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Effect of common *Brassica* vegetables (Brussels sprouts and red cabbage) on the development of preneoplastic lesions induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) in liver and colon of Fischer 344 rats

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Abstract

Aim of the present study was the investigation of effects of juices from commonly consumed *Brassica* vegetables (two cultivars of Brussels sprouts and two cultivars of red cabbage) on formation and development of preneoplastic lesions in colons (aberrant crypt foci, ACF) and livers (glutathione-*S*-transferase placental form, GST-P$^+$) in male F344 rats. The foci were induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), a widespread carcinogenic heterocyclic aromatic amine which is found in fried meats. Recently, we reported on pronounced protective effects in the two-organ foci model when the vegetable juices were given during the carcinogen treatment but several findings by other groups indicated that breakdown products of glucosinolates contained in *Brassica* vegetables cause tumour promotion in various organs of laboratory rodents. In the present study, the animals received the juices in the drinking water (5%) over a period of 20 days after treatment with IQ (100 mg/kg bw on 10 alternate days). To increase the foci yield (which facilitates the detection of modifying effects), the animals were fed with a modified (high fat, fibre free) AIN-76 diet. With exception of the sprout variety "Cyrus", all juices lowered the number of GST-P$^+$ foci as well as the foci area in the liver, but none of these effects was statistically significant. In the colon, none of the juices had an impact on crypt multiplicity (number of crypts/focus), whereas the number of ACF was decreased; only with the sprout variety Maximus the protective effect was significant (reduction 49%). The present findings show that administration of vegetable juices to the animals after the carcinogen does not increase the number and size of IQ-induced preneoplastic lesions in liver and colon.
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Keywords: *Brassica*; 2-Amino-3-methylimidazo[4,5-*f*]quinoline; Heterocyclic aromatic amines

1. Introduction

Several findings indicate that heterocyclic aromatic amines (HAs) which are contained in fried meats might be involved in the aetiology of human colon cancer [1]. As a consequence, strong efforts have been made to identify HA protective compounds in the human diet. An evaluation of the currently available data shows that more than 600 complex mixtures and individual compounds have been investigated in regard to antimutagenic and anticarcinogenic effects towards HAs (for reviews see [2,3]). In most of these experiments, in vitro models were used and only a few studies are available in which induction of preneoplastic foci and tumours were employed as endpoints. Of particular interest are dietary factors for which some evidence exists that their consumption is inversely related to the incidence of colon cancer in humans. This

Abbreviations: HA, heterocyclic aromatic amine; ACF, aberrant crypt foci; GST-P$^+$ foci, glutathione-*S*-transferase (placental form) positive liver foci; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline

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is for example the case for fibres, coffee, fermented foods which contain lactobacilli, and cruciferous vegetables [4,5]. Recently, we reported on the strong chemopreventive effects of commonly consumed *Brassica* vegetables towards induction of preneoplastic lesions by the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), in livers and colons of laboratory rats which occurred when the animals received juices of the vegetables before and during treatment with the carcinogen [6]. IQ is a potent rodent carcinogen that is abundant in fried fish [7] and beef [8]. In these experiments, Fischer 344 rats received juices of different vegetables (two varieties of red cabbage "Roxy" and "Reliant" and two cultivars of Brussel sprouts "Cyrus" and "Maximus") in the drinking water. Based on the pronounced reduction of the preneoplastic lesions which was seen in these experiments (in particular with the Brussel sprouts), we concluded that increased consumption of *Brassica* vegetables might protect also humans against the health risks of heterocyclic amines.

However, numerous earlier publications indicate that breakdown products of glucosinolates, which are considered to be responsible for the chemopreventive properties of cruciferous vegetables [9,10] act as tumour promotors (for review see [3]). Most experiments were carried out with indole-3-carbinole (I3C), a metabolite of glucobrassicin [10] and it was shown in different cancer models with animals that post-initiation treatment promotes tumour formation in a variety of organs (including liver, colon, thyreoidea, mammary gland, skin and tongue) [11–13]. Also with other glucosinolate breakdown products, such as phenethyl- isothiocyanate and benzyl-isothiocyanate and with sinigrin, one of the most widespread glucosinolates in *Brassica* vegetables [10], an increase in tumour incidence was seen in animal experiments when the compounds were given after the carcinogens [14]. These observations indicate that intake of individual glucosinolates and their breakdown products as well as increased consumption of glucosinolate-containing vegetables may lead to adverse effects. However, also antipromoting effects have been observed with glucosinolates and their breakdown products in a number of studies [15–19]. To our knowledge, only one promotion study with a *Brassica* vegetable has been carried out. Birt et al. [20] fed dried cabbage in high amounts (9–11% in the diet) to mice and hamsters and studied the effects on the development of chemically induced skin and pancreatic cancer. The authors found a significant increase of the tumour yield in both organs. In the present experiments we wanted to find out if consumption of *Brassica* vegetables has an impact on the development of IQ induced preneoplastic foci in the liver (glutathione-*S*-transferase positive foci; GST-P^+ foci) and in the colon (aberrant crypt foci; ACF) in the early post-carcinogen treatment phase. This should show a possible effect of the juices in a phase in which a cell with freshly damaged DNA develops into a cell in which fixation of tumour initiation has been completed.

2. Experimental

2.1. Chemicals

Methylene blue and bovine serum albumin were supplied by Sigma (St. Louis, MO, USA). 2-Amino-3-methyl-imidazo[4,5-*f*]quinoline was purchased from Toronto Research Chemicals (Toronto, Canada). GST-π polyclonal antibody was bought from Novocastra (Newcastle, UK).

2.2. Juice preparation

Brussels sprouts (*B. oleracea* L. var. Gemmifera "Cyrus" and *B. olearacea* L. var. Gemmifera "Maximus") and red cabbage (*B. oleracea* L. var. Capitata subvar. Rubra "Roxy" and *B. olearacea* L. var. Capitata subvar. Rubra "Reliant") were obtained from Novartis Seeds BV (Enkhuizen, The Netherlands). The vegetables were grown on the field and stored at 4 °C in the dark after being harvested. Juices were prepared freshly every day from raw material. After discarding the outer leaves, the vegetables were cut into small pieces and juiced with a commercial machine (Elin T3232). On the basis of preliminary experiments, in which the palatability of the juices had been investigated, a 5% (v/v) concentration in the drinking water was chosen for the main experiments.

2.3. Analyses of glucosinolates

The glucosinolate contents of the vegetables were determined according to Wathelet et al. [21] and Spinks et al. [22] with slight modifications. Briefly, fresh plant tissue was extracted in boiling methanol (70%) in a water bath at 70 °C for 20 min. Subsequently, the extract was centrifuged (1000 × *g*, 10 min) and the supernatant collected. The pellet was reextracted twice following the same procedure. Aliquot of the supernatant was loaded onto ion-exchange mini-columns (DEAE Sephadex A-25) and the glucosinolates were desulphated on-column. The desulphoglucosinolates were eluted with water and separated by gradient system high performance liquid chromatography (Thermo Separation Products) using a Nova Pak C18 (5 mm) reverse phase column (3.9 mm × 159 mm; Waters Corporation, USA). The solvent programme consisted of water for 1 min and a linear gradient over 20 min to water/acetonitrile 80/20 (flow 1.0 ml/min). The desulphoglucosinolates were monitored by UV-absorption at 229 nm and quantified against the internal standard glucotropaeolin. Identification of the individual glucosinolates was done by comparing retention times with pure standards and with a standard rapeseed reference material (BCR 367, Commission of the European Community Bureau of References, Brussels, Belgium).

2.4. Animals and treatment

All experiments were carried out with 3 weeks old male Fischer 344 rats (body weight, 133–157 g). At the beginning

of the experiments, the rats were 6 weeks old and had an average body weight of 139 g. Each experimental group consisted of eight animals. The animals were purchased from Charles River Inc. (Borchen, Germany) and housed in groups of three in plastic cages under standard conditions (24±1 °C, humidity 50 ± 5%, 12 h light/dark cycle). After one week acclimatisation, rats were switched from Purina rat chow (Soest, Germany) to a modified (high fat, fibre free) AIN-76 diet (SDS, Witham, UK) on which they remained for the duration of the experiment. The rats were randomised into several groups (eight animals per group), namely (i) negative control, (ii) IQ control and (iii) combined treatment groups (IQ and vegetables). The group size was defined on the basis of the study design of earlier chemoprevention experiments in which the formation of preneoplastic lesions was used as an endpoint in rats (see for example [23–27]). The body weights of the animals were measured every week. The rats were treated with IQ by gavage on 10 alternate days in corn oil (100 mg/kg bw per day). The negative control group received corn oil and pure tap water. Experiments with IQ in rats showed that DNA-damage (measured in the single cell gel electrophoresis assay) declines as a function of the exposure and no significant effects could be seen after 24 h [28]. This observation indicates that DNA-damage takes place within the first hours after IQ treatment and justifies the beginning of the vegetable administration in the post-treatment study 1 day after the last carcinogen treatment. The rats received drinking water supplemented with 5% (v/v) juice prepared from the red cabbage and Brussels sprouts cultivars for 25 days. Thereafter, the animals received normal drinking water. During the entire length of the experiment (24 weeks), the animals were kept on a modified (high fat fibre free) AIN-76 diet (AIN-76–high fat modification). The composition of the diet was per 100 g as follows: casein 20.0 g, corn starch 35.0 g, sucrose 20.0 g, sunflower seed oil 10.0 g, lard 10.0 g, AIN-76 vitamin mix 1.0 g, AIN-76 mineral mix 3.5 g, D,L-methionine 0.3 g, choline bitartrate 0.2 g, wheat bran 0.0 g.

We showed earlier that the use of this diet increases the foci yield substantially (two-fold), which facilitates the detection of modifying effect caused by dietary factors [29].

Sixteen weeks after the last juice feeding the animals were sacrificed by decapitation after CO_2 asphyxiation.

2.5. Determination of preneoplastic lesions in colon and liver

The livers of the sacrificed animals were weighed and sections fixed in freshly prepared Carnoy·s solution (ethyl alcohol, chloroform, acetic acid, 6:3:1) and processed as described earlier [30]. GST-P$^+$ foci (≥3 cells) were identified by anti-placental GST-stain under a light microscope (125× magnification). The foci numbers were calculated per cm^2 tissue and at least 1 cm^2 per animal was evaluated with a light microscope (10–60-fold magnification). The total evaluated area of the liver was measured with an automatic image analyser (Lucia, Nikon; Meerbusch, Germany).

For the determination of ACF, the colon was removed and cleaned with Ringer's solution, then cut open along the longitudinal median and fixed flat in 10% buffered formalin (pH 7.5) for 24 h. The samples were stained in 0.2% methylene blue and the number of ACF per colon and the crypt multiplicity (numbers of crypts/focus) were evaluated for the entire length of the colons from each rat at a 60-fold magnification as described by Bird [31].

2.6. Statistical analysis

The numbers of ACF as well as the numbers and area of GST-P$^+$ foci were compared using ANOVA and linear contrasts after homogenizing the variance using logarithmic transformation. Relative liver weights and fluid consumption was compared using ANOVA followed by Dunnet's test.

3. Results and discussion

3.1. Glucosinolate content of the vegetables

The overall glucosinolate contents of the Brussels sprouts cultivars were two–three-fold higher than that of red cabbages (Table 1). The level of the different glucosinolates widely varied not only between the different vegetables but also between the different cultivars of the same vegetable. Sinigrin dominated in Brussels sprouts "Cyrus" (68% of the total glucosinolate content) whereas "Maximus" was additionally rich in iberin. In red cabbage cultivars, sinigrin and glucoraphanin were the most abundant glucosinolates in "Roxy" and "Reliant", respectively.

3.2. Effect of consumption of Brussels sprouts and red cabbage juices on the body weight and relative liver weight of the animals

The body and relative liver weights of the animals of the different groups are listed in Table 2. It can be seen, that supplementation of the drinking water had no significant effect on any of these parameters.

3.3. Influence of the Brassica vegetables on the frequency and multiplicity of aberrant crypts in the colon

The effect of the vegetable juices on the induction of ACF and on the number of crypts per focus are shown in Table 3 IQ alone induced on average 10 ± 3.8 ACF per animal. This number is approximately 50% higher than that seen under identical conditions in animals fed with conventional diet [29]. All vegetables reduced the foci yield, but only with Maximus (a Brussel sprout cultivar) the decrease was significant. The crypt multiplicity (last column of Table 3) was not markedly influenced by any of the vegetables.

Table 1
Glucosinolate content of Brussels sprouts ("Cyrus" and "Maximus") and red cabbage ("Roxy" and "Reliant")[a]

Glucosinolate	Brussels sprouts Cyrus	Brussels sprouts Maximus	Red cabbage Roxy	Red cabbage Reliant
Iberin	4.4[b]	10.8	1.13	0.7
Progoitrin	3.3	2.7	0.54	0.8
Sinigrin	22.7	9.1	5.16	0.7
Glucoraphanin	0.4	3.0	0.75	5.0
Gluconapin	3.0	2.0	2.20	0.7
4-OH-glucobrassicin	0.2	0.3	0.65	0.6
Glucobrassicin	3.2	2.9	0.47	2.1
4-Meglucobrassicin	0.8	0.7	0.33	0.2
Neoglucobrassicin	0.0	0	0.05	0.0
Total glucosinolate content	38.0	31.5	11.28	12.0

[a] Fresh plant tissue was extracted with methanol, the extract centrifuged and the resulting supernatant was desulphated. Subsequently, the de-sulphoglucosinolates were separated by high performance liquid chromatography. Glucotropaeolin was used as an internal standard. Identification of the individual glucosinolates was done by comparing the retention times with pure standards and with a standard rapeseed reference material.
[b] Values represent glucosinolate contents in μmol/g dry weight.

Table 2
Consumption of Brussels sprouts and red cabbage juices, body weight gain of rats and relative liver weight[a]

Treatment group	Consumption of juice-supplement water/rat per day[a] (ml)	Initial/final body weight[b] (g)	Relative liver weight[b] (g per animal weight)
Negative control	17.5 ± 3.4	135.0 ± 7.2/290.5 ± 11.9	2.7 ± 0.2
IQ	18.5 ± 4.0	136.9 ± 12.1/299.9 ± 25.6	2.8 ± 0.1
Red cabbage			
IQ + Roxy	13.2 ± 1.3	142.8 ± 7.1/283.6 ± 36.6	3.1 ± 0.5
IQ + Reliant	12.8 ± 2.3	139.4 ± 11.2/269.2 ± 23.2	2.9 ± 0.2
Brussels sprout			
IQ + Cyrus	12.2 ± 2.5	136.0 ± 11.6/266.8 ± 19.5	2.9 ± 0.2
IQ + Maximus	12.3 ± 0.5	140.8 ± 8.1/281.9 ± 19.7	3.0 ± 0.2

[a] The juices were prepared fresh every day and diluted 1:20 with tap water. The consumption of water was measured daily during the entire supplementation period. The negative control and IQ groups received tap water. Values indicated are means±standard deviations of eight animals per group.
[b] The relative liver weights were calculated on the basis of the body weights of the animals on the day of sacrifice. Values indicated are means±standard deviations of eight animals per group. There was no statistical significant difference between the vegetable groups and the negative control concerning the water consumption per day as well as relative liver weights (ANOVA followed by Dunnet's test).

3.4. Influence of the Brassica vegetables on the frequency and area of GST-P$^+$ foci in the liver

The impact of the juices on the formation and development of liver foci is summarised in Table 4. None of the vegetables had a significant impact on the number and size of IQ induced preneoplastic lesions.

Taken together, the present results indicate that the juices of commonly consumed *Brassica* vegetables do not enhance the foci yield in the early post-carcinogen treatment phase

Table 3
Influence of red cabbage ("Roxy" and "Reliant") and Brussels sprouts ("Cyrus" and "Maximus") juices on IQ-induced colonic ACF frequency and crypt multiplicity in F344 rats[a]

Treatment group	ACF/rat	Alteration (%)	Aberrant crypts/focus	Alteration (as compared to IQ alone animals) (%)
Negative control	0	–		–
IQ	10.33 ± 3.87	0	1.45 ± 0.24	0
Red cabbage				
IQ + Roxy	8.50 ± 4.95	−18	1.71 ± 0.43	+18
IQ + Reliant	8.00 ± 2.50	−23	1.56 ± 0.16	+8
Brussels sprouts				
IQ + Cyrus	7.25 ± 6.15	−30	1.49 ± 0.39	+3
IQ + Maximus	5.22 ± 1.85[b]	−49	1.38 ± 0.30	−5

[a] Rats were given juices of Brussels sprouts or red cabbage in the drinking water (5%, v/v) 24 h after the last treatment (100 mg/kg body weight, period of on 10 alternating days). Sixteen weeks after the last IQ treatment, the animals were killed, colons removed and the number of ACF and crypts/focus evaluated. Data are means ± S.D. from eight animals per group.
[b] Statistically significant relative to IQ controls as determined by analysis of variance ($P < 0.05$).

Table 4
Influence of red cabbage and Brussels sprouts juices on IQ-induced liver GST-P$^+$ foci[a]

Treatment group	Number of foci/cm^2	Alteration (%)	Total area (mm^2/cm^2)	Alteration (as compared to IQ alone animals) (%)
Negative control	0.10 ± 0.27	–	0.01 ± 0.03	–
IQ	13.00 ± 4.62	0	9.03 ± 9.31	0
Red cabbage				
IQ + Roxy	10.58 ± 4.64	−18	7.40 ± 4.59	−18
IQ + Reliant	11.28 ± 3.23	−13	4.26 ± 1.96	−53
Brussels sprouts				
IQ + Cyrus	15.09 ± 9.61	+16	6.20 ± 6.12	−31
IQ + Maximus	11.87 ± 4.74[b]	−8	7.22 ± 5.10	−20

[a] The animals were treated with IQ and the juices as described in the footnote of Table 2, 16 weeks after the last IQ treatment, the animals were killed and the livers removed. Subsequently, liver tissue sections were stained and the frequency and size of GST-P$^+$ foci registered. Data are means ± S.D. from eight animals per group.

[b] Statistically significant relative to IQ controls as determined by analysis of variance ($P < 0.05$).

in a two-organ foci model with the heterocyclic amine IQ (Tables 3 and 4).

As described in Section 1, we found previously that supplementation of the drinking water with Brussels sprouts and red cabbage juice (5%) during treatment with IQ leads to a pronounced (in many cases ≥50%) reduction of the foci frequencies in colon and liver. Furthermore, a drastic (≥80%) reduction of the foci size was seen in the hepatic tissue but not in the colon [6]. Since the animals were treated with low amounts of the juices, we concluded that these experimental conditions are also relevant for humans. However, men consume vegetables before, during and after carcinogen exposure. Therefore, we wanted to know if consumption of the *Brassica* juices after the treatment phase with the carcinogen may have an effect on the development and formation of preneoplastic lesions.

As mentioned above, different glucosinolate metabolites have been reported to cause tumour promotion in a variety of organs in laboratory rodents (see Section 1) but it was also found that I3C causes a drastic decline of the number of IQ and PhIP induced ACF and colon tumours in rats [3,32,33]. Also in the present study we observed a decrease in the frequency of colonic ACF with the sprouts (Table 3), only with one cultivar (Cyrus) the effect was statistically significant; the red cabbage varieties were ineffective in both organs.

In the simultaneous treatment experiments, alterations of detoxifying enzymes, in particular of glucuronosyltransferase, seem to account for the chemoprotective effects of cruciferous vegetables against heterocyclic amines [6]. This mechanism provides no plausible explanation for the results of experiments where the putative protective agent is given after the carcinogen. It has been shown earlier that sinigrin, which is present in the Brussels sprouts in much higher concentrations than in the red cabbage cultivars [6] causes apoptosis in the rat colon [34]. Since in the present experiments the sprouts were more protective than the cabbage varieties (Table 3) this can be taken as tentative indication that induction of programmed cell death may account for the reduction of the ACF frequency.

We have analysed the glucosinolate contents of the *Brassica* vegetables which were used in the present study. The results of the analyses show that the exposure levels of the animals to the glucosinolates and consequently to their breakdown products which are formed enzymatically by myrosinase [35] or by representatives of the intestinal microflora [36] are several orders of magnitude lower than those used in the tumour promotion studies mentioned above. For example the dose levels of I3C used in liver promotion studies with rats were 2.0 and 2.5 g/kg in the diet [12,36]. This corresponds to a daily intake of 120–150 mg/kg body weight per day. The highest content of glucobrassicin (GB) from which I3C is formed was seen in the Brussel sprout cultivar "Cyrus", which contained 3.2 μg/g dry weight. Provided that the total amount of GB is converted to I3C, the daily consumption per animal (250 g) required to cause a measurable I3C-related effect would be more than 10 kg of vegetable material per day. Also the levels of phenethyl-isothiocyanate, benzyl-isothicoyanate and sinigrin used in promotion studies with laboratory rodents were quite high [15,16], and effects with *Brassica* vegetables would take place only if unrealistically large amounts are consumed. These comparisons suggest that the lack of an effect in the present two-organ model is probably due to the fact that the dose levels of glucosinolate metabolites required to cause tumour promotion is not reached when crude vegetables (or their juices) are given to the animals under conditions which reflect the intake in humans. The daily juice consumption of the animals in the present experiments was between 0.6 and 0.7 ml per animal, which corresponds to an uptake of 180–210 ml juice per person (75 kg bw) per day. This amount of juice is contained in a regular size vegetable meal (300–350 g). It is generally assumed that threshold levels exist for tumour promoters [37]. Therefore, exposure to low levels of glucosinolate metabolites will not cause effects at the post-initiation level. The extrapolation of the results of the present animal experiments as well as the findings of epidemiolog-

ical studies [38–40] indicate that the human cancer risk is not increased by consumption of *Brassica* vegetables but rather suggest that cancer protective effects can be expected.

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References

[1] M. Nagao, T. Sugimura, in: M. Nagao, T. Sugimura (Eds.), Food Borne Carcinogens, Wiley, Toronto, 2000.
[2] C.E. Schwab, W.W. Huber, W. Parzefall, G. Hietsch, F. Kassie, R. Schulte-Hermann, S. Knasmüller, Crit. Rev. Toxicol. 30 (2000).
[3] R.H. Dashwood, Mutat. Res. 511 (2002) 89.
[4] K.A. Steinmetz, J.D. Potter, Cancer Causes Control 2 (1991) 325.
[5] I. Wollowski, G. Rechkemmer, B.L. Pool-Zobel, Am. J. Clin. Nutr. 73 (2002) 451.
[6] F. Kassie, M. Uhl, S. Rabot, B. Grasl-Kraupp, R. Verkerk, M. Kundi, M. Chabicovsky, R. Schulte-Hermannm, S. Knasmüller, Carcinogenesis 24 (2003) 255.
[7] H. Kasai, Z. Yamaizumi, K. Wakabayashi, M. Nagao, T. Sugimura, S. Yokoyama, K. Miyazawa, N.E. Spingarn, J.H. Weisburger, S. Nishimura, Proc. Jpn. Acad. 56 (1980) 278.
[8] Z. Yamaizumi, H. Kasai, S. Nishimura, C.G. Edmonds, J.A. McCloskey, Mutat. Res. 173 (1986).
[9] D.T. Verhoeven, H. Verhagen, R.A. Goldbohm, P.A. van den Brandt, G. van Poppel, Chem. Biol. Interact. 103 (1997) 79.
[10] R.F. Mithen, M. Dekkeer, M. Verkerk, S. Rabot, I. Johnson, J. Sci. Food Agric. 80 (2000) 967.
[11] R.H. Dashwood, A.T. Fong, D.E. Williams, J.D. Hendricks, G.S. Bailey, Cancer Res. 51 (1991) 2362.
[12] D.J. Kim, B.S. Han, B. Ahn, R. Hasegawa, T. Shirai, N. Ito, H. Tsuda, Carcinogenesis 18 (1997) 377.
[13] J.S. Kang, D.J. Kim, B. Ahn, K.T. Nam, K.S. Kim, M. Choi, D.D. Jang, Cancer Lett. 169 (2001) 147.
[14] M. Hirose, T. Yamaguchi, N. Kimoto, K. Ogawa, M. Futakuchi, M. Sano, T. Shirai, Int. J. Cancer 77 (1998) 773.
[15] T. Tanaka, T. Kojima, Y. Morishita, H. Mori, Jpn. J. Cancer Res. 83 (1992) 835.
[16] M.A. Morse, C.X. Wang, S.G. Amin, S.S. Hecht, F.L. Chung, Carcinogenesis 9 (1988) 1891.
[17] D.F. Birt, B. Walker, M.G. Tibbels, E. Bresnick, Carcinogenesis 7 (1986) 959.
[18] R.G. Mehta, J. Liu, A. Constantinou, C.F. Thomas, M. Hawthorne, M. You, C. Gerhuser, J.M. Pezzuto, R.C. Moon, R.M. Moriarty, Carcinogenesis 16 (1995) 399.
[19] B. Srivastava, Y. Shukla, Cancer Lett. 134 (1998) 91.
[20] D.F. Birt, J.C. Pelling, P.M. Pour, M.G. Tibbels, L. Schweickert, E. Bresnick, Carcinogenesis 8 (1987) 913.
[21] J.P. Wathelet, P.J. Wagstaffe, A. Boeke, Commun., EUR 13339 EN (1991) 67.
[22] A. Spinks, K. Sones, G.R. Fenwick, Fette Seifen Anstrichmittel 86 (1984) 228.
[23] D. Guo, H.A. Schut, C.D. Davis, E.G. Snyderwine, G.S. Bailey, R.H. Dashwood, Carcinogenesis 16 (1995) 2931.
[24] L.R. Ferguson, P.J. Harris, Chem. Biol. Interact. 114 (1998) 191.
[25] M. Takahashi, Y. Totsuka, M. Masuda, K. Fukuda, A. Oguri, K. Yazawa, T. Sugimura, K. Wakabayashi, Carcinogenesis 18 (1997) 1937.
[26] C. Liew, H.A. Schut, S.F. Chin, M.W. Pariza, R.H. Dashwood, Carcinogenesis 16 (1995) 3037.
[27] T.P. Pretlow, M.A. O'Riordan, G.A. Somich, S.B. Amini, T.G. Pretlow, Carcinogenesis 13 (1992) 1509.
[28] F. Kassie, S. Rabot, M. Uhl, W. Huber, H.M. Qin, C. Helma, R. Schulte-Hermann, S. Knasmüller, Carcinogenesis 23 (2002) 1155.
[29] F. Kassie, V. Mersch Sundermann, R. Edenharder, L. Karl, F. Platt, F. Darroudi, E.C. Lhoste, C. Humbolt, E. Muckel, M. Uhl, M. Kundi, S. Knasmüller, Mutat. Res. 523–524 (2003) 183.
[30] B. Grasl-Kraupp, T. Waldhör, W. Huber, R. Schulte-Hermann, Carcinogenesis 14 (1993) 2407.
[31] R.P. Bird, Cancer Lett. 37 (1987) 147.
[32] M. Xu, A.C. Bailey, J.F. Hernaez, C.R. Taoka, H.A. Schut, R.H. Dashwood, Carcinogenesis 17 (1996) 1429.
[33] M. Xu, G.A. Orner, G.S. Bailey, G.D. Stoner, D.T. Horio, R.H. Dashwood, Carcinogenesis 22 (2001) 309.
[34] T.K. Smith, E.K. Lund, I.T. Johnson, Carcinogenesis 19 (1998) 267.
[35] A. Pessina, R.M. Thomas, S. Palmieri, P.L. Luisi, Arch. Biochem. Biophys. 280 (1990) 383.
[36] S. Knasmüller, H. Steinkellner, A.M. Hirschl, S. Rabot, E.C. Nobis, F. Kassie, Mutat. Res. 480–481 (2001) 129.
[37] H.C. Pitot, The molecular biology of carcinogenesis, Cancer 72 (1993) 962.
[38] K.A. Steinmetz, J.D. Potter, J. Am. Diet. Assoc. 96 (1996) 1027.
[39] D.T.H. Verhoeven, R.A. Goldblohm, G. van Poppel, H. Verhagen, R.A. Goldblohm, Cancer Epidemiol. Biomark. Prev. 5 (1999) 733.
[40] G. van Poppel, D.T.H. Verhoeven, H. Verhagen, R.A. Goldblohm, Adv. Exp. Med. Biol. 472, Adv. Nutr. Cancer 2 (1999) 159.