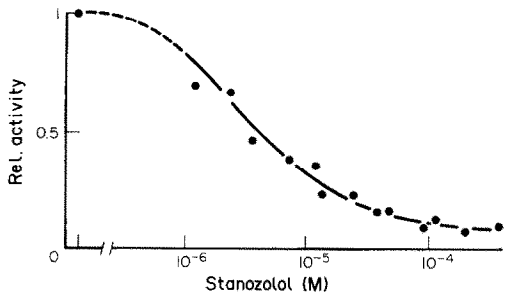

Fig. 3. Inhibition of *O*-dealkylation of 7-ethoxycoumarin in rat liver microsomes with Stanozolol. Stanozolol concentrations were in the range from 10^{-6} M to 5×10^{-4} M, 7-ethoxycoumarin was 10^{-3} M, cytochrome P-450 was 0.37 μM and protein 0.20 mg/ml. The enzymatic activity without Stanozolol was 2 nmol/min.

7-ethoxycoumarin to more than 90% at saturating concentration. This demonstrated interaction with the form(s) which catalyzed this monooxygenation in livers from rats pretreated with phenobarbital.

The data available shows differences in the tissue distribution when drug was administered subcutaneously to calves [13] in comparison to oral administration to rats [14]. In the first case only low level of the drug was found in liver (0.03–0.04 μg/g of tissue or 0.6% of dose in total organ). In rat liver, however, high tissue level was found (5.3 μg/g or 5% of dose). Although there are preliminary results showing that Stanozolol does not affect the microsomal activity in humans and does not influence the cytochrome P-450 content in rat liver *in vivo* [15], our data allow us to propose that Stanozolol may reduce the substrate interaction of other drugs, especially with such compounds which bind with low affinity to the enzyme. Further studies should be performed to determine the existence of species differences in the interactions with other forms of cytochrome P-450 and with the purified enzymes.

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Enzyme induction produced by *N*-(3,5-dichlorophenyl)succinimide (NDPS) in rats*

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N-(3,5-Dichlorophenyl)succinimide (NDPS) is an *N*-(haloaryl)succinimide which is active against many pathogenic plant fungi [1, 2]. NDPS-induced nephrotoxicity, however, limits the agricultural use of this fungicide. The mechanism of NDPS-induced nephrotoxicity is presently undetermined. Renal damage may be mediated by a toxic metabolite of NDPS generated by the hepatic mixed-function oxidase system. Previous work showed that deuterium labeling of the succinimide ring reduces NDPS nephrotoxicity [3]. These data suggest that oxidation of the carbon–carbon bridge is essential in the generation of nephrotoxic NDPS metabolites.

Many compounds present in the environment have the capacity to alter the activity of the mixed-function oxidase system. This paper focused on the capacity of NDPS to induce hepatic mixed-function oxidase enzymes. The following studies also determined if multiple exposures to NDPS may induce microsomal enzyme activity and modify NDPS nephrotoxicity.

Materials and methods

Male Fischer 344 rats (240–360 g) were maintained on a 12-hr light cycle at 23–25°. Animals were individually housed in metabolism cages to monitor daily urine output and food and water consumption [4]. Urine was semiquantitatively analyzed daily for protein, glucose, ketones and blood.

Rats (four/group) received a single intraperitoneal (i.p.)

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injection of NDPS [5, 6] (0.4 mmol/kg) or vehicle (sesame oil, 2.5 ml/kg). Control animals were pair-fed to the NDPS-treated groups to remove variability in data due to changes in food and water intake. *In vitro* hepatic metabolism was measured 24 and 72 hr post-injection of NDPS or vehicle. In a separate series of experiments, rats (four/group) were treated once daily for 3 days with NDPS (0.2 or 0.4 mmol/kg/day) or vehicle. Organic ion accumulation, BUN levels, kidney weight and *in vitro* hepatic metabolism were measured 24 hr following the last injection of NDPS or vehicle. Renal cortical slice accumulation of tetraethylammonium (TEA) as well as basal and lactate (10^{-2} M) stimulated *p*-aminohippurate (PAH) accumulation were measured as described previously [4, 5].

The livers were excised, and a 25% homogenate (w/v) was prepared in phosphate buffer (0.04 M, pH 7.4) and centrifuged (10,160 *g*) for 15 min at 4°. Samples were pre-incubated for 5 min at 37° prior to the addition of aniline [7], aminopyrine or hexobarbital [8, 9]. Cytochrome P-450 was measured by the method of Omura and Sato [10]. Protein was measured using the method of Lowry *et al.* [11].

Values were reported as mean ± SEM. Differences between control and NDPS-treated animals were quantitated using the two-tailed Student's *t*-test, at a 95% confidence interval.

Results and discussion

NDPS was shown in the following studies to induce hepatic microsomal enzymes. A single injection of 0.4 mmol/kg NDPS produced a moderate increase in aniline hydroxylase ($P < 0.05$) activity. Multiple treatments with NDPS increased ($P < 0.05$) hepatic aminopyrine demethylase and aniline hydroxylase metabolism relative to controls.

Liver weight was increased 23% ($P < 0.05$) above control by a 3-day treatment with 0.4 mmol/kg NDPS. NDPS (0.2 or 0.4 mmol/kg/day) increased aminopyrine demethylase activity ($P < 0.05$) 17 and 34%, respectively, above controls (Fig. 1A). Aniline hydroxylase activity (Fig. 1B) was increased 47 and 119% above the respective control by a 3-day pretreatment with NDPS (0.2 or 0.4 mmol/kg/day) ($P < 0.05$). Hexobarbital oxidase activity was not increased significantly by 3 days of treatment with NDPS. Cytochrome P-450 levels were 0.23 ± 0.065 ($P < 0.05$) and 0.04 ± 0.015 nmol P-450/mg protein in the NDPS (0.4 mmol/kg/day) and control groups respectively. Total cytochrome P-450 levels were not increased significantly by pretreatment with the low dose of NDPS.

Renal function was altered by multiple dosing with NDPS. Urine output was increased 2-fold above respective day 0 values ($P < 0.05$) for the NDPS (0.2 mmol/kg/day) treated rats (Fig. 2A). Daily urine output in the low dose NDPS group ($P < 0.05$) was increased 3-fold above vehicle controls. Food intake and body weight were decreased by NDPS (0.2 mmol/kg/day) treatment relative to day 0 values (Table 1). Three-day pretreatment with the high dose of NDPS (0.4 mmol/kg/day) increased urine output 2-fold above day 0 values ($P < 0.05$). Urine output was also enhanced relative to pair fed control 3- to 6-fold by NDPS (0.4 mmol/kg/day) treatment.

In vitro renal cortical slice accumulations of TEA and PAH (Fig. 2B) were altered by a 3-day pretreatment with NDPS (0.2 or 0.4 mmol/kg/day). NDPS (0.2 mmol/kg/day) treatment decreased basal and lactate-stimulated renal cortical PAH accumulation by 31% ($P < 0.05$) and 33% ($P < 0.05$), respectively, relative to pair-fed controls. TEA accumulation was not diminished by 3-day treatment with the low dose of NDPS. Three-day treatment with 0.4 mmol/kg/day NDPS did not alter organic ion accumulation. However, NDPS (0.4 mmol/kg/day, Table 1) increased kidney weight and BUN levels relative to controls ($P < 0.05$).

Three-day treatment with 0.4 mmol/kg NDPS resulted

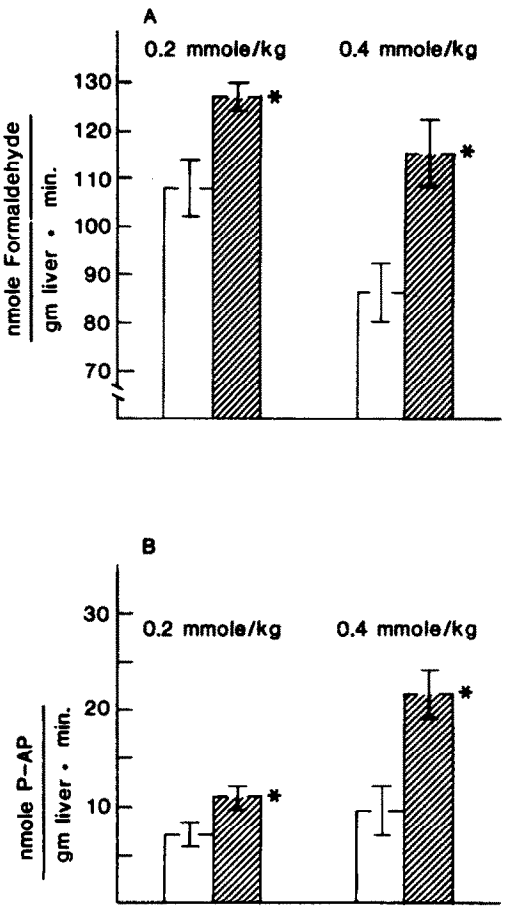

Fig. 1. Aminopyrine demethylase (A) and aniline hydroxylase (B) activities following a 3-day pretreatment with NDPS or vehicle. Values are reported as mean ± SEM; hatched bars denote NDPS-treated while open bars denote pair-fed controls ($N = 4$). An asterisk (*) denotes statistically significant ($P < 0.05$) using a Student's *t*-test, two-tailed.

in diuresis, increased BUN levels, and kidney weight relative to pair-fed controls. However, cortical slice accumulations of PAH and TEA were not diminished by NDPS pretreatment at the 0.4 mmol/kg dose. This is an interesting point since organic ion transport in renal cortical tissue is considered a sensitive parameter for detecting nephrotoxicity. These results suggest that multiple treatments with NDPS modify the nephrotoxicity of the fungicide.

Histological examination of kidneys from multiple treated (0.2 or 0.4 mmol/kg NDPS) and acutely treated (0.4 or 0.8 mmol/kg NDPS) rats revealed marked alterations in proximal tubules consistent with previous findings in NDPS-treated tissues [11]. However, histological examination of kidneys following multiple exposure to NDPS also demonstrated reduced morphological damage. In particular, there seemed to be less apical cytoplasmic tubular sloughing with most tubules free of luminal occlusions.

Although the mechanism of NDPS nephrotoxicity is not totally understood, pretreatment with the enzyme inducers phenobarbital and 3-methylcholanthrene (3-MC) modulates NDPS nephrotoxicity [12]. Phenobarbital pretreatment increases NDPS nephrotoxicity, whereas 3-MC pretreatment actually reduces the severity of NDPS-induced renal damage. These data suggest that renal

Table 1. Body weight, BUN levels, and kidney weight following a 3-day NDPS treatment

	Body weight (g) 0 hr	Body weight (g) 72 hr	BUN (mg/100 ml) 0 hr	BUN (mg/100 ml) 72 hr	Kidney weight (g/100 g body wt)
NDPS (0.2 mmol/kg)	325 ± 3.75	312 ± 2.50*	23.6 ± 0.33	37.9 ± 8.4	0.45 ± 0.03†
Control	319 ± 9.47	320 ± 8.89	23.7 ± 0.16	24.5 ± 1.47	0.38 ± 0.01
NDPS (0.4 mmol/kg)	262 ± 2.07†	224 ± 2.33*	23.6 ± 0.34	59.5 ± 20.1†	0.52 ± 0.02†
Control	250 ± 3.36	221 ± 2.81	23.2 ± 0.37	19.7 ± 0.45	0.37 ± 0.01

Values are expressed as mean ± SEM, N = 4.
* Statistically different from 0 hr ($P < 0.05$).
† Statistically different from control ($P < 0.05$).

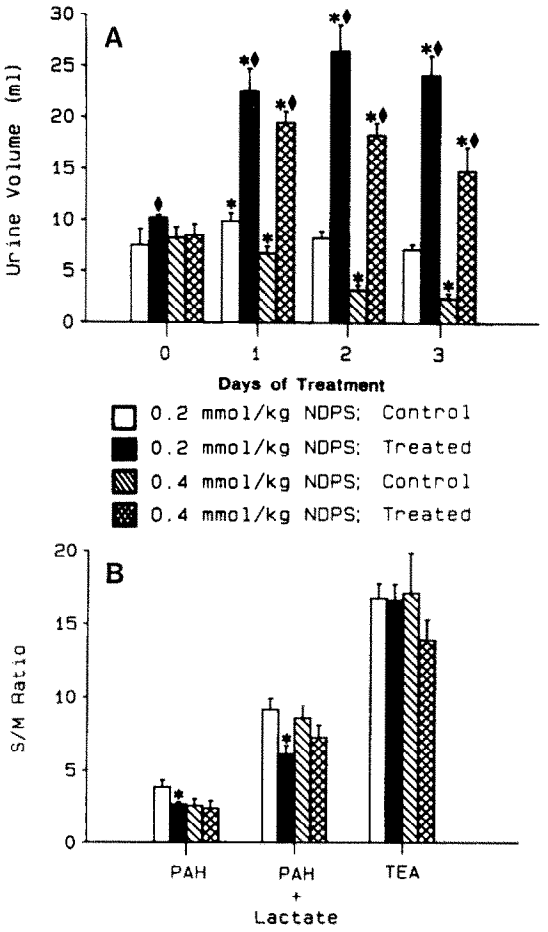

Fig. 2. Effect of NDPS (treated) or vehicle (control) administration for 3 days on urine volume (A) as well as PAH and TEA accumulation by renal cortical slices (B) in Fischer 344 rats. Panel A: Values are mean ± SEM; an asterisk (*) indicates significantly different ($P < 0.05$) from respective day 0. A diamond (◆) indicates that the treated group was significantly different ($P < 0.05$) from the appropriate pair-fed controls. Panel B: Kidneys used in this study were obtained at 72 hr after initiating treatments. Data are expressed as the mean ± SEM for $N \geq 4$. An asterisk (*) indicates $P < 0.05$ compared to pair-fed control animals.

damage produced by NDPS exposure may be due to a cytochrome P-450-mediated metabolite generated by the liver since phenobarbital pretreatment only induces hepatic microsomal enzymes in rats whereas 3-MC induces hepatic and renal enzymes [13]. Additionally, NDPS nephrotoxicity can be prevented by pretreatment with piperonyl butoxide or cobaltous chloride, two agents that inhibit cytochrome P-450 enzymes. Thus, inhibition of NDPS microsomal enzyme biotransformation may block the formation of some nephrotoxic metabolites of NDPS.

Repeated exposure to NDPS at levels of 0.2 mmol/kg or greater induces hepatic microsomal cytochrome P-450 and may have a very important role in modulating NDPS nephrotoxicity. Alteration of NDPS nephrotoxicity by phenobarbital or NDPS pretreatment also suggests that nephrotoxic metabolites are initiated by the hepatic microsomal enzymes. Further investigations will quantitate the ability of NDPS pretreatment to modulate the profiles of NDPS metabolites.

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