Chapter 7

Toxic effects

Adverse effects

The classic toxic effect of cruciferous vegetables is goitrogenicity, which has been described in cattle and humans and is caused by goitrin and other compounds derived from certain *Brassica* seeds.

Humans

Brassica vegetables

Owing to the high content of vitamin K in broccoli, drug interactions have been reported in patients receiving medication for hypoprothrombinaemia. Two such cases were reported in women who ate 230–450 g/day of broccoli (Kempin, 1983). One survey showed that most health-care professionals (> 87%) were aware of possible interactions between warfarin medication and the high content of vitamin K in certain *Brassica* vegetables, such as broccoli, whereas fewer [55%, read from figure] knew about the potential drug interaction with white cabbage (Couris *et al.*, 2000).

Drug interactions as a consequence of effects of cruciferous vegetables on xenobiotic metabolizing enzymes are also well known from controlled human intervention studies (see section 4 for more detail). In one study on the effects of eating 400 g/day of Brussels sprouts for 2 weeks on warfarin clearance, a 29% increase was observed (Ovesen *et al.*, 1988). The effect of eating Brussels sprouts and cabbage on the clearance of antipyrine, oxazepam and acetaminophen has also been assessed in controlled human intervention studies. The drugs were excreted significantly faster (by 10–20%) after intervention with cruciferous vegetables at a level of 250 g/day (Pantuck *et al.*, 1979, 1984).

Endemic goitre was common in many countries in the past century and in many European countries as late as 1985. Only the Nordic countries and the United Kingdom had succeeded by that time in decreasing the prevalence (European Thyroid Association, 1985). Goitre can be controlled by compulsory addition of iodide to all household salt. Several studies were conducted after 1947 on the goitrogenic effects of dietary components and drugs by injecting volunteers with radioiodine and determining uptake into the thyroid by external counting (van Etten *et al.*, 1969a).

A goitrogenic effect of cruciferous vegetables was hypothesized on the basis of field observations of Tasmanian schoolchildren and the source of milk in their homes. The most likely source of the goitrogenic compounds in the diets of these children was the milk of cows given *Brassica* forage crops, particularly during the winter months (Clements, 1957).

In Finland, when goitre was common, it was suspected to be due to low dietary iodine intake in combination with consumption of milk from cows grazing on fields rich in cruciferous vegetation. In an experiment with 22 healthy volunteers (7 women, 15 men) aged 22–46 years, radioiodine uptake into the thyroid was determined while they drank 1.5–2 l of milk containing 0–20.3 µg/l of goitrin, 2.1–8.0 mg/l of SCN^- and 8–142 µg/l of I^- (Vilkki *et al.*, 1962). No effects were found on radioiodine uptake, plasma-bound iodine or total serum iodine. In an experiment in which a single dose of 0.15–100 mg of goitrin was given to volunteers [number per group not given], a reduction in radioiodine uptake was seen at doses ≥ 50 mg.

Shortly after injection of ^{131}I and determinations of baseline uptake, groups of two men or women [sex not specified for each group] were given crystalline goitrin at one of six doses from 12.5 to 400 mg (Langer *et al.*, 1971). A dose-related decrease in radioiodine intake was observed, with 50% inhibition at around 300 mg [value read from curve]. In two further experiments described in the same publication, each individual served as his or her own control by undergoing two tests for radioiodine uptake 1 week apart. After a baseline test for radioiodine uptake 1 week earlier, six volunteers received 50 mg of crystalline goitrin, and 13 received 25 mg 1 h before the second test. A third group of 18 volunteers were given 10 mg of crystalline goitrin 12 h before and another 10 mg 1 h before the second test. Radioiodine uptake was statistically significantly decreased in the two former groups, whereas no decline was observed in the third group. In two individuals given 50 mg, uptake of radioiodine was completely blocked. Reduced iodine uptake by the thyroid gland was observed with the radioiodine test after a daily intake of 500–600 g of cabbage (raw, pickled, boiled or quick-steamed) for 2 weeks by a group of nine volunteers [previous work cited by Langer *et al.* (1971)]. Each individual served as his or her own control by comparison with a baseline test performed 6 months earlier.

In a parallel study of the effects of ingestion of 150 g of Brussels sprouts daily for 4 weeks by a group of three women and seven men, no effects were observed on serum concentrations of thyrotropic hormone, total or free thyroxine or total triiodothyronine (McMillan *et al.*, 1986). The sprouts variety had been selected for its high content of progoitrin and glucobrassicin, and the daily doses of these compounds were confirmed by food analyses to be 99 mg and 105 mg, respectively; however, the daily intakes were decreased to 69 mg and 39 mg, respectively, by cooking. The intake of progoitrin in this study could have led to an intake of 14 mg of the goitrogenic goitrin. The authors speculated that the low (2.4%) residual activity of myrosinase in the sprouts decreased the formation of goitrogenic derivatives and was indirectly the cause of the null effect observed in this study.

Indoles

Groups of 20 women at high risk for breast cancer were assigned to 400 mg/day indole-3-carbinol or placebo for 3 months. They were interviewed monthly about any adverse effects, and blood samples were collected for extensive clinical chemistry. No systematic adverse effects were reported, and there was no effect on plasma bilirubin and albumin or on serum glutamyl ornithine transaminase, suggesting no effect on liver function. Also, plasma estrogen and the number of days between menses were unaffected (Bradlow *et al.*, 1994).

In a double-blind placebo-controlled dose-ranging study, 57 women of an average age of 46.7 years (range, 22–74 years) who were at increased risk for breast cancer were randomized into groups of 7–10 and given indole-3-carbinol at a dose of 0–400 mg/day for 4 weeks (Wong *et al.*, 1997). No toxicity or consistent adverse effects were reported.

In a prospective open-label trial, indole-3-carbinol was given to 18 patients aged 1.2–66 years with recurrent respiratory papillomatosis; the children received a weight-related dose of 5–10 mg/kg bw per day, while the adults took 200 mg twice a day for 8–24 months (average, 14.6 months). Symptoms of disequilibrium developed in three persons who took excessive doses, one of whom was a male volunteer given 800 mg/day for 10 days. Light tremor was also observed. These symptoms disappeared when the dose was decreased to 400 mg/day. Two girls aged 2.5 years and 12 years who accidentally took triple doses both experienced unsteadiness for periods of 8–24 h (Rosen *et al.*, 1998).

Experimental animals

Acute toxicity

Glucosinolates and nitriles

The reported values for the median lethal dose (LD_{50}) of *Brassica* compounds in animals are shown in Table 64.

The acute toxic effects of subcutaneously injected *Brassica* glucosinolates and nitrites were studied in groups of 5–8 Holtzman rats of each sex, maintained on Teklad 4% mouse and rat diet and weighing 250–300 g (Nishie & Daxenbichler 1980). Some of the rats were pregnant. Indole-3-carbinol and 3-indolylacetonitrile induced sedation, ataxia, loss of righting reflex and sleep. In general, they were less toxic in pregnant females and more toxic in the younger male rats. Several other compounds were tested at only one or two doses, administered by a single or by two repeated subcutaneous injections. The kidneys, livers and adrenal and thyroid glands were examined histologically at necropsy or 12 days after dosing. 1-Cyano-3,4-epithiobutane (total dose, about 90 mg/kg bw) gave rise to increased renal weights and renal necrosis in pregnant dams, and signs of restorative growth were observed in survivors. Abnormal thyroids were observed in non-pregnant females given acutely toxic doses (150–650 mg/kg bw) of *epi*-progoitrin, 3-indolylacetonitrile, *S*-1-cyano-2-hydroxy-3-butene or *R*-goitrin, in the form of hyperplastic cells, smaller follicles, foamy areas and scanty colloid, whereas the thyroids of pregnant females were largely unaffected by these compounds. Slightly hyperplastic thyroids were observed in pregnant and non-pregnant rats after near-lethal doses (100–700 mg/kg bw; see Table 64) of 1-cyano-3,4-epithiobutane, sinigrin (including oral administration), sinalbin hydrate, *para*-hydroxyphenylacetonitrile, iberin nitrile, 3-indolylacetonitrile and indole-3-carbinol. Iberin at an acutely toxic dose (100 mg/ kg bw) did not affect thyroid tissue in rats. Sporadic changes in liver and adrenal weights were found with the nitrites. 1-Cyano-3,4-epithiobutane consistently increased adrenal weights of males and pregnant female rats by 25–50% at a dose of 95 mg/kg bw.

In order to determine the lethal dose, high single doses of the compounds were administered subcutaneously to Sprague-Dawley rats. The rats were observed for 12 days after dosing, at which time they were killed. The mean LD_{50} values were 90 mg/kg bw for iberin, 109 mg/kg bw for 1-cyano-3,4-epithiobutane, 200 mg/kg bw for *S*-1-cyano-2-hydroxy-3-butene and 255 mg/kg bw for 3-indolylacetonitrile; values could not be determined for indole-3-carbinol or *para*-hydroxyphenylacetonitrile, although their acute toxicity was similar to that of 3-indolylacetonitrile.

Isothiocyanates and derivatives

In the study of Nishie and Daxenbichler (1980), the mean LD_{50} value for allyl-ITC was reported to be 92 mg/kg bw (see Table 64). Mildly abnormal thyroid tissues were

Table 64. Median lethal doses (LD_{50}) of *Brassica* compounds

Compound	Species, strain	Route of administration	Duration of follow-up	LD_{50} (mg/kg bw)	Reference
1-Cyano-3,4-epithio-butane	Mouse	Oral	Not specified	178–240	van Etten *et al.* (1969a)
	Rat, Sprague-Dawley	Subcutaneous	12 days	109	Nishie & Daxenbichler (1980)
	Mouse	Oral	Not specified	170	van Etten *et al.* (1969a)
	Rat, Sprague-Dawley	Subcutaneous	12 days	200	Nishie & Daxenbichler (1980)
Allyl-ITC	Mouse (white)	Subcutaneous	14 days	80	Klesse & Lukoschek (1955)
	Rat	Oral		339	Jenner *et al.* (1964)
	Rat, Sprague-Dawley	Subcutaneous	12 days	92	Nishie & Daxenbichler (1980)
Phenethyl-ITC	Mouse (white)	Subcutaneous	14 days	250	Klesse & Lukoschek (1955)
	Mouse, Swiss-Webster	Subcutaneous	14 days	150	Lichtenstein *et al.* (1962)
		Oral	14 days	700	
		Intravenous	14 days	50	
Goitrin	Mouse	Oral	Not specified	1260–1415	van Etten *et al.* (1969a)
Iberin	Rat, Sprague-Dawley	Subcutaneous	12 days	90	Nishie & Daxenbichler (1980)
Indole-3-carbinol	Mouse, ICR	Oral	10 days cumulative	1670	Shertzer (1982)
		Intraperitoneal	10 days cumulative	500	
	Mouse, CD1	Oral	1 h	<750	Shertzer & Sainsbury (1991a)
	Rat, Wistar	Oral	10 days cumulative	1850	Shertzer (1982)
		Intraperitoneal	10 days cumulative	550	
	Rabbit, white	Oral	10 days cumulative	1400	
		Intraperitoneal	10 days cumulative	420	
3-Indolylacetonitrile	Rat, Sprague-Dawley	Subcutaneous	12 days	255	Nishie & Daxenbichler (1980)

observed in pregnant and non-pregnant rats after near-lethal doses. The LD_{50} of a 10% solution of allyl-ITC given subcutaneously in corn oil to mice was reported to be 80 mg/kg bw (IARC, 1985).

The acute toxicity of allyl-ITC in groups of five Fischer 344/N rats and five $B6C3F_1$ mice of each sex was examined in pilot studies before a long-term cancer bioassay (National Toxicology Program, 1982). After 16 days' administration of allyl-ITC in corn oil by gavage, growth retardation and dose-related signs of toxicity were seen at doses of 200 and 400 mg/kg bw in rats and 100–800 mg/kg bw in mice, evaluated on the basis of comparisons with the lowest dose (no controls). No female mice and only one male mouse survived the dose of 800 mg/kg bw.

Groups of five male and five female Osborne-Mendel rats were fasted for 18 h and then given a single high dose of allyl-ITC by gavage and observed for 14 days. An LD_{50} of 339 mg/kg bw (95% confidence interval, 318–361) was calculated (Jenner *et al.*, 1964). The animals were described as scrawny, with porphyrin-like deposits around the eyes and nose and rough fur.

The LD_{50} values for phenethyl-ITC in mice were reported to be 700 mg/kg bw after oral administration and 50 mg/kg bw after intravenous injection. In the same species, the LD_{50} of phenethyl-ITC administered subcutaneously was reported to be 150 mg/kg bw (Lichtenstein *et al.*, 1962) or 250 mg/kg bw (Klesse & Lukoschek, 1955).

In a study presented in an overview (van Etten *et al.*, 1969a), the LD_{50} values in mice were 1260–1415 mg/kg bw for goitrin (*R*-5-vinyloxazolidine-2-thione), 169 mg/kg bw for *S*-1-cyano-2-hydroxy-3-butene and 178–240 mg/kg bw for *S*-1-cyano-2-hydroxy-3,4-epithiobutane.

Indoles

Preliminary LD_{50} values were determined by Shertzer (1982) in male ICR mice, Wistar rats and New Zealand white rabbits treated with indole-3-carbinol for 10 days (Table 64). Male ICR mice, weighing about 30 g bw, were maintained on Purina animal chow and dosed orally or intraperitoneally for 10 consecutive days. The LD_{50} in male ICR mice was estimated to be 500 mg/kg bw after intraperitoneal administration and 1670 mg/kg bw when given by gavage. In male Wistar rats (weighing 300 g), the LD_{50} values for intraperitoneal and oral administration were 550 mg/kg bw and 1850 mg/kg bw, respectively, whereas the LD_{50} values for rabbits (male New Zealand white, weighing about 2 kg) were 420 mg/kg bw when given intraperitoneally and 1400 mg/kg when administered orally. No further details about the experiments were given.

The acute toxicity of indole-3-carbinol was studied in groups of male CD-1 mice weighing 30–35 g, maintained on Teklad standard rodent diet for 7 days and then given indole-3-carbinol orally at 50 mg/kg bw per day (dissolved in corn oil, 1.5 µl/g bw) for 10 days (Shertzer & Sainsbury, 1991a). Indole-3-carbinol did not change the body weight or the liver:body weight ratio. The activity of several plasma enzymes was determined 2 and 24 h after a single oral dose of 50–500 mg/kg bw. Plasma creatine phospho-kinase activity was not changed at doses up to 500 mg/kg bw, indicating a lack of severe cardiotoxicity; however, indole-3-carbinol caused a dose-dependent increase in plasma ornithine transcarbamylase activity (at doses > 100 mg/kg bw) and alanine transaminase activity (at doses > 250 mg/kg bw), indicating a hepatotoxic effect, 24 h after treatment. Indole-3-carbinol decreased the hepatic GSH concentration in a dose-dependent manner 2 h after oral doses > 100 mg/kg bw; the concentration had returned to background levels by 24 h at doses < 250 mg/kg bw. Leaking of hepatic enzymes into the bloodstream was seen at doses > 100 mg/kg bw. Neurological signs, including changes in posture and activity, were seen at doses ≥ 100 mg/kg bw 2 h after gavage. A dose of 500 mg/kg bw induced coma.

A single oral administration of indole-3-carbinol at a dose > 100 µmol per rat (15 mg/kg bw) to groups of four male Sprague-Dawley rats, 3–4 weeks old and maintained on a semi-purified diet for 7–8 days, led to toxic manifestations in the small intestine (Bradfield & Bjeldanes, 1987b).

Groups of six male guinea-pigs, weighing 400–500 g, were given two oral doses of indole-3-carbinol at 0.3 mg/kg bw per day for 4 days. The animals were killed after treatment on day 4, and four tissue samples were collected from the liver and lung of each animal and examined histopathologically. Indole-3-carbinol caused hepatic steatosis and interstitial pneumonia with septal hyperaemia (Gonzalez *et al.*, 1986).

Short-term effects

Brassicas

Crambe oilseed meals processed to increase or decrease the levels of epi-progoitrin, goitrin or total nitriles were given at 10% in the feed to groups of five weanling rats for 90 days, and the weight and survival of the animals were recorded as a measure of toxicity (van Etten *et al.*, 1969b). Fractions of *epi*-progoitrin (0.85%), *R*-goitrin (0.23%) and nitrile (0.1%) isolated from crambe seeds were mixed into the feed for additional groups. The nitrile-rich meals were the most toxic, leading to 100% mortality in a group fed 10% (w/w) meal treated to enrich nitriles, and a 40% reduction in body weight in a similar group in a second experiment. The weight of animals fed

0.1% nitrile fraction in the feed was only 17% that of controls (60% survival). Animals fed meal containing active myrosinase had only 40–60% weight gain and reduced survival. These meals had high concentrations of *epi*-progoitrin. The animals fed purified *epi*-progoitrin or *R*-goitrin reached 85% of the weight of controls.

Isothiocyanates and derivatives

WIST male rats weighing 70–90 g [number of animals per group not reported] were given allyl-ITC at a dose of 10, 20 or 40 mg/kg bw (5 days/week) by gavage for up to 6 weeks (Lewerenz *et al.*, 1988). The highest dose reduced body-weight gain and decreased blood glucose and serum globulin levels. Examination of the blood revealed an increased percentage of neutrophils and a decreased percentage of lymphocytes after treatment with the highest dose for 2 weeks. After 1–3 weeks, increased thymus, liver or adrenal weights (both relative and absolute) were found in all treated groups. Increasing the treatment period to 4 weeks resulted in no significant differences in thymus, liver or adrenal weights between treated and control animals. Renal dysfunction was indicated by increased urinary aspartate aminotransferase activity, reduced urine volume and changes in the specific gravity of the urine in the group of rats receiving the highest dose. Histopathological changes were observed in the kidneys of animals given 20 or 40 mg/kg bw per day and in the livers of animals at 40 mg/kg bw per day.

The toxicity of allyl-ITC after repeated doses was investigated in pilot studies before a long-term cancer bioassay in rats and mice of each sex. An experiment in which groups of five male and five female Fischer 344/N rats and B6C3F$_1$ mice received doses in corn oil of 25–400 mg/kg bw and 3–50 mg/kg bw, respectively, by gavage for 14 days resulted in 'adhesion of the stomach wall to the peritoneum' in rats and a dose-dependent thickening of the stomach mucosa in both species. Furthermore, mice given the highest dose developed thickening of the urinary-bladder wall. [The Working Group noted that histological details were not presented and that the significance of these findings is uncertain.] The doses of 200 and 400 mg/kg bw per day to rats and 50 mg/kg bw per day to mice were lethal. In a 13-week study in groups of 10 male and female Fischer 344/N rats and B6C3F$_1$ mice given a dose of 1.5, 3, 6, 12 or 25 mg/kg bw per day, no dose-related effects were observed on growth or gross morphology (National Toxicology Program, 1982).

Administration of phenethyl-ITC at a dose of 41, 82 or 122 mg/kg bw per day for 6 days by gavage to groups of 24 female and 24 male young A/J mice resulted in significantly reduced body-weight gains [absolute numbers not specified], hyperactivity, rough fur and emaciation (Adam-Rodwell *et al.*, 1993). A dose of 244 mg/kg bw per day was lethal after two to four doses and was discontinued.

The toxicity of benzyl-ITC was investigated in groups of 15 male rats weighing 85–110 g given the compound dissolved in sunflower oil at a dose of 50, 100 or 200 mg/kg bw per day by gavage for 4 weeks (Lewerenz *et al.*, 1992). Control rats were given the vehicle only. Body-weight gain and food consumption were decreased with increasing doses of benzyl-ITC. Haematological changes were observed at the highest dose, with increased serum cholesterol concentrations in all treated groups and decreased serum triglycerides at 200 mg/kg bw per day. Renal dysfunction was indicated by reduced urine volume, proteinuria and enhanced urinary lactate dehydrogenase activity. Benzyl-ITC at 200 mg/kg bw per day caused histological changes in the ductus choledochus, liver, ileum and mesenteric lymph nodes. The weights of the thymus (at 100 and 200 mg/kg bw per day) and spleen (at 200 mg/kg bw per day) were decreased in relation to body weight, whereas the weights of all other organs were increased.

Even at a low dose of 7.5 mg/kg bw per day to ACI rats for 53 weeks, benzyl-ITC resulted in a decrease in weight gain of 8–9% as compared with control animals (Sugie *et al.*, 1991).

Morse *et al.* (1989a) examined the toxicity of phenethyl-ITC at 0, 0.75, 1.5, 3 or 6 µmol/g of diet in male Fischer 344 rats (weighing 100–120 g) in a 13-week study. Dietary administration of phenethyl-ITC did not produce any deleterious effects on survival, body weight or food intake. Clinical evaluation showed no adverse changes suggesting toxicity. In addition, no significant differences from control animals were found on gross examination at necropsy or by determination of relative organ weights. Histopathological analyses, however, revealed centribular and mid-zonal fatty metamorphosis in the livers of rats exposed to phenethyl-ITC at all doses tested.

Indoles

Groups of eight male Sprague-Dawley rats weighing 70 g were given a control diet or control diet containing either glucobrassicin at 0.5 g/kg [30 mg/kg bw per day], sinigrin, gluconapin, glucosinalbin or glucotropaeolin each at 1.0 g/kg [60 mg/kg bw per day] or progoitrin at 3.0 mg/kg [160 mg/kg bw per day] for 29 days. Before treatment, the animals were held on control diet for 2 days, for 1 day on control diet containing 50% of the final glucosinolate level and for 1 day on control diet containing 75% of the final glucosinolate level. The dietary glucosinolates did not affect feed intake or body-weight gain

throughout the period. Only progoitrin affected the relative weights of the thyroid, liver and kidney (Vermorel *et al.*, 1986).

The effect of indoles on body weight was investigated in groups of six male weanling Sprague-Dawley rats fed AIN76 diet containing indole-3-carbinol at 50, 500, 5000 or 7500 mg/kg *ad libitum* for 3 weeks (Babish & Stoewsand, 1978). Significantly lower body-weight gain was observed with 5000 mg/kg [1500 mg/kg bw per day] and 7500 mg/kg [2250 mg/kg bw per day], representing 85% and 59% of the weight gain of untreated controls, respectively. The relative liver weights were increased by 1.6- and 1.8-fold, respectively, in these two groups. In another study, indole-3-carbinol was given by gavage at 5, 25, 50, 100 or 200 mg per day for 6 weeks to female Sprague-Dawley rats from 43 days of age (Grubbs *et al.*, 1995). The highest dose caused a 10% depression in body-weight gain and a 20% increase in liver weight. The modifying effect of indole-3-carbinol was investigated in a multi-organ carcinogenesis model in groups of 20 male Fischer 344 rats, 6 weeks of age, maintained on AIN-76A diet for 4 weeks and then switched to the same diet containing 0.5% indole-3-carbinol for 36 weeks [300 mg/kg bw per day]. An insignificant decrease in body weight was observed (Jang *et al.*, 1991).

The effect of dietary indole-3-carbinol on liver and body weight was investigated in groups of six female C3H/OuJ adult mice, 80–100 days old, maintained on Purina basal diet with 10% corn oil for 3 days and then given the same diet containing indole-3-carbinol at 0, 1000, 3000 or 5000 mg/kg [120, 360 or 600 mg/kg bw per day] for 3 weeks (Bradlow *et al.*, 1991). The average daily food consumption decreased significantly only in mice at the highest dose. The relative liver:body weight ratio increased with dose. Additionally, groups of six female SW mice, 80–100 days of age, were maintained on Purina basal diet with 10% corn oil for 3 days and then given the same diet containing indole-3-carbinol at 0, 250, 1000 or 2500 mg/kg [30, 120 or 300 mg/kg bw per day] for 3 weeks. No difference in body weight was observed. A slight increase in the liver:body weight ratio was seen in treated animals, but there was no gross evidence of hepatic toxicity. Two groups of 10 male and 10 female C3H/OuJ mice, 21–28 days of age, were kept on control Purina semi-synthetic diet or the same diet containing indole-3-carbinol at 2000 mg/kg [240 mg/kg bw per day] for 6 months. The average body weights of the females in each group were recorded about every 10–15 days. No differences in body weight were observed.

Groups of seven male Sprague-Dawley rats were fed diets containing indole-3-carbinol at concentrations providing a dose of 0, 50, 100 or 150 mg/kg bw per day, 5 days/week for 7 weeks (Exon *et al.*, 2001). The antibody response to an antigen challenge was significantly decreased at the highest dose. No effects were found on natural killer cell activity or delayed-type hypersensitivity.

Goitrogenic effects

Crucifers and unrefined fractions

A goitrogenic effect of crucifers was first noted after feeding *Brassica* seeds to rabbits (Chesney *et al.*, 1928). Several years later, the effect was found to be due mainly to the formation of organic and inorganic isothiocyanates and goitrins (oxazolidinethiones) (Astwood *et al.*, 1949). Goitrogenic effects in various domestic and laboratory animals have been reviewed (Fenwick *et al.*, 1989; Mawson *et al.*, 1994; Stoewsand, 1995).

Groups of 4–9 male Wistar rats were fed 10–15 g/day of a low-iodine diet (0.5–0.8 µg/day) with about 40 g of cabbage or carrots (both vegetables contained negligible amounts of iodine), or cabbage or carrots alone, for 60 days (Langer & Stolc, 1965). The thyroid glands were significantly enlarged and contained significantly less iodine per gram of organ. Plasma-bound iodine was also significantly decreased.

Pigs appear to be vulnerable to rapeseed meal glucosinolates. Groups of 7–10 male and female pigs, initially weighing 20–25 kg, received their ordinary diet containing 2–15% unrefined rapeseed meal to replace skim milk powder (Nordfeldt *et al.*, 1954). Thyroid and liver weights were increased in all treated animals. Adding an antibiotic (bacitracin), vitamin B_{12} or wheat bran to the diet did not alleviate the effects. Water extraction of the rapeseed meal significantly attenuated the effect.

Studies in pigs were reviewed by Mawson *et al.* (1994). A generally linear relationship was observed between glucosinolate intake from feed and thyroid weight at slaughter. Significant increases in thyroid weights were observed in pigs given feed with a total glucosinolate content of 2–3 mg/kg, particularly when progoitrin was one of the main glucosinolates.

Isothiocyanates and derivatives

The spontaneous formation of goitrin from 2-hydroxy-3-butenyl-ITC, resulting from degradation of progoitrogen catalysed by myrosinase, is one of the main causes of the goitrogenic action of rapeseed meal. Groups of eight female Sprague-Dawley rats given a diet containing about twice the minimal required iodine content were given 5-vinyl-2-thiooxazolidone intraperitoneally at a dose of 1–200 µg/day for 3 weeks (Elfving, 1980). Controls were injected with saline. Significant increases in thyroid weights were observed at doses > 5 µg/day. In the

thyroid, significantly increased ratios of 3′-monoiodothyronine:3,5-diiodothyronine and of 3,5,3′-triiodothyronine: 3,5,3′,5′-tetraiodothyronine were observed even at the lowest dose. Total ^{125}I uptake into the thyroid was unchanged at all doses, but iodine excretion into urine was decreased at the highest dose. The authors concluded that goitrin acts primarily on thyroxin biosynthesis.

Allyl-ITC has slight goitrogenic activity in rodents. Studies in rats (weighing 150–320 g) given allyl-ITC as a single dose of 2–4 mg in water by gavage showed 40–60% less iodine uptake into the thyroid gland. Oral administration of allyl-ITC at 2.5–5 mg/day for 60 days, however, did not decrease the total iodine content in the thyroid (Langer & Stolc, 1965).

The degree of goitrogenicity induced by allyl-ITC was found to be weaker than that induced by better-known goitrogens, such as thiouracil (Duncan, 1991).

Indoles

Groups of eight male Sprague-Dawley rats, weighing 70 g, were fed control diet or control diet complemented by glucobrassicin at 0.5 g/kg [30 mg/kg bw per day] for 29 days. Before treatment, the animals were given control diet for 2 days, then control diet containing 50% of the final glucosinolate level for 1 day and finally control diet containing 75% of the final glucosinolate level for 1 day. The animals were killed 29 days after the start of treatment. Neither thyroid weight nor thyroid hormone concentrations in the thyroid were affected (Vermorel *et al.*, 1986).

Haemolytic effects

Kale fed to ruminants in considerable quantities for 1–3 weeks led to haemolytic anaemia due to its content of *S*-methylcysteine sulfoxide (Smith, 1980). Groups of 52 and 103 goats were fed kale containing *S*-methylcysteine sulfoxide at 99 or 154 mg/kg. Heinz bodies reached their peak level at 9 and 33 days of the feeding period, respectively, and serum haemoglobin reached minimal levels at 20 and 44 days, respectively. Additional groups of goats received different concentrations: 10 animals received a fractionated kale extract containing *S*-methylcysteine sulfoxide at 285 mg/kg, 97 animals received *S*-methylcysteine sulfoxide at 150 mg/kg, and 104 animals received *S*-methylcysteine sulfoxide at 195 mg/kg. The number of days required to attain maximal formation of Heinz bodies was 7, 35 and 21, and the number of days to reach the lowest haemoglobin level was 15, 42 and 35, respectively.

The metabolite of *S*-methylcysteine sulfoxide, dimethyl disulfide, precipitates GSH to initiate the formation of Heinz–Ehrlich bodies in erythrocytes. According to a recent review, ruminants, fowl and rats are also vulnerable to anaemia after being fed kale or dimethyl disulfide, but guinea-pigs and rabbits are refractory (Stoewsand, 1995). Dosing of rats with feed containing *S*-methylcysteine sulfoxide at 2–4% (w/w) elicited the effect, but it was reversible after about 14 days.

Reproductive effects

Decreased fertility has been described repeatedly in domestic animals given rapeseed meal as part of their feed. Mawson *et al.* (1994) summarized published and anecdotal evidence and concluded that rats and pigs are the most vulnerable species, whereas ruminants and chicken are less sensitive. Pigs may be affected by total rapeseed glucosinolate concentrations exceeding 4 mg/kg of feed, whereas rats appear to tolerate even less.

Groups of 3–12 pregnant Holzman rats were given *Brassica* glucosinolates and degradation products at doses close to the LD_{50} on day 8 or 9 of gestation, and the numbers of live fetuses and resorptions were examined at necropsy or at termination 12 days later (Nishie & Daxenbichler, 1980). A significant 48% increase in fetal resorption was observed after a single dose of 1-cyano-3,4-epithiobutane at 95 mg/kg bw given on day 8 of gestation, whereas two doses of 44 mg/kg bw on days 8 and 9 did not affect fetal survival. A significant 19% increase in fetal resorption was observed after two doses of allyl-ITC at 100 mg/kg bw on days 8 and 9 of gestation, whereas one-half of this dose on the same days had no effect. Administration of iberin at 100 mg/kg bw on day 8 of gestation increased fetal resorption by 29%. None of these compounds resulted in malformations. Significant changes in mean fetal weights were recorded with epigoitrin, *S*-1-cyano-2-hydroxy-3-butene, *R*-goitrin, 1-cyano-3,4-epithiobutane, sinigrin, iberin nitrile, 3-indolylacetonitrile, indole-3-carbinol and *para*-hydroxyphenylacetonitrile when given at near-lethal doses to the pregnant dams. Sinalbin hydrate given at 300–600 mg/kg bw to pregnant dams did not lead to increased altered fetal weights, fetal resorption or malformations.

The teratogenic activity of allyl-ITC was evaluated in mice, rats, hamsters and rabbits in a study performed by the Food and Drug Research Laboratories in 1973 (IARC, 1985). Allyl-ITC dissolved in corn oil was administered by gavage in all studies. Groups of 23–25 CD-1 mice were given 0, 0.3, 1.3, 6 or 28 mg/kg bw per day on days 6–15 of gestation, and fetuses were examined on day 17 for malformations. Groups of 25 Wistar rats received a dose of 0, 0.2, 0.85, 4 or 18.5 mg/kg bw per day on days 6–15 of gestation, and fetuses were examined for malformations on day 20. Groups of 25–27 golden hamsters received a dose of 0, 0.2, 1.1, 5.1 or 23.8 mg/kg bw per day on days 6–10 of gestation, and fetuses were

examined for malformations on day 14. Groups of 11–14 Dutch-belted rabbits received a dose of 0, 0.123, 0.6, 2.8 or 12.3 mg/kg bw per day on days 6–18 of gestation, and fetuses were examined for malformations on day 29. No evidence of maternal toxicity or treatment-related malformations was found in any species. In mice, there appeared to be an increase in the number of dead and resorbed fetuses at the highest dose (28 mg/kg bw per day) (38/276 implantation sites had dead or resorbed fetuses, compared with 15/264 in the control group, and the average number of live pups per litter was 9.92 versus 11.3), although no statistical analysis of the data was presented.

Groups of pregnant Wistar rats were given allyl-ITC in corn oil by gavage at 0, 60 or 120 mg/kg bw per day on day 12 or 13 of gestation. Despite severe maternal toxicity at the higher dose, no adverse effect on the fetuses was reported (Ruddick *et al.*, 1976).

Groups of pregnant Sprague-Dawley rats were given indole-3-carbinol at a dose of 0 (vehicle, corn oil:acetone 19:1), 1 or 100 mg/kg bw on day 15 of gestation (Wilker et al., 1996). Each observation group consisted of three to five litters of male offspring, standardized at 10 per litter after whelping. Ano–genital distance and crown–rump length were decreased significantly at the higher dose on day 1 after birth. Several markers of sperm count and abnormalities were affected on day 62 after birth at either or both doses, including daily sperm production and epididymal transit time.

Groups of 6–8 weaned female Sprague-Dawley rats, 23 days of age (weighing 55–60 g) were given indole-3-carbinol (0.05, 0.5, 1 or 1.5 g/kg bw per day), 3,3′-diindolylmethane (0, 100, 200 or 400 mg/kg bw per day) or vehicle (dimethyl sulfoxide) by gavage (Gao *et al.*, 2002). Indole-3-carbinol at doses > 0.5 mg/kg bw per day decreased body-weight gain after four daily doses, whereas 3,3′-diindolylmethane had no effect on weight. The latter compound slightly decreased ovarian weight gain after a challenge with 5 IU of equine chorionic gonadotropin and reduced the number of ova shed after 72 h by about 30% in all treated groups. [The Working Group noted that these changes were not statistically significant]. Indole-3-carbinol decreased both markers significantly and in a dose-dependent manner, even at the lowest dose tested. Furthermore, the time courses of serum luteinizing hormone, follicle-stimulating hormone and progesterone were significantly decreased by indole-3-carbinol, but these end-points were tested only at the highest dose.

Groups of juvenile (12–18-month-old) rainbow trout were given diets containing indole-3-carbinol (providing five doses between 25 and 2000 mg/kg bw per day) or 3,3′-diindolylmethane (2.5–250 mg/kg bw per day) or control feed for 2 weeks (Shilling *et al.*, 2001). 3,3′-Diindolylmethane significantly increased vitellogenin production in the liver in a dose-dependent manner, showing saturation at around 25 mg/kg bw per day. In an additional experiment, the effect was shown to be additive to that of estradiol.

Cytotoxicity, genotoxicity and mutagenic and related effects

Cruciferous vegetables

Plants are known to contain a variety of bioactive substances with toxic effects that may be comparable to or greater than those of synthetic compounds (Ames *et al.*, 1990a,b). Thus, some studies have shown that cruciferous vegetables are cytotoxic, genotoxic and mutagenic. Wakabayashi *et al.* (1985) reported on the direct mutagenic effects of nitrite-treated Chinese cabbage and indoles in *Salmonella typhimurium*. They found that the contributions of indoles to the total mutagenic activity of nitrite-treated Chinese cabbage to *S. typhimurium* TA100 were 0.2% from indole-3-acetonitrile, 0.3% from 4-methoxyindole-3-acetonitrile and 17% from 4-methoxyindole-3-aldehyde (Wakabayashi *et al.*, 1986). Tiedink *et al.* (1988) studied the potential of 30 vegetables to form biologically active *N*-nitroso compounds after treatment with nitrite under acidic conditions. Although all the treated extracts contained *N*-nitroso compounds, only half of the vegetables were mutagenic after nitrite treatment. Extracts of cruciferous vegetables showed the highest mutagenic effect with respect to the number of *Salmonella* revertants induced; however, there was no significant correlation between the level of glucosinolates in these vegetables and the number of revertants. Subsequent detailed studies on the role of glucosinolates in the formation of *N*-nitroso compounds showed that only indole glucosinolates are involved in the formation of mutagenic agents (Tiedink *et al.*, 1991). Nevertheless, the contribution of these glucosinolates to the mutagenicity of nitrite-treated cruciferous vegetables appears to be negligible. For instance, although Brussels sprouts are one of the richest sources of indolyl glucosinolates (6.6 µmol/g of dry weight) they induced only 61 ± 2 revertants of *S. typhimurium* TA100 per 25 mg of dry matter, whereas radish, with an indolyl-glucosinolate concentration of 1 µmol/g of dry weight, induced 233 ± 63 revertants per 25 mg of dry matter (Tiedink *et al.*, 1988). Tiedink *et al.* (1990) found that the individual contribution of indole-3-acetonitrile to the total mutagenicity of green cabbage extracts treated with nitrite was about 2%. The most abundant

indole in the extract, indole-3-carboxyaldehyde, was not mutagenic. These studies therefore indicate that the contribution of indole compounds to the mutagenicity of cruciferous vegetables treated with nitrite is negligible.

The mutagenic effect of nitrosated indole compounds is not restricted to certain groups of indoles. Eight indole compounds, indole-3-acetonitrile, indole-3-carbinol, indole-3-acetamide, indole-3-acetic acid, 3-methylindole, indole-3-aldehyde, indole-3-carboxylic acid and indole, tested in *S. typhimurium* strains TA98 and TA100 and *Escherichia coli* WP2 uvr/PKM101 after nitrite treatment at pH 3 were mutagenic in all three strains in the absence of metabolic activation (at up to 0.5 μmol/plate). Indole-3-acetic acid had the strongest effect. Addition of a metabolic activation system decreased the effect (Sasagawa & Matsushima, 1991). None of the compounds was mutagenic without nitrite.

As the effect of vegetable extracts not treated with nitrite was not investigated in these studies, it is not clear whether other bioactive substances in cruciferous vegetables contributed to the observed effect. The genotoxicity and mutagenicity of eight cruciferous vegetables (Brussels sprouts, white cabbage, cauliflower, green cabbage, kohlrabi, broccoli, turnip and black radish) was tested in the *Salmonella*/microsome test, in the differential DNA repair assay with *E. coli* and in a test for chromosomal aberrations in Chinese hamster ovary cells (Kassie *et al.*, 1996). Brussels sprout juice had the strongest effect in all three assays, and exogenous metabolic activation was not required for induction of the genotoxic and mutagenic effects. Addition of Arochlor-induced rat liver microsomes reduced the mutagenicity of the juices to *S. typhimurium* TA100 by 20–50%. Tests carried out with two fractions of the juices, one containing isothiocyanate and the other phenolic compounds, showed that 70–80% of the mutagenicity of the crude juice was due to the isothiocyanate-containing portion. No correlation was found, however, between the mutagenicity of the juices and their histidine or total isothiocyanate content. Charles *et al.* (2002) studied the cytotoxic effect of Brussels sprouts, cauliflower, red cabbage, broccoli and turnip towards Chinese hamster ovary cells. The concentration of the crude extracts that produced a 50% reduction in the number of cells relative to the control was < 10 μl/ml of medium for broccoli and Brussels sprouts, 10–50 μl/ml of medium for turnips and 50–100 μg/ml for cauliflower and red cabbage. In the same study, broccoli juice significantly induced chromosomal aberrations at a concentration of 30 μl/ml of medium.

Purified glucosinolates do not have significant genotoxicity in vitro. Chromosomal aberrations were induced in Chinese hamster ovary cells by sinigrin and gluconasturtiin at concentrations > 2 mg/ml of medium (Musk *et al.*, 1995a).

Isothiocyanates

Isothiocyanates are biologically reactive compounds as a result of the highly electrophilic central carbon atom of their –N=C=S group. They react with oxygen-, sulfur- or nitrogen-centred nucleophiles to give rise to thiocarbamates, dithiocarbamates or thiourea, respectively.

Studies on the cytomorphological changes induced by benzyl-ITC–GSH, (10 μmol/ml of medium) and allyl-ITC–GSH (25 μmol/ml) in RL-4 rat hepatocytes showed that these compounds cause considerable toxicity, characterized by blebbing of the cells (Bruggeman *et al.*, 1986; Temmink *et al.*, 1986). At higher concentrations, distinct patches of dense heterochromatin in the nucleus, complete degranulation of the endoplasmic reticulum, disappearance of polysomes, high-amplitude swelling of the mitochondria, disappearance of blebs with simultaneous appearance of microvilli and concentration of intermediate filaments in the juxtanuclear region were observed. These changes are characteristic features of apoptosis, and it has been shown that isothiocyanates are potent inducers of apoptosis (see section 4). Addition of excess GSH or cysteine either abolished or diminished the cytotoxic effects of benzyl-ITC and allyl-ITC. The GSH and L-cysteine conjugates of benzyl-ITC and allyl-ITC gave effects comparable to the parent compounds, as a result of the reversibility of the reaction between thiols and isothiocyanates. In another study, in which the cytotoxic effect of benzyl-ITC and phenethyl-ITC was investigated in NR50 BALB/c mouse 3T3 fibroblasts (0.016–0.02 mmol/ml), heteropyknosis and vacuolization of the cytoplasm were observed (Babich *et al.*, 1993). Intravesicular instillation of benzyl-ITC and allyl-ITC (2.8 mg/kg bw) or the same molar quantity (37 μmol/kg bw) of benzyl-ITC metabolites conjugated either with GSH, cysteine–glycine, cysteine or mercapturic acid to the urinary bladder of Fischer 344 rats caused cytotoxicity. The effect of benzyl-ITC was greater than that of allyl-ITC. The benzyl-ITC metabolite mercapturic acid, which is thought to be the main final metabolite, had less effect than the other metabolites (Masutomi *et al.*, 2001).

The genotoxic and mutagenic effects of the predominant isothiocyanates contained in cruciferous vegetables are presented in Table 65. Ethyl-, *n*-butyl-, *tert*-butyl-, allyl-, benzyl- and cyclohexyl-ITCs were mutagenic to *S. typhimurium* TA100 after 1 h of preincubation at 37 °C (Yamaguchi, 1980). Allyl-ITC had the strongest effect. Sinigrin, the parent glucosinolate of allyl-ITC, was mutagenic to the same degree as allyl-ITC. Addition of exogenous metabolic

Table 65. Genotoxic and mutagenic effects of isothiocyanates (ITCs)

Isothio-cyanate	Concentration or dose	Genetic effect	Remarks	Reference
Allyl-ITC	100 µg/plate	Increase in number of *his*$^+$ *S typhimurium* TA100 revertants	S9 mix did not influence mutagenicity; weak effect in TA98 but no effect in TA1535, TA1536, TA1537 or TA1538	Yamaguchi (1980)
	0.05–500 µg/plate	Not mutagenic in *S. typhimurium* TA100 or TA98	No effects with or without S9 mix	Kasamaki *et al.* (1982)
	5 nmol/ml medium	Significant increase in structural and numerical chromosomal aberrations in Chinese hamster B241 cells	Colonies consisting of a pile of small cells were observed; S9 mix did not influence the result	
	0.06–0.5 µl/plate	Fivefold increase in number of *his*$^+$ *S. typhimurium* TA100 revertants	Mutagenic effect observed after preincubation for 2 h; mutagenicity only upon addition of S9 mix	Neudecker & Henschler (1985)
	0.2–3 µg/ml medium	Did not induce chromosomal aberrations or sister chromatid exchange in Chinese hamster ovary cells; no effect in SV40-transformed Indian muntjac cells	Parent glucosinolate, sinigrin, induced chromosomal aberrations at 4.6 mg/ml medium	Musk & Johnson (1993); Musk *et al.* (1995a)
	12–200 µg/plate	Marginal increase in number of *S. typhimurium* TA100 and TA98 revertants	S9 mix decreased effect	Kassie & Knasmüller (2000)
	1–25 µg/plate	Dose-dependent increase in reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A)	Genotoxicity attenuated by rat liver S9 mix, rat liver homogenate, bovine serum albumin, human saliva, vitamins E and C, β-carotene and sodium benzoate	
	0.2–4 µg/ml medium	Induction of micronucleus formation in human-derived HepG2 cells	Allyl-ITC less potent than phenethyl-ITC	
	90–270 mg/kg bw	Induction of reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A) in host-mediated assay in mice	Significant effect in liver, lung, colon, kidney and stomach	
	0.5–5 µmol/ml medium	Increased formation of 8-oxo-7,8-dihydro-2′-deoxy-guanosine in calf thymus DNA and human leukaemia cell line; increased DNA damage in *P53* tumour suppressor gene and *c-Ha-ras-1* proto-oncogene	No effect in hydrogen peroxide-resistant leukaemic cells; genotoxicity of allyl-ITC stronger than that of benzyl-ITC or phenethyl-ITC	Murata *et al.* (2000)
	10–50 nmol/ml medium	Induction of DNA strand breaks in human-derived HepG2 cells	Synergistic effect with benzo[*a*]pyrene	Uhl *et al.* (2003)
Benzyl-ITC	100 µg/plate	Increase in number of *his*$^+$ *S. typhimurium* TA100 revertants	S9 mix did not influence mutagenicity; weak effect in TA98 but no effect in TA1535, TA1536, TA1537 or TA1538	Yamaguchi (1980)
	0.22–0.88 µg/ml medium	Dose-related increase in his$^+$ *S. typhimurium* TA100 revertants		Musk & Johnson (1993)

Table 65 (contd)

Isothiocyanate	Concentration or dose	Genetic effect	Remarks	Reference
	0.3–1.2 µg/ml medium	Dose-dependent induction of chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells	Frequency of sister chromatid exchange much lower than that of chromosomal aberrations	Musk *et al.* (1995b)
	1–25 µg/ml medium	Dose-dependent increase in DNA strand breaks in Chinese hamster ovary cells	Loss of comet heads at highest concentration	
	12–200 µg/plate	Marginal increase in number of *S. typhimurium* TA100 and TA98 revertants	S9 mix decreased effect	Kassie *et al.* (1999)
	0.5–5.5 µg/ml medium	Dose-dependent increase in reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A)	Effect attenuated by rat liver S9 mix, rat liver homogenate, bovine serum albumin, human saliva, vitamins E and C, β-carotene and sodium benzoate	
	0.2–4 µg/ml medium	Dose-dependent induction of micronuclei in human-derived HepG2 cells		
	0.5–10 µg/ml medium	Dose-dependent induction of DNA strand breaks in primary rat hepatocytes and gastric mucosa cells	Genotoxic effect in gastric mucosa cells completely attenuated upon incubation of benzyl-ITC with gastric mucus	
	90–270 mg/kg bw	Moderate induction of reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A) in host-mediated assay in mice	Similar effects in liver, kidney, lung, stomach and intestine	
	110–220 mg/kg bw	Moderate increase in DNA strand breaks in rat gastric mucosa and colon in vivo	Strand break maximal 1 h after administration; after 4 h, it reached level in untreated animals	
	0.5–2 µmol/ml medium	Increased formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine in calf thymus DNA and human leukaemia cell line; increased DNA damage in *P53* tumour suppressor gene and *c-Ha-ras-1* proto-oncogene	Benzyl-ITC more genotoxic than phenethyl-ITC but less genotoxic than allyl-ITC	Murata *et al.* (2000)
	2.5–10 nmol/ml medium	Dose-related increase in DNA strand breaks in human-derived HepG2 cells	Synergistically genotoxic with benzo[*a*]pyrene at low concentrations	Kassie et al. (2003b)
Phenethyl-ITC	0.44–1.3 µg/ml medium	Dose-related increase in chomatid gaps and rearrangements in SV40-transformed Indian muntjac cell line and increased frequency of chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells		Musk & Johnson (1993); Musk *et al.* (1995a)

Table 65 (contd)

Isothiocyanate	Concentration or dose	Genetic effect	Remarks	Reference
	12–200 µg/ml medium	Marginal mutagenicity to *S. typhimurium* TA100 and TA98	Effect diminished by S9 mix	Kassie & Knasmüller (2000)
	1–25 µg/plate	Dose-dependent increase in reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A)	Genotoxicity attenuated by rat liver S9 mix; rat liver homogenate, bovine serum albumin, human saliva, vitamins E and C, β-carotene and sodium benzoate	
	0.2–4 µg/ml medium	Induction of micronucleus formation in human-derived HepG2 cells	Allyl-ITC less potent than phenethyl-ITC	
	90–270 mg/kg bw	Non-significant induction of reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A) in host-mediated assay in mice		
	0.5–2 µmol/ml medium	Increased formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine in calf thymus DNA and human leukaemia cell line; increased DNA damage in *P53* tumour suppressor gene and *c-Ha-ras-1* proto-oncogene	Less genotoxic than allyl-ITC or benzyl-ITC	Murata *et al.* (2000)
	2.5–10 nmol/ml medium	Dose-related increase in DNA strand breaks in human-derived HepG2 cells	Synergistically genotoxic with benzo[*a*]pyrene at low concentrations	Kassie *et al.* (2003b)
Methyl-ITC	12–200 µg/mplate	Marginal mutagenicity to *S. typhimurium* TA100 and TA98	Mutagenicity diminished by S9 mix	Kassie *et al.* (2001)
	0.5–4 µg/ml medium	Dose-dependent increase in reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A)	Genotoxicity attenuated by rat liver S9 mix; human saliva, bovine serum albumin and gastric juice	
	0.5–4 µg/ml medium	Dose-related increase in micronucleus frequency in human-derived HepG2 cells		
	3.6–5.4 µg/ml medium	Dose-related induction of DNA strand breaks in human-derived HepG2 cells		
	90 mg/kg bw	Moderate induction of reparable DNA damage in *E. coli* 343/753 (*uvr*B/*rec*A) in host-mediated assay in mice	Significant effect in liver	

S9 mix, 9000 × *g* supernatant of rodent liver

activation did not change the effect. The effect of allyl-ITC in *S. typhimurium* TA98 was weak, and no effect was seen in TA1535, TA1536, TA1537 or TA1538. In another study (Neudecker & Henschler, 1985), allyl-ITC had clear mutagenic effects only after preincubation for 120 min. Although exogenous metabolic activation was a prerequisite for mutagenicity, an excess reduced the mutagenic potency. Other workers reported only borderline mutagenic effects of allyl-ITC (Eder *et al.*, 1980), benzyl-ITC, phenethyl-ITC and methyl-ITC (Kassie *et al.*, 1999; Kassie & Knasmüller, 2000; Kassie *et al.*, 2001) in the standard test. In all these studies, addition of an exogenous metabolic activation system abolished the effect almost completely. Allyl-ITC was not mutagenic in *S. typhimurium* TA98 or TA100 in the presence or absence of metabolic activation (Kasamaki *et al.*, 1982).

Benzyl-ITC, allyl-ITC, phenethyl-ITC and methyl-ITC induced reparable DNA damage in *E. coli* in vitro and in vivo and DNA strand breaks and cytogenetic effects in various mammalian cells (Kasamaki *et al.*, 1982; Musk & Johnson, 1993; Musk *et al.*, 1995a,b; Kassie *et al.*, 1999; Kassie & Knasmüller, 2000; Kassie *et al.*, 2001, 2003b; Uhl *et al.*, 2003) (Table 65). Benzyl-ITC was the most potent of all the isothiocyanates. Allyl-ITC was more genotoxic than phenethyl-ITC in the bacterial assay, but the latter was more potent in cytogenetic tests. In an assay for differential DNA repair with *E. coli* in vivo, the pattern of genotoxicity was similar to that found in vitro, the effect of phenethyl-ITC being the weakest. The doses of isothiocyanates required to induce moderate genotoxic effects in mice were much higher (90–270 mg/kg bw) than those required for the highly cytotoxic and genotoxic effects in vitro. A similar observation was made in comet assays with benzyl-ITC, which weakly induced DNA damage at doses of 110 and 220 mg/kg bw, with a maximum effect 1 h after exposure; by 4 h after exposure, the damage was reduced to the level of that in untreated controls (Kassie *et al.*, 1999). The reduction in the effect of isothiocyanates in vivo could be due to non-specific binding to proteins, as witnessed by the pronounced reduction in the mutagenicity and genotoxicity of the compounds with addition of liver homogenate, an exogenous metabolic activation system, bovine serum albumin or saliva. Moreover, radical scavengers might also contribute to the weak effect of the compounds in vivo, as vitamin E and C, β-carotene and sodium benzoate reduced isothiocyanate-induced differential DNA damage in vitro. The effect of benzyl-ITC on gastric mucosa cells was drastically diminished by incubation of the compound with gastric mucus, corroborating the result observed in the differential DNA repair assay with *E. coli*.

The cytotoxic and genotoxic effects of isothiocyanates might be related to their potential to induce formation of reactive oxygen intermediates. Treatment of rat liver epithelial (RL34) cells with benzyl-ITC or allyl-ITC (10 nmol/ml) resulted in an immediate increase in reactive oxygen intermediates, which corresponded to induction of class πGST P1 (Nakamura *et al.*, 2000b). Depletion of GSH by diethyl maleate significantly increased the production of isothiocyanate-induced reactive oxygen intermediates (hydrogen peroxide, lipid hydroperoxide and peroxynitrite) and accelerated isothiocyanate-induced GST activity, while treatment of cells with GSH inhibited both reactions. Whereas benzyl-ITC was the most potent inducer of reactive oxygen intermediates, the effect of allyl-ITC was intermediate, and phenyl-ITC did not induce these compounds. Kassie and colleagues (1999, 2001) reported that benzyl-ITC, allyl-ITC, phenethyl-ITC and methyl-ITC induced thiobarbituric acid-reactive substances, indicators of lipid peroxidation, in HepG2 cells exposed to 0.5–4 μg/ml of the compounds for 1 h, the order of potency of induction being benzyl-ITC > allyl-ITC = methyl-ITC > phenethyl-ITC. In another study on the induction of reactive oxygen intermediates, allyl-ITC induced a significantly higher level of 8-oxodG in hydrogen peroxide-susceptible human myelogenous leukaemia cells than their resistant counterparts (Murata *et al.*, 2000). In the same study, the authors compared induction by allyl-ITC, benzyl-ITC and phenethyl-ITC of 8-oxodG in calf thymus DNA and DNA damage in ^{32}P-labelled DNA fragments obtained from the human *P53* tumour suppressor gene and the *c-Ha-ras-1* proto-oncogene. All the isothiocyanates caused Cu[II]-mediated formation of DNA damage and 8-oxodG, the order of potency being allyl-ITC > benzyl-ITC > phenethyl-ITC. Catalase and the Cu[II]-specific chelator bathocuproine, reduced 8-oxodG formation, suggesting the involvement of hydrogen peroxide and Cu[I], respectively. Superoxide dismutase was also inhibitory, indicating participation of superoxide in the DNA damage.

Another possible mechanism of the cytotoxicity and genotoxicity of isothiocyanates may be oxidative desulfuration of the compounds to reactive isocyanates by cytochrome P450 enzymes. Arochlor-inducible cytochrome P450 enzymes convert phenyl-ITC, benzyl-ITC, phenethyl-ITC and methyl-ITC to the respective isocyanates (Lee, 1992, 1996). Phenyl-ITC underwent the most metabolic conversion, followed by benzyl-ITC and phenethyl-ITC; methyl-ITC was only weakly metabolized. Isocyanates are reactive electrophilic agents capable of modifying nucleic acids in vitro and in vivo and cause

chromosome aberrations, sister chromatid exchange, mutations and cancer (Mason *et al.*, 1987; Bucher *et al.*, 1989).

Exposure of Chinese hamster B241 cells to allyl-ITC at 5 pmol/ml for 24 h and further cultivation for generations caused transformation of the cells (Kasamaki *et al.*, 1987). Subsequent isolation and subcutaneous injection of the anchorage-independent cell population resulted in tumour formation after 3–8 months. In another study, phenethyl-ITC failed to transform BALB/c 3T3 cells but enhanced the cell transforming potential of benzo[*a*]pyrene (Perocco *et al.*, 2002).

Indoles

With the exception of one report in which indole-3-carbinol was found to be cytotoxic to BALB/c 3T3 mouse fibroblasts at a concentration of 1 nmol/ml (Babich *et al.*, 1993), none of the studies of indole compounds contained in cruciferous vegetables has shown them to be genotoxic or mutagenic. After treatment with nitrite, however, most indole compounds become mutagenic. The first report on the mutagenicity of nitrite-treated indole-compounds was that of Wakabayashi *et al.* (1985).

Vegetables are the main sources of dietary nitrate (Meah *et al.*, 1994). Reduction of ingested nitrate to nitrite by bacteria in the oral cavity and reaction of nitrite with ingested vegetables at the acidic pH of the stomach may lead to formation of mutagenic indole compounds, which may be risk factors for stomach cancer. To shed light on the possible association between the risk for stomach cancer and exposure to nitrosated indoles, rats were given high doses of these compounds, and effects on the stomach mucosa were examined. 1-Nitrosoindole-3-acetonitrile, a nitrosated form of indole-3-acetonitrile, at 100 mg/kg bw caused the formation of DNA adducts in the forestomach and glandular stomach (Yamashita *et al.*, 1988), whereas indole-3-acetonitrile did not. 1-Nitrosoindole-3-acetonitrile also induced replicative DNA synthesis and ornithine decarboxylase activity in the gastric mucosa of rats treated at doses of 40–300 mg/kg bw (Furihata *et al.*, 1987), suggesting that the compound has tumour promoting activity.

Indolo[3,2-*b*]carbazole has some structural and functional properties in common with the tumour promoter 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), and some of the cellular changes induced by this indole in vitro might favour tumour promotion rather than chemoprevention. During carcinogenesis, the level of gap junction proteins is generally reduced, and Herrmann *et al.* (2002) found inhibition of gap-junction intracellular communication between primary rat hepatocytes co-cultured with a rat liver epithelial cell line, WB-F344, in response to indolo[3,2-*b*]carbazole at 0.1 nmol/ml for 8 or 12 h, with maximum inhibition after 24–48 h. Both plasma membrane staining and the mRNA levels of connexin 32 were reduced.

Prostaglandin E2 is believed to contribute to formation of tumours by increasing cell proliferation, preventing apoptosis and facilitating angiogenesis, particularly in the colon. In a study of the regulation of prostaglandin E2 in a colon carcinoma cell line, HCA7, Sherratt *et al.* (2003) found that indole[3,2-*b*]carbazole at 1 nmol/ml for 6 h increased COX-2 mRNA by 2.8-fold. Co-treatment with interleukin-1β increased the mRNA levels even further. Subsequent increases in prostaglandin E2 were also observed.

The Working Group concluded that exposure to indole compounds as a result of consumption of cruciferous vegetables does not appear to have adverse genetic effects.