IN VIVO INDUCTION AND IN VITRO INHIBITION OF HEPATIC CYTOCHROME P450 ACTIVITY BY THE BENZODIAZEPINE ANTICONVULSANTS CLONAZEPAM AND DIAZEPAM

RAYMOND W. NIMS, RUSSELL A. PROUGH, COLLINS R. JONES, DIANA L. STOCKUS, KONSTANTIN H. DRAGNEV, PAUL E. THOMAS, AND RONALD A. LUBET

Chemistry Section, Laboratory of Comparative Carcinogenesis (R.W.N., K.H.D., R.A.L.) and Biological Carcinogenesis and Development Program, PRI/DynCorp (C.R.J., D.L.S.), National Cancer Institute; Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine (R.A.P.); and Department of Chemical Biology, College of Pharmacy, Rutgers University (P.E.T.).

(Received November 1, 1996; accepted February 10, 1997)

ABSTRACT:

The ability of the benzodiazepines, as a chemical class, to cause the induction and/or inhibition of cytochromes P450 has not been well characterized. In the present study, the induction of the cytochrome P450 2B subfamily (CYP2B) in vivo and the inhibition of CYP2B activity in vitro by selected benzodiazepines was examined in hepatic tissues derived from male F344/NCr rats. Initial studies of the in vivo induction or in vitro inhibition of benzyloxyresorufin O-dealkylation activity revealed that both clonazepam and diazepam were relatively effective in vivo inducers of CYP2B when administered in the diet at 500 ppm for 5 days and also were fairly potent inhibitors of the activity of these hemoproteins in vitro. Oxazepam, in contrast, was ineffective as an inducer or an inhibitor of this activity. Further studies were performed to characterize the subfamily selectivity of the P450 induction and inhibition displayed by clonazepam. Specifically, microsomes from rats treated with clonazepam (1000 or 1800 ppm in the diet for 5 days) were found to be highly induced with respect to catalytic activities mediated by CYP2B, including benzyloxyresorufin and pentoxyresorufin O-dealkylation or testosterone 16β-hydroxylation, but other CYP proteins were minimally induced. In addition to inducing the CYP2B subfamily, clonazepam also induced the RNA encoding other drug metabolizing enzymes (e.g., epoxide hydrolase and the glutathione S-transferase α-subfamily) that are typically induced by phenobarbital-type inducers. Finally, clonazepam proved to be a potent noncompetitive or “mixed-type” competitive inhibitor of catalytic activities mediated by CYP2B, but not by other CYP proteins (e.g., CYP2A, CYP3A) in microsomes derived from phenobarbital-pretreated rats.

Clonazepam (CZP) is a clinically important benzodiazepine anticonvulsant that is employed for the treatment of absence, akinetic, and myoclonic seizures in children (1). The benzodiazepines diazepam and oxazepam are used clinically as sedative-hypnotics and anxiolytics (1). Several of the chemical classes that possess sedative/anticonvulsant or anxiolytic activity (e.g., barbiturates, hydantoin and dialkylacetyleurases) also induce hepatic cytochrome P450 subfamilies 2B and 3A (CYP2B and CYP3A) in the rat (2–6) and mouse (7, 8). It has been reported that induction of CYP2B-mediated O-dealkylation activity is caused in mice by diazepam (9–11), and, to a greater extent, by CZP (10). R. W. Nims and R. A. Lubet, unpublished data, 1989). There have been few and conflicting reports on the ability of the benzodiazepines, as a class, to induce P450 activities in rats. Hoogland et al. (12) demonstrated that the induction of drug tolerance in chlordiazepoxide-treated male rats was a result of induction of cytochromes P450. Similarly, Orme et al. (13) observed a decrease in plasma half-life of pentobarbital, a decrease in zoxazolamine paralysis time, and induction of hepatic ethylmorphine N-demethylation activity in chlordiazepoxide-pretreated rats compared with controls. However, in this study (13), pretreatment of rats with diazepam did not cause a significant change in any of these parameters. In fact, Vorne and Iländpää-Kiikkilä (14) concluded that diazepam was an inhibitor of hexobarbital hydroxylation, while having no apparent effect on N-methylaniline demethylation or diazepam metabolism. Similarly, Kapetanovic and Kuperberg (15) found diazepam to be an inhibitor of rat hepatic microsomal phenytoin p-hydroxylation. On the other hand, Heubel et al. have reported that serum phenobarbital levels are reduced (16) and hexobarbital sleep times are decreased (17) in diazepam-pretreated rats compared with controls. These findings, taken together, suggest that the benzodiazepines potentially may act as inhibitors or inducers of P450 activity.

A variety of structurally diverse compounds induce the CYP2B subfamily in the rat, including phenobarbital (PB), clotrimazole (CLOT), α-hexachlorocyclohexane, diallyl sulfide, and certain of the polychlorinated biphenyls (e.g., 2,2′,4,4′,5,5′-hexachlorobiphenyl) (18–20). Interestingly, a significant number of the compounds that are inducers of the CYP2B subfamily are also striking inhibitors of CYP2B-mediated catalytic activity in vitro. Thus, animal pretreatment with many of the known in vitro CYP2B-inhibitors, including SKF 525-A (21), CLOT (20, 22), chlorpromazine (23) and metyrapone (22), results in induction of hepatic CYP2B protein and catalytic activity. In fact, it has been hypothesized that interaction with the P450 active site may play a mechanistic role in the induction of the CYP2B proteins (24).
In the present study, the CYP2B-inducing properties of CZP, diazepam and oxazepam in vivo in the rat, as well as the abilities of the three benzodiazepines to inhibit rat hepatic CYP2B activity in vitro were examined, with benzylxoyresorufin (BZR) O-dealkylation as the endpoint. In addition, the potency and selectivity of CZP as an inhibitor of CYP-mediated catalytic activities (BZR O-dealkylation (CYP2B), testosterone 16β-hydroxylation (CYP2B), testosterone 6β-hydroxylation (CYP3A), and testosterone 7α-hydroxylation (CYP2A)) were examined. Spectral studies were performed to determine the ability of CZP to form a binary enzyme-ligand complex with P450, to form a metabolite complex with P450, or to inhibit the formation of a metabolite complex between benzphetamine and P450. Finally, the ability of CZP to induce in rat liver certain genes that constitute the pleiotropic response to structurally diverse PB-type inducers (18) was determined.

The results of these studies suggest that CZP and diazepam each have CYP2B-inducing properties in vivo in the rat, as well as the ability to preferentially inhibit certain rat hepatic CYP2B-mediated catalytic activities in vitro.

Materials and Methods

Chemicals. Oxazepam, CLOT, and PB were purchased from Sigma Chemical Co. (St. Louis, MO). Benzylxoyresorufin, pentoxyresorufin (PTR), methoxyresorufin (MTR), and ethoxyresorufin (ETR) were obtained from Molecular Probes, Inc. (Eugene, OR). Diazepam and CZP were from Roche Diagnostics (Nutley, NJ), while dicumarol and resorufin were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Animals and treatments. Male F344/Ncr rats were obtained from the Animal Production Area of the Frederick Cancer Research and Development Center, Frederick, MD. Animals were housed in polycarbonate cages on hardwood chip bedding and were given food (Purina Lab Chow #5010, St. Louis, MO) and water ad libitum. At 6 to 8 weeks of age, the rats were placed on normal diet or diet containing one of the benzodiazepines or PB at the indicated concentrations for a period of 5 days. The adulterated diets were prepared by combining the appropriate amount of neat xenobiotic with a pre-weighted amount of powdered diet in a V-blender.

Preparation of tissue samples and catalytic assays. At the end of treatment, each animal was killed by CO₂ asphyxiation and the entire liver was carefully removed from each, trimmed free of extraneous tissue, and homogenized in 0.15 M potassium chloride/0.2 M sucrose (3 ml/g wet weight). Post-mitochondrial (S9) supernatants and microsomes were obtained by sequential 9,000g and 105,000g centrifugation steps. Protein content in the S9 or microsomal samples was measured using fluorescamine (25) with bovine serum albumin as the standard. The O-dealkylation of alkoxresorufins by hepatic S9 subfractions was measured with a modification (26) of the assay developed originally by Burke and Mayer (27). The final substrate concentration used was 5.0 μM, the S9 concentrations used were between 100 and 1000 μg/ml, and reaction rates were determined during the linear portion of the reaction. In vitro inhibition studies were performed by adding various amounts of the benzodiazepines (in dimethylsulfoxide) to achieve the indicated concentrations (final dimethylsulfoxide concentration in the reaction mixtures = 1% v/v). The benzodiazepines were added simultaneously with the substrates and were not preincubated with the enzyme (S9). Reaction rates were determined as described above.

Testosterone hydroxylation assays. Analysis of testosterone metabolism in rat microsomes was performed with the HPLC methodologies that have been described previously (18, 28). In vitro inhibition studies with CZP were performed as described above. A single concentration of testosterone (250 μM) was employed throughout these studies.

Isolation of total cellular RNA. Liver tissue from individual rats was minced and homogenized in guanidine isothiocyanate/mercaptoethanol and total cellular RNA was isolated by centrifugation through cesium chloride as described previously (29).

RNA slot-blot analysis. Total cellular RNA from at least 4 individual chemically treated or control animals was pooled and loaded at 10, 3.3, and 1.1 μg onto supported nitrocellulose membranes. Hybridization was performed as described previously (18, 29, 30). The probe for CYP2B1 is a oligonucleotide sequence (5’-d(GGT TGG TAG CCG GTG TGA)-3’) initially described by Omiecinski et al. (31). The probes for the glutathione transferase a-subfamily as well as for microsomal epoxide hydrolase were kindly provided by Dr. C. Pickett (32, 33). The intensities of the resulting slots were measured by laser densitometry (LKB Ultrascan XL). For determination of RNA loading, the membranes were hybridized to a 0.6-kb fragment of β-actin cDNA (Lofstrand Labs, Ltd., Gaithersburg, MD). In cases where apparent differences in loading were observed, the densitometry results were corrected for the β-actin signal.

Immunodetection of CYP2B1 and CYP3A2 with monoclonal antibodies. Methodologies for the detection of CYP2B in hepatic microsomes with monoclonal antibodies to rat CYP proteins have been described previously (29, 30). The specific antibodies employed were B50 (CYP2B1) (34) and L181 (CYP3A2) (35). An LKB Ultrascan XL laser densitometer was used to scan the resulting blots, and band intensities were compared between the various control and treated groups.

Formation of metabolite complexes with CYP2B. Liver microsomes (1 mg/ml) in 0.1 M Tris-HCL buffer, pH 7.4, containing 1 mM EDTA and 150 mM KCl were placed in matched cuvettes and a baseline of equal light absorbance obtained with an Aminco DW2 spectrophotometer at 37°C. Benzphetamine (50 μM) or CZP (1 mM) was added to the sample cuvette and an equal volume of buffer added to the reference cuvette. The difference spectra of a P450-ligand complex were recorded from 360 to 520 nm. To measure the formation of potential metabolite complexes, 200 μM benzphetamine or CZP were added and a baseline of equal light absorbance obtained. The reaction was initiated by adding 0.5 mM NADPH and after 4.5 min, the spectra were recorded. No unique spectral intermediates were formed in the presence of low (50 μM), intermediate (200 μM) or high (1 mM) concentrations of CZP (data not shown). To measure the effect of CZP on formation of the benzphetamine-metabolite complex (36, 37), benzphetamine (200 μM) was added to the microsomal reaction mixture, and after addition of 0.5 mM NADPH, metabolite complex formation was measured at A454nm - A490nm, as a function of time in the presence of 1 mM CZP.

Statistical analyses. O-Dealkylation data were subjected to ANOVA. Where variances between treatment groups were homogeneous, pairwise comparisons to determine statistically significant differences were performed using Student’s t test. Multiple comparisons were performed with the Dunnett’s t test. Where variances were nonhomogeneous, pairwise comparisons were performed using the Mann-Whitney U test.

Results

The induction in male rats of CYP2B-mediated catalytic activities by a single dietary concentration (500 ppm, ~40 mg/kg body weight/day) of CZP, diazepam, oxazepam, or PB is displayed in table 1 (experiment 1). This concentration of PB has been shown previously to induce maximally CYP2B activity in male rats (38). The results demonstrate that while oxazepam failed to induce CYP2B activity, both CZP and diazepam (500 ppm) were effective inducers, resulting in a level of induction which was 30–37% of that obtained with the same dietary concentration of PB. In addition, the ability of various concentrations of the individual benzodiazepines to inhibit in vitro metabolism of a single concentration (~0.33 μM) of benzylxoyresorufin was determined (fig. 1). Oxazepam caused limited inhibition at the concentrations investigated. Diazepam was a moderately potent inhibitor (IC₅₀ = 110 μM), while CZP was the most potent inhibitor (IC₅₀ = 35 μM). Oxazepam was not fully soluble in the reaction mixture, as judged by visual inspection, at concentrations >250 μM.

The in vivo inducing and in vitro inhibitory effects of CZP in rats were examined in greater detail in further experiments. The subfamily selectivity of the CYP induction was assessed with several catalytic assays which are relatively selective probes for various CYP isoforms or subfamilies [MTR: CYP1A2 (39, 40), ETR: CYP1A1 (27), PTR: CYP2B (40, 41); BZR: CYP2B (40, 41)] in the rat. As shown in table 1 (experiment 2), CZP caused a concentration-dependent induction of
The ability of CZP to induce testosterone hydroxylation at various positions was examined (table 2) in rats exposed to the indicated dietary concentrations of the agents for 5 days. A number of the stereospecific hydroxylations of testosterone have been shown to be catalyzed by specific CYP subfamilies (28). Thus, 16β-hydroxylation is mediated by CYP2B; 6β- and 2β-hydroxylation by CYP3A, and 7α-hydroxylation by CYP2A. Clonazepam pretreatment markedly enhanced the levels of testosterone 16β-hydroxylation but failed to increase levels of 6β-hydroxylation. In contrast, PB pretreatment induced the 2β, 16β- and 6β-hydroxylation of testosterone, demonstrating induction of both the CYP2B and CYP3A subfamilies by PB.

To confirm the induction of CYP2B and the lack of induction of CYP3A by CZP determined with the catalytic endpoints, the levels of CYP2B1 and CYP3A2 proteins were determined with monoclonal antibodies (fig. 2). Immunoreactive CYP2B1 protein was clearly induced by CZP (~5-fold), although this induction was substantially less than that elicited by PB (24-fold) or CLOT (17-fold). Clonazepam caused no induction of immunoreactive CYP3A2 protein in contrast with PB (~6-fold) or CLOT (~4-fold).

The ability of CZP to inhibit the metabolism of specific CYP substrates in vitro was determined. Clonazepam caused a concentration-dependent inhibition of BZR metabolism (CYP2B), with $K_i = 33–36 \mu M$ (fig. 3). The inhibition pattern displayed was that of either a noncompetitive inhibitor or a “mixed-type” competitive inhibitor/inhibitory action of CZP was a result of its ability to act as either a noncompetitive inhibitor or a “mixed-type” competitive inhibitor/

---

**TABLE 1**

**Induction of alkoxyresorufin O-dealkylation activities by benzodiazepines**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Methoxy-</th>
<th>Ethoxy-</th>
<th>Benzyloxy-</th>
<th>Pentoxy-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>27 ± 4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Clonazepam (500 ppm)</td>
<td>ND</td>
<td>ND</td>
<td>300 ± 21$^c$</td>
<td>150 ± 14$^c$</td>
</tr>
<tr>
<td>Oxazepam (500 ppm)</td>
<td>ND</td>
<td>ND</td>
<td>37 ± 6</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>Diazepam (500 ppm)</td>
<td>ND</td>
<td>ND</td>
<td>330 ± 16$^c$</td>
<td>170 ± 20$^c$</td>
</tr>
<tr>
<td>Phenobarbital (500 ppm)</td>
<td>ND</td>
<td>ND</td>
<td>1000 ± 41$^c$</td>
<td>480 ± 26$^c$</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19 ± 3</td>
<td>26 ± 4</td>
<td>24 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Clonazepam (1000 ppm)</td>
<td>33 ± 8$^c$</td>
<td>97 ± 14$^c$</td>
<td>540 ± 48$^c$</td>
<td>280 ± 31$^c$</td>
</tr>
<tr>
<td>Clonazepam (1800 ppm)</td>
<td>48 ± 8$^c$</td>
<td>100 ± 22$^c$</td>
<td>750 ± 77$^c$</td>
<td>390 ± 25$^c$</td>
</tr>
<tr>
<td>Phenobarbital (500 ppm)</td>
<td>72 ± 7$^c$</td>
<td>120 ± 11$^c$</td>
<td>980 ± 56$^c$</td>
<td>470 ± 36$^c$</td>
</tr>
</tbody>
</table>

---

$^a$ Three male F344/NCR rats were exposed to the indicated dietary concentrations of the agents for 5 days.

$^b$ ND, not determined.

$^c$ p < 0.05, significantly greater than the respective control group (Mann-Whitney U test).
substrate for CYP2B and not to the formation of an abortive complex with CYP2B.

The ability of CZP to induce certain non-P450 drug metabolizing enzymes that are typically induced by PB-type inducers was examined. As shown in fig. 6a-c, CZP induced RNA coding for CYP2B1 as well as RNA coding for the glutathione S-transferase alpha subfamily (Ya/Yc) and for microsomal epoxide hydrolase. In general, CZP was less effective than PB (500 ppm) at inducing the latter genes.

Discussion

Many known in vitro inhibitors of CYP proteins actually serve to induce those proteins when administered in vivo. Thus, clotrimazole and other imidazoles (18, 20, 22), SKF 525-A (21), chlorpromazine (23) and metyrapone (22), all of which are inhibitors of CYP2B and CYP3A in vitro, induce these proteins in vivo. Similarly, troleandomycin, a specific inhibitor of CYP3A, highly induces this protein in vivo (28). Finally, isosafrole, which forms a metabolic complex with CYP1A2, strongly induces this CYP (42). This relationship is not invariant, however, since 7,8-benzoflavone, a relatively specific inhibitor of the CYP1A subfamily, fails to induce these proteins (43). In fact, 7,8-benzoflavone seems to be not only a strong direct inhibitor of the CYP1A proteins, but is a potent antagonist for the Ah receptor, which normally mediates the induction of these specific cytochromes (43). This latter property may account in large part for its lack of inducing properties.

The induction of CYP2B by equivalent dietary concentrations of three benzodiazepines (diazepam, CZP, and oxazepam) as well as the prototype CYP2B inducer, PB was examined. The concentration of PB employed causes maximal induction of CYP2B activity (BZR O-dealkylation) in male F344 rats (38). Diazepam and CZP were effective inducers of CYP2B activity, causing at 500 ppm in the diet 25% to 35% of maximal induction. In contrast, oxazepam had mini-

TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testosterone Hydroxylase Activity (pmol/min/mg microsomal protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2β-</td>
</tr>
<tr>
<td>Control</td>
<td>356</td>
</tr>
<tr>
<td>Clonazepam (1000 ppm)</td>
<td>312</td>
</tr>
<tr>
<td>Clonazepam (1800 ppm)</td>
<td>342</td>
</tr>
<tr>
<td>Phenobarbital (500 ppm)</td>
<td>873</td>
</tr>
</tbody>
</table>

*a* Three male F344/NCr rats were exposed to the indicated dietary concentrations of the agents for 5 days.

*b* Activities were determined in microsomes pooled from the individual groups. Hydroxylation of testosterone at the indicated positions has been shown to be associated with specific CYP subfamilies (2β, CYP3A; 6β, CYP3A; 2α, CYP2A, 16β, CYP2B).

![Fig. 2.](image) Levels of immunoreactive CYP2B1 and CYP3A2 protein in hepatic microsomes from male F344/NCr rats exposed to control diet (CTL), or diet containing 1000 ppm clonazepam (CZP_Low), 1800 ppm clonazepam (CZP_High), 500 ppm phenobarbital (PB) or clotrimazole (CLOT).

![Fig. 3.](image) Double-reciprocal plot of the inhibition, by clonazepam (CZP), of benzyloxyresorufin O-dealkylation activity of S9 from phenobarbital-induced rats.

![Fig. 4.](image) Specificity associated with the inhibition, by various concentrations of clonazepam, of the hydroxylation of testosterone at the 7α- (CYP2A), 6β- (CYP3A), 2β- (CYP3A) or 16β- (CYP2B) positions.

The hydroxylation activity is expressed as percentage of activity remaining (uninhibited activities for phenobarbital-induced microsomes are given in table 2).
mal effects on the levels of this CYP2B activity. Whether these differences reflect specific structural requirements for an effective inducer of CYP2B or rather reflect primarily pharmacokinetic differences is not known. Interestingly, oxazepam at dietary concentrations lower than 2500 ppm is a relatively weak inducer of CYP2B in mice, as well (R. W. Nims, unpublished data, 1987, 1989).

Based on the observation that many in vitro inhibitors of CYP2B are inducers of this P450 subfamily in vivo and on previous reports (14, 15) suggesting that diazepam might be an inhibitor of P450 activity, the inhibitory properties of the benzodiazepines were examined. A single concentration of benzylxoyresorufin (0.33 μM) that was slightly below the \( k_m \) for this substrate (0.35 μM) was used since it was expected to prove useful when examining either competitive or noncompetitive inhibitors. Oxazepam proved to be relatively ineffective as an inhibitor at any of the concentrations employed. In contrast, both diazepam and clonazepam caused concentration-dependent inhibition of BZR O-dealkylation activity. Clonazepam was somewhat more potent as an inhibitor (IC\(_{50}\) = 35 μM) than was diazepam (IC\(_{50}\) = 110 μM). Interestingly, PB, which is by far the most effective CYP2B inducer, was a less potent inhibitor than clonazepam and diazepam (IC\(_{50}\) > 500 μM; R. A. Lubet, unpublished data, 1989).

Examination of the effects of CZP on in vivo induction of CYP activity indicated a concentration-dependent induction of CYP2B. In contrast, CZP had minimal effects on the levels of CYP1A1 or CYP1A2. The induction of CYP2B was confirmed by the increases in relative levels of testosterone 16β-hydroxylase as well as increases in the levels of immunoreactive CYP2B1 protein. Interestingly, CZP failed to induce CYP3A activity. However, even at the highest concentration of CZP, the resulting induction of CYP2B was submaximal, and it has been shown for inducers such as phenobarbital that the concentration required for half-maximal CYP2B induction seems to be lower than the concentration required for half-maximal CYP3A induction (30, 44).

In vitro inhibition studies showed that CZP caused concentration-dependent decreases in the rate of dealkylation of BZR. When plotted as a Lineweaver-Burk plot, the data seemed to be consistent with CZP functioning as either a noncompetitive or a “mixed-type” competitive
induced epoxide hydrolase and the glutathione S-transferase 
hydrolase, NAD) in a more limited number of strains of rats (18, 32, 33, 47, 
ety of other drug metabolizing enzymes, including epoxide hydrolase,

zoxazolamine (85 mg/kg body weight) was significantly 
greater 

in vivo 
inducer 
inhibition data to the ability of a compound to serve as an 
inducer in vivo. An interesting aspect of CZP is that it is also an 
also CYP2B inducer (EC50 

b 
hydroxylation (CYP2B), it 

b 
alkoxyresorufin 
O-dealkylates, epoxide hydrolase, and liver weight 
gain: correlation with liver tumor-promoting potential in a series of 

3. F. Heubel, F. Kehl and A. von Maxen: Is there a pleiotropic response in 

P-450 enzyme induction by 5-ethyl-5-phenylhydantoin and 5,5-dieth-

ylhydantoin, analogues of barbiturate tumor promoters phenobarbital 
and barbital, and promotion of liver and thyroid carcinogenesis initiated 

Tumor promoting activities of ethylenylactyleurea and diethylactyla-
turea, the ring hydrolysis products of barbiturate tumor promoters 
barbital and barbital, in rat liver and kidney initiated by N-

Enhancement of hepatocarcinogenesis and induction of specific cyto-

chromes P450-dependent monoxygenase activities by the barbiturates 
alloabarbital, apropabital, pentobarbital, secobarbital, and 5-phenyl- 

differences in susceptibility to liver carcinogenesis initiated by N-
nitrosodimethylamine and its promotion by phenobarbital in C57BL/ 
6Ncr, C3H/HeNMTV- and DBA/2Ncr mice. Carcinogenesis 7, 215– 
220 (1986).

promotion by an anticovulsant agent, phenytoin, in mouse liver: 
correlation with CYP2B induction. Carcinogenesis 14, 2227–2231 
(1993).

benzodiazepine tranquilizers, diazepam and oxazepam, in mouse liver. 

10. B. A. Diwan, R. A. Lubet, R. W. Nims, J. E. Klaunig, C. M. Weghoest, 
of clonazepam on the development of N-nitrosodimethylamine-initiated 
hepatocellular tumors in mice is correlated with its inability to inhibit 
cell-to-cell communication in mouse hepatocytes. Carcinogenesis 10, 

11. F. Heubel, T. Reuter, and E. Gerstner: Differences between induction 
effects of 1,4-bis[(3,5-dichlorophenyl)oxy]benzene and phenobarbi-

12. D. R. Hoogland, T. S. Miwa, and W. F. Bousquet: Metabolism and 
tolerance studies with chlorodiazepoxide-2-14C in the rat. Toxicol. Appl. 

13. M. Orme, A. Breckenridge, and R. V. Brooks: Interactions of benzodiazep-

14. M. Vorne and J. Idanpää-Heikkilä: Inhibition of drug metabolizing 
enzymes by diazepam in rat liver. Experientia 31, 962–963 (1975).

15. I. M. Kapetanovic and H. J. Kuperberg: Inhibition of microsomal phe-

nytoin metabolism by nafimidone and related imidazoles. Potency and 

16. F. Heubel and R. Frank: Zur induktiven Wirkung von Diazepam. Arzneim-


