Synergism between Cyclopropenoid Fatty Acids and Chemical Carcinogens in Rainbow Trout (Salmo gairdneri) 1,2

D. J. Lee, J. H. Wales, J. L. Ayres, 3 and R. O. Sinnhuber

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon 97331

SUMMARY

The synergistic activities of cyclopropenoid fatty acids (CPFA), epoxysterolic acid, sesame seed oil, and autoxidized salmon oil with aflatoxin B1 were studied in rainbow trout. Sterculia foetida oil (49% sterculic, 7% malvalic acids) and Hibiscus syriacus oil (2% sterculic and 19% malvalic acids) were used as sources of CPFA. The combination of both 112 and 56 ppm CPFA procured from S. foetida oil and 210 ppm CPFA procured from H. syriacus oil promoted early tumor development, increased tumor incidence, and caused a several-fold increase in tumor growth rate over the positive control. Trout fed 100 ppm 2-acetylaminofluorene for 15 months did not develop hepatomas, but the addition of H. syriacus oil to the diet induced a 20% incidence of liver tumors.

Diet containing 200 ppm epoxysterolic acid (in Vernonia anthelmintica oil), 5% sesame seed oil, or 5% autoxidized salmon oil (peroxide value 200-300) did not alter the carcinogenicity of aflatoxin B1. Some unusual lipid deposits were noted in the livers of fish receiving sesame seed oil.

INTRODUCTION

The economically important cotton plant and other species included in the order Malvales contain cyclopropenoid fatty acids (CPFA) in their triglycerides. The unusual physiologic properties of these fatty acids were the subject of a review in 1965 by Phelps et al. (13). They reported that eggs from hens ingesting CPFA developed a pink discoloration of the whites during storage. Other physiologic effects noted were growth depression in rats, an alteration of body lipids to ones containing more stearic and less oleic acids than normal, and delayed sexual maturity in pullets and female rats.

Studies of the effect of CPFA on the fatty acid dehydrogenase enzyme system have provided a better understanding of the biochemical alteration responsible for the change in fatty acid composition. Reiser and Raju (14) found that CPFA inhibited the desaturation of stearic to oleic acid in intact rats, while Johnson et al. (7) reported that in hen liver tissue this enzyme system was inhibited by malvalic and sterculic acids.

In a previous feeding trial at our laboratory (19), CPFA markedly increased the severity of aflatoxin B1-induced hepatomas in rainbow trout. The source of CPFA was Sterculia foetida seed oil which contained 49% sterculic and 7% malvalic acids. The cyclopropenoids increased the incidence of hepatomas, promoted the growth rate of tumors, and hastened the appearance of liver nodes. This synergistic activity is of both practical and academic interest since cyclopropenoid-containing cottonseed meals are subject to contamination by aflatoxin-producing Aspergillus flavus.

In addition to investigations of CPFA-aflatoxin relationships, several other compounds were of interest because of possible synergism with CPFA or aflatoxin B1. Epoxides, lactones, and hydroperoxides have been suggested as having carcinogenic or cocarcinogenic properties (21, 22), and the presence of peroxides and polymers in heated and oxidized oils has prompted a number of investigations into their physiologic effects. Sugai et al. (20) found heated vegetable oil to enhance the carcinogenicity of 2-acetylamino-fluorene (AAF) in rats.

Sesame seed oil, with its unusual cyclic compounds, has been cited as a possible cytotoxic or carcinogenic substance. Mirand et al. (11) found sarcomas in mice previously injected with desoxycorticosterone acetate in sesame seed oil. Bischoff (4) reported sesame seed oil to enhance the carcinogenicity of the oxidation products of cholesterol when used as a vehicle for subcutaneous injections in mice. Ambrose et al. (2) found sesamol, a component of sesame seed oil, to have carcinogenic properties when fed to rats.

Gossypol, a cottonseed pigment, has been reported to increase the incidence and growth of aflatoxin-induced liver tumors in rainbow trout (19) and to accumulate in body tissues of the fish (16).

Acetylamino-fluorene was reported to induce liver tumors in rainbow trout when fed as 0.015 and 0.060% of the diet for 12 months (5). Lotlikar et al. (9) found that 5- and 7-hydroxy AAF were the primary excretory products after feeding AAF to trout; they were unable to detect the active carcinogen, N-hydroxy-AAF in either in vivo or in vitro experiments.

This report describes additional studies on the synergistic properties of CPFA when fed with a carcinogen. Feeding trials were designed to determine the synergism between CPFA and aflatoxin B1 at different dietary levels to compare the activity of malvalic and sterculic acids, to determine the effect of the

1 This work was supported in part by USPHS Grants No. CA-06285 and ES-00263.
2Paper No. 2503, Oregon Agricultural Experiment Station, the third communication of a series on dietary factors and hepatoma in rainbow trout.
3Present address: Biological Research Division, R. J. Reynolds Tobacco Co. Co., Winston-Salem, N. C.
cyclopropenoids when fed with AAF, and to study the effect of epoxycarbons, sesame seed oil, and autoxidized salmon oil on aflatoxin-induced hepatomas.

MATERIALS AND METHODS

The fish used in this study were hatched in our laboratory from rainbow trout eggs obtained from the Mt. Shasta brood stock of the California Department of Fish and Game. The fry were fed the control diet for 90 days before initiation of the experimental diets. The diets were formulated by adding CPFA and aflatoxin B₁ to the lipid portion of the semipurified diet described by Lee et al. (8). The AAF was incorporated into the diet by feeding the oxidized oil with and without 4 ppb aflatoxin B₁.

Aflatoxin B₁ was produced by culturing Aspergillus flavus (ATCC 15517) on rice. The toxin was purified according to the method of Shotwell et al. (17).

The Hibiscus syriacus and S. foetida oils were extracted from the seed of the plant. Extracted oils were analyzed for cyclopropene content using the modified Halphen test described by Bailey et al. (3). The amounts of malvalic and sterculic acids present in the oils were determined by preparing methyl esters (10) and selectively hydrogenating to the corresponding cyclopropanes by the method of Roehm (15). Percentages of component fatty acids were determined by gas-liquid chromatography using an Aerograph Model 600-B Hi-Fi gas chromatograph with a flame ionization detector with a 12 foot x 1/8 inch aluminum column packed with 10% diethyleneglycol succinate on Celite (Johns-Manville Corporation).

The synergism between CPFA and aflatoxin B₁ was investigated by feeding S. foetida oil at dietary levels of either 56 or 112 ppm CPFA. H. syriacus oil was fed at a level to supply 210 ppm CPFA in the diet. Two compounds fed in addition to the cyclopropenoids were gossypol and AAF. The possible role of gossypol-acetic acid (200 ppm) and CPFA (H. syriacus oil) in modifying the development of hepatoma was investigated by feeding these compounds with 4 ppb aflatoxin B₁. The sensitivity of the trout to AAF and the possible effect of CPFA on tumor development was determined by feeding 100 ppm AAF with and without H. syriacus oil.

Sesame seeds were ground and extracted at room temperature with hexane. The content of sesamol or other cyclic components in the oil was not measured. The effect of sesame seed oil in the diet of trout was investigated by replacing the 5% corn oil in the control diet with 5% sesame oil. Possible cocarcinogenicity was investigated by incorporating 4 ppb aflatoxin B₁ into the sesame seed oil diet.

Vernonia anthelmintica seeds were ground and extracted with pentane at room temperature. The levels of epoxycarbons in the oil were determined by the oxirane oxygen method described in the American Oil Chemist's Society method Cd 9-57. The response of rainbow trout to an epoxide was investigated by adding 0.033% V. anthelmintica oil to the control diet. This supplied 200 ppm and was fed with and without 4 ppb aflatoxin B₁.

The role of oxidized oil in carcinogenesis was evaluated by replacing the 5% fresh salmon oil in the control diet with 5% salmon oil air oxidized to a peroxide value of 200–230. As an added stress, the vitamin E supplement was omitted from the diet, although the 5% corn oil supplied considerable amounts of tocopherols. Possible cocarcinogenic activity was investigated by feeding the oxidized oil with and without 4 ppb aflatoxin B₁.

After 3, 6, 9, 12, and 15 months on the experimental diets, 10 fish were randomly sampled and examined for gross and microscopic abnormalities in the manner previously described (19). When the incidence of hepatoma in a group of fish approached 100%, they were killed and weighed, liver weights were recorded, and the total volume of tumor tissue was calculated. In calculating total volume of tumor tissue, each individual was treated as a sphere.

RESULTS AND DISCUSSION

Analysis of the S. foetida and H. syriacus seed oils used in this study showed them to contain 56% and 21% CPFA respectively. The oil from S. foetida seeds contained 49% sterculic and 7% malvalic acids. Two percent sterculic and 19% malvalic acids were present in the H. syriacus oil. The V. anthelmintica oil contained 61% epoxycarbons.

Results of the feeding trials presented in Table 1 clearly show the increased carcinogenicity of aflatoxin B₁ when CPFA is included in the diets. Diets 3 through 9 were terminated after 9% to 10 months because of the high incidence of hepatoma. In comparison, fish on 4 ppb aflatoxin B₁ with no CPFA (Diets 2) had only a 40% incidence at 9 months and 50% incidence at 12 months. The low incidence of hepatoma found in groups receiving CPFA but no aflatoxin cannot be readily explained. Eighteen of 385 fish receiving CPFA but no aflatoxin (Diets 3, 5, and 7) had liver tumors for a 5.6% incidence. A possible explanation for this low incidence is that the cyclopropenoids themselves are weak carcinogens in rainbow trout. However, in a previous experiment, trout were fed CPFA for as long as 20 months without tumor development (18). Another hypothesis is that a small amount of a carcinogenic substance, possibly aflatoxin, was present in one of the diet's ingredients, e.g., the corn oil. In a study of the aflatoxin content in contaminated vegetable oils, Parker and Melnick (12) found that alkali refining and washing of a highly contaminated oil would reduce aflatoxin B₁ levels to 10 to 14 ppb. Present-day bleaching processes were reported (12) to further reduce the level to less than a few ppb.

The oil used in this work was of the unbleached type and was consumed before analytical procedures for aflatoxin could be initiated. Five percent of such a contaminated unbleached corn oil could conceivably contribute 0.5–0.7 ppb aflatoxin to the diet. The results of this study would indicate that less than 0.4 ppb aflatoxin would produce such an incidence. As in any feeding trial, the chance of an error in feeding a carcinogenic diet cannot be overlooked, but this possibility seems remote since animals on 3 separate treatments developed tumors. Finally, the possibility that the oil seeds used as sources for CPFA were contaminated with aflatoxin was considered. This seems an unlikely explanation, as clean, bright seeds were extracted with hexane, a solvent in which...
D. J. Lee, J. H. Wales, J. L. Ayres, and R. O. Sinnhuber

Table 1

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Diet description</th>
<th>Hepatoma No.</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0/50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control + 4 ppb aflatoxin B₁</td>
<td>10/20</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Control + 0.02% Sterculia foetida oil</td>
<td>12/116</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Control + 0.02% Sterculia foetida oil + 4 ppb aflatoxin B₁</td>
<td>57/58</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>Control + 0.01% Sterculia foetida oil</td>
<td>1/136</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>Control + 0.01% Sterculia foetida oil + 4 ppb aflatoxin B₁</td>
<td>112/112</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>Control + 0.10% Hibiscus syriacus oil</td>
<td>5/133</td>
<td>3.7</td>
</tr>
<tr>
<td>8</td>
<td>Control + 0.10% Hibiscus syriacus oil + 4 ppb aflatoxin B₁</td>
<td>119/119</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>Control + 0.10% Hibiscus syriacus oil + 200 ppm gossypol + 4 ppb aflatoxin B₁</td>
<td>117/119</td>
<td>98</td>
</tr>
<tr>
<td>15 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0/50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control + 4 ppb aflatoxin B₁</td>
<td>65/108</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>Control + 0.4 ppb aflatoxin B₁</td>
<td>15/106</td>
<td>14</td>
</tr>
<tr>
<td>11</td>
<td>Control + 0.10% Hibiscus syriacus oil + 0.4 ppb aflatoxin B₁</td>
<td>42/105</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>Control + 100 ppm acetylaminofluorene</td>
<td>0/95</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Control + 0.10% Hibiscus syriacus oil + 100 ppm 2-acetylaminofluorene</td>
<td>20/102</td>
<td>20</td>
</tr>
</tbody>
</table>

Hepatoma incidence in rainbow trout fed carcinogenic diets with and without cyclopropenoid fatty acids.

*a* Experiments 3 through 9 were terminated at 9½ months.

*b* Sterculia foetida oil contained 49% sterculic and 7% malvalic acids.

*c* Hibiscus syriacus oil contained 19% malvalic and 2% sterculic acids.

Aflatoxin B₁ is insoluble, and aflatoxin analyses of the extracted seeds were negative. With the highest level of *H. syriacus* oil fed being 1000 ppm and *S. foetida* oil being 200 ppm, the aflatoxin B₁ content of these oils needed to produce these results would be 100—200 and 500—1000 ppb respectively. Experiments are underway to determine if purified methyl esters of CPFA function as synergists with aflatoxin B₁ and to investigate their possible role as weak carcinogens.

Gossypol fed with both CPFA and aflatoxin B₁ (Diet 9) and CPFA plus aflatoxin (Diet 8) produced 98% and 100% incidence of hepatoma respectively. Although there was no difference in incidence of hepatoma between diets, the addition of 200 ppm gossypol inhibited tumor growth. As shown in Table 2, average node size was 176 and 42 cu mm for Diets 8 and 9 respectively. The nature of this retardation of tumor growth is not known. Two hundred ppm gossypol did not inhibit growth of the fish.

Table 2 illustrates another aspect of cyclopropenoid involvement in carcinogenesis. The tumors found in livers of fish receiving CPFA and aflatoxin were much larger than in fish on aflatoxin alone. After 15 months, trout fed 0.4 ppm aflatoxin B₁ (Diet 10) exhibited a 14% incidence of hepatoma with an average volume/node of 123 cu mm. When *H. syriacus* oil, supplying 210 ppm CPFA, was added to this diet (Diet 11), the incidence increased from 14 to 40%, and the average size of the liver nodes increased to 434 cu mm. The fish receiving 4 ppb aflatoxin B₁ with cyclopropenoids (Diets 4, 6, 8, and 9) also showed increased tumor growth over the positive control.

Sterculic acid apparently promoted more rapid liver tumor growth in the trout than did malvalic acid. *S. foetida* oil supplying 93 ppm sterculic acid and 14 ppm malvalic acid produced the same size tumors as did *H. syriacus* oil, which provided 20 ppm sterculic and 190 ppm malvalic acids. Allen et al. (1) reported that sterculic acid was a more effective inhibitor of the liver fatty acid desaturase system, indicating a variation in the physiologic activity of these two compounds. Studies to determine if a difference in the ability to promote tumor growth exists between these two cyclopropenoid compounds are in progress. It should be noted, however, that if malvalic acid has only one-half the tumor growth-promoting activity of sterculic, it is still a serious consideration in the consumption of cottonseed products. Jackson et al. (6), in work carried out at our laboratory, reported that feeding a contaminated foodgrade cottonseed flour which supplied 0.4 ppb aflatoxin B₁ and 100 ppm CPFA (malvalic acid) to the diet resulted in a 20% incidence of hepatoma in rainbow trout after 9 months. Earlier feeding trials with a similar brand of cottonseed flour were negative (18).

Feeding AAF at 100 ppm (Diet 12) did not produce liver tumors in trout during the 15 months feeding trial, indicating that it is a very weak carcinogen toward this species. This result was not expected since Halver (10) reported a 5—15% incidence with 150 ppm after 12 months. In contrast, when 210 ppm CPFA were fed together with 100 ppm AAF (Diet 13), 20% of the fish had hepatoma after 15 months. These results demonstrate the ability of CPFA to act synergistically with carcinogenic compounds other than aflatoxin B₁.

The results of feeding epoxyoleic acid (in the triglycerides of *V. anthelmintica* oil) are presented in Table 3, along with data from feeding trials with sesame seed oil and oxidized salmon oil. None of these dietary compounds altered the number or growth of liver tumors from that found in the positive control. Unusual lipid deposits were found in the liver tissue of some of the fish receiving sesame seed oil (Fig. 1). These occurred in the normal liver parenchyma of fish receiving aflatoxin as well as those fed diets without aflatoxin. The tumors did not contain lipid droplets regardless of the diets. Aside from the histologic features just mentioned, the liver tissues of groups listed in Table 3 were essentially similar.

Cyclopropenoid triglycerides containing sterculic and malvalic fatty acids in the diet of rainbow trout cause cytotologic damage of liver parenchymal cells characterized by an accumulation of lipid globules and the development of unique "fibers" within these cells (Figs. 2—4). The typical features of this degeneration appear to be proportional to the dietary level of the cyclopropene, and they are transitory. In the rainbow trout the peak may be reached at about 2—3 months, after which the damaged cells are replaced by a new generation. Although the presence of cyclopropene in the diet can be detected by the cellular inclusions noted above, the individual variation in reaction of the animals, in extent and in time, tend to confuse an analysis of dietary level of these fatty acids. The alterations in liver cytology induced by *H. syriacus* oil and by *S. foetida* oil were essentially alike. Diet No. 7 (control diet with 0.1% *H. syriacus* oil but no aflatoxin) produced addi-
### Table 2

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Description</th>
<th>Cyclopropenoid content</th>
<th>Hepatoma incidence</th>
<th>Total No. of nodes</th>
<th>Average node size (cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0</td>
<td>0/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.4 ppb aflatoxin B₁</td>
<td>0</td>
<td>8/20</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>4 ppb aflatoxin B₁</td>
<td>112</td>
<td>57/58</td>
<td>127</td>
<td>336</td>
</tr>
<tr>
<td>6</td>
<td>4 ppb aflatoxin B₁</td>
<td>56</td>
<td>112/112</td>
<td>299</td>
<td>66</td>
</tr>
<tr>
<td>8</td>
<td>4 ppb aflatoxin B₁</td>
<td>210</td>
<td>119/119</td>
<td>215</td>
<td>176</td>
</tr>
<tr>
<td>9</td>
<td>4 ppb aflatoxin B₁ + 200 ppm gossypol HAc</td>
<td>210</td>
<td>117/119</td>
<td>199</td>
<td>42</td>
</tr>
<tr>
<td>15 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0</td>
<td>0/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.4 ppb aflatoxin B₁</td>
<td>0</td>
<td>65/108</td>
<td>310</td>
<td>202</td>
</tr>
<tr>
<td>10</td>
<td>0.4 ppb aflatoxin B₁</td>
<td>0</td>
<td>15/106</td>
<td>15</td>
<td>123</td>
</tr>
<tr>
<td>11</td>
<td>0.4 ppb aflatoxin B₁</td>
<td>210</td>
<td>42/105</td>
<td>53</td>
<td>434</td>
</tr>
<tr>
<td>12</td>
<td>100 ppm 2-acetylaminofluorene</td>
<td>0</td>
<td>0/95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>100 ppm 2-acetylaminofluorene</td>
<td>210</td>
<td>20/102</td>
<td>23</td>
<td>182</td>
</tr>
</tbody>
</table>

Hepatoma incidence and liver tumor size in fish fed carcinogenic diets.

*Experiments 4, 6, 8, and 9 were terminated at 9½ months.*

### Table 3

<table>
<thead>
<tr>
<th>Diet No.</th>
<th>Description</th>
<th>Hepatoma Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Control + 0.033% Vernonia anthelmintica oil</td>
<td>0/102</td>
</tr>
<tr>
<td>15</td>
<td>Control + 0.033% Vernonia anthelmintica oil + 4 ppb aflatoxin B₁</td>
<td>62/103</td>
</tr>
<tr>
<td>16</td>
<td>Control with 5% sesame seed oil</td>
<td>1/117</td>
</tr>
<tr>
<td>17</td>
<td>Control with 5% sesame seed oil + 4 ppb aflatoxin B₁</td>
<td>60/113</td>
</tr>
<tr>
<td>18</td>
<td>Control with 5% oxidized salmon oil and low vitamin E&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0/104</td>
</tr>
<tr>
<td>19</td>
<td>Control with 5% oxidized salmon oil, low vitamin E, and 4 ppb aflatoxin B₁</td>
<td>51/102</td>
</tr>
</tbody>
</table>

Hepatoma incidence in trout fed unusual lipids in aflatoxin-containing diets for 12 months.

*Vernonia anthelmintica oil supplied 200 ppm 12,13-epoxyoleic acid.*

*Source of vitamin E was the 5% corn oil in the diet.*

### Acknowledgments

The authors express their thanks to Dr. L. A. Goldblatt, Southern Utilization Research and Development Division, U.S. Department of Agriculture, New Orleans, Louisiana, for the gift of gossypol-acetic acid; Bumble Bee Seafoods, Astoria, Oregon, for the salmon oil; Distillation Products, Rochester, New York, for the vitamin E; Dr. R. A. Phelps, Anderson, Clayton & Co., Houston, Texas, for the sesame seeds; and Dr. I. A. Wolff, Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Illinois, for the Vernonia anthelmintica seeds. We also thank Mrs. L. J. Hunter, Theodore Will, and Richard A. Foster, Jr. for their excellent technical assistance.

### References

D. J. Lee, J. H. Wales, J. L. Ayres, and R. O. Sinnhuber


---

Fig. 1. Section of liver of trout fed Diet No. 15 containing 5% sesame seed oil (no aflatoxin). Note lipid globules present in most livers of this dietary group. H & E, × 128.

Fig. 2. Section of liver of trout fed Diet No. 7 containing 0.1% Hibiscus syriacus oil (no aflatoxin). Note random mixture of degenerating parenchymal cells containing lipid globules and "fibers," and islets of deeply basophilic regenerating cells. Arrow indicates cell with "fibers" and lipid shown in Fig. 3. H & E, × 128.

Fig. 3. Detail of Fig. 2 showing degenerating parenchymal cells containing lipid globules and fibers characteristic of diets containing cyclopropenoid fatty acids. H & E, × 320.

Fig. 4. Section of liver of fish fed Diet No. 7 containing 0.1% Hibiscus syriacus oil (no aflatoxin) showing peribiliary deposit of unidentified material together with hyperplastic connective tissue and pseudo bile ducts (arrow). H & E, × 128.

Fig. 5. Section of liver of trout fed Diet No. 19 containing 5% oxidized salmon oil and 4 ppb aflatoxin B₁, showing edge of typical, small hepatoma node (lower). The liver tissue (upper) are similar to those found in trout fed unoxidized salmon oil (Diet No. 1). H & E, × 320.

Fig. 6. Section of liver of trout fed Diet No. 9 containing 0.1% Hibiscus syriacus oil, 200 ppm gossypol-acetate, and 4 ppb aflatoxin B₁. Typical hepatoma (lower) and injured nonneoplastic liver (above). Arrow indicates characteristic, enlarged parenchymal cell filled with lipid globules and "fibers." Regenerating parenchyma appear as scattered, small basophilic islets. H & E, × 128.

Fig. 7. Interior of relatively large (15 mm diameter) hepatoma tumor showing typical, broad cords. Fish had received Diet No. 13, 100 ppm 2-acetylaminofluorene and 1000 ppm Hibiscus syriacus oil for 12 months. This tumor is indistinguishable from tumors of the same size produced by aflatoxin. H & E, × 128.

Fig. 8. Section of liver of fish fed diet No. 3 containing 0.02% Sterculia foetida oil without added aflatoxin. Arrows indicate margin of typical "spontaneous" hepatoma node (lower) distinguishable from the deeply staining normal liver tissue (above) by its much broader cords, smaller cells, and relative absence of blood vessels. This tumor is similar to tumors of the same stage of development in diets such as No. 2 (4 ppb aflatoxin B₁). H & E, × 128.

2316 CANCER RESEARCH VOL. 28
Fatty Acids and Carcinogens in Trout

1

2

3

4
Synergism between Cyclopropenoid Fatty Acids and Chemical Carcinogens in Rainbow Trout (Salmo gairdneri)

D. J. Lee, J. H. Wales, J. L. Ayres, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/28/11/2312

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/28/11/2312.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.