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Estimates of heterocyclic amine intake in the US population

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Abstract

HA-specific meat concentration estimates using a method that combines laboratory data to predict HA concentrations from meat type, cooking method and meat doneness were used with national dietary data to estimate daily HA intake for segments of the US population. PhIP was found to comprise ∼70% of US mean dietary intake of total HAs, with pan-frying and chicken being the single cooking method and meat type contributing the greatest to total estimated HA exposures. This analysis demonstrated significantly higher concentrations in grilled/barbecued meats than in other cooked meats. African-American males were estimated to consume nearly twofold and ∼35 to 40% more PhIP (and total HAs) than white males at ages <16 and >30 years, respectively.

Keywords: Heterocyclic amine intake

1. Introduction

Heterocyclic amines (HAs) are potent mutagens formed at particularly elevated levels in well-done meats and fish [1]. HAs also cause cancer at a variety of sites in multiple bioassay animal species/strains/sexes [2]. HAs are found in a wide range of commercially and domestically prepared meats on the order of 1–100 ng/g cooked meat [3–7] and HA urinary metabolites are detected in the population [8]. Factors associated with human exposure to HAs are the consumption of meats prepared by broiling, grilling/barbecuing or pan-frying (high-heat cooking methods demonstrated to form HAs in meats) and a preference for meats cooked well done. Recent case–control studies have shown that estimated human dietary HA intakes (categorized crudely, based on self-reported preferences for meat type, cooking method and/or doneness) are associated with elevated risks of colon, stomach, lung, breast and prostate cancer [9–18].

Estimates of human exposure to HAs are complicated by the absence of HA concentration data on meats consumed by the public at large. Absent such information, experimentally derived HA concentrations provide the best available data with which to estimate HA concentrations in cooked meats consumed by the public. To date, assessments of HA dietary intake have relied on such experimental data, notwithstanding up to 30-fold variations in measured HA concentrations for many commonly consumed meats [1]. Accordingly, estimates of population-average dietary HA intake vary considerably, ranging from ∼2 to >25 ng/kg per day [19–22]. A major methodological difference between these assessments is the selection of HA concentrations considered representative for meats consumed by the population. Whereas some studies conducted a review of the literature and used expert opinion to estimate HA concentrations for cooked meats consumed by the population, other studies obtained concentrations from laboratory cooking trials designed to represent common cooking conditions. Recently, an approach was developed to systematically incorporate experimental data on HAs from laboratory cooking trials into a methodology for estimating HA levels in cooked meats [23]. Using this methodology, an integrated approach to HA-exposure assessment was developed for estimating dietary HA exposures in a way that reflects individual meat-specific consumption preferences, intake rates, cooking methods and doneness preferences [24]. In this study, the approach is updated with new laboratory cooking data on HAs and new US cooking survey data that character-

Abbreviations: AαC, 2-amino-9*H*-pyrido[2,3-*b*]indole: 2-amino-α-carboline (CAS #26148-68-5); BBQ, grill/barbeque; CSFII, Continuing Survey of Food Intakes by Individuals; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (95896-78-9); HAs, heterocyclic amines; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline (76180-96-6); IT, maximum internal temperature; MeIQx, 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (77500-04-0); PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (105650-23-5)

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ize cooking-method preferences by race-ethnicity to derive new exposure estimates for segments of the US population.

2. Experimental

2.1. Update of HA database

The database of HA concentrations in cooked meats used previously [23] was updated through a review of the literature. The database consists of studies reporting HA concentrations in cooked meats and fish that: (1) used cooking methods consistently found to generate HAs in meat (oven broiling, pan-frying and grilling/barbecuing); (2) involved common HA-forming meat types, namely, beef (steak and beef cubes), hamburger, chicken (leg and breast), pork (chop, fillet, and ham slice), bacon, and fish (6 species); (3) did not involve uncontrolled (non-laboratory) cooking conditions (e.g., fast-food or cook-to-order restaurant sources), unique ethnic or regional meats (e.g., reindeer, Swedish sausage and bonito) or cooking practices shown to attenuate HA formation in cooked meats (e.g., marinade, microwave pre-cooking); (4) analyzed for $\geq$2 of the 5 major HAs (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine [PhIP], 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline [MeIQx], 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline [DiMeIQx], 2-amino-1,6-dimethylfuro[3,2-*e*]imidazo[4,5-*b*]pyridine [IFP], and 2-amino-9*H*-pyrido[2,3-*b*]indole [AαC] and; (5) reported either the internal temperature (IT) or weight loss (WL) of the cooked meat at termination of cooking or the cooking surface temperature and cooking time used to prepare the meat.

2.2. Estimation of total HA and HA-specific concentrations in meats

The methodology for estimating HA concentrations in cooked meats developed previously [23] was applied to the updated HA database for those meats for which new HA data were identified (beef steak, beef-hamburger, and chicken). Briefly, studies were sorted into meat-specific categories for exploratory regression analyses. These analyses examined the relationship between total HA concentration and IT conditional on cooking method. For studies not reporting IT, meat-specific IT was estimated from reported WL using an IT versus WL model [23]. HA-specific concentrations (ng/g) for AαC, IQ, MeIQx, DiMeIQx, and PhIP were estimated for total HA values by constrained least-squares linear regression using the model $HA_i = f_i$ HA to estimate HA-specific fractions of total method- and meat-specific HA concentrations independent of IT. Stepwise analysis of covariance was used to combine homogeneous values of f_i (for each HA) within method-specific meat categories. The results of these analyses were used to estimate total HA concentrations in each meat type at IT values of 71.6, 76.6, 82.2 and 87.7 °C. The first three IT values correspond to meats cooked to a medium (M), well (W), and very-well (VW) level of doneness, according to guidelines established by the Food Safety Inspection Service of the USDA [25]. The 87.7 °C level represents an extreme IT value selected to represent a charred, blackened, or "extra-well" (XW) meat doneness level.

2.3. Dietary HA analysis

Data from our previous study [24] was modified to reflect newly acquired data. US dietary consumption of cooked meats and fish was estimated from the US Department of Agriculture's 1989–1991 and 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII) database [26,27]. The CSFII surveys obtained multi-day food diaries that categorized the type and amount of meat consumed by the individual and its method of preparation. We previously identified 21,780 records in the CSFII databases corresponding to HA-containing food items and used these data to estimate US dietary HA intakes. The CSFII surveys categorized reports of grilled meat as "broiled" so for this analysis, race-specific frequencies for the preparation of hamburger by outdoor grill/barbeque and indoor grill/broil reported in the FDA/USDA Consumer Food Safety Surveys ([28], Fig. 1) were used to adjust CSFII reported national consumption patterns for broiled hamburger and other non-poultry meats among blacks versus whites. Meat-specific doneness preference distributions estimated without reference to race from available survey data [24] were applied to 24,790 individual-specific sets of CSFII records, a total of 20,185 of which pertain to US African-Americans ("blacks") and whites excluding all (−10%) those who during the survey days reported consuming meat(s) cooked using only non-HA-forming methods or only from fast-food sources (which generate minimal HAs). Meat intake records from the latter 20,185 sets were assigned total and individual HA quantities based on the corresponding estimated meat type/cooking method/IT value and the individual's intakes summed to provide the daily HA intake (ng/kg per day), scaled to adjust for caloric under-reporting with corresponding weighted mean intakes then obtained using CSFII sample weights, as previously described [24]. In the present study, however, blacks were assumed to grill/barbeque (versus pan-fry or broil) a 20% greater fraction of all hamburger and beefsteak they consume compared to US whites, based on cooking-method data obtained from the FDA/USDA Consumer Food Safety Surveys [28] showing a substantially greater difference in the cooking methods of hamburgers between African-Americans and whites (Fig. 1).

2.4. Data and statistical analysis

Pairwise comparisons of means were done by two-tailed *t*-tests, using Welch's approximate *t*-test in each case involving unequal variances as first assessed by an *F*-test [29]. All calculations were done using Mathematica and related software [30,31].

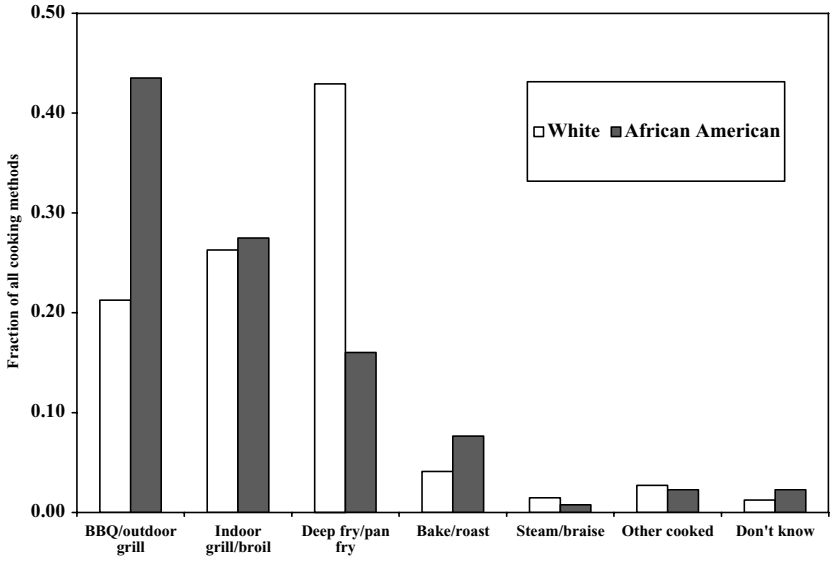

Fig. 1. Distribution of hamburger cooking methods by US blacks and whites reported in FDA/USDA survey (adapted from [28]).

3. Results

3.1. Update of HA database

Three studies meeting the database criteria were identified from the literature (Table 1). These studies added 16 measurements of HA concentrations in chicken and beef to the HA database. Studies not added to the database did not provide sufficient information on cooking conditions and efforts to obtain this information from the authors were unsuccessful. Absence of data on the weight loss of the cooked meat was the most common reason for excluding studies from the analysis.

3.2. Estimation of total HA concentrations in meats

Regression analysis of the updated data set identified significant relationships between IT and total HA con-

Table 1
New IT data and HA concentrations used in regression analyses

Meat type	Cooking temperature (°C)	Cooking time (min)	Weight loss (%)	Internal temperature (°C)[a]	HA concentrations (ng HA/g cooked meat)		
					MeIQx	DiMeIQx	PhIP
Pan fried chicken[b]	140	14	18	63.8	0.1	0.1	Trace
	170	16	21	68.3	0.3	0.1	0.7 ± 0.2
	190	18	26	75.4	1	0.6	10.5 ± 3
	220	12	25	74.0	1 ± 0.3	0.5	29.7 ± 1.4
	190	34	36	87.0	0.3 ± 0.1	0.3	38.2 ± 2
	190	31	38	88.7	1.8 ± 0.3	0.4	12.2 ± 0.4
	190	31	38	88.7	1.7 ± 0.4	0.4	19.3 ± 0.1
	170	20	20	66.8	0.2	0.1	Not detected
	220	20	29	79.3	1.5 ± 0.2	0.4	1.8 ± 0.1
Grilled chicken[c]	365	10	20	66.8	Not detected	Not detected	0.68 ± 0.48
	339	20	38	88.7	0.74 ± 0.19	0.54 ± 0.29	54.3 ± 32.2
	339	30	51	96.3	0.35 ± 0.43	0.72 ± 0.89	156.5 ± 122.4
	340	40	58	98.2	0.19 ± 0.17	1.93 ± 0.74	327.6 ± 128.9
Grilled beef[d]	250	3	31.3	82.1	0.2	0.1	0.8
	250	5	47.0	94.6	0.2	0.2	1.2
	250	7	55	98.2	1.3	0.4	2.0

[a] Internal temperature (IT, °C) of cooked meats estimated as the function, $IT = 100\,^{\circ}C(1 - \exp\{-(0.53[\pm 0.75] + 1.2[\pm 4.4]WL + 8.3[\pm 6.2]WL^2)\})$, of corresponding weight-loss (WL) due to cooking, by unweighted least-squares linear-quadratic regression of $-\log[1 - (IT/100)]$ on WL ($R^2 = 0.64$, $F(2, 50) = 45.3$, $P < 10^{-11}$ [24]).

[b] [33].

[c] [32].

[d] [34]. Values estimated from bar plots.

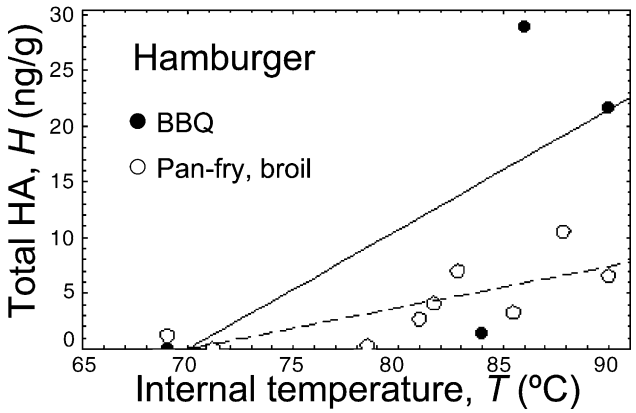

Fig. 2. Total HA concentration vs. internal temperature for hamburger by cooking method. Least-squares regressions are shown for BBQ ($H = 1.07R$) and for pan-fry/broil ($H = 0.371R$), where $R = [T - 70]$ and $[z] = \mathrm{Max}(0, z)$. The coefficient in R in the BBQ fit is significantly greater than that in the non-BBQ fit ($P = 0.028$, by two-tail Welch's t-test). BBQ: grill/barbeque.

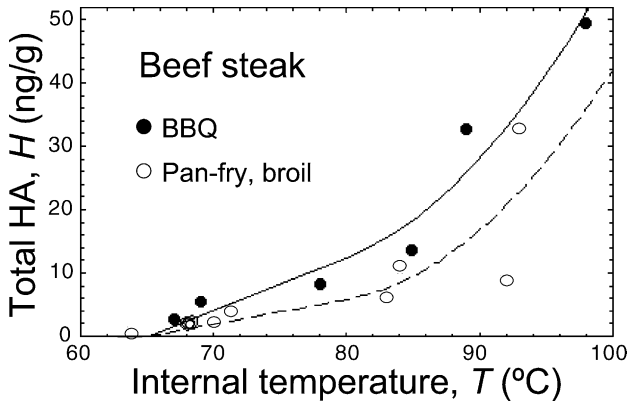

Fig. 3. Total HA concentration vs. internal temperature for beef steak by cooking method. Least-squares regressions are shown for BBQ ($H = 0.832R + 0.0718S^2$) and for pan-fry/broil ($H = 0.388R + 0.0706S^2$), where $R = [T - 65]$, $S = [T - 80]$, and $[z] = \mathrm{Max}(0, z)$. The coefficient in S in the BBQ fit is significantly greater than that in the non-BBQ fit ($P = 0.011$, by two-tail t-test). BBQ: grill/barbeque.

centration for each meat type, unconditional of cooking method. Analysis of the different cooking methods within each meat type identified significant differences between grilling/barbecuing and the other combined cooking methods. Grilling/barbeque was observed to be associated with approximately 1.4- to 2-fold higher HA levels than either pan-frying or oven broiling in hamburger (Fig. 2), beef steak (Fig. 3), and chicken (Fig. 4).

3.3. Dietary HA analysis

Using CSFII data adjusted for different cooking method preferences together with the cooking method-specific HA estimation equations as described above, PhIP was found to comprise ~70% of US mean dietary intake of total HAs, with pan-frying and chicken being the single cooking method and meat type contributing the greatest to total estimated HA exposures (data not shown). African-American males were estimated to consume nearly twofold and ~35–40% more PhIP (and total HAs) than white males at ages <16 and >30 years, respectively (Table 2). Although estimated total HA intakes were found to differ little by age and by sex, African-American males were estimated to consume significantly more PhIP and total HAs than white males: at ages <16 years this difference was found to be approximately twofold ($P = 2.7 \times 10^{-5}$), and at ages of >30 years about 1.4-fold ($P = 0.029$) (Table 2). The percents of weighted-mean values of total PhIP intakes es-

Table 2
Estimated HA intake of African-American and white males and females in US[a]

Age	Race	Sex	n	Major dietary HAs (ng/kg per day)			Total[b] (five HAs) (ng/kg per day)
				PhIP	MeIQx	DiMeIQx	
Child (15 years)	White	Male	2478	7.7*c*	1.3	0.27	11.0
		Female	2233	8.1*d*	1.2	0.28	11.4
	African-American	Male	520	14.7*c*, *g*	1.8	0.67	19.9
		Female	608	10.8*d*, *g*	1.5	0.42	14.8
Adult (>30 years)	White	Male	4734	9.2*e*	1.4	0.28	13.4
		Female	4955	9.2*f*	1.4	0.28	13.6
	African-American	Male	546	12.6*e*	2.1	0.47	18.4
		Female	858	12.7*f*	1.6	0.41	18.4

Statistical significance of differences (by Welch's two-tail t-test) between indicated pairs of estimated mean PhIP intakes: (c) $P = 2.7 \times 10^{-5}$; (d–g) $0.01 < P < 0.05$; $P > 0.05$ for all other pairs.

[a] Based on USDA CSFII survey data for 1989–1991 and 1994–1996; n: number of participants. HA estimates listed are weighted mean values using CSFII survey-sample weights scaled for underreported energy intake without adjustment for any doneness-preference differences by race as described previously [24], but US African-Americans ("blacks") were assumed for the present analysis to grill/barbeque (versus pan-fry or broil) a 1.2-fold greater fraction of all hamburger and beefsteak compared to US whites (see Methods). Coefficient of variation values corresponding to the 32 estimated mean intakes listed range from 2.0% (MeIQx for white/female adults) to 13% (DiMeIQx for black/female children), but for both PhIP and total intakes are all ≤2.5% (white adults), ≤5.0% (white children), or ≤10 and ≤11% (black males and females, respectively).

[b] Total HA includes estimated intakes of AαC and IQ.

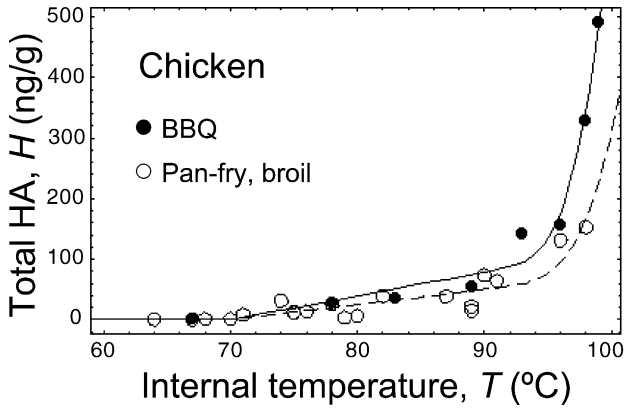

Fig. 4. Total HA concentration vs. internal temperature for chicken by cooking method. Least-squares regressions are shown for BBQ ($H = 3.96R + 0.0559S^4$) and for pan-fry/broil ($H = 2.51R + 0.0230S^4$), where $R = [T - 70]$, $S = [T - 90]$, and $[z] = \mathrm{Max}(0, z)$. The coefficients in R and S in the BBQ fit are significantly greater than those in the non-BBQ fit ($P < 10^{-6}$, by two-tail t-tests). BBQ: grill/barbeque.

timated to be due to intake of chicken, beefsteak, and fish for all black and white participants combined ($n = 20{,}185$) are approximately 50, 20 and 15%, respectively, with relatively little variation ($<\pm 10\%$) by age, sex and race. In marked contrast, of the total estimated mean PhIP intakes for black and white men under 30 years of age ($n = 3867$ and 703, respectively), the percent attributable to meat that is pan-fried is significantly greater among blacks (-75%) compared to whites (-45%) ($P = 0.00062$). These findings are consistent with our previous analysis [24] that attributed greater PhIP intake among African-American males to greater consumption of chicken and pan-fried meats than other ethnic groups.

4. Discussion

Our review of the literature identified many studies reporting HA concentrations in cooked meats since our previous analysis. Unfortunately, these studies did not report either IT or WL and so did not provide sufficient data for inclusion in the regression analysis. WL is relatively easy to determine and integrates multiple cooking variables such as cooking surface temperature and so offers a potentially useful parameter for normalizing different HA cooking studies. By adding newly acquired data from controlled cooking studies in which WL was recorded [32–34] to our original database, and by then re-analyzing the expanded database, grilling/barbecuing was shown to produce significantly higher HA levels than the other cooking methods. When cooking methods were compared within studies, grilling/barbecuing was found to produce higher HA levels than either pan-frying or broiling [34–37]. The analysis for grilled/barbecued hamburger relied on the fewest data and clearly has the poorest fit of the equations. Given the prevalence of hamburger consumption and the preference for grilling/barbecuing as a cooking method among segments of the population, our methodology would benefit most from more cooking studies of grilled/barbecued hamburger.

In this re-analysis of CSFII data, new laboratory data on HA levels in cooked meats were used [32–34] as well as a greater African-American versus white preference for grilling/barbequing based on recent US cooking-survey data that characterize cooking-method preferences by race-ethnicity [28]. Using the updated cooked meat database, our current analysis produced HA intake estimates for children and <30-year-old adults that were approximately 16 and 50% greater than our previous estimates [24], respectively. Without considering any racial differences in meat-doneness preferences, our current results again support the hypothesis that PhIP intake is about twofold higher by male African-American versus white children in the US. A preference for more well-done hamburger among African-Americans also indicated by recent survey data [38] was not even considered in our present analysis. We showed previously that taking this factor into account is likely to increase the expected ratio of PhIP intake by African-American to white male children (<15 years old) to be about 3.0 [24]. The 75% versus 45% fraction of total estimated PhIP due to pan-frying as a cooking method for African-American versus white men <30 years old reported here is similar to a corresponding difference for children <15 years old we reported previously [24]. This intake difference is interesting given recent laboratory studies showing that PhIP is capable of mutating prostate DNA and causes prostate tumor in rats [39,40] and the elevated rate of prostate cancer in African-Americans. HA dietary exposure may be a factor in the higher incidence of prostate cancer among African-Americans, especially so since HA exposure is highest in males during puberty, a period of gland development during which the prostate may be more sensitive to mutation by HAs.

Our estimates of daily intake of PhIP, MeIQx and DiMeIQx for an adult fall within the range of estimates reported by others. Estimates for the US population range from 6.3 ng/kg per day [21] to 20.1 ng/kg per day [19] whereas estimates for European populations range from 2.3 ng/kg per day [20] to 6.6 ng/kg per day [22]. Estimates for the US population from this analysis range from 11.0 to 19.9 ng/kg per day. Comparison of our estimates with that of [19] is particularly relevant as that study used select laboratory-derived HA values and the 1989–1991 CSFII survey to estimate HA intake and did not account for meat doneness as a variable in estimating HA concentrations. The two European studies analyzed commercial and home-cooked meats prepared to the cooking standards of the country of origin and used these concentrations to estimate HA intake. These estimates may reflect cultural differences in meat preferences and preparation that lead to lower HA intake in these countries. For example, Zimmerli [22] reports a 7% prevalence for charcoal

grilling in the Swiss population whereas in the US the prevalence of this cooking method is considerably greater [28]. It should be noted that estimates of HA intake have relied either exclusively or predominantly on HA concentrations from laboratory cooking studies and therefore may be unrepresentative of actual HA intake by various populations. Studies of HA concentrations in meats prepared domestically are needed to verify these intake estimates.

In conclusion, we present a method for estimating HA concentrations in cooked meats and fish that integrates data from different cooking studies. We applied the method in its present state to estimate daily HA intake based on a national survey of food consumption and internal temperatures selected as representative of meat doneness levels consumed by the public. We believe the method and corresponding HA estimates can and need to be improved using more and improved data on HA concentrations in cooked meats and fish and on dietary information pertaining to cooking practices and preferences. We encourage investigators to record IT and WL in laboratory studies of HAs in cooked meats.

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