Research Article

Protective effect of dietary tomatine against dibenzo[*a,l*]pyrene (DBP)-induced liver and stomach tumors in rainbow trout

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The potential anti-carcinogenic effects of tomatine, a mixture of commercial tomato glycoalkaloids α-tomatine and dehydrotomatine (10:1), were examined in the rainbow trout chemoprevention model. Prior to the chemoprevention study, a preliminary toxicity study revealed that tomatine in the diet fed daily at doses from 100 to 2000 parts per million (ppm) for 4 weeks was not toxic to trout. For the tumor study, replicate groups of 105 trout were fed diets containing dibenzo[*a,l*]pyrene (DBP) alone (224 ppm), (N = 3), DBP plus tomatine at 2000 ppm (N = 2), tomatine alone (N = 2), or control diet (N = 2) for 4 weeks. The fish were then returned to control diet for 8 months and necropsied for histopathology. Dietary tomatine was found to reduce DBP-initiated liver tumor incidence from 37.0 to 19.0% and stomach tumor incidence from 46.4 to 29.4%. Tomatine also reduced stomach tumor multiplicity. The tomatine-containing diets did not induce mortality, change in fish weights, or liver weights. No adverse pathological effects in the tissues of the fish on the tomatine diets were observed. Dose-response and chemopreventive mechanisms for tomatine protection remain to be examined. This is the first report on the anticarcinogenic effects of tomatine *in vivo*.

Keywords: Dibenzopyrene / Rainbow trout / Tomatine / Tomatoes / Tumor prevention

Received: May 16, 2007; revised: July 12, 2007; accepted: July 14, 2007

1 Introduction

Tomato plants (*Lycopersicon esculentum*) synthesize the glycoalkaloids dehydrotomatine and α-tomatine, possibly as a defense against bacteria, fungi and viruses, and insects. [1–3], as reviewed in [4]. Commercial tomatine used in this study is a ~10:1 mixture of α-tomatine and dehydrotomatine (Fig. 1) [5–7]. The structure of dehydrotomatine is similar to that of α-tomatine, in that the former molecule has a double bond in the steroidal ring B of the aglycone. Note that both tomato glycoalkaloids have the same tetrasaccharide side chain. The tomato glycoalkaloid α-tomatine has a tetrasaccharide side chain attached to the aglycone tomatidine, whereas the second glycoalkaloid present in tomato plants called dehydrotomatine has the same tetrasaccharide side chain attached to the aglycone tomatidenol.

Beneficial effects of tomatine include lowering cholesterol and triglycerides, enhancing the immune system, and antibiotic activities. We previously reported that dietary tomatine decreased plasma LDL cholesterol in hamsters fed a high saturated fat, high-cholesterol diet by 41% and plasma triglyceride concentrations by 47% [8]. Similar beneficial effects were observed with high-tomatine green tomato diets [9]. Because tomatine alone reduced both dietary cholesterol bioavailability and endogenous cholesterol, the data suggests that tomatine forms an insoluble complex with cholesterol from both dietary cholesterol and from endogenous cholesterol produced by the liver, which enters the digestive tract via the enterohepatic circulation.

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Abbreviations: **DBP**, dibenzo[*a,l*]pyrene; **OTD**, Oregon Test Diet; **ppm**, parts per million

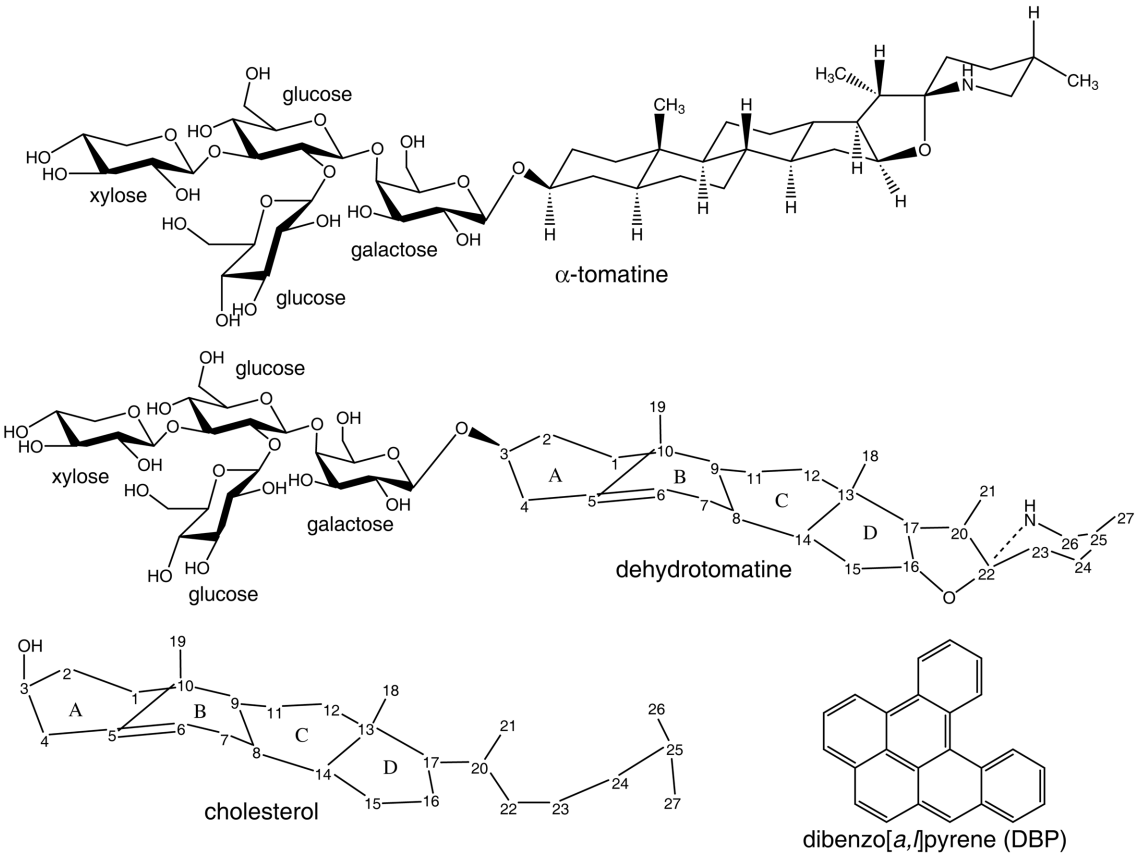

Figure 1. Stereochemistry of the tetrasaccharide side chain of tomatine attached to the aglycone tomatidine, of the identical tetrasaccharide side chain of dehydrotomatine attached to the aglycone tomatidenol, and of cholesterol. Anticarcinogenic activities of tomatine may result from formation of 1:1 complexes with cholesterol [4], disruption of cancer cell membranes [11, 39], and stimulation of the immune system [10].

The reported immunopotentiating effect of tomatine of T-cell mediated regression of lymphoid experimental tumors (EG7-Ova) may be the result of costimulation of CD80 and CD86 to induce antigen-specific cellular immunity [10]. Because tomatine induced antigen-specific cellular immunity in mice, the authors suggest that tomatine possesses remarkable potential as a vaccine adjuvant for infectious diseases as well as for cancer immunotherapy.

Recently, Ito *et al*. [1] found that the antibiotic effect of tomatine against the fungal pathogen *Fusarium oxysporum* involves activation of phosphotyrosine kinase and G-protein signaling pathways leading to Ca^{2+} elevation and accumulation of reactive-oxygen species (ROS). In related studies, Simons *et al*. [2] found that the mode of action of α-tomatine towards yeast cells involving cell membrane permeilization is distinct from that of the aglycone tomatidine that lacks the tetrasaccharide side chain.

Using a microculture tetrazolium (MTT) *in vitro* assay, we previously reported that tomatine is a strong inhibitor of growth for both human colon and liver cancer cell lines, as evidenced by the concentration-dependent (0.1 to 100 μg/mL) inhibition of HT29 colon cancer cells at levels ranging from 38.0 to 81.5%, and of human HepG2 cancer cells, from 46.3 to 89.2% [11]. The antiproliferative activity against human liver cancer cells at a tomatine concentration of 1 μg/mL was higher than the corresponding activity observed with the commercial anticancer drug doxorubicin.

To further define the potential value of tomatine in the *in vivo* chemoprevention of cancer, the objective of the present study was to determine the ability of dietary tomatine to inhibit dibenzo[*a,l*]pyrene (DBP)-induced liver and stomach tumors in the trout model determined in a long-term feeding study. As a prerequisite to the tumor study, the acute toxicity of tomatine added to a control diet fed orally to rainbow trout was determined. DBP, a planar polyaromatic hydrocarbon, is a potent environmental hydrocarbon [12] and has been identified as a combustion product in coal smoke [13] and tobacco smoke [14]. DBP is a potent tumor initiator in mouse skin and rat mammary gland [12, 15, 16]. In the rainbow trout model, DBP initiates tumors in multiorgans; liver, stomach and swimbladder [17 – 19].

The rainbow trout model is highly sensitive to diverse chemical carcinogens and is a statistically powerful vertebrate model used in many comparative studies of chemical

carcinogenesis and its modulation by dietary inhibitors [17 – 27].

2 Materials and methods

2.1 Test compounds

Tomatine was purchased from Sigma Chemical Company (St. Louis, MO, USA). Dibenzo[*a,l*]pyrene was obtained from the National Cancer Institute (NCI) Reference Standard Repository in Kansas City, MO, USA. Handling and storage of this potent multi-organ carcinogen was in accordance with National Institutes of Health and Oregon State University guidelines for Moderate Hazard Carcinogens. Both tomatine and DBP were dissolved in the oil component of the trout semi-synthetic Oregon Test Diet (OTD), [23, 25]. The concentrations of tomatine and DBP are expressed in parts per million (ppm) relative to the dry weight portion of the diet. DBP is light sensitive and was handled in subdued lighting. Diets with test compounds were prepared every 2 weeks and stored at -20°C until a day prior to feeding when the diets were moved to 4°C. DBP is stable in diets stored at -20°C for up to 2 years [28].

2.2 Animals

Shasta strain rainbow trout were spawned, reared and treated at the Sinnhuber Aquatic Research Laboratory (SARL), Oregon State University as described [23, 26] under protocols from the National Institute of Health (NIH) and received approval from our Institutional Animal Care and Use Committee. Fry were fed the OTD from onset of feeding to dietary initiation [25].

2.3 Tomatine acute toxicity study

Because 2000 ppm tomatine in the diet was a dose tolerated by hamsters, we carried out a range-finding experiment up to this level in the trout diet to guide selection of future doses for a dose-response tumor chemoprevention study in trout. Tomatine over a range of doses (100, 500, 1000, and 2000 ppm) was added to the oil component of the diet and fed daily for 4 weeks to groups of 50 trout each. One tank of 50 trout was fed OTD alone as a control group. During the exposure, mortalities were recorded on a daily basis. At the end of the 4 weeks, 10 trout per tank were removed and overdosed with tricaine methanesulfonate, MS-222 [17]. The livers were removed, weighed, and examined for abnormality.

2.4 Cancer chemoprevention study

A total of 945 trout were allocated to 9 tanks, N = 105 each tank. Trout acclimatized to the tanks for one week prior to start of the carcinogen exposure. Duplicate tanks received the control diet (OTD), tomatine (2000 ppm), tomatine (2000 ppm) and DBP (224 ppm), and triplicate tanks received only DBP (224 ppm). After 4 weeks of dietary exposure, all groups were returned to OTD for 8 months and necropsied for gross pathology and histopathological examination.

2.5 Tumor histology

Trout were sacrificed by MS-222 overdose and liver and stomach tumor development were quantified as described [24, 25]. Tissues were examined under a dissecting scope for gross tumors (≥0.5 mm diameter), fixed in Bouin's solution and processed by routine histological procedures. Numerous studies over the past twenty years have shown that 100% of stomach and 95% of liver tumors are surface-oriented outgrowths that are easily detected at gross necropsy [24, 26, 29]. From each organ having one or more suspect tumors at necropsy, one slide was prepared for histology. Tumor incidence is expressed as the percentage of fish with one or more confirmed tumors per tank.

2.6 Statistical analysis

Logistic regression was used to compare tumor incidences between treatment groups (Genmod procedure in SAS for Windows version 9.1.3). There was no evidence of extrabinomial variation between replicate tanks within treatment groups (deviance/df <1, $p > 0.47$ for both liver and stomach). Therefore, binomial variation was assumed and likelihood ratio tests used to compare treatment groups. More conservative tests (*e.g.* quasilikelihood *t*-tests using observed tank-to-tank variation) would also indicate significant differences ($p < 0.03$ for both liver and stomach).

Exact two-sided rank tests (Kruskal-Wallis and Wilcoxon) [30] were used to compare tumor multiplicity (per gross tumor bearing animal) between treatment groups (Npar1way procedure in SAS). There was no or little evidence of differences between replicate tanks ($p > 0.4$ for stomach in both treatment groups, $p > 0.14$ for liver in both treatment groups). Therefore, data were pooled over replicate tanks and comparisons between treatment groups were based on individual fish multiplicity. More conservative tests (*e.g.* two-sided *t*-tests on tank means with 3 and 2 tanks per group) would give the same conclusions.

3 Results

Results of the acute toxicity studies with dietary tomatine concentrations ranging from 100 to 2000 ppm show that there were no significant differences in either the fish mortalities, body weights or the liver weights between groups (Table 1). The livers of trout fed tomatine showed no gross pathology. These observations suggest that tomatine at

Table 1. Tomatine is not acutely toxic to trout in 4-week exposure

Group	Mortality[a)]	# Fish	Average fish weight (g) ± SD	Average liver weight (g)
OTD (control diet)	0%	10	2.7 ± 0.5	0.03
100 ppm tomatine	2%	10	3.0 ± 0.8	0.03
500 ppm tomatine	0%	10	3.2 ± 1.0	0.04
1000 ppm tomatine	2%	10	2.8 ±1.1	0.03
2000 ppm tomatine	0%	10	2.9 ± 1.1	0.02

a) Mortality is defined as the number of trout that died during the 4-week exposure divided by the number of trout per tank. The only reported mortalities, 2%, were for the doses of 100 and 1000 ppm of tomatine.

Table 2. Tomatine-reduced liver tumor incidence, stomach tumor incidence and stomach tumor multiplicity in rainbow trout initiated with dibenzo[*a,l*]pyrene

Treatment		# Trout per tank	Tumor incidence%		Tumor multiplicity[a)]	
			Liver	Stomach	Liver	Stomach
DBP 224 ppm		96	32.2	52.1	1.6	1.8
		98	37.8	45.9	2.1	1.8
		95	41.1	41.1	2.0	2.1
	Mean ± SE		**37.0 ± 2.6**	**46.4 ± 3.2**	**1.9 ± 0.2**	**1.9 ± 0.2**
DBP 224 ppm		96	16.7	28.1	1.6	1.5
Tomatine 2000 ppm		75[b)]	21.3	30.7	2.0	1.4
	Mean ± SE		**19.0 ± 2.3**	**29.4 ± 1.5**	**1.8 ± 0.2**	**1.5 ± 0.1**
			p = <0.0001[c)]	p = 0.0003[c)]	p = 0.83[c)]	p = 0.030[c)]
		% Reduction by tomatine	48.7	36.6	5.3	21.1
Controls						
Oregon Test Diet		97	0	0	–	–
		89	0	0	–	–
Tomatine 2000 ppm		100	0	0	–	–
		99	0	0	–	–

a) Apparent tumor multiplicity was calculated by dividing the total number of grossly observed tumors in a tank by the total number of tumor bearing fish in the tank. The endpoint is termed apparent tumor multiplicity because not every lesion in organs exhibiting multiple lesions at gross necropsy was examined histologically.
b) Mortality due to human error in tank maintenance. Note tumor incidence for both liver and stomach are similar to the tank without the loss.
c) *p*-value from asymptotic chi-square distribution.

70 mg/100 g wet weight is not acutely toxic to rainbow trout.

Table 2 shows tumor incidences and tumor multiplicities for both liver and stomach. Co-feeding tomatine and DBP significantly reduced the incidence of liver tumors by 48.7% and the incidence of stomach tumors by 36.6% compared to DBP alone ($p = 0.01$, $p = 0.03$ one-sided *t*-test), respectively. Control treatments, OTD and tomatine, showed no liver or stomach tumors.

Tumor multiplicity (total number of tumors per tank divided by the number of tumor-bearing trout in the tank) in the liver did not change significantly with the addition of tomatine. However, Table 2 shows that stomach tumor multiplicity did drop significantly ($p = 0.04$, one-sided *t*-test). Figure 2 depicts the change in stomach tumor multiplicity by rank order. Of the tumor bearing trout fed only DBP, 24.7% had three to five tumors compared to 10% for those co-fed DBP and tomatine. Of the tumor-bearing trout fed DBP, 50.7% had only one tumor compared to 64.0% for those co-fed DBP and tomatine. Independently, neither change was statistically significant but the overall change in stomach tumor multiplicity was.

4 Discussion

4.1 Anticarcinogenic effects of tomatine

This is the first report on the anticarcinogenic effects of tomatine *in vivo*. Results of this initial study demonstrate that a moderate dietary dose of 2000 ppm tomatine provides anti-tumorigenic protection with potency similar to that previously observed for chlorophyll [19], chlorophyllin [18], and indole-3-carbinol [31] in the trout model. The mechanism(s) of the anticarcinogenic effect of tomatine remain to be investigated. Tomatine is known, however, to bind to cholesterol in the digestive tract [9, 32], suggesting

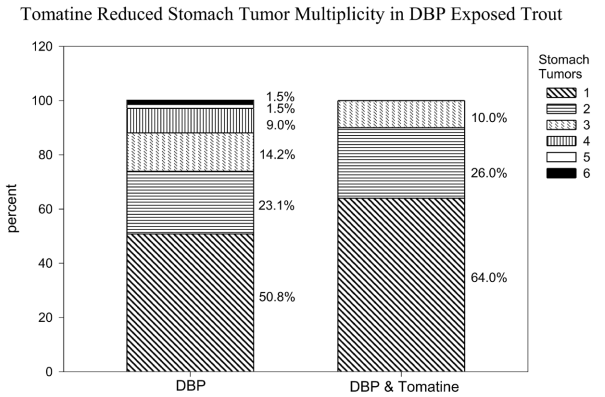

Figure 2. Tomatine reduced the percentage of fish with four or more tumors to zero and increased the percentage of fish with a single tumor. Evidence of a difference in stomach tumor multiplicity (p = 0.030) using Exact Wilcoxon test [30] in a fish-level analysis.

that its protective mechanism could be similar to those of chlorophyll or chlorophyllin. Studies in trout and rats suggest that non-covalent complexes formed between chlorophyll and chlorophyllin and carcinogens including aflatoxin B1 [21, 22, 33 – 35], DBP ([17, 18] and Simonich, M. T., *et al*., *submitted*) and heterocyclic amines [20, 36 – 38] are poorly absorbed from the digestive tract, thus substantially blocking initiation of chemical carcinogenesis. The more-recent experiments show that this kind of protective mechanism for chlorophylls extends to human volunteers (Bailey *et al*., unpublished results). However, experiments analogous to those mentioned above for chlorophyll (results not shown) designed to find out whether tomatine binds to DBP were negative. These observations suggest that the mechanism of the tomatine effect probably differs from that proposed for chlorophyll.

We suggest that the mechanism(s) of the chemo-preventive effect of tomatine may be the result of multiple molecular events including formation of complexes with cholesterol [8], potentiation of the immune system [10], and direct destruction of cancer cells via disruption of cell membranes [11, 39, 40]. This latter process is initiated by binding (intercalation) of tomatine to cholesterol located within cell membranes [4, 41].

The bioavailability and *in vitro* binding of carcinogens by both tomatine and dehydrotomatine require further study.

4.2 Relationship to tomato-based diets

Consumption of tomato products containing high levels of lycopene [42] is reported to be associated with lowered cancer risk [43], including colorectal adenomas [44]. Tomato-containing diets and lycopene also protected against N-methyl-N-nitrosourea (NMU)-induced prostate cancer in a rat model [45].

Our studies showed that the tomatine content of fresh tomatoes is quite low, ranging from ~4 to 42 mg/kg on a dry weight basis [7]. By contrast, tomatine levels of green tomatoes, including pickled green and fried green tomatoes are 50 to 100 times higher than those of the standard red varieties [7]. It is also relevant that red tomato varieties grown in the mountains of Peru contain high levels of tomatine [46]. These considerations suggest that (i) reported effects of red tomato-based diet against cancers and cholesterol may at least be due in part to tomatine; (ii) it would be of interest to ascertain whether high-tomatine green tomatoes exhibit anticarcinogenic properties *in vivo*; and (iii) there is a need to develop high-tomatine red tomatoes by suppressing the genes in the tomato plant that govern the formation of enzymes that degrade tomatine during post-harvest ripening of tomatoes.

We especially thank Eric Johnson, Greg Gonnerman, and Sheila Cleveland of the Sinnhuber Aquatic Research Laboratory for their excellence in fish rearing, necropsy, and histology. This work was partly supported through NIH grants CA90890, ES00210, ES03850, and funds from the Linus Pauling Institute at Oregon State University.

5 References

[1] Ito, S., Ihara, T., Tamura, H., Tanaka, S., *et al*., α-tomatine, the major saponin in tomato, induces programmed cell death mediated by reactive oxygen species in the fungal pathogen *Fusarium oxysporum, FEBS Lett.* 2007, *581,* 3217 – 3222.

[2] Simons, V., Morrissey, J. P., Latijnhouwers, M., Csukai, M., *et al*., Dual effects of plant steroidal alkaloids on *Saccharomyces cerevisiae*, *Antimicrob. Agents Chemother.* 2006, *50,* 2732 – 2740.

[3] Thorne, H. V., Clarke, G. F., Skuce, R., The inactivation of herpes simplex virus by some *Solanaceae* glycoalkaloids, *Antiviral Res.* 1985, *5,* 335 – 343.

[4] Friedman, M., Tomato glycoalkaloids: role in the plant and in the diet, *J. Agric. Food Chem.* 2002, *50,* 5751 – 5780.

[5] Friedman, M., Levin, C. E., Dehydrotomatine content in tomatoes, *J. Agric. Food Chem.* 1998, *46,* 4571 – 4576.

[6] Kozukue, N., Han, J. S., Lee, K. R., Friedman, M., Dehydrotomatine and α-tomatine content in tomato fruits and vegetative plant tissues, *J. Agric. Food Chem.* 2004, *52,* 2079 – 2083.

[7] Friedman, M., Analysis of biologically active compounds in potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), and jimson weed (*Datura stramonium*) seeds, *J. Chromatogr. A* 2004, *1054,* 143 – 155.

[8] Friedman, M., Fitch, T. E., Yokoyama, W. E., Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkaloid tomatine, *Food Chem. Toxicol.* 2000, *38,* 549 – 553.

[9] Friedman, M., Fitch, T. E., Levin, C. E., Yokoyama, W. H., Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides, *J. Food Sci.* 2000, *65,* 897 – 900.

[10] Morrow, W. J. W., Yang, Y.-W., Sheikh, N. A., Immunobiology of the tomatine adjuvant, *Vaccine* 2004, *22,* 2380 – 2384.

[11] Lee, K. R., Kozukue, N., Han, J. S., Park, J. H., *et al*., Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells, *J. Agric. Food Chem.* 2004, *52,* 2832 – 2839.

[12] Cavalieri, E. L., Rogan, E. G., Higginbotham, S., Cremonesi, P., Salmasi, S., Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of dibenzo[a]pyrenes: the very potent environmental carcinogen dibenzo[*a,l*]pyrene, *J. Cancer Res. Clin. Oncol.* 1989, *115,* 67 – 72.

[13] Mumford, J. L., Li, X., Hu, F., Lu, X. B., Chuang, J. C., Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke, *Carcinogenesis* 1995, *16,* 3031 – 3036.

[14] Snook, M. E., Severson, R. F., Arrendale, R. F., Higman, H. C., Chortyk, O. T., The identification of high molecular weight polynuclear aromatic hydrocarbons in a biologically active fraction of cigarette smoke condensate, *Beitraege zur Tabakforschung* 1977, *9,* 79 – 101.

[15] Cavalieri, E. L., Higginbotham, S., RamaKrishna, N. V., Devanesan, P. D., *et al*., Comparative dose-response tumorigenicity studies of dibenzo[alpha,l]pyrene versus 7,12-dimethylbenz[alpha]anthracene, benzo[alpha]pyrene and two dibenzo[alpha,l]pyrene dihydrodiols in mouse skin and rat mammary gland, *Carcinogenesis* 1991, *12,* 1939 – 1944.

[16] Higginbotham, S., RamaKrishna, N. V., Johansson, S. L., Rogan, E. G., Cavalieri, E. L., Tumor-initiating activity and carcinogenicity of dibenzo[*a,l*]pyrene versus 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene at low doses in mouse skin, *Carcinogenesis* 1993, *14,* 875 – 878.

[17] Pratt, M. M., Reddy, A. P., Hendricks, J. D., Pereira, C., *et al*., The importance of carcinogen dose in chemoprevention studies: quantitative interrelationships between, dibenzo[*a,l*]pyrene dose, chlorophyllin dose, target organ DNA adduct biomarkers and final tumor outcome, *Carcinogenesis* 2007, *28,* 611 – 624.

[18] Reddy, A. P., Harttig, U., Barth, M. C., Baird, W. M., *et al*., Inhibition of dibenzo[*a,l*]pyrene-induced multi-organ carcinogenesis by dietary chlorophyllin in rainbow trout, *Carcinogenesis* 1999, *20,* 1919 – 1926.

[19] Harttig, U., Bailey, G. S., Chemoprotection by natural chlorophylls *in vivo*, inhibition of dibenzo[*a,l*]pyrene-DNA adducts in rainbow trout liver, *Carcinogenesis* 1998, *19,* 1323 – 1326.

[20] Dashwood, R. H., Breinholt, V., Bailey, G. S., Chemopreventive properties of chlorophyllin: inhibition of aflatoxin B1 (AFB1)-DNA binding *in vivo* and anti-mutagenic activity against AFB1 and two heterocyclic amines in the *Salmonella* mutagenicity assay, *Carcinogenesis* 1991, *12,* 939 – 942.

[21] Breinholt, V., Hendricks, J., Pereira, C., Arbogast, D., Bailey, G., Dietary chlorophyllin is a potent inhibitor of aflatoxin B1 hepatocarcinogenesis in rainbow trout, *Cancer Res.* 1995, *55,* 57 – 62.

[22] Dashwood, R., Negishi, T., Hayatsu, H., Breinholt, V., *et al*., Chemopreventive properties of chlorophylls towards aflatoxin B1: a review of the antimutagenicity and anticarcinogenicity data in rainbow trout, *Mutat. Res.* 1998, *399,* 245 – 253.

[23] Sinnhuber, R. O., Hendricks, J. D., Wales, J. H., Putnam, G. B., Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis, *Ann. NY Acad. Sci.* 1978, *298,* 389 – 408.

[24] Hendricks, J. D., Meyers, T. R., Shelton, D. W., Histological progression of hepatic neoplasia in rainbow trout (*Salmo gairdneri*), *Natl. Cancer Inst. Monogr.* 1984, *65,* 321 – 336.

[25] Lee, B. C., Hendricks, J. D., Bailey, G. S., Toxicity of mycotoxins in the feed of fish, in: Smith, J. E. (Ed.), *Mycotoxins and animal feedingstuff: Natural occurrence, toxicity and control*, CRC Press, Boca Raton, FL 1991, pp. 607 – 626.

[26] Bailey, G. S., Williams, D. E., Hendricks, J. D., Fish models for environmental carcinogenesis: the rainbow trout, *Environ. Health Perspect.* 1996, *104 (Suppl. 1),* 5 – 21.

[27] William, D. E., Bailey, G. S., Reddy, A., Hendricks, J. D., *et al*., The rainbow trout (*Oncorhynchus mykiss*) tumor model: recent applications in low-dose exposures to tumor initiators and promoters, *Toxicol. Pathol.* 2003, *31 (Suppl.),* 58 – 61.

[28] Loveland, P. M., Reddy, A. P., Pereira, C. B., Field, J. A., Bailey, G. S., Application of matrix solid-phase dispersion in the determination of dibenzo[*a,l*]pyrene content of experimental animal diets used in a large-scale tumor study, *J. Chromatogr. A* 2001, *932,* 33 – 41.

[29] Hendricks, J. D., Shelton, D. W., Loveland, P. M., Pereira, C. B., Bailey, G. S., Carcinogenicity of dietary dimethylnitrosomorpholine, N-methyl-N'-nitro-N-nitrosoguanidine, and dibromoethane in rainbow trout, *Toxicol. Pathol.* 1995, *23,* 447 – 457.

[30] Agresti, A., Mehta, C. R., Patel, N. R., Exact inference for contingency tables with ordered categories, *J. Am. Statistical Assoc.* 1990, *85,* 453 – 458.

[31] Gallagher, E. P., Using salmonid microarrays to understand the dietary modulation of carcinogenesis in rainbow trout, *Toxicol. Sci.* 2006, *90,* 1 – 4.

[32] Stine, K. J., Hercules, R. K., Duff, J. D., Walker, B. W., Interaction of the glycoalkaloid tomatine with DMPC and sterol monolayers studied by surface pressure measurements and Brewster angle microscopy, *J. Phys. Chem. B Condens Matter Mater. Surf. Interfaces Biophys.* 2006, *110,* 22220 – 22229.

[33] Breinholt, V., Arbogast, D., Loveland, P., Pereira, C., *et al*., Chlorophyllin chemoprevention in trout initiated by aflatoxin B(1) bath treatment: An evaluation of reduced bioavailability *vs*. target organ protective mechanisms, *Toxicol. Appl. Pharmacol.* 1999, *158,* 141 – 151.

[34] Simonich, M. T., Egner, P., Roebuck, B. D., Orner, G., *et al*., Natural chlorophyll inhibits aflatoxin B1 induced multiorgan carcinogenesis in the rat, *Carcinogenesis* 2007, *28*, 1294 – 1302.

[35] Breinholt, V., Schimerlik, M., Dashwood, R., Bailey, G., Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B1: complex formation with the carcinogen, *Chem. Res. Toxicol.* 1995, *8,* 506 – 514.

[36] Mata, J. E., Yu, Z., Gray, J. E., Williams, D. E., Rodriguez-Proteau, R., Effects of chlorophyllin on transport of dibenzo(*a,l*)pyrene, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine, and aflatoxin B(1) across Caco-2 cell monolayers, *Toxicology* 2004, *196,* 117 – 125.

[37] Dashwood, R., Yamane, S., Larsen, R., Study of the forces of stabilizing complexes between chlorophylls and heterocyclic amine mutagens, *Environ. Mol. Mutagen.* 1996, *27,* 211 – 218.

[38] Guo, D., Schut, H. A., Davis, C. D., Snyderwine, E. G., *et al*., Protection by chlorophyllin and indole-3-carbinol against 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced DNA adducts and colonic aberrant crypts in the F344 rat, *Carcinogenesis* 1995, *16,* 2931 – 2937.

[39] Blankemeyer, J. T., White, J. B., Stringer, B. K., Friedman, M., Effect of α-tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin, *Food Chem. Toxicol.* 1997, *35,* 639 – 646.

[40] Friedman, M., Lee, K. R., Kim, H. J., Lee, I. S., Kozukue, N., Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells, *J. Agric. Food Chem.* 2005, *53,* 6162 – 6169.

[41] Friedman, M., Potato glycoalkaloids and metabolites: roles in the plant and in the diet, *J. Agric. Food Chem.* 2006, *54,* 8655 – 8681.

[42] Kozukue, N., Friedman, M., Tomatine, chlorophyll, β-carotene and lycopene content in tomatoes during growth and maturation, *J. Sci. Food Agric.* 2003, *83,* 195 – 200.

[43] Matulka, R. A., Hood, A. M., Griffiths, J. C., Safety evaluation of a natural tomato oleoresin extract derived from food-processing tomatoes, *Regul. Toxicol. Pharmacol.* 2004, *39,* 390 – 402.

[44] Erhardt, J. G., Meisner, C., Bode, J. C., Bode, C., Lycopene, beta-carotene, and colorectal adenomas, *Am. J. Clin. Nutr.* 2003, *78,* 1219 – 1224.

[45] Boileau, T. W., Liao, Z., Kim, S., Lemeshow, S., *et al*., Prostate carcinogenesis in N-methyl-N-nitrosourea (NMU)-testosterone-treated rats fed tomato powder, lycopene, or energy-restricted diets, *J. Natl. Cancer Inst.* 2003, *95,* 1578 – 1586.

[46] Rick, C. M., Uhlig, J. W., Jones, A. D., High α-tomatine content in ripe fruit of Andean *Lycopersicon esculentum var. cerasiforme*, developmental and genetic aspects, *Proc. Natl. Acad. Sci. USA* 1994, *91,* 12877 – 12881.