Initiation, Promotion, and Inhibition of Carcinogenesis in Rainbow Trout

by George Bailey,* Daniel Selivonchick,* and Jerry Hendricks*

The identification of etiological agents in feral fish neoplasia epizootics has been hampered in part by the lack of suitable fish models, and complicated by the likely existence of environmental agents which can act to stimulate or reduce population responses to genotoxin insult. The response of fish to tumor inhibitors and promoters, and the underlying mechanisms of modulation, have been studied in the rainbow trout model. Dietary treatment of trout with the compounds indole-3-carbinol (I3C), β-naphthoflavone (BNF), or the polychlorinated biphenyl (PCB) complex Aroclor 1254, before and during exposure to aflatoxin B1 (AFB1), was shown to reduce the final incidence of hepatocellular carcinoma after 12 months, compared to fish receiving AFB1 only. By contrast, treatment of trout with BNF or I3C following AFB1 initiation led to a significant enhancement of ultimate tumor response. Similarly, simultaneous treatment of trout with PCB and the carcinogen N-nitrosodiethylamine led to syncarcinogenic enhancement, rather than inhibition, of tumor response.

Mechanisms of inhibition of AFB1 carcinogenesis by PCB, BNF, and I3C were investigated. PCB and BNF, but not I3C, are known to be strong inducers of trout cytochrome P450 and associated activities. Dietary induction by BNF or PCB was shown to be accompanied in isolated hepatocytes by considerably altered AFB1 metabolism, and by significantly reduced rates of DNA adduct formation for all three agents. All agents differentially altered in vivo AFB1 pharmacokinetics, enhanced bile elimination of AFB1 as the aflatoxicol-M1 glucuronide, and significantly reduced peak levels of liver DNA adduct formation. No effects were seen on repair of AFB1-DNA adducts, which was very slow in trout. Detailed studies demonstrated that glutathione detoxication of the AFB1-2,3-oxide is not a significant pathway in trout fed control or inhibitor diets. The precise means by which I3C reduces adduct formation are presently unclear.

Introduction

Aquatic pollutants including polycyclic aromatic hydrocarbons and nitrogen heterocycles have been associated with elevated incidences of neoplasia in feral fish populations (1-3). However, direct demonstration of causal relationships remain to be established. While a number of studies have demonstrated the responsiveness of laboratory fish to carcinogens, most studies have concentrated on carcinogens such as aflatoxin B1 (AFB1) and N-nitrosodiethylamine (DEN), which have more relevance to neoplasia in man than in feral fish (4,5). An exception is the environmental pollutant benzo(a)pyrene (BP), which was recently shown to be carcinogenic to rainbow trout (6).

Identification of etiological agents in feral fish neoplasia is further complicated by the almost certain presence of compounds which may act as modulators (promoters, inhibitors, cocarcinogens) of environmental carcigenic response. Recent studies have begun to examine the possible effects on tumor response and mechanisms of action of carcigenesis modulators in fish.

We review here the studies of carcigenesis and its modulation in a convenient laboratory fish model, the rainbow trout. Most of these studies have examined modulation of AFB1 hepatocarcigenesis by polychlorinated biphenyls (PCBs) and other agents. AFB1, a highly potent mycotoxin, was initially identified as a human carcigen following its discovery as the etiological agent in outbreaks of hepatocellular carcinoma in trout hatcheries (7). Though it is a dietary rather than an aquatic pollutant, studies on AFB1 carcigenesis modulation in trout by PCB and other agents do serve to indicate the extent to which carcigenesis by genotoxic pollutants may be modulated in fish, and the possible mechanisms through which modulation can occur. Clearly, there is a need for similar studies using BP and other carcigenes directly relevant to the aquatic environment.

Tumor Studies in Trout and Other Laboratory Fish Models

The response of rainbow trout and other laboratory fish models to known mammalian carcigenes has been recently reviewed (4,5,8,9), and will only be summa-
rized here. Carcinogen classes tested have included: my-
cotoxins; polycyclic aromatic hydrocarbons (PAHs); ni-
trosamines, nitrosamides, and other alkylating agents;
heterocyclic amines; steroids and cyclic lipids; chlori-
nated hydrocarbon pesticides; and azo compounds. Sev-
eral species of small aquarium fish have been tested for
carcinogen response. Early studies of the zebra danio
(Brachydanio rerio) and the guppy (Lebistes reticula-
tus) (10–17), and later experiments with the Japanese
medaka (Oryzias latipes), the species Poeciliopsis,
and other aquarium fish (18–22), have revealed liver to be
a major target organ and hepatocellular carcinoma to
be the most prevalent tumor type in fish carcinogenic
response. Hepatocellular carcinoma is also the most
common neoplastic response in trout (9, 24–28), though
other neoplasms, including nephroblastoma, gastric and
swim bladder papillary adenoma, and fibrosarcoma can
be induced, depending on carcinogen used, exposure
route, and dose. For example, dietary exposure to N-
methyl-N'-nitro-N-nitrosoguanidine (MNNG) induces
only gastric papillary adenomas in rainbow trout, whereas
embryonic or fry water exposure to a solution of
MNNG also induces hepatocellular carcinoma, neph-
roblastoma, and swim bladder papillary adenoma (8).
The malignancy of some of these neoplasms has been
demonstrated by transplantation into isogenic hosts (29,
and Hendricks and Bailey, unpublished results). A sum-
mary of all reported tissue lesions in aquatic animals
resulting from controlled xenobiotic exposures has re-
cently been compiled (30), and the histological progres-
sion of liver neoplasms in rainbow trout has been re-
viewed (31,32).

Modulation of Carcinogenesis in
Rainbow Trout

The response of rainbow trout to modulators of car-
cinogenesis depends on the initiator (carcinogen) used,
the nature and dose of the modulator, and the relative
timing of initiator and modulator exposure. As shown in
Table 1, experiment 1, dietary treatment with the
synthetic BNF or the naturally occurring indole I3C
before, during, and after AFB1 exposure reduces the
tumor incidence of hepatocellular carcinoma at 12
months, compared to controls receiving AFB1 alone.
This effect is also seen if I3C or BNF exposure is re-
stricted to the period before and during AFB1 treat-
ment. By contrast, exposure to these modulators after
AFB1 initiation significantly enhances tumor response
(experiment 4). Several additional compounds, includ-
ing 17-β-estradiol, DDT, and cyclopropenoid fatty acids,
can promote hepatocellular carcinoma and other tumor
types, following initiation by AFB1 or other carcinogens
in trout (25,36,37, and Hendricks and Bailey, unpub-
lished results).

The ability of compounds to inhibit carcinogenesis in
troun can be observed over a range of carcinogen doses.
Experiment 2, Table 1, shows that coexposure of trout
to AFB1 and the PCB Aroclor 1254 results in a parallel
shift in the AFB1 dose-response curve toward higher
AFB1 dose. Higher doses of Aroclor have been shown
to provide more extensive inhibition of AFB1 carcino-
genesis (38). However, PCBs do not provide universal
protection against all carcinogens in trout. As shown in
experiment 3, coexposure to Aroclors 1254 or 1242 ac-
tually enhances DEN hepatocarcinogenesis in trout.
In this context it is interesting that the incidence of neo-
plasias in English sole in the Puget Sound shows a neg-
ative correlation with PCB levels in the sediment (39),
suggesting that the initiator(s) of these tumors may be
susceptible to PCB anticarcinogenesis rather than en-
hancement. It is important to note that PCBs have not
proven to be carcinogenic to trout, or to any other fish
species to our knowledge (e.g., experiment 2 PCB con-
trol), nor have we yet demonstrated promotion of car-
cinogenesis in trout by these compounds.

Mechanisms of Tumor Modulation
in Trout

The tumor results outlined in Table 1 indicate that
modulation of the carcinogenic process can occur during
the initiation or post-initiation (promotional) phases of
tumorigenesis in fish, as in mammalian test systems.
Mechanisms of promotion of carcinogenesis in trout are
currently poorly understood, and are under active in-
vestigation in our laboratory. By comparison, mecha-
nisms of anticarcinogenesis involving the initiation pro-
cess have been more thoroughly studied in this model
fish system. For example, several previous studies have
demonstrated that PCBs and BNF (33,40–43), but not
I3C (44), are potent inducers of cytochrome P448 and
associated hepatic mixed function oxidase activities in
troun. The following studies examined the extent to
which these responses could be correlated with altered
AFB1 pharmacokinetics in vivo, cellular activation and
detoxication reactions, and formation and/or persistence
of liver DNA adducts, as possible mechanisms for in-
hibition during the initiation phase of carcinogenesis.

The effects of dietary modulators on cellular carcin-
genesis metabolism and mutagenesis can be readily as-
sessed using freshly isolated trout hepatocytes in short-
term culture (40,45–47). Hepatocytes have been is-
olated from trout prefed control diet or diet containing
appropriate levels of BNF, PCB, or I3C, and incubated
under standard conditions with [3H]AFB1. The results
of these studies are summarized in Table 2. The known
enzyme-inducing behavior of dietary BNF and PCB is
accompanied by major changes in AFB1 metabolism.
Both compounds cause a massive increase in the pro-
duction of the relatively less carcinogenic metabolite
afatoxin M1 (AFM1), a substantial decrease in the pro-
duction of the highly carcinogenic afatoxicol (AFL), and
a decrease of 40 to 50% in the rate of formation of DNA
adducts, compared to control hepatocytes. The comp-
ounds were not equivalent, however, since only PCB
elevated the hourly rate of total AFB1 metabolism. By
comparison, dietary pretreatment with I3C caused little
perturbation in production of AFB1 metabolites, but did cause a significant decrease in DNA adduct formation. Thus the three anticarcinogens appear capable of reducing initial AFB1-DNA damage at the cellular level, but not by the same mechanisms.

At the whole organism level, alterations in cellular carcinogen metabolism, along with modified transport processes or other effects, may operate to alter carcinogen distribution and elimination kinetics. The effects of PCB, I3C, and BNF on AFB1 pharmacokinetics in trout have been extensively studied (48,49, unpublished results). Figure 1 summarizes some of the results of these studies. The major effect of all three inhibitors is a significant increase in vivo in the rate of elimination of AFB1 polar metabolites in bile, with I3C showing the smallest effect. (Effects of these dietary compounds on another potentially important pharmacokinetic pool, urine, were not included and are currently under study in our laboratory.) As in the hepatocyte studies, effects of these compounds were not identical, but were studied at only one dose each, that showing inhibition in the tumor studies. Though pharmacokinetic studies are highly laborious, it would be interesting to know if differences reported here are consistently maintained over a range of dietary concentrations of the three anticarcinogens.

The structures of the AFB1 metabolites found in rainbow trout bile have been previously established in our laboratory, and found to consist almost entirely of glucuronides of AFB and AFL-M1 (50). Bile collected from the same fish depicted in Figure 1 was analyzed on HPLC as published (50). As summarized in Figure 2, alterations in the levels of bile radioactivity in fish fed control, BNF, PCB, and I3C diets were accounted for by increases in AFL-M1 glucuronide, with I3C again showing the smallest effect.

### Table 1. Modulation of hepatocarcinogenesis in rainbow trout by tumor promoters and inhibitors.

| Experiment | Exposure protocol | Tumor incidence (%) | Reference |
|------------|------------------|---------------------|-----------|
| 1. Inhibition, varying dose of inhibitor | AFB1 (dietary, 20 ppb, days 57–66) | 45/118 (38) | (35) |
| | AFB1, plus BNF (50 ppm, days 1–114) | 21/117 (18) | |
| | AFB1, plus BNF (500 ppm, days 1–114) | 7/120 (6) | |
| | AFB1, plus I3C (1000 ppm, days 1–114) | 5/118 (4) | |
| | Control diet | 0/118 (0) | |
| 2. Inhibition, varying dose of carcinogen | AFB1 (1 ppb, 12 months) | 27/121 (22) | (34) |
| | AFB1 (4 ppb, 12 months) | 68/126 (64) | |
| | AFB1 (8 ppb, 12 months) | 98/118 (83) | |
| | AFB1 (1 ppb) plus Aroclor 1254 (50 ppm) | 14/120 (12) | |
| | AFB1 (4 ppb) plus Aroclor 1254 (50 ppm) | 38/122 (51) | |
| | AFB1 (8 ppb) plus Aroclor 1254 (50 ppm) | 88/118 (75) | |
| | Aroclor 1254 (50 ppm) | 0/180 (0) | |
| | Control diet | 0/120 (0) | |
| 3. Cocarcinogenesis, different carcinogen | DEN (1100 ppm, 12 months) | 12/118 (10) | (25) |
| | DEN (1100 ppm) plus Aroclor 1242 | 37/92 (40) | |
| | DEN (1100 ppm) plus Aroclor 1254 | 25/116 (22) | |
| 4. Inhibition or promotion, with timing of exposure varied | AFB1 (20 ppb, weeks 9–12) | 9/99 (9) | (5) |
| | AFB1, plus BNF (500 ppm, weeks 1–12) | 1/100 (1) | |
| | AFB1, plus BNF (500 ppm, weeks 13–24) | 30/100 (30) | |
| | AFB1, plus I3C (2000 ppm, weeks 1–12) | 1/66 (1) | |
| | AFB1, plus I3C (2000 ppm, weeks 13–24) | 51/100 (51) | |

*AFB1 = Aflatoxin B1, BNF = naphthoflavone, I3C = indole-3-carbinol, DEN = diethylnitrosamine.
*All tumor incidences were taken 12 months after initiation commenced.

### Table 2. AFB1–DNA binding and metabolite distribution in hepatocytes from trout fed control and inhibitor diets.

| Diet* | Relative distribution of recovered unbound isotopeb | DNA adducts* | Reference |
|-------|-----------------------------------------------|-------------|-----------|
|       | Polar | AFB1–M1 | AFL | AFB1 |       |
| **Experiment 1** | | | | | |
| Control | 21 (12) | 2.1 (.5) | 18 (4.3) | 45 (20) | 1.52 (.19)c | (46) |
| BNF | 29 (8.3) | 31 (9) | 6.2 (2.2) | 33 (18) | 0.96 (.4) | |
| **Experiment 2** | | | | | |
| Control | 3.6 (.6) | 1.6 (.3) | 11 (1.2) | 84 (1.5) | 1.13 (.13) | (48) |
| PCB | 5.8 (.5) | 27 (9) | 5.2 (.9) | 62 (2.9) | 0.66 (.19) | |
| **Experiment 3** | | | | | |
| Control | 2.6 (.4) | 1.8 (.2) | 8.1 (.6) | 86 (1) | 0.96 (.06) | (49) |
| I3C | 2.6 (.3) | 3.8 (.9) | 7.8 (5.5) | 85 (1) | 0.67 (.03) | |

*Diets were 500 ppm BNF for 6 weeks, 100 ppm Aroclor 1254 for 12 weeks, or 2,000 ppm I3C for 7 weeks. n = 7, 5, and 9 for each dietary group in experiments 1, 2, and 3, respectively.
*Expressed as a percent of total isotopes recovered as aflatoxins from HPLC.
*Expressed as µmole AFB1 adduct/m DNA/mg AFB1 metabolized. All values in parentheses represent (± SEM).
We were concerned that our data did not indicate directly the extent of involvement of glutathione (GSH) conjugation as a detoxication pathway for AFB1 in trout. This reaction may be especially significant since it traps the activated AFB1-2,3-epoxide and prevents its interaction with DNA. Since only a small percentage of ingested AFB1 (<13%) forms liver DNA adducts, inhibitor-mediated alterations in even a minor amount of detoxication by this pathway could be significant. This reaction has further been implicated as significant in anticarcinogenesis and species differences to AFB1 (51,52). To investigate more directly the possible role of this pathway in trout AFB1 carcinogenesis and its modulation, authentic AFB1-GSH conjugate was synthesized and used as an HPLC marker (53). Table 3 depicts the most significant results of these studies. Formation of AFB1 was found not to exceed 1% of total AFB1-derived metabolites in bile of trout fed control...
or inhibitor diets (Table 3), nor could it be produced in \textit{in vitro} incubations using trout microsomes from any source, under conditions where mouse microsomes (or mouse-trout mixtures) produced large quantities of the conjugate (53). Further, we were able to demonstrate that diethyl maleate treatment could substantially deplete GSH levels in trout and coho salmon hepatocytes without significant effect on AFB1-DNA adduct formation (data not shown). We conclude that GSH conjugation is not an important constitutive or inducible pathway for AFB1 detoxication in rainbow trout. The involvement of this detoxication pathway for benzo(a)pyrene is under investigation in this species (Varanasi, personal communication).

The final questions to be investigated were whether the inhibitor-mediated reduction in DNA adduct formation observed in incubations with isolated hepatocytes would also be seen \textit{in vivo}, and whether inhibitors might alter overall adduct persistence or repair. Liver nuclei were isolated from fish fed control, PCB, or I3C diets, and the DNA purified for determination of total level of adducts at various times after exposure. The results are depicted in Figure 3. As previously observed, peak adduct formation occurred in control trout 24 to 48 hr after AFB1 exposure. Dietary pretreatment with each of the inhibitors significantly reduced the level of peak adduct formation compared to control. [Only the 24-hr data point has been studied for BNF, with a reduction of adducts to 44% of control (8)]. Surprisingly, I3C, the inhibitor with the weakest effects on enzyme

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induction, hepatocyte metabolism, or bile conjugate stimulation, had the strongest effect on reduction of DNA adduct formation in vivo. In no case was there any indication that these compounds alter the persistence of DNA adducts in vivo. Indeed, as previously reported (8), AFB1-DNA adducts were unusually persistent in trout liver compared to rates of rodent repair. This repair deficiency may account in part for the high sensitivity of this species to AFB1 carcinogenesis.

Summary

Chemical pollutants are thought to be responsible for tumor epizootics in a number of feral fish populations, but specific etiological agents have yet to be identified. Tumor studies in laboratory fish models provide one approach toward identification of carcinogens, and of environmental parameters which may influence the response of fish to genotoxins. Extensive evidence from tumor studies in mammalian models, and in the rainbow, suggest that a range of nongenotoxic dietary and environmental agents may intervene in the carcinogenesis process, to act as stimulators or inhibitors of tumor response. Although studies of tumor modulation with aquatic carcinogenic pollutants in fish models have not been conducted, extensive studies of modulation of AFB1 carcinogenesis in the rainbow trout may serve as a model for understanding mechanisms of modulation in fish. Studies in this system have shown that (a) a wide range of compounds can act as inhibitors, promoters, and co-carcinogens in trout; (b) particular modulators can act alternatively as inhibitors or promoters, depending on the carcinogen used and the relative timing of carcinogen and modulator exposure; (c) the magnitude of the effect does not appear to depend critically on carcinogens dose, but does depend on modulator dose.

Specific studies on the mechanisms of anti-initiation by BNF, I3C, and PCB for AFB1 carcinogenesis in trout have shown that dietary pretreatment by each of these three modulators leads to reduced initial formation of AFB1-DNA adducts in vivo and in vitro. The precise mechanisms by which this is achieved differ. BNF and PCB inhibit at least in part through induction of cytochrome P448, and associated enhancement of AFM1 and AFL-M1-glucuronide detoxication reactions. PCB also appears to enhance overall rates of AFB1 metabolism in intact trout and in isolated hepatocytes. I3C shows the weakest effects on these pathways but, at the doses studied, had the strongest effects on reducing DNA adduct formation. Addition of I3C itself does not alter AFB1 metabolism or DNA binding in control hepatocytes (49). Hence the mechanism(s) through which I3C-mediated AFB1 binding reduction occurs are not clearly understood, but may involve direct or indirect effects of I3C metabolites on AFB1 metabolism or transport.

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REFERENCES

1. Black, J. J., Evans, E. D., Harshbarger, J. C., and Ziegel, R. F. Epizootic neoplasms in fishes from a lake polluted by copper mining wastes. J. Natl. Cancer Inst. 68: 915–926 (1982).
2. Malins, D. C., Krahn, M. M., Brown, D. W., Rhodes, L. D., Myers, M. S., McCain, B. B., and Chan, S.-L. Toxic chemicals in marine sediment and biota from Mukilteo, Washington: relationships with hepatic neoplasms and other hepatic lesions in English sole (Parophrys vetulus). J. Natl. Cancer Inst. 74: 487–494 (1985).
3. Kimura, I., Taniguchi, N., Kumai, H., Tomita, I., Kinase, N., Yoshizaki, K., Ito, M., and Ishikawa, T. Correlation of epizootiological observations with experimental data: chemical induction of chromatophoromas in the croaker, Nibea mitsukurii. In: Use of Small Fish Species in Carcinogenicity Testing (Natl. Cancer Inst. Monographs, Vol. 65), National Cancer Institute, Bethesda, MD, 1984, pp. 139–154.
4. Hoover, K. L. (Ed.). Use of Small Fish Species in Carcinogenicity Testing (Natl. Cancer Inst. Monographs, Vol. 65) National Cancer Institute, Bethesda, MD, 1984, 409 pp.
5. Bailey, G. S., Goeger, D. E., and Hendricks, J. D. Factors influencing carcinogenesis by polynuclear aromatic hydrocarbons or other genotoxins in laboratory fish models. In: Metabolism of Polynuclear Hydrocarbons in the Aquatic Environment (U. Varanasi, Ed.), CRC Press, Boca Raton, FL, in press.
6. Hendricks, J. D., Meyers, T. R., Shelton, D. W., Casteel, J. L., and Bailey, G. S. Hepatocarcinogenicity of benzo(a)pyrene to rainbow trout by dietary exposure and intraperitoneal injection. J. Natl. Cancer Inst. 74: 839–851 (1985).
7. Wales, J. H., and Sinnhuber, R. O. An early hepatoma epizootic in rainbow trout, Salmo gairdneri. Calif. Fish Game 52: 85–91 (1966).
8. Bailey, G. S., Hendricks, J. D., Nixon, J. E., and Pawloski, N. E. The sensitivity of rainbow trout and other fish to carcinogens. Drug Metab. Rev. 15: 725–750 (1984).
9. Stanton, M. F. Chemical carcinogenesis in fish. In: Aquatic Toxicology, Vol. 1 (L. J. Weber, Ed.), Raven Press, New York, 1982, pp. 149–211.
10. Stanton, M. F. Diethylnitrosamine-induced hepatic degeneration and neoplasia in the aquarium fish, Brachidionus rierio. J. Natl. Cancer Inst. 54: 117–130 (1965).
11. Stanton, M. F. Hepatocarcinomas of aquarium fish exposed to Cycas circinalis. Fed. Proc. Am. Soc. Exp. Biol. 25: 661 (1966).
12. Khudoley, V. V. The induction of hepatic tumors by nitrosamines in aquarium fish (Lebistes reticulatus). Vop. Onkol. 17: 67–72 (1971).
13. Khudoley, V. V. Induction of liver tumors by some azo compounds in aquarium fish (Lebistes reticulatus). J. Ichthyol. 12: 319–324 (1972).
14. Sato, S., Matsushima, T., Tanaka, N., Sugimura, T., and Takashima, F. Hepatic tumors in the guppy (Lebistes reticulatus) induced by aflatoxin B1, dimethyl nitrosamine, and 2-acetylaminofluorene. J. Natl. Cancer Inst. 50: 767–778 (1973).
15. Kimura, M., and Kubota, S. S. Effects of carcinogens on guppy. Proc. Japan. Soc. Sci. Fish. 1: 40 (1972).
16. Matsushima, T., Sato, S., Hara, K., Sugimura, T., and Takashima, F. Bioassay of environmental carcinogens with guppy (Lebistes reticulatus). Mutat. Res. 31: 265 (1975).
17. Plass, G. B., and Khudoley, V. V. Tumor induction by carcinogenic hydrocarbons in aquarium fish. J. Natl. Cancer Inst. 55: 129–136 (1975).
18. Ishikawa, T., Shimamine, T., and Takayama, S. Histologic and electron microscopy observations on diethylnitrosamine-induced hepatomas in small aquarium fish (Oryzias latipes). J. Natl. Cancer Inst. 55: 909–916 (1975).
19. Aoki, K., and Matsudaira, H. Induction of hepatic tumors in a tautog (Oryzias latipes) after treatment with methylazoxymethanol acetate: brief communication. J. Natl. Cancer Inst. 58: 1747–1749 (1977).
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20. Hawkins, W. E., Overstreet, R. M., Fournie, J. W., and Walker, W. W. Development of aquarium fish models for environmental carcinogenesis: tumor induction in seven species. J. Appl. Toxicol. 5: 261–264 (1985).

21. Park, E.-H., and Kim, D. S. Hepatocarcinogenicity of diethyl-nitrosamine to the self-fertilizing hermaphrodite fish Rutilus marmoratus (Teleostomi: Cyprinodontidae). J. Natl. Cancer Inst. 73: 871–876 (1984).

22. Ishikawa, T., and Takayama, S. Importance of hepatic neoplasms in lower vertebrate animals as a tool in cancer research. J. Toxicol. Environ. Health 5: 537–550 (1979).

23. Schultz, R. J., and Schultz, M. E. Characteristics of a fish colony of Poeciliopsis and its use in carcinogenicity studies with 7,12-dimethylbenzanthracene and dimethylnitrosamine. In: Use of Small Fish Species in Carcinogenicity Testing (Natl. Cancer Inst. Monographs, Vol. 65), National Cancer Institute, Bethesda, MD, 1984, pp. 5–13.

24. Sinnhuber, R. O., Hendricks, J. D., Wales, J. H., and Putnam, G. B. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. Ann. N.Y. Acad. Sci. 298: 389–408 (1977).

25. Hendricks, J. D. The use of rainbow trout (Salmo gairdneri) in carcinogen bioassay, with special emphasis on embryonic exposure. In: Phytoxic Approaches to Cancer (C. J. Dawe, et al., Eds.), Japan. Soc. Sci. Fish, pp. 297–300 (1981).

26. Schoenhard, G. L., Hendricks, J. D., Nixon, J. E., Lee, D. J., Wales, J. H., Sinnhuber, R. O., and Pawlowski, N. E. Aflatoxicol induced hepatocellular carcinoma in rainbow trout (Salmo gairdneri). J. Natl. Cancer Inst. 60: 561–564 (1981).

27. Grieco, M. P., Hendricks, J. D., Scanlan, R. A., Sinnhuber, R. O., and Pierce, D. A. Carcinogenicity and acute toxicity of diethylnitrosamine in rainbow trout (Salmo gairdneri) exposed as embryos. J. Natl. Cancer Inst. 64: 1511–1519 (1980).

28. Schultz, M. E., and Schultz, R. J. Transplantable chemically-induced liver tumors in the viviparous fish Poeciliopsis. Exptl. Mol. Pathol. 42: 320–330 (1985).

29. Meyers, T. R., and Hendricks, J. D. A summary of tissue lesions in aquatic animals induced by controlled exposures to environmental contaminants, chemotherapeutic agents, and potential carcinogens. Marine Fish. Rev. 44: 1–17 (1982).

30. Hendricks, J. D., Meyers, T. R., and Shelton, D. W. Histological progression of hepatic neoplasia in rainbow trout (Salmo gairdneri). In: Use of Small Fish Species in Carcinogenicity Testing (Natl. Cancer Inst. Monographs, Vol. 65), National Cancer Institute, Bethesda, MD, 1984, pp. 321–336.

31. Hinton, D. E., Lantz, R. C., Hampton, J. A., McCuskey, P. R., and McCusky, R. S. Normal versus abnormal structure: considerations in morphologic responses of teleosts to pollutants. Environ. Health Perspect. 71: 139–146 (1987).

32. Nixon, J. E., Hendricks, J. D., Pawlowski, N. E., Pereira, C. B., Sinnhuber, R. O., and Bailey, G. S. Inhibition of aflatoxin B1 carcinogenesis in rainbow trout by flavone and indole compounds. Carcinogenesis 5: 615–619 (1984).

33. Shelton, D. W., Hendricks, J. D., Coulombe, R. A., and Bailey, G. S. Effect of dose on the inhibition of carcinogenesis/mutagenesis by Aroclor 1254 in rainbow trout fed aflatoxin B1. J. Toxicol. Environ. Health 13: 649–657 (1984).

34. Shelton, D. W., Hendricks, J. D., and Bailey, G. S. The hepatocarcinogenicity of diethylnitrosamine to rainbow trout and its enhancement by Aroclors 1242 and 1254. Toxicol. Letters 22: 27–31 (1984).

35. Sinnhuber, R. O., Lee, D. J., Wales, J. H., Landers, M. K., and Keyl, A. C. Hepatic carcinogenesis of aflatoxin M1 in rainbow trout (Salmo gairdneri) and its enhancement by cyclopropene fatty acids. J. Natl. Cancer Inst. 53: 1285–1288 (1974).

36. Pawlowski, N. E., Hendricks, J. D., Bailey, M. L., Nixon, J. E., and Bailey, G. S. Structural-bioactivity relationship for tumor promotion by cyclopropenes. J. Agric. Food Chem. 33: 767–770 (1985).

37. Shelton, D. W., Coulombe, R. A., Pereira, C. B., Casteel, J. L., and Hendricks, J. D. Inhibitory effect of Aroclor 1254 on aflatoxin-initiated carcinogenesis in rainbow trout and mutagenesis using a Salmonella/trout hepatic activation system. Aquat. Toxicol. 3: 229–238 (1983).

38. Malins, D. C., McCain, B. B., Myers, M. S., Brown, D. W., Krahm, M. M., Roubal, W. T., Schieve, M. H., Landahl, J. T., and Chan, S.-L. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environ. Health Perspect. 71: 5–16 (1987).

39. Bailey, G. S., Taylor, M. J., Selivonchick, D. P., Eisele, T., Hendricks, J., Nixon, J., Pawlowski, N., and Sinnhuber, R. Mechanisms of dietary modification of aflatoxin B1 carcinogenesis. In: Genetic Toxicology, an Agricultural Perspective (R. Fleck and A. Hollaender, Eds.), Plenum Press, New York, 1982, pp. 149–164.

40. Lech, J. J., and Bend, J. R. Relationship between biotransformation and the toxicity and fate of xenobiotic chemicals in fish. Environ. Health Perspect. 34: 115–131 (1980).

41. Egaas, E., and Varanasi, U. Effects of polychlorinated biphenyls and environmental temperature on in vitro formation of benzo(a)pyrene metabolites by liver of trout (Salmo gairdneri). Biochem. Pharmacol. 31: 561–566 (1982).

42. Williams, D. E., and Buhrer, D. L. Purified form of cytochrome P-450 from rainbow trout with high activity toward conversion of aflatoxin B1-3,3'-epoxide. Cancer Res. 43: 4752–4756.

43. Eisele, T. A., Bailey, G. S., and Nixon, J. E. The effect of indole-3-carbinol, an aflatoxin B1 hepatocarcinoma inhibitor, and other indole analogs on the rainbow trout hepatic mixed function oxidase system. Toxicol. Letters 19: 133–138 (1983).

44. Bailey, G. S., Taylor, M. J., and Selivonchick, D. P. Aflatoxin B1 metabolism and DNA binding in isolated hepatocytes from rainbow trout (Salmo gairdneri). Carcinogenesis 3: 511–518 (1982).

45. Bailey, G. W., Taylor, M. J., Loveland, P. M., Wilcox, J. S., Sinnhuber, R. O., and Selivonchick, D. P. Dietary modification of aflatoxin B1 carcinogenesis: mechanism studies with isolated hepatocytes from trout. In: Use of Small Fish Species in Carcinogenicity Testing (Natl. Cancer Inst. Monograph, Vol. 65), National Cancer Institute, Bethesda, MD, 1984, pp. 379–385.

46. Coulombe, R. A., Jr., Bailey, G. S., and Nixon, J. E. Comparative activation of aflatoxin B1 to mutagens by isolated hepatocytes from rainbow trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch). Carcinogenesis 5: 29–33 (1984).

47. Shelton, D. W., Goeger, D. E., Hendricks, J. D., and G. S. Bailey. Mechanisms of anti-carcinogenesis: the distribution and metabolism of aflatoxin B1 in rainbow trout fed Aroclor 1254. Carcinogenesis 7: 1065–1071 (1986).

48. Goeger, D. E., Shelton, D. W., Hendricks, J. D., and Bailey, G. S. Mechanisms of anti-carcinogenesis by indole-3-carbinol: effect on the distribution and metabolism of aflatoxin B1 in rainbow trout. Carcinogenesis 7: 2025–2031 (1986).

49. Loveland, P. M., Nixon, J. E., and Bailey, G. S. Glucuronidases in bile of rainbow trout (Salmo gairdneri) injected with [3H]aflatoxin B1 and the effects of dietary B-naphthoflavone. Comp. Biochem. Physiol. 78C: 13–19 (1984).

50. Sparnins, V. L., Venegas, P. L., and Wattenberg, L. W. Gluthathione S-transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. J. Natl. Cancer Inst. 68: 493–496 (1982).

51. Deen, G. H., and Neumann, H.-G. Differences in aflatoxin B1-susceptibility of rat and mouse as correlated with the capability in vitro to inactivate aflatoxin B1 epoxide. Carcinogenesis 2: 307–306 (1981).

52. Valsta, L. The significance of glutathione conjugation for aflatoxin B1 metabolism in rainbow trout and coho salmon. Master of Science thesis, Oregon State University, 1985.