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Heterocyclic amines formed by cooking food: comparison of bioassay results with other chemicals in the Carcinogenic Potency Database

Lois Swirsky Gold*[a,b], Thomas H. Slone[a,b], Neela B. Manley[b], Bruce N. Ames[b]

[a]*Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720, USA*
[b]*Division of Biochemistry and Molecular Biology, Barker Hall, University of California, Berkeley, CA 94720, USA*

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Abstract

Results in the Carcinogenic Potency Database (CPDB) on 11 mutagenic heterocyclic amines (HA) tested for carcinogenicity in rats, mice and cynomolgus monkeys are compared to results for other chemicals. An analysis of strength of evidence of carcinogenicity for HA vs. other mutagenic carcinogens and vs. all rodent carcinogens, indicates strong carcinogenicity of HA in terms of positivity rates and multiplicity of target sites. The liver is the most frequent target site in each species. Despite several target sites in each species, concordance in target sites between rats and mice is restricted to the liver for each HA except one. In cynomolgus monkeys, liver tumors have been induced rapidly by 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ). Human exposures to HA in cooked animal foods are small, in the low ppb range. A comparison of possible carcinogenic hazards from a variety of exposures to rodent carcinogens in the American diet is presented, using an index (Human Exposure/Rodent Potency, HERP) that relates human exposure to carcinogenic potency in rodents. Results indicate that there is a large background of exposures to naturally-occurring rodent carcinogens in typical portions of common foods, and that possible hazards from HA rank below those of most natural pesticides and products of cooking or food preparation; synthetic pesticide residues also rank low.

Keywords: Carcinogenic potency; Dietary heterocyclic amine; Carcinogenic hazards, ranking of; Species comparison

1. Introduction

Seventeen years ago Sugimura et al. showed that charred parts of broiled meat and fish were mutagenic [24]. Subsequently, several mutagenic heterocyclic amines (HA) formed from cooking have been identified; most of them are potent mutagens, as shown by Sugimura [25]. Eleven have been tested in long-term rodent carcinogenesis bioassays, and 10 induce tumors in rodents. Like other products of cooking (e.g., furfural) and naturally occurring chemicals in plant food (e.g., caffeic acid), there can be widespread human exposure in the diet. Two analyses of HA are reported

* Corresponding author.

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in this paper, based upon results of the long-term cancer bioassays in the Carcinogenic Potency Database [6–9,13,16]: (1) bioassay results on 11 HA are compared to results on other chemicals that are mutagenic in *Salmonella*, as well as to the overall CPDB in terms of the proportion of chemicals that are carcinogenic, strength of evidence of carcinogenicity, target sites, and carcinogenic potency; (2) possible carcinogenic hazards from HA are compared to other rodent carcinogens in the American diet by ranking on an index, HERP (Human Exposure/Rodent Potency), which indicates what percentage of the tumorigenic dose-rate for 50% of rodents, a person receives from typical dietary exposures to naturally occurring and synthetic chemicals that are rodent carcinogens.

2. Comparison of bioassay results on heterocyclic amines to other chemicals in the CPDB

The CPDB is a compendium of analyses of chronic, long-term carcinogenesis bioassays including standardized results of approximately 4000 tests of 1100 chemicals. All experiments meet a set of inclusion criteria that are designed to permit the estimation of carcinogenic potency; therefore reasonable consistency of experimental protocols is assured. Tests on HA are included for 2-amino-*9H*-pyrido(2,3-*b*)indole (A-alpha-C), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imida-(Glu-P-2), 2-amino-3-methylimidazo[4,5-*f*]-quinoline (IQ), 2-amino-3-methyl-9*H*-pyrido-[2,3-*b*]-indole (MeA-alpha-C), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), *9H*-pyrido(3,4-*b*)indole (norharman), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP), 3-amino-1,4-dimethyl-*5H*-pyrido[4,3-*b*]indole acetate (Trp-P-1 acetate) and 3-amino-1-methyl-*5H*-pyrido[4,3-*b*]indole acetate (Trp-P-2 acetate). The maximum tolerated dose of each HA was typically administered in the diet to a single dose group of rats (usually Fischer 344) and mice (usually CDF_1). Animals were fed ad libitum. Positive results are also included in the CPDB for the bioassay of IQ in cynomolgus monkeys that is in progress at the National Cancer Institute (NCI) [1].

There is strong evidence of carcinogenicity in rodents for these mutagenic HA by several criteria including positivity rates, number of target organs and tumor yields [6–9,13,16]. Classification of a positive result at each site is based on the evaluation of the published author. Of the 45 experiments on HA in rodents, 41 are positive; the exceptions are A-alpha-C (negative in male and female rats), Trp-P-2 (negative in male ACI rats, though positive in females) and norharman (tested only in male rats and negative).

Overall in rodent bioassays, chemicals that are mutagenic (in *Salmonella*) compared to non-mutagens, are (a) more likely to be carcinogenic; (b) more likely to induce tumors at multiple target sites; and (c) more likely to be carcinogenic in 2 species [14]. Therefore, Table 1 compares the mutagenic HA to the subset of chemicals that have been evaluated as mutagenic, as well as to the overall database.

2.1. Positivity

Although the numbers of HA are small for detailed analyses, Table 1 indicates that positivity rates are high for chemicals tested for carcinogenicity: HA are more often positive, positive in both rats and mice, and in all four sex-species groups. Among chemicals tested in both species, only one HA, A-alpha-C, is positive in only one species. For norharman, the only negative HA (which is also a very weak mutagen [21]), more thorough animal testing is needed since the experiment was conducted for only 80 weeks in rats, while mice have not been tested. For the few chemicals tested at two doses, there is a dose-response curve at each positive site that is consistent with linearity [9,16].

2.2. Target sites

Compared to other chemicals, HA more often induce tumors at multiple target sites. For example in rats, 8/9 (89%) HA induce tumors at more than one site compared to 103/174 (59%) of mutagens in the CPDB (Table 1). Moreover, in rats 7/9 (78%) of carcinogenic HA induce tumors at three or more sites, compared to 67/174 (39%) of mutagenic

Table 1
Comparison of carcinogenicity in rodents between heterocyclic amines, other mutagens, and all chemicals in CPDB

Positivity	Proportion positive					
	Heterocyclic amines		Mutagens CPDB[a]		CPDB[a]	
	N	(%)	*N*	(%)	*N*	(%)
All chemicals in CPDB						
Rats or mice	10/11	(91%)	229/305	(75%)	584/1117	(52%)
Rats	9/11	(82%)	185/267	(69%)	426/859	(50%)
Mice	10/10	(100%)	140/223	(63%)	324/745	(43%)
Chemicals tested in rats and mice						
Positive in both species	9/10	(90%)	99/189	(52%)	167/481	(35%)
Positive in one species	1/10	(10%)	52/189	(27%)	123/481	(25%)
Negative	0/10	(0%)	39/189	(21%)	191/481	(40%)
Tested in four sex-species						
Positive in all 4	8/9	(89%)	52/154	(34%)	72/379	(19%)
All Rodent Carcinogens in CPDB[b]						
Positive at more than one target site						
Rats	8/9	(89%)	103/174	(59%)	202/375	(54%)
Mice	7/10	(70%)	70/136	(51%)	134/306	(44%)
Chemicals positive in rats and mice[b]						
No target site in common	1/9	(11%)	25/90	(28%)	51/150	(34%)
Liver is the only site in common	7/9	(78%)	22/90	(24%)	40/150	(27%)
Site(s) other than the liver are in common	1/9	(11%)	43/90	(48%)	59/150	(39%)

[a]The difference between mutagens and the CPDB overall does not represent nonmutagens since some chemicals have not been evaluated in *Salmonella*.
[b]Some experiments have been deleted because histopathology was restricted, the number of target sites could not be evaluated.

carcinogens. Induction of tumors at multiple sites is somewhat more frequent in rats than mice for HA, for other mutagens, and for all chemicals (Table 1) [14].

Tumors have been induced by HA at five different sites in mice and 12 in rats (Table 2). Except for liver and hematopoietic system, however, HA induce tumors at different sites in rats and mice. In mice, the target sites are among the most frequently-occurring targets in the CPDB [10]. In rats, however, several sites are infrequent targets. The liver is the most frequent target site among HA for each species, and it is a target more often for HA than other mutagens or the overall CPDB. For example, in mice nine of ten carcinogenic HA induce liver tumors compared to 56% of all mutagenic carcinogens and 57% of the CPDB (Table 2). Some of the infrequent target sites that are targets for HA in rats are colon, Zymbal's gland, skin, clitoral gland, oral cavity, nervous system and pancreas (Table 2).

The ratio of the high dose administered in a bioassay to the TD_{50} value reflects the increase in the proportion of animals with tumors and the experiment length at which that yield was obtained. The higher the ratio, the higher the proportion of induced tumors estimated for a standard lifespan. In rats and in mice, the mean ratio for HA is higher than for other mutagens or the overall CPDB, indicating that HA tend to induce high tumor yields.

We have examined in detail the chemicals that induce tumors in the rat Zymbal's gland [14]. The five HA positive at that site are similar to other Zymbal's gland carcinogens: they are mutagenic,

Table 2
Frequency of rodent target organs for heterocyclic amines, mutagenic carcinogens and all CPDB carcinogens, by species

Tissue	Heterocyclic amines		Mutagens		CPDB	
Mice	(N = 10)	(%)	(N = 140)	(%)	(N = 322)	(%)
Liver	9	(90)	79	(56)	184	(57)
Vascular system	4	(40)	27	(19)	51	(16)
Hematopoietic system	2	(20)	20	(14)	42	(13)
Lung	2	(20)	40	(29)	90	(28)
Stomach	2	(20)	27	(19)	44	(14)
Rats	(N = 9)	(%)	(N = 181)	(%)	(N = 402)	(%)
Liver	7	(78)	68	(38)	162	(40)
Clitoral gland	5	(56)	8	(4)	10	(2)
Large intestine	5	(56)	13	(7)	20	(5)
Zymbal's gland	5	(56)	24	(13)	34	(8)
Mammary gland	4	(44)	52	(29)	81	(20)
Small intestine	4	(44)	15	(8)	22	(5)
Skin	3	(33)	16	(9)	24	(6)
Hematopoietic system	2	(22)	21	(12)	41	(10)
Nervous system	2	(22)	12	(7)	17	(4)
Oral cavity	2	(22)	10	(6)	19	(5)
Pancreas	2	(22)	7	(4)	16	(4)
Urinary tract	2	(22)	23	(13)	40	(10)

For heterocyclic amines, all stomach are forestomach, all nervous system are brain and all large intestine are colon.

cause tumors at multiple target sites, and are positive in the mouse at multiple sites (though not in the Zymbal's gland of the mouse). The induction of tumors in the rat colon by five HA has been a subject of interest in the literature because of the frequency of such tumors in humans [22]. In the CPDB, colon tumors are induced in rats but not mice, are rarely induced by non-mutagens, and are induced by chemicals that are positive at other sites as well [14]. A list of chemicals in the CPDB that induce tumors at each target site can be found in references 10 and 14.

2.3. Prediction between species

A comparison of results in rats vs. mice provides information about interspecies extrapolation. If prediction is good between these closely related species, then confidence is strengthened in the possible extrapolation to humans [11]. There is good interspecies prediction of positivity for the HA (Table 1): nine of ten chemicals are positive in both species, and eight have a target site in common between rats and mice. When considering concordance between species, however, it is noteworthy that despite the several target sites in each species, concordance in target sites between rats and mice for each chemical is restricted to the liver in all but one case. (PhIP induced hematopoietic tumors in both species.) Moreover, interspecies concordance in target sites between rats and mice is more often restricted to the liver for HA than for other chemicals or other mutagens (Table 1).

2.4. Carcinogenic potency

Our numerical index of carcinogenic potency, TD_{50}, is calculated for each target site in the CPDB. TD_{50} is defined as the chronic dose rate (mg/kg/day) that will induce tumors in half the animals that would have remained tumor-free at zero dose at the end of a standard lifespan [23]. TD_{50} values, like other measures of potency, are restricted to a narrow range about the dose tested,

and do not indicate anything about carcinogenic effects at low doses. Many of the HA experiments were terminated before the standard lifespan of 2 years in rodents, and our convention in such cases is to adopt as a correction factor f^2, where f = experiment time/standard lifespan; this has the desired effect of lowering the TD_{50} (making it more potent) to reflect early deaths and the fact that tumor incidence increases as a function of age. In this analysis of HA, the potency value in each species for each chemical is the harmonic mean of the TD_{50} values in positive tests, using the lowest TD_{50} (most potent) from each test.

In the overall CPDB, TD_{50} values span a range of more than 10 million-fold. In comparison, for the 10 HA the range of potency is quite narrow, less than 100-fold (Table 3). This reflects the similarity of administered doses of the various HA [4]. As in the CPDB, values in rats tend to be more potent than in mice (Table 3). The average TD_{50} value for HA in each species is more potent than the average value in the CPDB. In rats, 16% of carcinogens in the CPDB are more potent than the most potent HA and 32% are less potent than the least potent HA. In mice, the corresponding percentages are 20 and 46%.

2.5. *IQ test in cynomolgus monkeys*

Three HA are being tested at NCI in cynomolgus monkeys by gavage. Tests of MeIQx and PhIP have been in progress for 6 and 3 years, respectively, and no tumors have been reported to this time [1]. IQ has been on test for 8 years at dose rates averaging 7 and 14 mg/kg body weight/day [16]. The experiment is still in progress, but to date 18/18 high dose and 14/20 low dose cynomologus monkeys have developed hepatocellular carcinomas, compared to 0/9 concurrent vehicle controls (U. Thorgeirsson, personal communication). No hepatocellular carcinomas have been found spontaneously in 32 years in the NCI colony control. The first tumor appeared early, at 27 months (the standard lifespan for cynomolgus monkeys is more than 20 years). Only one other chemical which has been tested in cynomolgus, *N*-nitrosodiethylamine, induced tumors in such a short time [26].

Nine chemicals have been shown to be carcinogenic in cynomolgus monkeys [26], and six of these induced hepatocellular carcinoma, the most frequently induced tumor: aflatoxin B_1, IQ, cycasin and MAM acetate, *N*-nitrosodiethylamine, *N*-nitrosopiperidine, and sterigmatocystin. The liver is the only target site in four of the six, including IQ.

The TD_{50} value for IQ in cynomologus monkeys is 0.577 mg/kg/day for the test in progress. In rodents, a comparison of the harmonic mean of the most potent TD_{50} values for IQ indicates that IQ is about 10 times more potent in rats than mice (Table 3). The TD_{50} for IQ in cynomologus monkeys is about 3 times more potent than in rats, and about 34 times more potent than in mice.

Table 3
TD_{50} values for heterocyclic amines, by species

Chemical	TD_{50} (mg/kg/day)	
	Rats	Mice
A-alpha-C	–	49.8
Glu-P-1	4.69	5.40
Glu-P-2	42.3	16.0
IQ[a]	1.89	19.6
MeA-alpha-C	8.70	22.2
MeIQ	0.539	12.3
MeIQx	1.99	24.3
Norharman	–	?
PhIP	4.29	28.6
Trp-P-1 acetate	0.576	40.7
Trp-P-2 acetate	6.66	12.6

–, negative in cancer test. ?, not tested for carcinogenicity.
[a]TD_{50} for IQ in cynomolgus monkeys is 0.577 mg/kg/day.

3. Ranking possible carcinogenic hazards

In several papers we have emphasized the importance of comparing possible carcinogenic hazards from the enormous background of natural chemicals to the synthetic chemicals that have been the focus of cancer testing [2,3,12]. Natural chemicals make up the vast bulk of chemicals that humans are exposed to, and the toxicology of natural and synthetic chemicals is not fundamentally different [12]. Therefore, it is important to set research and regulatory priorities by gaining a broad perspective on possible hazards, especially because

of the large extrapolation usually made from the results of high dose rodent tests to the low doses of most human exposures. One reasonable strategy is to use a simple index to rank possible carcinogenic hazards from a wide variety of exposures to rodent carcinogens at concentrations that humans typically receive, and then to focus on those that rank highest. We have used as an index, HERP (Human Exposure/Rodent Potency), which is not a direct estimate of risk because bioassay results do not provide sufficient information to estimate human risk at low dose. Although one cannot say whether the ranked chemical exposures are likely to be of major or minor importance in human cancer, it is not prudent to focus attention on the possible hazards at the bottom of a ranking if the same methodology indicates numerous common human exposures with much greater possible hazards. In general, one would expect a similar rank order of 'risk estimates' with the use of current regulatory risk assessment methodology for the same exposures and cancer tests because linear extrapolation from the TD_{50} generally leads to low-dose slope estimates similar to those determined on the basis of the linearized multistage model [19].

In this paper we compare possible carcinogenic hazards from daily lifetime consumption of naturally occurring HA in cooked animal food to other exposures in the American diet, including natural pesticides in plant foods, products of cooking other than HA, contaminants, synthetic pesticide residues and food additives. Our earlier work indicated that some occupational exposures and intake of pharmaceuticals rank high, and that the widespread exposures to naturally occurring rodent carcinogens cast doubt on the relevance to human cancer of far lower exposures of the general population to synthetic rodent carcinogens.

3.1. Human exposures to heterocyclic amines

Humans can be regularly exposed to HA from eating cooked meat. The most commonly investigated food has been pan fried hamburger; HA have also been identified in chicken, fish, lamb, pork and broiled beef [27]. Cooking at higher temperatures for longer periods of time generally tends to produce higher concentrations [18,24], although concentrations have sometimes been reported as decreasing at the highest times and temperatures investigated [17,18]. Concentrations vary more by cooking method, time and temperature than by type of food. Several papers on HA concentrations do not report cooking times and temperatures. Most of the laboratory measurements of HA concentrations are reported for higher cooking temperatures or longer cooking times than are typically used in American cooking for consumption. Reported concentrations in cooked food range from undetectable (<0.1 ppb of cooked weight) to concentrations in the low ppb range [18,27]. Our HERP ranking for rodent carcinogens in the American diet is reported in Table 4. Exposures are for 3-ounce portions of cooked hamburger and salmon, the foods for which studies reported in the literature are more similar to usual cooking of food [17,18]. Our estimates are reasonable concentrations of 3 HA in the diet, based on the best available data. We assumed that 5% of Americans eat their pan fried hamburger or baked salmon steak rare, 75% medium, and 20% well done. Further research is needed on concentrations of HA in foods as commonly cooked and consumed, perhaps using commercial samples.

3.2. Ranking of HERP values in the American diet

Possible carcinogenic hazards in the American diet from typical exposures to rodent carcinogens are ranked in Table 4. There are uncertainties in both the exposure estimates and the potency estimates used in HERP; the value of Table 4 is in the relative ranking of typical intakes rather than in the exact values of HERP. For synthetic pesticide residues HERP is for average daily intake from all foods combined. For other rodent carcinogens we report only the food with the largest HERP value even if the chemical has been identified in many foods; values for additional foods have been reported in Gold et al. [12]. Table 4 includes exposures to natural pesticides (the chemicals plants produce to defend themselves e.g., caffeic acid, allyl isothiocyanate); products of cooking and food preparation (e.g., HA, furfural, ethyl alcohol, urethane); synthetic pesticide residues (e.g., carbaryl, folpet); contaminants (aflatoxin B_1) and others (saccharin). Two convenient

Table 4
Ranking possible carcinogenic hazards: rodent carcinogens in the American diet (heterocyclic amines in italics)

Possible hazard: HERP (%)	Daily human exposure	Human dose of rodent carcinogen	TD_{50} (mg/kg) Rats	Mice
4.7	Wine (250 ml)	Ethyl alcohol, 30 ml	9110	(–)
0.3	Lettuce, 1/8 head (125 g)	Caffeic acid, 66.3 mg	284	(4970)
0.1	1 Mushroom (15 g)	Mix of hydrazines, etc.	(?)	20,300
0.1	Basil (1 g of dried leaf)	Estragole, 3.8 mg	(?)	52
0.07	Mango, 1 whole (245 g; pitted)	D-Limonene, 9.8 mg	204	(–)
0.07	Brown mustard (5 g)	Allyl isothiocyanate, 4.6 mg	96	(–)
0.06	Diet cola (12 oz; 354 ml)	Saccharin, 95 mg	2143	(–)
0.06	Parsnip, 1/4 (40 g)	8-Methoxypsoralen, 1.28 mg	32	(?)
0.03	Safrole: US avg from spices	Safrole, 1.2 mg	(436)	56.2
0.03	Peanut butter (32 g; 1 sandwich)	Aflatoxin, 64 ng	0.003	(+)
0.03	Comfrey herb tea (1.5 g)	Symphytine, 38 μg	1.91	(?)
0.006	Bacon, pan fried (85 g)	Diethylnitrosamine, 85 ng	0.02	(+)
0.005	Coffee, 1 cup (from 4 g)	Furfural, 630 μg	(679)	197
0.003	1 Mushroom (15 g)	Glutamyl *p*-hydrazino-benzoate, 630 μg	(?)	277
0.003	Bacon, pan fried (85 g)	*N*-nitrosopyrrolidine, 1.45 μg	(1.05)	0.679
0.002	Apple juice (6 oz; 177 ml)	UDMH, 5.89μg (from Alar, 1988)	(–)	3.94
0.002	Bacon, pan fried (85 g)	Dimethylnitrosamine, 255 ng	(0.2)	0.2
0.002	Coffee, 1 cup (from 4 g)	Hydroquinone, 100 μg	82.8	(225)
0.002	Coffee, 1 cup (from 4 g)	Catechol, 400 μg	336	(–)
0.001	Tap water, 1 liter	Chloroform, 83 μg (US avg)	(262)	90
0.001	Heated sesame oil (15 g)	Sesamol, 1.13 mg	1540	(4490)
0.0005	1 Mushroom (15 g)	*p*-Hydrazinobenzoate, 165 μg	(?)	454[a]
0.0003	Carbaryl: daily dietary avg	Carbaryl, 2.6 μg (1990)*	14.1	(–)
0.0002	Toxaphene: daily dietary avg	Toxaphene, 595 ng (1990)*	(–)	5.57
0.0001	*Salmon steak, baked (3 oz; 85 g)*	*PhIP, 306 ng*	4.29[a]	(28.6)[a]
0.00008	*Salmon steak, baked (3 oz; 85 g)*	*MeIQx, 111 ng*	1.99	(24.3)
0.00008	DDE/DDT: daily dietary avg	DDE, 659 ng (1990)*	(–)	12.5
0.00006	*Hamburger, pan fried (3 oz; 85 g)*	*PhIP, 176 ng*	4.29[a]	(28.6)[a]
0.00003	Whole wheat toast, 2 slices (45 g)	Urethane, 540 ng	(41.3)	22.1
0.00003	*Hamburger, pan fried (3 oz; 85 g)*	*MeIQx, 38.1 ng*	1.99	(24.3)
0.00002	Dicofol: daily dietary avg	Dicofol, 544 ng (1990)*	(–)	32.9
0.00002	Cocoa (4 g)	α-Methylbenzyl alcohol, 5.2 μg	458	(–)
0.000005	*Hamburger, pan fried (3 oz; 85 g)*	*IQ, 6.38 ng*	1.89[a]	(19.6)
0.000001	Lindane: daily dietary avg	Lindane, 32 ng (1990)*	(–)	30.7
0.0000004	PCNB: daily dietary avg	PCNB (Quintozene), 19.2 ng (1990)*	(?)	71.1
0.0000001	Chlorobenzilate: daily dietary avgan	Chlorobenzilate, 6.4 ng (1989)*	(–)	93.9
<0.00000001	Chlorothalonil: daily dietary avg	Chlorothalonil, <6.4 ng (1990)*	828	(–)
0.000000008	Folpet: daily dietary avg	Folpet, 12.8 ng (1990)*	(?)	2280
0.000000006	Captan: daily dietary avg	Captan, 11.5 ng (1990)*	2690	(2730)

Daily human exposure: reasonable daily intakes are used to facilitate comparisons; references are reported in [15]. *Possible hazard:* the human dose of rodent carcinogen is divided by 70 kg to give a mg/kg of human exposure, and this dose is given as the percentage of the TD_{50} in the rodent (mg/kg) to calculate the Human Exposure/Rodent Potency index (HERP). TD_{50} values used in the HERP calculation are averages calculated by taking the harmonic mean of the TD_{50} values of the positive tests in that species from the Carcinogenic Potency Database. Average TD_{50} values have been calculated separately for rats and mice and the more sensitive species is used for calculating possible hazard. A number in parentheses indicates a TD_{50} value not used in HERP calculation because it is the less sensitive species; (–), negative in cancer test. (+), positive in cancer test(s) not suitable for calculating a TD_{50}. (?), not adequately tested for carcinogenicity.

[a]The CPDB includes experiments on the hydrochloride salt. The TD_{50} value reported is expressed as the free base.

*Estimate is based on average daily dietary intake for 60–65 year old females, the only adult group reported for 1990. Because of the agricultural usage of these chemicals and the prominence of fruits and vegetables in the diet of older Americans, the residues are generally slightly higher than for other adult age groups.

reference points are the HERP of 0.001% for average exposure to chloroform (a by-product of water chlorination) in a liter of tap water, and the upper bound risk estimate used by regulatory agencies of one in a million (using the potency value q^*_1 derived from the linearized multistage model), which converts to a HERP of 0.00003% for rats and 0.00001% for mice. Natural chemicals in the diet have not been a focus of carcinogenicity testing, except for HA. Given the finding that about half of natural chemicals that are tested are carcinogenic, it is reasonable to assume that the background of natural exposures to rodent carcinogens is enormous, considering that each plant food has many untested natural pesticides and that there are about 1000 untested chemicals in roasted coffee.

In comparison to other dietary exposures to rodent carcinogens, HA rank low in HERP. Values for HA in a typical serving range from 0.0001% for PhIP in baked salmon steak to 0.000005% for IQ in pan fried hamburger. For hamburger, average US consumption among those who eat hamburgers is similar to the value in Table 4; for people in the top 10% of hamburger consumption, the values would be about twice those in the table. HERP values for HA rank similarly to those for dietary consumption of synthetic pesticides that are rodent carcinogens. For many natural chemicals HERP values are above the median of 0.001% in Table 3. These include natural pesticides in lettuce (caffeic acid), mushrooms (various hydrazines), basil (estragole), mango (D-limonene), mustard (allyl isothiocyanate), parsnip (8-methoxypsoralen) and coffee (hydroquinone, catechol). Additionally, products of cooking or food preparation other than HA rank above the median: wine (ethyl alcohol), bacon (nitrosamines) and coffee (furfural).

4. Discussion

Several factors are relevant in analysing animal data to see if a chemical may plausibly be a major cause of human cancer.

(1) How is the carcinogen causing cancer? Is it primarily working through increasing cell division, e.g., rat bladder tumors from sodium saccharin [5] or mouse liver tumors from chloroform [20], or is it primarily acting as a mutagen? Is it causing tumors at many sites? HA are potent mutagens causing tumors at many sites and are positive in more than one species, reasons for not dismissing them lightly.

(2) Is the HERP of the chemical high relative to the back-ground of chemicals to which we are exposed? The data on HA are not impressive because the exposure levels are low. For some subgroups of the population e.g., people eating a regular diet of blackened meats, HERP values would be higher.

It is always possible that humans are more (or less) sensitive than rodents. In this connection, it is worth noting that at the high doses used in bioassays, the TD_{50} value for IQ in monkeys is about 3-times more potent than the rodent species with the more potent TD_{50} (rats). However, even if the HERP value for each HA were 3 times higher, the HERP values would only be moderate or low.

In conclusion, HA are worthy of serious consideration, but because of the low levels of exposure, the evidence suggests that they are not a high priority of concern as possible carcinogenic hazards to humans. Further research is needed on concentrations of HA in foods as commonly consumed.

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