Effects of Coumarin Following Perinatal and Chronic Exposure in Sprague-Dawley Rats and CD-1 Mice

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Coumarin, as a class, comprised numerous naturally occurring compounds with important and diverse physiological activities. The parent compound, coumarin, is used as an additive to perfumes, soaps, detergents, toothpaste, and some tobacco products and alcoholic beverages outside the United States. It is used clinically in the treatment of high-protein edema (Jamal et al., 1989), chronic brucellosis, and immune suppression (Moran et al., 1987). In isolation (Thomes et al., 1989) or in combination with cimetidine (Marshall et al., 1987a, b, c; Dexeus et al., 1990), coumarin is currently undergoing several clinical trials for the treatment of several types of malignancies in humans, including the treatment of lung and kidney carcinoma. Due to its use as a drug, there is information in the literature concerning its toxicity, metabolism, and pharmacokinetics in man. While several studies have been conducted to examine the chronic toxicity and carcinogenicity of coumarin, none were conducted in accordance with current scientific guidelines. A GLP-compliant study was therefore undertaken. (Since this study was completed, the U.S. National Toxicology Program has conducted a bioassay of coumarin in F344 rats and B6C3F1 mice.)

MATERIALS AND METHODS

Chemicals

Coumarin (CAS 91-64-5) (>98% pure) was supplied by Rhone-Poulenc France as a white crystalline powder. Coumarin was admixed with Spratt's Laboratory Animal Diet No. 2 to form a premix. The premix was then diluted with diet to the appropriate dose levels. Diets were prepared weekly.

Animals and Treatments

Rats. Rats were housed five per cage in suspended wire-mesh cages. Animal rooms were maintained at 22°C and 50% relative humidity, with a 12-hr/12-hr light/dark cycle. Each cage was identified by cage card and each rat was individually identified by earmark and tattoo. Ad libitum access to food and tap water was provided.

All animals for the chronic study were derived from Sprague-Dawley rats received from Charles River (Charles River, Manston, Kent, UK) and bred at Huntingdon Research Centre, Huntingdon, Cambridgeshire, England. A preliminary study (Hunter et al., 1980, unpublished data) conducted in pregnant Sprague-Dawley rats demonstrated that 3000 and 5000 ppm resulted in large weight gain decrements in the exposed dams and consequent decreases in the number of viable young. Therefore, in the main study described here, rats in the control, 3000-, and 5000-ppm dose groups...
received control diet in utero and during the lactational phase until weaning at 21–28 days of age, when the main study phase dosing was initiated. Animals in the 333-, 1000-, and 2000-ppm dose groups received coumarin-dosed feed at those dose levels throughout the perinatal and chronic periods.

A total of 390 male and 390 female rats were used in this main chronic phase of the study. Each rat was allocated to one of six treatment groups, each comprising a main group (50 rats/sex) and a satellite group (15 rats/sex). Animals in the main group were given ophthalmoscopic evaluations and were used for complete chronic toxicity/carcinogenicity evaluation. The satellite groups were used for hematology, clinical chemistry, and urinalysis determinations. They were killed at Week 104 and received a gross necropsy and organ weight determinations. Only gross lesions were examined microscopically from the satellite animals, however. All male rats in the main groups were terminated after 104 weeks of postweaning exposure; female rats were terminated after 110 weeks. A complete gross necropsy with organ weights was conducted for all main group animals. The following organs were weighed: adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid, and uterus. Tissues for a complete histopathologic evaluation were collected and preserved in neutral-buffered formalin.

The following observations and measurements were recorded. Mortality and morbidity (recorded twice daily), clinical observations, body weight, food and water consumption, ophthalmoscopic evaluations, hematology, clinical chemistry, plasma cholinesterase, urinalysis, selected organ weights, and gross and microscopic pathology.

Mice. Animals were housed four per cage in solid bottom polypropylene cages with autoclaved, sifted sawdust as bedding. Animal rooms were maintained at 22°C and 50% relative humidity, with a 12-hr/12-hr light/dark cycle. Each cage was identified by colored cage label and each mouse was individually identified by earmark. Ad libitum access to food and tap water was provided.

A total of 416 male and female CD-1 mice (Charles River, Manston, Kent, UK) 28 ± 2 days of age were randomly assigned to four coumarin-treatment groups comprising 52 mice/sex/dose. Animals received 0, 300, 1000, or 3000 ppm coumarin in the diet. Male mice were terminated at Week 101 and female mice at Week 109. A complete gross necropsy, with organ weight determinations, was conducted for all animals. The following organs were weighed: brain, heart, kidneys, liver, testes. Tissues for a complete histopathologic evaluation were collected and preserved in neutral-buffered formalin.

The following observations and measurements were recorded: mortality and morbidity (recorded twice daily), clinical observations, body weight, food consumption, hematology, selected organ weights, and gross and microscopic pathology.

RESULTS

Rats

The only clinical observation potentially dose-related was a temporary loss of fur among males and females receiving 5000 ppm and among females receiving 3000 ppm.

Survival was decreased in animals receiving 333 ppm, but significantly increased among male and female rats receiving 3000 and 5000 ppm coumarin when compared with controls. These data are presented in Table 1.

Body weight gains and food consumption were significantly reduced among male and female rats receiving 2000, 3000, and 5000 ppm. These data are presented in Tables 2 and 3. Growth of all groups of male rats was essentially similar between Weeks 52 and 78. From Weeks 78 through study termination, control rats lost weight, as commonly occurs for male Sprague–Dawley rats at this age. Body weights of male rats in the three highest dose groups remained relatively constant, reflecting the generally better condition of males receiving higher levels of coumarin. Female rats receiving 2000, 3000, or 5000 ppm continued to gain less weight than controls during Weeks 52–78. From Week 78 onward, bodyweight gain by all groups of female rats was essentially similar.

The efficiency of food utilization by rats receiving 5000 ppm was impaired during Weeks 1–6. From Weeks 6–26, the efficiency of food utilization by these rats was superior to that of controls, indicating a degree of adaptation. From Weeks 13–26, efficiency of food utilization by males and females receiving 1000 or 2000 ppm was marginally impaired by treatment. Calculated group mean achieved intakes are described in Table 4.

Significant clinical chemistry findings included a treatment-related anemia, principally characterized by low hemoglobin levels among males and females from Week 6 onward. This effect was noted primarily among rats in the three highest dose groups. Minimally elevated white cell and lymphocyte counts were recorded among 5000 ppm females from Week 25 to the end of the study.

Treatment-related decreases in glucose and protein were found at Weeks 4 and 13 among 5000-ppm dose group animals, but the changes became less pronounced as the study progressed. Treatment-related increases in blood potassium levels, alkaline phosphatase, and glutamic–pyruvic transaminase were recorded throughout the study. Cholesterol levels of all groups of treated rats except for males in the highest dose group were increased throughout the study. Plasma cholinesterase, but not red blood cell cholinesterase, levels were decreased among females treated with coumarin, but not males.

At necropsy, an increased incidence of liver masses was noted for male rats in the 5000-ppm dose group. Increased liver weights were recorded among males and females receiving 3000 and 5000 ppm and among females at 1000 and 2000 ppm. There was a twofold increase in relative liver weight observed at the highest doses. Decreased absolute brain, pituitary, heart, kidney, and adrenal weights were recorded for males in the 5000-ppm dose group. Heart weights were also decreased for males in the 2000- and 3000-ppm dose groups. Decreased absolute adrenal weights also were recorded for males receiving 1000, 2000, or 3000 ppm and among females receiving 3000 or 5000 ppm.

Increased incidences of cholangiofibrosis, cholangiocarcinoma, and parenchymal liver cell tumors were recorded among male and female rats receiving 5000 ppm coumarin, although tumor incidence had no impact on survival. A single cholangiocarcinoma reported for a male rat receiving 3000 ppm was also considered to be potentially treatment-related. Rats receiving coumarin at 2000 ppm or less showed no evidence of increased hepatic tumor prevalence. No dose-
TABLE 1
Survival of Male and Female Sprague–Dawley Rats Receiving Coumarin in the Diet for 104–110 Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>0 ppm</th>
<th>333 ppm</th>
<th>1000 ppm</th>
<th>2000 ppm</th>
<th>3000 ppm</th>
<th>5000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males Weeks 0–104</td>
<td>21/50</td>
<td>11/50*</td>
<td>17/50</td>
<td>22/50</td>
<td>27/50*</td>
<td>33/50**</td>
</tr>
<tr>
<td>Females Weeks 0–110</td>
<td>13/50</td>
<td>11/50*</td>
<td>17/50</td>
<td>19/50</td>
<td>25/50*</td>
<td>32/50**</td>
</tr>
</tbody>
</table>
<pre><code>           | 26%   | 22%     | 34%      | 38%      | 50%      | 64%      |
</code></pre>

* $p \leq 0.05$.  
** $p \leq 0.001$.  

related increases in renal tumors or tumors at other tissue sites were observed. Histopathology results are presented in Table 5.

Mice

Body weight gain by males receiving 3000 ppm was significantly reduced compared to controls, demonstrating an 18% reduction over the first 52 weeks of the study. Body weight gain was reduced by 10% relative to controls among males in the 1000-ppm dose group. Body weights were similar across all female dose groups. Food utilization by males, but not females, in the 3000-ppm dose group was marginally inferior to that of controls. Achieved average coumarin intakes were calculated and are shown in Table 6.

No treatment-related changes in survival, clinical observations, hematology, or clinical chemistry were observed among males or females receiving coumarin in the diet. Females receiving 3000 ppm coumarin showed marginally, but statistically significant, increases in liver weight, although there were no associated changes at the cellular level. Males were not similarly affected. No other differences in organ weights were observed across dose groups. No increases in tumors were observed in liver, lung, or other tissues (Table 7). Gross and microscopic pathology was unchanged by dose exposure.

DISCUSSION

Concerns about coumarin's carcinogenic potential arose from a series of studies which started in 1967 with reports by Bär and Griepentrog (1967). These authors reported that lifetime exposure of rats to doses of 5000 ppm or higher

TABLE 2
Body Weight Gain for Male and Female Sprague–Dawley Rats during the Chronic Bioassay of Coumarin
(Weight Gain in Grams ± SD)

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>333 ppm</th>
<th>1000 ppm</th>
<th>2000 ppm</th>
<th>3000 ppm</th>
<th>5000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–13</td>
<td>230 ± 34</td>
<td>234 ± 31</td>
<td>228 ± 24</td>
<td>216 ± 25**</td>
<td>197 ± 23***</td>
<td>128 ± 21***</td>
</tr>
<tr>
<td>13–26</td>
<td>56 ± 29</td>
<td>56 ± 24</td>
<td>46 ± 18**</td>
<td>40 ± 16***</td>
<td>47 ± 18*</td>
<td>53 ± 16</td>
</tr>
<tr>
<td>26–52</td>
<td>113 ± 58</td>
<td>108 ± 47</td>
<td>93 ± 41**</td>
<td>71 ± 35***</td>
<td>68 ± 37***</td>
<td>41 ± 18***</td>
</tr>
<tr>
<td>52–78</td>
<td>80 ± 45</td>
<td>68 ± 63</td>
<td>71 ± 43</td>
<td>56 ± 38**</td>
<td>52 ± 44**</td>
<td>20 ± 16***</td>
</tr>
<tr>
<td>78-term</td>
<td>18 ± 106</td>
<td>33 ± 102</td>
<td>42 ± 78</td>
<td>39 ± 57</td>
<td>40 ± 56</td>
<td>26 ± 36</td>
</tr>
</tbody>
</table>

* $p \leq 0.05$.  
** $p \leq 0.01$.  
*** $p \leq 0.001$.  

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TABLE 3
Food Consumption (g/Rat) for Male and Female Sprague–Dawley Rats in the Chronic Bioassay of Coumarin

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>333 ppm</th>
<th>1000 ppm</th>
<th>2000 ppm</th>
<th>3000 ppm</th>
<th>5000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–13</td>
<td>2236</td>
<td>2272</td>
<td>2176</td>
<td>1883***</td>
<td>1757***</td>
<td>1209***</td>
</tr>
<tr>
<td>SD</td>
<td>88</td>
<td>65</td>
<td>68</td>
<td>82</td>
<td>68</td>
<td>103</td>
</tr>
<tr>
<td>% Control</td>
<td>102%</td>
<td>97%</td>
<td>94%</td>
<td>84%</td>
<td>79%</td>
<td>54%</td>
</tr>
<tr>
<td>13–26</td>
<td>2419</td>
<td>2459</td>
<td>2362</td>
<td>2103***</td>
<td>2069***</td>
<td>1602***</td>
</tr>
<tr>
<td>SD</td>
<td>132</td>
<td>89</td>
<td>48</td>
<td>73</td>
<td>88</td>
<td>113</td>
</tr>
<tr>
<td>% Control</td>
<td>102%</td>
<td>98%</td>
<td>87%</td>
<td>86%</td>
<td>86%</td>
<td>66%</td>
</tr>
<tr>
<td>27–78</td>
<td>9246</td>
<td>9452</td>
<td>8922</td>
<td>8225***</td>
<td>8209***</td>
<td>6772***</td>
</tr>
<tr>
<td>SD</td>
<td>626</td>
<td>369</td>
<td>265</td>
<td>376</td>
<td>357</td>
<td>415</td>
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<tr>
<td>% Control</td>
<td>102%</td>
<td>96%</td>
<td>89%</td>
<td>89%</td>
<td>89%</td>
<td>73%</td>
</tr>
<tr>
<td>79–term</td>
<td>5056</td>
<td>5096</td>
<td>5094</td>
<td>4492**</td>
<td>4369***</td>
<td>3644***</td>
</tr>
<tr>
<td>SD</td>
<td>283</td>
<td>394</td>
<td>461</td>
<td>242</td>
<td>183</td>
<td>243</td>
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<tr>
<td>% Control</td>
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<td>89%</td>
<td>86%</td>
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<td>72%</td>
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<td>Females</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–13</td>
<td>1637</td>
<td>1666</td>
<td>1626</td>
<td>1487***</td>
<td>1394***</td>
<td>1027***</td>
</tr>
<tr>
<td>SD</td>
<td>76</td>
<td>77</td>
<td>62</td>
<td>46</td>
<td>63</td>
<td>84</td>
</tr>
<tr>
<td>% Control</td>
<td>102%</td>
<td>99%</td>
<td>91%</td>
<td>85%</td>
<td>85%</td>
<td>63%</td>
</tr>
<tr>
<td>13–26</td>
<td>1669</td>
<td>1693</td>
<td>1629</td>
<td>1544***</td>
<td>1507***</td>
<td>1249***</td>
</tr>
<tr>
<td>SD</td>
<td>75</td>
<td>112</td>
<td>78</td>
<td>51</td>
<td>79</td>
<td>69</td>
</tr>
<tr>
<td>% Control</td>
<td>101%</td>
<td>98%</td>
<td>93%</td>
<td>90%</td>
<td>90%</td>
<td>75%</td>
</tr>
<tr>
<td>27–78</td>
<td>6959</td>
<td>6973</td>
<td>6734</td>
<td>6376***</td>
<td>6229***</td>
<td>5104***</td>
</tr>
<tr>
<td>SD</td>
<td>425</td>
<td>571</td>
<td>287</td>
<td>198</td>
<td>392</td>
<td>263</td>
</tr>
<tr>
<td>% Control</td>
<td>100%</td>
<td>97%</td>
<td>92%</td>
<td>90%</td>
<td>90%</td>
<td>73%</td>
</tr>
<tr>
<td>79–term</td>
<td>4849</td>
<td>4674</td>
<td>4894</td>
<td>4405*</td>
<td>4354**</td>
<td>3550***</td>
</tr>
<tr>
<td>SD</td>
<td>500</td>
<td>217</td>
<td>326</td>
<td>271</td>
<td>414</td>
<td>344</td>
</tr>
<tr>
<td>% Control</td>
<td>96%</td>
<td>101%</td>
<td>91%</td>
<td>90%</td>
<td>90%</td>
<td>73%</td>
</tr>
</tbody>
</table>

* p ≤ 0.05
** p ≤ 0.01
*** p ≤ 0.001

induced bile duct carcinoma. Cohen (1979) reviewed these slides and cast doubts on the interpretation of the histopathology and the reality of these carcinomas. Reevaluation of the original histopathology slides indicated that cholangiofibrosis and not cholangiocarcinoma was present (Evans et al., 1989). Evans reported that cholangiofibrosis is not a prerequisite for hepatocellular carcinoma and is not part of the carcinogenic process. Hagan et al. (1967) reported that groups of five to seven Osborne–Mendel rats given up to 5000 ppm coumarin in the diet were unaffected at 1000 ppm. Hepatotoxicity was noted at 2500 ppm. Animals fed 5000 ppm had decreased hemoglobin levels; enlarged livers with fatty metamorphosis, masses, and pale foci; and cholangiofibrosis. No cholangiocarcinomas were reported.

The National Toxicology Program (NTP) (1993) conducted 2-year bioassays of coumarin in F344 rats and B6C3F1 mice. Dose levels for these corn oil gavage studies were 0, 25, 50, and 100 mg/kg/day for rats and 0, 50, 100, and 200 mg/kg/day for mice, doses which are comparable to, or slightly lower than, the doses used in our studies. In the NTP study, survival of the male rats was severely compromised. Some hepatocellular necrosis and bile duct hyperplasia was found, but no increase in hepatocellular neoplasia was reported at any dose. The highest dose level in the NTP rat study was approximately equivalent to the 2000-ppm dose group reported here (achieved intake 87 mg/kg/day in males; 107 mg/kg/day in females). Hepatocellular findings were similar at this dose level, where no increase in tumor incidence was observed. In contrast to findings of
### TABLE 5
Liver Weights and Histopathologic Findings for Male and Female Sprague–Dawley Rats during the Chronic Bioassay of Coumarin (65 male and 65 female per group)

<table>
<thead>
<tr>
<th>Animals (65/sex/group)</th>
<th>Finding</th>
<th>Control</th>
<th>333 ppm</th>
<th>1000 ppm</th>
<th>2000 ppm</th>
<th>3000 ppm</th>
<th>5000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Liver weight (grams ± SD)</td>
<td>28.3 ± 5.91</td>
<td>29.4 ± 7.03</td>
<td>29.3 ± 5.37</td>
<td>29.7 ± 3.87</td>
<td>34.3 ± 10.55</td>
<td>44.6 ± 18.85</td>
</tr>
<tr>
<td>Cholangiocarcinoma (nonmetastasizing)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Cholangiocarcinoma (metastasizing)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Parenchymal tumors (benign + malignant)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>Liver weight (g ± SD)</td>
<td>18.8 ± 4.8</td>
<td>21.7 ± 4.1</td>
<td>23.4 ± 4.3</td>
<td>20.6 ± 4.5</td>
<td>2.2 ± 4.6</td>
<td>22.9 ± 5.5</td>
</tr>
<tr>
<td>Cholangiocarcinoma (nonmetastasizing)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cholangiocarcinoma (metastasizing)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Parenchymal tumors (benign + malignant)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Marginal increases in renal adenoma in the NTP study, no increase was observed in the Rhône-Poulenc (RP) study. No renal tumors were reported in females and only 1 renal adenoma was observed in a 2000-ppm dose group males.

The NTP bioassay in B6C3F1 mice reported an increase in alveolar/bronchiolar adenoma and carcinoma among male and female mice (14/50, 9/50, 15/50, 25/51 males; 2/51, 5/49, 7/49, 27/50 females). Although an increase in pulmonary tumors was noted in the current study, all tumor incidences were within laboratory historic control ranges for the age and strain of mouse. There were no significant dose-related increases in tumors of any type in the CD-1 mice receiving coumarin in the diet at doses slightly higher than those given the mice treated by gavage in the NTP study (achieved high doses 280 mg/kg/day males; 271 mg/kg/day females).

Chronic toxicity/carcinogenicity studies conducted in numerous species indicate that the liver is the main target organ for coumarin toxicity, but the severity of the effect appears to be dependent on the prevalent metabolic pathway. The 7-hydroxylation pathway appears to be a more efficient detoxification/elimination pathway. Chronic toxicity/carcinogenicity studies conducted in species with significant 7-hydroxylation capacity such as baboons (Gangolli et al., 1974; Evans et al., 1979), hamsters (Ueno and Hirono, 1980), and DBA/2J mice (Wood and Conney, 1974) have demonstrated significantly less hepatotoxicity and, in the bioassays, no increases in tumors at any site. DBA/2J mice are one of the few nonprimate species so far shown to possess a significant 7-hydroxylating capability (Wood and Conney, 1974). They appear to be more resistant to the hepatotoxic action of coumarin than is the CH3/HeJ mouse strain which possesses only a limited 7-hydroxylating potential (Endell and Seidel, 1978). Similarly, a bioassay study by Ueno and Hirono (1980) suggests that hamsters, which possess some 7-hydroxycoumarin (7-OHC) capacity, are refractory to coumarin-induced hepatotoxicity, although limited conclusions can be drawn due to the small number of animals. In addition, no carcinogenic or adverse toxicologic effects were found in this study when groups of 10–13 Syrian Golden hamsters were fed diet containing 0, 1000, and 5000 ppm coumarin for 2 years.

When administered as a single high dose to the rat, coumarin has been shown to produce hepatic centrilobular necrosis. Lake (Lake, 1984; Lake et al., 1989) demonstrated that coumarin-induced hepatotoxicity in rats is likely to be mediated via one or more reactive metabolites generated by cytochrome P450-dependent enzymes. Hepatotoxicity of coumarin was decreased by pretreatment with cobaltous chloride, elliptycine, and metyrapone and potentiated by pretreatment with diethyl maleate (known to reduce hepatic-reduced glutathione concentration). In in vitro studies, reactive [3-14C]-coumarin metabolite(s) generated by cytochrome P450-dependent enzyme was found to bind covalently to microsomal proteins. The authors suggest that the toxic compound may be a coumarin 3-4 epoxide intermediate.

Lake et al. (1989) compared the metabolism of [3-14C]-
current Sprague-Dawley rat study is likely due to the hepa-
dose male rats, clearly indicating that the maximum tolerated
responses were comparable at similar doses in the NTP and
toxicology incurred following high-dose exposure. Tumors
dose was exceeded, the carcinogenic response seen in the
were not metastatic and survival was significantly
increased
This is consistent with a rapid absorption by the gut in man
 metabolites. (Shilling et al., 1966).
35% is found in the urine of rats given an equivalent dose
administration, with 83% of the absorbed dose (200 mg/kg)
excretion of the coumarin metabolites in the rat. No such
important to note that the main feature in chronic liver toxic-
ity is bile duct alteration, which may correspond to the biliary
excretion of the coumarin metabolites in the rat. No such
toxicity is observed with species metabolizing through 7-
OHC, which is not significantly excreted through the bile.
In humans, coumarin is rapidly eliminated after oral ad-
ministration, with 83% of the absorbed dose (200 mg/kg)
found in the urine within 24 hr (79% as 7-OHC), while only
35% is found in the urine of rats given an equivalent dose
(<0.1% as 7-OHC) (Shilling et al., 1969; Feuer et al., 1966).
This is consistent with a rapid absorption by the gut in man
and with an absence of enterohepatic circulation of the
metabolites.
Given body weight decrements up to 60% among high-
dose male rats, clearly indicating that the maximum tolerated
dose was exceeded, the carcinogenic response seen in the
current Sprague-Dawley rat study is likely due to the hepato-
toxicity incurred following high-dose exposure. Tumors
were not metastatic and survival was significantly increased
among rats in the two highest dose groups. While hepatic
responses were comparable at similar doses in the NTP and
RP rat studies, findings of minimal increases in renal adeno-
mas only in the NTP study may indicate a strain susceptibili-
ty to renal abnormalities among F344 rats. Similarly, the
increase in lung tumors observed among B6C3F1 mice in
the NTP study was not confirmed in the RP study. This may
reflect a dose-route-dependent phenomenon (NTP corn oil
gavage vs RP dietary exposure) or a strain-related difference
in metabolism or susceptibility. The significance of any of
these positive carcinogenic results to man is highly question-
able in view of man’s much lower exposure potential and
the large metabolic differences between species.

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TABLE 7
Liver and Lung Histopathologic Findings for Male and Female CD-1 Mice during the Chronic Bioassay of Coumarin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Finding*</th>
<th>Control</th>
<th>300 ppm</th>
<th>1000 ppm</th>
<th>3000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver—male</td>
<td>Benign + malignant parenchymal tumors</td>
<td>20</td>
<td>22</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Liver—female</td>
<td>Benign + malignant parenchymal tumors</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Lung—male</td>
<td>Pulmonary adenoma</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Lung—female</td>
<td>Pulmonary adenocarcinoma</td>
<td>11</td>
<td>12</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

* All tumor incidences were within laboratory historic control values for this age and strain of mice.


