CHINESE-STYLE SALTED FISH

Chinese-style salted fish was considered by a previous IARC Working Group in 1992 (IARC, 1993). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Mode of production

In southern China, about 20 different fish, such as red snapper, threadfin, Spanish mackerel, croaker, Japanese mackerel, are used to prepare salted fish (Armstrong & Eng, 1983; Poirier *et al.*, 1987). Procedures for preparation of salted fish have been described in detail previously (IARC, 1993). Briefly, salted fish are prepared by salting, brining, dry-salting, pickle curing, or a combination of these treatments. In brining, fish are placed in a solution of crude salt in water until the fish tissue has absorbed the required amount of salt. For dry-salting, fish are mixed with dry salt and the resultant brine (from dissolution of the salt in the water present in the fish) is allowed to drain away. When pickling or pickle curing the fish is mixed with salt and stored under the brine (pickle) formed when the salt dissolves in the water extracted from the fish.

In southern China, fish are generally not gutted before salting, and only when bigger fish such as red snapper are salted are the guts drawn out through the throat, without making an incision in the belly of the fish. Salting is done with crude salt in wooden vats. After a few days, the fish are immersed in brine and weights (often large stones placed on top of grass mats) are placed on the surface to prevent the fish from floating, for one to five days. After this the fish are dried under the sun for one to seven days, depending on the size of the fish and the weather. Salted fish prepared in this way are called 'tough' or 'hard meat' salted fish. Sometimes, fish is allowed to soften by decomposition before salting, to produce 'soft meat' salted fish (Poirier *et al.*, 1989; Yu *et al.*, 1989a). During drying salted fish, insect infestation can be a serious problem, especially in damp weather. In southern China, the average annual temperature and humidity are high and are favourable for the growth of bacteria such as *Staphylococci* (Armstrong & Eng, 1983; Zou *et al.*, 1994). Salted fish are stored for 4 to 5 months before being consumed.

1.2 Compounds present in salted fish

The previous *IARC Monograph* (IARC, 1993) reviewed levels of *N*-nitrosamines reported for uncooked salted fish obtained from different countries. The levels of *N*-nitrosodimethylamine in uncooked salted fish ranged from not detected to 388 µg/kg (Poirier *et al.*, 1989). Some other volatile nitrosamines such as *N*-nitrosodiethylamine, *N*-nitrosopyrrolidine and *N*-nitrosopiperidine were also reported, their levels ranged between

not detected and about 30 µg/kg (Poirier *et al.*, 1989). Twenty samples of salted fish purchased in high- and low-risk areas for nasopharyngeal carcinoma (NPC) were analysed for four volatile *N*-nitrosamines; the highest levels of the sum of the four *N*-nitrosamines (373 µg/kg) were found in samples from the area with the highest NPC mortality (Zou *et al.*, 1992). Salted fish samples were also analysed for total *N*-nitroso compounds determined as the amount of nitric oxide (NO) released from the compounds after treatment with bromhydric acid (HBr) (Haorah *et al.*, 2001). Six types of dried salted fish purchased in the Fujian province of China, on the coast ~500 miles south of Shanghai, contained 3.9 ± 2.0 (range: 1.8–6.0) µmol/kg *N*-nitroso compounds. Upon steaming *N*-nitrosodiethylamine was detected in more samples than in uncooked or fried fish; *N*-nitrosodimethylamine was detected in all of the samples, whether cooked or uncooked (Huang *et al.*, 1981). The average levels of *N*-nitrosamines in steam-cooked salted fish collected from areas with high NPC mortality (1.51 ± 0.23 mg/kg) were significantly higher than those from areas with lower NPC mortality (0.60 ± 0.14–0.83 ± 0.18) (Zou *et al.*, 1994).

Fish are rich sources of secondary and tertiary amines, and nitrate and possibly nitrite occur in the crude salt used to pickle them. Steam-cooked, salted fish purchased in various areas in China have been found to contain nitrites (0.15 ± 0.24 mg/kg) and nitrates (6.54 ± 0.43 mg/kg) (Zou *et al.*, 1994). No differences were found in the levels of nitrites or nitrates between areas with different NPC mortality rates.

N-nitroso compounds, including *N*-nitrosamines, can form during the preparation of salted fish. Several factors may affect the levels of *N*-nitroso compounds, including levels of nitrites and nitrates in crude salt, those of nitrogen oxide in the air (when the preparation took place in open air), the growth of nitrate-reducing bacteria and pH. *N*-Nitroso compounds can also be formed after ingestion of foods by chemical nitrosation under acidic conditions in the stomach (IARC, 2010).

Aqueous food extracts of 116 samples of salted fish from China were analysed for four volatile *N*-nitrosamines before and after strong acid-catalysed nitrosation *in vitro*. After nitrosation, *N*-nitrosodimethylamine levels were increased about 70-fold, while *N*-nitrosopiperidine levels were increased nearly 200-fold (Zou *et al.*, 1994). Six types of dried salted fish purchased in the Fujian province of China contained 6000 ± 3200 (range: 4200–12300) µmol/kg precursors of total *N*-nitroso compounds determined as the amount of NO released after HBr treatment (Haorah *et al.*, 2001). These results confirm that salted fish contains high concentrations of precursors of *N*-nitroso compounds.

1.3 Prevalence of use of Chinese-style salted fish

Chinese-style salted fish is popular in Chinese populations along the south China coast and South-eastern Asian countries, where it is often used as an accompaniment to other dishes or rice. Although the amount consumed at any one time is small (not more than 10 g), the dish may appear at every meal; some people prefer the spoiled parts (Fong & Chan, 1973). Salted fish mixed with rice has also been used as a traditional weaning food, and was often given to infants early and frequently in their life (Topley, 1973; Yu *et al.*, 1981, 1989b). In three studies, 6–53% of individuals reported use during weaning; use in the post-weaning period was slightly lower in each subsequent study (Yu *et al.*, 1986, 1988, 1989b).

Data on prevalence of use are mainly derived from studies on the association with NPC, but in most studies the type of salted fish is not specified. Prevalence of use varies significantly (Table 1.1); in southern Chinese populations 4 to 48% of the adult population have reported eating

Table 1.1 Prevalence of salted fish consumption once weekly or more in Chinese populations[a]

Reference	Region or country	Data collection	Number of adult controls[b]	Consumption of salted fish	
				Childhood[c]	Adulthood
Armstrong & Eng (1983)	Malaysia	1980	100	[47%]	[20%]
Yu *et al.* (1986)	Hong Kong Special Administrative Region	1981–NR	250	[16%]	[8%]
Yu *et al.* (1988)	Guangxi, Southern China	1984–86	174	4%	NR
Yu *et al.* (1989b)	Guangzhou, Southern China	1983–85	304	47%	33%
Ning *et al.* (1990)	Tianjin, Northern China	1985–86	300	3%	NR
Zheng *et al.* (1994a)	Guangzhou, Southern China	1985–88	195	10%	1–3%
Lee *et al.* (1994)	Singapore	1988–90	369	16%	4%
Yuan *et al.* (2000)	Shanghai	1988–91	1032	NR	2%
Zou *et al.* (2000)	Yangjiang, Southern China	1987–95	192	NR	48%
Ward *et al.* (2000)	Taiwan, China	1991–94	327	NR	< 5%
			110	31%	NR
Yang *et al.* (2005)	Taiwan, China	1996–NR	1636	2%	NR
Guo *et al.* (2009)	Guangxi, Southern China	2004–05	758	NR	4%

[a] Prevalence in the control groups from the studies
[b] Number of controls with information on salted fish consumption
[c] Age 10 years, except Armstrong & Eng (1983) ('Childhood'); Zheng *et al.* (1994a) (0–3 years). Childhood and adulthood population are the same but were asked their consumption at different time points.
NR, not reported

salted fish more than once weekly. Comparing earlier and later studies shows a decreasing trend in the prevalence of use. Consumption of salted fish in Chinese populations has been declining since the second half of the 20th century, and consumption in weaning and early childhood is now rare (Zheng *et al.*, 1994a; Yu & Yuan, 2002). Both cultural changes and other methods of preserving food may be responsible for the decrease.

2. Cancer in Humans

2.1 Nasopharyngeal carcinoma

2.1.1 Overview of studies

Ho (1967) estimated that the Tankas (boat people), who consumed Chinese-style salted fish in their daily diet, had twice the incidence of nasopharyngeal carcinoma (NPC) compared with the land-dwelling Cantonese in Hong Kong Special Administrative Region. Subsequent studies demonstrated that the distinct pattern of NPC incidence among different ethnic or dialect groups in southern China coincided with the prevalence of their consumption of salted fish (Ho, 1978; Yu *et al.*, 1981), and that high incidence rates of NPC were retained in the Chinese who continued consuming salted fish after they migrated to Malaysia (Armstrong *et al.*, 1979; Armstrong & Eng, 1983). The peak in incidence rates at ages 45–54 years and decline thereafter suggested that the consumption of salted fish occurred early in life. [Salted fish mixed with soft rice was commonly fed to infants in the weaning and post-weaning period.]

Eight case–control studies on the association of salted fish with NPC, conducted between the 1970s and 1980s, were reviewed in the previous *IARC Monograph* (IARC, 1993) and are summarized

in Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-07-Table2.1.pdf) (Henderson *et al.*, 1976; Henderson & Louie, 1978; Geser *et al.*, 1978; Armstrong & Eng, 1983; Yu *et al.*, 1986, 1988, 1989b; Ning *et al.*, 1990; Sriamporn *et al.*, 1992). All but one were conducted on Chinese subjects and consistently demonstrated that consumption of Chinese salted fish was associated with increased risk for NPC. There was a dose-dependent relationship between frequency and duration of consumption and NPC risk. The association was stronger for intake of salted fish during childhood up to 10 years of age compared with intake at older ages.

Since the publication of the previous *IARC Monograph* (IARC, 1993), an additional 11 case–control studies on the association of Chinese-style salted fish with NPC association have been published in English or Chinese-language articles, all but one in Chinese populations (see Table 2.1 on-line). No cohort studies have been performed. In six studies a significant association between salted fish and NPC was observed (Huang *et al.*, 1993; Zheng *et al.*, 1994a, b; Armstrong *et al.*, 1998; Zou *et al.*, 2000; Guo *et al.*, 2009), in two the association was of borderline significance (Yuan *et al.*, 2000; Yang *et al.*, 2005), while lack of an association was observed in three studies (West *et al.*, 1993; Lee *et al.*, 1994; Ward *et al.*, 2000). Two of the negative studies were performed in populations with a low consumption of Chinese-style salted fish (West *et al.*, 1993; Ward *et al.*, 2000). In the positive studies, the strongest association was seen for intake in early childhood and during weaning, while the association with adult consumption was weaker. Only modestly increased risks were found in the majority of studies, and in the three largest studies (with more than 500 cases), increased risks were only observed for the most exposed individuals.

There are several possible reasons for the smaller risk observed in more recent studies. First, the consumption of salted fish by Chinese populations, especially feeding young children, has declined in parallel with economic development (Lee *et al.*, 1994; Zheng *et al.*, 1994b). While NPC incidence in certain areas of Southern China has remained stable in recent decades (Jia *et al.*, 2006), the incidence of NPC has declined significantly in Hong Kong Special Administrative Region and Singapore, and a preceding decrease in salted fish consumption may be a contributing factor (Yu & Yuan, 2002). Second, in recent decades the consumption of commercially produced salted fish and other preserved foods has increased and the consumption of home-preserved foods with possible higher nitrite and nitrosamine levels has declined (Ward *et al.*, 2000). Third, compared with later studies (Yuan *et al.*, 2000; Guo *et al.*, 2009; Yang *et al.*, 2005), the cases in some of the earlier studies were younger (Yu *et al.*, 1986, 1988). This is relevant as the effect of salted fish on the risk of NPC seems to be most pronounced in younger onset cases (Yang *et al.*, 2005).

2.1.2 Interaction with other risk factors

(a) Genetic risk factors

The involvement of a genetic factor in the development of NPC is likely and the familial risk of NPC in endemic areas is among the highest of any malignancy (IARC, 1997; Ung *et al.*, 1999) compared to those reported for other cancers (Goldgar *et al.*, 1994). Yang *et al.* (2005) found that the risk of NPC associated with salted fish consumption was strongest in families with three or more affected members in Taiwan, China; however, both genetic factors and shared environment could be responsible. In a study from Guangzhou comparing familial cases of NPC with sporadic cases, no significant differences in salted fish consumption between the two case groups were found (Luo *et al.*, 2009).

(b) Epstein-Barr virus

The association between Epstein-Barr virus (EBV) and undifferentiated NPC is firmly established and EBV is found in all tumour cells from NPC in endemic areas (Hjalgrim *et al.*, 2007). A synergistic effect between EBV and salted fish intake on the risk of NPC is suggested from a study where the association between salted fish and NPC was stronger in EBV VCA IgA positive individuals (Zheng *et al.*, 1994a). In a study of Caucasian NPC patients in the USA, intake of preserved meats with high levels of added nitrites increased the risk of undifferentiated NPC, while the risk of differentiated NPC was unaffected (Farrow *et al.*, 1998). In areas with low NPC incidence, undifferentiated, but not differentiated, NPC is associated with EBV (Hjalgrim *et al.*, 2007).

In studies attempting to control for EBV-infection status, the association between Chinese-style salted fish and NPC remained (Zheng *et al.*, 1994a; Guo *et al.*, 2009).

2.2 Cancer of the stomach

2.2.1 Overview of studies

A total of five case–control studies have investigated the association between Chinese-style salted fish and development of stomach cancer (Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-07-Table2.2.pdf). Two of the studies were conducted in Southern Chinese populations (Ye *et al.*, 1998; Cai *et al.*, 2003), two studies in Northern Chinese populations (You *et al.*, 1988; Takezaki *et al.*, 2001a) and one study in Malaysia (33% of the controls were Chinese) (Goh *et al.*, 2007). In the two largest studies, with 564 and 272 cases, modest increased risks around 1.4–1.6 were found in the most exposed group (You *et al.*, 1988; Ye *et al.*, 1998). However, the amount of fish consumed in the study from Shandong was small (You *et al.*, 1988). Higher risks were found in two of the smaller studies (Cai *et al.*, 2003; Goh *et al.*, 2007). A dose–response relationship was found in two smaller studies, with odds ratios ranging from 3.4 to 5.7 in the most exposed individuals (salted fish at least three times/week) (Takezaki *et al.*, 2001a; Cai *et al.*, 2003). Adjustments for smoking and alcohol were missing in two studies (You *et al.*, 1988; Ye *et al.*, 1998), while adjustment for *Helicobacter pylori* status was only performed in one study (Goh *et al.*, 2007).

An increased risk for stomach cancer associated with intake of highly salty foods has been observed in other populations (You *et al.*, 1988; Tsugane & Sasazuki, 2007).

2.2.2 Histology and topography

In the single study reporting histology, all cases were adenocarcinomas (Goh *et al.*, 2007). An equal effect of salted fish consumption was observed on cardia and non-cardia stomach cancer (Cai *et al.*, 2003).

2.2.3 Interactions

Interactions between salted fish consumption and other risk factors for stomach cancer have not been reported. The possible significance of early age at consumption and risk for stomach cancer has not been investigated. Growing evidence has associated EBV infection with a subset (5–10%) of all gastric carcinomas globally (Hjalgrim *et al.*, 2007). Analogous to nasopharyngeal carcinoma, the virus in EBV-positive gastric carcinomas is found in all tumour cells (Imai *et al.*, 1994), and EBV-antibodies are elevated in patients before diagnosis (Levine *et al.*, 1995). However, no studies have investigated the association between salted fish and EBV-positive gastric carcinomas. Nor has a possible interaction between salted fish intake and *Helicobacter pylori* infection been investigated.

2.3 Cancer of the oesophagus

Three studies have investigated the association between Chinese-style salted fish and cancer of the oesophagus (see Table 2.2 on-line). In Hong Kong Special Administrative Region, frequent consumption of salted fish, especially early in life, was associated with an increased risk for oesophageal cancer in univariate analyses, but was much weakened when alcohol and other confounders were taken into account (Cheng *et al.*, 1992). In a Northern Chinese population consumption of salted fish more than once weekly (the most exposed individuals) was associated with a non-significant 80% increased risk, and there was no significant trend (Takezaki *et al.*, 2001a). In a Southern Chinese population an increased risk for oesophageal cancer was associated with adult salted fish consumption in women, but not in men, and there was no dose–response relationship from both sexes combined (Li *et al.*, 2001). In the one study reporting histology, 85% of tumours were squamous cell carcinomas (Cheng *et al.*, 1992). Information on topography was not provided.

2.4 Other cancers

Consumption of salted fish in Chinese populations has also been associated with an increased risk for cancer of the lung (Wang *et al.*, 1996; Lu *et al.*, 2003), brain (Hu *et al.*, 1999), and prostate (Jian *et al.*, 2004); no such association was seen for lung cancer in two studies in Japan (Takezaki *et al.*, 2001b, 2003). Studies at these sites are too sparse to allow for a systematic evaluation.

2.5 Synthesis

In all five case–control studies salted fish consumption in adulthood is associated with an increased risk for stomach cancer. However, the effect in the largest studies is modest, and adjustment for important confounding risk factors (including smoking, alcohol and *Helicobacter pylori* status) were missing in several of the studies.

3. Cancer in Experimental Animals

Cantonese-style salted fish and salted fish extracts have been tested for carcinogenicity in three studies in rats and in one study in Syrian golden hamsters. Investigators administered specifically Cantonese-style salted fish to experimental animals (Table 3.1).

3.1 Oral administration

3.1.1 Rat

In one study, carcinomas of the nasal or paranasal regions developed in 4/10 [not significant] female rats fed steamed Cantonese-style salted fish for six months followed by extract of Cantonese-style salted fish heads as drinking-water for 1–2 years. No such tumours developed in similarly treated males (0/10) or in controls of either sex (0/3 and 0/3) (Huang *et al.*, 1978). [The working group noted the small number of animals.] In a larger study, malignant nasal cavity tumours of various kinds developed in male and female rats (4/148) fed Cantonese-style salted fish mixed in powdered diet for 18 months and observed until three years of age, but not in controls (0/73) (Yu *et al.*, 1989a).

Groups of 40–41 offspring (male and female) of rats were exposed to Cantonese-style salted fish mixed in the dams' diet during pregnancy and lactation and were themselves fed Cantonese-style salted fish mixed in diet after weaning for two years; 5 rats of both sexes developed malignant nasal and nasopharyngeal tumours of various kinds. Two offspring of rats exposed to control diet during pregnancy and lactation that were given Cantonese-style salted fish-containing diet after weaning also developed

Table 3.1 Carcinogenicity studies of oral administration of Cantonese-style salted fish in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Inbred WA albino (M, F) up to 24 mo Huang *et al.* (1978)	Steamed salted fish (30 g/d) for 6 mo, 5 d/wk, followed by salted fish soup (20 mL, 0.2 g fish/mL), 5 d/wk, for 1–2 yr 10 M, 10 F 3 M, 3 F (controls)	Adenocarcinoma of the nasal cavity: M–0/10 F–2/10	NR [NS]	Small number of animals
		Undifferentiated carcinoma of the paranasal sinus: M–0/10 F–1/10		
		Highly invasive squamous carcinoma in the upper posterior part of the right buccoalveolar sulcus: M–0/10 F–1/10		
		No nasal cavity tumours in controls (0/6)	-	
Rat, Inbred Wistar-Kyoto (M, F) 3 yr Yu *et al.* (1989a)	Steamed Cantonese-style salted fish (48% soft- & 52% hard-type):rat chow, 1:3 or 1:5, for 18 mo Controls given rat chow only 36–37 F or 37 M	Undifferentiated carcinoma in the mid-and left portions of the nasal cavity: (M–1/37) high dose diet		Positive (one sided $P = 0.02$), 4/148 vs historical controls.
		Moderately differentiated squamous cell carcinoma in the left lateral nasal cavity: (F–1/37) high dose diet		
		Spindle cell carcinoma in the left lateral nasal cavity: (F–1/37) high dose diet	NS	
		Spindle cell tumour in the left posterior nasal cavity: (M–1/37) low dose diet		
		No tumours in 73 controls		
Rat, Sprague-Dawley (M, F) 2 yr Zheng *et al.* (1994c)	Steamed & dried Cantonese-style salted fish (50% soft- & 50% hard-type), 0, 5 or 10% in the diet 40–41 M, F		Positive (P for trend = 0.041)	Positive, 7/122 vs historical controls (one tailed, $P = 0.004$)
	Pregnant rats fed 10% salted fish, 41 new born rats fed 10% salted fish (Group 1)	One squamous cell carcinoma (M) and 1 poorly differentiated carcinoma (F) of the nasopharynx; 1 adenocarcinoma (F) and 1 fibrosarcoma (M) of the nasal cavity		
	Pregnant rats fed control pellets, 41 new born rats fed 10% salted fish (Group 2)	One squamous cell carcinoma (M) of the nasopharynx and 1 rhabdomyosarcoma (F) of the nasal cavity		
	Pregnant rats fed 5% salted fish, 40 new born rats fed 5% salted fish (Group 3)	One soft tissue sarcoma (F) of the nasal cavity		
	40 untreated controls	No nasal cavity or nasopharygeal tumours in controls	-	

d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; vs, versus; wk, week or weeks; yr, year or years

malignant nasal or nasopharyngeal tumours. No nasal or nasopharyngeal tumours were found in control offspring born to untreated dams and fed regular pelleted diet throughout life (Zheng *et al.*, 1994c).

3.1.2 Hamster

No nasal or paranasal tumours were observed in eight male and six female Syrian golden hamsters fed steamed Cantonese-style salted fish for six months and then an extract of Cantonese-style salted fish heads as drinking-water five times per week for 1–2 years (Huang *et al.*, 1978).

3.2 Synthesis

In three studies in rats fed Cantonese-style salted fish, there was a consistent increased frequency of nasal cavity tumours, which are uncommon neoplasms in rats.

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genotoxicity and mutagenicity of Chinese-style salted fish in experimental systems has been reviewed in detail (IARC, 1993).

(a) Genotoxicity and mutagenicity in bacteria

DMSO extracts of 4 samples of different species of salted fish and 2 samples of dried shrimps were mutagenic in *Salmonella typhimurium* TA 100 and TA 98 in the presence of a metabolic activation system (Fong *et al.*, 1979). However, n-hexane and ethyl acetate extracts of hard and soft salted dried fish samples obtained in a high risk area for NPC in China were not mutagenic in *S. typhimurium* TA 100 and TA 98 in the absence or presence of rat liver metabolic activation system. Nevertheless, these salted fish samples contained high levels of precursors that upon nitrosation *in vitro* with sodium nitrite under acidic conditions yielded directly-acting genotoxic (probably *N*-nitroso) compounds (Tannenbaum *et al.*, 1985; Poirier *et al.*, 1989). Mutagenicity on *S. typhimurium* TA 100 of salted fish obtained from Hong Kong Special Administrative Region increased with increasing nitrite concentration (Weng *et al.*, 1992).

In one study, urine samples collected from WA rats fed Chinese-style salted fish showed mutagenic activity on *S. typhimurium* TA 100 and TA 98 (Fong *et al.*, 1979).

(b) Genotoxicity and mutagenicity in experimental animals

(i) DNA adduct

In one study, the levels of 7-methylguanine in the liver and nasopharynx of rats fed 5% or 10% steamed and dried Chinese-style salted fish were analysed by a post-labelling method. There was no significant difference in adduct levels between exposed and control animals, the levels ranging between 3.2–1.2 and 3.3–1.4 per 10^7 nucleotides, respectively (Widlak *et al.*, 1995).

(ii) EBV-activation activity

Aqueous extracts of Cantonese-style salted dried fish from China showed a strong activity in EBV reactivation when assayed in Raji cells (Shao *et al.*, 1988). EBV-reactivation activity was

decreased or showed no change after chemical nitrosation, but it was not correlated with the genotoxicity or nitrosamine levels of the samples (Poirier *et al.*, 1989).

4.3 Mechanistic considerations

The mechanisms by which consumption of Cantonese-style salted fish induces NPC remain unresolved.

NPC has been classified into three histologic types: keratinizing squamous cell carcinoma (class I), nonkeratinizing carcinoma (class II) and basaloid squamous-cell carcinoma (class III) (Chan *et al.*, 2005). Distinct etiological factors could be responsible for the three types of NPC. In high incidence areas such as southern China, 99% of NPC are class II whereas class I NPC is predominant in low-incidence regions. The etiological factors of NPC in high incidence areas include EBV, environmental risk factors and genetic susceptibility.

EBV has been classified as a Group 1 carcinogen by IARC, based on sufficient evidence for its carcinogenicity in humans, namely for NPC (IARC, 1997, 2012). EBV infects primarily B lymphocytes, but also epithelial cells such as oropharyngeal cells, essentially in the lymphoepithelium of the palatine tonsils from Waldeyers ring. The etiological association of NPC with EBV was first suggested on the basis of serological evidence (Old *et al.*, 1966). Circulating cell-free EBV DNA is detected in the plasma and serum of NPC patients, but not in healthy individuals, and its levels are positively correlated with disease stage and prognosis (Lin *et al.*, 2004). EBV DNA, RNA and gene products are also present in most tumour cells (zur Hausen *et al.*, 1970). EBV is detected in cancer cells of virtually all cases of class II NPC in endemic regions. In addition, NPC tumour cells were shown to be clonal expansions of a single EBV-infected progenitor cell (Raab-Traub & Flynn, 1986). EBV infection alone is, however, not a sufficient cause of NPC: the ubiquitous EBV infects and persists latently in over 90% of the world population, yet only a small proportion of individuals develop NPC. Although there is little variation in the prevalence of infection or the age at primary infection with EBV throughout China, risk for NPC is more than 20-fold higher in three provinces in southern China (Zeng, 1985). Therefore environmental and/or genetic factors may also contribute to NPC risk.

On the basis of studies on the natural history of NPC from southern Chinese populations, the following pathogenesis model for NPC has been proposed (Lo & Huang, 2002; Young & Rickinson, 2004). Clonal cell proliferation with 3p and 9p deletion is frequently detected in dysplastic lesions and even in histologically normal nasopharyngeal epithelia in the absence of EBV infection; loss of heterozygosity (LOH) appear to be an early event in the pathogenesis of NPC in this high-risk area (allelic loss may confer growth advantage and cells may expand to form multiple clonal population within the nasopharynx). These genetic events could result from the consumption of Cantonese-style salted fish and other traditional foods. Samples of Chinese-style salted fish contain high concentrations of several *N*-nitrosamines and their precursors such as *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopyrroline and *N*-nitrosopiperidine (see Section 1.2) which were all shown to be carcinogenic in animals (IARC, 1978, 1993; Tricker & Preussmann, 1991). In addition, increased formation of *N*-nitrosamines occurs after endogenous chemical nitrosation of salted fish with nitrite under acidic conditions (see Section 1.2). *N*-nitrosamino acids excreted in the urine were shown to be increased in subjects living in the high-risk areas of NPC in southern China, compared to those living in the low-risk areas (Yi *et al.*, 1993). These results suggest exposure to carcinogenic *N*-nitroso compounds, preformed in salted fish or formed endogenously by nitrosation of their precursors.

Polymorphisms in *cytochrome P450* (CYP) *2E1* (*CYP2E1*) (Hildesheim *et al.*, 1995, 1997; Kongruttanachok *et al.*, 2001) and *CYP2A6* (Tiwawech *et al.*, 2006) and the absence of *glutathione-S-transferase M1* (*GSTM1*) and/or *GSTT1* (Guo *et al.*, 2008; Zhuo *et al.*, 2009) have been associated with increased risk of NPC in Southern China. Polymorphisms in genes encoding for enzymes involved in *N*-nitrosamine metabolism and detoxification could affect carcinogenesis but exact mechanisms have not been elucidated.

Aqueous extracts of Cantonese-style salted dried fish from China can activate EBV-reactivation (Shao *et al.*, 1988). This is important, since EBV can persist benignly in the body unless it is reactivated. Active EBV can induce many different cellular processes that may lead to carcinogenesis (IARC, 2012). It can for instance, induce genomic instability (Fang *et al.*, 2009) and activation of the NADPH oxidase (Gruhne *et al.*, 2009) and increased expression of inducible nitric oxide synthase (Yu *et al.*, 2002). These enzymes produce reactive oxygen and nitrogen species that damage DNA through formation of 8-oxo-deoxyguanosine and 8-nitroguanine in NPC (Ma *et al.*, 2008; Segawa *et al.*, 2008; Gruhne *et al.*, 2009). Increased lipid peroxidation product (malondialdehyde) was also detected in the blood of NPC patients (Gargouri *et al.*, 2009). These findings indicate that reactivation by Chinese-style salted fish of latent EBV in infected cells may play a substantial role in NPC, by promoting genomic instability via induction of oxidative and nitrative DNA damage. Interestingly, epidemiological data showed that both EBV and Chinese-style salted fish are also associated with gastric carcinoma.

4.4 Synthesis

Possible mechanisms for the association of consumption of Cantonese-style salted fish with risk of NPC are the formation endogenously of *N*-nitroso compounds in the human body and/or their formation due to the processing of the fish — i.e. a reaction between secondary and tertiary amines in the fish and nitrate/nitrite in the crude salt used — and activation of the oncogenic Epstein-Barr virus. These two mechanisms are not mutually exclusive.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of Chinese-style salted fish. Chinese-style salted fish causes cancer of the nasopharynx. Also, a positive association has been observed between consumption of Chinese-style salted fish and cancer of the stomach.

There is *sufficient evidence* in experimental animals for the carcinogenicity of Cantonese-style salted fish.

Chinese-style salted fish *is carcinogenic to humans (Group 1).*

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