas P450b mRNA was increased only slightly (Fig. 1).

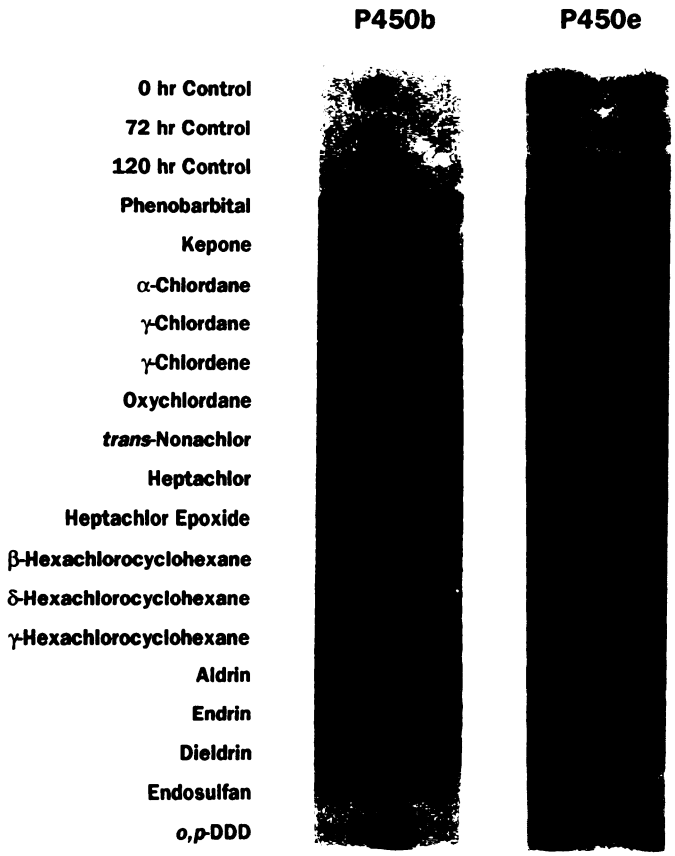


**Fig. 1.** Effects of organochlorine pesticides on the amounts of P450b and P450e mRNAs in cultured rat hepatocytes. Freshly isolated hepatocytes prepared from a single male rat were incubated for 3 days in standard, serum-free medium and then treated for 48 hr with medium containing PB ($10^{-4}$ M) or one of 16 organochlorine pesticides (all at $10^{-5}$ M, except γ-chlordane, at $3 \times 10^{-6}$ M). Total cellular RNA was extracted from three pooled dishes of cells for each treatment group and was analyzed by Northern blot hybridization using oligonucleotides specific to P450b and P450e, as described in Experimental Procedures. The autoradiographs displayed for P450b and P450e mRNAs resulted from film exposures of 1 and 3 days, respectively.

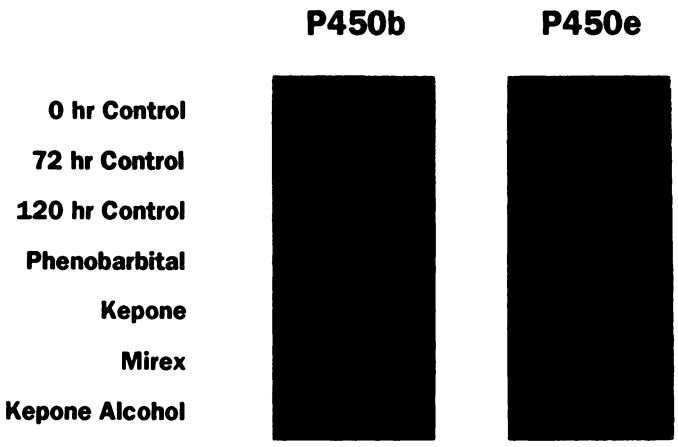


**Fig. 2.** Effects of kepone, mirex, or kepone alcohol on the amounts of P450b and P450e mRNAs in cultured rat hepatocytes. Three-day-old cultures, prepared as described in Fig. 1, were treated for 48 hr with medium containing PB ($10^{-4}$ M), kepone ($10^{-5}$ M), mirex ($10^{-5}$ M), or kepone alcohol ($10^{-5}$ M). Total cellular RNA was extracted from three pooled dishes of cells for each treatment group and was analyzed by Northern blot hybridization using oligonucleotides specific to P450b and P450e, as described in Experimental Procedures. The autoradiographs displayed for P450b and P450e mRNAs each resulted from film exposures of 1 day.

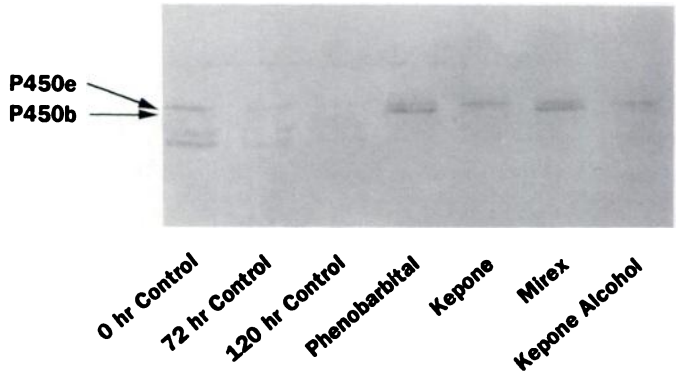


**Fig. 3.** Effects of kepone, mirex, or kepone alcohol on the amounts of immunoreactive P450b and P450e proteins in cultured rat hepatocytes. Three-day-old cultures were treated for 48 hr with medium containing PB ($10^{-4}$ M), kepone ($10^{-5}$ M), mirex ($10^{-5}$ M), or kepone alcohol ($10^{-5}$ M). Microsomes were isolated from eight pooled dishes of cells for each treatment group and were analyzed by Western blot hybridization, as described in Experimental Procedures.

In contrast, mirex, a maximally chlorinated cyclopentadiene derivative structurally similar to kepone, induced both P450b and P450e mRNAs to similar magnitudes as PB, when added to the hepatocyte cultures at $10^{-5}$ M (Fig. 2). Kepone alcohol, which contains a secondary alcohol instead of a ketone function at the bridgehead carbon, was a weaker inducer than kepone but appeared to selectively induce P450e mRNA (Fig. 2). These results were not restricted to mRNA, because analysis of cellular microsomal proteins by Western blot analysis revealed that PB and mirex induced P450b and P450e immunoreactive proteins, whereas kepone and kepone alcohol induced P450e immunoreactive protein selectively (Fig. 3). Similar selective induction by kepone of P450e mRNA and protein was also observed in a culture of female rat hepatocytes (data not shown).

Kepone selectively induced P450e mRNA over the entire dose-response range (Fig. 4). Thus, whereas PB treatment produced similar dose-dependent inductions of both P450b and P450e mRNAs (>10-fold), additions of kepone resulted in substantial dose-dependent increases only for P450e mRNA. In this experiment, kepone was a 10-fold more potent and equally efficacious (at the optimally effective doses of $10^{-5}$ M and $10^{-4}$ M for kepone and PB, respectively) inducer of P450e mRNA, compared with PB. In contrast, maximal induction of P450b mRNA by kepone was only 17% of that produced by PB (Fig. 4).

Time courses for the induction of P450b and P450e mRNAs differed somewhat among PB, kepone, and mirex (Fig. 5). PB treatment ($10^{-4}$ M) produced induction of both P450b and P450e mRNAs that was readily apparent after only 12 hr of treatment and was near maximal by 48 hr. The onset of detectable induction of P450b and P450e mRNAs by mirex ($10^{-5}$ M) and of P450e mRNA by kepone ($10^{-5}$ M) was somewhat slower, becoming apparent only after 24 hr of incubation. Again, kepone treatment produced essentially no induction of P450b mRNA at any time point examined (Fig. 5).

**Effects of kepone on mRNAs for other PB-responsive liver enzymes.** The lack of induction of P450b by kepone is even more remarkable because, like PB, kepone, when added

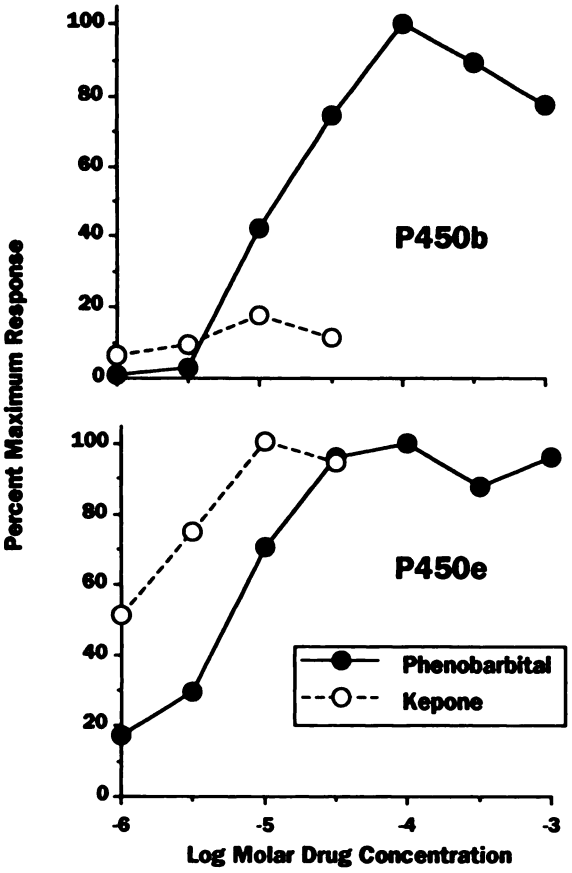


**Fig. 4.** Dose-response analysis of P450b and P450e mRNAs in cultured rat hepatocytes treated with PB or kepone. Three-day-old cultures were treated for 48 hr with medium containing PB ($10^{-6}$ to $10^{-3}$ M) or kepone ($10^{-6}$ to $3 \times 10^{-5}$ M). Total cellular RNA was extracted from three pooled dishes of cells for each treatment group and was analyzed by Northern blot hybridization using oligonucleotides specific to P450b and P450e, as described in Experimental Procedures. Autoradiographic data were densitometrically quantified and expressed as percentages of the responses obtained with $10^{-4}$ M PB.

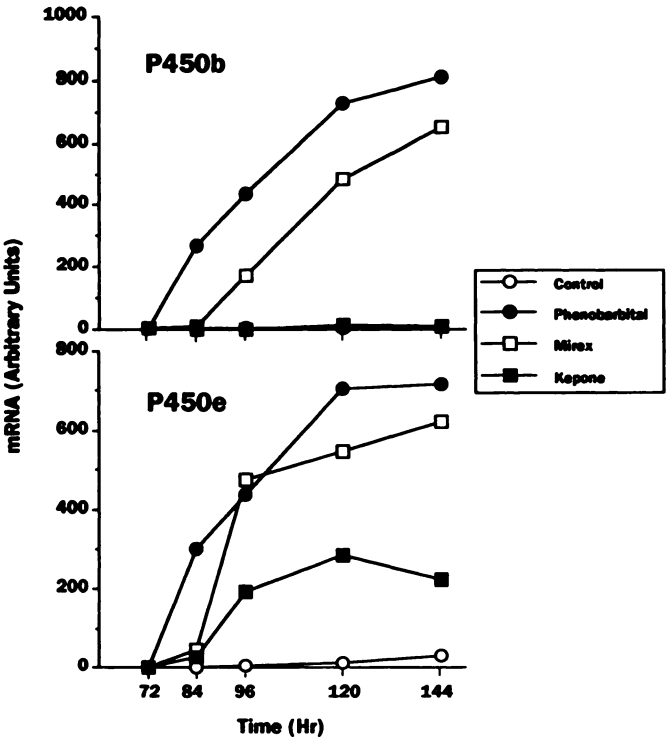


**Fig. 5.** Comparison of the time courses for induction of P450b and P450e mRNAs in cultured rat hepatocytes treated with PB, kepone, or mirex. Cultures incubated for 3 days in standard, serum-free medium were treated for 12–72 hr with medium containing PB ($10^{-4}$ M), kepone ($10^{-5}$ M), or mirex ($10^{-5}$ M). Total cellular RNA was extracted from three pooled dishes of cells for each treatment group and was analyzed by Northern blot hybridization using oligonucleotides specific to P450b and P450e, as described in Experimental Procedures. Autoradiographic data were quantified by scanning densitometry.

to hepatocyte cultures, produced prominent dose-dependent inductions of the mRNA for P450p, the major steroid-inducible P450 of the class IIIA gene subfamily (Fig. 6) (6, 9). As we have previously reported (36), the dose-response curve for induction of P450p mRNA by PB or by kepone was positioned clearly to the right of that for P450e induction. Thus, whereas P450e mRNA induction was maximal at $10^{-5}$ M kepone (Fig. 5), P450p mRNA induction was only just becoming apparent at this dose (Fig. 6). Also consistent with our previous studies of PB in hepatocyte cultures (36), kepone treatment slightly induced the mRNA for P450pcn2 (Fig. 6), a class IIIA P450 gene subfamily member that shares 90% nucleotide sequence similarity with P450p (34). Moreover, in cultures exposed to PB or to kepone there were dose-dependent increases in the mRNAs for P450f and $P450_{PB\text{-}1}$, two constitutive P450 gene subfamily IIC members (Fig. 6) (11, 12, 35). $P450_{PB\text{-}1}$ and P450f have previously been shown to be modestly induced in rats by PB (11) and kepone (12), respectively. Finally, kepone effectively induced the mRNA for P450 OR (Fig. 6), whereas in the same cultures P450c mRNA, the major aromatic hydrocarbon-inducible P450 form, remained unaffected, as expected (data not shown).

**Effect of kepone on P450b and P450e mRNA induction by PB.** To test whether kepone might actively repress P450b mRNA induction rather than simply fail to stimulate its accumulation in the cells, we incubated cultured hepatocytes with PB plus kepone. In the presence of increasing doses of the pesticide ($10^{-7}$ to $10^{-5}$ M), induction of P450b by PB ($10^{-4}$ M) became progressively inhibited (Fig. 7). The combined treatments with PB and kepone were not grossly toxic to the cells, as judged by total cellular mRNA content and lactate dehydrogenase leakage (data not shown). PB induction of P450e mRNA was less sensitive to inhibition by kepone cotreatment than was P450b mRNA induction, inasmuch as an inhibitory effect on the former was only apparent at a kepone dose of $10^{-5}$ M (Fig. 7).

**Effects of kepone and mirex on P450b and P450e mRNAs and proteins *in vivo*.** Finally, to examine the effects of kepone on P450b/e induction in whole animals, we administered PB, kepone, or mirex to groups of three male rats and assessed the effects on liver P450b and P450e mRNAs (Fig. 8) and proteins (data not shown). PB and mirex treatments each produced marked and equal relative inductions of P450b and P450e mRNAs. In contrast to its effects *in vitro*, kepone induced both P450e and P450b mRNAs, albeit weakly, to equal extents, relative to PB. Induction of the P450b and P450e proteins by the three compounds was consistent with the results obtained for the mRNAs (data not shown).

## Discussion

In this report, we have demonstrated that, among a variety of organochlorine pesticides tested, kepone alone selectively

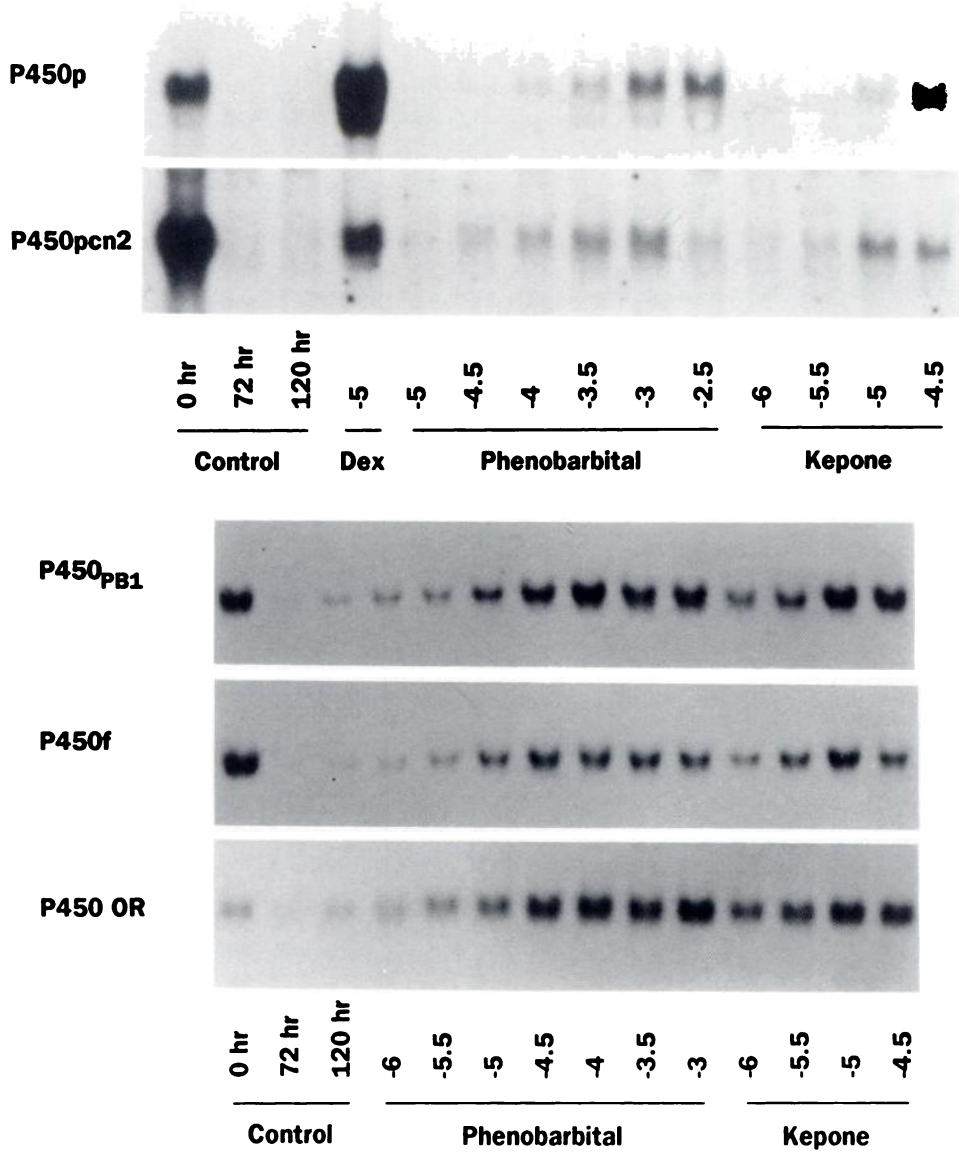


**Fig. 6.** Northern blot analysis of effects on mRNAs for PB-responsive genes in cultured rat hepatocytes treated with PB or kepone. Three-day-old cultures were treated for 48 hr with medium containing PB ($10^{-5}$ to $3 \times 10^{-3}$ M) or kepone ($10^{-6}$ to $3 \times 10^{-5}$ M). Total cellular RNA was extracted from three pooled dishes of cells for each treatment group and was analyzed by Northern blot hybridization using oligonucleotides specific to P450p and P450pcn2, as described in Experimental Procedures. The filters used to obtain the blots described in Fig. 4 were boiled in water, to remove bound oligonucleotides, and then rehybridized with cDNAs to P450f, $P450_{PB-1}$, and P450 OR. The autoradiographs displayed for P450p, P450pcn2, P450PB-1, P450f, and P450 OR mRNAs resulted from film exposures of 1, 3, 1, 1, and 5 days, respectively. *DEX*, dexamethasone.

induces expression of the P450e gene in primary rat hepatocytes cultured on Matrigel. Kepone induced P450e mRNA with considerable potency, being maximally effective at a dose ($10^{-5}$ M) 10-fold lower than that of PB. The maximal induction of P450e mRNA by kepone, although somewhat variable among cell preparations, was often quantitatively equal to that in cultures exposed to PB, whereas the induction of P450b mRNA (or protein) at the optimally effective dose of kepone, although often detectable, was never more than a small fraction of the response produced by PB in any of our experiments. To put the effective dose range of kepone into some perspective, a preliminary experiment in which cultured hepatocytes were treated with $10^{-5}$ M [$^{14}$C]kepone resulted in a cellular pesticide concentration that was within the range previously measured in liver biopsy samples taken from industrial workers who had been heavily exposed to kepone (39).

Although there is evidence for differential regulation of P450b and P450e in terms of basal expression and tissue specificity (40–44), kepone treatment provides the first clear example of the highly selective induction by a xenobiotic of both the mRNA and protein for one of these two remarkably similar structural genes. Previously, Scholte *et al.* (45) reported that 2,4,5,2′4′5′-hexachlorobiphenyl treatment of rats resulted in selective induction of P450b mRNA. However, a later study found, instead, coordinate induction of P450b and P450e immunoreactive proteins in rats treated with this polychlorinated biphenyl (46). Recently, Marcus *et al.* (47) reported that several 4-*n*-alkyl-substituted methylenedioxybenzenes selectively induced P450e protein in rats. These compounds did not, however, selectively induce P450e mRNA, suggesting that the discriminative effect was mediated through effects on synthesis or stabilization of the protein. Kepone differentially induced both P450e mRNA and protein in the hepatocyte cultures, suggesting that the pesticide acts at a pretranslational step in gene expression. Moreover, the methylenedioxybenzenes strongly induce P450c mRNA and protein (47), whereas kepone was without effect on P450c mRNA in the cultured hepatocytes and has been reported by others not to induce P450c-mediated monooxygenase activities in rats (48). Indeed, except for its failure to induce P450b, kepone functioned as a typical PB-like P450 agent, inducing mRNAs for P450 OR, a class IIIA P450 (P450p), and two members of the P450 IIC gene subfamily

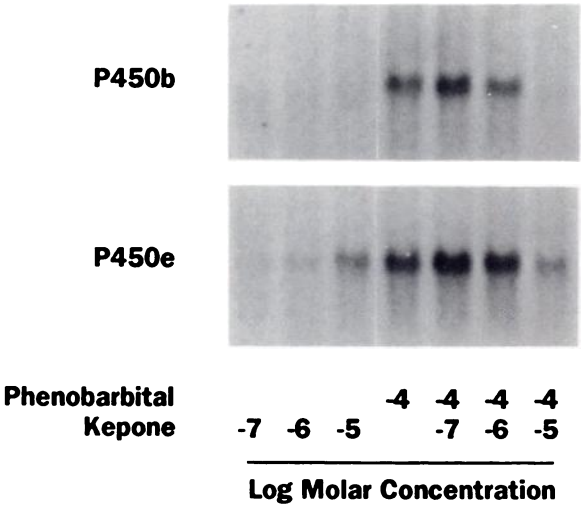


**Fig. 7.** Northern blot analysis of P450b and P450e mRNAs in cultured rat hepatocytes cotreated with PB and kepone. Three-day-old cultures were treated for 24 hr with medium containing PB ($10^{-4}$ M) or PB plus kepone ($10^{-7}$ to $10^{-5}$ M). Total cellular RNA was extracted from three pooled dishes of cells for each treatment group and was analyzed by Northern blot hybridization using oligonucleotides specific to P450b and P450e, as described in Experimental Procedures. The autoradiographs displayed for P450b and P450e mRNAs each resulted from film exposures of 2 days.

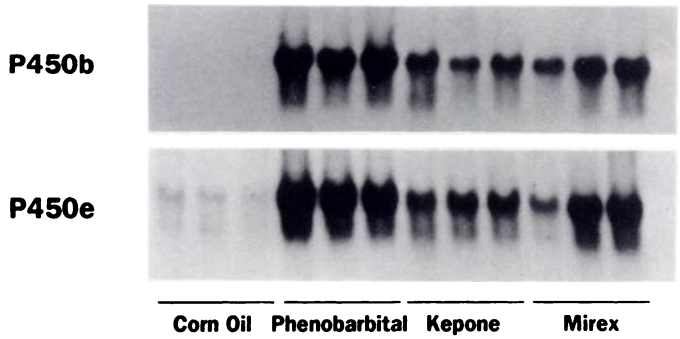


**Fig. 8.** Northern blot analysis of liver P450b and P450e mRNAs in male rats treated with PB, kepone, or mirex. Groups of three male Sprague-Dawley rats (280–300 g) were treated with PB, kepone, mirex, or corn oil, as described in Experimental Procedures. Total RNA was extracted from the livers and analyzed by Northern blot hybridization using oligonucleotides specific to P450b and P450e, as described in Experimental Procedures. Results for each individual rat are shown. The autoradiographs displayed for P450b and P450e mRNAs resulted from film exposures of 4 hr and 1 day, respectively.

(P450f and $P450_{PB-1}$). The latter effect of kepone, taken together with its selective induction of P450e, indicates that kepone treatment affects the hepatocyte in a manner that promotes the expression of several constitutive P450 forms. This finding is consistent with a previously reported induction profile of P450 protein, which more closely resembled that of control rats than PB- or methylcholanthrene-treated rats (49).

The efficacy of kepone in the induction of P450e, as well as P450p, P450pcn2, P450f, P450k, and P450 OR, eliminates the possibility that the failure of kepone to induce P450b was a result of deficient uptake of the drug into the hepatocytes cultured on Matrigel. Several possible explanations can be envisioned for the selective induction of P450e versus P450b mRNA by kepone, including 1) selective activation of transcription of the P450e gene, 2) selective stabilization of the P450e mRNA or pre-mRNA, and 3) typical PB-like induction of both P450b and P450e but simultaneous selective repression of P450b through another mechanism exerted by the drug.

PB has been shown to induce P450b and P450e by transcriptional activation of the genes (50–52). It is not yet known whether the organochlorine pesticides induce P450b and P450e in this manner also or through another mechanism, such as stabilization of the mRNAs or nuclear pre-mRNA transcripts, as has been suggested for the induction and potentiation of induction of these P450 forms by dexamethasone in rats (53, 54). Kepone and mirex induced P450b and P450e mRNAs with somewhat slower onset than occurred for PB. This finding may reflect distinct inductive mechanisms of the drugs or may, rather, be due to differences in their distributions in the cell cultures. Preliminary experiments using [$^{14}$C]kepone and [$^{14}$C] mirex revealed that these highly lipophilic compounds were extensively bound to the Matrigel matrix and may, therefore, more slowly accumulate to effective concentrations within the hepatocyte than does PB.

Accompanying the selective induction of P450e by kepone, we found an apparent selective repression by kepone of the PB-mediated induction of P450b mRNA. Possible explanations for this effect include 1) selective repression of PB-induced transcription of the P450b gene, 2) selective destabilization of the P450b mRNA or pre-mRNA, and 3) repression of PB induction of both P450b and P450e mRNAs but simultaneous induction of P450e through another mechanism exerted by kepone.

Our experiments also demonstrated an apparent discrepancy between the effects of kepone in whole rats and its effects in our cultured hepatocytes. One possible explanation for this finding would be the metabolism of kepone *in vivo* to a species that induces both P450b and P450e. This appears unlikely, however, because kepone has been shown not to be substantially metabolized in rat (55). Other explanations for the *in vivo/in vitro* difference include the possibilities that 1) kepone induces P450b indirectly *in vivo* by exerting an extrahepatic effect, 2) kepone induces P450b by acting directly on the liver but the cultured hepatocytes lack some permissive factor that is essential for the action of this compound and that is present *in vivo*, and 3) the single dose and regimen employed for kepone in the *in vivo* study resulted in intrahepatocellular kepone concentrations and distributions unlike those obtained *in vitro*. Whichever explanation is correct, our results do not reflect an inability of our cultured hepatocytes to respond appropriately to organochlorine pesticides in general, because these cells faithfully produced the usual coordinate induction of P450b and P450e mRNAs when treated with 15 other organochlorines, including mirex.

Although other selective inducers of P450e or P450b mRNAs and proteins will undoubtedly be found, at present kepone appears to be unique in this regard. Use of a responsive hepatocyte cell culture system and selective probes for P450b and P450e has allowed us to demonstrate pharmacologically that regulation of the induction of these two closely related P450 forms by xenobiotics is not identical. Although questions regarding the precise molecular mechanisms of the selective induction of P450e mRNA by kepone as well as the inhibition of PB-mediated P450b mRNA induction remain to be addressed, this hepatocyte system, coupled with techniques allowing further molecular analyses, should provide the appropriate answers.

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