Toxicity and Carcinogenicity Studies of Oxazepam in the Fischer 344 Rat

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Oxazepam and related benzodiazepines are used in the treatment of anxiety. Carcinogenicity studies of oxazepam were performed with the F344 rat because of marked differences in tumor responses observed in NTP studies with B6C3F1 and Swiss-Webster mice compared to the results of Sprague-Dawley rat studies submitted to the FDA by a manufacturer to support registration of the drug. Groups of 50 male and 50 female F344/N rats were fed diets containing 0, 625, 2500, or 5000 ppm oxazepam for up to 105 weeks. A stop-exposure group of 50 males and 50 females received 10,000 ppm oxazepam in diet for 26 weeks, after which animals received control diet. All 5000- and 10,000-ppm stop-exposure males died before the end of the study. Survival of 2500-ppm males and females was lower than that of controls. Body weight gains of 2500- and 5000-ppm males and females were less than those of controls. Male rats exposed to 2500 ppm had an increased incidence of renal tubule adenoma and hyperplasia. In addition, the incidences of renal tubule adenoma and hyperplasia were increased in the 10,000-ppm stop-exposure group. The incidences of nephropathy in exposed females were greater than in controls, and the severity of nephropathy increased in exposed males. Epithelial hyperplasia and chronic inflammation of the nonglandular stomach were increased in males given 2500 and 5000 ppm and the incidence of ulcers of the nonglandular stomach in 2500-ppm males was also greater than that in controls. In males exposed to 5000 ppm, mineralization of the glandular stomach and erosion of the duodenum were observed. In females exposed to 2500 ppm, the incidences of epithelial hyperplasia, chronic inflammation, and ulcers of the nonglandular stomach and the incidence of erosion in the glandular stomach were increased. The incidences of centrilobular hepatocyte hyperplasia in males and females given 2500 and 5000 ppm were greater than those in controls. In summary, there was equivocal evidence of carcinogenicity in males based on increased renal tubule adenomas in groups which also had significantly enhanced nephropathy. There was no evidence of carcinogenicity of oxazepam in females given a diet containing 625, 2500, or 5000 ppm for 2 years or 10,000 ppm for 6 months. © 1998 Society of Toxicology.

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Benzodiazepines are primarily used to treat anxiety (Hardman et al., 1996). Oxazepam is a short-acting agent given orally at doses of 10, 15, or 30 mg, three or four times per day (PDR, 1996). It was first produced by Wyeth Laboratories and has been marketed since 1965 under the trade name Serax.

Benzodiazepine use by the general population has been reported as 8% in the United Kingdom, 7% in the United States, and 8 to 10% in Norway (Pedersen and Lavik, 1991). In 1983, 2.6 million prescriptions for oxazepam were written in the United States (Anonymous, 1986). Oxazepam is also a common metabolite of several other benzodiazepines, some of which have been more widely used, including diazepam (Valium).

The toxicokinetics and metabolism of oxazepam in F344 rats and B6C3F1 and Swiss-Webster mice have been described by Yuan et al. (1994) and Griffin and Burka (1993, 1995). Oxazepam is fairly well absorbed by rodents after oral administration, with peak blood concentrations achieved within 2 to 3.5 h. Elimination from plasma is first order and best described by a two-compartment model with terminal elimination half-lives of 4 to 5 h for rats and 5 to 7 h for mice. The bioavailability of oxazepam given in the diet is not complete, and steady-state blood concentrations, reached after about 4 days on dosed feed, are not proportional to dose (Yuan et al., 1994).

The metabolism of oxazepam in rodents is more complex than that in humans (Griffin and Burka, 1995). In rodents, ring oxidation is a major pathway coincident with conjugation of the oxidation products or the parent with glucuronic acid or sulfate. A nonenzymatic condensation of the benzodiazepine ring also occurs. Not all metabolites have been identified. In rodents, metabolites are excreted primarily in feces and urine and appear in differing amounts depending on the dose and the time of collection. In contrast to the mouse, pretreatment of rats with oxazepam did not significantly alter metabolism or elimination profiles (Griffin and Burka, 1995). Pretreatment of mice tended to shift metabolites from feces to urine and increased excretion of glucuronide and unchanged drug, a pattern more resembling that of the human (Griffin and Burka, 1993; Griffin et al., 1995a).

Toxic effects in Swiss-Webster and B6C3F1 mice in 13-
14-week diet studies at concentrations from 625 to 10,000 ppm were marked liver weight increases and centrilobular hepatocellular hypertrophy (NTP, 1993). Fox and Lahcen (1974) observed liver neoplasms in oxazepam-treated Swiss-Webster mice in reproductive toxicity studies. In a National Toxicology Program (NTP) study, male and female Swiss-Webster mice were given diets containing 0, 2,500, or 5000 ppm oxazepam for 57 weeks. The study was terminated because of poor survival of exposed groups. Mice receiving oxazepam had high rates of hepatocellular neoplasms as well as exacerbated amyloidosis. In a similar study with B6C3F1 mice receiving 2500 or 5000 ppm, exposed mice had poor survival and high rates of hepatocellular neoplasms. Thyroid gland follicular cell adenomas were also increased in females (NTP, 1993; Bucher et al., 1994).

Unpublished carcinogenicity studies with Sprague-Dawley rats are cited in the Physician’s Desk Reference (1996) and report increases in benign thyroid follicular cell tumors, testicular interstitial cell tumors, and prostatic adenomas, although no experimental details are given. Because of the marked neoplastic responses found in the two mouse strains and indications of a less severe carcinogenic response in the Sprague-Dawley rat, the NTP performed additional 2-year studies of oxazepam using the F344/N rat.

Prechronic studies were not performed in rats because it was determined that the prior 13-week studies with mice gave very little information on which to base dose selection for the 2-year studies in that species. For this reason, five rather than the typical three exposure concentrations were chosen for the 2-year rat study. After 26 weeks, one exposure group was terminated for reasons of cost, and the highest exposure group was removed from dosed feed for the duration of the study. The range of doses selected was based on our prior experience with the two mouse strains and literature reports of chronic benzodiazepine studies in rats (de la Iglesia et al., 1981). The full results of this study have been reported in NTP Technical Report 468 (NTP, 1996) and form the basis of this paper.

**MATERIALS AND METHODS**

**Chemical.** Oxazepam was obtained from Roussel Corporation (Englewood Cliffs, NJ). Information on purity (>99%) stability, and dose preparation was previously reported (Bucher et al., 1994; NTP, 1996).

**Study design.** Groups of 50 male and 50 female F344/N rats (Taconic Farms, Germantown, NY) were fed diets containing 0, 625, 1250, 2500, 5000, or 10,000 ppm oxazepam for up to 105 weeks. Based on body weight gains and clinical observations, the 5000-ppm exposure concentration was determined during the course of the study to be the minimally toxic dose. The concentrations of 0, 625, 2500, and 5000 ppm were selected for the continuous-exposure study. After 26 weeks of exposure, rats in the 1250-ppm group were eliminated during the course of the study to be the minimally toxic dose. The concentrations of 0, 625, 2500, and 5000 ppm were selected for the continuous-exposure study. After 26 weeks of exposure, rats in the 1250-ppm group were eliminated because it was anticipated that this group would provide insufficient information to justify their cost. Rats in the 625-, 2500-, and 5000-ppm groups received dosed feed throughout the 2-year study. Dosing of rats in the 10,000-ppm group was stopped at 26 weeks because of poor body weight gain. These animals remained on a control diet until study termination.

Rats were quarantined for 2 weeks and were approximately 6 weeks old at the beginning of the study. Five male and five female rats were randomly selected for parasite evaluation and gross observation of disease at the end of the quarantine period. The health of the animals was monitored during the study according to the protocols of the NTP Sentinel Animal Program. There were no findings suggestive of infection during the study (NTP, 1996).

Rats were housed five per cage. Feed and water were available ad libitum. Feed consumption was measured for a 7-day interval during weeks 1 and 4 and every 4 weeks thereafter by cage. Cages were changed twice weekly, and racks were rotated every 2 weeks. Animals were observed twice daily. Clinical findings were recorded every 4 weeks and at study termination. During study week 105 (1 to 3 days prior to necropsy), blood samples were collected from the retroorbital sinus of 6 males and 6 females in the 625-, 2500-, and 5000-ppm groups at either 6:00 AM or 6:00 PM. On the days of necropsy, blood samples were similarly collected from up to 15 randomly selected females that had not been bled earlier. Plasma oxazepam concentrations were measured using an HPLC method.

A complete necropsy and microscopic examination were performed on all rats in the 0-, 625-, 2500-, and 5000-ppm groups. Histopathologic evaluation of rats in the 10,000-ppm group was limited to gross lesions, stomach (glandular and nonglandular), small intestine, kidney, thyroid gland, and liver. At necropsy, all organs and tissues were examined for grossly visible lesions. All major tissues were fixed and preserved in 10% neutral buffered Formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For extended evaluation of renal tubule proliferative lesions in male rats, kidneys were step-sectioned at 1-mm intervals to obtain a maximum of four additional sections per kidney. For all paired organs (i.e., adrenal gland, kidney, ovary) samples from each organ were examined. Tissues examined microscopically are listed in NTP (1996). The 2-year study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58).

**Statistical methods.** The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Statistical analyses for possible dose-related effects on survival used Cox’s (1972) method for testing dose-related trends. All reported p values for the survival analyses are two sided. The majority of neoplasms in these studies were considered incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. Further details can be found in Bucher et al. (1994) or NTP (1996).

**RESULTS**

**Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings**

Estimates of 2-year survival probabilities for male and female rats are shown in the Kaplan–Meier survival curves (Figs. 1 and 2). All 5000-ppm continuous-exposure and 10,000-ppm stop-exposure males died before the end of the study. Survival of 2500-ppm continuous-exposure males and females but not 5000-ppm females, was significantly lower than that of the controls.

The mean body weights of 2500- and 5000-ppm males and females were lower than those of the controls throughout the study (Fig. 3). The mean body weights of 10,000-ppm stop-exposure males were also generally lower than those of controls. The mean body weight of 10,000-ppm stop-exposure females was approximately 18% lower than that of the controls.
FIG. 1. Kaplan–Meier survival curves for males and females administered oxazepam in diet for 2 years.
FIG. 2. Kaplan–Meier survival curves for males and females in the 2-year stop-exposure diet study of oxazepam.
FIG. 3. Growth curves for males and females administered oxazepam in diet for 2 years.
sections (combined) were increased in the 2500- and 10,000-

incidences of renal tubule adenoma in the standard and step

the step section evaluation and combined incidences of stan-

plasia were identified. The incidences of proliferative lesions in

and numerous additional occurrences of renal epithelial hyper-

exposed to 2500 ppm suggested a possible compound-related

This neoplasm in 2-year NTP diet studies (Table 2). Initially, a

controls and was at the upper limit of the historical range for

concentrations of males and females were similar at each diet

Plasma oxazepam concentrations were measured at the end

Plasma oxazepam concentrations were measured at the end

Pathology and Statistical Analyses

After histopathologic examination of the standard kidney

sections, the incidence of renal tubule adenoma in male rats

exposed to 2500 ppm was slightly greater than that of the controls

and was at the upper limit of the historical range for

this neoplasm in 2-year NTP diet studies (Table 2). Initially, a

single section of each kidney was examined microscopically.

The increased incidences of renal tubule adenoma in male rats

exposed to 2500 ppm suggested a possible compound-related

effect; therefore, an extended step-section evaluation of kid-

neys was performed (male rats only) using the residual For-

malin-fixed kidneys. Additional rats with renal tubule adenoma

and numerous additional occurrences of renal epithelial hyper-

plasia were identified. The incidences of proliferative lesions in

the step section evaluation and combined incidences of stan-

dard and step sections in male rats are given in Table 2. The

incidences of renal tubule adenoma in the standard and step

sections (combined) were increased in the 2500- and 10,000-

ppm (stop-exposure) groups. The incidences of renal tubule

hyperplasia in standard and step sections (combined) in the

male rats exposed to 2500, 5000, or 10,000 ppm (stop-exposure)

were significantly greater than those in controls.

One adenoma in a control, 2500- and 5000-ppm-treated rat

was grossly visible at necropsy. Microscopically, approxi-

mately 50% of the adenomas in exposed rats were approxi-

mately 1 mm in diameter. All were discrete, well-circum-

scribed lesions, five or more tubule diameters in size, and were

distinguished from hyperplasia by having a more complex

structure. Most hyperplasias were generally minimal to mild

focal lesions consisting of tubules with diameters that were two
to three times larger than a normal tubule.

The incidences of nephropathy in exposed males were sim-
lar to those in controls (Table 2). However, there was a

concentration-related increase in the severity of nephropathy.
The incidences of nephropathy in exposed females were sig-
nificantly greater than those in controls; severity was increased

only in the 5000-ppm group (Table 2). Nephropathy in males

was generally mild in controls and 625-ppm groups and mod-
erate to marked in 2500-, 5000-, and 10,000-ppm groups.

Parathyroid gland hyperplasia and fibrous osteodystrophy of

the bone (secondary to renal lesions) were increased in exposed

males; the incidences of parathyroid gland hyperplasia (0 ppm,

3/39; 625 ppm, 6/41; 2500 ppm, 9/46; 5000 ppm, 16/40) and

fibrous osteodystrophy of the bone (0/50, 1/50, 6/50, 8/50)

occurred with positive trends.

In male rats, there were positive trends in the incidences of

epithelial hyperplasia and chronic inflammation of the non-
glandular stomach. The incidences of epithelial hyperplasia

and chronic inflammation of the nonglandular stomach of

males exposed to 2500 or 5000 ppm and the incidence of ulcers

in 2500-ppm males were significantly greater than those in

controls (Table 3). In 10,000-ppm stop-exposure males, min-
eralization of the glandular stomach was significantly in-

creased. In females given 2500 ppm, epithelial hyperplasia,

chronic inflammation, and ulcers in the nonglandular stomach

and the incidence of erosion of the glandular stomach were

greater than those in controls (Table 3).

In males and females, there was a positive trend in minimal
to mild centrilobular hepatocyte hypertrophy (Table 4), and

incidences in 2500- and 5000-ppm males and females were

greater than those in controls. Centrilobular hypertrophy was

characterized by enlargement of hepatocytes around central

veins. In females, the incidences of clear cell foci in the 2500-

and 5000-ppm groups were increased; however, the incidence

in females in the 10,000-ppm stop-exposure group was less

than that in controls. The incidences of basophilic foci in

2500-ppm males and females, 5000-ppm females, and 10,000-

ppm stop-exposure females were less than those in controls.

In females exposed to 2500 and 5000 ppm, the incidences of

fibroadenoma of the mammary gland were lower than those in

controls. The incidences of fibroadenoma; carcinoma; and fi-

broadenoma, adenoma, or carcinoma (combined) occurred

with negative trends (0 ppm 27/50, 625 ppm 23/50, 2500 ppm
FIG. 4. Growth curves for males and females in the 2-year stop-exposure diet study of oxazepam.
TABLE 1
Plasma Concentrations of Oxazepam in Rats in the 2-Year Diet Study of Oxazepam

<table>
<thead>
<tr>
<th>Parts per million</th>
<th>Male</th>
<th>625</th>
<th>2500</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>104 weeks</td>
<td>10</td>
<td>6</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104 weeks</td>
<td>25</td>
<td>19</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± standard errors, µg/ml.

* No values were collected because of complete mortality at this dose.

9/50, 5000 ppm 13/50). All incidences were within historical control ranges from NTP 2-year feed studies and were consistent with incidences seen in control groups of comparable body weight. The incidences of mammary gland neoplasms in stop-exposure females (23/50), which had regained body weight, were similar to those in controls.

There were negative trends in pituitary gland (pars distalis) adenoma in males (17/49, 12/50, 10/50, 2/48) and females (31/50, 28/50, 21/50, 12/50). In females exposed to 5000 ppm, the incidence of adenoma or carcinoma (combined) was also less than that in controls. There was a negative trend in the incidence of hyperplasia in male rats (9/49, 5/50, 3/50, 1/48).

DISCUSSION

Evaluation of the toxicity and carcinogenicity of oxazepam in F344/N rats was prompted by the finding of a marked hepatocellular neoplasm response in similar studies with B6C3F1 and Swiss-Webster mice (NTP, 1993; Bucher et al., 1994). Studies of the carcinogenicity of oxazepam in Sprague–Dawley rats were performed previously (PDR, 1996). However, given the widespread use of benzodiazepines and the fact that oxazepam is a common metabolite of several of the more widely used variants of this drug class, carcinogenesis studies in a second strain of rat were deemed necessary to examine more fully the possible strain differences in response.

Thirteen-week rat studies were not performed prior to the 2-year studies summarized in this report because relatively little useful information had been obtained from the 13-week studies conducted in preparation for the 2-year mouse studies. Instead, a five-dose chronic study was begun with doses selected based on information from the literature, in anticipation that sufficient information would be gained during the first 6 months of the 2-year study to determine which exposure groups would be allowed to proceed to study termination. Based on low body weight gains, dosing of male and female rats fed diets containing 10,000 ppm was discontinued at 26 weeks and the animals were given a control diet until the end of the study. Groups fed the diet containing 1250 ppm were terminated based on cost considerations, because it was predicted that relatively little additional information would be gained from these animals.

In retrospect, the decision to forego performance of 13-week studies was incorrect. The 5000- and 2500-ppm exposure concentrations selected for the continuous administration portion of the study were sufficiently high to result in substantial reductions in both body weight gains and survival of male rats. Survival was somewhat reduced in the 625-ppm male group, and mean body weights were also slightly lower (2–8%) in this group during most of the study. Based on body weight and survival data, an adequate chronic toxicity and carcinogenicity study in male rats could have been performed using 625 ppm as the highest dose. However, serum levels of oxazepam at this dose were within the therapeutic target levels in humans, providing no margin of safety for cross-species comparison. Although these data represented single time point plasma levels, based on the modeling work of mouse oxazepam toxicokinetics by Yuan et al. (1994), it is unlikely that there was marked variation in the blood oxazepam concentrations in rats throughout the day given the similar feeding habits and k2 values for elimination of oxazepam in mice and rats.

In females, adverse effects on body weights and survival were not nearly as severe as those in males, although both were reduced in an exposure-related fashion. Nephropathy was increased in females, but the severity was mild, in contrast to the situation in males. Varying degrees of nephropathy normally develop in the aging rat, and this condition is worsened when the animals are maintained on a relatively high-protein diet, such as the NIH-07 diet used in these studies (Rao et al., 1993). Nephropathy is considered a major contributor to early mortality in rats and likely accounts for the pattern of deaths observed in this study. It is possible that enhanced nephropathy would have been seen in certain exposure groups in 13-week studies because this lesion was more severe in the 10,000-ppm stop-exposure group of males, 18 months after exposure ceased. However, enhanced nephropathy was not seen in mice receiving oxazepam, nor was it reported in mice or rats in chronic studies with prazepam (de la Iglesia et al., 1981), temazepam (Robinson et al., 1984), or ripazepam (Fitzgerald et al., 1984), although some renal tubule dilatation in rats was reported with the last compound.

The parathyroid gland hyperplasia and fibrous osteodystrophy of bone that occurred in the males are consistent sequelae to severe nephropathy and secondary hyperparathyroidism of chronic renal failure (Leininger and Riley, 1990; Seely and Hildebrandt, 1990; Capen, 1994). It has been shown experimentally that stress-related ulceration of the glandular stomach can be enhanced by either acidic or nonacidotic renal insufficiency (Fischer et al., 1974), and mineralization of the glandular stomach and nonglandular stomach can be secondary to uremia (Brown and Hardisty, 1990).

Males exposed to 2500 or 5000 ppm and females exposed to....
TABLE 2

Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney of Rats in the 2-Year Feed Study of Oxazepam

<table>
<thead>
<tr>
<th>Parts per million</th>
<th>0</th>
<th>625</th>
<th>2500</th>
<th>5000</th>
<th>(Stop-exposure)</th>
</tr>
</thead>
</table>

**Male**

<table>
<thead>
<tr>
<th>Number examined microscopically</th>
<th>50</th>
<th>50</th>
<th>50</th>
<th>50</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephropathy</td>
<td>49 (1.9)*</td>
<td>44 (2.3)</td>
<td>49 (2.7)**</td>
<td>50 (3.2)**</td>
<td>42 (3.3)**</td>
</tr>
<tr>
<td>Renal tubule hyperplasia</td>
<td>0</td>
<td>1 (1.0)</td>
<td>3 (2.3)</td>
<td>1 (2.0)</td>
<td>0</td>
</tr>
<tr>
<td>Renal tubule adenoma</td>
<td>1†</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
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</table>

**Step sections (extended evaluation)**

<table>
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<th>Number examined microscopically</th>
<th>50</th>
<th>50</th>
<th>50</th>
<th>50</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal tubule hyperplasia</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>21**</td>
</tr>
<tr>
<td>Renal tubule hyperplasia, oncocytic</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Renal tubule adenoma, multiple</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Renal tubule adenoma, all</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>6*</td>
</tr>
<tr>
<td>Renal tubule, oncocytoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Single sections and step sections (combined)**

<table>
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<th>Number examined microscopically</th>
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<th>50</th>
<th>50</th>
<th>50</th>
<th>45</th>
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<tr>
<td>Renal tubule hyperplasia</td>
<td>5</td>
<td>6</td>
<td>12*</td>
<td>9*</td>
<td>21**</td>
</tr>
<tr>
<td>Renal tubule hyperplasia, oncocytic</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Renal tubule adenoma, multiple</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Renal tubule adenoma, all</td>
<td>2</td>
<td>1</td>
<td>7*</td>
<td>6</td>
<td>6*</td>
</tr>
<tr>
<td>Renal tubule, oncocytoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Female**

<table>
<thead>
<tr>
<th>Number examined microscopically</th>
<th>50</th>
<th>50</th>
<th>50</th>
<th>50</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephropathy</td>
<td>32 (1.1)</td>
<td>43** (1.3)</td>
<td>41** (1.3)</td>
<td>48** (1.7)**</td>
<td>1 (2.0)</td>
</tr>
</tbody>
</table>

* Number of animals with lesion.

† Average severity of lesions in affected animals: 1, minimal; 2, mild; 3, moderate; 4, marked.

‡ Historical dosed feed study control incidence 0.7% ± 1.5%, range 0 to 6%.

* Significantly different (p < 0.05) from the control group by the logistic regression test (incidence) or by the Mann-Whitney U test (severity)

**p < 0.01

2500 ppm had increased epithelial hyperplasia, ulceration, and inflammation of the nonglandular stomach. Degenerative and proliferative forestomach lesions are relatively common in studies in which chemicals are administered orally (Gopinath et al., 1987). Papillomas were not noted in this study; however, severe papillary hyperplasia was observed in some animals. The increased incidences of these lesions in the nonglandular stomach of continuously exposed animals suggest that their development was probably a direct effect of chemical administration. In the stop-exposure study, the increased incidence of hyperplasia in the nonglandular stomach indicates a failure of this lesion to resolve during the prolonged recovery period.

There were a few renal tubule adenomas noted principally in the 2500-ppm group in the initial evaluation of kidneys of exposed male rats, although a dose response was not evident. To more fully evaluate the possibility that this was a treatment-related finding, the remaining kidney tissues were step-sectioned and additional microscopic adenomas were observed. The NTP experience with multiple-step sectioning of kidneys has been reported by Eustis et al. (1994). In 13 prior studies, additional renal tubule neoplasms and oncocytomas were observed with step sectioning in both control and exposed groups. Although additional neoplasms were not found in the control rat kidneys for many of the studies, as many as six additional neoplasms were found in the control group for one study. Even greater numbers of additional neoplasms were found in some exposure groups. Eustis et al. (1994) noted that the studies in which additional renal neoplasms were found were also those in which nephropathy was more severe than in typically seen or those in which the chemical enhanced the nephropathy. A similar situation occurred in the present study with oxazepam.

Although the renal tubule neoplasms occurred with a positive trend, the incidence of 14% in the 2500-ppm group is similar to the upper range of neoplasms found in historical controls after step sectioning (Eustis et al., 1994). There was no increase in renal tubule neoplasms in the 625-ppm group. Whether these renal neoplasms represent a true carcinogenic effect of oxazepam or are secondary to the oxazepam-enhanced nephropathy cannot be determined from these studies. There is no convincing evidence to suggest that oxazepam is mutagenic or has the ability to induce chromosomal aberrations or other adverse genetic effects (NTP, 1993).
The effects of oxazepam on the liver of male and female rats were limited to centrilobular hepatocyte hypertrophy, which is commonly seen with a wide variety of agents, including other benzodiazepines and barbiturates, and changes in the incidences of basophilic and clear cell foci. There was no evidence of an increase in hepatocellular neoplasms in male or female rats exposed to oxazepam. This is in sharp contrast to the increases in liver neoplasms reported with the two mouse strains studied earlier (NTP, 1993; Bucher et al., 1994, 1995). Oxidative metabolism (of the phenyl ring) occurs in both mice and rats (Griffin et al., 1995b), but is more pronounced in rats. There are also differences in the major conjugation reactions with glucuronic acid and sulfate, as well as differences in fecal and urinary excretion patterns. On repeated dosing, there is a shift in the metabolite pattern in mice, but not rats, suggesting an induction of enzymes which are involved in oxazepam metabolism. Serum concentrations of oxazepam were higher at comparable dosed feed concentrations in mice (NTP, 1993) than in rats in the current study, but the differences appeared due at least in part to the relatively greater consumption of dosed feed by mice than rats on a body weight basis. Mice also eliminated less oxazepam in the feces than did rats and had a slightly longer terminal elimination half-life from plasma, suggesting that enterohepatic circulation may be greater in mice (Yuan et al., 1994; Griffin and Burka, 1995).
TABLE 4

Incidences of Nonneoplastic Lesions of the Liver of Rats in the 2-Year Feed Study of Oxazepam

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number examined microscopically</td>
<td>Number examined microscopically</td>
</tr>
<tr>
<td>Basophilic focus</td>
<td>21*</td>
<td>44</td>
</tr>
<tr>
<td>Clear cell focus</td>
<td>2</td>
<td>6</td>
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<tr>
<td>Eosinophilic focus</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Mixed cell focus</td>
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<td>5</td>
</tr>
<tr>
<td>Hepatocyte, centrilobular, hypertrophy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>44</td>
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<tr>
<td></td>
<td>50</td>
<td>10*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3*</td>
</tr>
<tr>
<td></td>
<td>10,000 (Stop-exposure)</td>
<td>7**</td>
</tr>
</tbody>
</table>

* Number of animals with lesion.

** p < 0.01.

1993, 1995). This may indicate a greater degree of exposure of the liver of mice to oxazepam and its metabolites when compared to rats. Nonetheless, despite considerable effort devoted to examining the basis for the differential carcinogenic response in rats and mice, no clear biochemical basis for this effect has been identified.

Two recent epidemiological studies have evaluated the association between human cancers and benzodiazepine use. Neither examined oxazepam specifically. Rosenberg et al. (1995) did not find an association between sustained benzodiazepine use (at least 4 days per week for at least 1 month, initiated at least 2 years prior to hospital admission) and any one of 11 cancers (breast, large bowel, malignant melanoma, lung, uterine endometrium, ovary, non-Hodgkin’s lymphoma, testis, Hodgkin’s disease, thyroid gland, and liver) in a large United States hospital-based surveillance study. Harlow and Cramer (1995) reported an association between prior use of benzodiazepines exceeding “one to six months” with subsequent development of ovarian cancer (adjusted odds ratio 1.8, 95% CI 1.0–3.1). These authors proposed that the induction of hepatic microsomal enzymes by benzodiazepines might enhance the metabolism of estrogen, thus stimulating higher gonadotropin levels. Ovarian neoplasms were not increased in female rats in the present studies or in studies with mice reported earlier (NTP, 1993).

In summary, under the conditions of these 2-year dosed feed studies, there was equivocal evidence of carcinogenic activity in male F344/N rats based on increased incidences of renal tubule adenomas in exposed groups also exhibiting significantly enhanced nephropathy. There was no evidence of carcinogenic activity of oxazepam in female F344/N rats exposed to feed containing 625, 2500, or 5000 ppm for 2 years or 10,000 ppm for 6 months.

REFERENCES


