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DIFFERENTIAL METABOLISM OF *O*-ETHYL *O*-4-NITROPHENYL PHENYLPHOSPHONOTHIOATE BY RAT AND CHICKEN HEPATIC MICROSOMES

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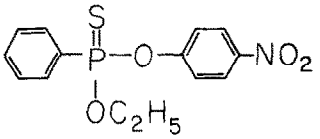
Abstract—The *in vitro* hepatic metabolism of *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN) was investigated in the hen (a species that is sensitive to EPN delayed neurotoxicity) and the rat (an insensitive species). EPN, which produced a Type I binding spectrum on incubation with cytochrome P-450, was converted by liver microsomes from both species to its oxygen analog, *O*-ethyl *O*-4-nitrophenyl phenylphosphonate (EPNO), and to *p*-nitrophenol (PNP). The formation of EPNO and PNP was dependent on the presence of NADPH in the reaction mixture and could be inhibited by either SKF-525A or by anaerobic conditions. The rates of EPNO and PNP formation by rat liver microsomes were, however, 3- and 20-fold higher, respectively, than the rates of formation by chicken liver microsomes. There was also a 4-fold difference in the cytochrome P-450 contents of the liver microsomes. The EPNO-hydrolyzing activity of rat liver microsomes was much greater than that of chicken liver microsomes. EPNO metabolism, in contrast to EPN metabolism, did not require NAPDH nor was it inhibited by SKF-525A or by anaerobic conditions. Prior exposure of rats to phenobarbital (PB) or Arochlor 1254 resulted in an increase in hepatic microsomal EPN metabolism and cytochrome P-450 content. On the other hand, 3-methylcholanthrene (3-MC) treatment elevated microsomal cytochrome P-450 but did not increase EPNO or PNP formation. Pretreatment with EPN did not alter either microsomal EPN metabolism or cytochrome P-450 levels. In chickens, prior exposure to PB, 3-MC or 100 mg/kg EPN increased EPNO and PNP formation by liver microsomes as well as cytochrome P-450 levels; prior exposure of chickens to 15 mg/kg EPN did not alter these variables. The λ_{max} Soret bands of the reduced hepatic cytochrome P-450 complexes from these animals differed as follows (rat then chicken): untreated, 450 vs 452 nm; PB-treated, 450 vs 451 nm; and 3-MC-treated, 448 vs 449 nm. None of the above treatments had an effect on EPNO metabolism by liver microsomes.

Various organophosphorus esters with anticholinesterase activity can cause delayed neurotoxicity in certain animal species, including humans [1]. Among these compounds is EPN‡ (Fig. 1) [2], a phenylphosphonothioate insecticide and acaricide which has been marketed worldwide for over 25 years [2–5]. Administration of a single oral dose (25–500 mg/kg) as well as multiple daily oral or dermal doses (0.1–10.0 mg/kg) of EPN caused delayed neurotoxicity in hens similar to that reported for other neurotoxic organophosphorus compounds [6, 7]. After a typical delay period of 7–21 days, degeneration of both central and peripheral nerve axons with secondary degeneration of the myelin sheath was observed, resulting in progressive ataxia and paralysis. EPN also has been implicated as the cause of the neuropathy, weakness, easy fatigability and weight loss in workers who have been exposed to it on multiple occasions [8]. Rats and mice, however, were not susceptible to the delayed neurotoxic effects of phenylphosphonothioate esters [9].



EPN

Fig. 1. Chemical structure of EPN.

Currently, it is poorly understood why EPN, as well as other neurotoxic organophosphorus compounds, induces delayed neuropathy only in certain animal species. Recent evidence [10–14] suggests that quantitative differences in biotransformation and pharmacokinetics of these compounds may contribute to the species-selective delayed neurotoxic effect. EPN initially is metabolized to its oxygen analog, EPNO [15, 16], by NADPH-dependent mixed-function oxidases present in mammalian liver. This oxygen analog, which is a potent inhibitor of acetylcholinesterase as well as a phosphorylating agent [17], is a more potent neurotoxic agent than its parent compound [18]. EPNO subsequently is hydrolyzed to *p*-nitrophenol and ethyl phenylphosphonic acid by a microsomal arylesterase [16, 19]; these latter compounds may be metabolized further and are excreted in either free or conjugated form [20, 21].

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‡ Abbreviations: EPN, *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate; EPNO, *O*-ethyl *O*-4-nitrophenyl phenylphosphonate; PB, phenobarbital; 3-MC, 3-methylcholanthrene; and PNP, *p*-nitrophenol.

The present study was undertaken to investigate species differences of the *in vitro* metabolism of EPN and EPNO in the chicken, a species susceptible to the delayed neurotoxic effects of these compounds, and the rat, a nonsusceptible species. The effect of previous exposure to xenobiotics on the metabolism of EPN and EPNO also was examined.

MATERIALS AND METHODS

Chemicals and treatments

3-Methylcholanthrene, atropine sulfate, NADPH and *p*-nitrophenol were purchased from the Sigma Chemical Co. (St. Louis, MO). Sodium phenobarbital was obtained from the Mallinckrodt Chemical Works (St. Louis, MO). Arochlor 1254 was a gift of Dr. Joyce Goldstein, National Institute of Environmental Health Sciences, Research Triangle Park, NC. EPN and related compounds were provided by E. I. DuPont de Nemours & Co., Inc. (Wilmington, DE). All other chemicals used were of the highest grade commercially available.

Animals

Laying hens (*Gallus gallus* domesticus), Leghorn (Featherdown Farms, Raleigh, NC), each weighing approximately 1.5 kg, and male CD rats (200–250 g, Sprague–Dawley, Madison, WI) were used. Water and food were provided *ad lib.* until immediately prior to killing the animals. Following a 1-week acclimatization period, treatments were administered on three consecutive days and dosing was staggered so that one animal in each treatment group was killed on each of 4 consecutive days. Treatments administered were: sodium phenobarbital, $80\ mg \cdot kg^{-1} \cdot day^{-1}$ i.p. in saline; 3-methylcholanthrene, $40\ mg \cdot kg^{-1} \cdot day^{-1}$ in corn oil; EPN, $15\ mg \cdot kg^{-1} \cdot day^{-1}$ i.p. in corn oil; chickens also received EPN, $100\ mg \cdot kg^{-1} \cdot day^{-1}$ in corn oil. Arochlor 1254 was administered 4 days prior to sacrifice in a single oral dose of 500 mg/kg. Animals receiving EPN were given atropine sulfate concurrently to protect against the severe cholinergic side effects of EPN. Controls received either an equivalent volume of vehicle alone or vehicle together with atropine sulfate.

Enzyme preparation

Animals were decapitated and the livers were quickly removed and rinsed in ice-cold 1.15% KCl. All subsequent procedures were performed at 4°. After being weighed, the livers were coarsely chopped with scissors and a 25% (w/v) homogenate was made in 66 mM Tris–HCl buffer, pH 7.4, containing 1.15% KCl using a polytron-type homogenizer for 30 sec. Microsomes were then prepared by differential centrifugation as previously described [22]. Microsomes were suspended in 0.25 M sucrose at a final protein concentration of 20 mg/ml and frozen at −70° under N_2 until use. Protein concentration was measured by the method of Lowry *et al.* [23] using bovine albumin as a standard. Cytochrome P-450 concentration was determined from the reduced CO difference spectrum by the method of Omura and Sato [24] using an Aminco DW-2a spectrophotometer (American Instrument Co., Silver Springs, MD) in the split beam mode. An extinction coefficient of $91\ mM^{-1}\ cm^{-1}$ between 450 and 490 nm was used.

EPN and EPNO metabolism

Microsomes equivalent to 1–10 mg protein were suspended in 2 ml of 66 mM Tris–HCl buffer, pH 7.4. After an initial preincubation period of 3 min at 37°, the reactions were begun with either 1 μmole EPN plus 5 μmoles NADPH or 1 μmole EPNO. The reactions were terminated at various times (see Results) by the addition of 2 ml of ice-cold acetone, followed by 0.4 ml of 1 N HCl. The mixtures were then extracted with two 5-ml portions of H_2O-saturated ethyl acetate, which were combined, dehydrated with anhydrous $MgSO_4$, and concentrated to

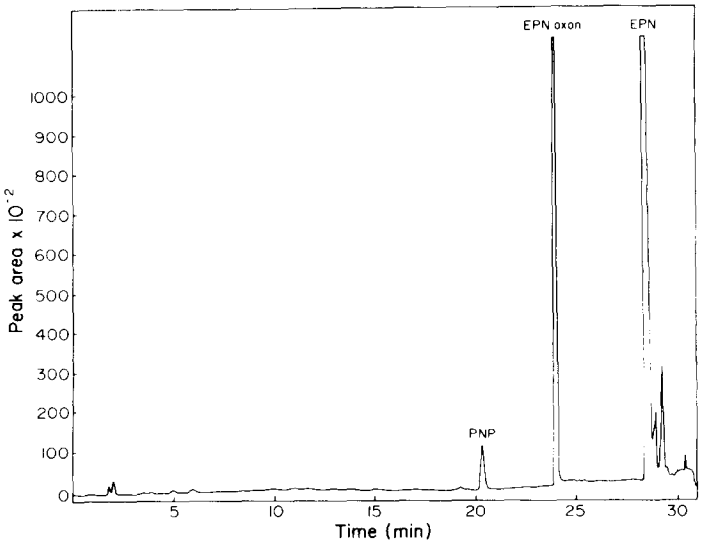

Fig. 2. Separation of EPN, EPN oxon and PNP by reverse-phase HPLC. Elution from a Lichrosorb RP-8 column, using a gradient of 1–95% methanol in water (initial solvent also contained 5% glacial acetic acid), in 30 min after a 10-min isocratic delay. Flow rate was 1.2 ml/min at 25°.

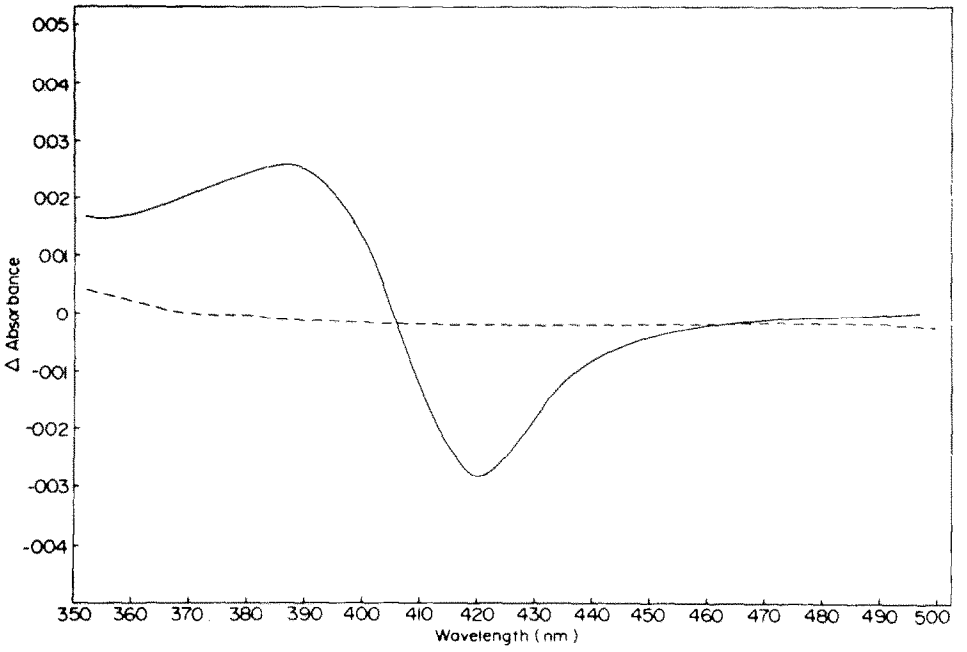

Fig. 3. Optical-difference spectrum of EPN. Liver microsomes from phenobarbital-treated rats (2 mg protein/ml) were suspended in 66 mM Tris–HCl buffer, pH 7.4. The final concentration of EPN was 1 mM.

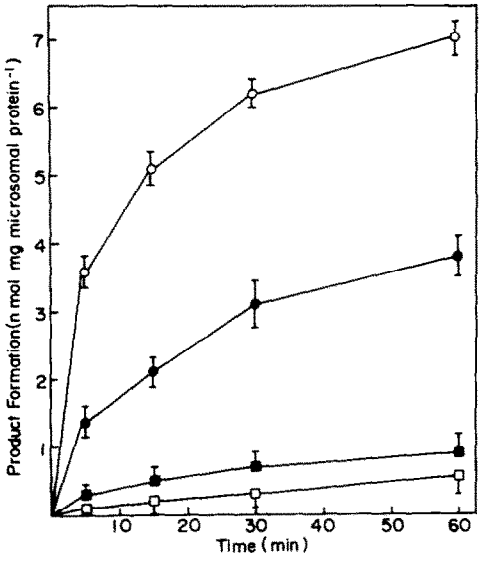

Fig. 4. Formation of EPNO and PNP by chicken and rat liver microsomes. Two milligrams of microsomal protein was suspended in 2 ml of 66 mM Tris–HCl buffer, pH 7.4, containing 2 μmoles EPN plus 5 μmoles NADPH. After 30 min at 37°, the reactions were terminated, and metabolites were analyzed by HPLC as described under Materials and Methods. Key: (○——○) PNP formation by rat liver microsomes; (●——●) EPNO formation by rat liver microsomes; (■——■) EPNO formation by chicken liver microsomes; and (□——□) PNP formation by chicken liver microsomes.

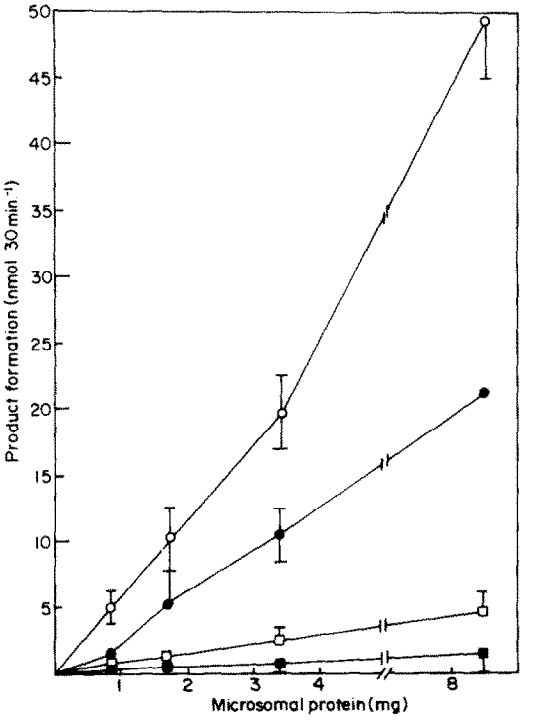

Fig. 5. Effect of protein concentration on EPNO and PNP formation by rat and chicken liver microsomes. Microsomal protein (amounts indicated in figure) was suspended in 2 ml of 66 mM Tris–HCl buffer, pH 7.4, containing 2 μmoles EPN plus 5 μmoles NADPH. After 30 min at 37°, the reactions were terminated, and metabolites were analyzed by HPLC as described under Materials and Methods. Key: (○——○) PNP formation by rat liver microsomes; (●——●) EPNO formation by rat liver microsomes; (■——■) EPNO formation by chicken liver microsomes; and (□——□) PNP formation by chicken liver microsomes.

Table 1. Effects of inducers of microsomal drug-metabolizing activities on EPN metabolism by chicken and rat liver microsomes

Species	Treatment	Cytochrome P-450* [nmoles · (mg protein)$^{-1}$]	Apparent λ_{max} (nm)	EPN metabolism* [nmoles · (mg protein)$^{-1}$ · (30 min)$^{-1}$]	
				EPNO	PNP
Rat	Corn oil	0.47 ± 0.2	450	3.1 ± 0.2	6.2 ± 0.4
Rat	PB†	0.99 ± 0.3‡	450	7.6 ± 0.2‡	19.0 ± 0.3‡
Rat	3-MC§	0.83 ± 0.5‡	448	2.5 ± 0.2	5.8 ± 0.6
Rat	Arochlor‖	1.47 ± 0.4‡	449	4.7 ± 0.2	12.5 ± 0.6‡
Rat	EPN¶	0.45 ± 0.1	450	3.0 ± 0.1	6.0 ± 0.1
Chicken	Corn oil	0.13 ± 0.1	452	0.7 ± 0.1	0.32 ± 0.1
Chicken	PB	0.35 ± 0.2‡	451	4.0 ± 0.2‡	1.19 ± 0.1‡
Chicken	3-MC	0.50 ± 0.2‡	449	1.2 ± 0.3‡	0.8 ± 0.1‡
Chicken	EPN¶	0.14 ± 0.1	452	0.7 ± 0.1	0.32 ± 0.1
Chicken	EPN**	0.24 ± 0.4‡	452	1.3 ± 0.1‡	0.9 ± 0.2‡

* Each value is the mean ± S.E. of at least three separate experiments.
† Eighty mg per kg per day × 3.
‡ Significantly different from corresponding control value ($P < 0.05$) by Student's t-test.
§ Forty mg per kg per day × 3.
‖ Five hundred mg per kg.
¶ Fifteen mg per kg per day × 3.
** One hundred mg per kg.

dryness with nitrogen gas. Residues were then resolubilized in 50 μl methanol for high-pressure liquid chromatography (HPLC) analysis. A Waters Associates liquid chromatograph (Milford, MA) was employed, consisting of two M6000A pumps, a M660 solvent programmer, a M440 u.v. detector and a U6-K injection system. EPN and metabolites were separated by gradient elution at room temperature according to the method of Lasker *et al.* [25]. Detection and quantification were performed by measuring the u.v. absorbance of the column eluates at 254 nm. Peak areas were measured with a Shimadzu chromatopak E1A reporting integrator (Kyoto, Japan). Standard concentration curves at this u.v. wavelength were constructed using authentic metabolite standards.

RESULTS

Cytochrome P-450-dependent metabolism of EPN

Incubation of EPN with chicken or rat liver microsomes in the presence of NADPH resulted in the formation of EPNO and PNP. No other EPN-related products were detected by either of these microsomal preparations when analyzed by HPLC. The HPLC ultraviolet detection limit was 0.01 μg for EPN and related compounds. The recovery efficiencies of ethyl acetate extraction of the incubation mixture of EPN and the rat and chicken liver microsomes ranged from >95% for both EPN and EPN oxon to 85% for PNP. A typical HPLC metablic profile of EPN is illustrated in Fig. 2. EPNO and PNP production did not occur when NADPH was omitted from the incubation mixture or when heat-denatured microsomal protein was used. Because this dependence on NADPH indicated the involvement of cytochrome P-450, the participation of this hemeprotein in EPN hepatic microsomal metabolism was investigated further. Figure 3 shows the binding spectrum observed when EPN was added to a suspension of rat liver microsomes. The difference spectrum is indicative of Type I spectral change, with a peak at about 386 nm and a trough at about 421 nm. Cytochrome P-450-dependent metabolism of EPN was indicated by the complete inhibition of EPNO and PNP formation when 1 mM SKF-525A was included in the microsomal incubation mixtures or anaerobic conditions were employed (data not shown).

Species differences in EPN metabolism

Figure 4 illustrates the observed species difference in hepatic microsomal EPN metabolism. At each time point examined, the formation of EPNO and PNP by rat liver microsomes was significantly greater than their formation by chicken microsomes. Approximately 5-fold more EPNO and 20-fold more PNP were formed by the rat tissue (on a per mg protein basis) at the end of a 60-min incubation period. This species difference in hepatic microsomal EPN metabolism is also shown in Fig. 5, where the formation of EPNO and PNP is plotted as a function of microsomal protein concentration. While the rates of both EPNO and PNP formation in each species exhibited a linear dependence on the amount of hepatic microsomal protein present in the reaction mixture, the EPN-metabolizing activity of rat liver microsomes was again significantly greater than that of chicken liver microsomes at each protein concentration tested.

Species differences of hepatic cytochrome P-450

The species difference in hepatic microsomal EPN metabolism may be a result of the difference in native hemeprotein content of the two microsomal preparations. As shown in Table 1, the specific content of cytochrome P-450 present in rat liver was 3.6-fold greater than in chicken liver microsomes. The Soret peaks of the CO difference spectra of the reduced cytochromes represent another distinction between these two species. The λ_{max} of the reduced cytochrome P-450 complex from untreated chickens

Table 2. EPN- and EPNO-dependent PNP formation by chicken and rat liver microsomes*

Tissue	NADPH	PNP† [nmoles · (mg protein)$^{-1}$ · (30 min)$^{-1}$] Substrate EPN	EPNO
Rat liver microsomes	+	6.2 ± 0.4	95.2 ± 7.7
Rat liver microsomes	−	0	94.6 ± 8.4
Chicken liver microsomes	+	0.3 ± 0.1	0.1 ± 0.0
Chicken liver microsomes	−	0	0.1 ± 0.0

* Procedures for incubation and for isolation and measurement of metabolites are given under Materials and Methods.

† Each value is the mean ± S.E. of at least three separate experiments.

was at 452 nm, whereas cytochrome P-450 from untreated rats exhibited a λ_{max} at 450 nm.

In addition, the λ_{max} of the Soret peak of the reduced cytochrome P-450 CO complex in phenobarbital-treated chicken liver microsomes shifted from 452 to 451 nm, while that of the rats remained centered at 450 nm.

Effect of prior exposure to xenobiotics on EPN metabolism

Rat liver microsomes. The effect on the hepatic microsomal metabolism of EPN after exposure to foreign compounds also was investigated (Table 1). Rats treated with phenobarbital or Arochlor 1254 (a mixture of polychlorinated biphenyl congeners) showed a significant increase in the rate of hepatic EPN metabolism compared to those that were untreated. These increased rates of EPNO and PNP formation were accompanied by an increase in the microsomal cytochrome P-450 content. Formation of EPNO and PNP, however, increased to a greater extent in phenobarbital-treated rats than in Arochlor 1254-treated rats, even though the hepatic cytochrome P-450 content of the latter animals was about 50% higher than the hemeprotein of the former. These results are not surprising since Arochlor 1254 contains a variety of congeners some of which induce cytochrome P-450 and some of which induce cytochrome P-448. Some of the increase in cytochrome P-450 may have resulted from an increase in cytochrome P-448 which, as shown by the results for induction with 3-MC, was apparently not greatly involved in EPN metabolism. Thus, treatment with 3-MC elevated the cytochrome P-450 content 60% over control values but did not increase the rate of microsomal EPN metabolism. As shown in the table, the λ_{max} of the reduced cytochrome P-450 CO complex was different with each of these three inducers of monooxygenase activity. Table 1 also shows that neither hepatic microsomal EPNO and PNP formation nor cytochrome P-450 content was altered by 15 mg/kg EPN treatment in the rat.

Chicken liver microsomes. Treatment of chickens with phenobarbital resulted in a greater increase in both EPNO and PNP formation than the treatment with 3-MC, although the hemeprotein content of chicken liver microsomes increased to a greater extent (Table 1) by pretreatment with 3-MC. As was observed with rats, treatment of chickens with 15 mg/kg EPN had no effect on microsomal EPNO and PNP formation or microsomal hemeprotein content. Upon increasing the dose of EPN to 100 mg/kg, however, significant increases in both these variables were noted. These results are noteworthy, since EPN would be expected to cause a reduction in cytochrome P-450 levels and oxidative activity through release of "active" sulfur. However, oxidative activity in the chicken liver microsomes seems to be so low that the amount of sulfur released had no observable inhibitory effect. The λ_{max} of the Soret peak of the reduced P-450 CO complex remained at 452 nm after pretreatment with this compound.

Hepatic metabolism of EPN oxon

The metabolism of EPNO, the oxon analog of the parent compound, by chicken and rat liver microsomes is presented in Table 2. With both of these microsomal preparations, the only polar metabolite detected from EPNO was PNP. As shown, the EPNO-hydrolyzing activity of rat liver microsomes was substantial. PNP formation from EPNO by rat liver microsomes was 15.4 times greater than that from EPN. This hydrolytic activity resulted in the metabolism of about 60% of the total EPNO in 30 min. On the other hand, chicken liver microsomes displayed extremely low EPNO-hydrolyzing activity. More PNP was formed by chicken liver microsomes when EPN rather than EPNO was used as substrate. In the case of both of these, NADPH was not required for EPNO hydrolysis to occur. Boiling of the microsomes abolished any EPNO-hydrolyzing activity. Additionally, none of the treatments described above which increased hepatic microsomal EPN metabolism was found to affect the EPNO-hydrolyzing activity of either chicken or rat liver microsomes.

DISCUSSION

EPN is a member of a group of neurotoxic organophosphorus compounds which elicits a characteristic delayed neuropathological effect in only certain animal species [5]. Humans, dogs, cats, cows and chickens are susceptible to organophosphate-induced delayed neurotoxicity while rodents are notably resistant [1].

In this study, qualitative differences in hepatic microsomal EPN metabolism were not observed

between the chicken and the rat. Both species converted EPN to its oxygen analog, EPNO, and hydrolyzed it, generating PNP. There was no evidence for dealkylation of EPN to the desethyl derivative by either species *in vitro* [16].

EPN metabolism by liver microsomes *in vitro* from both chickens and rats was found to be a cytochrome P-450-mediated process, as substantiated by several lines of evidence: (1) conversion of EPN to EPNO and PNP did not occur in the absence of NADPH; (2) addition of EPN to a rat liver microsomal suspension gave a typical Type I spectral change (almost all compounds which exhibit Type I binding spectra are substrates for mixed-function oxidation by the cytochrome P-450 monooxygenase system [26]; and (3) EPN metabolism was inhibited by SKF-525A, an inhibitor of many cytochrome P-450-mediated monooxygenation reactions. These results are similar to those of other investigators examining oxidative EPN metabolism by subcellular fractions of total homogenates of liver [15, 16], in addition to the fact that the data strongly support the direct participation of cytochrome P-450 in the reaction.

In contrast to the lack of qualitative differences in hepatic microsomal EPN metabolism observed between the two species, definite quantitative differences were found. Using EPN as a substrate, EPNO and PNP formation by rat liver microsomes proceeded at significantly higher rates than that observed in chicken liver microsomes. This difference was apparently due, in part, to the variation in cytochrome P-450 content of the two microsomal preparations, since both hemeprotein content and EPNO formation were 4-fold less with chicken than with rat liver microsomes. However, the native cytochrome P-450 hemeproteins found in rat and chicken liver microsomes had different spectral properties (the λ_{max} of the Soret band of the reduced cytochrome P-450 CO complex in chicken liver microsomes was centered at 452 nm while that of the rat liver microsomes was at 450 nm). It is therefore reasonable to suspect that these hemeproteins may exhibit different EPN turnover rates in addition to different substrate specificities [27].

Regarding EPNO metabolism, the two species again varied in their ability to hydrolyze this compound. EPNO hydrolysis to PNP was rapidly catalyzed by rat liver microsomes. The inability of chicken liver microsomes to hydrolyze EPNO suggests a deficiency of arylhydrolase activity in this cellular fraction. However, the observation that EPN can be hydrolyzed to PNP by chicken liver microsomes in the presence of NADPH suggests a direct oxidative pathway for EPN dearylation by this species, similar to that reported for the metabolism of EPN as well as of other structurally similar compounds [16, 28]. Whether an oxidative dearlyation pathway for EPN also exists in rat liver cannot be determined at present, since we were unable to inhibit the formation of PNP via EPNO in this tissue.

Several interesting differences as well as similarities in the monooxygenase systems of mammalian and avian liver were noted in this study. First, the levels of native cytochrome P-450 found in chicken liver were 4-fold less than those found in rat liver. As mentioned above, the nature of these hemeproteins was different, e.g. the λ_{max} of the Soret band of the reduced cytochrome P-450 complex of chicken liver microsomes was at 452 nm while that of rat liver microsomes was at 450 nm. Whether there exists a true native cytochrome P-450 in chickens or a mixture of cytochromes which gives rise to the 452 nm peak can only be determined using purification procedures which are beyond the scope of this investigation. Second, the avian monooxygenase system was capable of responding to inducers of microsomal drug-metabolizing activity in a manner similar to that of mammals. Prior exposure to PB or 3-MC caused increased levels of hepatic microsomal cytochrome P-450 in both rats and chickens. As in the case of the untreated animals, however, the microsomal hemeproteins induced by these compounds were of a different nature, since the λ_{max} of the reduced cytochrome P-450 complexes were different in each case. Third, hepatic microsomal EPN metabolism, as well as hemeprotein content in chickens, was increased by previous exposure to a large oral dose of EPN (100 mg/kg). Prior exposure to smaller doses of this compound produced neither effect in chickens or rats. These results suggest that EPN may be able to stimulate its own metabolism in the chicken in a dose-dependent manner. It should be noted here that a dose of EPN larger than 15 mg/kg could not be utilized with rats without killing them (even with concurrent administration of atropine sulfate).

If the data in Table 1 are recalculated on a per nmole cytochrome basis rather than on a per mg microsomal protein basis, then the only treatment causing a significant increase in hepatic EPN metabolism was phenobarbital. On this basis, the other treatments actually caused a decrease in EPNO and PNP formation. This suggests that the cytochrome P-450 induced by phenobarbital displays the highest activity towards EPN. In rats, the microsomal cytochromes P-450 in PB-induced animals are supposedly present in proportions similar to that of microsomes from control animals [29, 30], while in chickens the data presented here suggest that PB treatment slightly changes this proportion. Therefore, the quantitative distinctions between the avian and mammalian monooxygenase systems most probably represent differences in the relative proportions of the component enzymes before and after inductions, since the evidence for the multiplicity of heterogenous enzymes within the monooxygenase system is not considerable [31].

Some aromatic organophosphorus esters which produce delayed neurotoxicity undergo metabolic conversion to more potent neurotoxic metabolites. One such example is provided by tri-*o*-cresyl phosphate (TOCP), which is metabolized *in vivo* to the active neurotoxic agent *o*-cresyl saligenin phosphate [32]. A 500 mg/kg dose of the parent compound is required to produce delayed neurotoxicity while the metabolite causes the effect at a dose of 1 mg/kg [33]. EPN also appears to undergo a metabolic biotransformation which produces an active neurotoxic agent, the oxygen analog EPNO [18]. Therefore, the more EPNO that is formed *in vivo* and accumulated at the neurotoxicity target sites by an animal species, the more susceptible that species would be to delayed

neurotoxicity (assuming that the target sites in nervous tissue are similar in both species). This hypothesis is supported by the results presented here.

Although the rat liver formed greater amounts of EPNO than the chicken liver, microsomal enzymes from the rat also hydrolyzed (and therefore detoxified) this compound at a much faster rate than microsomal enzymes from the chicken. This may contribute, at least in part, to the sensitivity of hens to delayed neurotoxicity induced by EPN. In assessing these results, however, it should be noted that liver cytosol and serum also contain arylhydrolase activity that may be capable of hydrolyzing EPN and/or EPNO. Preliminary studies with cats, another species susceptible to EPN-delayed neurotoxicity, indicated that the capacity of the species to detoxify EPNO is also low. Experiments are now in progress to test this hypothesis using other organophosphorus compounds capable of producing delayed neurotoxicity.

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