FINAL

Report on Carcinogens Background Document for

2-Amino-3-methylimidazo [4,5-*f*]quinoline (IQ)

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Prepared for the:
U.S. Department of Health and Human Services
Public Health Service
National Toxicology Program
Research Triangle Park, NC 27709

Prepared by:
Technology Planning and Management Corporation
Canterbury Hall, Suite 310
4815 Emperor Blvd
Durham, NC 27703
Contract Number NOI-ES-85421

Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

US Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

2-Amino-3-methylimidazo[4,5-*f*]quinoline, IQ

CASRN 76180-96-6

Carcinogenicity

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ), is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of benign and malignant tumor formation at multiple tissue sites in multiple species of experimental animals (IARC 1993). Oral exposure of rats to IQ induces neoplasms of the mammary gland, liver, small intestine, clitoral gland, oral cavity and Zymbal gland in females and neoplasms of the liver, skin, colon, small intestine, oral cavity and Zymbal gland in males. Oral exposure of mice to IQ induces increased incidences of neoplasms of the lung, liver, and forestomach in males and females. Intraperitoneal exposure to mice and oral exposure to cynomologus monkeys causes liver tumors.

Epidemiology studies have provided some indication that human cancer risk is related to consumption of broiled or fried foods, but there is inadequate evidence that human cancer risk is specifically associated with exposure to IQ or other heterocyclic amines.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Studies of the genotoxicity of IQ have given uniformly positive results in a wide variety of bacterial, plant, and animal assays, predominately in systems providing metabolic activation (IARC 1993). IQ induces mutations, chromosomal aberrations, sister chromatid exchanges, micronuclei and unscheduled DNA synthesis in various human cells in culture. Metabolic activation of IQ to reactive intermediates involves acetylation and hydroxylation. *N*-acetoxy-IQ degrades to an unstable nitrenium ion that can bind to DNA. IQ-DNA adducts have been demonstrated in many tissues in animals receiving IQ, including those where IQ-induced tumors occur. All animal species studied have been found to metabolize IQ to DNA-reactive products, and cells from human mammary gland and microsomes from human liver have been shown to accomplish these reactions *in vitro*.

No data are available that would suggest that the mechanisms thought to account for tumor induction by IQ in experimental animals also would not operate in humans.

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1 Introduction

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was nominated for listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences (NIEHS) Report on Carcinogens (RoC) Review Group (RG1) based on review of a 1993 International Agency for Research on Cancer (IARC) monograph which indicated that there is sufficient evidence in experimental animals for the carcinogenicity of IQ and that IQ is *probably carcinogenic to humans* (Group 2A).

1.1 Chemical identification

IQ ($C_{11}H_{10}N_4$, mol wt 198.23, CASRN 76180-96-6) is a light tan, crystalline solid also known as: 3-methyl-3*H*-imidazo[4,5-*f*]quinolin-2-amine. The structure of IQ is illustrated in Figure 1-1.

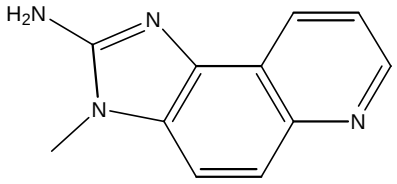

Figure 1-1. Structure of IQ

1.2 Physical-chemical properties

IQ is stable under moderately acidic and alkaline conditions and in cold, dilute aqueous solutions when protected from light (IARC 1986). It is rapidly degraded by dilute hypochlorite (IARC 1993). The RTECS number for IQ is NJ5910000. The physical and chemical properties of IQ are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of IQ

Property	Information	Reference
Molecular weight	198.23	Chemfinder (1999), Budavari *et al.* (1996)
Color	light tan	Chemfinder (1999), Budavari *et al.* (1996)
Physical state	crystalline solid	Chemfinder (1999), Budavari *et al.* (1996)
Melting point (^oC)	> 300	IARC (1993)
Solubility:		
Water at 20°C	insoluble	CSL (1987)
Dimethylsulfoxide	soluble	IARC (1993)
95% Ethanol at 16°C	1 - 10 mg/mL	Radian (1991)
Methanol	soluble	IARC (1993)
Acids (dilute)	soluble	CSL (1987)
Alcohols (dilute)	soluble	CSL (1987)

IQ is one of a number of heterocyclic amines found in cooked food, primarily in meats and fish. IQ has no commercial uses; however, some is synthesized for research purposes, and the majority of the studies reviewed in this document were performed using chemically synthesized IQ.

2 Human Exposure

2.1 Use

IQ has no known commercial uses (Radian 1991, IARC 1993).

2.2 Production

IQ is produced commercially in small quantities for research. Chemical synthesis was first reported by Kasai *et al.* (1980, cited by IARC 1993). 5,6-Diaminoquinoline was reacted with cyanogen bromide, which produced a cyclic intermediate that was converted to the tetramethyl ammonium salt and then heated under reduced pressure to form IQ. Sublimation, silica-gel column chromatography, and crystallization from aqueous methanol were used to purify the IQ (IARC 1993).

2.3 Analysis

IQ is found predominantly in cooked foods. Table 2-1 describes the various methods of analysis for determining concentrations of IQ.

Table 2-1. Analytical methods for determining IQ concentration

Sample matrix	Assay procedure[a]	Preparation	Reference
Broiled, sun-dried sardines	NMR and high-resolution mass spectral analysis	extracted with methanol and purified by Diaion PH-20 column chromatography, chloroform-methanol-water partitioning and Sephadex LH-20 column chromatography, silica-gel column chromatography and reverse-phase HPLC	Kasai *et al.* (1980, 1981)
Beef extract	mass spectrometry and ultraviolet spectrophotometry and/or mutagenic assays	isolated by dichloromethane extraction, column chromatography on Adsorbosil-5 and Sephadex LH-20 and HPLC	Hargraves and Pariza (1983); Turesky *et al.* (1983)
Fried ground beef	off-line mass spectrometry	dichloromethane extraction, chromatography on XAD-2 resin and three different HPLC separations	Felton *et al.* (1984)
Aqueous solutions	HPLC	adsorbed onto cellulose or cotton to which C.I. reactive blue 21 has been covalently bound (blue cotton adsorption technique); eluted with an ammonia-methanol solution	Hayatsu *et al.* (1983)
Food and food extracts	HPLC	solid-phase extraction and subsequent analysis allow IQ determination at a level of 1 ng/g food from only 10 g of food	Gross (1990); Gross and Gruter (1992)

Source: IARC (1993).
[a] NMR: nuclear magnetic resonance; HPLC: high-performance liquid chromatography.

2.4 Environmental occurrence

IQ is one of many heterocyclic amines (HCAs) formed when various meats and fish are cooked. Originally, it was isolated from broiled fish, fried ground beef, and beef extracts. No uses have

been identified that would be expected to lead to the release of IQ into the environment. Environmental occurrence of IQ may arise from food waste and disposal in landfills.

IQ was found when certain compounds were mixed and heated, such as in meat extracts. Meat extracts typically are formed by heating a source of amino acids, reduced sugars, fats, and other ingredients at temperatures greater than 100°C for times sufficient to develop flavor. Aeschbacher *et al*. (1987, cited in Jackson *et al.* 1994) reported that commercial meat extracts can contain up to 100 ng/g of HCAs (including IQ, methyl-IQ, and dimethyl-IQ) (Jackson *et al.* 1994). Creatine and proline heated to 180°C will produce IQ. IQ also was found in mixtures of creatinine and phenylalanine or creatinine, phenylalanine, and glucose, heated to 200°C and in a dry mixture of serine and creatinine heated to 200°C (IARC 1993). Table 2-2 summarizes IQ concentrations in various products.

Table 2-2. Concentrations of IQ in foods and in cigarette smoke condensate

Sample	Concentration wet weight (ng/g)	No. of samples[a]	Reference
Chicken leg, with skin, fried for 15 minutes			
100°C	0.13	10	Chiu *et al.* (1998)
150°C	0.14	10	Chiu *et al.* (1998)
200°C	0.51	10	Chiu *et al.* (1998)
Egg, fried at 325°C	0.1	1	Grose *et al.* (1986)[b]
Fish, fried at 260°C	0.16	1	Zhang *et al.* (1988)[b]
Ground beef, fried			
240°C	0.5 – 20[c]	2	Barnes *et al.* (1983)[b]
250°C	0.02	1	Felton *et al.* (1984)[b]
275°C	0.3 - 1.9	3	Turesky *et al.* (1988)[b]
Ground beef, broiled	0.5	1	Yamaizumi *et al.* (1986)[b]
Beef, broiled	0.19	1	Wakabayashi *et al.* (1992)[b]
Beef extract, food-grade	<0.2	1	Takahashi *et al.* (1985)[b]
Beef extract, food-grade	< 0.1 - 6.2	3	Turesky *et al.* (1989)[b]
Minute steak cooked at 150°C for 2.5 min	0.1	—	Skog *et al.* (1995)
Meat balls cooked at 175°C for 7.5 min	0.05	—	Skog *et al.* (1995)
Pork chops cooked at 175°C for 11 min	0.1	—	Skog *et al.* (1995)
Salmon, broiled	0.3 - 1.8	2	Yamaizumi *et al.* (1986)[b]
Sardines, sun dried, broiled	~ 20	1	Kasai *et al.* (1980)[b]
Sardines, sun dried, broiled	158	1	Sugimura *et al.* (1981)[b]
Cigarette smoke condensate	0.26 per cigarette	—	Yamashita *et al.* (1986)[b]

[a] (—), number of samples not given.
[b] Cited in IARC (1993).
[c] 20 ng/g in a high-fat sample, 0.5 ng/g in a low-fat sample.

2.5 Environmental fate

No environmental fate data could be found. IQ's persistence in the environment after any potential release is unknown.

IQ degrades in light and air. It may be sensitive to prolonged heat exposure. A solution of IQ in water (concentration not specified), dimethylsulfoxide (DMSO), 95% ethanol, or acetone is stable for 24 hours under ambient conditions (Radian 1991).

2.6 Environmental exposure

The most likely mode of exposure is ingestion of cooked food products containing IQ, such as broiled or fried beef, fish, or eggs (Radian 1991). IQ also is present in cigarette smoke (Yamashita *et al.* 1986). Based upon analysis of various foodstuffs and analysis of HCA content (including IQ), estimated daily exposure of the U.S. population to heterocyclic aromatic amines ranges from 100 ng to 10 µg per day. It is difficult to quantify overall U.S. exposure, because IQ content depends on the meat, cooking temperature, and manner of preparation (Turesky *et al.* 1993). Individual IQ intake would depend upon these factors.

Another study estimated human exposure to five heterocyclic amines in an elderly population in Stockholm. After a semi-quantitative food survey was administered, 22 dishes were cooked and chemically analyzed. The total daily dietary intake of the heterocyclic amines ranged from 0 to 1,816 ng, with a mean intake of 160 ng. Of this mean daily HCA intake, IQ intake was < 1 ng/day (Augustsson *et al.* 1997).

2.7 Occupational exposure

Occupational exposure to IQ may occur where employees work with broiled or fried foods, such as beef, fish, or eggs. No studies were found that dealt with IQ intake among workers who prepared or served such foods. It is unknown whether exposure by routes other than ingestion (i.e., dermal or inhalation) would occur in this setting.

2.8 Biological indices of exposure

IQ undergoes metabolic activation to yield the reactive metabolite *N*-acetyl-IQ, which binds to DNA (Probst *et al.* 1992). Using the ^{32}P-postlabeling method, Fan *et al.* (1995) found IQ-DNA adducts in human mammary gland epithelial cells following *in vitro* exposure to IQ. Leong-Morganthaler *et al.* (1998) also found IQ-DNA adducts in TK6 human lymphoblastoid cells following *in vitro* exposure to IQ, using the same method.

Ten urinary IQ metabolites have been identified in animal studies (see Section 6), but no studies demonstrating the occurrence of these metabolites in humans were located.

2.9 Regulations

No IQ-specific regulations were found. One regulation (40 CFR 721) states that significant new uses for substituted quinoline must be reported. Currently, however, IQ has no known commercial uses.

3 Human Cancer Studies

A substantial body of literature suggests that risk of several cancers may be related to consumption of meat, particularly red meat, and to methods of food preparation, particularly grilling and frying (IARC 1993). The mechanism underlying this risk is as yet unclear. One possibility is that HCAs formed when meat is cooked are involved. This section reviews studies that assessed the relationship of human cancer risk to exposure to IQ or to total HCAs. The major problem encountered in these studies was the difficulty of quantifying HCA exposure. The studies are summarized in Table 3-1.

De Stefani *et al.* (1997) conducted a hospital-based case-control study in Uruguay to determine the relationship of IQ exposure to risk of breast cancer in pre- and post-menopausal women. Between May 1994 and November 1996, 352 newly diagnosed breast cancer patients in six major hospitals were enrolled in a case-control study. During the same time period and in the same hospitals, 382 patients with non-neoplastic diseases were enrolled as controls. Controls were frequency-matched to cases by age, sex, and residence. In-person interviews were used to collect information on demographic and anthropometric factors, occupational history, alcohol and tobacco use, and family history of cancer. The interview also included a food-frequency questionnaire, which collected information on consumption of 64 food items over the two years prior to the interview or the onset of symptoms. Data on food consumption and cooking practices were used to estimate total IQ intake, which was then categorized into quartiles. Risks were adjusted for age, residence, family history of breast cancer, age at menarche, parity, history of benign breast disease, total calories, vegetable intake, and fat intake. Considering both pre- and post-menopausal women, odds ratios (ORs) increased from 1.22 (95% CI 0.75 - 1.99) for quartile II to 1.87 (95% CI 1.10 - 3.15) for quartile III and to 3.34 (95% CI 1.85 - 6.02) for quartile IV (P for trend < 0.001). Similar results were seen in post-menopausal women, but the association was weaker in pre-menopausal women, possibly because of the smaller sample size. The strengths of this study are large sample size, ability to account for other breast cancer risk factors, and the attempt to quantify IQ exposure. Limitations include the hospital-based design and difficulties in estimating IQ exposure, including poor recall of usual diet by hospitalized participants and failure to account for smoking as a source of IQ exposure.

Probst-Hensch *et al.* (1997) conducted a sigmoidoscopy-based case-control study in California to determine the relationship of dietary HCA exposure to prevalence of distal colorectal adenomas. A total of 488 individually matched case-control pairs were selected from two southern California medical centers where they had undergone sigmoidoscopy between January 1, 1991, and August 25, 1993. Cases had a first diagnosis of histologically confirmed adenoma. Controls had no current or past polyp of any kind and were individually matched to cases by age, gender, date of sigmoidoscopy, and medical center. In-person interviews were used to collect data on smoking, therapeutic drug use, physical activity, height, weight, and family history of cancer. A semiquantitative food-frequency questionnaire was administered, which used nine categories, ranging from “never or less than once per month” to “six or more a day,” to determine food consumption during the previous year. Percent of time meat was fried was associated with adenoma prevalence (P for trend = 0.004), but degree of doneness, darkening of meat surface, and percent of time meat was barbecued were not. Only total HCA and not IQ exposure was estimated. Two estimates of HCA exposure were made, based on degree of doneness or on

surface darkening of red meat. Participants who ate red meat at most once per week, fried it at most 10% of the time, and preferred meat that was not well done or darkly browned, respectively, were classified as having low exposure. Those who ate red meat more than once per week, who fried it more than 10% of the time, and who preferred it well done or darkly browned, respectively, were classified as having high exposure. Others were classified as having intermediate exposure. After adjustment for smoking, calories, and fruit and vegetable intake, HCA exposure calculated by either method was associated with adenoma prevalence: for doneness, OR for high vs low exposure = 1.7 (95% CI 0.9 - 3.3), *P* for trend = 0.05; for darkening, OR = 2.2 (95% CI 1.1 - 4.3), *P* for trend = 0.05. The strengths of the study include large sample size, selection of cases and controls from the same series of patients, ability to account for other colorectal cancer risk factors, and the attempt to quantify HCA exposure. Limitations include examination of adenomas only in the left side of the colon and difficulties in estimating HCA exposure.

Augustsson *et al.* (1999) conducted a population-based case-control study to assess the relationship of daily intake of total or specific HCAs to the risk of colon, rectum, bladder, and kidney cancers in Sweden. Subjects were identified from the population-based Swedish cancer registry and included 352 with colon cancer, 249 with rectal cancer, 273 with bladder cancer, and 138 with kidney cancer. Controls (553) were randomly selected from a population register and frequency-matched to colon cancer cases by age and sex. A semiquantitative food-frequency questionnaire that included 188 food items was used to determine food consumption for the previous five years. Color photographs were used to determine the degree of surface browning of fried meats. Daily intakes of specific HCAs were estimated based on type of meat or fish eaten, frequency of consumption, portion size, cooking methods, and degree of surface browning. Estimates were based on concentrations of HCAs measured in previous studies of foods cooked in standard ways. Median daily intake of total HCAs was less than 100 ng. The highest daily intake reported by controls was 1,816 ng; seven subjects with cancer reported a higher intake. Intakes were categorized into quintiles, and risks were adjusted for age, sex, and smoking (for bladder and kidney cancer only). For total HCAs, weak associations were observed for bladder and kidney cancer, but no dose response was evident. No association with IQ was observed for any cancer. Strengths of the study include the large sample size, the population-based design, and the use of detailed data on diet coupled with measurements of HCAs in cooked foods to estimate HCA intake. Limitations include the restricted range of HCA intake. The results of this study suggest that low levels of HCA intake are not associated with increased cancer risk, but do not exclude the possibility that very high levels of intake may present a risk.

In summary, evidence relating IQ consumption to cancer risk is limited. Of the studies reviewed here, one suggests a relationship of IQ to breast cancer risk, another suggests a relationship of total HCAs to colorectal cancer risk, and the third provides little evidence for a relationship of IQ or total HCAs to risk of cancer of colon, rectum, bladder, or kidney. Although all studies were well conducted, each was hampered by difficulties in estimating exposure to IQ or total HCAs. Thus, the evidence from human studies regarding the relationship of IQ exposure to cancer risk remains inconclusive.

Table 3-1. Case-control studies of HCA or IQ exposure and human cancer risk

Reference	Population	Exposure	Effects	Potential confounders
De Stefani *et al.* (1997) Uruguay	352 breast cancer cases and 382 controls with non-neoplastic diseases recruited from 6 major hospitals between May 1994 and November 1996	IQ intake estimated using a food-frequency questionnaire and information on cooking practices, and then categorized into quartiles: the lowest quartile is the referent category. quartile I: ≤ 0.42 ng/g quartile II: 0.43 - 0.66 ng/g quartile III: 0.67 - 1.01 ng/g quartile IV: ≥ 1.02 ng/g	**breast cancer OR for all subjects** quartile II: 1.22 (95% CI 0.75 - 1.99, 77 cases) quartile III: 1.87 (95% CI 1.10 - 3.15, 92 cases) quartile IV: 3.34 (95% CI 1.85 - 6.02, 130 cases) *P* for trend < 0.001 **breast cancer OR for premenopausal women** quartile II: 1.86 (95% CI 0.54 6.52, 18 cases) quartile III: 2.70 (95% CI 0.76 - 9.53, 18 cases) quartile IV: 2.15 (95% CI 0.58 - 8.05, 32 cases) *P* for trend = 0.24 **breast cancer OR for postmenopausal women** quartile II: 1.11 (95% CI 0.64 - 1.92, 59 cases) quartile III: 1.75 (95% CI 0.96 - 3.19, 74 cases). quartile IV: 3.80 (95% CI 1.90 - 7.60, 98 cases) *P* for trend < 0.001	risks adjusted for age, residence, family history of breast cancer in a first-degree relative, age at menarche, parity, previous history of benign breast disease, total energy, vegetable intake, and fat intake

Reference	Population	Exposure	Effects	Potential confounders
Probst-Hensch *et al.* (1997) United States	488 individually matched pairs of cases and controls recruited from sigmoidoscopy patients at two California medical centers; cases had first diagnosis of histologically confirmed adenoma; controls were free of past or current polyps.	HCA intake estimated from a semiquantitative food-frequency questionnaire and information on cooking practices; estimates based on degree of doneness or surface darkening of meat, and categorized as low, intermediate, or high exposure; low exposure is the referent category.	**colorectal adenoma OR based on meat doneness:** **intermediate**: 1.4 (95% CI 1.0 - 2.0, 307 cases) **high**: 1.7 (95% CI 0.9 - 3.3, 31 cases) *P* for trend = 0.05 **colorectal adenoma OR based on surface darkening:** **intermediate**: 1.3 (95% CI 0.9 - 1.9, 297 cases) **high**: 2.2 (95% CI 1.1 - 4.3, 37 cases) *P* for trend = 0.02	risks adjusted for total calories, smoking, and fruit and vegetable intake

Reference	Population	Exposure	Effects	Potential confounders
Augustsson *et al.* (1999) Sweden	source population of people in Sweden born between 1918 and 1942, who had a permanent address in Stockholm at least one month between November 1, 1992, and December 31, 1994 352 cases of colon cancer, 249 cases of rectal cancer, 273 cases of bladder cancer, and 138 cases of kidney cancer identified from population-based cancer registry; 553 controls randomly selected from population register and frequency matched to colon cancer cases	semiquantitative food-frequency questionnaire used to determine food consumption 5 years previously; color photographs used to determine degree of surface browning of fried meats; daily intake of IQ estimated based on type of meat or fish eaten, frequency of consumption, portion size, cooking methods, and degree of surface browning; estimates were based on concentrations of IQ measured in previous studies of foods cooked in standard ways; intake categorized into quintiles; quintile 1 is the referent category.	**colon cancer OR**: **quintile 2**: 1.1 (95% CI 0.7 - 1.7) **quintile 3**: 0.8 (95% CI 0.5 - 1.3) **quintile 4**: 1.4 (95% CI 0.9 - 2.1) **quintile 5**: 1.1 (95% CI 0.7 - 1.6) **rectum cancer OR**: **quintile 2**: 0.9 (95% CI 0.5 - 1.4) **quintile 3**: 0.8 (95% CI 0.5 - 1.3) **quintile 4**: 1.4 (95% CI 0.9 - 2.2) **quintile 5**: 0.8 (95% CI 0.5 - 1.3) **bladder cancer OR**: **quintile 2**: 0.9 (95% CI 0.5 - 1.5) **quintile 3**: 1.1 (95% CI 0.7 - 1.9) **quintile 4**: 1.0 (95% CI 0.6 - 1.8) **quintile 5**: 1.1 (95% CI 0.7 - 1.9) **kidney cancer OR**: **quintile 2**: 1.0 (95% CI 0.5 - 1.8) **quintile 3**: 0.6 (95% CI 0.3 - 1.2) **quintile 4**: 1.3 (95% CI 0.7 - 2.3) **quintile 5**: 0.9 (95% CI 0.5 - 1.6)	risks adjusted for age, sex, and smoking (bladder and kidney cancer only)

OR: Odds ratio

4 Studies of Cancer in Experimental Animals

4.1 Orally administered IQ

4.1.1 Studies in mice

IARC reviewed studies of IQ carcinogenicity in experimental animals (IARC 1993). In one of these studies, groups of 40 male and 40 female CDF_1 mice ([BALB/cAnN x DBA/2N]F_1), seven weeks of age, were fed either basal diet or diet containing IQ (> 99.6% pure) at a concentration of 300 ppm for 675 days. Survival of animals administered IQ was similar to that of controls. Body weights of females receiving IQ were slightly less than those of controls. Administration of IQ caused significant increases in the incidences of hepatocellular adenomas and carcinomas (combined), adenomas and adenocarcinomas (combined) of the lung, and papillomas and squamous cell carcinomas (combined) of the forestomach in both sexes (Ohgaki *et al.* 1984, 1986, cited in IARC 1993). Table 4-1 presents data from this study.

Table 4-1. Tumor incidences in CDF_1 mice fed a diet containing IQ at a concentration of 300 ppm for up to 675 days

Tumor type	Tumor incidence/number examined			
	Males		Females	
	Control	IQ treated	Control	IQ treated
Liver: adenoma	2/33	8/39	0/38	5/36
carcinoma	0/33	8/39	0/38	22/36**
adenoma and carcinoma (combined)[a]	2/33	16/39*	0/38	27/36**
Lung: adenoma	4/33	13/39	3/38	7/36
adenocarcinoma	3/33	14/39	4/38	8/36
adenoma or carcinoma	7/33	27/39**	7/38	15/36*
Forestomach: papilloma	1/33	11/39	0/38	8/36
squamous cell carcinoma	0/33	5/39	0/38	3/36
papilloma or carcinoma	1/33	16/39*	0/38	11/36*

Source: Ohgaki *et al.* (1984 1986, cited in IARC 1993).
[a]Statistics calculated for combined tumors.
*$P < 0.05$; **$P < 0.01$.

Tudek *et al.* (1989, cited in IARC 1993) administered IQ by gavage to groups of 10 or more female CDF_1 mice, 27 to 31 days old, at doses of 200 or 400 mg/kg bw, one-half the LD_{50}, twice at a four-day interval. The numbers of aberrant colonic crypts, considered a precursor lesion for colon carcinogenesis, were increased in a dose-related manner 21 days after the initial IQ dose. Crypts were found most frequently in the cecal end of the colon.

4.1.2 Studies in rats

A group of 32 female Sprague-Dawley rats, six weeks old, received IQ hydrochloride by gavage at a dose of 0.35 mmol body weight (70 mg/kg) in 5% Emulphor. The dosing regimen was three doses per week during weeks 1 through 4, two doses per week during weeks 5 through 8, and

one dose per week during weeks 9 through 31. Animals were maintained without further dosing until study termination at week 52. Vehicle-control rats were dosed on the same schedule, and a group of nine animals served as untreated controls. Administration of IQ caused increased incidences of mammary gland tumors (14/32); primarily adenocarcinomas, neoplastic nodules (3/32), hepatocellular carcinomas (2/32), and hemangioendotheliomas of the liver (2/32), and squamous cell carcinomas of the Zymbal gland (11/32). No tumors of the mammary gland, Zymbal gland, or liver were observed in control animals (Tanaka *et al.* 1985, cited in IARC 1993).

Groups of 40 male and 40 female Fischer 344 rats, eight weeks of age, were fed pelletized diets containing IQ at a concentration of 300 ppm (purity not stated) for 104 weeks. Groups of 50 males and 50 females served as controls. The animals fed IQ had significantly increased incidences of sacrifices in moribund condition and early deaths due to hepatocellular carcinomas, squamous cell carcinomas of the Zymbal gland, and adenocarcinomas of the intestine. One control male was reported to have a hepatocellular carcinoma. No other controls were found to have tumors at any of the sites listed in Table 4-2. A summary of the tumor incidences is presented in Table 4-2 (Takayama *et al.* 1984, Ohgaki *et al.* 1986, both cited in IARC 1993).

Table 4-2. Tumors observed in Fischer 344 rats fed a diet containing IQ at a concentration of 300 ppm for up to 104 weeks

Tumor type	Tumor incidence/number examined			
	Males		Females	
	Control	IQ treated	Control	IQ treated
Zymbal gland: squamous cell carcinoma	0/50	36/40*	0/50	27/40*
Large intestine: adenocarcinoma	0/50	25/40*	0/50	9/40*
Small intestine: adenocarcinoma	0/50	12/40*	0/50	1/40
Liver: hepatocellular carcinoma	1/50	27/40*	0/50	18/40*
Skin: squamous cell carcinoma	0/50	17/40*	0/50	3/40
Oral cavity: squamous cell carcinoma	0/50	2/40	0/50	1/40
Preputial/Clitoral gland: squamous cell carcinoma	NR	NR	0/50	20/40*

Source: Takayama *et al.* (1984), Ohgaki *et al*. (1986), both cited in IARC (1993).
*$P < 0.01$ Fisher's exact test, calculated by NTP (RG1).
NR: not reported

Weisburger *et al.* (1995) investigated the carcinogenicity of IQ in 30 male F344 rats, six weeks old, given IQ by intrarectal infusion at a dose of 35 mmol/kg in DMSO, three times per week for four months, followed by two times per week for 14 more months. Tumor incidences were compared with those in a group of 50 male control rats; unspecified numbers of which were untreated or received the vehicle. Increased incidences of carcinomas of the colon (13/30), squamous cell carcinomas of the skin (11/30), and liver adenomas (5/30) were observed in rats receiving IQ. Controls had incidences of 1/50 for each tumor site and type. In another experiment designed to evaluate the enhancement of the action of IQ by dietary fat, 34 to 35 male and 34 female F344 rats were fed 75 ppm IQ in 5% or 23.5% corn–oil-diets (10% and 40%

of fat calories) for 12 months. Males were sacrificed at 15 months and females at 18 months. Complete necropsies were performed. Increased incidences of tumors, identified as Zymbal gland/earduct neoplasms, multiple sebaceous malignant and benign skin tumors (in males), liver tumors, mammary gland carcinomas, colon tumors, and preputial gland tumors, were observed. Corresponding tumor incidences in groups of 34 controls were reported as 0 or 1. These results are summarized in Table 4-3 (Weisburger *et al.* 1995).

Table 4-3. IQ-induced cancers observed in rats fed 75 ppm IQ in 5% or 23.5% corn-oil-diets

Tumor type	Tumor incidence/corn oil (%)			
	Males[a]		Females[a]	
	5%	23.5%	5%	23.5%
Zymbal gland[b]	14	14	12	4
Lip[b]	5	0	2	3
Skin[b]	16	20	1	0
Liver: carcinoma	2	17	6	6
adenoma	0	5	3	2
Mammary tumors	—	—	10	17
Preputial/clitoral tumors	3	2	9	13
Colon tumors	2	3	0	1
Lung tumors	1	6	1	1
Lung metastases	5[c]	2[d]	0	0

Source: Weisburger *et al.* (1995)
[a] 34 rats examined/group, except 35 in males on 23.5% fat diet; tumor incidence in controls was 0 or 1.
[b] In many cases, these were multiple malignant or benign neoplasms of sebaceous nature.
[c] From sebaceous skin tumors
[d] Carcinomas: one from skin, one from liver.
—, not given.

Tudek *et al.* (1989, cited in IARC 1993) administered IQ by gavage to groups of 10 or more female Sprague-Dawley rats, 21 days old, at doses of 200 or 400 mg/kg body weight, a total of five times at four-day intervals. The numbers of aberrant colonic crypts observed 21 days after the initial IQ dose were dose-related. Crypts were found most frequently in the cecum.

4.1.3 Gavage study in monkeys

Groups of 20 cynomologus monkeys (*Macaca fascicularis*) were administered IQ by gavage in hydroxypropyl cellulose at doses of 10 mg/kg (14 males, 6 females) or 20 mg/kg (8 males, 12 females), five times per week for 60 months. The monkeys were one year old at the initiation of the study. Hepatocellular carcinomas were found in all 20 animals that had received IQ at 20 mg/kg and in 15 animals that had received IQ at 10 mg/kg. Metastases to the lung were observed in several animals. No liver tumors were observed in an unspecified number of control animals from the same colony (Adamson *et al.* 1990, 1991, cited in IARC 1993; Thorgeirsson *et al.* 1996).

4.2 Intraperitoneally administered IQ

Newborn B6C3F$_1$ mice received intraperitoneal injections of IQ (total doses of 0, 0.625, or 1.25 μmol [125 - 250 μg] dissolved in DMSO) on days 1, 8, and 15 after birth. The initial number of mice used in this study was not stated. Animals were sacrificed after 8 and 12 months. The incidence of hepatocellular adenomas was significantly higher ($P < 0.005$) in IQ treated mice than in controls surviving to both sacrifice periods (1/44 controls, 5/24 low-dose mice and 5/16 high-dose mice at 8 months; 5/44 controls, 7/19 low-dose mice and 14/20 high-dose mice at 12 months). Additionally, two hepatocellular carcinomas were found in high-dose animals at 12 months. No carcinomas occurred in controls examined at 8 or 12 months (Dooley *et al.* 1992, cited in IARC 1993).

4.3 Other studies

Numerous investigators have used IQ to induce preneoplastic events or tumors in animals in studies designed to examine the pro- or anti-carcinogenic activities of other substances. Orally administered IQ has been employed to induce hepatic cytochrome P-450 1A-2 (Nerurkar *et al.* 1993; Xu *et al.* 1997); colonic neoplasms, aberrant colonic crypts, and colonic foci of aberrant crypts in rodents (Kristiansen *et al.* 1996; Xu *et al.* 1997; Ferguson and Harris 1998); DNA adducts in rodents (Davis *et al.* 1994; Nerurkar *et al.* 1995, 1996; Turesky *et al.* 1995, 1996a, 1997; Schut *et al.* 1997a, 1997b; Xu *et al.* 1997) and nonhuman primates (Snyderwine *et al.* 1993b; Turesky *et al.* 1997, 1996b); and mammary gland neoplasms in mice (Weisburger *et al.* 1997).

IQ also has been used to induce preneoplastic events or tumors in studies of oncogene mutations in mice (Herzog *et al.* 1993), rats (Makino *et al.* 1992; Kakiuchi *et al.* 1993; Takahashi *et al.* 1993; Makino *et al.* 1994; Tachino *et al.* 1995), and primates (Fujimoto *et al.* 1994). Mutations were detected in colonic tumors and aberrant crypts and in hepatocellular carcinomas, and carcinomas of the Zymbal gland in animals dosed with IQ. In a study in which IQ was used comparatively with 2-amino-1-methyl-6-phenylimidazo-[4,5,-*b*]pyridine to evaluate mutations in induced rat colon tumors, microsatellite instability was increased in the rat colon adenocarcinomas induced by 2-amino-1-methyl-6-phenylimidazo-[4,5,-*b*]pyridine, but not IQ, suggesting impaired DNA mismatch repair (Canzian *et al.* 1994). In other studies, IQ-induced colorectal tumors in F344/N rats showed inhibition of cell death (apoptosis). Colorectal tumors were found to exhibit increased expression of *bcl-2*, an anti-apoptosis protein, and decreased *bax*, an apoptosis activator (Hayashi *et al.* 1996; Dashwood *et al*. 1998). The results of these studies consistently have shown that relatively short periods of administration of IQ cause preneoplastic or neoplastic changes in experimental animals. Mutations in catenins, a tumor suppressor protein, also were noted in IQ-induced colon tumors (Dashwood *et al.* 1998).

4.4 Summary

The carcinogenicity of IQ has been demonstrated in studies with mice, rats, and nonhuman primates. Rats orally exposed to IQ developed neoplasms of the mammary gland, liver, small intestine, skin, oral cavity, preputial/clitoral gland, and Zymbal gland. Mice orally exposed to IQ had increased tumors of the lung, liver, and forestomach. Nonhuman primates administered IQ by gavage developed liver tumors. Intraperitoneal administration of IQ to newborn mice resulted in liver tumors, and intrarectal administration to rats resulted in intestine and liver neoplasia.

5 Genotoxicity

5.1 Prokaryotic Systems

5.1.1 Induction of mutation in Salmonella typhimurium

In studies reviewed by IARC (1993), IQ was consistently found to be mutagenic in a variety of *Salmonella typhimurium* tester strains with metabolic activation.

In more recent studies, IQ induced a significant increase in the induction of *His*+ revertants in *S. typhimurium* strain YG1012 (TA1538 1,8-DNP pYG213) in an assay in which human P-450-1A2-containing microsomes and hydrogen peroxide were used as a metabolic activation system. The mutagenic response was found to depend on the concentrations of microsomal proteins, IQ, and hydrogen peroxide. Addition of peroxides greatly enhanced the induction of *His*+ revertants (Morrison *et al.* 1993; Anari *et al.* 1997). IQ also was mutagenic in *S. typhimurium* strain TA98 in the presence of metabolic activation provided by microsomal protein (from rat, cynomologus monkey, or human liver) and NADPH. No statistically significant differences in species activation potential were noted (Davis *et al.* 1993).

Coincubation of IQ and ram seminal vesicle microsomes (RSVM), metabolically activated by prostaglandin H synthase and supplemented with arachidonic acid, with *S. typhimurium* strain YG1024 (TA98 pYG219) yielded a dose-dependent increase in the frequency of revertants. Omission of the arachidonic acid supplement inhibited the mutagenicity of IQ. Heating of the RSVM abolished the mutagenicity of IQ in this test system (Wolz *et al.* 1995).

IQ induced a significant dose-dependent increase in base-pair substitution mutations at the *his*G46 allele in *S. typhimurium* strain TA100 in the presence of exogenous rat-liver S9 metabolic activation. The observed mutations were predominantly GC→TA transversions with a pronounced preference for the second codon position, CCC→CAC. IQ was, however, not mutagenic in *S. typhimurium* strain TA1535 in the presence of rat-liver S9 metabolic activation. The dose used was 30-fold that needed to induce a three fold increase in reversion in *S. typhimurium* strain TA100 (Koch *et al.* 1998).

Studies have been conducted with antimutagenic agents to determine the mechanism of IQ mutagenesis. In one of these studies, the use of theafulvins in a *Salmonella* assay (strain TA98 in the absence of metabolic activation) provided evidence that the inhibition of cytochrome P-450 by theafulvins correlated, in a concentration-dependent manner, with decreased mutagenic activity of IQ. Theafulvins were shown to inhibit *O*-dealkylation of methoxy-, ethoxy-, and pentoxy-resorufin (chemical probes for CYP1A2, CYP1A1, and CYP2B proteins, respectively, of the cytochrome P-450 system) and thereby inhibit the bioactivation of IQ to its mutagenic metabolites (Catterall *et al*. 1998).

Studies in which green tea or black tea was used in conjunction with IQ or its mutagenic metabolite (2-hydroxyamino-3-methylimidazo[4,5-*f*]quinoline, or *N*-hydroxy-IQ) in a *Salmonella* assay (strain TA98) in the absence of metabolic activation provided evidence that scavenging of *N*-hydroxy-IQ by the green tea or black tea caused a concentration-dependent inhibition of the mutagenic effect of IQ and its metabolite *N*-hydroxy-IQ on *Salmonella* (Chen and Yen 1997; Hernaez *et al*. 1998).

5.1.2 Induction of mutation in Escherichia coli

IQ caused prophage λ induction in *Escherichia coli* K12 in the presence of exogenous metabolic activation (Nagao *et al.* 1983, cited in IARC 1993).

The mutational specificity of IQ was evaluated in *E. coli* by testing the ability of IQ to inactivate the *URA3*-gene of yeast (*Saccharomyces cerevisiae*). The *URA3*-gene obtained from *S. cerevisiae* was randomly modified with *N*-hydroxy-IQ and transferred into *E. coli* (DB6656). Following incubation for one hour in a 5-fluoro-orotic acid (toxic to *URA3+*, selects for *URA3-* mutant clones), a spectrum of mutations was observed. These mutations included base-pair substitution (~70%), transversions, and transitions, as well as frameshift mutation, gene deletions and insertions, and gross alterations. More than 97% of the base-pair substitutions occurred at the GC pairs. In the *URA3* gene, the most common base substitution induced by IQ was found to be GC→AT transitions (52%), followed by GC→CG (25.9%) and GC→TA (18.5%) transversions (Broschard *et al.* 1998).

5.2 Plants

5.2.1 Chromosomal aberrations (CA)

5.2.1.1 Somatic mutation

A dose-dependent increase in the frequency of somatic mutations (yellow, dark green, and twin mutational spots on the leaves) was observed in the heterozygous strain T-219 of the soybean plant ($Y_{11}Y_{11}$) following treatment with IQ in the presence of exogenous metabolic activation. Preincubation of IQ with rat S9 liver homogenate increased the frequency of yellow spots by as much as 2 to 4 times. Treatment with the highest concentration (0.1 μg/mL) caused growth inhibition with deformed leaf development (Katoh *et al.* 1992).

5.3 Eukaryotic Systems

5.3.1 Mutagenicity in Drosophila melanogaster

5.3.1.1 Sex-linked recessive lethal assay

IQ induced sex-linked recessive lethal mutations in *Drosophila melanogaster* in two studies (Wild *et al.* 1985, Graf *et al.* 1992, both cited in IARC 1993).

5.3.1.2 Somatic mutation and recombination

IQ induced somatic mutations and recombination in *D. melanogaster* in two studies (Yoo *et al.* 1985, Graf *et al.* 1992, both cited in IARC 1993).

5.4 Mammalian Systems

5.4.1 In vitro *assays*

5.4.1.1 hprt *locus forward mutation test*

In two studies, IQ induced hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus forward mutations in Chinese hamster ovary (CHO) cells (uv5) in the presence of exogenous metabolic activation (Thompson *et al.* 1983, Brookman *et al.* 1985, both cited in IARC 1993). In two other studies, IQ induced gene mutations (gene locus unspecified) in Chinese hamster lung cells with exogenous metabolic activation (Nakayasu *et al.* 1983, Sugimura *et al.* 1989, both cited in IARC 1993).

In other studies, IQ did not increase the frequency of forward mutations at the adenine phosphoribosyl transferase (*aprt*) or *hprt* loci in CHO cells (*AA*8), in the presence of exogenous metabolic activation (Thompson *et al.* 1983, cited in IARC 1993). The mutation frequency at the *hprt* locus in Chinese hamster lung V79 cells was not increased following exposure to IQ (Loprieno *et al.* 1991, cited in IARC 1993). Similarly, IQ did not induce mutations in the *ouabain* locus in Chinese hamster lung cells in the presence of exogenous metabolic activation (Takayama and Tanaka 1983, cited in IARC 1993). *In vitro* exposure of human peripheral blood lymphocytes to IQ with exogenous metabolic activation also failed to induce forward mutations at the *hprt* locus (McManus *et al.* 1988b, cited in IARC 1993).

In more recent studies, treatment of CHO-K1 cells with IQ in the presence of exogenous S9 metabolic activation induced a significantly increased frequency of mutations at the *hprt* locus (Lee and Shih 1995).

In repair-deficient CHO (UV5P3) cells that express cytochrome P-450-1A2 and had been transfected with cDNA of either human *N*-acetyltransferase-2 or *Salmonella O*-acetyltransferase, IQ increased the incidence of mutations at the *aprt* locus about 3.1 x 10^3-fold, compared with the mutation rate in the parental CHO (UV5P3) cell line (Wu *et al.* 1997).

IQ induced mutations at the thymidine kinase and *hprt* loci in human lymphoblastoid cells, in the presence of exogenous rat-liver S9 metabolic activation. IQ significantly increased *hprt* mutant ion frequencies over those seen in untreated cells. This study also showed that the IQ metabolite IQ-nitrene was a ≥ 50-fold more potent a mutagen in human lymphoblastoid cells. IQ-nitrene is formed by the photoactivation of N3-IQ [an azido derivative (2-azido-3-methylimidazo[4,5-*f*]quinoline) of IQ] (Leong-Morgenthaler *et al.* 1998).

5.4.1.2 Genetic changes in animal tumor cells

Activated *c*-Ha-*ras* proto-oncogenes were found in four of seven IQ-induced Zymbal gland tumors in rats. The mutations were G→C transversions at the first base of codon 13 (two tumors), a G→T transversion at the second base of codon 13 (one tumor), and an A→T transversion at the second base of codon 61 (Kudo *et al*. 1991, cited in IARC 1993). Gene mutations also were found in the p53 gene in four of 15 IQ-induced Zymbal gland tumors in rats. These mutations were CGT→GGT, TGC→TTC, and GTG→TTG transversions, and GAA deletions at codons 156, 174, 214, and 256, respectively (Makino *et al*. 1992, cited in IARC 1993).

5.4.1.3 Chromosomal aberration tests

Chromosomal aberrations

Exposure of CHO cells to IQ in the presence of exogenous metabolic activation induced CA (Lopreino *et al.* 1991, cited in IARC 1993). Exposure of CHO (*uv5*) cells or CHO (AA8) cells to IQ did not induce an increased frequency of CA (Thompson *et al.* 1983, cited in IARC 1993).

Exposure of human peripheral blood lymphocytes to IQ in the presence of exogenous metabolic activation induced CA (Loprieno *et al.* 1991, cited in IARC 1993). However, negative results were obtained in another study using a higher IQ concentration in the presence of exogenous metabolic activation (Aeschbacher and Ruch 1989, cited in IARC 1993).

In other studies, IQ was evaluated for the ability to induce CA in Chinese hamster lung fibroblast (CHL/IU) cells. A significant increase in chromosomal aberrations was observed in cells exposed to IQ in the presence of metabolic activation. With metabolic activation, longer exposure to IQ significantly increased the frequency of aberrant cells only at 10 μg/mL. Only a weak induction of aberrations was observed at the highest concentration tested without metabolic activation (Miura *et al.* 1993). IQ did not induce CA in the CYP-deficient (V79-MZ and V79-NH) and XEMd-MZ cell lines. The results in the XEMd-NH cells were equivocal (Rodrigues *et al.* 1994).

Micronucleus test

IQ induced a small increase in micronucleus formation in human peripheral blood lymphocytes *in vitro* in the presence of exogenous metabolic activation (McManus *et al.* 1988b, cited in IARC 1993).

Exposure of ovine seminal vesicle cells with IQ in the presence of a prostaglandin H synthase metabolic activation system induced micronuclei in a statistically significant concentration-dependent manner (Degen *et al.* 1998).

5.4.1.4 Sister chromatid exchanges (SCEs)

An increased frequency of SCEs was observed in CHO (uv5) cells after exposure to IQ in the presence of exogenous metabolic activation. However, only a weak induction of SCEs was observed in CHO (AA8) cells exposed to IQ in the presence of exogenous metabolic activation (Thompson *et al.* 1983, cited in IARC 1993).

An increased frequency of SCEs was observed in human peripheral blood lymphocytes exposed to IQ in the presence but not in the absence of exogenous metabolic activation (Aeschbacher and Ruch 1989, cited in IARC 1993).

The genetically engineered V79 cell lines V79-MZ (CYP-deficient), V79-NH (CYP-deficient with endogenous acetyl transferase activity), XEMd-MZ (expressing rat CYP 1A2), and XEMd-NH (CYP 1A2 with endogenous acetyltransferase activity) were exposed to IQ at concentrations of 10, 30, and 90 μM in the absence of metabolic activation. SCEs were not increased in any cell line at any concentration tested (Rodrigues *et al.* 1994).

5.4.1.5 DNA damage/repair test

IQ induced DNA single-strand breaks in mouse hepatocytes without exogenous metabolic activation (Hayashi *et al.* 1985, cited in IARC 1993) and in radiation-induced mouse leukemic cells with exogenous metabolic activation (Caderni *et al.* 1983, cited in IARC 1993). IQ also induced DNA single-strand breaks in rat hepatocytes without exogenous metabolic activation (Caderni *et al.* 1983; Holme *et al.* 1987, both cited in IARC 1993).

IQ caused a significant concentration-dependent increase in the incidence of DNA strand breaks and percent tail DNA in cultured T-antigen immortalized human liver epithelial cells tested in the Comet assay (Barcelo *et al.* 1998). Human T5-neo (parental cell line) and T5-1A2 (human cytochrome CYP1A2-positive cell line) cells were exposed to IQ without exogenous metabolic activation. A significant dose-dependent increase of percent tail DNA was observed in the T5-

1A2 cells relative to untreated controls at all IQ concentrations tested. IQ did not induce DNA strand breaks in the T5-neo cells, although there was a slight increase in the percent tail DNA at the highest IQ concentration tested. IQ was not cytotoxic to the T5-neo and T5-1A2 cells.

5.4.1.6 Unscheduled DNA synthesis (UDS)

IQ induced UDS in both Syrian hamster and mouse hepatocytes *in vitro* in the absence of exogenous metabolic activation (Yoshimi *et al.* 1988, cited in IARC 1993). Exposure of primary rat hepatocytes to IQ without exogenous metabolic activation also induced UDS (Barnes and Weisburger 1985; Yoshimi *et al.* 1988, both cited in IARC 1993).

UDS induction was assessed in liver slices of rat, mouse, and human origin. Increased UDS was observed in both mouse and human cultured liver slices at the concentration ranges tested. Induction of UDS was not observed in the cultured rat liver slices (Beamand *et al.* 1998).

5.4.2 In vivo *assays*

5.4.2.1 Mouse spot test

A single intraperitoneal (i.p.) injection of IQ at a dose of 20 mg/kg body weight did not induce mutations in the mouse coat color spot test. A single *in utero* IQ dose of 400 mg/kg maternal body weight also failed to cause mutation in mouse melanocytes (Wild *et al.* 1985, cited in IARC 1993).

5.4.2.2 Host-mediated assay

IQ-induced mutagenicity was observed in two host-mediated assays with mice. In the first assay, a single i.p. dose of 0.198 mg/kg of IQ was administered to intrasanguinous NMRI mouse hosts to *S. typhimurium* strain TA98 (Wild *et al.* 1985, cited in IARC 1993). In the second assay, a single peroral dose of 2.3 mg/kg of IQ was administered to intrasanguinous Swiss albino mouse hosts to *E. coli* strains M343/753 and M343/765 (Knasmüller *et al.* 1992, cited in IARC 1993).

5.4.2.3 Chromosomal aberrations

A single IQ dose of 160 mg/kg administered i.p., did not increase the frequency of CA in the bone marrow cells of mice (Minkler and Carrano 1984, cited in IARC 1993). However, a single IQ dose of 50 mg/kg administered i.p. increased the frequency of CA in rat hepatocytes (Minkler and Carrano 1984, cited in IARC 1993).

5.4.2.4 DNA damage/repair test

Single-strand breaks

An IQ dose of 10 mg/kg (delivered via an unspecified route) induced DNA strand breaks in mouse liver cells (Hayashi *et al.* 1985, cited in IARC 1993).

Male CD-1 mice were given a single i.p. injection of IQ at a dose of 13 mg/kg. The stomach, liver, lung, kidney, brain, spleen, and bone marrow of the animals were examined at various time intervals (0, 1, 3, and 24 hours) after the injection. DNA damage (fragmentation) was observed in the liver, lung, and brain three hours after the injection and in the stomach and kidney 24 hours after the injection. The DNA damage observed in the liver and brain returned to control

levels at 24 hours, but persisted in the lungs. Significant DNA damage could be observed in the stomach and kidney at 24 hours but not at three hours (Sasaki *et al.* 1997).

Micronucleus test

IQ did not induce an increased frequency of micronuclei in mice administered a single i.p. injection at a dose of 594 mg/kg (Wild *et al.* 1985, cited in IARC 1993) or with a single peroral dose of 40 mg/kg (Loprieno *et al.* 1991, cited in IARC 1993).

5.4.2.5 Sister chromatid exchanges (SCEs)

IQ did not increase the frequencies of SCEs in bone marrow cells of mice administered a single i.p. injection dose of 20 mg/kg (Minkler and Carrano 1984, cited in IARC 1993) or in hepatocytes of rats administered a single i.p. injection at a dose of 50 mg/kg bw (Sawada *et al.* 1991, cited in IARC 1993).

5.5 Summary

IQ induces mutations in *S. typhimurium, E. coli*, and meristematic cells of soybean plants. IQ induces mutations and DNA damage in *D. melanogaster* and is both clastogenic and mutagenic in mammalian cells *in vitro* and *in vivo*. Mutations in c-Ha-*ras* and p53 genes were found in some IQ-induced Zymbal gland carcinomas in rats. Mammalian cells rich in cytochrome P-450-1A2 and *N*-acetyltransferase-2 are more susceptible to IQ induction of mutations, SCEs, CA, and DNA breaks, than cells not expressing these proteins.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and elimination (ADME)

6.1.1 Absorption, distribution, and elimination

After oral administration (gavage or dietary) to rats, IQ is rapidly absorbed, primarily from the small intestine, metabolized, and excreted in the urine and feces (Sjödin and Jägerstad 1984, Alldrick and Rowland 1988, and Inamasu *et al.* 1989, all cited in IARC 1993).

Intravenously administered ^{14}C-labeled IQ was widely distributed in male NMRI, pregnant NMRI, and female C3H mice (Bergman 1985, cited in IARC 1993). Although IQ crossed the placental barrier, it did not accumulate or persist in fetal tissues.

6.1.2 Metabolism

Human liver microsomes activate IQ to DNA-reactive metabolites, including *N*-hydroxy-IQ; the isozyme involved has been tentatively identified as CYP1A2 (Butler *et al.* 1989, Shimada *et al.* 1989, and McManus *et al.* 1990, all cited in IARC 1993). In human fetal liver tissue, cytochrome P-450 HFLa is the main enzyme involved in activation of IQ (Kitada *et al.* 1990, cited in IARC 1993). IQ is oxidatively metabolized to *N*-hydroxy-IQ by rat and rabbit hepatic microsomal enzymes (Yamazoe *et al.* 1983, Kato 1986, and McManus *et al.* 1988a, all cited in IARC 1993). DNA-reactive IQ metabolites also were found in human mammary epithelial cells cultured in the presence of IQ (Pfau *et al.* 1992, cited in IARC 1993). IQ also is subject to oxidation via a prostaglandin hydroperoxide-dependent pathway present in microsomes isolated from ram seminal vesicles (Wild and Degen 1987, Petry *et al.* 1989, both cited in IARC 1993). Turesky *et al.* (1991, cited in IARC 1993) reported that cytosols from human liver and colon are able to catalyze the 7-hydroxylation of IQ. The hydroxy-IQ metabolite can be further esterified by *O*-acetyltransferase to *N*-acetoxy-IQ and sulfated by sulfotransferase to IQ-*N*-sulfate (Kato and Yamazoe 1987, cited in IARC 1993; Snyderwine *et al.* 1992a).

Another study reports that the initial step in IQ metabolism can be either *N*-hydroxylation or *N*-acetylation. In studies using monkey kidney COS cells transiently transfected with human CYP1A1, CYP1A2, and *N*-acetyltransferase (NAT)1 or NAT2, hydroxylation is catalyzed by either cytochrome P-450 isozyme (predominantly CYP1A1), while NAT1 or NAT2 carries out the *N*-acetylation reaction (NAT2 > NAT1). *N*-acetylated IQ can undergo *N*-hydroxylation (catalyzed by CYP1A1 or CYP1A2) to IQ-hydroxamic acid. The two pathways converge through *N*,*O*-transacetylation and *O*-acetylation, both of which yield *N*-acetoxy-IQ. It is presumed that the spontaneous degradation of *N*-acetoxy-IQ leads to the formation of an unstable nitrenium ion capable of binding to DNA (Figure 6-1) (Probst *et al.* 1992).

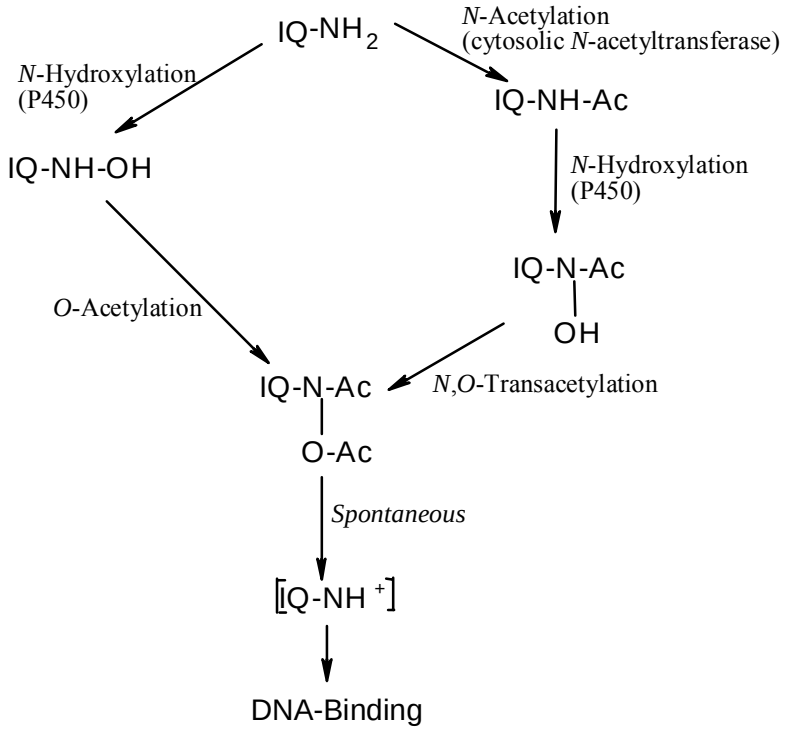

Figure 6-1. Metabolic activation of IQ

Source: Snyderwine *et al.* (1992b, 1995)

A recent study (Liu and Levy 1998) has confirmed the earlier findings that a prostaglandin hydroperoxide-dependent pathway can activate IQ to DNA-binding metabolites (Wild and Degen 1987, Petry *et al.* 1989, both cited in IARC 1993). Figure 6-2 presents the major routes of IQ metabolism in monkeys (Synderwine *et al.* 1992b, 1995).

In addition to *N*-oxidation, metabolites were formed from cytochrome P-450-mediated ring oxidation (at position C-5) and *N*-demethylation. Turesky *et al*. (1993) administered IQ to rats and isolated ring (C-5) oxidation metabolites. The glucuronide of *N*-hydroxy-IQ was detected in the urine of IQ-dosed rats (Turesky *et al.* 1993). *In vitro*, glucuronidation of 7-hydroxy-IQ was mediated by uridine 5′-diphosphoglucuronic acid-dependent glucuronyl transferase isolated from human and rat livers (Kaderlik *et al.* 1994).

Enteric bacterial flora from mice, rats, and humans also may contribute to the conversion of IQ to the directly mutagenic 7-hydroxy derivative (Bashir *et al.* 1987, Rumney *et al.* 1993a). Intestinal bacteria from rats and mice convert IQ to 7-hydroxy-IQ more rapidly than bacteria isolated from human fecal samples. In rats, consumption of diets supplemented with beef drippings increased the rates of enteric-bacteria-mediated conversion of IQ to 7-hydroxy-IQ, relative to low-fat diets (Rumney *et al.* 1993b).

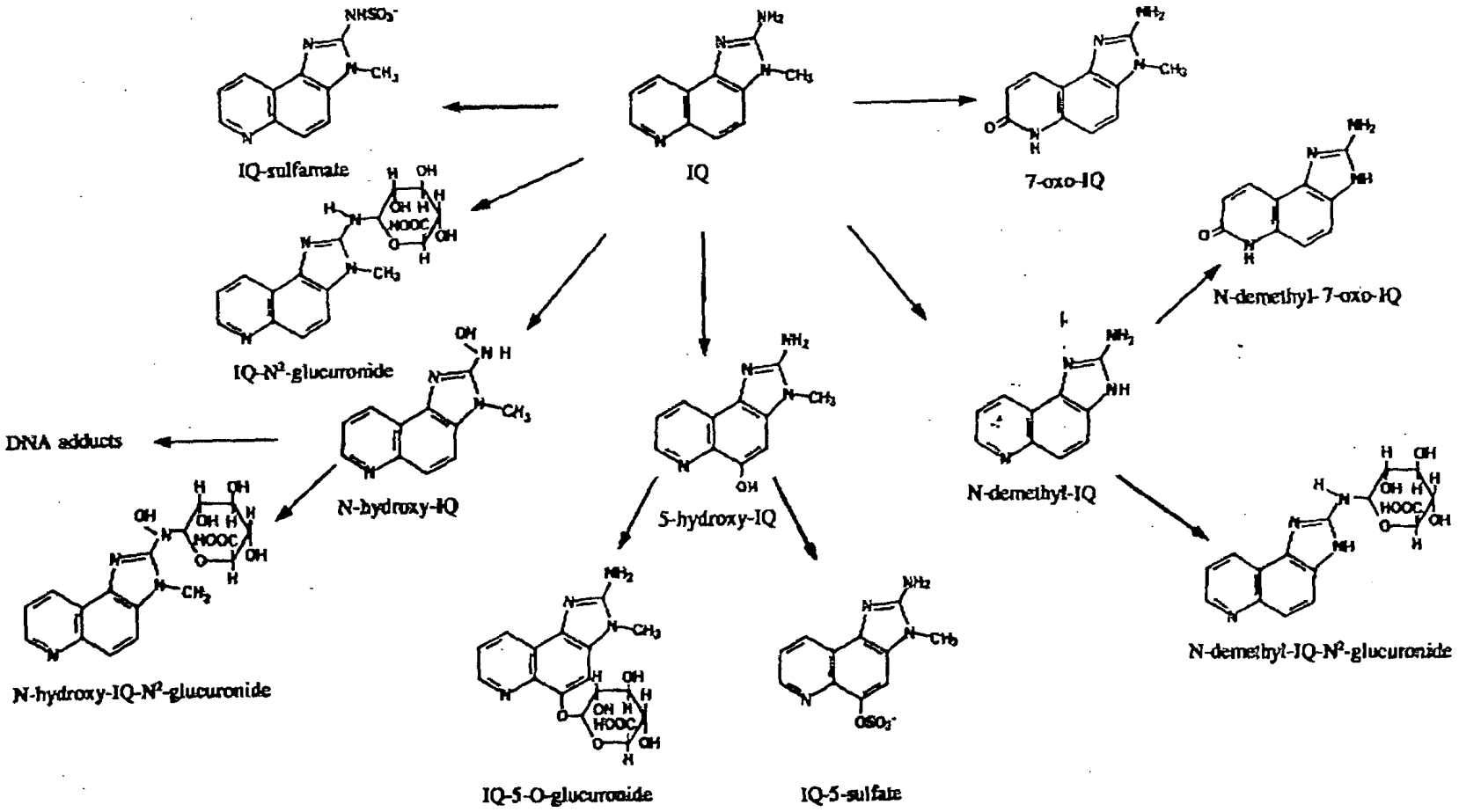

Figure 6-2. Major routes of IQ metabolism in the monkey

Source: Snyderwine *et al.* (1995)

6.2 Formation of DNA adducts

Metabolic activation of IQ via cytochrome P-450-mediated *N*-hydroxylation and subsequent esterification yields two reactive metabolites, *N*-hydroxy-IQ and *N*-acetoxy-IQ (Snyderwine *et al.* 1988a, 1988b, cited in IARC 1993). These reactive metabolites bind to DNA (Figure 6-3) (Snyderwine *et al.* 1988a, cited in IARC 1993). *N*-hydroxy-IQ binds nonenzymatically with polyguanylic acid *in vitro* (pH 7.4) in the presence of polynucleotides (Snyderwine *et al.* 1988a, 1988b, cited in IARC 1993).

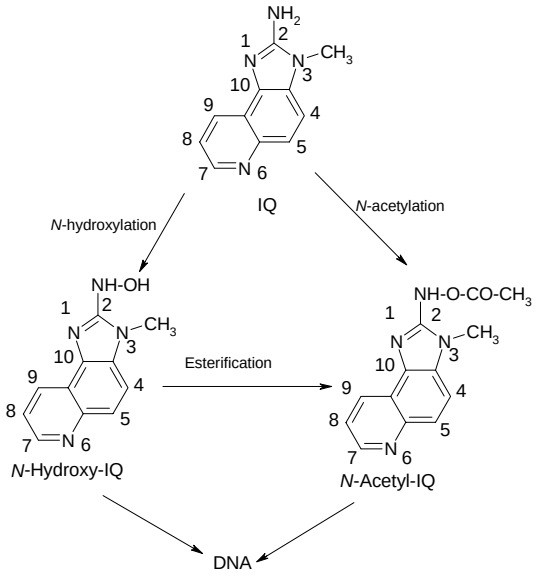

Figure 6-3. Structures of DNA-adduct-forming metabolites of IQ

Source: Snyderwine et al. 1988a, cited in IARC 1993)

IQ-DNA adducts were detected in bacteria exposed to IQ with exogenous metabolic activation by means of the ^{32}P-postlabeling method (Asan *et al.* 1987, cited in IARC 1993).

More recently, DNA adducts formed in *S. typhimurium* strain YG1024 (TA98 pYG219) (which overexpresses acetyltransferases) after treatment with IQ have been detected with the ^{32}P-postlabeling method. Exogenous metabolic activation was provided by prostaglandin H synthase (Probst *et al.* 1992, cited in Degen *et al.* 1998).

The ^{32}P-postlabeling method also has been used to detect IQ-DNA adducts in Syrian hamster embryo cells exposed to IQ in the presence of exogenous metabolic activation (Asan *et al.* 1987, cited in IARC 1993). IQ-DNA adducts also were detected in rat hepatocytes exposed to IQ at a concentration of 10 μg/mL in the absence of exogenous metabolic activation (Dirr *et al.* 1989, Wallin *et al.* 1992, both cited in IARC 1993).

In mammalian *in vivo* systems, the ^{32}P-postlabeling and ^{14}C-labeling methods have been used to detect formation of IQ-DNA adducts in multiple organs and species. Adducts were found in the liver and several other organs of rats following peroral and i.p.

administration of IQ at doses ranging from 5 mg/kg (Zu and Schut 1991a, 1991b, both cited in IARC 1993) to 100 mg/kg (Yamashita *et al.* 1988, cited in IARC 1993), in the liver and heart of rats following dietary administration of IQ at a dose of 36 mg/kg for 4 weeks (Övervik *et al.* 1991, cited in IARC 1993), in the liver and several other organs of mice after peroral and i.p. administration of IQ at doses ranging from 5 mg/kg (Zu and Schut 1991a, cited in IARC 1993) to 40 mg/kg (Loprieno *et al.* 1991, cited in IARC 1993), and in the liver of cynomologus monkeys following 15 peroral administrations of IQ at a dose of 20 mg/kg (Snyderwine *et al.* 1988c, cited in IARC 1993). The major product identified cochromatographs with *N*-(deoxyguanosi-8-yl)-IQ (Schut *et al.* 1991, cited in IARC 1993).

More recently, CDF_1 mice were administered IQ in the diet at a concentration of 0.01% for three weeks or by gavage at single doses of 50 mg/kg of IQ to test for the ability of IQ to form DNA adducts. During the 12 days of monitoring, IQ-DNA adducts formed from dietary IQ, and removal of adducts from the liver, lungs, and stomach was slow, with 40.8% to 64.5% of the day-1 adducts remaining on day 12. In contrast, IQ-DNA adducts formed after single gavage administration of IQ were removed from the liver and stomach much more rapidly; only 10.3% to 14.3% of day-1 adducts remained on day 8. The rate of removal of IQ-DNA adducts from the lungs was independent of the mode of IQ administration. The principal adduct in mammalian species, as detected by the ^{32}P-postlabeling method, is *N*-(deoxyguanosine-8-yl)-IQ (68.9% to 83.4%) (Schut *et al.* 1997a). IQ also forms a minor adduct with the exocylic amino group (N^2) of guanine. Although analysis with the ^{32}P-postlabeling method indicates that IQ forms additional minor adducts, the structures of these adducts have not been elucidated (Schut *et al.* 1997b).

Snyderwine *et al.* (1992b, 1997) reported ten (and identified nine) IQ metabolites in the urine, bile, and feces of monkeys following IQ administration in a cancer bioassay. The derivatives identified for IQ included both primary metabolites and their sulfate or β-glucuronic acid conjugates. Both *N*-hydroxyl-IQ and its glucuronide also have been detected in the urine of IQ-dosed monkeys. *N*-hydroxyl-IQ has been shown to form adducts with DNA in a number of organs, particularly the liver (a carcinogenesis target organ for IQ in monkeys), kidney, and heart (Snyderwine *et al.* 1992a).

IQ-DNA adducts are formed in human mammary epithelial cells incubated with IQ (Pfau *et al.* 1992, cited in IARC 1993). In more recent studies, the ^{32}P-postlabeling method was used to detect IQ-DNA adducts following *in vitro* IQ exposure of human mammary gland epithelial cells (Fan *et al.* 1995) and TK6 human lymphoblastoid cells (Leong-Morganthaler *et al.* 1998). IQ-DNA adducts also have been detected with the ^{32}P-postlabeling method in a variety of tissues in mice following oral (Davis *et al.* 1996; Schut *et al.* 1997a,b) or i.p. exposure (Nerurkar *et al.* 1995), in rats following oral exposure (Davis *et al.* 1994; Schut *et al.* 1994, 1997a; Turesky *et al.* 1996a, 1997; Turesky and Markovic 1995; Xu *et al.* 1996, 1997), and in nonhuman primates following oral exposure (Turesky *et al.* 1996b, 1997).

In a subsequent study, Stone *et al.* (1998) found that DNA adduct levels were consistently higher (>10-fold) in mammary epithelial cells after incubation with *N*-

hydroxy-IQ than after incubation with IQ *per se*. The results of these experiments indicate that *N*-hydroxylation may be the rate-limiting step in the activation of IQ to DNA-adduct-forming products. Since *N*-acetylation appears to be a metabolic step in activation of IQ, it was proposed that the formation of IQ-DNA adducts in human cells is influenced by acetylator phenotype (i.e., rapid or slow acetylation). When human mammary epithelial cells of known acetylator phenotype (NAT2) were incubated with IQ, the mean level of IQ-DNA adducts was higher in cells from rapid acetylator donors than in cells from slow acetylator donors. However, the difference in adduct level was not statistically significant, probably because of the large variability in adduct levels (Stone *et al.* 1998).

6.3 Summary

IQ is rapidly absorbed and distributed in mammalian systems following oral administration. The initial step in IQ metabolism is either *N*-hydroxylation or *N*-acetylation, mediated by cytochrome P-450 isozymes and *N*-acetyltransferase to yield *N*-acetoxy-IQ and *N*-hydroxy-IQ, respectively. These mutagenic metabolites bind covalently to DNA, forming DNA adducts *in vitro* and *in vivo*, inducing base-pair substitution, transversion, transition, frameshift, and deletion/insertion mutations. For the optimal metabolic activation of IQ, mammalian cells require the presence of cytochrome P-450-1A2 and *N*-acetyltransferase-2 to metabolize IQ into genotoxic forms that induce mutations, SCEs, CA, and DNA breaks. Cells not expressing these proteins are less sensitive to IQ genotoxicity/mutagenesis. *N*-acetoxy-IQ also may spontaneously degrade to form an unstable nitrenium ion capable of binding to DNA.

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Appendix A: IARC. (1993). *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines, and Mycotoxins.* Monographs of Evaluation of Carcinogenic Risks to Humans. IQ (2-Amino-3-methylimidazo[4,5-*f*]quinoline). Lyon, France. World Health Organization. Volume 56 pp. 166-195.

IQ (2-AMINO-3-METHYLIMIDAZO[4,5-*f*]QUINOLINE

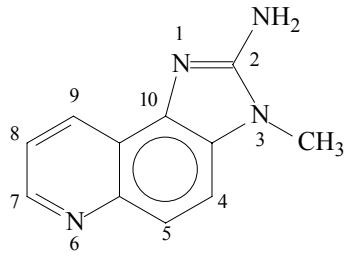
This substance was considered by a previous Working Group, in October 1985 (IARC, 1986a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Services Reg. No.: 76180-96-6
Chem. Abstr. Name: 3-Methyl-3*H*-imidazo[4,5-*f*]quinolin-2-amine
IUPAC Systematic Name: 2-Amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline



$C_{11}H_{10}N_4$ Mol. wt: 198.23

1.1.2 *Chemical and physical properties*

(*a*) *Description*: Crystalline solid (Kasai *et al.*, 1981)
(*b*) *Melting-point*: > 300 °C (Kasai *et al.*, 1981)
(*c*) *Spectroscopy data*: Ultraviolet (Sugimura *et al.*, 1981), proton nuclear magnetic resonance (Kasai *et al.*, 1980a), infrared absorbance (Kasai *et al.*, 1981) and mass spectral data (Spingarn *et al.*, 1980) have been reported.
(*d*) *Solubility*: Soluble in methanol, ethanol and dimethyl sulfoxide (Kasai *et al.*, 1980a, 1981; Lee *et al.*, 1982; Schunk *et al.*, 1984)
(*e*) *Stability*: Stable under moderately acidic and alkaline conditions and in cold dilute aqueous solutions protected from light (Sugimura *et al.*, 1983)
(*f*) *Reactivity*: Rapidly degraded by dilute hypochlorite; not deaminated by weakly acidic nitrite solutions (Tsuda *et al.*, 1985)

1.1.3 Trade names, technical products and impurities

No data were available to the Working Group.

1.1.4 *Analysis*

The complex matrix of cooked foods makes analysis of IQ difficult. IQ was originally isolated from broiled, sun-dried sardines, extracted with methanol and purified by Diaion HP-20 column chromatography, chloroform-methanol-water partitioning and Sephadex LH-20 column chromatography, silica-gel column chromatography and, finally, reverse-phase high-performance liquid chromatography (HPLC). The structure was deduced mainly from data obtained by proton nuclear magnetic resonance and high-resolution mass spectral analysis (Kasai *et al.*, 1980b, 1981).

IQ has been isolated from beef extract by dichloromethane extraction, column chromatography on Adsorbosil-5 and Sephadex LH-20 and HPLC, with analysis by mass spectro-metry, ultraviolet spectrophotometry and/or mutagenesis assay (Hargraves & Pariza, 1983; Turesky *et al.*, 1983). IQ was detected in fried ground beef following dichloromethane extraction, chromatography on XAD-2 resin, three different HPLC separations and off-line mass spectrometry (Felton *et al.*, 1984).

IQ can be adsorbed from aqueous solutions onto cellulose or cotton to which CI Reactive Blue 21, a trisulfo-copper phthalocyanine dye, has been bound covalently (referred to as the 'blue cotton' adsorption technique). The adsorbed IQ is eluted with an ammonia-methanol solution and quantified by HPLC (Hayatsu *et al.*, 1983).

More sophisticated methods of detecting IQ in cooked foods using deuterium-labelled internal standards and HPLC–thermospray–mass spectrometry have been devised (Yamaizumi *et al.*, 1986; Turesky *et al.*, 1988). Although they differ in detail, these methods involve methanol extraction, acid-base partitioning and 'blue cotton' adsorption prior to analysis by HPLC-thermospray-mass spectrometry.

Monoclonal antibodies to IQ have been developed (Vanderlaan *et al.*, 1988), and Yanagisawa *et al.* (1990) showed that their antibodies bound to IQ and related heterocyclic amines with varying specificities. Turesky *et al.* (1989) used monoclonal antibodies immobilized on a support for selective immunoaffinity chromatography as a clean-up procedure in the analysis of beef extracts.

A practical, solid-phase extraction and HPLC (Kieselgur-Sephasorb) method for the analysis of IQ and other heterocyclic amines in foods and food extracts was devised by Gross *et al.* (1989). Improvements to the method (Gross, 1990; Gross & Grüter, 1992) allow determination of IQ and most of the other known heterocyclic amines at a level of 1 ng/g from only 10 g of food sample. Replicate samples and spiking allow accurate determination of extraction losses; chromatographic peak identities are confirmed using a diode array–ultraviolet detector.

1.2 Production and use

1.2.1 *Production*

The isolation and identification of IQ were first reported by Kasai *et al.* (1980b). Its structure was confirmed by chemical synthesis, in which 5,6-diaminoquinoline was reacted

with cyanogen bromide and the resulting cyclic intermediate was converted to IQ by heating the tetramethylammonium salt under reduced pressure. Final purification was accomplished by sublimation, silica-gel column chromatography and crystallization from aqueous methanol (Kasai *et al.*, 1980a, 1981).

Improved synthetic routes were devised by Lee *et al.* (1982), Adolfsson and Olsson (1983) and Waterhouse and Rapoport (1985). Synthesis of ^{14}C-labelled IQ was reported by Adolfsson and Olsson (1983) and of tritium- and deuterium-labelled IQ by Waterhouse and Rapoport (1985).

IQ is produced commercially in small quantities for research purposes.

1.2.2 *Use*

IQ is not used commercially.

1.3 Occurrence

As cooking terms such as broiling and grilling have different meanings in different parts of the world, the authors' terminology has been retained.

IQ is formed during the cooking of various meats and fish. It was originally isolated from broiled fish and has also been quantified in fried ground beef and beef extracts. The amounts found in these and other samples are listed in Table 1. IQ was also detected but not quantified in fried ground pork (Gry *et al.*, 1986), in beef extracts used for bacteriological media and in food-grade beef extracts (Hargraves & Pariza, 1983).

Table 1. Concentrations of IQ in foods

Sample	Concentration (ng/g)	No. of samples	Reference
Sardines, sun-dried, broiled	~ 20	1	Kasai *et al.* (1980b)
	158	1	Sugimura *et al.* (1981)
Ground beef, fried			
240 °C	0.5–20[a]	2	Barnes *et al.*(1983)
250 °C	0.02	1	Felton *et al.* (1984)
275 °C	0.3–1.9	3	Turesky *et al.*
Ground beef, broiled	0.5	1	Yamaizumi *et al.* (1986)
Beef, broiled	0.19	1	Wakabayashi *et al.* (1992)
Beef, extract, food-grade	< 0.2	1	Takahashi *et al* (1985)
	< 0.1–6.2	3	Turesky *et al.* (1989)
Salmon, broiled	0.3–1.8	2	Yamaizumi *et al*. (1986)
Fish, fried at 260 °C	0.16	1	Zhang *et al.* (1988)
Egg, fried at 325 °C	0.1	1	Grose *et al* (1986)

[a]20 ng/g in a high-fat sample, 0.5 ng/g in a low-fat sample

IQ was found in a mixture of creatine and proline heated to 180 °C (Yoshida *et al.*, 1984), in a mixture of glycine, fructose and creatinine heated to 128 °C (Grivas *et al.*, 1986), in dry mixtures of creatinine and phenylalanine or of creatinine, phenylalanine and glucose heated

to 200 °C (Felton & Knize, 1990) and in a dry mixture of serine and creatinine heated to 200 °C (Knize *et al.*, 1988). Quantities of mixtures of heterocyclic amines found in different foods are listed in Table 2.

Table 2. Representative concentrations of heterocyclic amine (ng/g) in various food samples and in cigarette smoke condensate

Sample	PhIP (ng/g)	MeIQx (ng/g)	IQ (ng/g)	MeIQ (ng/g)	Reference
Beef, fried at 190 °C	48.5	8.3	ND[a]	ND[a]	Gross (1990)
Walleye pollack, fried at 260 °C	69.2	6.44	0.16	0.03	Zhang *et al.* (1988)
Ground beef, fried at 250 or 300 °C	15	1.0	< 0.1	Trace	Felton *et al.* (1986)
Chicken, broiled	38.1	2.33	NA	NA	Hayatsu *et al*. (1991)
Ground beef, fried at 250 °C	NA	1.0	0.02	ND	Felton *et al*. (1984)
Salmon, baked at 200 °C for 30′	18	4.6	NA	ND	Gross & Grüter (1992)
Cigarette smoke condensate	NA	ND	0.26 ng per cigarette	ND	Yamashita *et al*. (1986)

ND, not detected by method used; NA, not analysed

[a]Limit of detection 1 and 2 ng/g according to the method used

1.4 Regulations and guidelines

No data were available to the Working Group.

2. Studies of Cancer in Humans

No epidemiological study was available that addressed the carcinogenic risk to humans of IQ itself. Cancer risks associated with consumption of broiled and fried foods, which may contain IQ as well as other heterocyclic amines, have, however, been addressed in a number of case–control studies. Several of these are summarized below. IQ is also a component of tobacco smoke, which has been considered in a previous IARC monograph (IARC, 1986b).

A large number of studies on diet and cancer have been conducted, most of which addressed specific hypotheses (e.g., dietary fat intake and risk of colorectal cancer) (see Tomatis *et al.*, 1990). Many of the investigators, however, either failed to collect data on methods of food processing, or, if they collected such data, did not analyse the findings at all or reported only summary findings. Thus, it is possible that the studies reviewed below are only a small segment of the data potentially available on the effects in humans of heterocyclic amines formed in cooking, and a positive reporting bias cannot be ruled out.

2.1 Cohort studies

A study by Ikeda *et al.* (1983) involved 11 203 subjects already enrolled in the Adult Health Study of the Radiation Effects Research Foundation in Hiroshima and Nagasaki

between 1968 and 1970. Complete personal histories and information on current dietary and other habits were available for 7553 of these subjects (1781 men and 3341 women in Hiroshima; 965 men and 1466 women in Nagasaki). After 11 years of follow-up, there were 244 deaths from cancer, 79 of which were from stomach cancer. Intake of five foods was assessed in a multivariate analysis: the relative risks associated with consumption of broiled fish were 1.7 for gastric cancer and 1.3 for cancers at all sites, after other food variables had been taken into account (both $p < 0.05$).

The report of a large prospective study of 88 751 nurses followed up from 1980, when they answered a mailed questionnaire, to 1986 mentions that no association was found between 150 incident cases of colon cancer and the degree of cooking of red meat, but no data were shown (Willett *et al.*, 1990).

2.2 Case–control studies

2.2.1 *Cancer of the colon and rectum*

A case–control study included 340 colon and rectum cancer patients and 1020 hospital controls enrolled during 1959 from seven hospitals in Kansas City, USA (Higginson, 1966). A food frequency questionnaire was used. Unadjusted proportions of subjects in different categories were presented. No difference was observed between cases and controls with respect to consumption of fried potatoes, fried meats, fried food for breakfast or method of cooking meats. [The Working Group noted that confounding by intake of other nutrients was not controlled.]

Wynder *et al.* (1969) carried out a hospital-based case–control study in two cancer hospitals in Tokyo, Japan, in which 157 cases of colonic or rectal cancer and 307 sex- and age-matched controls were interviewed concerning their usual adult intake of a number of foods, including fried foods and charcoal-broiled fish. No consistent difference was found. [The Working Group noted that confounding by intake of other nutrients and method of cooking was not controlled.]

Phillips (1975) interviewed 41 Seventh-day Adventists with colonic–rectal cancer and 77 with breast cancer discharged from two Adventist-operated hospitals in 1969–73, each matched by age, sex and race with three Adventist controls: two hospitalized for hernia or osteoarthritis and the third from the general population [details not given]. Consumption of beef, meat in general and several sources of saturated fat were positively associated with the incidence of colonic but not breast cancer. Fried foods were positively associated with cancers at each site; the association with fried potatoes was statistically significant at the 5% level (odds ratio, 2.7 for colonic and 2.4 for breast cancer). [The Working Group noted that only a crude, unmatched analysis was presented and that confounding by intake of other nutrients and method of cooking was not controlled.]

Young and Wolf (1988) reported a case–control study of 353 cases of colonic cancer (152 proximal and 201 distal subsites) in people aged 50–89, drawn from the Wisconsin (USA) Cancer Reporting System, and 618 general population controls. The study was population-based, but 62.2% of the potential study subjects were alive and could be contacted and interviewed; 17.7% refused to participate, 13 patients were < 50 years old, thus leaving 353 cases for the study. Information on the frequency of consumption of foods

in 27 groups and replies to questions about eating habits and cooking styles were requested for three periods: before the age of 18, between 18 and 35 years of age and after the age of 35. Consumption of broiled foods increased markedly with age and was consistently lower among cases than controls; on the contrary, the frequency of consumption of pan-fried foods decreased with age but was higher among cases. Consumption of processed meat and pan-fried foods was consistently associated with increased risks for cancers at each site. A significant risk for the upper versus the lowest quartile of consumption of pan-fried foods was observed for cancer of the proximal colon in association with diet in young adults (18–35 years old) (odds ratio, 1.79; 95% confidence interval [CI], 1.15–2.80, adjusted for age and sex). The overall association (for both proximal and distal subsites) was not, however, significant. [The Working Group noted that confounding by intake of other nutrients was not controlled.]

Peters *et al.* (1989) reported a study of 147 men with colorectal carcinomas among 232 eligible cases, all of whom were aged 24–44 years at diagnosis and were identified through the Los Angeles County (USA) Cancer Surveillance Program, and 147 neighbourhood controls who were compared in terms of occupational exposure, tobacco and alcohol use and usual consumption of foods grouped into a few broad categories. There was no significant difference between cases who were interviewed and those not interviewed with regard to marital status, religion, birth place, social class or subsite. Elevated risks for tumours located in the right (ascending) side of the colon were associated with heavy consumption (five or more times per week) of deep-fried foods (odds ratio, 3.9; 95% CI, 1.4–10.7), fried bacon or ham (2.6; 0.9–7.9) and barbecued or smoked meats (2.9; 1.2–7.3). The only item associated with risk for rectal cancer was deep-fried food (4.3; 1.5–12.1). These findings did not change after control for physical activity, body mass and occupational exposure to dust or fumes in a multivariate analysis. [The Working Group noted that it was not clear whether each food was adjusted for the others.]

Lyon and Mahoney (1988) identified all histologically confirmed cases of adenocarcinoma of the colon occurring in the population covered by the Utah (USA) Cancer Registry from 1976 to 1978 and aged 40–74 at diagnosis. Out of 348 eligible cases, 246 (71%) were interviewed. Controls were chosen by random-digit dialling: 484 subjects were interviewed out of 560 eligible people identified through a census of all adult householders from 92% of all residential telephone numbers selected at random. The food frequency questionnaire, which focused on diet five years before the interview, included questions on the method of food preparation, from which a score was derived for the frequency of consumption of broiled (including barbecued) foods and deep-fat and/or pan-fried foods. A slight increase in risk was seen with increasing level of ingestion of fried meats, which, however, was present only in women and was no longer significant after adjustment for total caloric intake. The age- and calorie-adjusted odds ratios for the upper tertile versus the lower tertile of intake were 1.3 (95% CI, 0.8–2.1) for women and 1.2 (0.8–1.9) for men. No association was found with eating broiled meat.

In a population-based case–control study of colorectal adenocarcinoma conducted in Stockholm, Sweden, in 1986–88 (Gerhardsson de Verdier *et al.*, 1990a,b, 1991), fairly high participation rates were obtained for both cases (559 interviewed; 78% of incident cases) and controls (505 interviewed; 81% of an age- and sex-stratified random sample of the resident population). Diet was investigated by replies to a self-administered food frequency

questionnaire, supplemented, when necessary, by interview by a nurse's aide for cases and by telephone for controls. The usual portion size was estimated from a photograph to be small, moderate or large. The overall results of the study showed that protein, fat intake and body mass index increased risk, while dietary fibre and physical activity decreased risk—each factor having different effects on cancers at different colorectal subsites. The questions regarding meat (Gerhardsson de Verdier *et al.*, 1991) focused on bacon/smoked ham, beef/pork and sausages; for beef/pork and sausages, separate questions were asked foreach of three cooking methods: fried, oven-roasted and boiled. A significantly increased risk, systematically higher for rectal than for colonic cancer, was observed for high consumption of each type of meat; the odds ratios were higher, however, for eating boiled meat than for fried or roasted meat. The risk associated with consumption of fried or roasted meat disappeared after adjustment for protein. Respondents who ever ate fried meat were also asked about browning of the meat surface during the previous five and previous 20 years, with three response alternatives: preference for meat with a light, moderately or heavily browned surface. They were also asked if they preferred fried meat to be prepared by high- or low-temperature frying. The risks for both colonic and rectal cancer were significantly higher for frequent consumption (more than once a week) of brown gravy (odds ratio, 1.8 for colon and 2.1 for rectum), for preference for heavily browned meat surface, especially in the past five years (odds ratios, 2.3 and 3.7) and for high-temperature frying (odds ratios, 1.9 and 1.6). The association was slightly reduced when total fat was adjusted for and when browned meat was adjusted for high frying temperature and vice versa, indicating that these questions may partly reflect the same exposure. The adjusted risk estimates nevertheless remained elevated and statistically significant. Adjustment for other potential confounding factors, namely total energy, dietary fibre, body mass and physical activity, had little or no effect on the magnitude of the association. The authors stated that restriction of the analysis to patients without gastrointestinal symptoms at diagnosis or to patients who had not required help in filling in the questionnaire did not alter the overall findings. People with a preference for heavily browned meat surface and with high consumption of fried meat and brown gravy had higher risks than people with a preference for moderately browned meat and low consumption of fried meat and brown gravy. Fat intake but not protein was adjusted for in the analysis. [The Working Group noted that the finding of a higher risk with boiled than with other methods of cooking meat makes these results difficult to interpret with respect to the carcinogenicity of heterocyclic amines formed in cooking.]

Schiffman *et al.* (1989) and Schiffman and Felton (1990) reported the results of a case–control study of adenocarcinoma of the colon and rectum, diet and faecal mutagenicity carried out in three hospitals in Washington DC, USA, which was based on 50 cases diagnosed in 1985–87 and 96 age- and sex-matched surgical controls. The response rates were about 30%. Virtually all subjects reported frequent consumption of cooked meats, but cases were more likely than controls to report that they usually ate their red meat well done (medium to medium-well done, odds ratio, 0.9; 95% CI, 0.4–2.5; well done, odds ratio, 3.5; 95% CI, 1.3–9.6; compared with rare to medium rare). [The Working Group noted that only a crude analysis was reported and that details on how information was collected was not provided.]

2.2.2 Other sites

In a population-based case–control study carried out in Stockholm, Sweden, in 1985–87 (Steineck *et al.*, 1990), information was collected from 323 patients (78% response rate) with urothelial cancer and/or squamous-cell carcinoma of the lower urinary tract (94% bladder cancers) and from 392 subjects (77% response rate) randomly sampled within strata of gender and year of birth. Diet was investigated on the basis of replies to a mailed food frequency questionnaire supplemented by a telephone interview. Usual portion size was estimated from a photograph to be small, moderate or large. Information on intake of fried meat was obtained by asking questions about consumption of fried and oven-cooked meat/pork/sausages, smoked ham and bacon. An increased odds ratio was seen for eating fried eggs (1.8; 95% CI, 1.0–3.1, for weekly versus less often), gravy (1.6; 1.0–2.4) and fried potatoes (1.6; 1.1–2.6); increased odds ratios were also suggested to be associated with consumption of fried meat and grilled foods, but not fried fish. Collating the data on fried eggs, fried potatoes, fried meat and gravy in a single variable and adjusting for age, gender, smoking habits and average daily intake of fat gave an odds ratio for moderate intake (exposed to two or three of the fried foods) of 1.7 (95% CI, 0.9–3.0) and an odds ratio for high intake (exposed to all four fried foods) of 2.2 (1.2–4.1).

In the study from Kansas City, described in detail above, a series of 93 gastric cancer patients were also compared to 279 controls (Higginson, 1966). Cases reported slightly greater consumption of fried potatoes, fried meats and fried food for breakfast, but the difference was not significant. No difference was suggested according to the method of cooking meat.

Norell *et al.* (1986) carried out a case–control study in Sweden on dietary habits and tobacco smoking among 99 cases of pancreatic cancer (out of 120 eligible patients) and 138 population controls (out of 162 eligible subjects). As an additional control group, 163 patients hospitalized for inguinal hernia were interviewed, out of 179 eligible subjects. Cases were drawn from all patients diagnosed in 1982–84 at the three surgical departments in Stockholm and Uppsala where suspected pancreatic cancer cases are referred. Frequent consumption of a number of meat items was associated with an increased risk, whatever control group was used for comparison, but most of the effect was confined to fried/grilled meat. Subjects who ate meat at least twice a week and grilled/fried meat at least twice a week showed an odds ratio of 2.5 (95% CI, 1.2–5.3; population controls) in comparison with subjects who ate meat less often. Eating meat at least twice a week but grilled/fried meat less often was not associated with increased risk. Controlling for tobacco smoking and a number of other food items associated with the risk of pancreatic cancer was stated to increase slightly the association with fried/grilled meat, but data were not shown. [The Working Group noted that adjustment for nutrients was not attempted.]

A case–control study from Ankara, Turkey, included 100 cases of adenocarcinoma of the stomach, enrolled during 1987–88 from seven hospitals, 61 controls from the same hospitals with no cancer and 39 healthy controls (Demirer *et al.*, 1990). Cases and controls did not differ in their consumption of fried potatoes, fried meat or fried fish.

Kono *et al.* (1988) conducted a study in a low-risk area for stomach cancer in rural northern Kyushu, Japan. Between 1979 and 1982, 139 newly diagnosed cases of gastric

cancer (85% histologically confirmed) were identified among 4729 subjects who had visited a referral centre in the area for the diagnosis of gastrointestinal diseases. Cases were compared with two sets of controls: 2574 hospital controls free of gastrointestinal disease and 278 general population controls who were similar to the cases by sex, year of birth and residence. Two different groups of people, using a standard questionnaire, interviewed patients before diagnostic procedures at the referral centre and the general population about dietary habits in the year preceding the interview or before a change in dietary habits. Consumption of broiled fish or grilled meat was not associated with an increased risk.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

A group of 40 male and 40 female CDF_1 mice [(BALB/cAnN × DBA/2N)F_1], seven weeks of age, were fed a pelleted diet containing 300 mg/kg IQ (purity, > 99.6%) for 675 days, at which time the experiment was terminated. A group of 40 males and 40 females fed basal diet alone served as controls. The numbers of survivors on day 394, when leukaemia was found in a female control, were similar in the four groups: 39/40 treated males, 36/40 treated females, 33/40 control males and 38/40 control females. The number of mice with liver tumours (hepatocellular adenomas and hepatocellular carcinomas) was significantly higher in treated groups than in controls: 16/39 versus 2/33 in males; 27/36 versus 0/38 in females; hepatocellular carcinomas occurred in eight treated males and 22 treated females. One male and three female controls developed haemangioendotheliomas. The incidences of tumours of the lung and forestomach were also significantly higher in treated mice than in controls: combined incidences of adenoma and adenocarcinoma of the lung, 27/39 *versus* 7/33 in males and 15/36 versus 7/38 in females; incidences of adenocarcinoma of the lung, 14/39 versus 3/33 in males and 8/36 versus 4/38 in females; combined incidences of papilloma and squamous-cell carcinoma of the forestomach, 16/39 versus 1/33 in males and 11/36 versus 0/38 in females; and incidences of squamous-cell carcinoma of the forestomach, 5/39 versus 0/33 in males and 3/36 versus 0/38 in females (Ohgaki *et al.*, 1984, 1986).

Groups of 10 or more female CDF_1 mice, 27–31 days old, were treated with IQ (purity, > 98%; dissolved in 55% ethanol in 0.9% sodium chloride solution) at 200 or 400 mg/kg bw (one-half of the LD_{50}) by gavage twice at a four-day interval. The numbers of aberrant crypts in the colon scored 21 days after the first IQ treatment were dose-related. Crypts were found more frequently in the caecal end (Tudek *et al.*, 1989).

3.1.2 *Rat*

A group of 32 female Sprague-Dawley rats, six weeks of age, received IQ hydrochloride at 0.35 mmol [70 mg]/kg bw in 5% Emulphor by gavage three times per week during weeks 1–4, twice per week during weeks 5–8 and weekly during weeks 9–31 and were maintained without further treatment until sacrifice at week 52. A group of 27 rats received 0.25 mL 5% Emulphor according to the same schedule, and a further group of nine animals served as

untreated controls. Treated rats showed a 94% weight gain by the end of the experiment compared with controls. Twenty-one adenocarcinomas of the mammary gland were observed in 14/32 ($p < 0.05$) treated animals. No such tumour was observed in controls. Liver tumours were observed in 6/32 (three neoplastic nodules, two hepatocellular carcinomas and two haemangioendotheliomas) treated animals, but in none of the controls. Twelve squamous-cell carcinomas of the Zymbal gland were found in 11/32 treated animals, and no such tumour occurred in controls [$p = 0.002$; Fisher's exact test]. The treated group also had altered liver-cell foci (17/32), atypical hyperplastic acinar-cell lesions in the pancreas (19/32) and altered proliferative foci in the adrenal cortex (5/32), none of which was present in the control group (Tanaka *et al.*, 1985).

Groups of 40 male and 40 female Fischer 344 rats, eight weeks of age, were fed a pelleted diet containing 300 mg/kg IQ (purity confirmed by HPLC [percentage not indicated]) for 104 weeks. Control groups of 50 males and 50 females were fed basal diet alone. The times of appearance of the first tumours in males were day 255 in the colon, day 239 in the small intestine and day 288 in the liver. Twenty males and four females from the treated group [but no control] were killed at 300 days, because the animals were moribund owing to the occurrence of tumours. Treated animals had a significantly increased incidence of tumours of the liver, Zymbal gland, colon and small intestine during the 104-week study (Table 3). No such tumour, except one hepatocellular carcinoma in a male, occurred in concurrent controls (Takayama *et al.*, 1984; Ohgaki *et al.*, 1986).

Table 3. Tumour incidence in Fischer rats fed a diet containing IQ at 300 mg/kg for 104 weeks

Tumour type	Males	Females
Squamous-cell carcinoma, Zymbal gland	36/40	27/40
Adenocarcinomas, colon	25/40	9/40
Adenocarcinomas, small intestine	12/40	1/40
Hepatocellular carcinoma	27/40	18/40
Carcinoma, skin	17/40	3/40
Squamous-cell carcinoma, oral cavity	2/40	1/40
Squamous-cell carcinoma, clitoral gland	-	20/40

From Ohgaki *et al.* (1986)

Groups of 10 or more female Sprague-Dawley rats, 21 days old, were treated with IQ (purity, > 98%; dissolved in 55% ethanol in 0.9% sodium chloride solution) at 200 or 400 mg/kg bw (one-half of the LD_{50}) by gavage twice at a four-day interval. The numbers of aberrant crypts in the colon scored 21 days after the first IQ treatment were dose-related. Crypts were found more frequently in the caecal end (Tudek *et al.*, 1989).

3.1.3 *Monkey*

Twenty cynomolgus monkeys (*Macaca fascicularis*) (14 males, six females), about one year old, were administered 10 mg/kg bw IQ (purity, > 99.9%) suspended in hydroxypropyl cellulose by gavage five times a week for up to 60 months. Another 20 monkeys (8 males,

12 females) were administered IQ by gavage five times a week at a dose of 20 mg/kg bw. Hepatocellular carcinomas were found in 13 monkeys which were necropsied; 10 had received 20 mg/kg (average latent period, 37 months) and three, 10 mg/kg (first tumour seen after 30 months; average latent period, 45 months). Metastases to the lung occurred in several monkeys. No such tumour occurred in colony controls (Adamson *et al.*, 1990, 1991).

3.2 Intraperitoneal administration

Mouse

Groups [initial number unspecified] of newborn male B6C3F$_1$ mice were injected intraperitoneally with IQ (> 98% pure) at total doses of 0, 0.625 or 1.25 μmol [125–250 mg] (maximal tolerated dose) dissolved in 5, 10 or 20 mL dimethyl sulfoxide and administered on days 1, 8 and 15 after birth, respectively. Animals were sacrificed at 8 and 12 months. The incidence of hepatocellular adenomas was significantly higher in treated mice than in controls: at eight months, 1/44 in controls, 5/24 at the low dose and 5/16 at the high dose; at 12 months, 5/44 in controls, 7/19 at the low dose and 14/20 at the high dose. Two hepatocellular carcinomas were found in the high-dose group at 12 months (Dooley *et al.*, 1992).

3.3 Administration with known carcinogens

3.2.1 *Mouse*

In a two-stage skin carcinogenesis study, a group of 20 female CD-1 mice, seven weeks of age, received topical applications on the dorsal skin of 0.75 mg IQ in 0.1 mL dimethyl sulfoxide twice weekly for five weeks, followed one week later by topical applications of 2.5 μg 12-*O*-etradecanoylphorbol 13-acetate (TPA) twice weekly for 47 weeks. A positive control group received applications of 7,12-dimethylbenz[*a*]anthracene (DMBA; total dose, 100 μg) plus TPA. Skin tumours were found in 0/20 mice treated with IQ, 1/20 mice treated with IQ and TPA, 4/19 mice treated with DMBA, 17/18 treated with DMBA and TPA and 0/20 solvent controls (Sato *et al.*, 1987).

3.2.2 *Rat*

Groups of 40 male Wistar rats, six weeks old, were given IQ at 10 mg/kg bw or solvent (water, acidified to pH 3.5 with citric acid) by gavage every day for two weeks. One week later, the rats were divided into two groups and received either no additional treatment or 500 ppm [mg/L] phenobarbital sodium in the drinking-water for the remainder of the study. Ten animals from each group were sacrificed at week 42, and the study was terminated after 58 weeks. Zymbal gland carcinomas were found in 2/40 rats that received IQ only and in 2/40 that received IQ plus phenobarbital but not in the untreated group or in the group that received phenobarbital only. A hepatocellular adenoma was found in one rat given IQ alone, and a hepatocellular adenoma and a tumour diagnosed as a cystic cholangiocarcinoma occurred in one rat in the group treated with IQ plus phenobarbital. Treatment with IQ and phenobarbital significantly increased ($p < 0.01$) the number of γ-glutamyl transpeptidase-

positive foci of altered hepatocytes in comparison with the respective controls; some such foci were also found after administration of phenobarbital alone (Kristiansen *et al.*, 1989).

In a short-term assay for tumour-initiating activity in the liver, groups of 20 male Fischer 344 rats, five weeks of age, each received a two-week dietary treatment with IQ at doses of 0.025, 0.05 and 0.1% (250, 500 and 1000 mg/kg of diet) and were then maintained on a diet supplemented with either 500 mg/kg phenobarbital or 24 mg/kg 3′-methyl-4-dimethyl-aminoazobenzene (3′-Me-DAB) from week 3 until final sacrifice at week 86. One group received only 0.1% IQ (1000 mg/kg of diet). All animals underwent a two-thirds partial hepatectomy at week 1. There were 14–19 effective animals per group, including both animals that survived to the end of the experiment and those that died with tumours after week 52. Administration of IQ alone or IQ at 0.025–0.1% with either phenobarbital or 3′-Me-DAB caused hyperplastic liver nodules in all animals; and subsequent administration of phenobarbital or 3′-Me-DAB caused a significant increase in the incidence of hepatocellular carcinomas: IQ alone, 0/19; 3′-Me-DAB with 0, 0.025, 0.05 and 0.1% IQ, 0/18, 1/17, 3/18 and 5/16; phenobarbital with 0, 0.025, 0.05 and 0.1% IQ, 0/18, 6/17, 7/18 and 7/14. Administration of IQ plus phenobarbital increased the incidence of thyroid adenomas and carcinomas: IQ alone, 1/19; phenobarbital plus 0, 0.025, 0.05 and 0.1% IQ, 2/18, 8/17, 8/18 and 9/14. IQ alone caused squamous-cell carcinomas or keratoacanthomas of the Zymbal gland (5/19; 26%), but the only effect of subsequent treatment with phenobarbital or 3′-Me-DAB was with 0.1% IQ plus phenobarbital (6/14; 43%). Animals that received IQ, phenobarbital or 3′-Me-DAB alone or in combination had preputial gland tumours at incidences ranging from 22 to 50%, but there did not appear to be a dose-response relationship (Tsuda *et al.*, 1988).

In a short-term assay for tumour-initiating activity in the liver, a group of 10 male Fischer 344 rats, five weeks of age, each received a single intragastric dose of 80 mg/kg bw IQ and, two weeks later, were fed a diet containing 0.05% phenobarbital for six weeks. Rats received a two-thirds partial hepatectomy three weeks after the IQ treatment. The number and total area of foci of phenotypically altered hepatocytes in the liver were scored using expression of placental-form glutathione *S*-transferase (GST-P) as the marker. Treated rats had a significant, two-fold greater number of foci than five vehicle-treated control rats; IQ without subsequent phenobarbital treatment did not induce a significant increase in the number of foci in five rats. Another group of 10 rats received a two-thirds partial hepatectomy 12 h before IQ treatment, two weeks later was fed the diet containing phenobarbital as above, and then received a single intraperitoneal injection of 300 mg/kg D-galactosamine one week after the phenobarbital treatment. Administration of IQ increased the number and area of foci more than 10 fold over that in five vehicle-treated control rats. IQ without subsequent phenobarbital treatment, but with galactosamine, produced a smaller, nonsignificant increase in the number and area of foci. These results suggest that IQ has tumour-initiating activity in rat liver, especially if combined with partial hepatectomy (Tsuda *et al.*, 1990).As part of a medium-term carcinogenicity study on the synergistic effects of five hetero-cyclic amines, groups of 13–15 male Fischer 344 rats, six weeks of age, each received a single intraperitoneal injection of 200 mg/kg bw *N*-nitrosodiethylamine and, two weeks later, were fed a diet containing IQ at 12, 60 or 300 ppm (mg/kg). A two-thirds partial hepatectomy was performed in week 3 of the experiment; all animals were killed after eight weeks. Fifteen rats

treated only with the nitrosamine served as controls. The effects were assessed by counting the numbers of GST-P-positive foci in the liver. IQ alone at the mid- and high-dose levels significantly increased the area of GST-P-positive foci (Ito *et al.*, 1991).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

The toxicology and metabolism of heterocyclic aromatic amines have been reviewed (Övervik & Gustafsson, 1990; Aeschbacher & Turesky, 1991).

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The absorption and excretion of [^{14}C]- and [^{3}H]IQ have been studied by fluorescence in rats and mice following gavage (Sjödin & Jägerstad, 1984; Alldrick & Rowland, 1988; Inamasu *et al.*, 1989) and that of IQ after intraperitoneal administration (Størmer *et al.*, 1987). IQ was absorbed rapidly, mainly from the small intestine (Alldrick & Rowland, 1988), metabolized and excreted almost quantitatively within three days; in rats, 36-49% and 46–68% of the administered dose was recovered in the urine and faeces, respectively (Sjödin & Jägerstad, 1984). The excretion pathways were essentially similar following dietary exposure (Inamasu *et al.*, 1989).

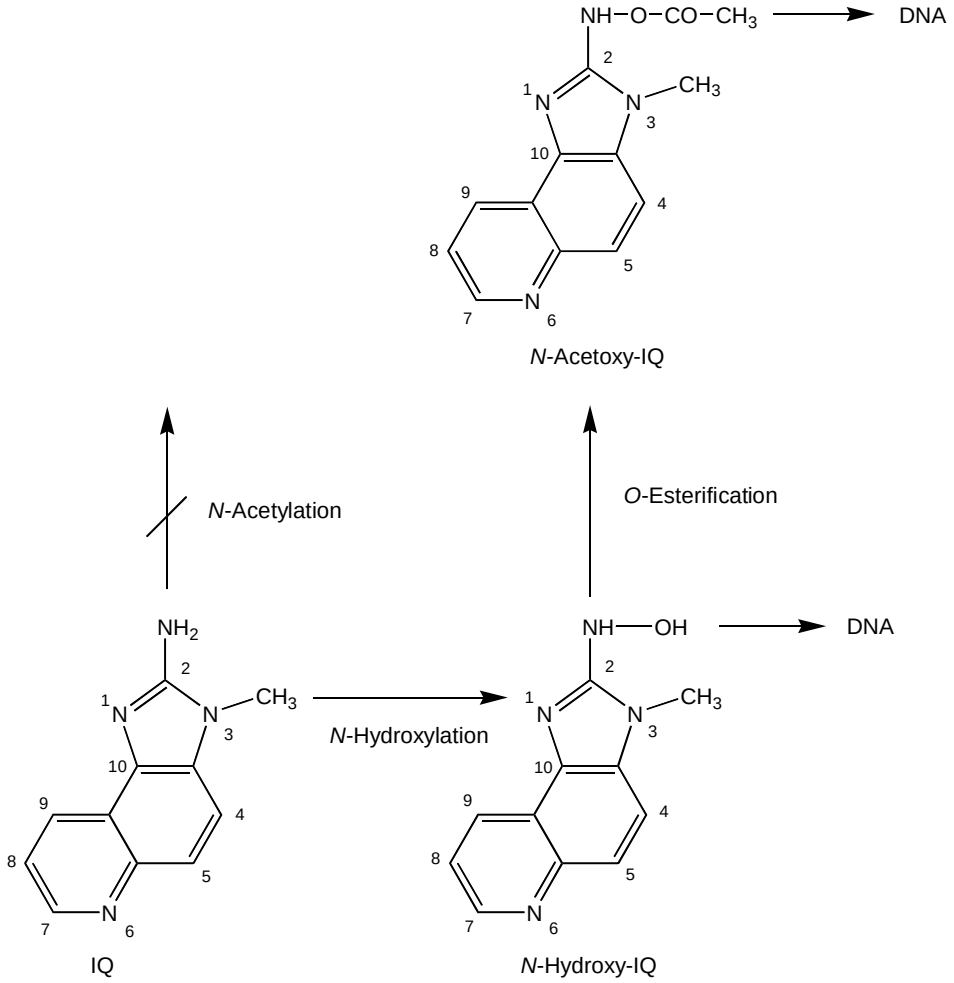
The fate of intravenously injected [^{14}C]IQ was studied in male NMRI, pregnant NMRI and female C3H mice. Whole-body autoradiograms were characterized by an accumulation of radiolabel in metabolic and excretory organs (liver, kidney, bile, urine, gastric and intestinal contents, salivary glands, nasal mucosa and Harder's gland) and in lymphomyeloid tissues (bone marrow, thymus, spleen and lymph nodes) and endocrine and reproductive tissues (adrenal medulla, pancreatic islets, thyroid, hypophysis, testis, epididymis, seminal vesicles, ampulla and prostate). The liver and kidney cortex were identified as sites of retention of nonextractable radiolabel. IQ crossed the placenta, but no radiolabel was retained in fetal tissues (Bergman, 1985).

IQ was oxidized to *N*-hydroxy-IQ in the presence of rat and rabbit liver microsomal homogenates (Yamazoe *et al.*, 1983; Kato, 1986; McManus *et al.*, 1988a). The activity of cytochrome P450 IA2 isozyme in the liver was induced in rats by prior intraperitoneal injection of IQ and other heterocyclic amines (Degawa *et al.*, 1989); the activity of this enzyme was induced in cultured rat hepatocytes by β-naphthoflavone and polychlorinated biphenyls (Wallin *et al.*, 1992). IQ can also be oxidized via a prostaglandin hydroperoxidase- dependent pathway, as shown in microsomes isolated from ram seminal vesicles (Wild & Degen, 1987; Petry *et al.*, 1989).

N-Hydroxy-IQ can be esterified by *O*-acetyltransferase, sulfotransferase and prolyl-tRNA synthetase but at much lower rates than aromatic amines (Kato & Yamazoe, 1987).

N-Acetylation of IQ may not be important for DNA binding (Fig. 1; Snyderwine *et al.*, 1988a). Human liver microsomes could activate IQ into a DNA-reactive species. The isozyme involved was tentatively identified as CYP IA2 (P450 IA2) (Shimada *et al.*, 1989). The same enzyme was shown to be responsible for the formation of *N*-hydroxy-IQ in human hepatic cytosols (Butler *et al.*, 1989; McManus *et al.*, 1990). Human liver and colon cytosols catalysed the formation of *N*-hydroxylated IQ into a DNA-binding form, but *N*-acetylation was not observed under the same conditions (Turesky *et al.*, 1991). DNA-binding products were also found in human mammary epithelial cells cultured in the presence of IQ (Pfau *et al.*, 1992). In human fetal liver tissue, cytochrome P450 HFLa was the main activating enzyme of IQ (Kitada *et al.*, 1990).

Fig. 1. Schematic pathway of activation of IQ to DNA-binding products



Adapted from Snyderwine *et al.* (1988a)

IQ binds to rat haemoglobin and albumin *in vivo*. One of the haemoglobin products was identified as a sulfinamide at a cysteine residue (Turesky *et al.*, 1987).

Mixed and pure cultures of human intestinal anaerobic bacteria metabolized IQ to IQ-7-one (Carman *et al.*, 1988). In rats, the routes of detoxication of IQ include cytochrome

P450-mediated ring hydroxylation at the C5 position, followed by conjugation to a sulfate or glucuronic acid (Luks *et al.*, 1989; Vavrek *et al.*, 1989). Another pathway involves conjugation of the exocyclic amine group to a glucuronic acid or sulfate (Inamasu *et al.*, 1989). Conjugated 5-hydroxy-IQ accounted for about 40% of urinary and biliary metabolites in rats; *N*-sulfamates are another major group of excretion products (Inamasu *et al.*, 1989; Turesky *et al.*, 1986; Luks *et al.*, 1989). In the urine of monkeys, sulfate and glucuronide conjugates predominated (Snyderwine *et al.*, 1991). Treatment of rats with polychlorinated biphenyls increased the excretion of sulfate conjugates into the urine (Vavrek *et al.*, 1989).

Constituents of feed may play a role in the metabolism of IQ. Dietary fibres can bind IQ *in vitro* (Sjödin *et al.*, 1985), and a high-fat diet increased the capacity of rat liver microsomes to activate IQ (Alldrick *et al.*, 1987).

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental toxicity

No data were available to the Working Group.

4.4 Genetic and related effects

The genetic effects of IQ have been reviewed (Sugimura, 1985; Hatch, 1986; de Meester, 1989; Sugimura *et al.*, 1989).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 4 and Appendices 1 and 2)

N-Hydroxy-IQ binds nonenzymatically to DNA *in vitro* at pH 7.4. In the presence of polynucleotides, the *N*-hydroxy-IQ was bound particularly extensively with polyguanylic acid (Snyderwine *et al.*, 1988a,b). *N*-Hydroxy-IQ reacts with DNA to form up to five adducts; the major one co-chromatographs with *N*-(deoxyguanosin-8-yl)-IQ (Schut *et al.*, 1991).

IQ induced prophage, SOS repair and mutation in bacteria. Bacterial mutations were also induced in the intrasanguinous mouse host-mediated assay and following exposure to the urine of IQ-dosed rats.

IQ induced somatic and sex-linked recessive lethal mutations in *Drosophila melanogaster*. It formed DNA adducts and DNA strand breaks in cultured mammalian cells and induced unscheduled DNA synthesis in primary hepatocytes cultured from mice, rats and Syrian hamsters, but not in those from guinea-pigs. Responses in other cultured mammalian cell assays were complex. IQ induced gene mutation to diphtheria toxin resistance and at the *Hprt* locus; mutations at the *Hprt* locus were observed only in single studies in repair-deficient cell lines and in a repair-proficient cell line co-cultured with hepatocytes

Table 4. Genetic and related effects of IQ

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, *Escherichia coli* K12, prophage λ induction	0	+	1.0000	Nagao *et al.* (1983a)
PRB, *Salmonella typhimurium* TA1535, SOS repair	0	+	0.0300	Nakamura *et al.* (1987)
PRB, *Salmonella typhimurium*, SOS repair, with human microsomes	0	+	2.0000	Kitada *et al.* (1990)
PRB, *umu* expression, *Salmonella typhimurium* TA1535/pSK 1002	0	+	2.0000	Shimada *et al.* (1989)
ERD, *Escherichia coli rec* strains, differential toxicity	-	+	0.2600	Knasmüller *et al.* (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0500	Nagao *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	0.0050	Wild *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0000	Barnes *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+	0.0000	Felton & knize (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	-	+	0.0000	Grivas & Jägerstad (1984)
SA2, *Salmonella typhimurium* TA102, reverse mutation	0	+	0.0000	Felton & Knize (1990)
SA4, *Salmonella typhimurium* TA104, reverse mutation	0	+	0.0000	Felton & Knize (1990)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	-	-	25.0000	Wild *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	0.0025	Wild *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	0.0005	Thompson *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	0.0003	Wild *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+	0.1500	Felton & Knize (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0050	Nagao *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	0.0003	Wild *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0015	Barnes *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+[b]	0.0050	Ishida *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	0.3125	Loprieno *et al.* (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+[c]	0.2000	Holme *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	0.0017	Lin *et al.* (1992)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0000	Felton & Knize (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0010	Nagao *et al.* (1983b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.1250	Hayashi *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0003	Wild *et al.* (1991)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+	0.0005	Buonarati & Felton (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	-	+	0.0010	Grivas & Jägerstad (1984)
SA5, *Salmonella typhimurium* TA96, reverse mutation	0	+	0.0000	Felton & Knize (1990)

Table 4 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAS, *Salmonella typhimurium* TA97, reverse mutation	0	+	0.0000	Felton & Knize (1990)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	0.0017	Lin *et al.* (1992)
SAS, *Salmonella typhimurium* TA97, reverse mutation[d]	+	+	0.0017	Lin *et al.* (1992)
SAS, *Salmonella typhimurium* TA98, reverse mutation[d]	+	+	0.0017	Lin *et al.* (1992)
SAS, *Salmonella typhimurium* TA98/1,8-DNP_6, reverse mutation	0	-	0.0250	Nagao *et al.* (1983b)
SAS, *Salmonella typhimurium* TA98/1,8-DNP_6, reverse mutation	0	-	0.0005	Buonarati & Felton (1990)
SAS, *Salmonella typhimurium* TA98/1,8-DNP_6, reverse mutation	0	-	0.0015	Wild *et al.* (1991)
SAS, *Salmonella typhimurium* TA1535/pSK 1002	0	+	2.0000	Ubukata *et al.* (1992)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	0	+	0.0500	Thompson *et al.* (1983)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	0	(+)	0.0050	Wild *et al.* (1985)
SAS, *Salmonella typhimurium* YG1024, reverse mutation	0	+	0.0001	Wild *et al.* (1991)
DMM, *Drosophila melanogaster*, somatic mutation and recombination	+	0	25.0000	Yoo *et al.* (1985)
DMM, *Drosophila melanogaster*, somatic mutation and recombination	+	0	250.0000	Graf *et al.* (1992)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	200.0000	Wild *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+	0	200.0000	Graf *et al.* (1992)
DMM, *Drosophila melanogaster*[f], wing spot test	+	0	250.0000	Graf *et al.* (1992)
DIA, DNA strand breaks, radiation-induced mouse leukaemic cells *in vitro*	-	+	1.9800	Caderni *et al.* (1983)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	0	0.9900	Caderni *et al.* (1983)
DIA, DNA strand breaks, mouse hepatocytes *in vitro*	+	0	2.0000	Hayashi *et al.* (1985)
DIA, DNA strand breaks, Chinese hamster V79 cells *in vitro*	-	-[c,e]	100.0000	Holme *et al.* (1987)
DIA, DNA strand breaks, rat hepatocytes[e] *in vitro*	+	0	20.0000	Holme *et al.* (1987)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.0250	Barnes *et al.* (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	0.1000	Yoshimi *et al.* (1988)
UIA, Unscheduled DNA synthesis, mouse hepatocytes *in vitro*	+	0	1.0000	Yoshimi *et al.* (1988)
UIA, Unscheduled DNA synthesis, Syrian hamster hepatocytes *in vitro*	+	0	1.0000	Yoshimi *et al.* (1988)
GCL, Gene mutation, Chinese hamster lung cells, DT^r, *in vitro*	-	+	5.0000	Nakayasu *et al.* (1983)
GCL, Gene mutation, Chinese hamster lung cells *in vitro*	0	+	1.0000	Sugimura *et al.* (1989)
GCO, Gene mutation, Chinese hamster ovary cells (*uv-5*) *hprt* locus *in vitro*	0	+	50.0000	Thompson *et al.* (1983)
GCO, Gene mutation, Chinese hamster ovary cells (*uv-5*) *aprt* locus *in vitro*	0	+	50.0000	Thompson *et al.* (1983)

Table 4 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GCO, Gene mutation, Chinese hamster ovary cells (AA8) *hprt* locus *in vitro*	0	–	300.0000	Thompson *et al.* (1983)
GCO, Gene mutation, Chinese hamster ovary cells (AA8) *aprt* locus *in vitro*	0	–	300.0000	Thompson *et al.* (1983)
GCO, Gene mutation, Chinese hamster ovary cells (*uv-5*) *in vitro*	0	(+)	26.0000	Brookman *et al.* (1985)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	0	(+)[c,e]	20.0000	Holme *et al.* (1987)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	0	–	25.6000	Loprieno *et al.* (1991)
G90, Gene mutation, Chinese hamster lung V79 cells, ouabain[f] *in vitro*	0	–	50.0000	Takayama & Tanaka (1983)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	+[c,e]	20.0000	Holme *et al.* (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary cells (*uv-5*) *in vitro*	0	+	50.0000	Thompson *et al.* (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary cells (AA8) *in vitro*	0	(+)	300.0000	Thompson *et al.* (1983)
CIC, Chromosome aberration, Chinese hamster ovary cells *in vitro*	0	+	12.8000	Loprieno *et al.* (1991)
CIC, Chromosome aberration, Chinese hamster ovary cells (*uv-5*) *in vitro*	0	–	80.0000	Thompson *et al.* (1983)
CIC, Chromosome aberration, Chinese hamster ovary cells (AA8) *in vitro*	0	–	300.0000	Thompson *et al.* (1983)
G1H, Gene mutation, human lymphocytes, *hprt* locus *in vitro*	0	–	200.0000	McManus *et al.* (1988b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	+	0.2000	Aeschbacher & Ruch (1989)
MIH, Micronucleus test, human lymphocytes *in vitro*	0	(+)	200.0000	McManus *et al.* (1988b)
CHL, Chromosomal aberration, human lymphocytes *in vitro*	0	–	1000.0000	Aeschbacher & Ruch (1989)
CHL, Chromosomal aberration, human lymphocytes *in vitro*	–	+	116.2000	Loprieno *et al.* (1991)
BFA, Body fluids from rats, mutagenicity to *S. typhimurium* TA98, TA100	–	+	7.0000	Barnes & Weisburger (1985)
HMM, Host-mediated assay, intrasanguinous NMRI mouse, *S. typhimurium* TA98	+	0	0.198 × 1 ip, po	Wild *et al.* (1985)
HMM, Host-mediated assay, intrasanguinous Swiss albino mice, *Escherichia coli* strains M343/753, M343/765	+	0	2.3 × 1 ip, po	Knasmüller *et al.* (1992)
DVA, DNA strand breaks, mouse liver cells *in vivo*	+		10.0000	Hayashi *et al.* (1985)
GVA, Gene mutation, mouse melanocytes *in vivo*	–		400.0 × 1 in utero	Wild *et al.* (1985)
MST, Mouse coat colour spot test *in vivo*	–		20.0 × 1 ip	Wild *et al.* (1985)
GVA, Gene mutation, rat granuloma cells, *hprt* locus *in vivo*	+		10.0 into pouch	Radermacher *et al.* (1987)

Table 4 (contd)

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, mouse bone-marrow cells[e] *in vivo*	+		20.0 × 1 ip	Minkler & Carrano (1984)
SVA, Sister chromatid exchange, rat hepatocytes *in vivo*	+		50.0 × 1 ip	Sawada *et al.* (1991)
MVM, Micronucleus test, mouse bone-marrow cells *in vivo*	-		594.0 × 1 ip	Wild *et al.* (1985)
MVM, Micronucleus test, mouse bone-marrow cells *in vivo*	-	0	40.0 × 1 po	Loprieno *et al.* (1991)
CBA, Chromosomal aberration, mouse bone-marrow cells[e] *in vivo*	-		160.0 × 1 ip	Minkler & Carrano (1984)
CVA, Chromosomal aberration, rat hepatocytes *in vivo*	+		50.0 × 1 ip	Sawada *et al.* (1991)
BID, Binding (covalent) to DNA in *S. typhimurium*[g] *in vitro*	+	0	0.0000	Schut *et al.* (1991)
BID, Binding (covalent) to DNA *in vitro* (bacterial DNA)[g]	0	+	100.0000	Asan *et al.* (1987)
BID, Binding (covalent) to DNA *in vitro* (rat hepatocytes)[g]	+		24.0000	Dirr *et al.* (1989)
BID, Binding (covalent) to DNA *in vitro* (Syrian hamster embryo)[g]	0	+	10.0000	Asan *et al.* (1987)
BID, Binding (covalent) to DNA *in vitro* (rat hepatocytes)	+	0	10.0000	Wallin *et al.* (1992)
BVD, Binding (covalent) to DNA in rats (multiple organs) *in vivo*[g]	+		5.00 × 1 ip	Schut *et al.* (1988)
BVD, Binding (covalent) to DNA in mice (multiple organs) *in vivo*[g]	+		25.00 × 1 po	Hall *et al.* (1990)
BVD, Binding (covalent) to DNA in mice (multiple organs) *in vivo*[g]	+		5.00 × 1 po	Zu & Schut (1991a)
BVD, Binding (covalent) to DNA in rats (multiple organs) *in vivo*[g]	+		5.00 × 1 po	Zu & Schut (1991b)
BVD, Binding (covalent) to DNA in rats liver and heart *in vivo*[g]	+		36.0000 × 4 wk diet	Övervik *et al.* (1991)
BVD, Binding (covalent) to DNA in liver of cynomolgus monkeys *in vivo*[g]	+		20.00 × 15 po	Snyderwine *et al.* (1988c)
BVD, Binding (covalent) to DNA in mice (multiple organs) *in vivo*[h]	+		40.0 × 1 po	Loprieno *et al.* (1991)
BVD, Binding (covalent) to DNA in rat liver *in vivo*[g]	+		100.0 × 1 po	Yamashita *et al.* (1988)
BVD, Binding (covalent) to DNA in mouse liver *in vivo*[g]	+		50.0 × 1 po	Schut *et al.* (1991)
BVD, Binding (covalent) to DNA in rat liver *in vivo*[g]	+		50.0 × 1 po	Schut *et al.* (1991)

+, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable response in several experiments within an adequate study)

[a]In-vitro tests, μg/ml; in-vivo tests, mg/kg bw; 0.0000, not given

[b]Rhesus liver S9

[c]Hepatocytes

[d]IQ reacted with nitrite (not on profile)

[e]Polychlorinated biphenyl-treated

[f]Nitro-IQ synthesized

[g]^{32}P-Postlabel

[h]^{14}C-Label

from polychlorinated biphenyl-treated rats. No hp*rt* locus or ouabain-resistance mutations were observed in other studies, in which exogenous metabolic activation systems were provided by rat liver homogenates. IQ induced sister chromatid exchange in Chinese hamster cells. Chromosomal aberrations were induced in one study with Chinese hamster ovary cells but not in studies in which a repair-deficient cell line and a repair-proficient cell line were used (Thompson *et al.*, 1983).

In cultured human lymphocytes, IQ did not induce *hprt* locus mutations but did induce sister chromatid exchange and micronucleus formation. Inconsistent results were obtained for chromosomal aberrations in metaphases.

IQ-DNA adducts were formed *in vivo* in multiple organs of rats and mice and in the liver (only organ examined) of cynomolgus monkeys given oral doses of IQ. These results obtained *in vivo* confirm that the major DNA adduct co-chromatographs with *N*-(deoxyguanosin-8-yl)-IQ (Schut *et al.*, 1991).

After administration *in vivo*, IQ induced DNA strand breaks in mouse liver, but it did not induce unscheduled DNA synthesis in rat stomach. Gene mutations were induced in neither the mouse coat colour spot test nor in a transplacental assay in mice, but IQ induced *hprt* locus mutations in a single granuloma pouch assay. Sister chromatid exchange was induced in mouse bone marrow and rat liver. Whereas chromosomal aberrations were induced in rat liver, neither these nor micronuclei were induced in mouse bone marrow.

(*a*) *IQ–nitrite interaction*

Reaction mixtures of IQ and nitrite were mutagenic to *Salmonella typhimurium* strains TA97 and 98 both in the absence and presence of an exogenous metabolic activation system. Nitro-IQ induced somatic mutation in *Drosophila melanogaster*.

(*b*) *Genetic changes in animal tumours*

Activated c-Ha-*ras* proto-oncogenes were found in four of seven Zymbal gland tumours induced in rats by IQ. The mutations were G to C transversions at the first base of codon 13 (two tumours), a G to T transversion at the second base of codon 13 (one tumour) and an A to T transversion (one tumour) at the second base of codon 61 (Kudo *et al.*, 1991). p53 Gene mutations were found in four of 15 Zymbal gland tumours induced in rats by IQ. These involved changes of *C*GT to *G*GT, T*G*C to T*T*C, *G*TG to *T*TG and *G*AA deletion at codons 156, 174, 214 and 256, respectively (Makino *et al.*, 1992).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

IQ (2-Amino-3-methylimidazo[4,5-*f*]quinoline) has been found in cooked meat and fish. A few determinations indicated that the levels of IQ were lower than those of MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline) and PhIP (2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine). IQ was reported in the only sample of cigarette smoke condensate tested.

5.2 Human carcinogenicity data

No data directly relevant to an evaluation of the carcinogenicity to humans of IQ were available; however, several studies that were potentially relevant were considered.

The only cohort study in which detailed results were presented showed a significantly increased risk for cancers at all sites and for gastric cancer associated with the consumption of broiled fish.

Two case–control studies, in Sweden and the USA, in which consumption of meat cooked in different ways was addressed and in which consumption of a number of nutrients was controlled did not show increased risks for colorectal cancer associated with consumption of fried meat; however, the study from Sweden showed an association with a preference for browned meat. One case–control study on gastric cancer in Japan showed no association with consumption of broiled fish or grilled meat.

The available information was insufficient to establish whether cooking methods that result in the formation of heterocyclic amines are a risk factor for cancer independent of the food item itself.

5.3 Animal carcinogenicity data

IQ was tested for carcinogenicity by oral administration in one experiment in mice, in two experiments in rats and in one study in monkeys. Hepatocellular adenomas and carcinomas, adenomas and adenocarcinomas of the lung and squamous- cell papillomas and carcinomas of the forestomach were produced in mice. In rats, hepatocellular carcinomas, adenocarcinomas of the small and large intestine, and squamous-cell carcinomas of the Zymbal gland were produced in animals of each sex. A high incidence of mammary adenocarcinomas was observed in females. In addition, squamous-cell carcinomas were found in the skin of males and in the clitoral gland of females. Hepatocellular carcinomas were produced in one study in monkeys.

Intraperitoneal injection of IQ to newborn male mice increased the incidence of hepatic adenomas.

Single dose or short-term oral treatment of rats with IQ followed by phenobarbital, with or without further modulating procedures, increased the numbers of foci of altered hepatocytes and of carcinomas in the liver. Sequential administration of IQ after *N*-nitroso-diethylamine enhanced the appearance of foci of altered hepatocytes in rats.

5.4 Other relevant data

No data were available on the genetic and related effects of IQ in humans.

IQ bound to DNA in many organs of cynomolgus monkeys and rodents dosed *in vivo*. In rodents treated *in vivo*, IQ induced DNA damage, gene mutation and chromosomal anomalies. It induced chromosomal anomalies in human cells *in vitro* and chromosomal anomalies, gene mutation and DNA damage in animal cells *in vitro*. It induced mutations in *Drosophila melanogaster* and DNA damage and mutations in bacteria. Gene mutations in c-Ha-*ras* and p53 genes were found in some Zymbal gland carcinomas induced in rats by IQ.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of IQ.

There is *sufficient evidence* in experimental animals for the carcinogenicity of IQ.

Overall evaluation

IQ (2-Amino-3-methylimidazo[4,5-*f*]quinoline*) is probably carcinogenic to humans (Group 2A)*.

In arriving at the overall evaluation, the Working Group took into consideration the following contributory information:

IQ is comprehensively genotoxic, and this activity can be expressed *in vivo* in rodents. IQ can be metabolized by human microsomes to a species that damages bacterial DNA.

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[1]For definition of the italicized terms, see Preamble.

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