Chapter 6

Carcinogenicity

Humans

In the epidemiological studies of cancer reviewed in section 4, some of the relative risks for high versus low consumption of cruciferous vegetables were above unity, and a few were significantly greater than 1.0: i.e., in one cohort study of colorectal cancer, one case–control study of breast cancer and one case–control study of thyroid cancer. These were extreme examples of estimates that tended to centre close to the null. None of these results was considered by the Working Group to represent evidence of carcinogenicity in humans.

Experimental studies

Cruciferous vegetables

The fact that control animals given cruciferous vegetables alone did not show tumour enhancement indicates that these vegetables alone are not carcinogenic. Nevertheless, in a few studies, dietary *Brassica* vegetables may have enhanced the tumour response in carcinogen-treated animals (Table 61).

Colon

In two studies, Temple and Basu (1987) and Temple and El-Khatib (1987) examined the potential inhibitory effect of dietary cabbage on the formation of colon tumours in female Swiss mice treated with dimethylhydrazine. Unexpectedly, cabbage elicited some enhancement of tumour response when given throughout the experiment or during carcinogen treatment, although these increases were not statistically significant.

Pancreas and gall-bladder

Birt *et al.* (1987) evaluated the ability of dried cabbage supplements in the diet to inhibit pancreatic carcinogenesis in hamsters. Pancreatic cancer was induced by treatment with *N*-nitroso-bis(2-oxopropyl)amine (BOP) at 40 mg/kg bw. Before receiving the carcinogen, the animals were given a low-fat diet containing 9% (w/w) dried cabbage; from 1 week after BOP treatment, cabbage was given in low- (at 9%) and high-fat (at 11%) diets. The high-fat cabbage-containing diet increased the yield of BOP-induced pancreatic ductule carcinoma (1.6 carcinomas per animal) in comparison with that observed in hamsters fed the low-fat diet cabbage-containing or a high-fat diet without cabbage (0.6–0.8 carcinomas per animal; $p < 0.05$). The incidence of BOP-induced gall-bladder adenocarcinoma was increased in cabbage-fed hamsters irrespective of dietary fat.

Skin

Birt *et al.* (1987) evaluated the ability of dietary dried cabbage supplements to inhibit skin tumorigenesis in mice. Skin tumours were induced in SENCAR mice with 10 nmol of 7,12-dimethyl-benz[*a*]anthracene (DMBA) and promoted beginning 1 week later by twice weekly applications of 2 µg of 12-*O*-tetradecanoylphorbol 13-acetate (TPA). Dried cabbage was incorporated into AIN semi-purified diets before DMBA and throughout TPA treatment. The skin papilloma yield was increased in DMBA-initiated TPA-promoted mice fed diets containing 10% cabbage, from 7.25 papillomas per mouse in mice given control diet to an average of 8.45 papillomas per mouse after 22 weeks of promotion ($p < 0.001$).

Spermatic cord and others

Srisangnam *et al.* (1980) fed diets containing dehydrated cabbage to weanling male C57BL/6 mice injected subcutaneously with 20 mg/kg bw of dimethylhydrazine at weekly intervals for 36 weeks. Diets known to be adequate in all nutrients for mice were modified to include ground dehydrated cabbage leaves to 10%, 20% and 40% of diet, protein, crude fibre and lipids being held at constant levels. The diets containing 10% or 20% cabbage enhanced dimethylhydrazine-induced tumorigenicity, while the diet containing cabbage at 40% had a protective effect, which was not significant. Tumours of the spermatic cord were the most frequent, with occasional kidney and liver tumours.

Glucosinolates

Morse *et al.* (1988) studied the effects of long-term dietary administration of sinigrin (2-propenyl glucosinolate) at 3 µmol/g of diet to Fischer 344 rats before and during treatment with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (1.76 mg/kg subcutaneously three times a week for 20 weeks). After 104 weeks, there was no effect of sinigrin on tumorigenesis in liver, lung or nasal cavities; however, a significant increase in pancreatic tumours was observed in rats treated with both sinigrin and NNK.

Table 61. Enhancement of carcinogen-induced tumours by dietary cruciferous vegetables in experimental animals

Species, strain (sex)	Age at start (weeks)	No. of animals per group	Carcino-gen, route, dose, duration	Cruciferous vegetable, amount, duration	Timing of treatment	Enhancement of tumour incidence (TI) or multiplicity (TM)	Summarized effect	Reference
Colon Mouse, ICR (F)	5–7	20–40	Dimethylhy-drazine, starting at 17 mg/kg bw and then increasing by 21%, weekly x 8 (total, 291 mg/kg bw), s.c., killed 27 weeks after first dose	Cabbage, 12.8% of diet, purchased locally, starting 5 weeks before or after the carcinogen	Initiation, post-initiation or throughout	Adenoma TM: dimethylhydrazine alone, 1.85; + cabbage during initiation, 2.00; + cabbage after initiation, 0.92 ($p < 0.05$); + cabbage throughout, 2.00 Adenocarcinoma TM: dimethylhydrazine alone, 0.85; + cabbage during initiation, 1.15; + cabbage after initiation, 0.75; + cabbage throughout, 1.42	Cabbage increased colon tumours, particularly adeno-carcinomas, in initiation period but reduced adenomas in promotion period	Temple & Basu (1987)
Mouse, Swiss (M, F)	5–7	14–17	Dimethyl-hydrazine, starting at 23 mg/kg bw and then increasing weekly x 7, s.c., killed 17 weeks after first dose	Cabbage, 13% of diet, 21 weeks, purchased locally, starting 31 days before carcinogen	Through-out	Adenoma incidence (%) Females: dimethylhydrazine alone, 42.9; + cabbage, 62.5 Males: dimethylhydrazine alone, 47.0; + cabbage, 37.5 Adenocarcinoma TI (%) Females: dimethylhydrazine alone, 35.7; + cabbage, 68.7 Males: dimethylhydrazine alone, 64.7; + cabbage, 37.5 Total tumours per tumour-bearing mouse Females: dimethylhydrazine alone, 3.17; + cabbage, 4.92 Males: dimethylhydrazine alone, 3.08; + cabbage, 2.89	Although males were little affected by cabbage, females fed cabbage showed a tendency for increase in colon tumours	Temple & El-Khatib (1987)

Table 61 (contd)

Species, strain (sex)	Age at start (weeks)	No. of animals per group	Carcinogen, route, dose, duration	Cruciferous vegetable, amount, duration	Timing of treatment	Enhancement of tumour incidence (TI) or multiplicity (TM)	Summarized effect	Reference
Pancreas and gall-bladder								
Hamster, Syrian golden (M)	4	15–30	BOP, 40 mg/kg bw x 1 s.c., killed at 52 weeks	Dried cabbage, 9.1% of diet, starting 4 weeks before carcinogen or 10.9% starting 1 week after carcinogen	Through-out	Pancreatic ductule carcinomas per animal: high fat, 0.6; cabbage + high fat, 1.6 ($p < 0.05$) Gall-bladder adenocarcinoma TI (%): no cabbage, 3; cabbage, 12 ($p < 0.05$)	Cabbage diet enhanced pancreatic and gall-bladder tumours	Birt *et al.* (1987)
Skin								
Mouse, Sencar (F)	5	10–30	DMBA, 10 nmol x 1 + TPA, 2 μg twice per week beginning 1 week after DMBA, topically, killed at 24 weeks	Dried cabbage, 10% of diet, starting 4 weeks before carcinogen	Through-out	Skin papillomas per mouse: TPA, 7.25 after 22 weeks of promotion; cabbage + TPA, 8.45 ($p < 0.001$)	Cabbage diet enhanced skin tumours	Birt *et al.* (1987)
Spermatic cord and others								
Mouse, C57/Bl/6 (M)	Weanling	19–89	Dimethylhy-drazine, 20 mg/kg bw weekly x 36, s.c., killed at 36 weeks	Dehydrated cabbage, 10%, 20%, 40% of diet	Through-out	TI (%): dimethylhydrazine alone, 30.5; + 10% cabbage, 40.4; + 20% cabbage, 46.1; + 40% cabbage, 15.8 Spermatic cord TI (%): dimethylhydrazine alone, 23.2; + 10% cabbage, 29.8; + 20% cabbage, 34.9; + 40% cabbage, 15.8	Diets containing 10% and 20% cabbage enhanced dimethyl-hydrazine-induced tumorigenicity, while cabbage at 40% provided a protective effect	Srisangnam *et al.* (1980)

s.c., subcutaneously; DMBA, 7,12-dimethylbenz[a]anthracene; M, male; F, female; TPA, 12-O-tetradecanoyl-13-phorbol acetate; BOP, *N*-nitrosobis(2-oxo-propyl)amine

Isothiocyanates

Like most agents that disrupt or impair biological processes, isothiocyanates can also have adverse effects, including tumour promotion, weak tumorigenicity, genotoxicity and slight, transient organ toxicity. Whether an isothiocyanate inhibits or enhances tumorigenesis depends on its alkyl chain length, the animal species, the target organ, the time and duration of treatment, the dose and the carcinogen used to induce the tumours. The studies in which enhancement of carcinogenicity was observed are summarized in Table 62.

Tumour promotion

Oesophagus

Stoner et al. (1995) reported that oesophageal carcinogenesis was enhanced in male Fischer 344 rats by 6-phenylhexyl-ITC, a synthetic ITC, given in the diet for 2 weeks before challenge with N-nitrosomethylbenzylamine (NMBA) (0.5 mg/kg bw once a week for 15 weeks by subcutaneous injection), and continued in the diet for the remainder of the 21-week experimental period. 6-Phenylhexyl-ITC increased the number of NMBA-induced oesophageal tumours at all dietary concentrations (0.4, 1.0 and 2.5 µmol/g of diet), with significant increases at 1.0 and 2.5 µmol/g: the tumour multiplicity increased from 7.2 papillomas per oesophagus in controls given NMBA alone to 11.6 and 12.2, respectively. There was no effect on tumour incidence or size.

Colon

Dietary 6-phenylhexyl-ITC given in the diet at 640 or 320 mg/kg 2 weeks before treatment with azoxymethane (subcutaneous injection of 15 mg/kg bw, once a week for 2 weeks) and for the duration of the experiment (52 weeks) increased tumorigenicity in the intestine of male Fischer 344 rats (Rao et al., 1995). At the higher dietary concentration, the incidence of intestinal adenocarcinomas (small intestine and colon) was significantly increased (81% to 97%), as was the multiplicity of invasive and non-invasive adenocarcinomas of the colon (0.53 to 0.86 and 0.97 to 1.67, respectively). At the lower concentration of 6-phenylhexyl-ITC, the multiplicity of non-invasive adenocarcinomas was significantly increased in the colon (0.97 to 1.46) but not in the small intestine. The tumour volume in the colon was increased by approximately twofold (at 320 mg/kg) and 4.3-fold (at 640 mg/kg), and at the higher concentration the tumour size was significantly increased (tumours > 1 cm increased from 10 to 35). In addition, COX-2 and LOX activities were increased by approximately twofold at the higher concentration of 6-phenylhexyl-ITC in both colon mucosa and colon tumours.

Liver and urinary bladder

Phenethyl-ITC and benzyl-ITC were reported to have promoting activity on bladder carcinogenesis in male Fischer 344 rats (Hirose et al., 1998). Rats were pretreated with a single intraperitoneal injection of N-nitrosodiethylamine (NDEA) at 200 mg/kg bw and 2 days later were given drinking-water containing N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) for 4 weeks. Three days after the end of BBN treatment, phenethyl-ITC or benzyl-ITC was added to the powdered diet at 0.1%, and animals were maintained on this diet until they were killed at 32 weeks. The number of rats with papillary and nodular hyperplasia or carcinoma of the bladder was increased from 57% to 100% and from 24% to 100%, respectively, with both compounds. Phenethyl-ITC, but not benzyl-ITC, also significantly increased the number of altered liver cell foci > 0.5 mm in liver, from 1.05 to 1.45 per cm².

Using the same dosing schedule but varying the concentration of phenethyl-ITC, Ogawa et al. (2001) also found an increased incidence of papillary or nodular hyperplasia (73% to 100%) with phenethyl-ITC at a concentration of 0.1%, 0.05% or 0.01% in the diet and an increase in the frequency of dysplasia (33% to 73% and 100% at 0.01%, 0.05% and 0.1%, respectively). The frequency of transitional-cell carcinoma increased from 20% to 100% at 0.1% and 0.05%. More importantly, phenethyl-ITC at the two highest concentrations increased the incidence of invasive carcinoma from 0% to 67% and 93%. In the liver, both the number (per cm²) and area (mm²/cm²) of GST-P⁺ foci were increased with phenethyl-ITC at 0.1% and 0.05% of diet (in number from 5.8 to 17.8 and 11.9 and in area from 0.4 to 1.18 and 0.8, respectively).

Mammary gland

Lubet et al. (1997) found that phenethyl-ITC actually decreased the latency for mammary tumours in female Sprague-Dawley rats, slightly increased the tumour incidence and increased tumour multiplicity. Phenethyl-ITC was given to rats in the diet at a concentration of 1200 or 600 mg/kg, beginning 1 week before dosing with 12 mg of DMBA by gavage. Phenethyl-ITC at 1200 mg/kg also weakly induced hyperplasia in the bladders of all rats and increased the liver:body weight ratio. Using a different chemoprevention scheme, Ino et al. (1996) also found that benzyl-ITC lacked inhibitory activity for mammary tumorigenesis. Benzyl-ITC at 400 mg/kg in a high-fat diet (23.5% corn oil) was fed to Sprague-Dawley rats, beginning 1 week before dosing with the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) at 100 mg/kg bw eight times over 16 days by gavage. One week after the final PhIP dose, the rats were given the high-fat diet only. After 31 weeks, rats given PhIP alone had a tumour incidence of 74.2% and a tumour

Table 62. Promoting effect of isothiocyanates (ITCs) on carcinogen-induced tumours in male Fischer 344 rats

Organ site	Age at start (weeks)	No. per group	Carcinogen, route, dose, duration	ITC, dose, route	Timing of treatment	Effect on tumour incidence (TI) or multiplicity (TM)	Reference
Oesophagus	6–7	15	NMBA, s.c., 0.5 mg/kg bw, once a week for 15 weeks	6-Phenylhexyl-ITC, 0.4, 1, 2.5 µmol/g diet	2 weeks before, during and after NMBA	At 21 weeks, no effect on TI; at 1 and 2.5 µmol/g, significantly enhanced TM	Stoner *et al.* (1995)
Colon	5	12–36	Azoxymethane, s.c., 15 mg/kg bw, once a week for 2 weeks	6-Phenylhexyl-ITC, 320 or 640 mg/kg diet	2 weeks before azoxymethane to termination at 52 weeks	At 640 mg/kg, significantly enhanced TI, TM and tumour volume. At 320 mg/kg, increased TM and tumour volume	Rao *et al.* (1995)
Urinary bladder, liver	6	21	NDEA, i.p., 200 mg/kg bw once, after 2 days BBN, drinking-water, 0.05%, 4 weeks	Phenethyl-ITC, 0.1% or benzyl-ITC, 0.1% in diet for 32 weeks	3 days after BBN	Both ITCs increased incidences of papillary or nodular hyperplasia and carcinoma of bladder. Phenethyl-ITC, but not benzyl-ITC, increased foci in liver	Hirose *et al.* (1998)
Urinary bladder, liver	6	15	NDEA, i.p., 200 mg/kg bw, once, after 2 days BBN in drinking-water at 0.05%, 4 weeks	Phenethyl-ITC, 0.01%, 0.05%, 0.1%, in diet for 32 weeks	3 days after BBN	At 0.05% and 0.1%, significantly increased papillary or nodular lesions, dysplasia and carcinomas of bladder in carcinogen-promoted animals. Also weakly promoted hepato-carcinogenesis	Ogawa *et al.* (2001)

s.c., subcutaneously; i.p., intraperitoneally; NMBA, *N*-nitrosomethylbenzylamine; NDEA, *N*-nitrosodiethylamine; BBN, *N*-butyl-*N*-(4 hydroxybutyl)-nitrosamine

multiplicity of 1.71, and those given benzyl-ITC and PhIP had an incidence of 62.5% and a multiplicity of 1.91; the mean size of tumours in benzyl-ITC-treated rats increased, although not statistically significantly, from 9.9 mm to 10.8 mm.

Carcinogenicity

Hirose et al. (1998) found that feeding a diet containing phenethyl-ITC or benzyl-ITC at 1000 mg/kg without carcinogen treatment increased the incidence of papillary or nodular hyperplasia in the bladder of Fischer 344 rats (from 0 to 100%) but did not increase the number of foci in the liver.

Similarly, Ogawa et al. (2001) found that phenethyl-ITC in the diet at 500 or 1000 mg/kg without carcinogen pretreatment increased the frequencies of simple hyperplasia (from 0 to 100%), papillary or nodular hyperplasia (from 0 to 100%) and dysplasia (from 0 to 53% and 80%).

Okazaki et al. (2002) found that benzyl-ITC in the diet at 100 or 1000 mg/kg resulted in an increased frequency of epithelial hyperplasia in the bladder and suggested that benzyl-ITC not only did not inhibit bladder carcinogenesis but might also have a weak carcinogenic effect.

In another study with phenethyl-ITC, Sugiura et al. (2003) reported that feeding Fischer 344 rats a diet containing phenethyl-ITC at 1000 mg/kg for 48 weeks or for 32 weeks with normal diet for 1, 3 or 7 days or 16 weeks before death resulted in high incidences of simple and papillary or nodular hyperplasia, dysplasia and carcinoma (transitional-cell carcinoma with squamous-cell carcinoma and adenocarcinoma components). Phen-ethyl-ITC at 1000 mg/kg of diet for 32 weeks and then basal diets increased the incidence of simple and papillary or nodular hyperplasia from 0 to 100% and that of dysplasia from 0 to 67% and 92% when animals were killed 7 days and 16

weeks after removal of phenethyl-ITC from the diet. Furthermore, the incidence of carcinoma increased from 0 to 33% and 58%, respectively. Treatment of animals for 48 weeks with phenethyl-ITC increased the incidence of simple and papillary or nodular hyperplasia and dysplasia from 0 to 100% and the incidence of carcinoma from 0 to 92% (11/12 animals). Although the area of bladder mucosa occupied by hyperplasia was reduced after removal of phenethyl-ITC after 32 weeks, the numbers of animals with dysplasia and carcinoma actually increased, showing tumour progression in the absence of phenethyl-ITC. The areas of hyperplasia that were reversible were those showing little atypia.

Allyl-ITC was administered at 12 or 25 mg/kg bw in corn oil five times per week by gavage to groups of 50 Fischer 344 rats and 50 $B6C3F_1$ mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex received corn oil alone and served as vehicle controls. Transitional-cell papillomas in the urinary bladder occurred in treated male rats with a statistically significant trend ($p < 0.05$; controls, 0/49; lower dose, 2/49, 4%; higher dose, 4/49, 8%). Administration of allyl-ITC also increased the prevalence of epithelial hyperplasia in the urinary bladder in male rats. No evidence of an association between administration of allyl-ITC and increased tumour incidence was seen in the mice (Dunnick et al., 1982).

Indoles

The initial studies on the chemopreventive effects of indole-3-carbinol (Wattenberg & Loub, 1978; Bailey et al., 1982) addressed its efficacy as a 'blocking agent', that is, when given before or during carcinogen treatment. These and subsequent studies showed that indole-3-carbinol is an effective chemopreventive agent

against a variety of chemical carcinogens and in a variety of target organs and animal models (see section 4). Studies of its efficacy against estrogen-driven cancers, especially breast cancer, have attracted special attention; however, with few exceptions, these studies did not address its efficacy in animals in which cancer had already been initiated, that is, post-initiation or 'suppression' effects. Several such studies suggest that certain protocols result in enhanced tumour response in carcinogen-treated animals (Table 63).

Protocol-dependent tumour promotion in liver and mammary gland

The first evidence that dietary indole-3-carbinol treatment might have deleterious consequences came from studies with models of colon and liver carcinogenesis. In a multi-factorial design for studying colon cancer (Pence et al., 1986), indole-3-carbinol fed to mice before, during and after dimethylhydrazine was found to act synergistically with tallow and cholesterol to increase the tumour response. More direct evidence that indole-3-carbinol promotes tumours was provided by studies of liver carcinogenesis. While dietary co-treatment with indole-3-carbinol resuted in strong, dose-dependent decreases in aflatoxin B_1–DNA adduction and hepatocarcinogenesis in rainbow trout (Bailey et al., 1982; Dashwood et al., 1988, 1989b), subsequent treatment with indole-3-carbinol of trout previously initiated with aflatoxin B_1 resulted in potent tumour promotion (Bailey et al., 1987; Dashwood et al., 1990, 1991; Dashwood, 1998). These experiments provided reproducible evidence that indole-3-carbinol has multi-functional activity in vivo, with protocol-dependent potential to enhance as well as reduce cancer risk.

The finding of promotion in trout was confirmed and extended in

Table 63. Promoting effect of indole-3-carbinol on carcinogen-induced tumours in rats

Strain (sex), organ	Age at start (weeks)	No. per group	Carcinogen, route, dose, duration	Indole-3-carbinol dose, route	Timing of treatment	Effect observed on tumour incidence (TI) or multiplicity (TM)	Reference
Sprague-Dawley (M), liver	6	11–15	NDEA, 200 mg/kg bw, i.p.	0.25%, diet	2 weeks before or after NDEA for 6 weeks	Decreased hepatic GST-P$^+$ foci before initiation; increased number and area after initiation	Kim et al. (1994)
Sprague-Dawley (M), multiple organs	6	10–20	NDEA, MNU, DHPN	0.25%, diet	1 week after carcinogen for 20 weeks	Significant GST-P$^+$ foci promotion at week 24, non-significant liver adenoma promotion at week 52, significant enhancement of thyroid tumours at week 52	Kim et al. (1997)
Sprague-Dawley (F), multiple organs	54 days	20	DMBA, aflatoxin B$_1$, azoxymethane	2000 mg/kg, diet, 25 weeks, weeks 5–30	After last carcinogen treatment on day 29	500% increase in hepatic GST-P$^+$ foci	Stoner et al. (2002)
Sprague-Dawley (F), mammary gland	7	19	DMBA, 20 mg, orally	250 mg/kg bw, three times a week, 12 weeks	3 weeks after DMBA	No inhibition	Malejka-Giganti et al. (2000)
Sprague-Dawley (F), mammary gland	4	24–34	MNU, 50 mg/kg bw, i.p.	100 or 300 mg/kg, diet, 24 weeks	1 week after MNU	Increased TI and TM at 300 mg/kg bw	Kang et al. (2001)
Fischer 344 (M), colon	4–5	20–23	Dimethyl-hydrazine, 20 mg/kg bw, once a week, 5 weeks, s.c.	1000 mg/kg, 1 year, diet	1 week after last carcinogen treatment	No effect	Xu et al. (2001)

NDEA, N-nitrosodiethylamine; MNU, N-methyl-N-nitrosourea; DHPN, dihydroxy-di-N-propylnitrosamine; DMBA, 7,12-dimethylbenz[a]anthracene; i.p., intraperitoneally; S.C., subcutaneously; F, female; M, male

conventional rodent models. Kim et al. (1994) used a medium-term bioassay with male Sprague-Dawley rats to show that 0.25% indole-3-carbinol in the diet for 2 weeks before a single initiation with NDEA significantly decreased the number of hepatic GST-P^+ foci, whereas treatment at this dose for 6 weeks after the carcinogen significantly promoted the number and mean area of hepatic GST-P^+ foci. A study with a modified, multi-organ protocol showed that indole-3-carbinol caused significant promotion of GST-P^+ foci by week 24, non-significant hepatic adenoma promotion by week 52 and significant enhancement of thyroid gland tumours by week 52 in rats (Kim et al., 1997).

Bailey and colleagues (Stoner et al., 2002) developed a three-organ Sprague-Dawley rat model for simultaneous comparison of the effects of indole-3-carbinol post-initiation on mammary, colon and liver carcinogenesis in the same animals. In this model, feeding a diet containing indole-3-carbinol at 2000 mg/kg for 25 weeks post-initiation induced a modest delay in the latency of mammary tumour formation, no effect on mammary tumour incidence or multiplicity, a 40% decrease in the number of aberrant crypt foci in the colon, but a 500% increase in the volume per cent of hepatic GST-P^+. Because of the different kinetics of tumour development in the liver and mammary gland, it has not been possible to compare the effects of indole-3-carbinol in these two organs directly in this multi-organ model.

Studies of the effects of administering indole-3-carbinol post-initiation on the suppression of mammary carcinogenesis in rodents had mixed results. Treatment of rats with high, sustained doses of indole-3-carbinol (250 mg/kg bw three times a week for 12 weeks) failed to suppress mammary tumour development in DMBA-initiated Sprague-Dawley rats (Malejka-Giganti et al., 2000). This finding was suggested to be due to a persistent increase in putative estrogenic and carcinogenic estrogen metabolites in this model (Ritter et al., 2001). Longer, 25-week dietary treatment with indole-3-carbinol at 100 or 300 mg/kg similarly failed to suppress mammary tumour development in MNU-initiated Sprague-Dawley rats (Kang et al., 2001); on the contrary, the mean tumour incidence and multiplicity at the higher concentration were higher than those in the group receiving the carcinogen alone.

[The Working Group concluded that these studies indicate that high, sustained doses of indole-3-carbinol post-initiation may be necessary for any measurable protection against mammary tumorigenesis, and that the dose–response potency of indole-3-carbinol for liver tumour promotion exceeds that for suppression of mammary tumorigenesis in rats. It is notable that the doses of indole-3-carbinol often used in human studies (5–10 mg/kg bw per day) are far below those (200–500 mg/kg bw per day) required to suppress mammary tumours in rats but are comparable to the doses (5–20 mg/kg bw per day) that potently promote liver tumours in trout.]

Dose-dependent protection versus promotion

Few quantitative assessments of the relative potency of dietary indole-3-carbinol for reducing (blocking or suppressing) and enhancing (promotion) tumours have been reported. In studies in rainbow trout, up to 9000 fish were used to quantify the effects of the dose of indole-3-carbinol on the position and slope of entire curves of carcinogen dose–tumour response, using the tumour dose index (TD_{50}), defined as the ratio between the dose of aflatoxin B_1 required to give a 50% tumour response in the absence of indole-3-carbinol and that required to give a 50% tumour response in the presence of indole-3-carbinol (Dashwood et al., 1989b, 1990; Bailey et al., 1991; Dashwood et al., 1991). These dose–dose matrix experiments showed that concomitant feeding of indole-3-carbinol with aflatoxin B_1 during a 4-week initiation period displaced the entire curve towards higher values for the TD_{50} of aflatoxin B_1 to an extent that increased with indole-3-carbinol dose and with an effective 50% inhibition concentration of 1400 mg/kg. Feeding indole-3-carbinol for 9 months after initiation with aflatoxin B_1, however, displaced the dose–response curves towards lower TD_{50} values for aflatoxin B_1, with an effective 50% promotion concentration of indole-3-carbinol of 1000 mg/kg. Indole-3-carbinol-mediated promotion was later shown to be non-reversible and directly proportional to the duration of treatment (Dashwood et al., 1991). Subsequent experiments to determine quantitative dose–response relationships, with as many as 9000 trout, failed to produce evidence for a threshold dose below which indole-3-carbinol given post-initiation would not promote tumours (Oganesian et al., 1999). The authors of these studies concluded that indole-3-carbinol provided as prolonged treatment is more potent in promoting initiating events than in blocking concurrently administered carcinogens in trout liver.

Mechanisms of tumour promotion in liver

The general concept that supplementation with indole-3-carbinol might provide a systemic, protective anti-estrogenic effect (Michnovicz & Bradlow, 1990, 1991; Bradlow et al., 1994) does not apply to all species, strains or tissues. In particular, indole-3-carbinol and 3,3′-diindolylmethane have potent estrogenic activity in vivo in trout,

inducing estrogen-responsive genes in the liver (Shilling *et al.,* 2001). Since trout liver tumorigenesis is promoted by estrogen treatment, the estrogenicity of indole-3-carbinol might account for its promotional activity in this species. Interestingly, indole-3-carbinol, like estrogen, suppresses liver tumour development in the infant C57BL mouse model (Oganesian *et al.,* 1997). Conversely, increased estrogen metabolism has been proposed to explain the suppression of spontaneous mammary tumours in CH3/OuJ mice by this compound (Bradlow *et al.,* 1991). [The Working Group concluded that these studies suggest that indole-3-carbinol can have pro- and anti-estrogenic, organ-specific effects in mammals. These mechanisms are further discussed in section 4.]

Risk enhancement in colon

Indole-3-carbinol has been reported to enhance development of colon tumours in rats when given before, during and after dimethylhydrazine (Pence *et al.,* 1986). Its effects in this organ appear, however, to depend on the end-point assessed, the carcinogen used and the timing and dose of indole-3-carbinol administration. Dietary administration of indole-3-carbinol post-initiation at 1000 mg/kg did not enhance but significantly suppressed colon tumours induced in Fischer 344 rats by IQ (Xu *et al.,* 2001), and indole-3-carbinol given only post-initiation with dimethylhydrazine had no effect on colon tumorigenesis. [The Working Group concluded that, on balance, the available studies suggest that prolonged treatment with indole-3-carbinol might provide protection against at least some colorectal cancers, rather than promotion.]

Carcinogenicity

There are no reports of tumour induction by treatment with indole-3-carbinol alone in any animal model system.