

A Current Genotoxicity Database for Heterocyclic Thermic Food Mutagens.

I. Genetically Relevant Endpoints

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Cooking, heat processing, or pyrolysis of protein-rich foods induce the formation of a series of structurally related heterocyclic aromatic bases that have been found to be mutagens. The primary genetic assay utilized to detect and isolate these mutagens has been the *his* reversion assay in *Salmonella typhimurium*. The classification and nomenclature of these chemicals is revised to reflect recent advances. The findings of short-term tests for genetic injury that have been applied to these agents are presented in a systematic way. Cell-free, bacterial, mammalian cell culture, and *in vivo* systems are included. Major results, the mutagens tested, and key references are presented in tabular form, with text commentary. Integrated conclusions on the state of current knowledge of the genetic toxicity of thermic food mutagens are presented. Areas in need of further research are defined. Finally, an outline is presented of a suggested path leading to the determination whether normal methods of food preparation and processing constitute a human health hazard.

Introduction

Cooking, heat processing, or pyrolysis of protein-rich foods induce the formation of a series of structurally related heterocyclic aromatic bases that have been found to be mutagens (1). Approximately 16 such structures have been partially or completely identified to late 1984 in extracts from cooked foods, heated amino acids or proteins, and model heating systems containing simple molecular precursors. The exclusive tool for detecting the presence and tracking the isolation of these substances has been the *Salmonella* mutagenesis assay of Ames et al. (2), primarily with the strains sensitive to frameshift mutations (TA 1538 and TA 98). Thus, the compounds are, by definition, bacterial mutagens, although their potency in the Ames assay varies over nearly five orders of magnitude (3). Some of them exhibit very high potency, second only to the dinitropyrenes in the magnitude of their mutagenic effect in *Salmonella*. Naturally, these potent compounds, when made available by organic synthesis in sufficient quantities for testing, have received the most attention to date in studies of their genotoxic effects in other short-term assays and in rodent carcinogenesis bioassays.

It is the objective of this review to summarize in a systematic way the current database of genetically relevant tests in cell-free, bacterial, mammalian cell culture, and *in vivo* systems. Our emphases will be on tabulation of key references, on major results, and the

mutagens from which they were derived, on integrated conclusions about genetic effects, and on areas in need of further research.

Revised Classification of the Heterocyclic Thermic Mutagens

The research effort on the heterocyclic thermic mutagens arose in a few laboratories in Japan from studies on the origin of mutagens in cigarette smoke condensate (4). The studies then turned to amino acids, proteins, and finally to protein-rich foods. In rapid succession twelve mutagens of this type were characterized chemically. Owing to the complexity of their proper chemical nomenclature, common names and abbreviations were assigned that have now permeated the literature and meeting discussions. Very recently several new compounds have been added to the list: some of them are methylated congeners of those already identified (in some cases with still unproven position assignments for the methyl group); others begin a new series of pyridine derivatives. It is now sadly apparent that some of the common names have undesirable features, e.g., trimethylated compounds being called "dimethyl—" when the parent already possessed an unnamed methyl group.

The present listing of the fully and provisionally identified heterocyclic thermic mutagens is presented in Table 1; structures are shown in Figure 1. The classification has been revised to reflect the new series of mutagens from beef that is being characterized in our

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Table 1. Revised classification of heterocyclic thermic mutagens.

Common name	Systematic name	CAS no.	Mol.wt	Formula	Methyl sites
I. Amino-carboline congeners					
A. Alpha carbolines					
1. AAC	2-Amino-9H-pyrido(2,3-b)indole	26148-68-5	183	C ₁₁ H ₉ N ₃	0
2. MeAC	2-Amino-3-methyl-9H-pyrido(2,3-b)indole	68006-83-7	197	C ₁₂ H ₁₁ N ₃	3
B. Gamma carbolines					
1. Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrido(4,3-b)indole	62450-06-0	211	C ₁₃ H ₁₃ N ₃	1,4
2. Trp-P-2	3-Amino-1-methyl-5H-pyrido(4,3-b)indole	62450-07-1	197	C ₁₂ H ₁₁ N ₃	1
C. Delta-azacarbolines					
1. Glu-P-1	2-Amino-6-methyldipyrido(1,2-a:3,2'-d)imidazole	67730-11-4	198	C ₁₁ H ₁₀ N ₄	6
2. Glu-P-2	2-Aminodipyrido(1,2-a:3,2'-d)imidazole	67730-10-3	184	C ₁₀ H ₈ N ₄	0
D. Other N-heterocyclics					
1. Lys-P-1	3,4-Cyclopentenopyrido(3,2-a)carbazole		258	C ₁₈ H ₁₄ N ₂	0
2. Orn-P-1	4-Amino-6-methyl-1H-2,5,10,10b-tetraazafluoranthene		237	C ₁₈ H ₁₁ N ₅	6
II. Aminoimidazo azaarenes					
A. Pyridine congeners					
1. Phe-P-1	2-Amino-5-phenylpyridine	33421-40-8	170	C ₁₁ H ₁₀ N ₂	0
2. 1-M-5-PhIP	2-Amino-1-methyl-5-phenylimidazoapyridine		224	C ₁₃ H ₁₂ N ₄	1
3. n,n,n-TMIP	2-Amino-n,n,n-trimethylimidazopyridine		176	C ₉ H ₁₂ N ₄	n,n,n
B. Quinoline congeners					
1. 3-MIQ (IQ)*	2-Amino-3-methylimidazo(4,5-f)quinoline	76180-96-6	198	C ₁₁ H ₁₀ N ₄	N3
2. 3,4-DMIQ (MeIQ)	2-Amino-3,4-dimethyl-3H-imidazo(4,5-f)quinoline	77094-11-2	212	C ₁₂ H ₁₂ N ₄	N3,4
C. Quinoxaline congeners					
1. 3,8-DMIQx (MeIQx)	2-Amino-3,8-dimethylimidazo(4,5-f)quinoxaline	77500-04-0	213	C ₁₁ H ₁₁ N ₅	N3,8
2. 3,4,8-TMIQx (DiMeIQx)	2-Amino-3,4,8-trimethylimidazo(4,5-f)quinoxaline	95896-78-9	227	C ₁₂ H ₁₃ N ₅	N3,4,8
3. 3,7,8-TMIQx (DiMeIQx)	2-Amino-3,7,8-trimethylimidazo(4,5-f)quinoxaline	92180-79-5	227	C ₁₂ H ₁₃ N ₅	N3,7,8

* Presently used common names in parentheses.

laboratory. A proposed revision of the common nomenclature is presented that corrects the undesirable features of the current names; this is done by assigning the abbreviations IP, IQ, and IQx to the aminoimidazo azaarene nuclei of the pyridine, quinoline, and quinoxaline series, respectively. Methyl groups are noted as M [1], DM [2], and TM [3]; and their positions are given if known.

Genetically Relevant Endpoints

Published experimental data will be summarized in a series of tables with commentary. The presentation will cover: the molecular level of DNA binding or other damage and its repair; mutagenesis in *Salmonella*, Chinese hamster cells, and *in vivo*; chromosomal effects; cell transformation and local tumor formation. Carcinogenesis bioassays have been described elsewhere (5) and will not be presented in this review. When appropriate, the information will be presented in the sequence: molecular studies in cell-free and cell culture systems, prokaryotic assays, mammalian cellular systems, and *in vivo* animal systems.

DNA Binding

Molecular and Subcellular Studies

DNA binding of thermic mutagens has been reported from *in vitro* experiments with calf thymus DNA as well as from all phylogenetic levels (Table 2). In the absence of metabolic activation, noncovalent interaction with DNA in solution (intercalation) has been studied

by UV, fluorescence, and dichroic spectroscopy for mutagens of the glutamic and tryptophan pyrolyzates and the IQ class. With the latter two classes interaction was said to be correlated with mutagenic potency in *Salmonella*, whereas this was not the case with the glutamic class. With microsomal activation radiolabeled mutagens were bound covalently, and in two cases the C8-guanine adducts of Glu-P-1 and Trp-P-2 were isolated and identified.

Plasmid/Viral DNA

Noncovalent and covalent binding to plasmid and viral DNA have been reported. The C8-G adduct of Glu-P-1 was isolated from a plasmid.

Salmonella

Covalent binding of Trp-P-2 and IQ to the DNA of *Salmonella* TA 1538 under Ames assay conditions has been reported only in abstract form. An additional reference is included on the binding of *N*-hydroxy-2AF and *N*-hydroxy-2AAF to *Salmonella* DNA because of the interesting observation that the C8-G adduct of the former exists in the usual *anti* configuration when linked to deoxyribose in DNA, whereas the latter exists in the abnormal *syn* form. The *syn* form is believed to be a potential cause of conversion of normal B DNA to the Z conformation, which could affect the regulation of genetic transcription (6).

Mammalian Cells

In repair-deficient Chinese hamster ovary cells, covalent DNA binding of Trp-P-2 and IQ was measured with

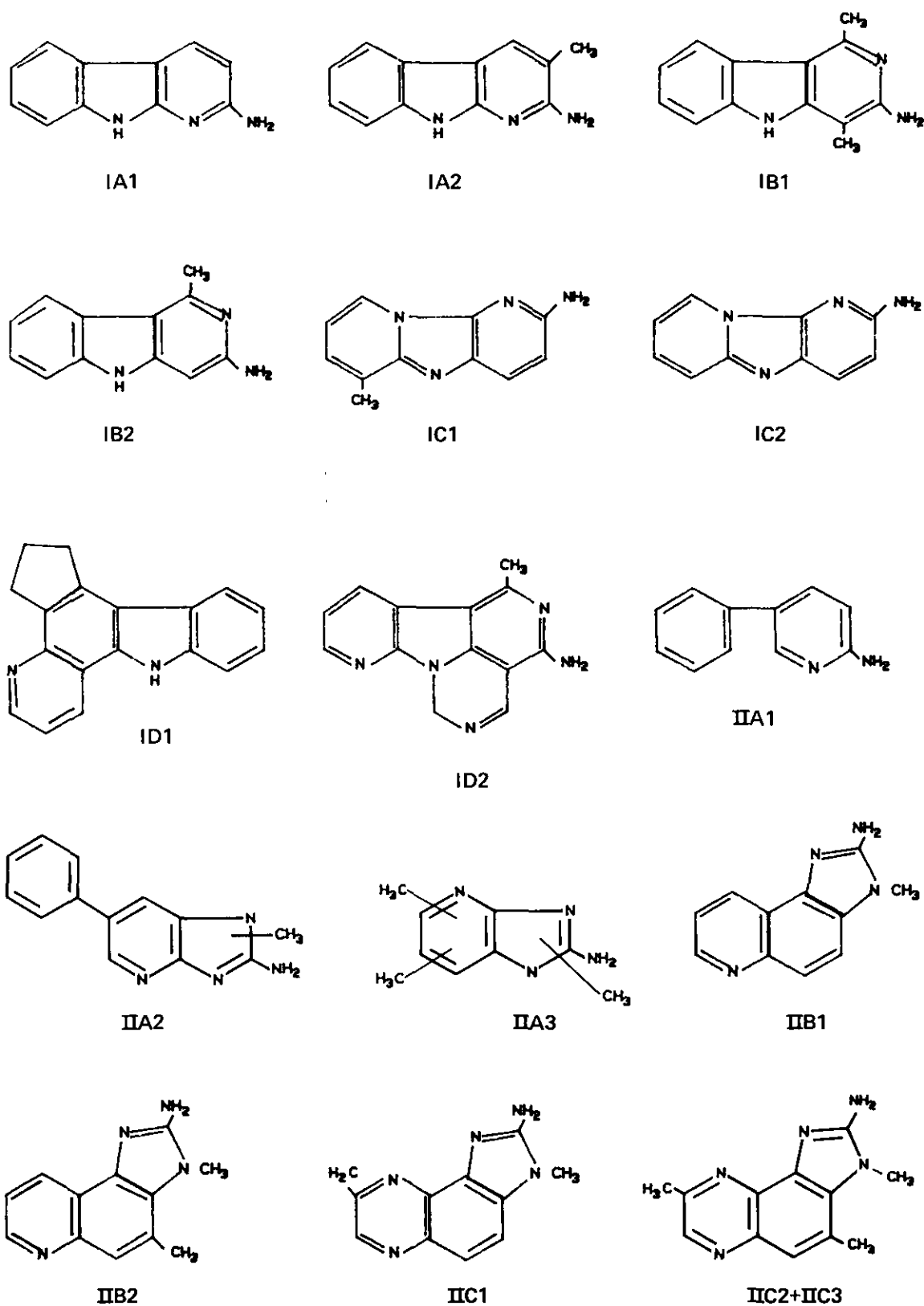


FIGURE 1. Structures of the known heterocyclic thermic food mutagens. Labels are keyed to Table 1. Note that a single structure is shown for compounds II C2 and II C3, which is that of II C2; in II C3 the methyl group at position 4 is absent and appears at position 7.

Table 2. DNA binding studies.

System	Mutagen	Method	Binding	Remarks	Reference
DNA <i>in vitro</i>					
Calf thymus DNA	Glu-P-1 and -2	UV fluorescence, dichroic spectra	Noncovalent binding, UV, fluorescence, dichroic spectra	Affinity not correlated to mutagenic potency	(7)
Calf thymus DNA	Trp derivs	Scatchard plot, with microsomes	Noncovalent, covalent	Noncovalent affinity correl. with mutagenic potency	(8)
Calf thymus DNA	IQ class	UV spectra	Scatchard plots	Intercalation = mutagenicity	(9)
DNA + mutagen + microsomes	Trp-P-1 and -2	3-H-Trp-P-2	<i>In vitro</i> binding	Rat PCB microsomes	(10)
Calf thymus DNA	Trp-P-2	Adduct identification	C8-G-Trp-P-2	Microsomal activation	(11)
Calf thymus DNA	Glu-P-1	Adduct identification	C8-G-Glu-P-1	Microsomal activation	(12)
Calf thymus DNA	Trp-P-2	N-OH-Trp-P-2 reaction adduct isolation	C8-G-Trp-P-2	Synthetic metabolite or microsomal	(13)
Plasmid/viral DNA					
Closed circ. E1 DNA	Glu-P-1 and -2	Intercalation	Noncovalent intercalation		(14)
32P-pBR322 DNA	Glu-P-1	Adduct identification	C8-G-Glu-P-1 isolated	GC-rich sequence pref.	(15)
SV40 DNA	Trp-P-1 and -2	<i>In vitro</i> reaction	Noncovalent binding	Variety of physical and biochem. effects without activation	(16)
Bacteria					
Salmonella TA 1538	Trp-P-2	DNA isolation, simultaneous mutation	9 adducts/10E6 bases, 0.0029 mutations/adduct/kbp	Hamster PCB S9	(17)
Salmonella TA 1538	IQ	DNA isolation, simultaneous mutation	0.18 adducts/10E6 bases, 0.0069 mutations/adduct/kbp	Hamster PCB S9	(17)
Salmonella	N-OH-2AF	Adduct identification	dG8-2AF, <i>anti</i> form	B-DNA conformation	(18)
Salmonella	N-OH-2AAF	Adduct identification	dG8-2-AAF, <i>syn</i> form	Possible Z-DNA conformation	(18)
Mammalian cells					
CHO UV-5 cells	Trp-P-2	DNA isolation, simultaneous mutation	29 adducts/10E6 bases 0.0036 mutations/adduct/kbp	Hamster PCB S9	(19)
CHO UV-5 cells	IQ	DNA isolation, simultaneous mutation	3.5 adducts/10E6 bases, 0.0036 mutations/adduct/kbp	Hamster PCB S9	(19)
Mouse leukemia cells, rat hepatocytes	IQ	Alkaline elution	Incr. at 10E-5M concn	PCB S9 for mouse leuk. cells	(20)
Rodent <i>in vivo</i>					
Mouse, C57B1/6J injected IP	Trp-P-2	DNA isolation	0.2-4 adducts/10E6 bases liver > other tissues, none in sperm	Uninduced mice	(21)
Rat, Wistar injected IP	Trp-P-2	DNA isolation	20 adducts/10E6 bases C8-G adduct		(22)
Rat, Wistar injected IP	Glu-P-1	DNA isolation	20 adducts/10E6 bases C8-G adduct		(22)

simultaneous measurement of induced mutations. Although binding of IQ was substantially less than of Trp-P-2 and required a much higher exposure concentration, the mutagenic efficiency (mutations induced per adduct bound) was virtually identical for the two agents.

DNA damage as studied by alkaline elution was measured for IQ in mouse leukemia cells and primary rat hepatocytes. Positive effects were obtained at or above 10E-5M IQ. Activation with PCB-induced S9 was required for the mouse cells.

Rodent *in Vivo*

The quantitative distribution of bound Trp-P-2 was measured in mouse tissues. Constitutive metabolism of the mutagen was involved since no inducer of mixed

function oxidases was given. Fifteen hours after intraperitoneal injection of labeled mutagen, the liver DNA contained 5- to 20-fold more label than DNA of other tissues; mature sperm were unlabeled. The binding level in liver DNA was similar to that found in CHO cell DNA at nearly equal exposure concentrations. Rats injected intraperitoneally with Glu-P-1 and Trp-P-2 also showed levels of binding (expressed as adducts per million nucleotides) similar to those reported in the mouse and CHO cells for Trp-P-2. The C8-G adducts of both mutagens were identified in rat liver.

DNA Repair

Unscheduled DNA synthesis assays have been reported in rat hepatocytes for several mutagens and in

human cells from xeroderma pigmentosum patients (Table 3). The hepatocyte assay is sensitive to the effect of these mutagens. The repair-deficient human cells showed a diminished amount of repair synthesis and also of single-strand breaks when compared with normal human diploid fibroblasts.

Mutagenesis

Salmonella

Potency data for the heterocyclic thermic mutagens that were identified earlier are not reported here from the original sources (primarily from Japan) since a number of revisions have taken place in subsequent reviews. References to recent summaries are given (Table 4).

In our laboratory the crude extract of fried ground beef shows nearly exclusive preference for the Ames frameshift-sensitive strains of *Salmonella* (TA 1538, TA 98, with lesser activity in TA 97). Activity is virtually undetectable in the base substitution strains TA 1535 and TA 100. Application of the newer Ames strains shows that the crude mutagen mixture has a distinct preference for affecting gene loci with GC-rich reversion sites, so that AT-rich sites and free radical mechanisms do not appear to be involved.

In view of the extraordinary potency of some of the heterocyclic thermic mutagens in the Ames assay, it was of interest to assay Trp-P-2 and IQ at additional *Salmonella* gene loci, in order to understand whether the mutagens exhibited special affinity for the DNA sequences in the *his* D gene. Studies were performed with the multilocus forward mutation assay for arabinose resistance developed by Ong et al. and with the forward mutation assay at the *hprt* locus developed by Skopek et al.; these assays indicated similar highly sensitive responses to both mutagens. At all three genetic loci IQ was more potent than Trp-P-2. Thus, no special affinity of the thermic mutagens for the *his* gene DNA sequence was shown.

Chinese Hamster Cells

Mutation induction by certain heterocyclic thermic mutagens has been studied in CHO (ovary) cells, both wild type and repair-deficient, at the *hprt* (TGr) and *aprt* (AAR) loci, and in CHL (lung) cells at the diphtheria toxin resistance (DTr) locus (Table 5). In CHO cells both

loci were similarly sensitive to Trp-P-2, with the repair-deficient line responding about twofold more than the repair-competent line. Although both loci in the repair-deficient line responded to IQ at much higher exposure concentration, the repair-competent line gave no significant response. Cytogenetic assays were reported in the same experiments, and the responses closely paralleled those for mutation induction (see section on Clastogenesis). A comparison of mutation induction in CHL cells by a variety of thermic and standard mutagens was reported in two articles. The responses varied over a broad range, and for the thermic mutagens were said to correlate better than potency in *Salmonella* with the results of carcinogenesis bioassays. There is, however, a moderately good correlation between the DTr responses and *his* reversion (F. T. Hatch, unpublished). Ouabain resistance mutation was reported in V79 cells for Trp-P-2 and Lys-P-1. Metabolic activation was provided by irradiated Syrian hamster embryo cells and was required for Lys-P-1, but not for Trp-P-2.

Drosophila

Somatic mutation and sex-linked recessive lethal assays are reported in *Drosophila melanogaster* for Trp-P-1 and Trp-P-2 (Table 6). For both mutagens somatic mutation was positive, and the heritable assay results were equivocal.

Mouse Spot Test

Several thermic mutagens have been tested in the mouse spot test (Table 6). Significant responses occurred with the carboline mutagens, but the aminoimidazo mutagens were so far negative—possibly owing to insufficient solubility to achieve high exposure doses.

Clastogenesis

Hamster Cells

As noted above, in repair-deficient CHO cells, SCE and chromosome aberration responses to Trp-P-2 and IQ mirrored those for induced mutation at two loci, with Trp-P-2 being substantially more potent (Table 7). IQ proved negative for chromosomal aberrations. Similar findings for Trp-P-2 were made in hamster Don-6 cells without exogenous metabolic activation.

Table 3. DNA repair.

System	Mutagen	UDS repair	Metabolism	Reference
Hepatocyte, rat	Trp-P-1 and -2	Trp-P-2 < Trp-P-1 << 2-AF and AFB1		(23)
Hepatocyte, rat	Glu-P-1 and -2	Glu-P-1 and -2 = Trp-P-2		(23)
Hepatocyte, rat	IQ and aryl amines	UDS above 10E-6M 4-aminobiphenyl similar		(24)
XP2BI cells-group G, Human fibroblast control	Trp-P-1	XP cells deficient in UDS and SS breaks	Rat/hamster PCB S9	(25)
XP2BI cells-group G, Human fibroblast control	Trp-P-2	XP cells deficient in UDS and SS breaks	Rat/hamster PCB S9	(25)

Table 4. Mutagenesis in *Salmonella* strains.

System	Mutagen	TA 1538	TA 98	TA 100	SV-50	TM-677	Comment	Reference
Salmonella, Ames strains	Trp-P-2	47,000 rev/ μ g, S9 = 1 mg	89,000 rev/ μ g, S9 = 1 mg	1600 rev/ μ g, S9 = 2 mg			Other Ames strains	(26)
Salmonella, forward	Trp-P-2				39,000 mut/ μ g arabinose-resistant	6200 mut/ μ g AG-resistant		(26)
Salmonella, Ames strain	IQ	272,000 rev/ μ g, S9 = 2 mg	94,000 rev/ μ g, S9 = 2 mg	3500 rev/ μ g, S9 = 2 mg			Other Ames strains	(26)
Salmonella, forward	IQ				34,000 mut/ μ g arabinose-resistant	88,000 mut/ μ g AG-resistant		(26)
Salmonella	MeIQx	75,000 rev/ μ g					PCB S9 required	(27)
Salmonella	MeIQx		130,000 rev/ μ g					(28)
Salmonella	7,8-DiMeIQx		163,000 rev/ μ g	9900 rev/ μ g			S9 required	(29)
Salmonella	DiMeIQx	310,000 rev/ μ g					Rat PCB S9	(27)
Salmonella	TMIP	100,000 rev/ μ g					Potent 2-ring thermic food mutagen	(30)
Salmonella	1-M-5-PhIP	4,000 rev/ μ g					Low mut. act., high mass in beef	(30)
Salmonella Ames strain	Protein, peptide pyrolysates		Assay data				S9 reqd., most pyrolysates active	(31)
Salmonella Ames strain	Amino acid pyrolysates		Assay data				Pyrolyzed @ 5-600C L-Trp most active	(32)
Salmonella Ames strain	Thermic, other mutagens		12 Thermic mutagens	12 Thermic mutagens			PCB S9 required	(3)
Salmonella Ames strains	Thermic	7 Thermic mutagens	12 Thermic mutagens	12 Thermic mutagens			2-3 labs compared	(1)
Salmonella Ames strains	Basic fraction of fried beef	Mixture positive	Mixture positive	Mixture negative			TA 1537 weak, TA 1535 negative	(33)

Human Cell lines

In a variety of human cell lines several carboline mutagens induced SCE at low exposure concentrations in the presence of PCB-induced rat liver S9. The induction of SCE by both direct and indirect mutagens (including carbolines) was inhibited by 3-aminoharman, a β -carboline derivative.

Mouse *in Vivo*

In bone marrow of mice preinduced with PCB, Trp-P-2 and IQ induced SCE, the former mutagen being somewhat more potent. Chromosomal aberrations were induced only by Trp-P-2.

Transformation, Local Tumors

In several hamster cell systems, morphologic transformation was caused by Trp-P-1 and -2 and by a crude tryptophan pyrolyzate (Table 8). Additional X-irradiation of cells caused a synergistic response with Trp-P-2. In a mouse system, IQ produced transformation at extremely low exposure concentrations, data which seem in need of confirmation in view of the weak responses to IQ in all other mammalian genotoxicity assays.

Trp-P-1 produced sarcomas at the site of subcutaneous injection in the Syrian hamster, mouse and rat. Enzyme-altered foci in the liver were also observed.

Genotoxicity Review Summaries

Broad summaries across genotoxicity assay systems, and including carcinogenesis bioassay results, have been written recently by Sugimura and Sato, by Hargraves and Pariza, and by Miller (Table 9). Reviews with evaluation of available data on Trp-P-1 and -2 have been published by IARC.

Discussion

Dietary Genotoxins

There are many sources of genotoxins in the human diet. Natural mutagens are contributed by flavonoids, pyrrolizidine alkaloids, hydrazines, alkylating agents, and nitrite or nitrate plus natural amines. Mycotoxins may be present in several plant crops. The subject of this review is those genotoxins formed during cooking and heat processing of foods. These include pyrolytic and low temperature thermic mutagens arising in high-protein foods, and products of browning reactions and caramelization arising in high-carbohydrate foods. We

Table 5. Mutagenesis in Chinese hamster cells.

System	Mutagen	Mutation		Result	Metabolism	Reference
		Loci	Dose			
CHO AA8 (wild type) UV-5 (repair-def.)	Trp-P-2	TGr and AAr Cytogen. data: see Table 7	0.5–2 µg/mL	UV-5 2x >AA8	Hamster PCB S9	(34)
CHO AA8 (wild type) UV-5 (repair-def.)	IQ	TGr and AAr Cytogen. data: see Table 7	20–100 µg/mL	UV-5 Pos. AA8 Neg.	Hamster PCB S9	(34)
CHL cells	Thermic	DTr		Trp-P-2 max. Glu-P-1 and -2 low	Rat PCB S9 reqd. Trp-P-1 direct	(35)
CHL cells	Thermic, etc.	DTr	Various	0.3–160 mutants/10E6 cells/µg	Rat PCB S9	(36)
CH V79 + or – irradi. SHE cells	Thermic	Ouar		Trp-P-2 Pos. Lys-P-1 Pos.	Irrad. SHE reqd. for Lys-P-1, not Trp-P-1	(37)
CHL cells and hamster <i>in vivo</i>	Trp pyrolysate	AGr and OUAr			No induction	(38)

Table 6. Mutagenesis in *Drosophila* and the mouse spot test.

System	Mutagen	Endpoints	Result	Dose	Time admin.	Induction	Reference
<i>Drosophila melanogaster</i>	Trp-P-1&2	Somatic mutation Sex-link rec. lethal	Positive Equivocal				(39)
C57BL/6J mouse	Glu-P-1	Mutation, spot test, recessive skin spots	Pos., $p < 0.01$	18 mg/kg × 3	Day 8,9,10		(40)
C57BL/6J mouse	Trp-P-2	Mutation, spot test, recessive skin spots	Pos., $p < 0.05$	4.2 mg/kg × 3	Day 8,9,10		(40)
C57BL/6J mouse	IQ	Mutation, spot test, recessive skin spots	Not signif.	20 mg/kg × 3	Day 8,9,10	+ or – pcb induction day 5	(41)
C57BL/6J mouse	MeIQ	Mutation, spot test, recessive skin spots	Not signif.	17 mg/kg × 3	Day 8,9,10	+ or – pcb induction	(41)

have focused here on the heterocyclic organic bases of thermic or pyrolytic origin.

Presentation of Database

This field is literally only 8 years of age. Yet, because of the apparent high mutagenic potency of many of the chemicals identified of this type, a data base on their genetic toxicity is rapidly forming. In presenting a summary of the current status of this database, we have followed a conventional scheme of covering genetic endpoints assumed to be relevant: including chemical modification of DNA, mutagenesis, clastogenesis, and effects associated with malignant transformation of cells. Results of carcinogenesis bioassays have been presented comprehensively elsewhere and are omitted here. For each genotoxic endpoint we have proceeded from molecular studies up the phylogenetic scale to *in vivo* studies in rodents, where information is available.

There is a further important aspect of a genetic toxicity database that is not presented here. This includes supporting data on cytotoxicity, pharmacodynamics, enzymatic metabolism, and modulation of toxicity. We are compiling a companion database on this subject.

The current database has become comprehensive only for a few mutagens, notably Trp-P-2. Most of the other

agents discovered earlier have only fragmentary data at this time. Since most of these agents have either not been actually found in cooked foods or do not appear to be the major mutagens therein, it is unclear whether further research on their genotoxicity will be given much priority. On the other hand, only recently have structures been established for the major thermic mutagens of important cooked foods and sufficient quantities made available for testing. Additional related mutagens are still having structures confirmed and syntheses carried out. For these agents of higher priority, genetic assay data are beginning to appear at accelerating frequency; but the information matrix is understandably still fragmentary.

Genotoxicity of Trp-P-2 and IQ

At this time a comprehensive conclusion about the genotoxicity of thermic heterocyclic mutagens is possible only for a few members of the carboline class. Trp-P-2 appears to be a completely classic mutagen, evoking responses in virtually all assays at low concentrations. On the other hand the first member of the aminoimidazoazaarene class to yield information, IQ, provides a paradoxical picture. Although extraordinarily potent in *Salmonella*, its activity in mammalian short-term tests

Table 7. Clastogenesis assays.

System	Mutagen	Dose range	SCE	Aberrations	Metabolism	Reference
CHO AA8 (wild type) UV-5 (repair-def.)	Trp-P-2	0.5–2 µg/mL	Positive at 0.4–1 µg/mL	Positive at 0.7–2 µg/mL	Hamster PCB S9	(34)
CHO AA8 (wild type) UV-5 (repair-def.)	IQ	20–100 µg/mL	Weak positive at 50 µg/mL	Negative	Hamster PCB S9	(34)
Diploid human fibroblast and lung, Hamster Don-6 cells	Trp-P-1 and -2		Increased except Trp-P-2 in D.H.F.	Chromatid breaks	None added	(42)
Hamster embryo cells	Trp pyrolysates			Chromatid breaks and exchanges	None added	(43)
Human lymphoblastoid and NL3 cells	Carbolines	SCE at 10E-7 or 10E-6M	Trp-P-2 > Trp-P-1 = Glu-P-1 > AAC		Rat PCB S9	(44)
Human lymphocyte NL3 cells	3-NH2 harman		3-NH2 harman inhibits direct and indirect mutagens			(45)
Human lymphocyte NL3 and CHO-K1 cells	3-NH2 harman carbolines		Trp-P-1 and -2 > 3-NH2-harman > AAC		Rat PCB S9	(46)
C57B1/6 mouse <i>in vivo</i> , bone marrow	Trp-P-2	4–20 mg/kg	Positive at 4–12 mg/kg, then plateau	Positive at 20 mg/kg, dose response	PCB-induced, BrdU implant	(47)
C57B1/6 mouse <i>in vivo</i> , bone marrow	IQ	20–160 mg/kg	Positive at 20–40 mg/kg, then plateau	Not significant	PCB-induced, BrdU implant	(47)
CHL cells	500 cpds.			Chromatid exchanges	With/without	(48)

Table 8. Transformation, local tumors.

System	Mutagen	Transformation/tumor		Comment	Reference
		Dose	Result		
Pienta SHE cell assay	Trp-P-1	0.5 µg/mL × 8 days	1.35% colony transformation		(49)
Pienta SHE cell assay	Trp-P-2	0.5 µg/mL × 8 days	1.5% colony transformation		(49)
Hamster embryo cells	Trp pyrolysate		Colony morphologic transformation	No activation system	(43)
Balb 3T3 cells	IQ	5 nM	Foci positive		(50)
SHE cells, X-ray + mutagen	Trp-P-2		150 rad X-ray + Trp-P-2 4 × > Trp-P-2 alone		(51)
Syrian hamster, Fischer rat, CDF1 mouse	Trp-P-1	1 mg SC/wk × 20	Local sarcomas	Enzyme-altered liver foci	(52)
Hamster embryo cells	Trp-P-2		<i>In vitro</i> transfmn.		(53)
Hamster embryo cells	Glu-P-2	10–20 µg/mL	Morphologic transformation		(54)
Hamster embryo cells	Trp-P-1&2	0.5 µg/mL	Trp-P-1 and -2 positive	Colonies form check-pouch tumors	(55)

is weak or negligible. An investigation of covalent DNA binding to discover the basis for this behavior suggests that active metabolites may have limited access for interactions with nuclear DNA in mammalian cells, in contrast to ready access in *Salmonella*. The mechanism for this is unclear: possibly highly reactive metabolites undergo competing reactions with other cellular constituents. However, when bound to DNA, IQ adducts appear to have the same quantitative propensity for

inducing mutation in cultured cells as do Trp-P-2 adducts. Further information on the genotoxicity of the aminoimidazo azaarene mutagens is, of course, badly needed, and should be available soon.

Carcinogenicity

Several of the carboline mutagens and two of the aminoimidazo azaarenes have been tested in Japan in

Table 9. Genotoxicity review summaries.

Systems	Mutagen	Reference
Salmonella, Chinese hamster lung cells, rodent bioassays	Thermic mutagens, etc.	(3)
Short-term tests and carcinogenesis bioassays	Thermic mutagens, etc.	(56)
Genetic toxicity profiles, modulation of activity	Thermic mutagens	(57)
Evaluates available data	Trp-P-1	(58)
Evaluates available data	Trp-P-2	(59)

single-dose-level rodent feeding carcinogenesis bioassays. All compounds tested have proven carcinogenic, generally in multiple organs and in both mice and rats. Of great potential importance is the induction of colon tumors in rats by the aminoimidazo azaarenes. Although no dose-response data are yet available, the tentative carcinogenic potency estimates (from effective tumor-inducing doses) cover a range of only a factor of four. Yet for the same series of agents the potency range in *Salmonella* is approximately twenty thousand. Practical significance of these observations is shown by an example from our own laboratory. One of several major mutagens isolated from fried ground beef is about 100-fold less potent in *Salmonella* than the most potent major mutagens; but it is present in 10- to 100-fold greater quantity. Thus, if its carcinogenicity is actually of the same order as the other compounds, it could be the most significant mutagen present.

Future Research Needs

Future research needs for the thermic food mutagens are many. Some will be obvious from what has been discussed above. There are two broad classes of these mutagens, with preliminary indications that they may behave very differently in mammalian systems, and therefore possibly in humans. There are seven subclasses based on chemical structures; only superficial genetic toxicity data are available for most of them. Even after the genetic matrix has begun to fill with data, there will be needs for information on metabolism, pharmacodynamics, etc., in order to understand the mechanisms by which genetic effects may occur and to facilitate extrapolation to possible effects in humans. All data so far pertain only to somatic effects. Yet after somatic effects are proven, there is a need for evaluation of heritable effects on germ line cells—not to mention the question of reproductive toxicity, which has not yet been addressed. Beside the genetic toxicity database, there are important needs for information, whether the same suite of mutagens will be found in all protein-rich foods or whether the elaborate processes of isolation and identification will have to be repeated for each kind of meat, fish, eggs, etc. Also, the ability to measure even the known mutagens in different foods is still in a primitive state. Quantitative measurement of complex organics in complex matrices at part per billion concen-

trations remains a major challenge to the analytical chemist. Finally, there is an area where research has been initiated, but little understanding achieved yet. It is known that different types of dietary factors can influence the *in vivo* metabolism and ultimate toxicity of ingested mutagens. These factors range from the overall composition of the diet, e.g., protein content, to the presence of specific trace substances, e.g., carotenoids and other vitamins capable of modulating the effects of genotoxins. Additives to the diet—for example, during cooking—might also play a role. Certain types of dietary fiber may bind mutagens in the gastrointestinal tract, leading to their excretion in the feces. Substantial further knowledge is required of how features of the total diet may interact with thermic mutagens before or after ingestion to modulate their genetic toxicity *in vivo*.

Significance

What is the significance of the accomplishments of the past eight years? We have evidence before us of the formation of a relatively small number of similar organic mutagens that are formed in trace quantities when protein-rich foods are cooked or heat-processed to a well done state. There is a wide spectrum of the potency of these mutagens in the *Salmonella* assay, but, from limited data, a rather narrow spectrum of potency for carcinogenesis. Available information from mammalian genetic assays *in vitro* and *in vivo* suggests paradoxical differences in potency for some mutagens in these systems when compared with both *Salmonella* mutagenicity and tumor induction in rodents. This inconsistency points to our current level of ignorance; but may be understood when the database expands. The introduction of genotoxins into the diet by normal practices of food preparation, in however small a quantity, is disturbing. Even though the amounts consumed by most individuals may be very small, the exposure covers most of a lifetime. The consistent carcinogenicity of these mutagens in rodents, in the assays completed to date, is further cause for concern. It would seem that we have no alternative to a thorough investigation of the problem.

The ultimate purpose of the genetic toxicity database and the carcinogenicity bioassays is to provide a foundation for analysis of the potential hazard to humans from the existence of thermic mutagens in their diet. In order to reach this goal, clearly it is not necessary to fill every box in the multidimensional matrix of mutagens vs. foods vs. genetic endpoints; but we must achieve a systematic understanding of both the quantitative prevalence and the genetic toxicity of the thermic mutagens. We are now rather distant from this objective, but the directions for additional research are known. At some time in the near future, it will become appropriate for leaders in this research to confer with members of the food processing and food service industries. Not only will information transfer be important, but the industries may wish to initiate applied

research directed to mitigation of the main areas of concern. A formal risk analysis for dietary thermic mutagens lies farther in the future. Public agencies such as the National Institutes of Health and the Food and Drug Administration will become involved at that time, as some consensus must be reached concerning the reality of any human health hazard and the feasibility of available measures for its mitigation.

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