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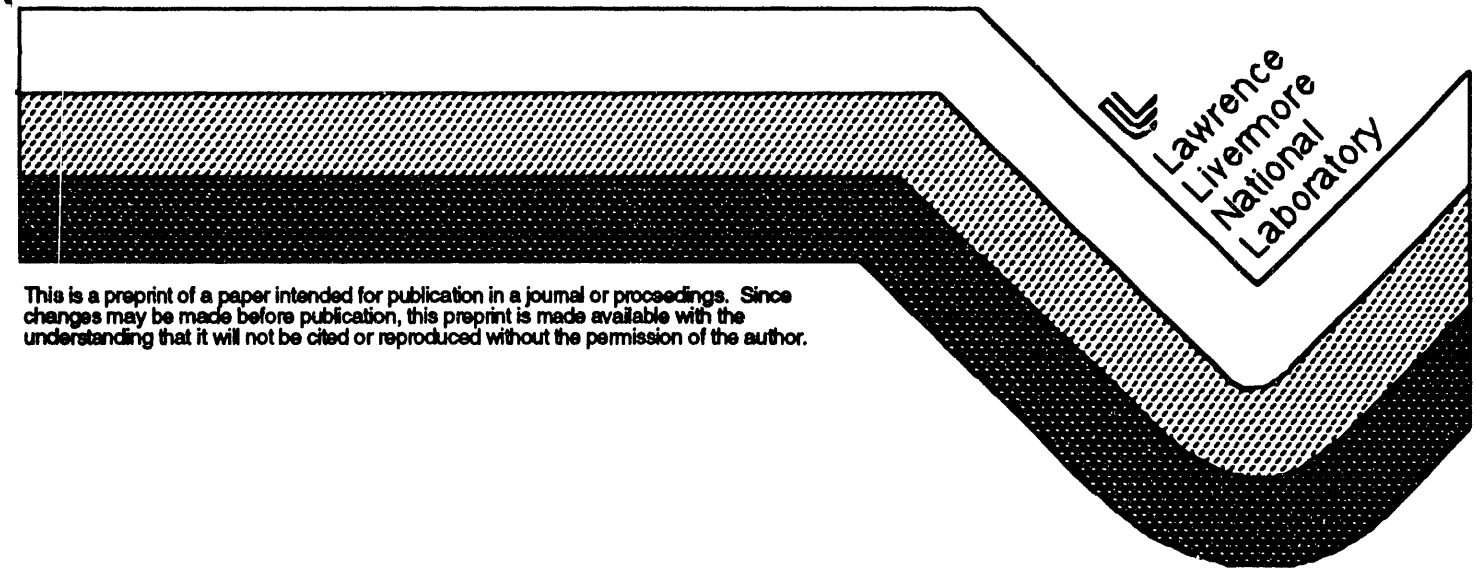

DISCLAIMER

Mutagenic Activity and Heterocyclic Amine Content of the Human Diet

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Running head: **Heterocyclic amines in the diet**

Abstract: The mutagenic activity and the mass amount of heterocyclic amines responsible for the mutagenic activity have been measured in some cooked foods. Cooked meats are the predominant source of mutagenic activity in the diet with values ranging from 0 to 10,000 revertants per gram reported in the Ames/Salmonella test with strain TA98. Several heterocyclic amines are present and have been quantified using solid-phase extraction followed by HPLC. Frying at higher temperatures and for longer times produces the greatest mutagenic response, and concomitantly, the largest amounts of heterocyclic amines. Most of the mutagenic activity in fried meat samples can be accounted for by MeIQx, DiMeIQx and IQ, although other heterocylic amines are present and PhIP mutagenic activity becomes significant at higher temperatures.

Non-meat products such as baked breads can also form significant mutagenic activity, particularly when overcooked. Commercially prepared hamburgers made from meat substitutes such as tofu, wheat gluten or tempeh and fried at 210°C have up to 10% of the mutagenic activity of a fried beef patty cooked under the same conditions.

When detected, amounts of heterocyclic amines in fried beef patties

range from a total of 0.35 ng/g for commercial beef hamburgers to 142 ng/g for a beef patty cooked over a barbecue. Dietary intake is expected to have a large range, from less than one microgram per day to over 50 micrograms per day based on current knowledge of known heterocyclic amine chemicals and heterocyclic amine-containing foods.

The human diet is complex, with one survey by the U.S. Department of Agriculture showing the diet contains more than 3500 items. Ninety percent of the bulk of the diet is from 500 different food items (1). The type of food, duration of cooking, and cooking temperature are important determinants in heterocyclic amine formation in foods.

The finding of potent mutagenic activity in foods was made by Sugimura et al. (2) using the Ames/*Salmonella* test. This test has been used extensively for: 1) the survey of foods for mutagenic activity, 2) the characterization of the mutagenic response using bacterial strain and metabolic activation differences, and 3) the detection of mutagenic activity to guide purification schemes used to isolate and identify a series of mutagenic heterocyclic amines from foods.

Recently published methods of heterocyclic amine extraction and analysis have allowed reproducible recovery-corrected analysis of foods for specific heterocyclic amine chemicals (3, 4, 5, 6).

This paper examines the amounts of mutagenic activity and heterocyclic amines present in a variety of foods. This information will help determine if the amount of heterocyclic amines consumed in foods is large enough to be responsible for carcinogenic effects observed in humans.

Mutagenic activity of foods

Measurement of mutagenic activity is a useful screening tool to determine

the foods and preparation conditions that need to be assessed for heterocyclic amine content. Table I shows mutagenic activity data from a variety of food types and cooking methods, and results from many different laboratories. For these data either strain TA98 or TA1538 was used. These two strains of frame-shift sensitive bacteria have been shown to give similar mutagenic responses to the purified heterocyclic amines discussed herein (7). The amount of mutagenic activity measured varies with cooking temperature, cooking method, and meat type.

Cooking methods have been compared by many researchers who have found that frying and broiling produce higher amounts of mutagenic activity while baking and deep-fat-frying produce lower amounts (9,10). Microwave cooking usually produces no mutagenic activity (10), although microwave cooked beef steak has produced mutagenic activity in one sample (11).

In contrast to meats, other cooked foods have shown little or no mutagenic activity. Cabbage and eggplant cooked at up to 400°C did not produce measurable mutagenic activity (16). A survey of 28 beverages revealed only three to have weak mutagenic activity (18). Springarn and Weisburger showed starchy foods like potatoes and breads heated to 230-265°C had mutagenic activity but only up to 10% of the mutagenic activity of beef cooked using similar conditions (19)

The generation of potent mutagenic activity measured in extracts of cooked meat has been explained by the condensation of creatine or creatinine with amino acids and sugars or their thermal decomposition products (23, this volume). These reactions form the basis for a series of aminoimidazoazaarenes that are potent mutagens in the Ames/Salmonella test.

Figure 1 is a graph of the mutagenic activity of a variety of bread products. These foods were toasted or baked in either of two ways, 1) according to package directions or normal cooking practices (Edible), or 2) toasted or baked for twice the normal time (Overcooked). Surprisingly, all samples showed an increase in

mutagenic activity with prolonged heating, with many samples having mutagenic activity approaching the values measured in cooked meats.

Table 2 shows mutagenic activity of meat substitutes fried as patties without added oil at 210°C for 6 min per side. All exhibit measurable mutagenic activity, up to 10% of the mutagenic activity of beef cooked in the same way. The high mutagenic activity in these non-meat foods cooked at normal temperatures is difficult to explain, because they lack the relatively high amount of creatine or creatinine found in muscle meats (0.4 to 0.5% by weight) These results suggest that creatine or creatinine are not required for generation of relatively high mutagenic activity in foods.

Mutagenic activity in the breads and meat substitute patties appears to be the result of heterocyclic amines, based on extraction and mutagenic activity characteristics. The contribution to cancer risk of the non-meat mutagenic activity will need to be determined from the amount and identity of the compounds responsible for the activity, and dietary consumption patterns for these foods.

We also note that reported mutagenic activities per gram of commercially-prepared foods are much lower than those shown in Table I; from 0 to 203 revertants per gram wee reported in a study of foods in Finland (21). A complete human diet prepared and cooked according to the usual practices in The Netherlands and then lyophilized showed 476-500 TA1538 revertants per gram. This compares to 0 to 3 revertants per gram for an uncooked diet (22). This result again shows that the cooking process is responsible for almost all of the mutagenic activity detected in human diets.

Quantitation of the mutagens in cooked foods

The compounds responsible for the mutagenic activity in cooked meats have been isolated from cooked meats and identified as a series of heterocyclic

amines. The number of compounds and the range of mutagenic activity per microgram make it necessary to determine both the mass amount and the toxicological potency of individual compounds for meaningful risk estimates to be made. Table 3 shows the results of several studies that determined the amount of several compounds in cooked meats. While a variety of methods have been used, the recent development of greatly simplified extraction procedures and the use of internal standards allows for repeated measurements and recovery corrected results (reviewed in ref. 25).

The results shown in Table 3 are generally consistent, showing PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) to be present in the highest amount and MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline) in an intermediate amount. Fewer measurements and lesser amounts of DiMeIQx (2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline), IQ (2-amino-3- methylimidazo[4,5-f]quinoline), Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3- b]indole), and Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole) are reported. The large differences in amounts measured in Table 3 can be explained in part by differences in cooking conditions used to prepare the samples. We have begun to analyze foods prepared by well-documented cooking procedures for known heterocyclic amines using the solid-phase extraction and HPLC method devised by Gross (5).

The sample of beef cooked over a barbecue (Table 3) was analyzed for polycyclic aromatic hydrocarbons (PAH) as well as heterocyclic amines. Six PAH were quantified and benzo[a]pyrene was found to be present at the highest concentration, at 17 ng/g. The total mass of the PAH measured was 34 ng/g. The contribution of PAH to the mutagenic activity of this barbecued beef sample would be less than 10 revertants per gram compared with over 2000 revertants contributed by the measured heterocyclic amines in this sample. Beef patties fried at 230°C had no detectable PAH (N. Rothman personal communication), but

contained four heterocyclic amines as shown in Table 3 .

A study of the formation of four heterocyclic amines in beef patties fried at 190°C for various lengths of time summarized in Figure 2. A large increase in all four amines is seen at 6 and 10 minutes. The low values at 2 and 4 minutes probably reflect a delay due to water loss causing the dehydration or precursor distillation to the meat surface, both of which appear to be necessary for heterocyclic amine formation.

The mutagenic activity of extracts of the fried meat also increases with frying time (fig. 2). Using the known specific activities of PhIP, MeIQx, DiMeIQx, and IQ of 20, 99, 320, and 200 TA98 revertants per nanogram, and multiplying by the nanograms of each heterocyclic amine measured (fig. 2), the expected mutagenic activities would be 30, 80, 434, 918 revertants per gram of meat for 2, 4, 6, and 10 min frying time per side, respectively. Measured mutagenic activities are 22, 153, 178, and 310 revertants per gram for the 2, 4, 6, and 10 min frying times. Mutagenic activity for standard solutions is additive and we are investigating other explanations for the apparent discrepancy between the measured and calculated mutagenic activities in extracts of fried beef patties.

The heterocyclic amines shown in Table 3 are responsible for most of the mutagenic activity in the food samples examined to date but some other heterocyclic amines have been reported. An amino-trimethylimidazopyridine (molecular weight 176) was reported in fried beef and a fried Norwegian meat product (26,27). IQx (2-amino-3-methylimidazo[4,5-f]quinoxaline, molecular weight 199) and DMIP (2-amino,1,6-dimethylimidazo[4,5-b]pyridine, molecular weight 162) were reported in a fried Norwegian meat product (27,28). A proposed amino-imidazofuropyridine (molecular weight 202) (29) was reported in cooked pork and beef (30). Of these, the amino-imidazofuropyridine may be present in the highest amount relative to known heterocyclic amines in pork cooked at 250°C. Synthetic

compounds to be used as standards are needed to develop methods for the analysis of these heterocyclic amines.

Mutagen exposure reduction

Since the discovery that heterocylic amines are produced during cooking, ideas for minimizing their formation have been explored. Lowering cooking temperatures to below 200°C and using cooking methods that reduce meat surface temperature reduces mutagenic activity and the amounts of heterocyclic amines formed (6). Shortening the cooking time also can also reduce the production of these compounds. As can be seen in Fig 2, cooking for six minutes per side instead of 10 minutes reduces the total heterocyclic amine content from 14.5 ng/g to 4.2 ng/g. Reducing the concentrations of heterocyclic amine precursors creatine, amino acids and sugars by microwave pretreatment of meat patties before frying has been shown to reduce mutagenic activity to 10% of meat patties fried as usual (31).

The addition of 2-4% of simple carbohydrates such as glucose or lactose reduces the mutagenic activity of subsequently-cooked ground meat (32). Tryptophan (75 mg) added to each surface of a meat hamburger before frying also lowers mutagenic activity (33). Such additives and procedures appear to be practical ways to reduce heterocyclic amine formation and thereby lower the human exposure to heterocyclic amines.

Estimates of dietary intake from food analysis

Dietary intake of heterocyclic amines will vary greatly depending on food preparation and the type of foods eaten. The 1985 USDA food intake survey reports the intake of meat, poultry and fish to be an average of 224.5 g per day. Using conservative values for meat cooked at 190°C for 6 min per side, PhIP and

MeIQx are present at 1.3 and 1.9 ng/g of meat respectively (data from Figure 2). Multiplying by the daily intake gives an average intake of 720 ng per day for just PhIP and MeIQx, and this figure accounts only for meats. For beef cooked at 250°C for 10 min per side, the average meat intake would result in 6650 ng per day for the 24 and 8 ng/g measured for PhIP and MeIQx, respectively (data not shown).

Estimates of dietary intake from human urine analysis

Since it appears that the human diet has many cooked foods that contain heterocyclic amines, methods have been developed to calculate the dietary dose from urinary excretion of the compounds after food ingestion and metabolism.

Baker et al.(34), showed mutagenic activity can be detected in human urine following the ingestion of cooked pork. Hayatsu et al. (35) determined that 2 to 19% of the consumed mutagenic activity was recovered in urine after a meal of cooked beef.

Recovery of a specific mutagenic compound, MeIQx, was determined in urine by Murray et al. to be 1.8 to 4.9% of the ingested amount (36). Recently, the amounts of four heterocyclic amines in urine from humans eating an unrestricted diet were determined by Ushiyama et al. (37). MeIQx, PhIP, Trp-P-1 and Trp-P-2 were detected in each sample. MeIQx was the most abundant compound. Adjusting for metabolic losses, the daily dose from MeIQx alone was determined to range from 0.2 to 2.6 micrograms per day for the 10 individuals participating in the study. It is noteworthy that heterocyclic amines were detected in all individuals on an unrestricted diet but were not detected in three hospital inpatients receiving parenteral alimentation with a solution of sugars, salts and vitamins.

DISCUSSION

Total amounts of heterocyclic amines in the diet appear to range from less

than one microgram per day for those eating a diet low in cooked foods, especially cooked meat, to greater than 10 micrograms per day based on the food and human urine analysis noted above. Heterocyclic amine amounts probably vary greatly from day-to-day and among individuals based on dietary practices over a lifetime because of dietary practices.

The measurement of mutagenic activity of food extracts is a useful screening tool to determine which food samples should be analyzed for heterocyclic amines. Most of the mutagenic activity of fried beef is contributed by the high specific activity compounds, MeIQx, DiMeIQx, and IQ, although in some food samples, such as barbecued beef patties, known heterocyclic amines only account for 25% of the mutagenic activity detected in the meat sample. Given the range in mutagenic potency of individual heterocyclic amines, quantitative measurement of amounts are needed. Unknown mutagenic heterocyclic amines are probably present in some foods, and their contribution to the risk of heated foods cannot be determined except by the evaluation of genotoxic effects of whole foods.

Many of the food studies reported in the literature have a bias toward the generation of high mutagenic activity using high temperatures. This was necessary because measurable mutagenic responses were required for mutagenic compound isolation and identification. For risk assessment of an entire population, realistic determinations for the individual compounds need to be made from foods cooked as they are normally eaten. Food analysis, combined with food consumption surveys, are needed to give accurate dietary dose assessments of these compounds. We believe that samples prepared at higher temperatures and cooking times are useful for determining risk of a selected population that prefers foods cooked to a well-done state.

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Figure 1. Mutagenic activity of bread products. "Edible" denotes cooking according to package directions. "Overcooked" denotes cooking for twice the normal cooking time.

Figure 2. Graph of heterocyclic amine content and mutagenic activity in beef patties fried at 190°C for 2, 4, 6, or 10 minutes per side.

Table 1 Mutagenic activity of Heated Foods and Ingredients

Food type and heat source temperature	rev/g (cooked weight)	reference
Beef hamburger, 275°C	750-9200	8
Beef, baked, fried, 121-204°C	100-994	9
Meats, various, 100-220°C	0-580	10
Pork products, 200-300°C	1-240[a]	15
Fish, Hawaiian, 190-280°C	1-85[a]	12
Cheese, baked or fried, 204-375°C	0-4	13
Pancakes, Swedish 160-175°C	80	14
Pancakes, 475°C	2	15
Eggs, fried 100-310°C	0-3	15
Oatmeal, 100°C	4	9
Beans, boiled/baked, boiled/fried	0-36	13
Tofu, 100-200°C	0-4	13
Rice, to 400°C	0	16
Wheat gluten	14,000	17

[a] revertants per gram uncooked weight.

Table 2. Mutagenic Activity of Fried Meat Substitutes

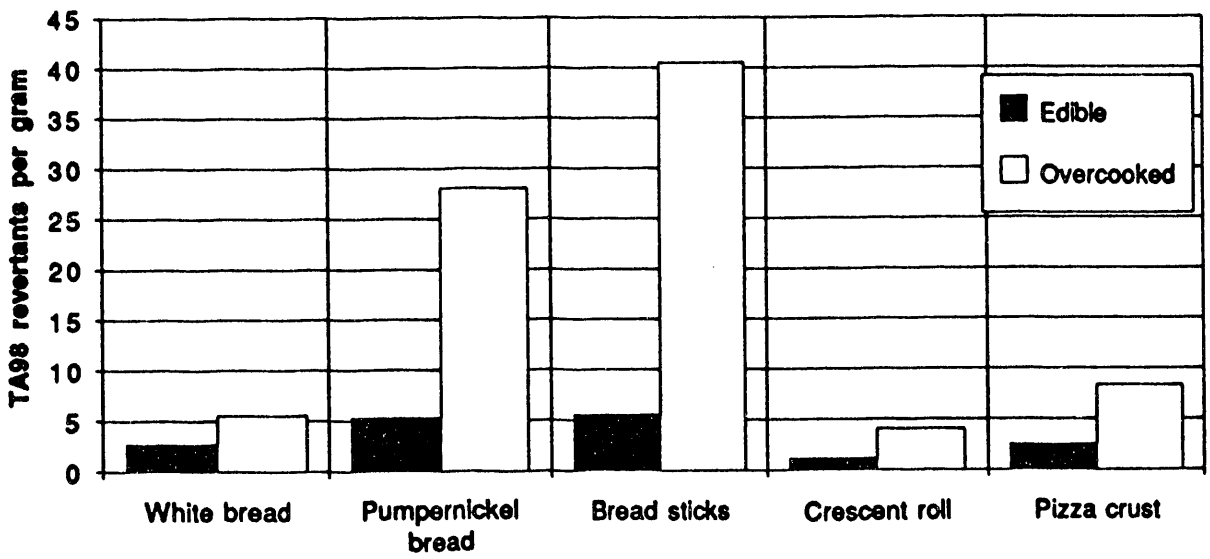
Sample [a]	TA98 revertants per gram
Gluten-based #1	7.7
Gluten-based #2	9.4
Tofu	nd[b]
Falafel	2.3
Tempeh-based patty	22.8
Tofu-based patty	6.6
Vegetable steak (gluten)	6.0
Ground beef patty	218

[a] All samples were formed into patties 1.5 cm thick, 9 cm in diameter and fried at 210°C,6 min per side.
[b] Mutagenic activity not detected.

Table 3. Amounts of Multiple Heterocyclic Amines in Cooked Meats

Food	PhIP	MeIQx	AAC	4,8-DiMeIQx	IQ	Trp-P-2	Trp-P-1	Ref.
Beef, broiled	15.7	2.11	1.20	nd[a]	0.19	0.25	0.21	23
Beef, fried	0.56	0.64	nd	0.12	nd	0.21	0.19	23
Fish, fried	69.2	6.44	nd	0.1	0.16	nd	nd	24
Beef, fried 190°C	48.5	8.3	8.9	2	nd	nd	nd	5
Salmon, fried 200°C, 12 min	23	5	4.6	nd	nd	nd	nd	6
Beef, barbecued	38	4.4	95	2.7	1.6	nd	nd	*c*
Beef, fried. 230°C, 12 min	4.1	1.1	nd	0.25	0.25	nd	nd	*c*
Commercial Hamburger #1	nd	0.68	--*b*	0.28	--	--	--	*c*
Commercial Hamburger #2	nd	0.26	--	0.1	--	--	--	*c*

a Not detected.
b Not determined, method used does not detect this compound.
c This laboratory, unpublished results.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35
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25
20
15
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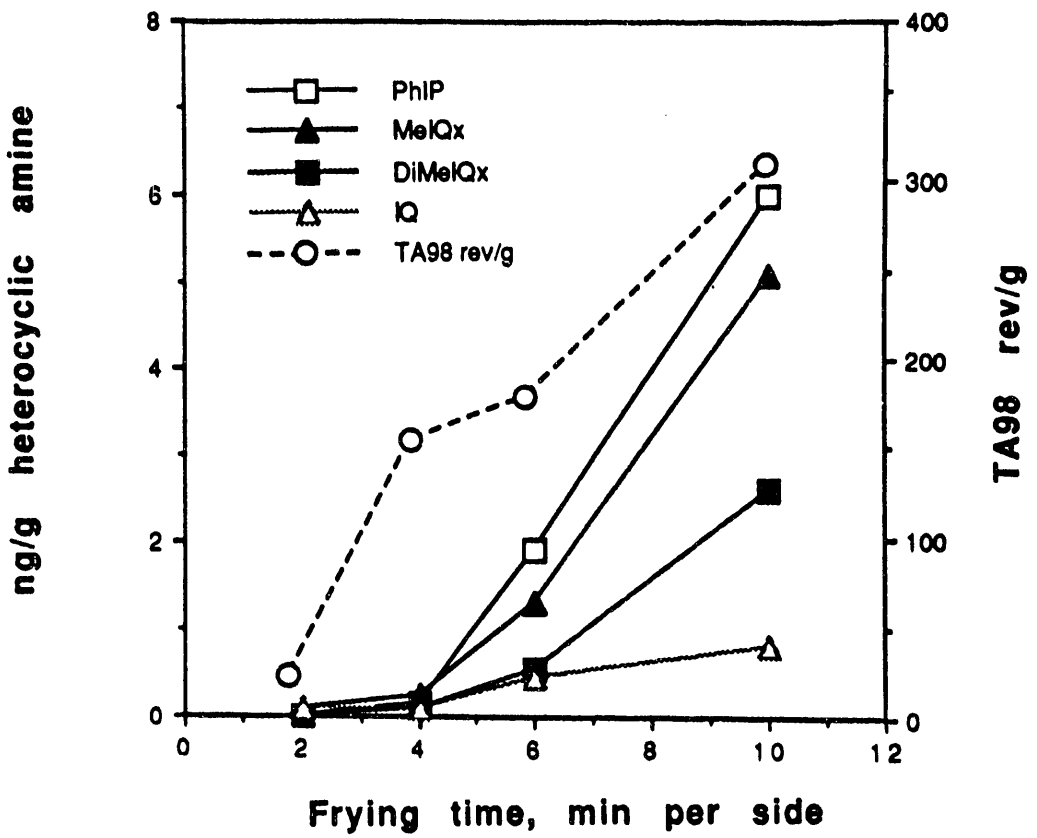
TA98 revertants per gram
Edible
Overcooked
White bread
Pumpernickel bread
Bread sticks
Crescent roll
Pizza crust

Fig 1


PhIP
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TA98 rev/g
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TA98 rev/g
Frying time, min per side
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fig 2

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