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# Protein Quality and the Effects of Processing

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## Thermally Induced Mutagens in Protein Foods

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### ABSTRACT

Thermally induced mutagens are formed in muscle foods as a result of common cooking practices, including frying, broiling, and baking. They are generated from endogenous precursors in muscle, such as amino acids and sugars, which have individually undergone chemical decomposition and rearrangement, or by the recombination of two or more of these substrates, as in the Maillard Browning reaction. The mutagenic compounds that have been identified to date are predominantly N-substituted aryl amines that exhibit extraordinarily potent frame shift mutagenic activity in the Ames *Salmonella* assay. The carcinogenicity of some specific mutagens are verified in multiple rodent species. The thermally induced mutagens are consumed daily at the low ppb level from ingestion of cooked vertebrate muscle, such as beef, pork, poultry, and fish. Although little is known about the toxicologic significance of exposure to mutagens, they have been associated with a variety of human diseases. For this reason we are attempting to develop methods to reduce or eliminate these compounds from the food supply.

### INTRODUCTION

Thermally induced mutagens are formed in many cooked protein foods. Concern about the routine ingestion of these dietary components

rests on the controversial association between mutagen exposure and chronic disease development. Mutagens induce heritable changes in DNA, and their effect on human ailments range from inconsequential to lethal. Nonetheless, there is considerable evidence to indicate that mutational events are generally deleterious, having been implicated in the etiology of a variety of diseases, including cancer. Epidemiologists have estimated that diet may contribute to the development of approximately 35% of all human cancers in the United States (Wynder and Gori, 1977; Doll and Peto, 1981). Evidence has been derived from a variety of population studies; the most illustrative data comes from the global variation in tumor frequencies and sites among diverse groups of people and the gradual changes in cancer incidence that occur after migration.

The link between mutational events and cancer is based on a turn-of-the-century theory that remains questioned, yet still merits support. The work of Ames et al. (1975) in particular demonstrates a high probability that a carcinogen (a cancer-causing agent) is also a mutagen. More recently, the observation that mutations and rearrangements in cellular oncogenes induce neoplastic characteristics in mammalian cells suggests further that these genetic events may initiate neoplasia.

Laboratory investigations have revealed that many foods contain mutagenic components (Williams, 1986). Most of these compounds are derived from plants and microorganisms; they have been referred to as "nature's pesticides" (Ames, 1983). These mutagens include flavonoids, hydrazines, methylenedioxybenzenes, pyrrolizidine alkaloids, nitrite, and mycotoxins (Knudsen, 1982; Ames, 1983; Lodge and Daniel, 1984; Prival, 1985), among others. In addition to the plant and microbial mutagens, synthetic food additives or contaminants and endogenously formed mutagens, such as fecapentaenes in human feces, represent other potential sources of diet-related mutagens. The final class, which shall be discussed in the present chapter, are mutagens formed during thermal processing of protein foods. Previous reviews have been published giving detailed information on earlier research (Hargraves and Pariza, 1984; Hatch et al., 1984; Miller, 1985).

## HISTORICAL OVERVIEW

Sir Perceval Pott's landmark observation in the eighteenth century provided the first evidence that combusted organic material contained carcinogenic substances. He concluded that scrotal cancers occurred often in adult males who, as child chimney sweeps, were exposed to coal and wood soot. More recently, air pollution and tobacco smoke, both the products of combustion, have been implicated as causes of

certain cancers. Therefore, it was no surprise to find that foods may contain carcinogens upon heating to high temperatures. In the 1950s nitrosamines were the first thermally induced carcinogens recognized in protein foods (Magee and Barnes, 1956). In 1964, polycyclic aromatic hydrocarbons, including the carcinogen benzo[a]pyrene, were detected in charcoal-broiled beef (Lijinsky and Shubik, 1964). Carcinogens including naphthylamines were also identified in pyrolysates of the amino acids glutamic acid and leucine (Masuda et al., 1967).

Using the Ames *Salmonella* mutagenicity assay (Ames et al., 1975), which will be described below, investigators demonstrated in charred muscle foods mutagenic activity not attributable to any previously identified mutagen (Sugimura et al., 1977a). Commoner et al. (1978a,b) reported that mutagens were generated in beef extract and hamburgers fried at normal household cooking temperatures. Those observations led to a worldwide effort to identify the thermally induced mutagens and carcinogens. In addition to identification of mutagenic components, investigators also have engaged in studies to establish mechanisms of formation, determine toxicity, identify precursors, and develop effective methods for their elimination from the food supply.

#### PROBING WITH *SALMONELLA* FOR MUTAGENIC ACTIVITY IN FOODS

It is fair to say that without the Ames test as a probe these compounds would remain undetected in the complex chemical milieu that exists in cooked foods. This could occur because some of these mutagens are among the most potent yet tested in this mutagenicity assay. *Salmonella typhimurium*, in fact, is a more sensitive detector for some of these compounds than is mass spectrometry.

The assay detects reverse mutations at the histidine operon and has a high degree of reliability in detecting carcinogens, since most have mutagenic activity (Ames et al., 1975). The tester strains are histidine-dependent auxotrophs and are used in conjunction with microsomes (S9) which simulate in vivo metabolic processes. Two strain types exist for detecting different types of mutations, but for this discussion only mutations detected by the frameshift-sensitive strains are described. Frameshift mutations occur after deletion or insertion of nucleotide pairs in DNA; they result in reading frame aberrations during transcription or DNA synthesis and, thus, produce an altered protein or daughter strand, respectively. TA98 and TA1538 are the strains used most frequently for frameshift mutagen detection.

To conduct the assay, the compound to be tested, the tester strain, and an S9 mixture are combined and plated on to a petri dish containing minimal growth medium. Reverted histidine-independent prototrophs (wild type) are then counted after incubation at 37°C for 48 hr. Colony counts relate quantitatively to dose and mutagenic potency of the test compound.

The Ames mutagenicity bioassay is an efficient and reliable screening method that has been applied to a host of complex environmental mixtures, including food and derived extracts. It is rapid compared to carcinogenicity feeding studies, having a two-day turnaround time after dosing. Most importantly, a high degree of correlation exists between *Salmonella* assay results and rodent carcinogenicity tests. These three assets—sensitivity, rapidity, and predictability—make the Ames test the best method currently available to monitor genotoxicity of these types of mutagenic compounds.

### THERMALLY INDUCED MUTAGENS IN PROTEIN FOODS

The use of *Salmonella* as a detection system enabled Japanese investigators to purify and identify nine mutagenic polar aromatic bases from smoke and amino acids which were heated to 500–600°C. A list, including formal and common names and physical properties, is found on Table 1, while structures are presented in Fig. 1. Pyrolysis products were identified as: Trp-P-1 and Trp-P-2 from tryptophan (Sugimura et al., 1977b); Phe-P-1 from phenylalanine (Sugimura et al., 1977b); Glu-P-1 and Glu-P-2 from glutamic acid (Yamamoto et al., 1978); Lys-P-1 from lysine (Wakabayashi et al., 1978); Orn-P-1 from ornithine (Yokota et al., 1981); and AAC and AMAC from smoke condensates of pyrolyzed proteins (Yoshida et al., 1978).

Novel mutagens in cooked protein foods were found in extracts derived from broiled fish and beef in the mid-1970s. Like the amino acid pyrolysates, activity in food toward the Ames strains TA98 and TA100 was observed only in the presence of an S9 metabolizing mixture (Nagao et al., 1977a; Sugimura et al., 1977a). The activity was greater on TA98, indicating that the compounds caused frameshift mutations predominantly. The mutagenic compounds were soon identified as the amino acid pyrolysis products described above (Sugimura et al., 1977b; Yamaguchi et al., 1980). Later three amino  $\alpha$ -carbolines were also identified from grilled foods (Yoshida et al., 1978; Matsumoto et al., 1981b). These compounds comprised only 1–5% of the total mutagenic activity in fish and beef samples (Kasai et al., 1979, 1980a; Yamaguchi et al., 1980a,b).

A second class of mutagenic compounds in protein foods was discovered after Commoner et al. (1978a,b) detected mutagenic activity in nutrient broth, commercial beef extract, and moderately heated

Table 1 Thermally Induced Mutagens and Carcinogens

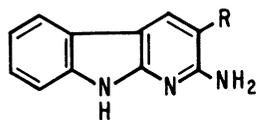
Common name	Formal name	Empirical formula	Molecular wt.
Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub>	211
Trp-P-2	3-Amino-1-methyl-5H-pyrido[4,3-b]indole	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub>	197
Glu-P-1	2-Amino-5-methyldipyrrodo[1,2-a:3',2'-d]imidazole	C <sub>11</sub> H <sub>10</sub> N <sub>4</sub>	198
Glu-P-2	2-Aminodipyrrodo[1,2-a:3',2'-d]imidazole	C <sub>10</sub> H <sub>8</sub> N <sub>4</sub>	184
Phe-P-1	2-Amino-5-phenylpyridine	C <sub>11</sub> H <sub>10</sub> N <sub>2</sub>	170
Lys-P-1	3,4-Cyclopentenopyrido[3,2-a]carbazole	C <sub>18</sub> H <sub>14</sub> N <sub>2</sub>	258
Orn-P-1	4-Amino-6-methyl-1H-2,5-10,10b]tetraazafluoranthene	C <sub>13</sub> H <sub>11</sub> N <sub>5</sub>	237
AAC	2-Amino-9H-pyridol[2,3-b]indole	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub>	183
AMAC	2-Amino-3-methyl-9H-pyridol[2,3-b]indole	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub>	197
IQ	2-Amino-3-methylimidazo[4,5-f]quinoline	C <sub>11</sub> H <sub>10</sub> N <sub>4</sub>	198
MeIQ	2-Amino-3,4-dimethylimidazo[4,5-f]quinoline	C <sub>12</sub> H <sub>12</sub> N <sub>4</sub>	212
MeIQx	2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline	C <sub>11</sub> H <sub>11</sub> N <sub>5</sub>	213
7,8-DiMeIQx	2-Amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub>	227
4,8-DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub>	227
PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine	C <sub>13</sub> H <sub>12</sub> N <sub>4</sub>	224
TMIP	2-Amino-n,n-trimethylimidazopyridine	C <sub>9</sub> H <sub>12</sub> N <sub>4</sub>	176

Two other mutagens were soon identified by Japanese investigators. MeIQ was identified from broiled, sun-dried sardines (Kasai et al., 1981b) after partial purification by eight isolation steps. MeIQx was isolated from fried beef using an identical procedure used for IQ, but had a UV absorption maximum at 274 nm, instead of 264 nm for the two preceding compounds. The quinoxaline structure was assigned from mass and NMR spectroscopic data. Confirmation was provided by comparison of structural data on isolated samples to synthesized MeIQx (Kasai et al., 1981a). Recently, additional mutagens have been identified in fried ground beef. These include 4,8 DiMeIQx, PhIP, and TMIP (Felton et al., 1983b,1986b). In all, a total of 16 heterocyclic mutagens have been isolated to date. A variety of approaches have been taken for the synthesis of the IQ-type mutagens and their derivatives (Akimoto et al., 1977,1985; Takeda et al., 1978,1981; Kasai et al., 1980a,b,c, 1981a; Lee et al., 1982; Adolfsson and Olsson, 1983; Grivas, 1985, 1986; Nyhammar and Grivas, 1986; Olsson and Grivas, 1986; Rapoport et al., 1986), including incorporation of radiolabels (Adolfsson and Olsson, 1983; Waterhouse and Rapoport, 1985). In ground beef fried at 300°C MeIQx contributed more than 20% of the mutagenic activity, TMIP (15%), IQ (6%), MeIQ(trace), DiMeIQx (20%), and PhIP (18%) (Felton et al., 1986b).

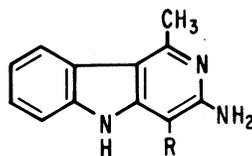
#### THE MAJOR CHALLENGE: EXTRACTION AND PURIFICATION

Isolation of the thermally induced mutagens present at parts-per-billion levels in complex food media has been the most difficult problem yet encountered by investigators. A variety of procedures were developed that ranged from direct solvent or acid extraction (Kasai et al., 1979; Nagao et al., 1977a; Uyeta et al., 1970) to acid-based partitioning between immiscible solvents (Commoner et al., 1978; Yamaizumi et al., 1980a; Felton et al., 1981). The key observation that the mutagens were most soluble in polar organic solvents, especially methanol, and were most extractable from media around pH 12 indicated that the compounds were organic bases and provided information that permitted development of better extraction methods.

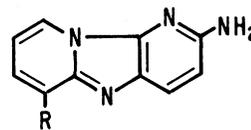
Improved extraction efficiency was realized by the use of two selective adsorbents. Bjeldanes et al. (1982b) reported yields in excess of 80% of the total mutagen load of an extract of fried ground beef sample by the use of XAD-2 resin (styrene-divinylbenzene copolymer) to adsorb mutagenic activity; the activity was eluted from the chromatographic column with acetone and methanol. This innovation exceeded by fivefold the yield of the then-best liquid-liquid partition method. A second extraction method employed trisulfocopper-phthalocyanine dye covalently bound to cotton (Hayatsu et al.,

$\alpha$ -CARBOLINES

AAC R = H  
AMAC R = CH<sub>3</sub>

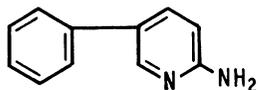
 $\gamma$ -CARBOLINES

TRP-P-1 R = CH<sub>3</sub>  
TRP-P-2 R = H

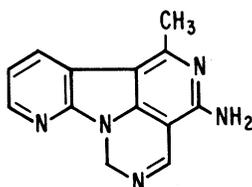
AZA- $\Delta$ -CARBOLINES

GLU-P-1 R = CH<sub>3</sub>  
GLU-P-2 R = H

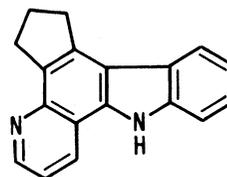
## MISC. N-HETEROCYCLICS



PHE-P-1



ORN-P-1



LYS-P-1

Fig. 1 Structure of the amino acid pyrolysis product mutagens.

(below 200°C) ground beef (Vithayathil et al., 1978). The observation was confirmed by several other investigators studying heated vertebrate animal foods, including beef (Pariza et al., 1979a,b; Spingarn and Weisburger, 1979; Felton et al., 1981), pork (Bjeldanes et al., 1982a; Miller and Buchanan, 1983a,b; Överik et al., 1984), lamb (Bjeldanes et al., 1982a), fish (Bjeldanes et al., 1982a), poultry (Bjeldanes et al., 1982a), and eggs (Grose et al., 1986). A comparative survey by Bjeldanes et al. (1982a) demonstrated that protein foods exhibited a wide range of mutagenic activity in *Salmonella* when 100-g portions were tested on TA1538: beef (6300 revertant colonies), pork (6000 revertants), chicken (1000 revertants), fish (1334 revertants).

IQ (2-amino-3-methylimidazo[4,5-f]quinoline) was identified in beef (Spingarn et al., 1980) and broiled sardines (Kasai et al., 1981b) after microgram quantities of the purified material were isolated from kilograms of starting material (Table 1, Fig. 2). Identification was based on reverse phase high performance liquid chromatography (HPLC), low and high resolution mass spectroscopy, and NMR. The structure was confirmed by synthesis (Kasai et al., 1980a) and X-ray crystallography (Yokoyama et al., 1980).

Table 2 Quantification of Mutagenic Pyrolysis Products in Foods

Product	Heat method	Temperature (°C)	Detected amount of compound (ng/g)								
			Trp-1	Trp-2	Glu-1	Glu-2	Phe-1	AAC	AMAC		
Beef	Broil	NR	50								
	Grill	NR							651		63
Squid	Broil	NR			+						
Sardines	Broil	NR	13	13				Tr			
Casein	Pyrolysis	300-400								+	
Onion	Grill	NR							1		ND
Chicken	Grill	NR							180		15
Mushroom	Grill	NR							47		5
Soybean globulin	NR	NR							+		+

Tr = Trace; ND = Not detected; NR = Not reported; + = Detected, but not quantified.  
 See references: Yoshida et al., 1978; Matsumoto et al., 1981b; Yamaguchi et al., 1979, 1980a,b;  
 Yamazumi et al., 1980a.

Table 3 Quantification of Creatine-Derived Mutagens in Heated Foods

Product	Temperature (°C)	Detected amount of compound (ng/g)						
		IQ	MeIQ	MeIQx	DiMeIQx	PhIP	TMIP	
Beef	192	ND			+			
	200	+		+				
	250	0.02		1				
	300				0.5	15	0.5	
Beef extract	NR	0.2						
	NR	42	Tr	59				
Sardine	NR	20-40	+	200-300				
	NR	5	17					
Salmon muscle skin	NR							
		0.3-2	0.6-3					
Eggs		1-2	1-31					
	325	0.1						

ND = Not detected; + = Detected, but not quantified; Tr = Trace, NR = not reported.  
 See References: Grose et al., 1986; Felton et al., 1983b, 1986a,b; Hayatsu et al., 1983b;  
 Takahashi et al., 1985; Hargraves and Pariza, 1983; Yamazumi et al., 1980b, 1986.

1983a,b). "Blue Cotton" adsorbs polycyclic planar compounds from dilute aqueous solutions; small quantities of polar organic solvents then elute the mutagenic concentrate. Recoveries of up to 93% in model systems were reported (Hayatsu et al., 1983a,b). This method has been recently adapted for use as a membrane electrode for rapid preliminary detection of polycyclic mutagens (Tomoda et al., 1986).

While thin layer or liquid chromatography were used in early studies for subsequent purification, these procedures have been generally replaced by sequential HPLC procedures. A preparative C-18 reverse phase column is the first step for isolation, which is followed by either amino or cyano-bonded silica normal phase columns. When necessary, an analytical reverse phase column separation is included to complete the isolation. Detection is either by ultraviolet absorbance, with a scanning diode array spectrophotometer (Felton et al., 1983b; Turesky et al., 1983), or electrochemical methods (Takahashi et al., 1985). Mass spectroscopy is used for identification of the mutagenic components, although NMR has been used when sufficient quantities (more than a microgram) have been isolated. Data on the quantification of the thermally induced mutagens from various sources are presented in Tables 2 and 3.

Although progress in analysis has been made to date, current research methods lack the ruggedness and simplicity required for routine quantitative screening or regulatory compliance purposes. The analytical methods still require comparatively large amounts of initial sample to perform the labor-intensive tasks associated with the necessary extraction and sequential chromatographic steps. In many cases quantitative data are questionable because of the significant losses associated with sample clean-up.

The promise of the future for improved analytical methods is encouraging, however. Several advanced technologies may provide quantitative information on a routine basis. These methods include gas chromatography/mass spectroscopy using isotope-labeled internal standards (Nishimura, 1986), HPLC/mass spectroscopy (Edmonds et al., 1986) and monoclonal antibodies (J. Felton, personal communication).

#### THE KEY TO ELIMINATION: PRECURSORS AND FORMATION MECHANISMS

Model systems have been employed, with varying degrees of success, to determine the precursors and establish the reaction mechanisms by which the thermally induced mutagens form (Masuda et al., 1967; Spingarn and Garvie, 1979; Shinohara et al., 1980a,b; Shibamoto et al., 1981; Toda et al., 1981; Ohe, 1982; Yoshida and Okamoto, 1982; Shinohara et al., 1983; Shibamoto, 1984; Kuroda et al., 1985). Results to date indicate that the amino acid pyrolysis products are produced by exposure of single amino acids, either in solution or dry, to

temperatures in excess of 300°C. Pyrolysis produces many reactive free radical fragments that condense onto intact portions of amino acids to form new heterocyclic structures. Hydroquinone may mediate this reaction since it is present in many pyrolysis reactions (Yoshida and Mizusaki, 1985).

Formation of IQ-type mutagens requires a mixture of soluble precursors, all of less than 500 daltons (Taylor et al., 1981, 1984a,b). Amino acids, sugars, and the guanidino compound creatine and its hydrolysis product creatinine are the most likely precursors. Model system experiments have demonstrated the potential for creatine and creatinine (most creatine is converted to creatinine during heating) to generate mutagenic components when refluxed or otherwise heated in the presence of amino acids or sugars, in combination or singly (Yoshida and Okamoto, 1980a,b; Jagerstad et al., 1982, 1984; Ohe, 1982; Negishi et al., 1984; Yoshida et al., 1984; Grivas et al., 1985; Nyhammer et al., 1986). All of the amino-imidazo mutagens contain the creatinine backbone structure (see Fig. 2). Condensed on this ring are a variety of single or double N-aromatic rings, including quinoline, quinoxaline, and pyridine, all of which are present in cooked muscle foods (Shibamoto, 1980). Although unconfirmed, vitamin K may be involved in the formation of these compounds (Vithayathil et al., 1983).

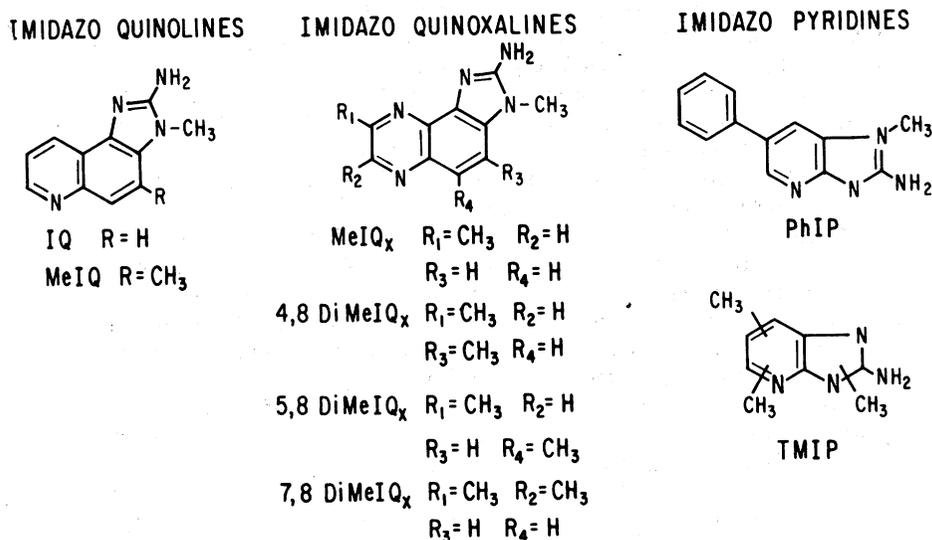


Fig. 2 Structure of the creatine-derived mutagens.

Creatine and creatinine's involvement as precursors for the IQ-type mutagens was demonstrated in our laboratory in a food system when we observed that fried shrimp muscle did not form mutagenic activity unless supplemented before cooking with creatinine. Most invertebrates lack creatine phosphate, but rather use arginine phosphate for muscle energy storage.

The formation of the creatine-derived mutagens has been studied in model systems, and it appears that Maillard nonenzymatic browning occurring at above 100°C is the most likely mechanism, although Taylor et al. (1986) questioned this assertion. Regardless of the mechanism of formation, the millimolar abundance of the precursor compounds in protein foods and the nanomolar yields of mutagenic end-product suggests that the reactions that form these mutagens are inefficient at best. Jagerstad (personal communication) indicated that yields are only 1% in a model system shown to generate MeIQx. The yield would be expected to be significantly lower in food.

#### FACTORS AFFECTING MUTAGEN FORMATION IN FOODS

Temperature, time, cooking method and surface, proximate composition, and precursor levels are all important in the generation of the thermally induced mutagens. Temperature is the most important extrinsic variable affecting their formation, with high surface temperatures inducing greatest mutagenic activity (Pariza et al., 1979b; Bjeldanes et al., 1982a). Generally, mutagens are found on the cooked surface of foods. Bacon is an ideal food product for these types of studies because the entire product approaches the temperature of the heating medium. We have demonstrated a 15-fold increase in mutagenic activity when nitrite-free bacon was heated from 125 to 225°C for 6 min (Miller and Buchanan, 1983a). Mutagenicity was also observed when low temperatures and extended cooking time were used (Commoner et al., 1978b; Pariza et al., 1979b).

Cooking time also has a significant effect on mutagen formation. After an initial lag time, the food surface is raised above 100°C and the rate of mutagen formation increases to a maximum and then decreases with longer cooking time (Dolara et al., 1979; Felton et al., 1981; Vithayathil et al., 1983). Bjeldanes et al. (1983) proposed that temperature and time were the most important variables for mutagen formation, as suggested by a derived regression equation. In general, increased cooking time and temperature result in higher levels of the heterocyclic mutagens.

Cooking method affects the heating rate and temperature of the food and, thus, can influence mutagen formation. In a study conducted in the author's laboratory (Miller and Buchanan, 1983b), it was determined that cooking methods such as baking, broiling, and

high temperature autoclaving (161°C) induced mutagen formation in nitrite-free bacon in a manner directly proportional to the heating temperature. Conversely, low temperature cooking methods such as conventional autoclaving (121°C), steaming, and microwave heating failed to produce mutagenic activity. Microwave heating has been studied by various investigators and all have concluded that no mutagens are formed during this cooking process (Commoner et al., 1978a,b; Nader et al., 1981; Baker et al., 1982). Taylor et al. (1986) found that fried hamburgers produced less than 10% of their potential mutagenic activity if they had been precooked in a microwave oven for 0.5–1.5 min. This was probably from soluble precursors that were lost as drip during the brief microwave cooking.

The cooking surface can affect final levels of mutagen formation by altering heating rate or temperature. Bjeldanes et al. (1983), for example, determined that mutagen formation was slower in hamburgers fried on a ceramic surface than stainless steel, but reached the same final level. A thin Teflon coating on the steel had little influence on the kinetics of mutagen generation. Studies of commercial heating processing of muscle foods revealed that mutagen formation was variable depending upon the product; fish are most variable and beef is most frequently mutagenic (Krone and Iwaoka, 1984; Krone et al., 1986). In general, cooking methods involving high heat or direct contact with a hot metal surface or radiant heat result in greatest mutagen formation.

Proximate composition of the product can affect mutagen formation, with protein level being most significantly related to mutagenicity in heated foods. Water also is important as a medium for mutagen formation to occur, since no activity was observed in heated ground beef until the moisture content was reduced from the fresh level (about 67%) to 55%. At that water level mutagen formation occurred rapidly as meat moisture decreased from 55 to 35% (Bjeldanes et al., 1983). Further moisture removal, however, may affect the type, more than the quantity, of mutagens formed (Taylor et al., 1986). An abundance of data indicates that fat level has little effect on mutagen formation. Carbohydrate levels affect mutagen formation by providing reducing sugars as carbonyl precursors. Complex carbohydrates also may bind these mutagens and thus prevent their absorption and further metabolism (discussed below).

## GENETIC TOXICOLOGY

The mutagenic activity of some of the thermally induced mutagens (Table 4) exceeds by logarithmic orders the common diagnostic compounds used in routine genetic toxicity testing. The genetic toxicology of the amino acid pyrolysis products has been studied extensively

Table 4 Mutagenicity of Thermally Induced Mutagens in *Salmonella typhimurium* TA98 + S9

Compound	Mutagenicity (Rev/ $\mu$ g TA98)	Reference
Trp-P-1	6,300-39,000	Sugimura et al., 1977b
Trp-P-2	13,600-104,200	Sugimura et al., 1977b
Glu-P-1	13,700-49,000	Yamamoto et al., 1978
Glu-P-1	245-1,900	Yamamoto et al., 1978
Phe-P-1	41	Sugimura et al., 1977b
Lys-P-1	87	Wakabayashi et al., 1978
Orn-P-1	28,200-57,000	Yokota et al., 1981
AAC	206-300	Yoshida et al., 1978
AMAC	32-200	Yoshida et al., 1978
IQ	118,000-433,000	Kasai et al., 1981b Felton et al., 1986a Nagao et al., 1981
MeIQ	253,000-700,000	Kasai et al., 1980b Grivas et al., 1985 Felton et al., 1986a Nagao et al., 1981
MeIQx	58,000-145,000	Felton et al., 1986a Jagerstadt and Grivas, 1985
7,8-DiMeIQx	150,000-163,000	Negishi et al., 1984
4,8-DiMeIQx	126,000-183,000	Grivas et al., 1985
PhIP	2350 <sup>a</sup>	Felton et al., 1986b
TMIP	100,000 <sup>a</sup>	Felton et al., 1986a

<sup>a</sup>TA1538 + S9.

Table 5 Qualitative Toxicology Profile of Creatine-Derived Mutagens

Assay	IQ	MeIQ	MeIQx	4,8-DiMeIQx	7,8-DiMeIQx	PhIP	TMIP
Bacterial point mutation	+	+	+	+	+	+	+
Mammalian in vitro point mutation	w	+	+	NR	NR	+	NR
<i>Drosophila</i> point mutation	+	+	+	NR	NR	NR	NR
DNA damage in vitro	+	+	-	NR	NR	NR	NR
Sister chromatid exchange:							
in vivo	w	+	NR	NR	NR	NR	NR
in vitro	+	NR	NR	NR	NR	NR	NR
Chromosomal aberration:							
in vitro	-	NR	NR	NR	NR	NR	NR
in vitro	-	NR	NR	NR	NR	NR	NR
Neoplastic transformation in vitro	+	NR	NR	NR	NR	NR	NR
Oncogene activation	+	NR	NR	NR	NR	NR	NR
Nuclear aberrations in vivo	+	NR	NR	NR	NR	NR	NR

NR = Not reported; + = positive; - = negative; w = weak positive.

and was reviewed by the author (Miller, 1985), while a comprehensive review was published more recently (Hatch, 1986).

Our understanding of the genotoxicity of the creatine derived mutagens has advanced considerably. The creatine derived mutagens showed marked activity with the frameshift-sensitive *Salmonella* strains TA98 and TA1538, which are sensitive to picogram amounts. This indicates that the SOS error-prone repair system present on a R<sup>+</sup> plasmid contained in TA98, does not influence sensitivity. Deletion of the excision repair pathway, which was carried out during construction of the tester strain, however, is an essential requirement for mutagenic activity (Felton et al., 1983a).

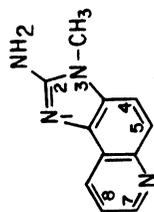
Unlike the mutagenic response in *Salmonella*, IQ is a weak mutagen in mammalian cells as indicated in Table 5 (Nakayasu et al., 1983; Takayama and Tanaka 1983; Thompson et al., 1983). The apparent inverse relationship between bacterial and mammalian activity was reinforced by results from in vivo mammalian assays, which demonstrated that IQ was weak in the induction of chromosomal aberrations, sister chromatid exchanges, in vivo point mutations, and transplacental mutations (Minkler and Carrano, 1984; Wild et al., 1985). IQ induced mutations in *Drosophila*, however (Wild et al., 1985), as did the tryptophan and glutamic acid pyrolysis products,  $\alpha$ -carbolines, as well as MeIQ and MeIQx (Yoo et al., 1985). Dolara et al. (1985) used an alkaline elution technique to demonstrate damage to mammalian cell DNA by IQ, MeIQ, and MeIQx. Although not a true genotoxic assay, neoplastic transformation of BALB 3T3 cells by IQ was demonstrated (Cortesi and Dolara, 1983). Moreover, Ishikawa et al. (1985) used IQ to activate a c-raf oncogene in rat hepatocellular carcinoma. This finding is of interest because it demonstrated that IQ can activate a gene responsible for cell proliferation.

#### STRUCTURE/ACTIVITY RELATIONSHIPS

The mutagenic structure/activity relationship of a number of the thermally induced mutagens was studied using *Salmonella*. The addition of methyl groups to Trp-P-2, other than at the number one carbon, reduced mutagenicity. Alterations to the exocyclic amino groups also resulted in reduced activity (Pezzuto et al., 1980, 1981). For the 2-aminodipyridoimidazole (glutamic acid) pyrolysates, methyl substitution dramatically altered mutagenic activity (Takeda et al., 1980). Bulkier alkyl substitution at the 3-position of the  $\alpha$ -carbolines produced decreased mutagenic activity compared to their methyl counterparts (Matsumoto et al., 1981a).

Structure/activity relationships of the creatine-derived compounds were investigated similarly (Table 6). Grivas and Jagerstad (1984) showed that both the imidazole ring and its 2-amino group were required for the high mutagenicity of the IQ compounds. Likewise,

Table 6 Mutagenic Structure-Activity Relationship of 2-Amino-3-Methylimidazo[4,5-f]Quinoline



Compound	Change	TA98 revertants/ nmol	Reference
2-Amino-3-methyl-IQ	Parent	80,000-85,700	Jägerstadt and Grivas, 1985 Wild et al., 1986
	<i>Alter 3-Me:</i>		
2-Amino-1-methyl-IQ	Move	617,000	Wild et al., 1986
2-Amino-4-methyl-IQ		79	Nagao et al., 1981
2-Amino-5-methyl-IQ		6	Nagao et al., 1981
2-Amino-3,4-dimethyl-IQ	Add	53,742-140,000	Wild et al., 1986 Nagao et al., 1981
2-Amino-3,5-dimethyl-IQ		30,000	Jägerstadt and Grivas, 1985
2-Amino-1,4-dimethyl-IQ	Replace	159,000	Nagao et al., 1981
2-Amino-1,5-dimethyl-IQ		98,000	Nagao et al., 1981
2-Amino-3-ethyl-IQ		54,000	Jägerstadt and Grivas, 1985
2-Amino-3-ethyl-4-methyl-IQ		8,678	Jägerstadt and Grivas, 1985

Table 6 (Continued)

Compound	Change	TA98 revertants/ nmol	Reference
2-Amino-IQ	Remove	0.12-55	Wild et al., 1986
	Alter 2-amino:		
3-Methyl-IQ	Remove	35	Grivas and Jägerstad, 1984
3,4-Dimethyl-IQ	Remove and add	0	Grivas and Jägerstad, 1984
	Alter 6-N:		
2-Amino-3-methylnaphthol [1,2-d]imidazole	Remove	3.6	Wild et al., 1986

IQ = Imidazo[4,5-f]quinoline.

the 3-methyl group was associated with the maximum amount of mutagenic activity, since 3-ethyl analogues had lower activity (Jagerstad and Grivas, 1985) and removal of the 3-methyl group from IQ or MeIQ virtually eliminated the original mutagenicity (Nagao et al., 1981). The number of methyl groups generally increased the mutagenicity of the IQ compounds, as evidenced by the greater potency of MeIQ above IQ. The position of the methyl groups also affected mutagenic potential (Nagao et al., 1981), with the N-1-MeIQ (IsoIQ) the most potent IQ form known (Wild et al., 1986). The same tendencies were observed for imidazoquinoxalines (Nyhammer et al., 1986), although, in general, imidazoquinolines were two to three times more potent than their respective quinoxaline analogues.

#### CARCINOGENICITY STUDIES

Rodent feeding studies have demonstrated that the nine thermally induced mutagens tested to date are all carcinogenic (Sugimura, 1985, 1986a,b; Sugimura et al., 1986). A summary of results is presented in Table 7. The compounds tested include the pyrolysis

Table 7 Carcinogenicity of Thermally Induced Mutagens

Compound	Dose (% diet)	Species	Sex	Tumor site
Trp-P-1	0.02	Mouse	M,F	Liver
	0.015	Rat	M	Liver, intestine
	0.02	Rat	F	Liver
Trp-P-2	0.02	Mouse	M,F	Liver
	0.02	Rat	M	Liver, intestine, urinary bladder
			F	Liver, intestine, zymbal gland, clitoral gland
AAC/AMAC	0.08	Mouse	M,F	Liver, blood vessels
	0.08	Rat	M,F	Inconclusive
Glu-P-1/ Glu-P-2	0.05	Mouse	M,F	Liver, blood vessels
	0.05	Rat	M	Liver, intestine, zymbal gland
			F	Liver, intestine, zymbal gland, clitoral gland

Table 7 (Continued)

Compound	Dose (% diet)	Species	Sex	Tumor site
IQ	0.03	Mouse	M,F	Liver, forestomach, lung
		Rat	M	Zymbal gland, liver, intestine, skin, oral cavity
	F		Zymbal gland, liver, intestine, skin, oral cavity, clitoral gland	
	a	Rat	F	Mammary gland, ear duct, liver, pancreas, urinary bladder
MeIQ	0.04	Mouse	M,F	Forestomach, liver
MeIQx	0.04	Mouse	M,F	Small intestine

<sup>a</sup>0.4 mmol/kg body weight by gavage weekly for 31 weeks.

products of tryptophan (Hosaka et al., 1981; Matsukura et al., 1981a,b; Takyama et al., 1985a) and glutamic acid (Takayama et al., 1984b; Ohgaki et al., 1984b); two amino- $\alpha$ -carbolines (Ohgaki et al., 1984b,c; Takayama et al., 1985a,b); IQ (Ohgaki et al., 1984a); MeIQ (Ohgaki et al., 1985) and MeIQx (Sugimura, 1985). Tumors developed on multiple sites including the liver, clitoral gland, and the zymbal gland of rats. Colon tumors formed in the rat but not in the mouse. Effective tumorigenic doses were closely clustered and generally not well correlated with bacterial mutation assay potency. This last point is of concern because carcinogens with low mutagenic potency will be certainly overlooked when screened with *Salmonella*.

#### METABOLISM

The fact that mutagenic activity was recovered from the urine of human volunteers who previously ate a fried pork or bacon meal (Baker et al., 1982, 1986) immediately galvanized the research community, since this was the first demonstration that thermally induced mutagens were absorbed and excreted by humans. Subsequently, others have confirmed that mutagenic activity is present in urine (Hayatsu et al., 1985a) and feces (Hayatsu et al., 1985b) when

volunteers ate either pork or beef. The mutagenic urinary metabolite was shown to be a MeIQx derivative (Hayatsu et al., 1985a). Human metabolism was established further when MeIQx was isolated from the dialysate fluid of uremic patients (Yanagisawa et al., 1986). Absorption, distribution, and excretion of the tryptophan pyrolysis products and IQ compounds have been studied in rodents (Brandt et al., 1983; Munzner and Wever, 1984; Sjödin and Jägerstad, 1984; Barnes and Weisburger, 1985; Kimura et al., 1985; Bergman, 1985). In general, results showed that absorption occurred in the intestine. The compounds were then distributed systematically, metabolized rapidly in the liver, and excreted predominantly in bile.

In order to exhibit their toxic effects, all of the thermally induced mutagens require biotransformation to their ultimate form. This is simulated *in vitro* by S9 and other preparations. Most species tested metabolized these compounds to active mutagens, including rat, mouse, hamster, rabbit, and human (Dolara et al., 1980; Hino et al., 1982; Kato et al., 1983; Waziers and Decloitre, 1983; Felton and Healy, 1984; Loretz and Pariza, 1984; Alldrick and Rowland, 1985; Aune and Aune, 1986). In addition, S9 preparations, organelles, or intact cells from lung, liver, and intestine were used to form the ultimate mutagens (Mita et al., 1981b; Niwa et al., 1982; Gayada and Pariza, 1983; Decloitre et al., 1984; Loretz and Pariza, 1984; Alldrick and Rowland, 1985).

Extensive molecular level studies have been performed to determine the mechanism of Trp-P-2 and Glu-P-1 activation (Hashimoto et al., 1978; Nemoto et al., 1979; Nebert et al., 1979; Ishii et al., 1981; Kato et al., 1983). N-hydroxylation by cytochrome P-448 is the first step in the activation process (Yamazoe et al., 1980a,b, 1981a,b; Mita et al., 1981a; Okamoto et al., 1981; Watanabe et al., 1982a; Kamataki et al., 1983; Kawajiri et al., 1983; Saito et al., 1983b). The ultimate mutagens appear to be the N-O-acyl forms (Hashimoto et al., 1979, 1980a,b,c, 1982a,b, 1984). While strand cleavage occurred when DNA was treated with N-OH-Trp-P-2, this activity was caused by active oxygen radicals and was distinct from the mutagenic activity (Wakata et al., 1985). Other metabolic pathways for Glu-P-1 and Trp-P-2 have been reported as well (Yamazoe et al., 1981b; Saito et al., 1983a,b; Nemoto and Takayama, 1984; Shinohara et al., 1984, 1985). AAC and IQ are also activated to the hydroxy-amino derivatives (Okamoto et al., 1981; Niwa et al., 1982). The ultimate forms of Glu-P-1 and Glu-P-2, IQ, MeIQ, and MeIQx may be sulfate esters of the N-hydroxy derivatives (Nagao et al., 1983; Loretz and Pariza, 1984). Turesky and Skipper (1986) determined that the major biliary IQ metabolite in the rat was the sulfamate derivative.

The specific interaction with DNA is not known with certainty, but for both Trp-P-1 and Trp-P-2 the binding site was established (Shishido et al., 1980). Glu-P-1 has the greatest affinity for

guanine residues in GC clusters (Hashimoto and Shudo, 1983) and is oriented parallel to the plane of the base pairs (Imamura et al., 1980). IQ, MeIQ, and MeIQx have DNA-binding constants that correspond to the order of bacterial mutagenic potency (Watanabe et al., 1982a,b), and IQ binding is stronger at GC pairs than at AT pairs (Watanabe et al., 1982c). The binding mechanism of IQ to DNA is currently under investigation. IQ causes DNA damage as shown by alkaline elution of mouse leukemia cell DNA (Caderni et al., 1983). Interestingly, ethanol and Aroclor pretreatment of animals prior to mutagen dosing enhanced the levels of DNA repair and hepatocellular genotoxicity by a variety of these compounds (Loury and Byard, 1983; Loury et al., 1985).

The disparity between mutagenic response of bacterial and cellular mutagenicity systems, referred to in a prior section, was observed by Brookman et al. (1985), who suggested that this may be due to the inability of the IQ metabolites to reach the target DNA. From this information we can conclude that control of the bioactivation of these compounds can be used to modulate genotoxic activity.

## MODULATION

Five strategies can be employed to prevent formation of or reduce or eliminate the genotoxic activity caused by the thermally induced mutagens. These include destruction of precursors, deterrence of promutagen formation, binding or degrading promutagens, preventing bioactivation to the ultimate mutagen, and blocking mutagen-DNA interactions. A variety of methods in each category has been identified and will be described.

Degradation of mutagen precursors is an important approach to reduce the thermally induced genotoxin burden, since mutagen formation would be virtually eliminated. Despite this, to date only one method has been identified. Low-glucose beef, resulting from a stress condition known as Dark Firm Dry (DFD), was shown to have reduced levels of mutagenic activity when compared to normal beef which contained about 0.1% glucose (Jägerstad et al., 1982). Presumably, the decreased mutagen load in DFD beef resulted from depletion of glucose as a mutagen precursor. Since destruction of precursors may be the most efficient means to eliminate thermally induced mutagens, more research should be directed toward this approach.

The most effective method yet identified to prevent promutagen (the mutagen before metabolic activation) formation is by careful heat application, as described in an earlier section. Furthermore, certain additives, when added to ground beef prior to frying, were inhibitory, including EDTA (1%), soy protein concentrate (5-10%),

or BHA (about 15 nM) (Wang et al., 1982). Finally, in a model system, polyphenolic antioxidants, including tannic acid, quercetin, rutin, chlorogenic acid, and catechin, at a level of 0.2 g/g protein, reduced by 20–76% the formation of mutagenic pyrolysis products when added to albumin before heating (Fukuhara et al., 1981). Additional research is warranted to verify these methods and to optimize their conditions. Moreover, research should be conducted to determine if classical nonenzymatic browning inhibitors and other antioxidants would effectively accomplish this task.

Thermally induced mutagens can be bound or destroyed once they are formed. Vegetable fibers, for example, tightly bound Trp-P-1, Trp-P-2, and Glu-P-1, which almost eliminated mutagenic activity (Kada et al., 1986). Similarly, cereal fibers, including corn and wheat bran and alfalfa meal bound 50% of IQ (Barnes et al., 1983). Other dietary fibers bound radiolabeled IQ, MeIQ, and MeIQx to varying degrees (Sjödén et al., 1985). Sorghum fiber, for example, bound 50% but 12 other fibers bound only 8–22% of the mutagen load. Vegetable and fruit extracts from numerous sources reduced mutagenic activity of the thermally induced mutagens. Cabbage, spinach, celery, apple, pineapple, eggplant, green pepper, rhubarb, and brussels sprouts suppressed tryptophan pyrolysate mutagenicity (Morita et al., 1978; Kushi et al., 1980). One desmutagenic (mutagen-inactivating) component found in cabbage (Inoue et al., 1981) and broccoli (Morita et al., 1982) is a hemo-protein exhibiting peroxidase activity. Yamada et al. (1979) previously demonstrated that peroxidases from a variety of sources together with peroxide destroyed Trp-P-1, Trp-P-2, and Glu-P-1. Protein binding of IQ was partially responsible for its inactivation by horseradish and rat intestinal mucosa peroxidase (Dolara et al., 1984). Human saliva was found to inhibit the mutagenic activity of Trp-P-1. The mechanism of the inactivation was hypothesized to entail either biochemical reactions with low molecular weight compounds, such as enzymes or vitamins, or by biophysical adsorption by bacteria and higher molecular weight substances (Nishioka et al., 1981).

Two methods have been reported that degraded preformed thermally induced mutagens and are also useful tools to differentiate between the two classes. The first method came from the observation that hypochlorite at the level of 1.5 ppm inactivated mutagenicity of both pyrolysis and creatine-derived compounds (Tsuda et al., 1983, 1985). Chlorinated tap water was equally effective, which suggests that consumption of water with meals may inhibit potential mutagenic effects. The second method was discovered upon the observation that acidic nitrite deaminated the pyrolysis products, but not the IQ-type mutagens, and formed the corresponding nonmutagenic hydroxy compounds (Yoshida and Matsumoto, 1978; Tsuda et al., 1980; Tsuda et al., 1985). Prolonged treatment of AAC with nitrite formed

a direct acting mutagenic nitroso derivative, however (Tsuda et al., 1981). A comparison of mutagenicity before and after treatment with nitrite and hypochlorite serves as a method to determine the relative percentage contributed by each category of mutagen.

Certain inhibitors were identified that interfered with the bioactivation of the thermally induced mutagens, with plant products being particularly effective. For example, the xanthine derivatives theophylline, caffeine, and 3-isobutyl-1-methylxanthine inhibited the metabolic activation of Trp-P-2 (Yamaguchi and Nakagawa, 1983). In addition, plant antioxidants such as vitamin A (Busk et al., 1982), quinones (Kushi et al., 1980; Yamaguchi, 1982), and chlorophyllin (Arimoto et al., 1980; Ong et al., 1986)—the sodium and copper salt of chlorophyll—were shown to be inhibitory. Heme pigments such as hemin (a metal chelate of heme) and biliverdin are animal-derived constituents that have been shown to be effective inhibitors (Arimoto et al., 1980). Furthermore, oleic acid from the acid fraction of ground beef, rat organ preparations, and human feces inhibited mutagenic activity of both classes of mutagens *in vivo* (Hayatsu et al., 1981a,b; Caderni et al., 1986). Oleic acid inhibits the formation of the active metabolite mainly by inhibition of hepatic microsomal oxidation systems (Saito et al., 1983a). It is noteworthy that oleic acid did not protect rats against 1,2-dimethylhydrazine-induced colon tumors (Nelson and Samuelson, 1984). Other animal products that inhibited metabolic activation of the thermally induced mutagens include a beef and pork muscle-derived mutagenesis inhibitor (Pariza et al., 1979b, 1983, 1986a,b), which was later shown to inhibit initiation of mouse epidermal tumors by 7,12-dimethylbenz[*a*]anthracene (Pariza and Hargraves, 1985). Inorganic inhibitors that were proven effective antimutagens are cobaltous chloride on Trp-P-1 (Mochizuki and Kada, 1982) and germanium oxide on Trp-P-2 (Kada et al., 1984). The antimutagenic activity of the metal salts was postulated to be due to interference with the error-prone DNA repair that is stimulated by the activated mutagens. Sister chromatid exchanges induced by OH-Trp-P-2 were suppressed more than 50% by addition of 3-aminoharman (3-amino-1-methyl-9H-pyrido[3,4-*b*]indole). Hemin and perhaps other pyrroles confer at least a portion of their antimutagenic activity by interfering with the bioactivated metabolites (Arimoto et al., 1980).

Three groups of enhancing agents or comutagens were identified that potentiated genetic effects of these mutagens. Organic solvents, including ethanol, increased the mutagenic activity of Trp-P-1 and Trp-P-2 (Arimoto et al., 1982). The  $\beta$ -carboline harman and norharman found in amino acid and protein pyrolysates also potentiated pyrolysate genotoxicity (Nagao et al., 1977b). Finally, cysteine, cysteine ethyl ester, and cysteamine (10 mM each) increased by several-fold mutagenicity of Trp-P-1 and Trp-P-2 (Negishi and

and Hayatsu, 1979). Interestingly, the cysteine derivatives did not affect the mutagenic activity of a beef-extract mutagen.

### EPIDEMIOLOGY

Little information is available concerning the relationship between food preparation techniques and the cancer patterns in populations. Limited data, however, exist from two human population studies by Japanese researchers. Kuratsune and coworkers (1986) determined that consumption of broiled fish two or more times per week increased stomach and liver cancer risks. This research group also studied Japanese Seventh Day Adventists and determined the abstinence from consumption of muscle foods reduced incidence of stomach cancer. From this information they speculated that pyrolysis products may be associated with gastric neoplasia, but acknowledged that the assertion is arguable. Hatch and Felton (1986) have challenged epidemiologists to interact with laboratory scientists in this fertile area of research. They added the caveat that "retrospective studies have limited power and prospective studies take many years."

### RISK

Without substantive epidemiological data in hand or imminent, an analysis of potential risk from ingestion of the thermally induced mutagens and carcinogens is limited to an evaluation of total exposure and potency in models. Exposure data will emerge as better quantitative methods become available. Since each of the 16 known thermally induced mutagens are generated in variable amounts, rigorous studies will continue to be required to compile a profile of their relative abundance in cooked protein foods. Likewise, the great variation in mutagenic potency needs to be explored further. Only upon obtaining this voluminous quantity of data can the two components of risk—exposure levels and potency—be better assessed.

An estimate of risk can be calculated using the Human Exposure/Rodent Potency (HERP) Quotient, developed by Ames et al. (1987). From Sugimura's data (Sugimura, 1986b) that the human intake of all of the thermally induced mutagens is 100 µg/day and that the average TD<sub>50</sub> (the dose required to induce cancers in 50% of test animals) obtained from mouse studies is 8 mg/kg/day, the calculated HERP value is 0.0125%. Felton et al. (1986a) calculated the human risk exposure to the creatine-derived compounds. Their calculation was based on an average human intake of 20 µg/day and a mouse TD<sub>50</sub> of 15 mg/kg/day (HERP = 0.00133%). Larger HERP values correspond to greater estimated risk rankings. The range of risks of the carcinogenic exposure was provided by Ames et al. (1987), who estimated

the daily exposure of formaldehyde in the air of mobile homes yields a HERP of 2.1%, while daily consumption of chloroform in contaminated well water has a quotient of 0.0002%. The thermally induced mutagens fall toward the lower end of this range. Such efforts are crude estimates, but, as more data become available, determination of a more refined risk analysis may be possible.

Risk estimates from rodents fed a single compound at the maximally tolerated dose (MTD) cannot be directly extrapolated to the human case, since human cancers generally develop only after prolonged exposure to carcinogens as complex mixtures. In order to more effectively determine risks associated with the thermally induced mutagens, they must be assessed in the context of our complex lifestyle. Mutagens formed during the cooking of protein food represent only a fraction of the total load of environmental challenges. To better assess their relative importance, Ames (1986) suggested a comparison of exposures to burned and browned material, from food, tobacco, and air pollution. Even with these data available, other sources of genotoxic material would be overlooked. The most comprehensive analysis would encompass exposure data from all sources: lifestyle, occupation, environment. Furthermore, it is abundantly clear that cancer development requires more than just mutational events.

If future information establishes that the thermally induced mutagens possess a significant human health risk, what do we do? Perhaps the most reasonable approach is to weight the benefits against risks and respond in a prudent fashion. The benefit of destruction of pathogens and the psychological well-being associated with thermal treatment of food may offset much of the potential risk. Nonetheless, it will be the shared responsibility of scientists and physicians, regulators and legislators, and, most importantly, the public, through changing food-purchasing patterns to contribute to the resolution of the risk/benefit question.

In the absence of data, at present we must conclude that the low concentrations of thermally induced mutagens and the known facts to date require little initiative for dietary modification. Moreover, even if a health problem exists, a total ban or regulation prohibiting heating of protein foods would be unacceptable, since cooking is a method of food preparation that permeates the lives of the public from infancy to old age. For the present, for individuals or institutions concerned about mutagen exposure, modification of heating practices, with emphasis on low temperatures and short heating times, such as microwave cooking, will reduce or eliminate the generation of these mutagens, without increasing the risk to microbial food safety. For the future, continued research is essential to develop:

1. rapid and selective isolation and quantification procedures
2. higher yielding synthesis

3. detailed understanding of formation mechanisms
4. comprehensive toxicology profiles, including pharmacokinetics and metabolism
5. refined risk assessments

Finally, continued research may reveal or verify mutagenesis modifiers to provide additional methods of elimination or reduction of the thermally induced mutagens from the food supply.

#### REFERENCES

- Adolfsson, L. and Olsson, K. 1983. A convenient synthesis of mutagenic 3H-imidazo[4,5-f]quinolin-2-amines and their 2-<sup>14</sup>C-labelled analogues. *Acta. Chem. Scand.* B37:157.
- Akimoto, H., Kawai, A., Nomura, H., Nagao, M., Kawachi, T., and Sugimura, T. 1977. Synthesis of potent mutagens in tryptophan pyrolysates. *Chem. Lett.* 9: 1061.
- Akimoto, H., Kawai, A., and Nomura, H. 1985. Synthesis of 3-amino-5H-pyrido[4,3-b]indoles, carcinogenic  $\gamma$ -carbolines. *Bull. Chem. Soc. Jpn.* 58:123.
- Alldrick, A. J. and Rowland, I. R. 1985. Activation of the food mutagens IQ and MeIQ by hepatic S9 fractions derived from various species. *Mutat. Res.* 144: 59.
- Ames, B. N., McCann, J., and Yamasaki, E. 1975. Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* 31: 347.
- Ames, B. N. 1983. Dietary carcinogens and anticarcinogens. *Science* 221: 1256.
- Ames, B. N. 1986. Food constituents as a source of mutagens, carcinogens, and anticarcinogens. In: *Genetic Toxicology*, Knudsen, I. (Ed.). Alan R. Liss, Inc., New York.
- Ames, B. M., Magaw, R. and Gold, L. S. 1987. Ranking possible carcinogenic hazards. *Science* 236: 271.
- Arimoto, S., Ohara, Y., Namba, T., Negishi, T., and Hayatsu, H. 1980. Inhibition of the mutagenicity of amino acid pyrolysis products by hemin and other biological pyrrole pigments. *Biochem. Biophys. Res. Comm.* 92: 662.
- Arimoto, S., Nakano, N., Ohara, Y., Tanaka, K., and Hayatsu, H. 1982. A solvent effect on the mutagenicity of tryptophan-pyrolysate mutagens in the *Salmonella*/mammalian microsome assay. *Mutat. Res.* 102: 105.
- Aune, T. and Aune, K. T. 1986. Mutagenic activation of IQ and Me-IQ by liver and lung microsomes from rabbit and mouse, and with isolated lung cells from the rabbit. *Carcinogenesis* 7: 273.
- Baker, R., Arlauskas, A., Bonin, A., and Angus, D. 1982. Detection of mutagenic activity in human urine following fried pork or bacon meals. *Canc. Lett.* 16:81.

- Baker, R. S. U., Darton-Hill, I., Bonin, A. M., Arlauskas, A., Braithwaite, C., Wootton, M., and Truswell, A. S. 1986. Urine mutagenicity as an indicator of exposure to dietary mutagens formed during cooking of foods. *Environ. Health Persp.* 67: 147.
- Barnes, W. S., Maiello, J., and Weisburger, J. H. 1983. In vitro binding of the food mutagens 2-amino-3-methylimidazo-[4,5-f]-quinoline to dietary fibers. *J. Natl. Canc. Inst.* 70: 757.
- Barnes, W. S. and Weisburger, J. H. 1985. Fate of the food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in Sprague-Dawley rats. I. Mutagens in the urine. *Mutat. Res.* 156: 83.
- Bergman, K. 1985. Autoradiographic distribution of <sup>14</sup>C-labelled 3-H-imidazo[4,5-g]quinoline-2-amines in mice. *Canc. Res.* 45: 1351.
- Bjeldanes, L. F., Morris, M. M., Felton, J. S., Healy, S., Stuermer, D., Berry, P., Timourian, H., and Hatch, F. T. 1982a. Mutagens from the cooking of food II. Survey by Ames/*Salmonella* test of mutagen formation in the major protein-rich foods of the American diet. *Fd. Chem. Toxic.* 20: 357.
- Bjeldanes, L. F., Grose, K. R., Davis, P. H., Stuermer, D. H., Healy, S. K., and Felton, J. S. 1982b. An XAD-2 resin method for efficient extraction of mutagens from fried ground beef. *Mutat. Res.* 105: 43.
- Bjeldanes, L. F., Morris, M. M., Timourian, H., and Hatch, F. T. 1983. Effects of meat composition and cooking conditions on mutagen formation in fried ground beef. *J. Agric. Food Chem.* 31: 18.
- Brandt, I., Gustafsson, J.-A., and Rafter, J. 1983. Distribution of the carcinogenic tryptophan pyrolysis product Trp-P-1 in control, 9-hydroxyellipticine and  $\beta$ -naphthoflavone pretreated mice. *Carcinogenesis* 4: 1291.
- Brookmán, K. W., Salazar, E. P., and Thompson, L. H. 1985. Comparative mutagenic efficiencies of the DNA adducts from the cooked-food-related mutagens Trp-P-2 and IQ in CHO cells. *Mutat. Res.* 149: 249.
- Busk, L., Ahlborg, U. G., and Albanus, L. 1982. Inhibition of protein pyrolysate mutagenicity by retinol (vitamin A). *Fd. Chem. Toxic.* 20: 535.
- Caderni, G., Kreamer, B. L., and Dolara, P. 1983. DNA damage of mammalian cells by the beef extract mutagen 2-amino-3-methylimidazo[4,5-f]quinoline. *Fd. Chem. Toxic.* 21: 641.
- Caderni, G., Lodovici, M., Salvadori, M., Bianchini, F., and Dolara, P. 1986. Inhibition of the mutagenic activity of some heterocyclic dietary carcinogens and other mutagenic/carcinogenic compounds by rat organ preparations. *Mutat. Res.* 169: 35.
- Commoner, B., Vithayathil, A. J., and Dolara, P. 1978a. Mutagenic analysis as a means of detecting carcinogens in foods. *J. Food Protect.* 41: 996.

- Commoner, B., Vithayathil, A. J., Dolara, P., Nair, S., Madyastha, P., and Cuca, G. C. 1978b. Formation of mutagens in beef and beef extract during cooking. *Science* 201: 913.
- Cortesi, E. and Dolara, P. 1983. Neoplastic transformation of Balb 3T3 mouse embryo fibroblasts by the beef extract mutagen 2-amino-3-methylimidazo-[4,5-f]quinoline. *Canc. Lett.* 2: 43.
- Cortesi, E. and Dolara, P. 1983. Neoplastic transformation of Balb 3T3 mouse embryo fibroblasts by the beef extract mutagen 2-amino-3-methylimidazo[4,5-f]quinoline. *Canc. Lett.* 20: 43.
- Decloitre, F., Hamon, G., Martin, M., and Thybaud-Lambay, V. 1984. Mutagenic activation of 3-amino-1,4-dimethyl-5H-pyrido-(4,3-b)indole-(Trp-P-1) and 3-amino-1-methyl-5H-pyrido(4,3-b)-indole(Trp-P-2) by primary cultures of adult rat hepatocytes: Effect of Aroclor induction in vitro. *Mutat. Res.* 137: 123.
- Dolara, P., Commoner, B., Vithayathil, A., Cuca, G., Tuley, E., Madyastha, P., Nair, S., and Driebel, D. 1979. The effect of temperature on the formation of mutagens in heated beef stock and cooked ground beef. *Mutat. Res.* 79: 213.
- Dolara, P., Barale, R., Mazzoli, S., and Benetti, D. 1980. Activation of the mutagens of beef extract in vitro and in vivo. *Mutat. Res.* 79: 213.
- Dolara, P., Caderni, G., and Lodovici, M. 1984. Inactivation of 2-amino-3-methyl-imidazo[4,5-f]quinoline by horse radish and intestinal peroxidase. *Arch. Toxicol. Suppl.* 7: 253.
- Dolara, P., Salvadori, M., Santoni, G., and Caderni, G. 1985. Mammalian cell DNA damage by some heterocyclic food mutagens is correlated with their potency in the Ames test. *Mutat. Res.* 144: 57.
- Doll, R. and Petro, R. 1981. The cause of cancer: quantitative estimate of avoidable risks of cancer in the United States today. *J. Nat. Canc. Inst.* 66: 1191.
- Edmonds, C. G., Sethi, S. K., Yamaizumi, Z., Kasai, H., Nishimura, S., and McCloskey, J. 1986. Analysis of mutagens from cooked foods by directly combined liquid chromatography-mass spectrometry. *Environ. Health Persp.* 67: 35.
- Felton, J. S., Healy, S., Stuermer, D., Berry, C., Timourian, H., Hatch, F. T., Morris, M., and Bjeldanes, L. F. 1981. Mutagens from the cooking of food. I. Improved extraction and characterization of mutagen fractions from cooked-ground beef. *Mutat. Res.* 88: 33.
- Felton, J. S., Hatch, F. T., Knize, M. G., and Bjeldanes, L. F. 1983a. Mutagens in cooked beef: characterization and genotoxic effects. In *Diet, Nutrition, and Cancer: From Basic Res. to Policy Implications*. Alan R. Liss, Inc., New York, p. 177.
- Felton, J. S., Knize, M. G., Wood, C., Wuebbles, B. J., Healy, S. K., Stuermer, D. H., Bjeldanes, L. F., Kimble, B. J., and Hatch, F. T., 1983b. Isolation and characterization of new mutagens from fried ground beef. *Carcinogenesis* 5: 95.

- Felton, J. S., and Healy, S. K. 1984. Activation of mutagens in cooked ground beef by human-liver microsomes. *Mutat. Res.* 140: 61.
- Felton, J. S., Knize, M. G., Shen, N. H., Andersen, B. D., Bjeldanes, L. F., and Hatch, F. T. 1986a. Identification of the mutagens in cooked beef. *Environ. Health Persp.* 67: 17.
- Felton, J. S., Knize, M. G., Shen, N. H., Lewis, P. R., Andresen, B. D., Happe, J., and Hatch, F. T. 1986b. The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PHIP). *Carcinogenesis* 7: 1081.
- Fukuhara, Y., Yoshida, D., and Goto F. 1981. Reduction of mutagenic products in the presence of polyphenols during pyrolysis of protein. *Agric. Biol. Chem.* 45: 1061.
- Gayada, D. P. and Pariza, M. W. 1983. Activation of 2-amino-3-methylimidazo-[4,5-f]quinoline and 2-aminofluorene for bacterial mutagenesis by primary monolayer cultures of adult rat hepatocytes. *Mutat. Res.* 118: 7.
- Grivas, S. and Jagerstad, M. 1984. Mutagenicity of some synthetic quinolines and quinoxalines related to IQ, MeIQ, or MeIQx in Ames test. *Mutat. Res.* 137: 29.
- Grivas, S. 1985. A convenient synthesis of the potent mutagen 3,4,8-trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine. *Acta Chem. Scand.* B39: 213.
- Grivas, S., Nyhammar, T., Olsson, K., and Jagerstad, M. 1985. Formation of a new mutagenic DiMeIQx compound in a model system by heating creatinine, alanine and fructose. *Mutat. Res.* 151: 177.
- Grivas, S. 1986. Efficient synthesis of mutagenic imidazo[4,5-f]-quinoxalin-2-amines via readily accessible 2,1,3-benzoselenadiazoles. *Acta Chem. Scand.* B40: 404.
- Grose, K. R., Grant, J. L., Bjeldanes, L. F., Andresen, B. D., Healy, S. K., Lewis, P. R., Felton, J. S., and Hatch, F. T. 1986. Isolation of the carcinogen IQ from fried egg patties. *J. Agric. Food Chem.* 34: 201.
- Hargraves, W. A. and Pariza, M. W. 1983. Purification and mass spectral characterization of bacterial mutagens from commercial beef extract. *Canc. Res.* 43: 1467.
- Hargraves, W. A. and Pariza, M. W. 1984. Mutagens in cooked foods. *Environ. Sci. Health* C2(1): 1.
- Hashimoto, Y., Takeda, K., Shudo, K., Okamoto, T., Sugimura, T. and Kosuge, T. 1978. Rat liver microsome-mediated binding to DNA of 3-amino-1-methyl-5H-pyrido[4,3-b]indole, a potent mutagen isolated from tryptophan pyrolysate. *Chem-Biol. Interactions* 23: 137.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1979. Structural identification of a modified base in DNA covalently bound with mutagenic 3-amino-1-methyl-5H-pyrido[4,3-b]indole. *Chem. Pharm. Bull.* 27: 1058.

- Hashimoto, Y., Shudo, K., and Okamoto, T. 1980a. Activation of a mutagen, 3-amino-1-methyl-5H-pyrido[4,3-b]indole. Identification of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole and its reaction with DNA. *Biochem. Biophys. Res. Comm.* 96: 355.
- Hashimoto, Y., Shudo, K., and Okamoto, T., 1980b. Metabolic activation of a mutagen, 2-amino-6-methyldipyrido-[1,2-a:3',2'-d]-imidazole. Identification of 2-hydroxyamino-6-methyldipyrido-[1,2-a:3',2'-d]imidazole and its reaction with DNA. *Biochem. Biophys. Res. Comm.* 92: 971.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1980c. Activation of a mutagen, 3-amino-1-methyl-5H-pyrido[4,3-b]indole. Identification of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole and its reaction with DNA. *Biochem. Biophys. Res. Comm.* 96: 355.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1982a. Modification of DNA with potent mutacarcinogenic 2-amino-6-methyldipyrido-[1,2-a:3',2'-d]imidazole isolated from a glutamic acid pyrolysate: Structure of the modified nucleic acid base and initial chemical event caused by the mutagen. *J. Am. Chem. Soc.* 104: 7636.
- Hashimoto, Y., Shudo, K., and Okamoto, T., 1982b. Modification of nucleic acid with muta-carcinogenic heteroaromatic amines in vivo. Identification of modified bases in DNA extracted from rats injected with 3-amino-1-methyl-5H-pyrido[4,3-b]indole and 2-amino-6-methyldipyrido-[1,2-a:3',2'-d]imidazole. *Mutat. Res.* 105: 9.
- Hashimoto, Y., and Shudo, K. 1983. Sequence selective modification of DNA with muta-carcinogenic 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole. *Biochem. Biophys. Res. Comm.* 116: 1100.
- Hashimoto, Y., Shudo, K., and Okamoto, T. 1984. Deoxyribonucleic acid modification by mutagenic 3-amino-1-methyl-5H-pyrido[4,3-b]indole: The chemical events. *Chem. Pharm. Bull.* 32: 4300.
- Hatch, F. T., Felton, J. S., Stuermer, D. H., and Bjeldanes, L. F. 1984. Identification of mutagens from the cooking of food. In *Chemical Mutagens*, Serres de, F. J., (Ed.), 9: 111. Plenum Publishing Corporation, New York.
- Hatch, F. T. 1986. A current genotoxicity database for heterocyclic thermic food mutagens. I. Genetically relevant endpoints. *Environ. Health Persp.* 67: 93.
- Hatch, F. T. and Felton, J. S. 1986. Toxicologic strategy for mutagens formed in foods during cooking: Status and needs. In *Genetic Toxicology of the Diet*, Knudsen, I., (Ed.), Progress in Clinical and Biological Research, 206: 109, Alan R. Liss, Inc., New York.
- Hayatsu, H., Arimoto, S., Togawa, K., and Makita, M. 1981a. Inhibitory effect of the ether extract of human feces on activities of mutagens: Inhibition of oleic and linoleic acids. *Mutat. Res.* 81: 287.
- Hayatsu, H., Inoue, K., Ohta, H., Namba, T., Togawa, K., Hayatsu, T., Makita, M., and Wataya, Y. 1981b. Inhibition of the mutagenicity of cooked-beef basic fraction by its acidic fraction. *Mutat. Res.* 91: 437.

- Hayatsu, H., Oka, T., Wakata, A., Ohara, Y., Hayatsu, T., Kobayashi, H., Arimoto, S. 1983a. Adsorption of mutagens to cotton bearing covalently bound trisulfo-copper-phthalocyanine. *Mutat. Res.* 119: 233.
- Hayatsu, H., Matsui, Y., Oharam, Y., Oka, T., and Hayatsu, T. 1983b. Characterization of mutagenic fractions in beef extract and in cooked ground beef. Use of blue-cotton for efficient extraction. *Gann* 74: 472.
- Hayatsu, H., Hayatsu, T., and Ohara, Y., 1985a. Mutagenicity of human urine caused by ingestion of fried ground beef. *Jpn. J. Canc. Res. (Gann)*, 76: 445.
- Hayatsu, H., Hayatsu, T., Wataya, Y., and Mower, H. F. 1985b. Fecal mutagenicity arising from ingestion of fried ground beef in the human. *Mutat. Res.* 143: 207.
- Hino, O., Nemoto, N., Nagao, M., Kosugi, A., and Kitagawa, T. 1982. Induction of drug-metabolizing enzymes in the rat liver by 3'-methyl-4-(dimethylamino)azobenzene. *Canc. Lett.* 15: 131.
- Hosaka, S., Matsushima, T., Hirono, I., and Sugimura, T. 1981. Carcinogenic activity of 3-amino-1-methyl-5H-pyrido[4,3-b]indole. (Trp-P-2), a pyrolysis product of tryptophan. *Canc. Lett.* 13: 23.
- Imamura, M., Takeda, K., Shudo, K., Okamoto, T., Nagata, C. and Kodama, M. 1980. Non-covalent interaction with DNA of the mutagens 2-amino-dipyrido[1,2-a:3',2'-d]imidazole and methyl-substituted isomers. *Biochem. Biophys. Res. Comm.* 96: 611.
- Inoue, T., Morita, K., and Kata, T. 1981. Purification and properties of a plant desmutagenic factor for the mutagenic principle of tryptophan pyrolysate. *Agric. Biol. Chem.* 45: 345.
- Ishii, K., Yamazoe, Y., Kamataki, T., and Kato, R. 1981. Metabolic activation of glutamic acid pyrolysis products, 2-amino-6-methyldipyrido-[1,2-a:3',2'-d]imidazole and 2-amino-dipyrido-[1,2-a:3',2'-d]imidazole, by purified cytochrome P-450. *Chem. Biol. Interactions* 38:1.
- Ishikawa, F., Takaku, F., Ochiai, M., Hayashi, K., Hirohashi, S. Terada, M., Takayama, S., Nagao, M., and Sugimura, T. 1985. Activated c-raf gene in a rat hepatocellular carcinoma induced by 2-amino-3-methylimidazo-[4,5-f]quinoline. *Biochem. Biophys. Res. Comm.* 132: 186.
- Jägerstad, M., Reuterswärd, A. L., Oste, R., Dahlqvist, A., Grivas, S., Olsson, K., and Nyhammar, T. 1982. Creatinine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. *ACS Symposium Series* 215: 507.
- Jägerstad, M., Olsson, K., Grivas, S. Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., and Sugimura, T. 1984. Formation of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in a model system by heating creatinine, glycine, and glucose. *Mutat. Res.* 26: 239.

- Jägerstad, M., and Grivas, S. 1985. The synthesis and mutagenicity of the 3-ethyl analogues of the potent mutagens IQ, MeIQ, MeIQx and its 3,7-dimethyl isomer. *Mutat. Res.* 144: 131.
- Kada, T., Mochizuki, H., and Miyao, K. 1984. Antimutagenic effects of germanium oxide on Trp-P-2 induced frameshift mutation in *Salmonella typhimurium* TA98 and TA1538. *Mutat. Res.* 125: 145.
- Kada, T., Inoue, T., Morita, K., and Namiki, M. 1986. Dietary desmutagens. In *Genetic Toxicity of the Diet*, Knudsen, I. (Ed.). Alan R. Liss, Inc., New York, p. 245.
- Kamataki, T., Maeda, K., Yamozoe, Y., Matsuda, N., Ishii, K., and Kato, R. 1983. A high-spin form of cytochrome P-450 highly purified from poly-chlorinated biphenyl-treated rats. Catalytic characterization and immunochemical quantitation in liver microsomes. *Mol. Pharmacol.* 24: 146.
- Kasai, H., Nishimura, S., Nagao, M., Takahashi, Y., and Sugimura, T. 1979. Fractionation of a mutagenic principle from broiled fish by high-pressure liquid chromatography. *Canc. Lett.* 7: 343.
- Kasai, H., Nishimura, S., Wakabayashi, K., Nagao, M., and Sugimura, T. 1980a. Chemical synthesis of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), a potent mutagen isolated from broiled fish. *Proc. Japan Acad.* 56: 382.
- Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., and Nishimura, S. 1980b. Structure and chemical synthesis of Me-IQ, a potent mutagen isolated from broiled fish. *Chem. Lett.* 1391.
- Kasai, H., Yamaizumi, Z., Wakabayashi, K., Nagao, M., Sugimura, T., Yokoyama, S., Miyazawa, T., Spingarn, N. E. Weisburger, J. H., and Nishimura, S. 1980c. Potent novel mutagens produced by broiling fish under normal conditions. *Proc. Japan Acad.* 56 Ser. B: 278.
- Kasai, H., Shiomi, T., Sugimura, T., and Nishimura, S. 1981a. Synthesis of 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (Me IQx), a potent mutagen isolated from fried beef. *Chem. Lett.* 675.
- Kasai, H., Yamizumi, Z., and Nishimura, S. 1981b. A potent mutagen in broiled fish. Part 1. 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline. *J. C. S. Perkin I*: 2290.
- Kato, R., Kamataki, T., and Yamazoe, Y. 1983. N-hydroxylation of carcinogenic amines. *Environ. Health Persp.* 49:21.
- Kawajiri, K., Yonekawa, H., Gotoh, O., Watanabe, J., Igarashi, S., and Tagashira, Y. 1983. Contributions of two inducible forms of cytochrome P-450 carcinogens. *Canc. Res.* 43: 819.
- Kimura, T., Nakayama, T., Kurosaki, Y., Suzuki, Y., Arimoto, S., and Hayatsu, H. 1985. Absorption of 3-amino-1-methyl-5H-pyridol-[4,3-b]-indole, a mutagen-carcinogen present in tryptophan pyrolysate, from the gastrointestinal tract in the rat. *Jpn. J. Canc. Res. (Gann)*, 76: 272.

- Knudsen, I. 1982. Natural, processed, and artificial mutagens in food-significance and consequences. In *Mutagens in Our Environment*, p. 315. Alan R. Liss, Inc., New York, NY.
- Krone, C. A. and Iwaoka, W. T. 1984. Occurrence of mutagens in canned foods. *Mutat. Res.* 141: 131.
- Krone, C. A., Yeh, S. M. J., and Iwaoka, W. T. 1986. Mutagen formation during commercial processing of foods. *Environ. Health Persp.* 67: 75.
- Kuratsune, M., Ikeda, M., and Hayashi, T. 1986. Epidemiologic studies on possible health effects of intake of pyrolyzates of foods, with reference to mortality among Japanese Seventh Day Adventists. *Environ. Health Persp.* 67: 143.
- Kuroda, M., Yoshida, D., and Mizusaki, S. 1985. Mutagenicity of pyrolyzates of natural substances toward *Salmonella typhimurium* TA97. *Agric. Biol. Chem.* 49: 1893.
- Kushi, A., Koiwai, A., Yoshida, D., and Goto, F. 1980. Effect of emodin on the mutagenicity of 3-amino-1-methyl-5H-pyrido[4,3-b]-indol toward *Salmonella*. *Agric. Biol. Chem.* 44: 2513.
- Lee, C.-S., Hashimoto, Y., Shudo, K., Okamoto, T. 1982. Synthesis of mutagenic heteroaromatics: 2-aminoimidazo[4,5-f]quinolines. *Chem. Pharm. Bull.* 30: 1857.
- Lijinsky, W. and Shubik, P. 1964. Benzy(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. *Science* 145: 53.
- Lodge, D. C. and Daniel, J. W. 1984. Mutagens in Foods. *British Nutrition Foundation Nutr. Bull.* 9: 32.
- Loretz, L. and Pariza, M. W. 1984. Effect of glutathione levels, sulfate levels, and metabolic inhibitors on covalent binding of 2-amino-3-methylimidazo[4,5-f]quinoline and 2-acetylaminofluorene to cell macromolecules in primary monolayer cultures of adult rat hepatocytes. *Carcinogenesis* 5: 895.
- Loury, D. J. and Byard, J. L. 1983. Aroclor 1254 pretreatment enhances the DNA repair response to amino acid pyrolysate mutagens in primary cultures of rat hepatocytes. *Canc. Lett.* 20: 283.
- Loury, D. J., Kado, N. Y., and Byard, J. L. 1985. Enhancement of hepto-cellular genotoxicity of several mutagens from amino acid pyrolysates and broiled foods following ethanol pretreatment. *Fd. Chem. Toxic.* 23: 661.
- Magee, P. H., and Barnes, J. M. 1956. The production of primary hepatic tumors in the rat by feeding dimethylnitrosamine. *Br. J. Canc.* 10: 114.
- Masuda, Y., Mori, K., and Kuratsune, M. 1967. Studies on bladder carcinogens in the human environment. I. Naphthylamines produced by pyrolysis of amino acids. *Int. J. Canc.* 2: 489.

- Matsukura, N., Kawachi, T., Morino, K., Ohgaki, H., and Sugimura, T. 1981a. Carcinogenicity in mice of mutagenic compounds from a tryptophan pyrolyzate. *Science* 231: 346.
- Matsukura, N., Kawachi, T., Wakabayashi, K., Ohgaki, H., Morino, K., Sugimura, T., Nukaya, H., and Kosuge, T. 1981b. Liver cancer and pre-cancerous changes in rats induced by the basic fraction of tryptophan pyrolysate. *Canc. Lett.* 13: 181.
- Matsumoto, T., Yoshida, D., Tomita, H. 1981a. Synthesis and mutagenic activity of alkyl derivatives of 2-amino-9H-pyrido[2,3-b]-indole. *Agric. Biol. Chem.* 45: 2031.
- Matsumoto, T., Yoshida, D., and Tomita, H. 1981b. Determination of mutagens, amino- $\alpha$ -carbonilines in grilled foods and cigarette smoke condensate. *Canc. Lett.* 12: 105.
- Miller, A. J. and Buchanan, R. L. 1983a. Reduction of mutagen formation in cooked nitrite-free bacon by selected cooking treatments. *J. Food Sci.* 48: 1772.
- Miller, A. J. and Buchanan, R. L. 1983b. Detection of genotoxicity in fried bacon by the *Salmonella*/mammalian microsome mutagenicity assay. *Fd. Chem. Toxic.* 21: 319.
- Miller, A. J. 1985. Processing-induced mutagens in muscle foods. *Food Technol.* (Feb.): 75.
- Minkler, J. L. and Carrano, A. V. 1984. In vivo cytogenetic effects of the cooked-food-related mutagens Trp-P-2 and IQ in mouse bone marrow. *Mutat. Res.* 140: 49.
- Mita, S., Ishii, K., Yamazoe, Y., Kamataki, T., Kato, R., and Sugimura, T. 1981a. Evidence for the involvement of N-hydroxylation of 3-amino-1-methyl-5H-pyrido[4,3-b]indole by cytochrome P-450 in the covalent binding to DNA. *Canc. Res.* 41: 3610.
- Mita, S., Yamazoe, Y., Kamataki, T., and Kato, R. 1981b. Metabolic activation of the tryptophan pyrolysis product, 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) by isolation rat liver nuclei. *Canc. Lett.* 14: 261.
- Mochizuki, H. and Kada, T. 1982. Antimutagenic action of cobaltous chloride on Trp-P-1-induced mutagens in *Salmonella typhimurium* TA98 and TA1538. *Mutat. Res.* 95: 145.
- Morita, K., Hara, M., and Kada, T. 1978. Studies on natural desmutagens; screening for vegetable and fruit factors active in inactivation of mutagenic pyrolysis products from amino acids. *Agric. Biol. Chem.* 42: 1235.
- Morita, K., Yamada, H., Iwamoto, S., Sotomura, M., Suzuki, A. 1982. Purification and properties of desmutagenic factor from broccoli (*Brassica Oleracea* Var. *Italica Plenck*) for mutagenic principle of tryptophan pyrolysate. *J. Food Safety* 4: 139.
- Müzner, R., and Wever, J. 1984. Investigations on the detection of mutagenic activity of beef extract in rats after oral administration. *Canc. Lett.* 23: 109.

- Nader, C. J., Spencer, L. K., and Weller, R. A. 1981. Mutagen production during pan-broiling compared with microwave irradiation of beef. *Canc. Lett.* 13: 147.
- Nagao, M., Honda, M., Seino, Y., Yahagi, T., and Sugimura, T., 1977a. Mutagenicities of smoke condensates and the charred surface of fish and meat. *Canc. Lett.* 2: 221.
- Nagao, M., Yahagi, T., Kawachi, T., Sugimura, T., Kosuge, T., Tsuji, K., Wakabayashi, K., Mizusaki, S., and Matsumoto, T. 1977b. Comutagenic action of norharman and harman. *Proc. Japan Acad.* 53: 95.
- Nagao, M., Wakabayashi, K., Kasai, H., Nishimura, S., and Sugimura, T. 1981. Effect of methyl substitution of mutagenicity of 2-amino-3-methylimidazo[4,5-f]quinoline, isolated from broiled sardine. *Carcinogenesis* 2: 1147.
- Nakayasu, M., Nakasato, F., Sakamoto, H., Terada, M., and Sugimura, T. 1983. Mutagenic activity of heterocyclic amines in Chinese hamster lung cells with diphtheria toxin resistance as a marker. *Mutat. Res.* 118: 91.
- Nagao, M., Fujita, Y., Wakabayashi, K., and Sugimura, T. 1983. Mutagenic activity of heterocyclic amines in Chinese hamster lung cells with diphtheria toxin resistance as a marker. *Mutat. Res.* 118: 91.
- Nebert, D. W., Bigelow, S. W., Okey, A. B., Yahage, T., More, Y., Nagao, M., and Sugimura, T. 1979. Pyrolysis products from amino acids and protein: highest mutagenicity requires cytochrome P<sub>1</sub>-450. *Proc. Nat. Acad. Sci.* 76: 5929.
- Negishi, T. and Hayatsu, H. 1979. The enhancing effects of cysteine and its derivatives on the mutagenic activities of the tryptophan-pyrolysis products Trp-P-1 and Trp-P-2. *Biochem. Biophys. Res. Comm.* 88: 97.
- Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., Sugimura, T., Saito, H., Maeda, M., and Jägerstad, M. 1984. Formation of 2-amino-3,7,8,-trimethylimidazo[4,5-f]quinoxaline, a new mutagen, by heating a mixture of creatine, glucose and glycine. *Mutat. Res.* 140: 55.
- Nelson, R. L. and Samuelson, S. L. 1984. Inability of the mutagen-blocking agent oleic acid to protect against colon carcinogenesis in the rat. *Mutat. Res.* 140: 155.
- Nemoto, N., Kusumi, S., Takayama, S., Nagao, M., and Sugimura, T. 1979. Metabolic activation of 3-amino-5H-pyrido[4,3-b]indole, a highly mutagenic principle in tryptophan pyrolysate, by rat liver enzymes. *Chem.-Biol. Interactions* 27: 191.
- Nemoto, N. and Takayama, S. 1984. Activation of 2-amino-6-methyl-dipyrido[1,2-a:3',2'-d]imidazole, a mutagenic pyrolysis product of glutamic acid, to bind to microsomal protein by NADPH-dependent and -independent enzyme systems. *Carcinogenesis* 5: 653.
- Nishimura, S. 1986. Chemistry of mutagens and carcinogens in broiled food. *Environ. Health Presp.* 67: 11.

- Nishioka, H., Nishi, K., and Kyokane, K. 1981. Human saliva inactivates mutagenicity of carcinogens. *Mutat. Res.* 85: 323.
- Niwa, T., Yamazoe, Y., and Kato, R. 1982. Metabolic activation of 2-amino-9H-pyrido[2,3-b]indole by rat-liver microsomes. *Mutat. Res.* 95: 159.
- Nyhammar, T. and Grivas, S. 1986. Synthesis of the potent mutagen 3,5,8-trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine. *Acta Chem. Scand. B* 40: 583.
- Nyhammar, T., Grivas, S., Olsson, K., and Jagerstad, M. 1986. Formation of 4,8-DiMeIQx from the model system fructose, alanine and creatine. Comparison with the isomeric 5,8-DiMeIQx. *Mutat. Res.* 174: 5.
- Ohe, T. 1982. Mutagenicity of pyrolysates from guanidine, ureide, secondary amines and polyamines found by the *Salmonella*/mammalian-microsome test. *Mutat. Res.* 101: 175.
- Ohgaki, H., Kusama, K., Matsukura, N., Marino, K., Hasegawa, H., Sato, S., Takayama, S., and Sugimura, T. 1984a. Carcinogenicity in mice of a mutagenic compound 2-amino-3-methylimidazo[4,5-f]quinoline, from broiled sardine, cooked beef, and beef extract. *Carcinogenesis* 5: 921.
- Ohgaki, H., Matsukura, N., Morino, K., Kawachi, T., Sugimura, T., and Takayama, S. 1984b. Carcinogenicity in mice of mutagenic compounds from glutamic acid and soybean globulin pyrolysates. *Carcinogenesis* 5: 815.
- Ohgaki, H., Hasegawa, H., Kato, T., Suenaga, M., Ubukata, M., Sato, S., Takayama, S., and Sugimura, T. 1985. Induction of tumors in the forestomach and liver of mice by feeding 2-amino-3,4-dimethylimidazo-[4,5-f]quinoline (MeIQ). *Proc. Jpn. Acad.* 61(B): 137.
- Okamoto, T., Shudo, K., Hashimoto, Y., Kosuge, T., Sugimura, T., and Nishimura, S. 1981. Identification of a reactive metabolite of the mutagen 2-amino-3-methylimidazo[4,5-f]quinoline. *Chem. Pharm. Bull.* 29: 590.
- Olsson, K., and Grivas, S. 1986. New synthetic routes to the potent mutagen 3,7,8-trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine. *Acta Chem. Scand. B* 40: 486.
- Ong, T.-M., Whong, W.-Z., Stewart, J., and Brockman, H. E. 1986. Chlorophyllin: a potent antimutagen against environmental and dietary complex mixtures. *Mutat. Res.* 173: 111.
- Overik, E., Nilsson, L., Fredholm, L., Levin, O., Nord, C.-E., and Gustafsson, J.-A. 1984. High mutagenic activity formed in pan-broiled pork. *Mutat. Res.* 135: 149.
- Pariza, M. W., Ashoor, S. H., and Chu, F. S. 1979a. Mutagens in heat-processed meat, bakery and cereal products. *Fd. Cosmet. Toxicol.* 17: 429.
- Pariza, M. W., Ashoor, S. H., Chu, F. S., and Lund, D. B. 1979b. Effects of temperature and time on mutagen formation in pan-fried hamburger. *Canc. Lett.* 7: 63.

- Pariza, M. W., Loretz, L. J., Storkson, J. M., and Holland, N. C. 1983. Mutagens and modulator of mutagenesis in fried ground beef. *Canc. Res. Suppl.* 43: 2444s.
- Pariza, M. W., and Hargraves, W. A. 1985. A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. *Carcinogenesis* 6: 591.
- Pariza, M. W., Hargraves, W. A., and Boissonneault, G. A. 1986a. Modulation of carcinogenesis by a beef-derived mutagenesis modulator and by dietary fat. In *Genetic Toxicology of the Diet*, Knudsen, I. (Ed.). Alan R. Liss, Inc., New York, p. 265.
- Pariza, M. W., Hargraves, W. A., Benjamin, H., Christou, M., Jefcoate, C. R., Storkson, J., Albright, K., Kraus, D., Sharp, P., Boissoneault, G. A., and Elson, C. E. 1986b. Modulation of carcinogenesis by dietary factors. *Environ. Health Persp.* 67: 25.
- Pezzuto, J. M., Lau, P. P., Luh, Y., Moore, P. D., Wogan, G. N., and Hecht, S. M. 1980. There is a correlation between the DNA affinity and mutagenicity of several 3-amino-1-methyl-5H-pyrido[4,3-b]indoles. *Proc. Natl. Acad. Sci.* 77: 1427.
- Pezzuto, J. M., Moore, P. D., and Hecht, S. M. 1981. Metabolic activation of 1-methyl-3-amino-5H-pyrido[4,3-f]indole and several structurally related mutagens. *Biochemistry* 20: 298.
- Prival, M. J. 1985. Carcinogens and mutagens present as natural components of food or induced by cooking. *Nutr. and Canc.* 6: 236.
- Rapoport, H., Waterhouse, A. L., Thompson, C. M., and O'Connell J. F. 1986. Synthesis and radiolabeling of heterocyclic food mutagens. *Environ. Health Persp.* 67: 41.
- Saito, K., Yamazoe, Y., Kamatki, T., and Kato, R. 1983a. Interactions between the active metabolite of tryptophan pyrolysate mutagens, N-hydroxy-Trp-P-2, and lipids: The role of lipid peroxides in the commision of N-hydroxy-Trp-P-2 to non-reactive forms. *Chem.-Biol. Interactions* 45: 295.
- Saito, K., Yamazoe, Y., Kamataki, T., and Kato, R. 1983b. Activation and detoxification of N-hydroxy-Trp-P-2 by glutathione and glutathione transferases. *Carcinogenesis* 4: 1551.
- Shibamoto, T. 1980. Heterocyclic compounds found in cooked meats. *J. Agric. Food Chem.* 28: 237.
- Shibamoto, T., Nishimura, O., and Mihara, S. 1981. Mutagenicity of products obtained from a maltol-ammonia browning model system. *J. Agric. Food Chem.* 29: 643.
- Shibamoto, T. 1984. Mutagen formation in browning model systems. *J. of App. Toxicol.* 4: 97.
- Shinohara, K., Wu, R.-T., Jahan, N., Tanaka, M., Morinaga, N., Murakami, H., and Omura, H. 1980a. Mutagenicity of the browning mixtures by amino-carbonyl reactions of *Salmonella typhimurium* TA100. *Agric. Biol. Chem.* 44: 671.

- Shinohara, K., Lee, J.-H., Tanaka, M., Murakami, H., and Omura, H. 1980b. Mutagenicity of intermediates produced in the early stage of the browning reaction of triose reductone with nucleic acid related compounds on bacterial tests. *Agric. Biol. Chem.* 44: 1727.
- Shinohara, K., Jahan, N., Tanaka, M., Yamamoto, K., Wu, R.-T, Murakami, H., and Omura, H. 1983. Formation of mutagens by amino-carbonyl reactions. *Mutat. Res.* 122: 279.
- Shinohara, A., Yamazoe, Y., Saito, K., Kamataki, T., and Kato, R. 1984. Species differences in the N-acetylation by liver cytosol of mutagenic heterocyclic aromatic amines in protein pyrolysates. *Carcinogenesis* 5: 683.
- Shinohara, A., Saito, K., Yamazoe, Y., Kamataki, T., and Kato, R. 1985. DNA binding of N-hydroxy-Trp-P-2 and N-hydroxy-Glu-P-1 by acetyl-CoA dependent enzyme in mammalian liver cytosol. *Carcinogenesis* 6: 305.
- Shishido, K., Tachibana, T., and Ando, T. 1980. Enzymatic studies on binding of mutagenic principles in tryptophan pyrolysate to DNA. *Agric. Biol. Chem.* 44: 1609.
- Sjödin, P. and Jägerstad, M. 1984. A balance study of <sup>14</sup>C-labelled 3-H-imidazo[4,5-f]quinolin-2-amines (IQ and MeIQ) in rats. *Fd. Chem. Toxicol.* 22: 307.
- Sjödin, P. B., Nyman, M. E., Nilsson, L., Asp, N. L., and Jägerstad, M. I. 1985. Binding of <sup>14</sup>C-labeled food mutagens
- Spingarn, N. E., and Garvie, C. T. 1979. Formation of mutagens in sugar-ammonia model systems. *J. Agric. Food Chem.* 27: 1319.
- Spingarn, N. E. and Weisburger, J. H. 1979. Formation of mutagens in cooked foods. I. Beef. *Canc. Lett.* 7: 259.
- Spingarn, N. E., Kasai, H., Vuolo, L. L., Nishimura, S., Yamaizumi, Z., Sugimura, T., Matasushima, T., and Weisburger, J. H. 1980. Formation of mutagens in cooked foods. III. Isolation of a potent mutagen from beef. *Canc. Lett.* 9: 177.
- Sugimura, T., Nagao, M., Kawachi, T., Honda, M., Yahagi, T., Seino, Y., Sato, S., Matsukura, N., Matsushima, T., Shirai, A., Sawamura, M., and Matsumoto, H. 1977a. Mutagen-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. In *Origins of Human Cancer*. Cold Spring Harbor Laboratory, New York, p. 1561.
- Sugimura, T., Kawachi, T., Nagao, M., Yahagi, T., Seino, Y., Okamoto, T., Shudo, K., Kosuge, T., Tsuji, K., Wakabayashi, K., Iitaka, Y., and Itai, A. 1977b. Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. *Proc. Japan Acad.* 53: 58.
- Sugimura, T. 1985. Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. *Mutat. Res.* 150: 33.
- Sugimura, T. 1986a. Studies on environmental chemical carcinogenesis in Japan. *Science* 233: 312.

- Sugimura, T., 1986b. Past, present, and future of mutagens in cooked foods. *Environ. Health Persp.* 67: 5.
- Sugimura, T., Sato, S., Ohgaki, H., Takayama, S., Nagao, M., and Wakabayashi, K. 1986. Mutagens and carcinogens in cooked foods. In *Genetic Toxicology of the Diet*, Knudsen, I. (Ed.). Alan R. Liss, Inc., New York, p. 85.
- Takahashi, M., Wakabayashi, K., Nagao, T., Yamamoto, M., Masui, T., Goto, T., Kinae, N., Tomita, I., and Sugimura, T., 1985. Quantification of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline (MeIQx) in beef extracts by liquid chromatography with electrochemical detection (LCEC). *Carcinogenesis* 6: 1195.
- Takayama, S. and Tanaka, M. 1983. Mutagenesis of amino acid pyrolysis products in Chinese hamster V79 cells. *Toxicol. Lett.* 17: 23.
- Takayama, S., Masuda, M., Mogami, M., Ohgaki, H., Sato, S., and Sugimura, T. 1984. Induction of cancers in the intestine, liver, and various other organs of rats by feeding mutagens from glutamic acid pyrolysate. *Gann* 75: 207.
- Takayama, S., Nakatsuru, Y., Ohgaki, H., Sato, S., and Sugimura, T. 1985a. Carcinogenicity in rats of a mutagenic compound, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, from tryptophan pyrolysate. *Jpn. J. Canc. Res. (Gann)*, 76: 815.
- Takayama, S., Natusuru, Y., Ohgaki, H., Sato, S., and Sugimura, T. 1985b. Atrophy of salivary glands and pancreas of rats fed a diet with aminomethyl- $\alpha$ -carboline. *Proc. Japan Acad.* 61: 277.
- Takeda, K., Shudo, K., Okamoto, T., and Kosuge, T. 1978. Synthesis of mutagenic principles isolated from L-glutamic acid pyrolysate. *Chem. Pharm. Bull.* 26: 2924.
- Takeda, K., Shudo, K., Okamoto, T., Nagao, M., Wakabayashi, K. and Sugimura, T. 1980. Effect of methyl substitution on mutagenicity of 2-amino-dipyrido[1,2-a:3',2'-d]imidazole, Glu-P-2. *Carcinogenesis* 1: 889.
- Takeda, K., Shudo, K., Okamoto, T., and Kosuge, T. 1981. Synthesis of mutagens isolated from tryptophan pyrolysate and of some analogs, 3-amino-5H-pyrido[4,3-b]indoles. *Chem. Pharm. Bull.* 295: 1280.
- Taylor, R. T., Fultz, E., and Shore, V. 1981. Food mutagen formation in model boiling systems. *Environ. Mutagen.* 3: 349.
- Taylor, R. T., Fultz, E., and Shore, V. 1984a. Mutagen formation in a model beef boiling system. I. Conditions with a soluble beef-derived fraction. *J. Environ. Sci. Health A19*: 791.
- Taylor, R. T., Shore, V., and Fultz, E. 1984b. Mutagen formation in a model beef boiling system. II. Effects of proteolysis and comparison of soluble fractions from several protein sources. *J. Environ. Sci. Health, A19*: 819.

- Taylor, R. T., Fultz, E., and Knize, M. 1986. Mutagen formation in a model beef supernatant fraction. IV. Properties of the system. *Environ. Health Persp.* 67: 59.
- Thompson, L. H., Carrano, A. V., Salazar, E., Felton, J. S., and Hatch, F. T. 1983. Comparative genotoxic effects of the cooked-food-related mutagens Trp-P-2 and IQ in bacteria and cultured mammalian cells. *Mutat. Res.* 117: 243.
- Toda, H., Sekizawa, J., and Shibamoto, T. 1981. Mutagenicity of the L-rhamnose-ammonia-hydrogen sulfide browning reaction mixture. *J. Agric. Food Chem.* 29: 381.
- Tomoda, R., Kusunoki, S., Nakashima, K., Matsunaga, T. 1986. Use of copper-phthalocyanine membrane electrode for rapid preliminary detection of polycyclic mutagens. *Mutat. Res.* 164: 203.
- Tsuda, M., Takahashi, Y., Nagao, M., Hirayama, T., and Sugimura, T. 1980. Inactivation of mutagens from pyrolysates from tryptophan and glutamic acid by nitrite in acidic solution. *Mutat. Res.* 78:331.
- Tsuda, M., Nagao, M., Hirayama, T., and Sugimura, T. 1981. Nitrate converts 2-amino- $\alpha$ -carboline, an indirect mutagen, into 2-hydroxy- $\alpha$ -carboline, a non-mutagen and 2-hydroxy-3-nitroso- $\alpha$ -carboline, a direct mutagen. *Mutat. Res.* 83: 61.
- Tsuda, M., Wakabayashi, K., Hirayama, T., Kawachi, T., and Sugimura, T. 1983. Inactivation of potent pyrolysate mutagens by chlorinated tap water. *Mutat. Res.* 119: 27.
- Tsuda, M., Negishi, C., Makino, R., Sato, S., Yamaizumi, Z., Hirayama, T., and Sugimura, T. 1985. Use of nitrite and hypochlorite treatments in determination of the contributions of IQ-type and non-IQ-type heterocyclic amines to the mutagenicities in crude pyrolyzed materials. *Mutat. Res.* 147: 335.
- Turesky, R. J., Wishnok, J. S., Tannenbaum, S. R., Pfund, R. A., and Buchi, G. H. 1983. Qualitative and quantitative characterization of mutagens in commercial beef extract. *Carcinogenesis* 4: 863.
- Turesky, R. J., and Skipper, P. L. 1986. Sulfamate formation is a major route for detoxification of 2-amino-3-methylimidazo[4,5-f]-quinoline in the rat. *Carcinogenesis* 7: 1483.
- Uyeta, M., Kanada, T., Mazaki, M., Taue, S., and Takahashi, S. 1979. Assaying mutagenicity of food pyrolysis products using the Ames test. In *Naturally Occurring Carcinogens-Mutagens and Modulators in Carcinogenesis*, Miller E. C. et al. (Eds.). Japan Sci. Soc. Press, Tokoyo/Univ. Park Press, Baltimore, p. 169.
- Vithayathil, A. J., Commoner, B., Nair, S., and Madyastha, P. 1978. Isolation of mutagens from bacterial nutrients containing beef extract. *J. Toxicol. Environ. Health* 4: 189.
- Vithayathil, A. J., Strasik, M., and Strasik, L. 1983. Heat-induced mutagen formation from creatine and fat-soluble constituents of food. *Mutat. Res.* 121: 167.

- Wakabayashi, K., Tsuji, K., Kosuge, T., Takeda, K., Yamaguchi, K., Shudo, K., Iitaka, Y., Okamoto, T., Yahagi, T., Nagao, M., and Sigimura, T. 1978. Isolation and structure determination of a mutagenic substance in L-lysine pyrolysate. *Proc. Japan Acad.* 54: 569.
- Wakata, A., Oka, N., Hiramoto, K., Yoshioka, A., Negishi, K., Wataya, Y., and Hayatsu, H. 1985. DNA strand cleavage in vitro by 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole, a direct-acting mutagen formed in the metabolism of carcinogenic 3-amino-1-methyl-5H-pyrido[4,3-b]indole. *Canc. Res.* 45: 5867.
- Wang, Y. Y., Vuolo, L. L., Spingarn, N. E., and Weisburger, J. H. 1982. Formation of mutagens in cooked foods. V. The mutagen reducing effect of soy protein concentrates and antioxidants during frying of beef. *Canc. Letts.* 16: 179.
- Watanabe, J., Kawajiri, K., Yonekawa, H., Nagao, M., and Tagashira, Y. 1982a. Immunological analysis of the roles of two major types of cytochrome P-450 in mutagenesis of compounds isolated from pyrolysates. *Biochem. Biophys. Res. Comm.* 104: 193.
- Watanabe, T., Yokoyama, S., Kasai, H., Nishimura, S., Miyazawa, T., 1982b. DNA-binding of IQ, MeIQ, MeIQx, strong mutagens found in broiled foods. *Nucleic Acids Res.* 11: 163.
- Watanabe, T., Yokoyama, S., Hayashi, K., Kasai, H., Nishimura, S., and Miyazawa, T. 1982c. DNA-binding of IQ, MeIQ, and MeIQx, strong mutagens found in broiled foods. *FEBS Letts.* 150: 434.
- Waterhouse, A. L. and Rapoport, H. 1985. Synthesis and tritium labeling of the food mutagens IQ and methyl-IQ. *J. of Labelled Compounds and Radio-pharmaceuticals* 22: 201.
- Waziers de, I., and Decloitre, F. 1983. Formation of mutagenic derivatives from tryptophan pyrolysis products (Trp-P-1 and Trp-P-2) by rat intestinal S9 fraction. *Mutat. Res.* 119: 103.
- Wild, D., Gocke, E., Harnasch, D., Kaiser, G., and King, M.-T. 1985. Differential mutagenic activity of IQ (2-amino-3-methylimidazo[4,5-f]-quinoline) in *Salmonella typhimurium* strains in vitro and in vivo, in *Drosophila*, and in mice. *Mutat. Res.* 156: 93.
- Wild, D., Kaiser, G., King, M.-T., and Harnasch, D. 1986. Genotoxic activity of IQ (2-amino-3-methylimidazo[4,5-f]quinoline) and structural analogs. In *Genetic Toxicology of the Diet*, Knudsen, I. (Ed.). Alan R. Liss, Inc., New York, p. 145.
- Williams, G. M. 1986. Food-borne carcinogens. In *Genetic Toxicology of the Diet*, I. Knudsen, (Ed.). Alan R. Liss, Inc., New York, p. 73.
- Wynder, E. L. and Gori, G. B. 1977. Contribution of the environment to cancer incidence: an epidemiologic exercise. *J. Nat. Canc. Inst.* 58: 825.

- Yamada, M., Tsuda, M., Nagao, M., Mori, M., and Sugimura, T. 1979. Degradation of mutagens from pyrolysates of tryptophan, glutamic acid and globulin by myeloperoxidase. *Biochem. Biophys. Res. Comm.* 90: 769.
- Yamaguchi, K., Zenda, H., Shudo, K., Kosuge, T., Okamoto, T., and Sugimura, T. 1979. Presence of 2-aminodipyrido[1,2-a:3',2'-d]imidazole in casein pyrolysate. *Gann* 70: 849.
- Yamaguchi, K., Shudo, K., Okamoto, T., Sugimura, T., and Kosuge, T. 1980a. Presence of 2-amino-dipyrido[1,2-a:3'-2'-d]-imidazole in ordinary broiled cuttlefish. *Gann* 71: 743.
- Yamaguchi, K., Shudo, K., Okamoto, T., Sugimura, T., and Kosuge, T. 1980b. Presence of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole in broiled beef. *Gann*. 71: 745.
- Yamaguchi, T. 1982. Reduction of induced mutability with biologically active quinones through inhibition of metabolic activation. *Agric. Biol. Chem.* 46: 2373.
- Yamaguchi, T. and Nakagawa, K. 1983. Reduction of induced mutability with xanthine and imidazole-derivatives through inhibition of metabolic activation. *Agric. Biol. Chem.* 47: 1673.
- Yamaizumi, Z., Shiomi, T., Kasai, H., Nishimura, S., Takahashi, Y., Nagao, M., and Sugimura, T. 1980a. Detection of potent mutagens, Trp-P-1 and Trp-P-2, in broiled fish. *Canc. Lett.* 9: 75.
- Yamaizumi, Z., Shiomi, T., Kasai, H., Nishimura, S., Takahashi, Y., Nagao, M., and Sugimura, T. 1980b. Detection of potent mutagens, Trp-P-1 and Trp-P-2, in broiled fish. *Canc. Lett.* 9: 75.
- Yamaizumi, Z., Shiomi, T., Kasai, H., Wakabayashi, K., Nagao, M., Sugimura, T., and Nishimura, S. 1980b. Quantitative analysis of a novel potent mutagen, 2-amino-3-methyl-imidazo[4,5-f]quinoline, present in broiled food by GC/MS. *Koenshu-lyo Masu Kenkuyaki.* 5: 245.
- Yamaizumi, Z., Kasai, H., Nishimura, S., Edmonds, C. G., and McCloskey, J. A. 1986. Stable isotope dilution quantification of mutagens in cooked foods by combined liquid chromatography-thermospray mass spectrometry. *Mutat. Res.* 173: 1.
- Yamamoto, T., Tsuji, K., Kosuge, T., Okamoto, T., Shudo, K., Takeda, K., Iitaka, Y., Yamaguchi, K., Seino, Y., Yahagi, T., Nagao, M., and Sugimura, T. 1978. Isolation and structure determination of mutagenic substances in L-glutamic acid pyrolysate. *Proc. Japan Acad.* 54: 248.
- Yamazoe, Y., Ishii, K., Kamataki, T., Kato, R., and Sugimura, T. 1980a. Isolation and characterization of active metabolites of tryptophan pyrolysate mutagen, Trp-P-2, formed by rat liver microsomes. *Chem. Biol. Interact.* 30: 125.
- Yamazoe, Y., Yamaguchi, N., Kamataki, T., and Kato, R. 1980b. Metabolic activation of Trp-P-2, a mutagenic amine from tryptophan-pyrolysate, by liver microsomes from 3-methylcholanthrene-responsive and non-responsive mice. *Xenobiotica* 10: 483.

- Yamazoe, Y., Kamataki, T., and Kato, R. 1981a. Species difference in N-hydroxylation of a tryptophan pyrolysis product in relation to mutagenic activation. *Canc. Res.* 41: 4518.
- Yamazoe, Y., Tada, M., Kamataki, T., and Kato, R. 1981b. Enhancement of binding of N-hydroxy-Trp-P-2 to DNA by seryl-tRNA synthetase. *Biochem. Biophys. Res. Comm.* 102: 432.
- Yanagisawa, H., Manabe, S., Kitagawa, Y., Ishikawa, S., Nakajima, K., and Wada, O. 1986. Presence of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in dialysate from patients with uremia. *Biochem. and Biophys. Res. Comm.* 138: 1084.
- Yokota, M., Narita, K., Kosuge, T., Wakabayashi, K., Nagao, M., Sugimura, T., Yamaguchi, K., Shudo, K., Iitaka, Y., and Okamoto, T. 1981. A potent mutagen isolated from a pyrolysate of L-ornithine. *Chem. Pharm. Bull.* 29: 1473.
- Yokoyama, S., Miyazawa, T., Kasai, H., Nishimura, S., Sugimura, T., and Iitaka, Y. 1980. Crystal and molecular structures of 2-amino-3-methylimidazo[4,5-f]quinoline, a novel potent mutagen found in broiled food. *FEBS Lett.* 122: 261.
- Yoo, M. A., Ryo, H., Todo, T., and Kondo, S. 1985. Mutagenic potency of heterocyclic amines in the *Drosophila* wing spot test and its correlation to carcinogenic potency. *Jpn. J. Canc. Res. (Gann)*, 76: 468.
- Yoshida, D. and Matsumoto, T. 1978. Changes in mutagenicity of protein pyrolyzates by reaction with nitrite. *Mutat. Res.* 58: 35.
- Yoshida, D., Matsumoto, T., Yoshimura, R., and Matsuzaki, T. 1978. Mutagenicity of amino- $\alpha$ -carboline in pyrolysis products of soybean globulin. *Biochem. Biophys. Res. Comm.* 83: 915.
- Yoshida, D. and Okamoto, H. 1980a. Formation of mutagens by heating creatine and glucose. *Biochem. Biophys. Res. Comm.* 96: 844.
- Yoshida, D. and Okamoto, H. 1980b. Formation of mutagens by heating the aqueous solution of amino acids and some nitrogenous compounds with addition of glucose. *Agric. Biol. Chem.* 44: 2521.
- Yoshida, D., and Okamoto, H. 1982. Mutagenicity of the pyrolysis products of ammonium salts. *Agric. Biol. Chem.* 46: 1067.
- Yoshida, D., Saito, Y., and Mizusaki, S. 1984. Isolation of 2-amino-3-methyl-imidazo-[4,5-f]quinoline as a mutagen from the heated product of a mixture of creatine and proline. *Agric. Biol. Chem.* 48: 241.
- Yoshida, D. and Mizusaki, S. 1985. Formation of mutagens by heating amino acids with addition of hydroquinone. *Agric. Biol. Chem.* 49: 1199.