Biochemical Pharmacology, Vol. 37, No. 13, pp. 2529–2535, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

INDUCTION OF XENOBIOTIC BIOTRANSFORMATION BY THE INSECTICIDE CHLORDIMEFORM, A METABOLITE 4-CHLORO-*O*-TOLUIDINE AND A STRUCTURALLY RELATED CHEMICAL *O*-TOLUIDINE

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(*Received* 12 *November* 1987; *accepted* 7 *January* 1988)

Abstract—Chlordimeform, 4-chloro-*o*-toluidine and *o*-toluidine have all been found to have carcinogenic properties. Due to an empirical link between such properties and alteration of some biotransformation enzymes, the abilities of these three chemicals to affect cytochrome P-450 mediated biotransformation, epoxide hydrolase and glutathione *S*-transferase have been examined. Chlordimeform had no effect on the cytochrome P-450 content, aniline *p*-hydroxylase or glutathione *S*-transferase activities, but induced ethoxyresorufin-*O*-deethylase, ethoxycoumarin-*O*-deethylase and epoxide hydrolase activities and decreased aldrin epoxidase and aminopyrine *N*-demethylase activities. The metabolite 4-chloro-*o*-toluidine increased cytochrome P-450, ethoxyresorufin-*O*- deethylase, ethoxycoumarin-*O*-deethylase, glutathione *S*-transferase and epoxide hydrolase activities. *o*-Toluidine induced cytochrome P-450, ethoxyresorufin-*O*-deethylase, ethoxycoumarin-*O*-deethylase, and aldrin epoxidase activities. Ethoxyresorufin-*O*-deethylase activity was induced approximately eight times by chlordimeform and 18 times by 4-chloro-*o*-toluidine and *o*-toluidine. Induction was seen at 50 mg/kg with chlordimeform and at 10 mg/kg with the other treatments. Chlordimeform increased the 7α and 16α androstenedione hydroxylase pathways. 4-Chloro-*o*-toluidine increased the 7α, 16β and 16α hydroxylase pathways, while *o*-toluidine increased the 7α, 6β, 16β and 16α hydroxylase pathways. All three chemicals marginally decreased the testosterone pathways. SDS-PAGE of rat microsomes revealed an increase in a protein band of MW c54,000 for the chlordimeform and 4-chloro-*o*-toluidine treated groups. Taken together with the increase in ethoxyresorufin-*O*-deethylase activity these observations are consistent with the induction of hepatic isozyme P-450d. Thus each chemical has been shown to induce various pathways of biotransformation with increases in the P-450c and P-450d specific substrate ethoxyresorufin-*O*-deethylase being a consistent finding.

Chlordimeform, *N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethyl formamidine, is an insecticide which is used particularly in the cotton industry. It has a role in prevention of the development of resistance to other insecticides. Recent studies have shown that, even with careful handling and protective equipment, workers still have significant exposure [1, 2]. Chlordimeform has been found to be carcinogenic in mice [3, 4]. One of its principal metabolites, 4-chloro-*o*-toluidine, has been found to be a more potent carcinogen than the parent compound in mice [3, 4] and to be associated with bladder tumours in man [5], while *o*-toluidine, a structurally related chemical, is carcinogenic in rats and mice [6]. Furthermore, human cancer has been linked with exposure to this chemical [6, 7]. It is of interest that one group of authors has suggested an empirical link between carcinogenicity of a compound and its ability to induce cytochrome P-450 mono-oxygenases in a specific manner [8–10]. Mammalian hepatic cytochrome P-450 is known to contain multiple isozymes which are subject to regulation by factors including steroid hormones and exposure to xenobiotics [11]. Studies [12, 13] have shown that these isozymes can be partly distinguished by substrate specificity. Many cytochrome P-450 isozymes exhibit a high degree of regio- and stereo selectivity as evidenced by the positional hydroxylation of the C19 steroid androst-4-ene-3,17-dione [13].

The following study was carried out to examine the relationship between chlordimeform, 4-chloro-*o*-toluidine and *o*-toluidine, and their capacity to affect xenobiotic biotransformation in the rat.

MATERIALS AND METHODS

Chemicals. Technical grade chlordimeform (CDF) (81.8%) CGS 800 SP, was obtained from Schering, Australia. Ethoxycoumarin, 7-hydroxycoumarin, resorufin, 1,2-dichloro-4-nitrobenzene (DCNB), 4-chloro-*o*-toluidine (5-chloro-2-aminotoluene) (5-CAT) (97%) and *o*-toluidine (O-TOL) (99%) were purchased from Aldrich Chemical Company (Milwaukee, WI); phenobarbitone (PB) from BDH (Poole, U.K.); 3-methylcholanthrene (3-MC), unlabelled androst-4-ene-3,17,dione (androstenedione), 6β-hydroxy- and 16α-hydroxyandrostenedione from Sigma Chemical Company (St Louis, MO); 7α-hydroxyandrostenedione was obtained from Professor D. N. Kirk and the MRC Steroid Reference Collection, Queen Mary's College, London, U.K.; isosafrole (ISF) was pur-

chased from Eastman Kodak Company (Rochester, NY) and redistilled prior to use; aldrin and dieldrin from Shell (Aust) Ltd; ethoxyresorufin from Pierce Chemical Company (Rockford, IL); and ^{14}C-styrene oxide and [4-^{14}C]-androstenedione from Amersham (Sydney). Chemicals for electrophoresis were obtained from Bio Rad (Sydney, N.S.W.). The preparation of 16β hydroxyandrostenedione was performed as described by Murray *et al.* [14]. All other chemicals used were of the highest quality commercially available.

Animals. Male Sprague–Dawley rats (240–400 g) were allowed food and water *ad libitum* until sacrifice. Rats received CDF (1, 10, 50 or 100 mg/kg) dissolved in saline or 5-CAT (10 or 100 mg/kg) or O-TOL (10 or 100 mg/kg) dissolved in corn oil via an intraperitoneal (i.p.) injection for 7 consecutive days. For the electrophoresis experiments rats received 3-MC (20 mg/kg) or ISF (150 mg/kg) in corn oil i.p. once daily for 3 consecutive days. All controls received 1 ml/kg of the vehicle. The animals were sacrificed 24 hr after the final dose.

Preparation of microsomes. Livers were perfused with ice-cold buffer (0.02 M Tris, 1.15% KCl, 3 mM EDTA, pH 7.6 at 0–4°) and then homogenized in 3 vol. of buffer w/v. The homogenate was centrifuged at 10,000 *g* for 15 min and the resultant supernatant centrifuged again at 105,000 *g* for 1 hr to obtain the microsomal pellet. Aliquots of the supernatant were taken and stored for glutathione *S*-transferase assays. The microsomal pellet was separated from the glycogen pellet, rinsed and then resuspended in storage buffer to a protein concentration of 20–50 mg/ml. The storage buffer was made up of the perfusion buffer at pH 7.4 with the addition of 20% glycerol. The cytosolic and microsomal samples were stored at −80° until required.

Protein and enzyme assays. Protein content was determined by the method of Lowry as modified by Chaykin [15], using bovine serum albumin as the standard. Cytochrome P-450 content was determined by the method of Omura and Sato [16] using a buffer containing 0.2 M potassium phosphate. 1 mM EDTA and 20% glycerol (pH 7.5). Ethoxyresorufin and ethoxycoumarin-*O*-deethylation were determined by spectrofluorimetric quantification of the product at 37° according to the method of Prough *et al.* [17]. Aldrin epoxidation was determined according to the procedure of Wolff *et al.* [18], with modifications. The 5 ml incubations contained 0.5 mg microsomal protein, cofactors, 274 nmol aldrin in 0.1 ml methyl cellosolve and buffer (pH 7.6 at 25°). Incubation time was 10 minutes at 37°. Aminopyrine *N*-demethylation was determined following the method of Mazel [19]. Aniline-*p*-hydroxylation was measured by the method of Mazel (19) with minor modifications. The reaction mixture contained 5 mM aniline, 3–4 mg microsomal protein, cofactors and buffer (pH 7.6) at 25° in a total volume of 4 ml. Glutathione *S*-transferase activity was determined by following the method of Kulkarni *et al.* [20] using 1,2-dichloro-4-nitrobenzene (DCNB) as the substrate. Epoxide hydrolase was determined by the method of Guengerich [21] using ^{14}C-styrene oxide as the substrate. Incubations contained 0.18 mg microsomal protein and 55,000 dpm of ^{14}C-styrene oxide. Androstenedione hydroxylase activity was determined as described [14] following the method of Gustaffson and Ingelman-Sundberg [22].

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was performed on 1 mm-thick 7.5% gels prepared according to the procedure of Laemmli [23]. Microsomes were incubated with 4% SDS and 5% 2-mercaptoethanol following Toftgard *et al.* [24]. Samples were boiled for 5–10 min. The gels were calibrated by electrophoresis of phosphorylase B (MW 92,500), bovine serum albumin (MW 66,200), ovalbumin (MW 45,000) and carbonic anhydrase (MW 31,000). Molecular weights of sample protein were determined by comparison of mobilities with the standards using a Hoefer 3000 Scanning Densitometer, interfaced with an Apple IIe computer.

Statistics. All results were evaluated by an analysis of variance and compared using the least significant difference. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of treatments on microsomal monooxygenase activities in rats

The effects of CDF, 5-CAT and O-TOL on xenobiotic biotransformation parameters are shown in Tables 1, 2 and 3 respectively. Only 5-CAT and O-TOL induced cytochrome P-450 content. All three chemicals induced ethoxyresorufin-*O*-deethylase and ethoxycoumarin-*O*-deethylase activities, the former showing the greater fold increase. 5-CAT and O-TOL induced ethoxyresorufin-*O*-deethylase and ethoxycoumarin-*O*-deethylase activities at a dose of 10 mg/kg while induction associated with CDF treatment was only evident at 50 mg/kg. At the 100 mg/kg dose, CDF induced ethoxyresorufin-*O*-deethylase activity 8-fold while 5-CAT and O-TOL caused an approximate 18-fold increase. CDF decreased aldrin epoxidation when administered to rats at a dose of 50 mg/kg, a finding in contrast to O-TOL which increased aldrin epoxidation at the same dose-level. Administration of 5-CAT was not associated with a change in aldrin epoxidation. Neither O-TOL nor 5-CAT (10 or 100 mg/kg) affected aminopyrine *N*-demethylation. In contrast, exposure of male rats to CDF resulted in a dose related decrease in aminopyrine *N*-demethylation. Aniline-*p*-hydroxylation was unchanged by any treatment.

Since individual cytochromes P-450 catalyze the position-specific hydroxylation of C19 steroids, the formation of hydroxyandrostenediones was measured in microsomes from rats treated with CDF, 5-CAT and O-TOL. As shown in Table 4, androstenedione 16α hydroxylase (16αOH) activity was reduced 2.3- and 1.6-fold in hepatic microsomes isolated from male rats treated with CDF and 5-CAT respectively. In contrast, treatment with O-TOL was associated with a 1.6-fold increase in 16αOH activity. The rate of microsomal androstenedione 6β-hydroxylation was increased by administration of O-TOL causing an approximate 1–7-fold induction. In contrast, administration of CDF and 5-CAT to male rats did not affect 6β hydroxylation. The adminis-

Table 1. Dose-dependent effects of chlordimeform on hepatic cytochrome P-450 monooxygenase activities and related enzymes

Enzyme parameter	Dose				
	Control*	1 mg/kg	10 mg/kg	50 mg/kg	100 mg/kg
Cytochrome P-450 (nmol/mg protein)	0.72 ± 0.02	0.71 ± 0.03	0.67 ± 0.04	0.64 ± 0.03 ABC	0.75 ± 0.04 ABCD
Ethoxyresorufin-*O*-deethylase (pmol/min/mg protein)	74 ± 3	71 ± 6	72 ± 4	447 ± 94 ABC	633 ± 64 ABCD
Ethoxycoumarin-*O*-deethylase (pmol/min/mg protein)	297 ± 19	296 ± 14	276 ± 27	491 ± 45 ABC	816 ± 69 ABC
Aldrin epoxidase (nmol/min/mg protein)	2.79 ± 0.13	2.62 ± 0.23	2.58 ± 0.24	1.34 ± 0.23	1.66 ± 0.16
Aniline hydroxylase (nmol/min/mg protein)	0.24 ± 0.01	0.18 ± 0.02	0.20 ± 0.02 AB	0.22 ± 0.02 AB	0.23 ± 0.02 AB
Aminopyrine *N*-demethylase (nmol/min/mg protein)	4.44 ± 0.26	4.13 ± 0.17	3.72 ± 0.19	3.45 ± 0.20 B	3.49 ± 0.22 ABC
Epoxide hydrolase (nmol/min/mg protein)	13.0 ± 0.3	11.7 ± 0.5	13.1 ± 0.5	16.7 ± 0.5	20.4 ± 0.7
Glutathione *S*-transferase (nmol/min/mg protein)	215.0 ± 18.3	ND	ND	235.0 ± 24.3	276.4 ± 15.6

Data are presented as means ± SEM of 6 rats.
Rats were dosed daily (i.p.) for 1 week.
* Control is 0.9% saline.
A Statistically different from controls.
B Statistically different from 1 mg/kg group, $P < 0.05$.
C Statistically different from 10 mg/kg group, $P < 0.05$.
D Statistically different from 50 mg/kg group, $P < 0.05$.
ND Not determined.

tration of both 5-CAT and O-TOL was associated with increases in 16β hydroxylation. Microsomes isolated from the livers of rats treated with O-TOL and 5-CAT catalysed microsomal 16β hydroxylation rates at 3- and 1.9-fold of control, respectively. CDF treatment was not associated with changes in androstenedione 16β hydroxylase (16βOH) activity. All three treatments induced androstenedione 7α hydroxylase (7αOH) activity 1.5-fold, while each caused a small decrease in testosterone formation via a ketosteroid 17β- oxidoreductase, a microsomal P-450 independent enzyme.

Table 2. Dose-dependent effects of 4-chloro-*O*-toluidine on hepatic cytochrome P-450 monooxygenase activities and related enzymes

Enzyme parameter	Dose		
	Control*	10 mg/kg	100 mg/kg
Cytochrome P-450 (nmol/mg protein)	0.81 ± 0.03	0.80 ± 0.03	1.15 ± 0.6 AB
Ethoxyresorufin-*O*-deethylase (pmol/min/mg protein)	90 ± 5.0	260 ± 30 A	1590 ± 100 AB
Ethoxycoumarin-*O*-deethylase (pmol/min/mg protein)	393 ± 19	581 ± 24 A	1961 ± 142 AB
Aldrin epoxidase (nmol/min/mg protein)	3.85 ± 0.30	3.81 ± 0.22	3.83 ± 0.26
Aniline hydroxylase (nmol/min/mg protein)	0.31 ± 0.02	0.29 ± 0.01	0.28 ± 0.01
Aminopyrine *N*-demethylase (nmol/min/mg protein)	9.74 ± 0.19	9.52 ± 0.47	10.63 ± 0.24
Epoxide hydrolase (nmol/min/mg protein)	24.4 ± 1.9	31.9 ± 4.0	46.9 ± 2.5 AB
Glutathione *S*-transferase (nmol/min/mg protein)	188 ± 14.9	223 ± 12.3	376 ± 33.7 AB

Data are presented as means ± SEM of 5 rats.
Rats were dosed daily (i.p.) for 1 week.
* Control is corn oil.
A Statistically different from controls, $P < 0.05$.
B Statistically different from 10 mg/kg group, $P < 0.05$.

Table 3. Dose-dependent effects of *O*-toluidine on hepatic cytochrome P-450 monooxygenase activities and related enzymes

Enzyme parameter	Dose		
	Control*	10 mg/kg	100 mg/kg
Cytochrome P-450 (nmol/mg protein)	0.68 ± 0.04	A 0.88 ± 0.04	A 0.92 ± 0.07
Ethoxyresorufin-*O*-deethylase (pmol/min/mg protein)	57 ± 7	A 103 ± 10	AB 1100 ± 97
Ethoxycoumarin-*O*-deethylase (pmol/min/mg protein)	420 ± 18	A 574 ± 62	AB 1852 ± 86
Aldrin epoxidase (nmol/min/mg protein)	2.95 ± 0.27	A 3.94 ± 0.35	A 4.05 ± 0.36
Aniline hydroxylase (nmol/min/mg protein)	0.27 ± 0.02	0.27 ± 0.02	0.25 ± 0.02
Aminopyrine *N*-demethylase (nmol/min/mg protein)	8.13 ± 0.45	9.23 ± 0.51	9.15 ± 0.37
Epoxide hydrolase (nmol/min/mg protein)	23.7 ± 2.4	31.6 ± 1.1	31.8 ± 2.1

Data are presented as means ± SEM of 5 rats.
Rats were dosed daily (i.p.) for 1 week.
* Control is corn oil.
A Statistically different from controls, $P < 0.05$.
B Statistically different from 10 mg/kg group, $P < 0.05$.

Effects of treatments on other hepatic enzymes

The three test compounds CDF, 5-CAT and O-TOL, were also investigated for their effects on glutathione *S*-transferase. 5-CAT (100 mg/kg) induced cytosolic glutathione *S*-transferase activity while CDF was not associated with changes in transferase activity. As only one substrate has been used to assay for this activity, it is possible that there has been a selective induction which is not evidenced in the present results. CDF at 50 mg/kg and 5-CAT at 10 mg/kg induced epoxide hydrolase activity but hepatic microsomes isolated from male rats treated with O-TOL showed no increases in activity of this enzyme at either dose level.

SDS-PAGE

Microsomes from CDF, 5-CAT and O-TOL treated rats were subjected to electrophoresis on polyacrylamide gels and the appearance of the cytochrome P-450 molecular weight regions were compared to controls, 3-MC and ISF treated animals (Figs 1 and 2). The densitometer scans suggest that CDF and 5-CAT induced a microsomal protein with MW approximately 54 kD. The apparent concentration of this protein band appears small in comparison to the concentration in microsomes from isofrole and 3-MC treated rats. Nevertheless, the densitometric analysis revealed small peaks for these two treatment groups with the highest concentration

Table 4. The effect of treatment by chlordimeform, 4-chloro-*O*-toluidine and *O*-toluidine on androstenedione hydroxylases and testosterone formation

Treatment	Hydroxylase activity—nmoles/min/mg protein				Testosterone formation (nmoles/min/mg protein)
	7αOHA*	6βOHA	16βOHA	16αOHA	
Control†	0.12 ± 0.01	0.16 ± 0.01	0.04 ± 0.01	0.44 ± 0.04	2.79 ± 0.08
Chlordimeform	A			A	A
100 mg/kg	0.19 ± 0.01	0.13 ± 0.02	0.04 ± 0.01	0.19 ± 0.03	2.15 ± 0.03
4-chloro-*o*-toluidine	A	B	AB	A	A
100 mg/kg	0.19 ± 0.01	0.18 ± 0.01	0.11 ± 0.01	0.26 ± 0.04	2.19 ± 0.06
O-toluidine	A	ABC	AB	ABC	A
100 mg/kg	0.18 ± 0.01	0.27 ± 0.02	0.12 ± 0.01	0.69 ± 0.09	2.34 ± 0.07

Data are presented as means ± SEM of 4 rats.
Rats were dosed daily (i.p.) for 1 week.
* 7αOHA = 7α-hydroxyandrost-4-ene-3,17 dione.
† Control is corn oil.
A Statistically different from control, $P < 0.05$.
B Statistically different from chlordimeform treatment, $P < 0.05$.
C Statistically different from 4-chloro-*o*-toluidine treatment, $P < 0.05$.

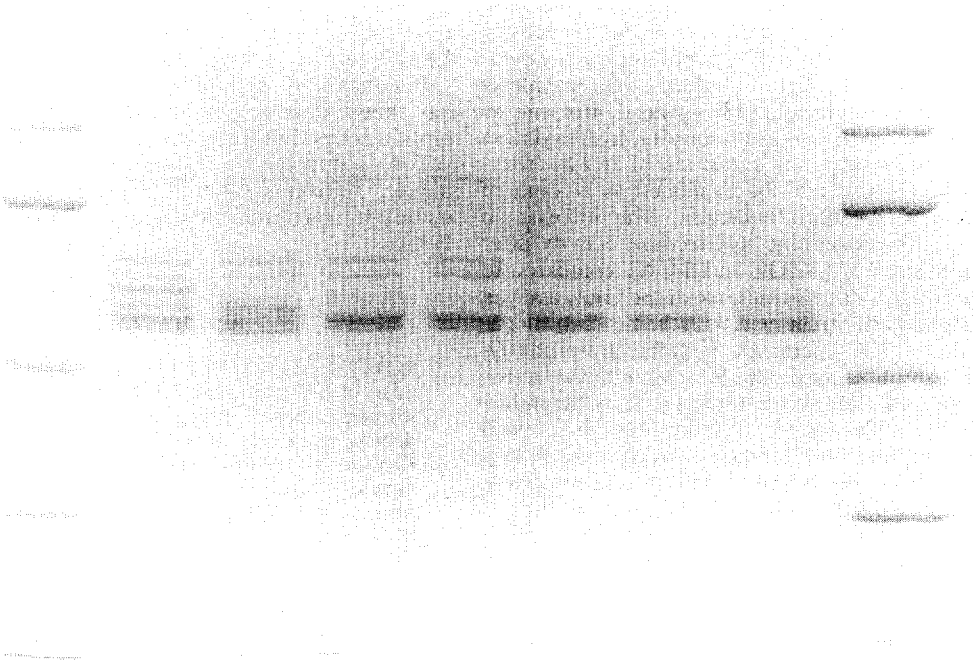

Fig. 1. Photograph of SDS-PAGE of microsomal proteins from male rats treated, from left to right, with 3-methylcholanthrene (20 mg/kg for 3 days); isosafrole (150 mg/kg for 3 days); corn oil control (2 wells) (1 ml/kg for 7 days); chlordimeform (100 mg/kg for 7 days); *o*-toluidine (100 mg/kg for 7 days) and 4-chloro-*o*-toluidine (100 mg/kg for 7 days). Standards were applied to the first and last wells. The anode is at the bottom of the photograph. Approximately 15 μg protein was loaded per sample.

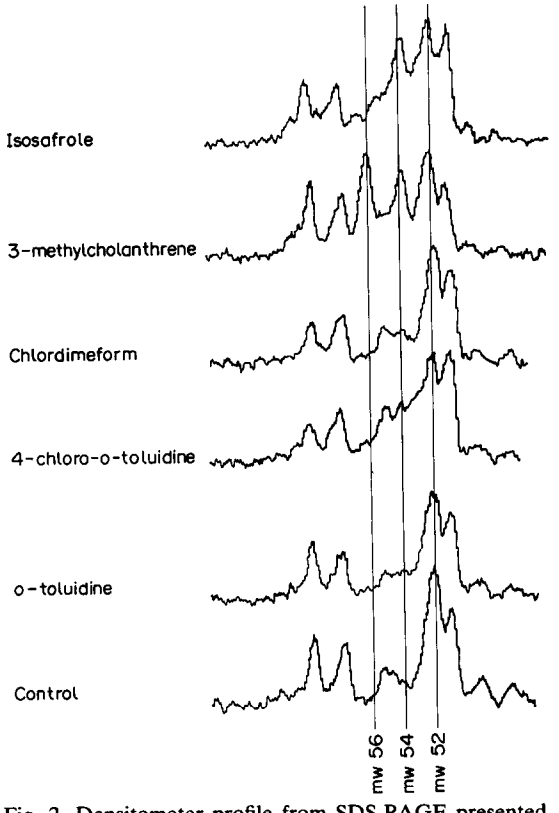

Fig. 2. Densitometer profile from SDS-PAGE presented in Fig. 1. The bottom of the gel is to the right of the photograph.

of this protein in 5-CAT induced microsomes (Fig. 2).

DISCUSSION

The results of this study indicate that administration of CDF, 5-CAT and O-TOL to male rats is associated with changes in the hepatic xenobiotic biotransformation system. This is indicated both by microsomal enzyme assays and SDS-PAGE. O-TOL and 5-CAT generally resulted in greater induction of xenobiotic biotransformation than CDF. A finding of particular interest was the induction of ethoxyresorufin-*O*-deethylase activity at a dose of 10 mg/kg by O-TOL and 5-CAT, whereas a CDF dose of 50 mg/kg was required before induction was observed. This is consistent with lower doses of 5-CAT than CDF being required to produce carcinomas. The relative activities of the various androstenedione hydroxylation pathways were assayed in our experiments in order to investigate further the P-450 isozymes affected by the induction regime. 16α-OH activity is associated with P-450$_{UT-A}$ (P-450h), a male specific constitutive enzyme [13]. The activity of 7α-OH is associated mainly with P-450a, androstenedione 6β hydroxylase (6β-OH) with P-450PCN-E and 16β-OH with P-450b/e [13]. The results presented in Table 4 therefore suggest that the administration of chlordimeform to male rats causes an increase in P-450a content and that 5-CAT increases both P-450a and P-450b/e. The data also suggest that O-TOL increases P-450a, P-450b/e and P-450PCN-E. The decrease in 16α-OH activity after

treatment with CDF and 5-CAT suggests a decrease in P-450h. An apparent induction of 16αOH activity after treatment with O-TOL is not necessarily cytochrome P-450h mediated. Indeed, this enzyme is apparently noninducible [13]. Instead, isozyme P-450b is also capable of catalysing 16α hydroxylation [13]. Thus the apparent induction of 16α hydroxylation by O-TOL may be explained as a result of cytochrome P-450b/e induction. The increase in overall androstenedione hydroxylase activity after administration of O-TOL and 5-CAT compared to CDF corresponds to an increase in spectrally assayed P-450 found after treatment with the first two chemicals only. The increases in ethoxyresorufin-*O*-deethylase and ethoxycoumarin-*O*-deethylase activities observed after all treatments suggest the induction of P-450c and/or P-450d [12]. It is well established that 3-MC induces cytochromes P-450c and P-450d. Cytochrome P-450c has a molecular weight of 56–58,000 while cytochrome P-450d has a molecular weight of 52–54,000 [11, 25]. ISF has been shown to induce primarily cytochrome P-450d [11]. Figures 1 and 2 show that hepatic microsomes isolated from 3-MC, ISF, CDF and 5-CAT treated male rats showed an increase in a protein staining band of molecular weight 54,000 when compared to controls. The increase observed for CDF treated animals was the least of those chemicals causing an induction.

The absence of an increase in overall P-450 content after treatment by CDF can be explained by the isozymic nature of P-450. It is possible that an increase in some isozyme(s) has been offset by a decrease in others. The decrease in aldrin epoxidation after treatment with CDF may be due to a decrease of the male specific constitutive isozyme P-450h as indicated by the 16αOH data. This is consistent with a previous study by Reidy *et al.* [26] where sex differences were observed in the response of aldrin epoxidase activity to picloram. The decrease in aminopyrine *N*-demethylation observed after CDF treatment may also be due to a decrease in cytochrome P-450h content as this enzyme has been shown to catalyse this reaction [12] and is probably a major contributor to this activity in microsomes from untreated rats.

The increase in aldrin epoxidation and 16αOH activity after O-TOL treatment may be due in part to a lack of effect of O-TOL on cytochrome P-450h compared with CDF and 5-CAT treatment. Thus, the apparent induction of cytochrome P-450b/e (as evidenced by the increase in 16βOH activity) after O-TOL treatment results in an increase of aldrin epoxidation and 16αOH activity, both of which have been shown to be catalysed by cytochrome P-450b/e [13, 18]. Alternatively, an induction of cytochrome P-450$_{PCN-E}$ as evidenced by an increase in 6βOHA may account for the elevation in aldrin epoxidation.

The concurrent elevation of ethoxyresorufin-*O*-deethylation and decrease in aldrin epoxidase activity characteristic of many carcinogens [8, 9] was observed after treatment with CDF, but not 5-CAT or O-TOL. Ioannides *et al.* [27], however, have suggested that the induction of ethoxyresorufin-*O*-deethylase activity alone (as found after 5-CAT and *O*-TOL) treatment, is sufficient to raise some suspicion regarding the carcinogenicity of a chemical. It is of interest that 5-CAT, which causes a carcinogenic response at doses lower than CDF also induces EROD activity at lower doses.

In conclusion, each of the three chemicals tested has been found capable of inducing various biotransformation pathways. A consistent observation among the three chemicals is an ability to induce ethoxyresorufin-*O*-deethylation, an enzymatic reaction specific to cytochrome P-450c and/or P-450d.

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