ACRYLAMIDE IN FOOD:
IS IT A REAL THREAT TO PUBLIC HEALTH?

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by

Joseph D. Rosen, Ph.D.
Department of Food Science
Rutgers University
New Brunswick NJ 08901-8520

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AMERICAN COUNCIL ON SCIENCE AND HEALTH
1995 Broadway, 2nd Floor, New York, NY 10023-5860
Tel. (212) 362-7044 • Fax (212) 362-4919
URL: http://www.acsh.org • E-mail: acsh@acsh.org

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EXECUTIVE SUMMARY

- There is no credible evidence that acrylamide in food poses a human cancer risk.
- Recent studies indicate that acrylamide, a known animal carcinogen, is formed in many foods when they are cooked.
- Acrylamide has not, even in high exposure occupational settings, been shown to cause cancer in humans. The high-dose rodent tests that concluded that acrylamide increases the incidence of tumors cannot be extrapolated directly to humans.
- Toxicity and carcinogenicity tests on rodents are performed using very high doses for much of the animals' lifespan. These doses may be hundreds or thousands of times greater than those to which humans are typically exposed.
- One hypothesis suggests that any chemical at high enough dose will kill some cells, thus causing an animal's body to increase proliferation of cells for replacement. This increased rate of cell division in and of itself makes the animal more susceptible to any carcinogen or mutagen. But this type of experimental approach skews the results of the tests, and artificially inflates the risk calculated from those results.
- The fact that a chemical causes cancer in one species, e.g. rats, does not necessarily mean it will be carcinogenic in other species like mice, let alone in humans. Sometimes only one sex of one species will be susceptible to the carcinogenic effects. This was evident in the case of the synthetic sweetener saccharin, which increased the risk of cancer only in male rats.
- There are many naturally occurring and cooking-induced chemicals in human foods that, like acrylamide, can cause tumors at high doses in rodent tests. Avoiding all of them would leave practically nothing for humans to eat.
- The risk that acrylamide (and most other rodent carcinogens) in our foods increases the risk of human cancer is hypothetical at best. ACSH does not advise consumers to alter either their food choices or food preparation methods on the basis of postulated cancer risks.

INTRODUCTION: WHAT IS ACRYLAMIDE?

Acrylamide is a chemical whose major use is to produce polyacrylamide, a coagulant used in drinking water and wastewater treatment. Acrylamide is also used in the construction of foundations for tunnels and sewers. In one such application, the construction of the Hallandsas railway tunnel in southern Sweden, acrylamide was used to repair water leaks that had developed in the tunnel. Not all of the acrylamide hardened into the polymer and some of the acrylamide seeped into an adjacent river with the result that fish were killed and several cows that drank from the river were paralyzed. Concern about the health of the tunnel workers (acrylamide was a known carcinogen in rats and a neurotoxin in occupationally-exposed workers) led a group of scientists headed by Margareta Tornnqvist, an associate professor of environmental chemistry at Stockholm University, to develop a test that measured the presence of an acrylamide-protein adduct in blood. The investigators not only found this adduct in the exposed tunnel workers, but also among members of the general population that had no known occupational exposure to acrylamide. Theorizing that acrylamide may be a food contaminant, they joined forces with the Swedish National Food Administration (NFA) to develop analytical methodology for the determination of acrylamide in food.

In April of 2002 the Swedish scientists and NFA called a press conference to announce that they had found "alarmingly" high quantities of acrylamide in bread, biscuits, cereal, potato chips and French fries. Lief Busk, head of Sweden's NFA, said, "I have been in this field thirty years and I have never seen anything like this before".[1] Adding to the worry was that the news stories covering this announcement noted that World Health Organization (WHO) regulations permitted only one microgram acrylamide in a liter of water. (A microgram is one-millionth of a gram; a liter of water weighs one kilogram or 1,000 grams or 2.2 pounds or 35.2 ounces by weight.) This translated to "an ordinary bag of potato chips may contain up to 500 times more" and French fries sold by "fast-food chains Burger King Corp and McDonalds contained about 100 times more"[1] acrylamide than permitted by WHO in drinking water. Dr. Tornqvist, said that consumption of a <u>single</u> potato chip "could take acrylamide intake up to the WHO maximum for drinking water.[2]

In late June 2002, because of the possible importance of these findings to human health, WHO and the United Nations Food and Agriculture Organization (FAO) convened a meeting of 23 scientific experts from all over the world to assess the situation. After 3 days of consultations, the experts agreed that they did not yet have enough information to assess how much risk, if any, acrylamide posed and they identified a number of important issues for which more research was needed.

Not content to wait for any new information that might allow scientists to provide meaningful advice, two California environmental groups filed suit under California's Proposition 65 to require fast food restaurants and snack food manufacturers to warn consumers that their products contained a chemical "known to the State of California to cause cancer".[3]

So, is acrylamide formation in food really a cause for concern? Or will it turn out to be another one of those periodic food scares such as saccharin, aminotriazine in cranberries, cyclamates in diet soda, nitrosamines in bacon and luncheon meats, Red Dye #2 in processed foods, Alar in apples and all the other chemicals that have been the subjects of the frequent news reports about something else in our food or water that causes cancer? Acrylamide, just like a large number of other rodent carcinogens, is formed during normal cooking of a wide variety of foods.

In the next few months a great deal of information about acrylamide will be published and broadcast. The aim of this article is to provide information on food chemistry, food toxicology, and animal carcinogenicity testing so that consumers can better interpret this information and better judge the possibility of risks to themselves and to their loved ones.

TOXICOLOGY 101

Perhaps the best way to start a toxicology discussion is to recall the teachings of Paracelsus, a 16th century physician who said, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy." Toxicologists determine the toxicity of a chemical by administering varying doses of the chemical to animals and then calculate the amount needed to kill 50% of the animals. This number is known as the lethal dose for half the animals (LD_{50}). A chemical with a low LD_{50} is more toxic than one with a high LD_{50} because it takes a smaller dose to kill half the animals in a group with the former chemical than with the latter. Clostridium botulinum toxin, the agent responsible for botulism, is considered to be the most toxic material known, with an LD_{50} of 400 picograms toxin per kilogram mouse[4] (a picogram is one trillionth of a gram). Sodium cyanide, while very toxic, is relatively benign, with an LD_{50} of 15 milligrams per kilogram[5] (there are 1,000 milligrams in a gram). In spite of the incredible toxicity of Clostridium botulinum toxin, thousands of people have had extremely dilute solutions of BoTox injected into their faces to remove wrinkles and have lived to tell the tale (although without too much expression).

The Food and Drug Administration (FDA) has used Paracelsus' doctrine in the regulation of food additives. Before a new food additive is approved for use, the manufacturers of these materials must submit toxicity data including dose–related effects, the identification of a target organ, and clearly defined "no observable adverse effect level" (NOAEL) for one or two different animal species. This is the lowest dose at which there are no signs of toxicity in **either** animal species. In order to account for the possibility that humans might be more susceptible to the food additive than either animal species, this NOAEL is divided by a factor of 100 to determine the dose that is considered safe for humans (Acceptable Dietary Intake, or the ADI). The FDA then takes into account how much of the additive people will be exposed to, and will approve the additive only if the human dose is less than the ADI. The Environmental Protection Agency (EPA), in general, follows the same procedures for pesticides, but may apply a safety factor of up to 1000 instead of 100 if it is determined that children are unduly exposed to that particular pesticide and the pesticide is thought to have adverse developmental effects.

Food additives and pesticides that are determined to be carcinogens in animal tests are regulated differently. A proposed food additive that was shown to cause cancer in animals would not be permitted under any circumstances under provisions of Section 409 C(3)(A) of the Food, Drug and Cosmetic Act (the Delaney Clause). Under certain circumstances however, such as when the food chemical is not *legally defined as a food additive* (such as a pesticide), the Delaney Clause does not apply. Instead, a human cancer risk is calculated from animal data (almost always rats and mice administered very high doses of test substance) and estimated human exposure data, which is very much lower. If it is calculated that the chemical will cause an increase of between 1 and 10 cancers per million people over a 70-year lifetime, the chemical is not approved for use. For chemicals that have been found to cause cancer in rodents after they have already been approved, government agencies may bring action to rescind the chemical's approval if the human risk calculation exceeds this standard.

Risk assessment for non-carcinogens and carcinogens are both based on Paracelsus' teaching that the dose makes the poison. However, whereas non-carcinogens have been shown to have a no observable adverse effect level, some scientists believe that no such level (dose) exists for carcinogens, especially if the carcinogen in question is also a mutagen. A mutagen is a material that can chemically react with DNA; such a reaction may cause irreversible changes in the cell. Should one of these changes in the cell's DNA impair the cell's ability to limit its growth, a tumor may eventually result, especially since the mutated cells can multiply much faster than normal cells. Thus, while the risk of cancer from a small dose of a mutagen may be small, it is not zero. In general, any cancer risk above the standard 1 to10 in a million over a 70-year lifetime exposure is a cause of concern to regulatory agencies. In 1989, the EPA proposed the banning of Alar because of a calculated cancer risk of 45 extra cancer cases over the lifetime of 1,000,000 exposed individ-

uals. No one seemed to care that, even if this calculation was correct, the chances of not getting cancer from the daily ingestion of tiny quantities of Alar for life was 999,955 out of 1,000,000.

Ever since the realization that exposure to some chemicals, primarily in high dose occupational settings, can cause cancer in humans, a great deal of time and money has been spent on trying to determine which of the thousands of chemicals in our environment are responsible for contributing to this disease. Since human testing is out of the question, we get our information from animal experiments with laboratory rats and mice, which, unfortunately, may not permit us to accurately predict human cancer risk.

Consider the testing of a chemical that will increase cancer incidence by only 0.1% if administered to animals at the doses humans would ordinarily be exposed to. That doesn't sound like much but it translates into an additional 280,000 cases of cancer in the United States alone. If we use rodents that have a 1% spontaneous cancer rate (many of the rodents used in standardized cancer assays have higher rates), then in order to prove, with 95% confidence in our results, that the test chemical is indeed a carcinogen, we will need to administer the chemical to *80,000 animals in each of 3 test groups*.[6] There aren't enough laboratories, cages, water bottles, technicians and veterinary pathologists to do the assay properly.

Since it is experimentally impractical to do a proper cancer assay, regulatory agencies have opted for a protocol that at least allows for determining if a chemical has any cancer potential at all. To do so, rats and/or mice are exposed to huge doses of the test substance every day for life, 18–24 months. This dose is determined by finding out what is the maximum dose of the test substance the animal can tolerate without dying or getting sick from causes other than cancer; hence the term maximum tolerated dose (MTD). Historically, according to National Cancer Institute/ National Toxicology Program (NCI/NTP) protocols, cancer tests for chemicals such as acrylamide were carried out at least three doses: the MTD, the control dose (zero amount of chemical) and at a dose or doses between the MTD and the control. But this regimen creates other experimental difficulties. Ames and Gold and their colleagues at the University of California (Berkeley) and many other scientists believe that dosing at such high levels causes chronic cell killing and increased cell division that, in turn, convert oxidative DNA lesions from normal metabolism in rodent cells to mutations and then to cancer.[7] Furthermore, mutagens (p.5) can both damage DNA and cause the death of other cells at high doses, thus creating a situation where normal cells are replaced by mutated (i.e., pre-cancer) cells. In other words, a greater number of rodents with cancer are found at high doses because of the synergy created by the combination of DNAdamage AND cell proliferation.[8] Because this synergy artificially inflates rodent cancer incidence, *the calculated cancer risks at the doses humans are ordinarily exposed to become much higher than they really are.* Waxing poetic, Ames and Gold[9] have warned:

"When cell proliferation is ignored,
then risk assessment is flawed."

The Carcinogenic Potency Database[7] is a standardized resource of chronic cancer bioassay results for 1298 chemicals found in both the general literature and over 400 reports from the NCI/NTP. Although it is a common perception that synthetic chemicals cause cancer and natural compounds do not, 59% of the synthetic compounds in the database were positive for cancer but *so were 57% of the naturally occurring chemicals*. It is quite possible that the large number of positive results may be due to additional factors, one of which is toxin-induced cell proliferation. Several scientific studies that support this contention are summarized by Ames *et al.*[10]

Another serious problem with cancer assays is that results from rodent assays may not be relevant to human cancer. Indeed, a positive assay in rats does not necessarily mean a positive assay in mice, that female rodents will react the same as male rodents, or that a positive result in one strain of mice can predict cancer in a different mouse strain. Saccharin was almost banned by the FDA in 1977 because it induced bladder cancer in male rats when fed at extremely high doses (5-7.5% of the diet, the equivalent of about 180 cans of diet soda per day). The ban was delayed by passage and by frequent renewal of the Saccharin Safety and Labeling Act, a law enacted as

a result of widespread consumer fears that the only approved non-caloric sweetener (Aspartame was not yet available) was about to be taken off the market. It turns out that saccharin was a bladder carcinogen only in male rats because of a combination of cell proliferation and a protein specific to the male rat. This combination led to the formation of bladder stones, which caused irritation[8] of the bladder tissue that contributed to the apparent carcinogenic effect of saccharin.

Limonene, a constituent of lemons and oranges, causes kidney tumors only in male rats by a mechanism[11] involving enhanced cell proliferation and accumulation of a similar protein present in male rats but not in female rats (or male and female humans for that matter). Stevens *et al.*[12] demonstrated that the carcinogenicity of atrazine (a common herbicide) was specific to the female Sprague-Dawley rat as a result of endocrine control mechanisms in that strain of rat that differed from the mechanisms operating in another female rat strain and female mice. No cancers of any type were found in male mice or rats as a result of atrazine administration. Partially because of this study, EPA has classified atrazine as "not a likely human carcinogen"[13]. Recently, the widely held belief that pesticides that caused cancer in rodents was responsible for the perceived high incidence of breast cancer on Long Island was shown (after an expenditure of $30,000,000) to be without factual basis.[14]

Studies on the concordance of rat and mouse cancer bioassays indicate that the results agree 75% of the time, but that 80% of this concordance can be explained by the toxic effects resulting from testing at the MTD.[7] So, if there is so much discordance between rat and mouse cancer bioassay results conducted at the MTD, what is the predictive value of such rodent bioassays conducted for human cancer risk? Recently published studies conducted at the molecular level indicate not much, as there is an important difference in how cancer is formed in humans and mice.[15] "Mounting evidence implied that the process of cancer can be different between humans and the animal commonly used to study cancer in the lab, the mouse", said the project's director, Dr. Christopher Counter of Duke University Medical Center. "We therefore asked, all things being equal, do mouse and human cells rely on identical pathways for tumor growth, and the answer was no." [16]

What About Acrylamide?

Acrylamide appears to be a rodent carcinogen. Studies in mice have revealed skin and lung tumors in Sencar and A/J mice, respectively[17] as well as in ICR-Swiss mice.[18] However, these studies were conducted at astronomical doses (as high as 75-300 mg/kg/day). Even at these doses, application of a powerful tumor promoter was needed to induce skin cancer.[17,18] Furthermore, the lung tumors were obtained by injecting acrylamide into the mouse body cavity or by inserting it directly, by tube, into the mouse stomach, hardly methods to determine acrylamide risk to humans. More applicable studies were performed on rats.

Johnson *et al.* [19] administered acrylamide to a 4 groups of Fischer 344 male rats in their drinking water every day for two years at doses of 2, 0.5, 0.1 and 0.01 milligrams (mg) acrylamide per kilogram (kg) body weight of rat. Some rats in the 2 and 0.5 mg/kg dose groups developed mesotheliomas in the scrotum. Mathematical analysis found the results to be statistically significant, i.e., they did not arise by chance. (Demonstration of statistical significance is of major importance in animal bioassays, because laboratory experiments are so variable.) There were no statistically significant tumors in the low dose groups. Johnson originally classified the scrotal mesotheliomas as malignant but a later analysis found that these tumors were benign.[20] Friedman and his coworkers,[21] also working with Fischer 344 rats, confirmed the presence of scrotal mesotheliomas at the 2 mg/kg dose but not at the 0.5 mg/kg dose. While this discrepancy does not necessarily negate Johnson's data, Friedman's data is statistically more powerful because Friedman *et al*. tested 102 rats at the 0.5 mg/kg dose while Johnson *et al.* tested only 60. In both studies, non-cancer mortality at the 2 mg/kg daily dose exceeded mortality of the controls by more than 10 %, suggesting (according to National Cancer Institute protocols) that the *maximum tolerated dose may have been exceeded.* Benign tumors of the adrenal medulla were found at the high dose in Johnson's laboratory but not in Friedman's. Conversely, Friedman found benign thyroid adenomas at 3 mg/kg

in female rats but Johnson did not, possibly because Johnson's highest dose was 2 mg/kg. In no animal group in either laboratory was there more than a 16 % tumor incidence above those found in animals that did not ingest acrylamide. Benign mammary gland fibroadenomas were found in female Fisher 344 female rats by both groups of investigators but this tumor rarely, if ever, progresses to a malignant tumor in animals. Statistically significant tumors of the central nervous system were found by Johnson (only after a statistically insignificant increase in brain cell tumors were combined with a statistically insignificant increase in spinal cord tumors) in female (but not male), rats at the highest dose tested. No statistically significant tumors of the central nervous system were found in the Friedman study.

While some of the bioassays appear to indict acrylamide as a tumor-causing chemical in rats, this doesn't necessarily mean that acrylamide will cause tumors in humans, especially at the lower doses to which humans are exposed.

Why not? First, acrylamide was administered to the rats in water in both studies. It is possible that this method of administration delivers a more rapid dose to the rat than if it was mixed in with the food where much of the acrylamide may be bound. Since the analytical method used for the determination of acrylamide[22] in foods cannot distinguish between free and bound acrylamide, the amounts reported for acrylamide in food may overestimate the amount available for toxic interactions in humans.

Second, the fact that all the statistically significant tumors were found in endocrine organs raises the likely possibility that hormonal mechanisms may be responsible. This is an important consideration in cancer risk assessment because hormonal mechanisms are believed to have a threshold value (i.e., there is a dose below which no adverse effect occurs). Tumors arising from a genotoxic mechanism are considered to have no threshold value, thus making them subject to linear extrapolation to the lowest doses.

Acrylamide is known to modulate dopamine in the brain, which in turn leads to decreased prolactin and testosterone production in male rats.[23] In turn, this may result in enhancing the formation of Leydig cell tumors (aging Fisher 344 male rats have high incidences of Leydig cell tumors even without acrylamide), which then physically abrade the scrotal lining resulting in the observed scrotal mesotheliomas.[24] Thus, the formation of Leydig cell tumors is an obligatory precursor step for the formation of scrotal mesotheliomas. It has also been hypothesized that acrylamide exacerbates age-related change in female F344 rats.[25] As they get older, female F344 rats tend to enter repetitive pseudopregnancy, which is considered to be an obligatory precursor step to the formation of mammary gland fibroadenomas. Repetitive pseudopregnancy is characterized by sustained and elevated progesterone levels. Consequently, the mammary gland, in response to this progesterone signal, lays down more fibrous connective tissue. Acrylamide contributes to this process in rats by enhancing progesterone production. Thus, the progesterone signal is increased and eventually results in the formation of mammary gland fibroadenomas by a mode of action unique to the aging female F344 rat. In women, fibroadenomas result from a decrease in progesterone, *the opposite of what happens in rodents.*[25]

There is also evidence that dopamine receptors are present on rat thyroid follicular cells and it is possible that acrylamide could activate a metabolic pathway that induces cell proliferation in the thyroid gland.[26] In addition, the EPA has noted that there are no chemicals that have been shown to be causally related to the formation of human thyroid tumors.[27]

Although by no means conclusive, human epidemiology data appear to support this view. A study (Collins *et al*.[28]) of 8,854 workers exposed to acrylamide between the years 1925 and 1976 found no significant difference in cancer incidence between these workers and the general population. In fact, there was a statistically significant decrease in deaths from all causes. Marsh and his co-investigators did a follow-up study of these workers through 1994 and corroborated "the original cohort study fundings of little evidence for causal relation between exposure to acrylamide and mortality from any cancer sites..."[29]

One area of concern is that an acrylamide metabolite, glycidamide, is a known genotoxin. Injection of acrylamide into rats results in its conversion to glycidamide, a very reactive material that then may rapidly react with DNA[30] to possibly initiate carcinogenesis. (A simplified explanation of why reaction of a mutagen with DNA is considered dangerous may be found on page 5). The good news is that glycidamide is formed more slowly in humans than in rats.[31] Glycidamide also reacts with glutathione, a tripeptide present in all mammals. Indeed, the products of these reactions have been found in the urine of mice dosed with acrylamide.[32] Reaction of glycidamide with glutathione and proteins leaves less of it to react with DNA, a protective mechanism that may be overwhelmed when high doses of acrylamide are administered. This is a biochemical explanation for Paracelsus' dictum that the dose makes the poison.

A close chemical relative of acrylamide, acrolein, is also converted to a reactive metabolite, glycidaldehyde,[33] by rats. Glycidaldehyde is a powerful mutagen and easily reacts with DNA.[34] Yet, acrylamide is a carcinogen in Fischer 344 rats but acrolein, tested at about the same dose in Sprague-Dawley rats, is not.[35] A reasonable explanation for this disparity is that acrylamide, unlike acrolein, is transported to the brain (it is a known nerve toxin at higher doses) where it can initiate the cascade of events leading to scrotal mesothelioma as outlined above. Another explanation is that differences between the two rat strains are enough to give different bioassay results. Both explanations reinforce the idea that the Fischer 344 rat acrylamide cancer bioassay may have no relevance for human cancer assessment.

In summary, there is substantial evidence that the rodent studies may not be accurately predicting relevance to human health: 1) Tumor formation has been demonstrated in only one species, the rat; 2) only one rat strain, the Fischer 344, has been studied; 3) the statistically significant tumors are hormonally mediated, raising the possibility that non-genotoxic mechanisms (with a threshold dose) may be involved.

FOOD CHEMISTRY 101

Food consists mainly of water, carbohydrates (polysaccharides and sugars), proteins, amino acids and fats. When food is heated, a number of complex chemical reactions involving these constituents occur and literally thousands of different compounds are formed. These compounds are responsible for the odor, taste, color and texture of boiled, poached, broiled, baked, micro waved, roasted, sautéed and fried foods. Caramel can be produced by heating sugar to a temperature of approximately 400° Fahrenheit. The sugar molecule is converted to smaller molecules, such as acetic acid (vinegar) and formic acid as well as larger molecules that are mainly responsible for caramel color and for the unique caramel flavor.[36]

The reactions are even more complex and many more reaction products are formed when a typical food is heated. Fats and proteins are also converted to a myriad of chemicals. These reaction products can, in turn, react with each other. Reactions between carbohydrates and amino acids (the constituents of proteins) were first discovered in 1912 by the French chemist, Louis-Camille Maillard, who observed the formation of a brown pigment when he heated a mixture of glucose with the amino acid, lysine.[37] The reaction has come to be known as either the Maillard reaction or non-enzymatic browning. (Enzymatic browning, exemplified by the browning of lettuce occurs by a different mechanism.) Since then, many of the wonderful odors that we associate with coffee, baked breads, butter, chocolate, grilled meat, baked and French fried potatoes, have been shown to be due to chemicals produced by the Maillard reaction and the chemicals produced by heating oils and proteins. An excellent review of many aspects of the Maillard reaction may be found in a book published by the American Chemical Society[38].

When Good Reactions Go Bad

In the 1970's, Bruce Ames and his colleagues developed an assay for mutagenesis.[39] It was becoming increasingly apparent that there was a correlation between mutagenicity and carcinogenicity and it was believed that a

short-term assay for mutagenicity would, at the very least, provide a basis for deciding which of the thousands of environmental chemicals to which we were exposed should be tested for cancer in animals. The test used bacteria that were incapable of synthesizing their own histidine, an amino acid necessary for growth. Incubation of these bacteria with a mutagenic chemical would cause a change in the bacteria's DNA that would now allow them to produce histidine on their own. Thus, a chemical could easily be tested for mutagenic activity by incubating it in a Petri dish with the histidine-deficient bacteria for 48 hours. The chemical would be deemed positive for mutagenic activity if the bacteria multiplied. Ninety percent (157) of the 175 known rodent carcinogens tested positive in the Ames assay while 94 of the 108 chemicals considered non-carcinogens tested negative (87%).[40] Although not perfect, the assay appeared to have a great deal of promise for determining the mutagenic activity and carcinogenic potential not only of individual chemicals but also of whole food extracts. In the mid-1970's, Japanese researchers led by Takashi Sugimura at the National Cancer Center Research Institute in Tokyo, found mutagenic activity in the charred portion of broiled sardines and beef. Using the Ames assay to guide them, they were able to isolate and identify the mutagenic materials and found that they were identical in structure to some of the chemicals arising from pyrolysis of amino acids[41,42]. Two tryptophan pyrolysis products (Trp-P-1, Trp-P-2), two glutamic acid pyrolysis products (Glu-P-1, Glu-P-2) and two mutagens (AaC, MeaC) identified among the pyrolysis products of soybean protein, as well as in grilled ground beef were later found to be carcinogenic in animal bioassays.[43-46]

At about the same time, American investigators found mutagenic activity to result from non-enzymatic browning in a model system consisting of ammonia and simple sugars.[47] Later, mutagenic activity was found in fried potatoes, toasted white bread, toasted pumpernickel and baked biscuits.[48] (None of this activity was from acrylamide because it is negative in the assay used to determine mutagenic activity, suggesting that there are other mutagens in these foods that have not yet been characterized). It was also observed that a boiled beef extract exhibited no mutagenic activity until just after the color of the broth had become dark brown,[49] an observation that further supported the theory that some of the chemicals produced by non-enzymatic browning were mutagens. It soon became apparent that the pyrolyzed amino acid mutagens discovered in broiled fish and beef accounted for only a small portion of the mutagenic activity. Broiling sardines at lower temperatures resulted in the discovery of two additional highly mutagenic/carcinogenic heterocyclic amines, IQ and MeIQ.[50] IQ was also found in heated beef extract.[51] A closely related material, MeIQx, was isolated from fried beef[52] and exhibited very high mutagenic activity as well. Subsequently, it was demonstrated that these materials were also formed as a result of non-enzymatic browning.[53] (The IQ-type compounds are also chemically classified as heterocyclic amines, but unlike the amino acid pyrolysates, contain an imidazole ring.)

Additional mutagenic and/or carcinogenic non-enzymatic browning reaction products are formed in bread and coffee. A major product of non-enzymatic browning, 5-hydroxymethyl furfural, is mutagenic[54] and is found in breakfast cereal at concentrations of 3.7-193 micrograms per gram (µg/g).[55] A closely related material, furfural, is a known rodent carcinogen that is found in bread[56]and potatoes[57]. Gold *et al*.[7] list 19 rodent carcinogens in roasted coffee, many of which, such as furan and furfural are formed by non-enzymatic browning. Several structurally–related furans and furaldehydes have also been found in bread and coffee but have not yet been tested for carcinogenicity.

FORMATION OFACRYLAMIDE IN FOOD

A number of theories have been proposed to account for the mechanism by which acrylamide is formed in food. One possible route to acrylamide formation is the reaction of ammonia with either acrolein or its oxidation product, acrylic acid. Acrolein and acrylic acid are likely decomposition products of simple sugars undergoing non-enzymatic browning. These materials may also be formed from the triglycerides liberated from the fats during frying or sautéing. Another possible mechanism envisions acrylic acid arising directly from the decomposition of two common amino acids, alanine and aspartic acid. Still another common amino acid, asparagine, could be directly converted to acrylamide by loss of two simple molecules, carbon dioxide and ammonia (a process whose

driving force is predicted from the laws of thermodynamics). Ammonia is a known decomposition product not only of asparagine but of glutamine, another amino acid commonly found in food. Asparagine and glutamine are found abundantly in wheat, corn and oats and contribute to non-enzymatic browning of these grains by release of ammonia.[58] Both of these amino acids are abundant in potatoes,[57,59] green beans,[59] kale,[59] spinach,[59] cauliflower,[59] broccoli florets[60] and in peanuts.[61] Asparagine is found in such abundance in asparagus that it was named for that vegetable.

Food scientists at two universities in the United Kingdom[62] and others at Nestle in Switzerland[63] have reported good evidence for a Maillard reaction involving, as a first step, a glucose decomposition product and asparagine. Since asparagine concentration varies widely[57] in different potato varieties, a possible method to reduce acrylamide formation is to use the variety with lowest asparagine content for the production of French fries and potato chips.

It's More Than Potato Chips and French Fries

Initial reports[1,2] focused on the presence of acrylamide in potato chips and French fried potatoes and to a lesser extent in bread and breakfast cereal. In their peer-reviewed paper,[22] the Swedish scientists reported that although commercial potato chips had the highest acrylamide concentration [an average of 1737 micrograms per kilogram (µg/kg)], beets heated even in the absence of oil had about half that (850 µg/kg); spinach heated the same way contained 112 µg/kg. Microwaving a grated potato for only 2 minutes resulted in an average acrylamide content of 447 µg/kg, slightly more than the French fried potato average of 424 µg/kg, suggesting that triglyceride decomposition may not be a major contributor to acrylamide precursors in potato. It does appear that heat, length of heating, and heat penetration efficiency (such as in grated or thinly sliced potatoes) are important factors in acrylamide formation.

German scientists found acrylamide in all 24 brands of ground coffee and 7 brands of espresso that they analyzed.[64] A list compiled by Dr. Detlef Muller[65] for the Conseil des Industries Agro-Alimentaires, a group combing all national and food associations in the European Union, included 13-162 µg/kg acrylamide in wheat bread; 196 µg/kg in baked pretzels; 30-2400 µg/kg in rye crisps; 50-79 µg/kg in English muffins; 207 µg/kg in oat cereal; 110-247 µg/kg in rice cereal; 183 µg/kg in boiled rice; 199 µg/kg in baked potato; 55-143 µg/kg in baked asparagus; 30 µg/kg in roasted peanuts; 30-39 µg/kg in fried fish. Contrary to perceptions from the reports of last April, fried meats are not immune: 64 µg/kg in (presumably Swedish) meatballs, 10-51 µg/kg in hamburger, 16-42 µg/kg in chicken patties, 53 µg/kg in pork patties. The Swiss Federal Office of Public Health[66] reported finding as much as 2300 µg/kg acrylamide in muesli, as much as 150 µg/kg in cornflakes, 40-120 µg/kg in baby cereal, 200-310 µg/kg in coffee, 670-700 µg/kg in chicory coffee, 70-2000 µg/kg in diet biscuits, 120-200 µg/kg in cocoa powder, 50 µg/kg in lightly toasted bread, 100-380 µg/kg in darkly toasted bread, 30-110 µg/kg in fruit tarts, 70-190 in caramel µg/kg, 30 mg/kg in pizza. The one tested sample of pasteurized milk contained about 7.5 µg acrylamide per liter (about 2 µg in an 8 ounce glass), 10 times more than the EPA or the WHO allows in a glass of water.

It is by now quite obvious that French fries and potato chips are not the only foods in which acrylamide is formed and that the original media reports singling out these foods were premature.

While this article has focused on acrylamide, let's not forget that a number of other carcinogens and mutagens have been identified in food: 5-hydroxymethyl furfural in breakfast cereal; furfural in bread, coffee and potatoes; the 20 rodent carcinogens in coffee (it used to be 19 until acrylamide was recently found); caffeic acid in a whole host of fruits and vegetables; nitrosamines[67] in bacon and cold cuts; amino acid pyrolysates and polycyclic aromatic hydrocarbons[68] in grilled beef, fish and chicken; imidazole heterocyclic amines in cooked beef, fish and chicken. *If we were to make a list of foods that do not contain something that has been shown to cause cancer*

in rodents, it would be a very short list indeed.

But before we all go on a very severe diet, keep in mind that the carcinogenic activities of these chemicals have been determined by feeding high doses of them to rodents, and direct extrapolation of cancer risk to humans who ingest much lower doses is questionable, as discussed in earlier sections of this article. In spite of the 20 known carcinogens found in coffee, no one has ever demonstrated that coffee can cause cancer in humans (and not for lack of trying). Caffeic acid is a known rodent carcinogen found in many fruits and vegetables; yet increased ingestion of fruits and vegetables (even those that contain pesticide residues) is known to prevent several types of cancer,[69] a finding that is the basis for the National Cancer Institute recommendation to eat more fruits and vegetables. There appears to be a disconnection between rodent bioassays and reality.

CANCER RISK AND ACRYLAMIDE

There are two ways to calculate cancer risk. An unofficial method compares the risk of all rodent carcinogens to each other based on the potency of the rodent carcinogen and human exposure to it. The official method uses linear extrapolation of the high dose rodent cancer bioassay results to the very low doses humans are exposed to.

The first is known as the Human Exposure/Rodent Potency Index (HERP)[70] and was developed by Bruce Ames and Lois Gold. HERP is determined by dividing the dose of rodent carcinogen a 70-kilogram human is exposed to every day by the dose that causes cancer in half the rodents (TD_{50}). The TD_{50} is determined by averaging all the experimental cancer values found in the scientific literature for either rats or mice, depending on which species is more susceptible. A lower TD_{50} means that the material is more carcinogenic since it takes a lower dose to give half the rodents cancer.

$$\text{HERP} = \frac{\text{mg of rodent carcinogen eaten every day divided by 70 kg}}{TD_{50} \quad \text{(in mg/kg/day)}}$$

Looking at this formula, one can see that the higher the amount ingested, the higher the HERP value. Similarly, a lower TD_{50} gives a higher HERP value. The higher the HERP value, the greater the level of concern about possible human carcinogenicity.

The TD_{50} value for acrylamide is 6.15 mg/kg/day while the value for caffeic acid, a rodent carcinogen present in many fruits and vegetables is 297 mg/kg/day. Even though the potency of acrylamide is 48 times greater than that of caffeic acid, the concentration of acrylamide is about 1000 times less in French fried potatoes (425 µg/kg) than the concentration of caffeic acid in lettuce (530,000 µg/kg). Thus, a supersize portion (a 6.2 ounce bag) of French fries has a HERP value of 0.02% while a half-ounce portion of lettuce gives a HERP value of 0.04%. Maybe the next time I'm in a restaurant I'll tell them to hold the lettuce.

Regulatory agencies calculate human cancer risk from a linear extrapolation of the animal cancer incidence data to low dose human exposure. The World Health Organization and government regulatory agencies from the USA, Sweden and Norway have calculated a human cancer risk ranging from 0.7 to 4.5 per 1000 from a daily acrylamide dose of 1 µg/kg body weight. So if you weigh 70 kg (154 lbs.) and you ingest 70 µg acrylamide every day for 70 years, your risk of cancer is anywhere from 0.7 to 4.5 per 1000, depending on which mathematical model you believe. If you ingest 140 µg a day, your risk is theoretically doubled. The calculated human cancer risk is an extrapolation from the rat cancer bioassay data and none of the possible mitigating circumstances discussed in this article is factored into the calculation. In addition, the wide range of values represents the uncertainty brought about by different mathematical models (none of which have ever been scientifically verified) based on different worst-case assumptions, all of which are most likely too conservative.

People in different countries have different eating patterns, but it appears that the average human dose of acrylamide for residents of Norway, Sweden, Germany and the UK is, at the most, no more than 2 µg/kg.[65,71] This translates to a *calculated* risk of about 2 in 1000, which is about the same as the risk calculated for the heterocyclic amines.[72] You can use this data in several ways. The HERP index value is 0.046 %, which is about the same as the HERP index values for 1 apple, 1 pear, a smear of mustard, and 1 cup of coffee (from caffeic acid alone) and a lot less than a glass of wine or a bottle of beer.[70] As a regulator, you can use the risk estimates to decide what, if any, action should be taken. As a food activist, you can do the arithmetic and assert in your press release that acrylamide causes several thousand cancers per year in the USA.[73] As an individual, you can calculate your odds and decide that they are too low for you to worry about. In California, you can sue.

A law commonly known as Proposition 65 allows groups or individuals to demand that the state require sellers of products that contain chemicals "known to the State of California to cause cancer" to so inform their customers. Failure to comply with the law results in fines of $ 2500 a day for each infraction, payable to the plaintiffs. Considering the number of fast food restaurants in California, pretty soon that can add up to real money. California's Office of Environmental Health Hazard Assessment (OEHHA) has determined that an exposure of only 0.2 µg per day poses a "significant risk" to humans. That's 0.00286 µg/kg for a 70 kg person, 175,000 times lower than the 500 µg/kg dose that *did not* cause scrotal mesotheliomas in the Friedman study,[21] or 25,000 times lower than the dose that *did not* cause the same tumors in the Johnson investigation.[19] A "significant risk" in Prop 65 parlance is defined as a cancer risk of 1 cancer case per 100,000 people. Two groups have now come forward to take advantage of this law. One group, Environmental World Watch, has alleged that Frito-Lay, Wendy's, General Mills, Heinz, Proctor & Gamble, Kellogg and KFC have failed to warn of acrylamide exposures. Environmental World Watch is one of three groups that is responsible for filing a total of approximately 5000 Notices of Intent to Sue under Proposition 65 in just 2 months.[74] The second group, Council for Education and Research on Toxics (CERT), has targeted McDonald's and Burger King.

However, if these groups are really interested in protecting public health within the California definition of significant risk, they should also sue every restaurant (including the ones that serve only organic food), supermarket, diner, luncheonette, bodega and hot dog stand in the state. Using the acrylamide concentrations found in various foods by European government and industrial scientists, a significant risk, by Proposition 65 standards, is about 1/15 of a potato chip (don't laugh, you can't make this stuff up), 1/8 of a French fry or 1/8 of an asparagus spear, a spinach leaf or two, a sip of coffee, one bite of an English muffin, a few crumbs of toast and 3/4 ounce of pasteurized milk. Unpasteurized milk would not require a cancer warning. And please, don't "buy me some peanuts and crackerjacks" at the old ballgame unless its no more than 9 peanuts.

WELL, IS IT A REAL THREAT TO PUBLIC HEALTH?

No. The data suggesting that acrylamide may cause cancer in humans is derived from only *one* strain of *one* animal species. Analyses of the types of tumors found in these animals suggest that the effects of acrylamide may be on the animal's endocrine system (which differs considerably from that of humans) and not on the animal's DNA. Calculated cancer risks for acrylamide are based on mathematical models that have never been verified and are based on overly conservative assumptions. The WHO and the regulatory agencies of the United States, United Kingdom, Sweden, Norway and Germany, while still trying to obtain more data, have not recommended any changes in dietary habits on the basis of current data.

What we really should worry about is how the media report rodent bioassay results and cancer risk calculations unfettered by any explanation as to how these conclusions are arrived at. These periodic episodes lead to unnecessary anxiety about what is safe or unsafe to eat and absurd laws such as California's Proposition 65 while at the same time diverting us from real diet and nutrition-related risks, such as not eating enough fruits and vegetables.

REFERENCES

1. Starck, P, Reuters. Cancer risk found in French fries, bread. Apr 24, 2002
2. BBC News, Bread and crisps in cancer risk scare. Apr 25, 2002
3. Fulmer, M. Sellers of French fries may face suits, *Los Angeles Times*, July 3, 2002.
4. Gill, DM. Bacterial toxins: A table of lethal amounts. *Microbiol. Rev.* 46:86-94, 1982.
5. Budavari, S, ed, The Merck Index, 11th Edition., Merck and Co., Rahway NJ (1989)
6. Schlatter, J, Lutz, WK. The carcinogenic potential of ethyl carbamate (urethane): Risk assessment at human dietary exposure levels. *Food Chemical Toxicology.* 28:205-211, 1990.
7. Gold, LS, Slone, TH, Ames, BN. What do animal cancer tests tell us about human cancer risk?: Overview of analyses of the carcinogenic potency database. *Drug Metabolism Reviews.* 30:359-404, 1988.
8. Cohen, SM, Ellwein, LE. Cell proliferation in carcinogenesis. *Science.* 249:1007-1011, 1990.
9. Ames, BN, Gold, LS. The rodent high-dose cancer test is limited at best: When cell division is ignored, then risk assessment will be flawed. *Comments Toxicol.* 6(4):271-276, 1998.
10. Ames, BN. Gold, LS. Shigeaga, MK. Cancer prevention, rodent high-dose cancer tests and risk assessment, *Risk Analysis.* 16:613-617, 1996. And on the internet at http://potency.berkeley.edu/text/riskanaleditorial.html.
11. Whysner, J, Williams, GM. D-limonene mechanistic data and risk assessment: Absolute species-specific toxicity, enhanced cell proliferation, and tumor promotion. *Pharmacol. and Therap.* 71:127-136, 1996.
12. Stevens, JT, Breckenridge, CB, Wetzel, L. A risk characterization of atrazine: oncogenicity profile. *J. Toxicol. Environ. Health.* 56:69-109, 1999.
13. EPA Summary of Atrazine Risk Assessment May 2, 2002 http://www.epa.gov/pesticides/reregistration/atrazine
14. Gammon, MD *et. al*, Environmental toxins and breast cancer on Long Island. II.

    Organochlorine levels in blood. Cancer Epidemiology Biomarkers & Prevention. 11: 686-697 2002.
15. Hamad, NM, Elconin, JH, Karnoub, AE, Bai, W, Rich, JN, Abraham, RT, Der, CJ, Counter, CC. Distinct requirements for Ras oncogenesis in human versus mouse cells. *Genes & Development.* 16:2045-2057, 2002.
16. http://www.eurekaalert.org/pub_release/2002-08cshl-oma080202.php
17. Bull, RJ, Robinson, M, Laurie, RD, Stoner, GD, Greisiger, E, Meier, JR, Stober,J. Carcinogenic effect of acrylamide in Sencar and A/J mice. *Cancer Research* 44: 107-111, 1984.
18. Bull, RJ, Robinson, M, Stober, JA. Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Letters* 24: 209-212 (1984).
19. Johnson, K, Gorzinski, S, Bodnar, K, Campbell, R, Wolf, C, Friedman, M, Mast, R. Chronic toxicity and oncogenicity study in acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol. Appl. Pharmacol.* 85:154-168, 1986.
20. Damjanov, I, Friedman, MA. Mesotheliomas of tunica vaginalis testis of Fischer 344 rats treated with acrylamide: a light and electron microscope study. *In Vitro*, 12: 495-502, 1998.
21. Friedman, M, Dulak, L, Stedham, M. A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl.*

Toxicol. 27:95-105, 1995.

22. Tareke, E, Rydberg, P, Karlsson, P, Eriksson, S, Tornqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agr. Food Chem.* 50:4998-5006, 2002.

23. Ali, SF, Hong, JS, Wilson, WE, Uphouse, LL, Bondy, SC. Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. *Arch. Toxicol.* 52:35-43, 1983.

24. Crump, KS, Consideration of the potency classification of acrylamide based on the incidence of tunica vaginalis mesotheliomas (TVMs) in Male Fischer 344 Rats. Prepared for The Acrylamide Monomer Producers Group. The K.S. Crump Group, Inc., Ruston, LA. June 11, 1999.

25. Crump, KS, Mechanism of Acrylamide Induction of Benign Mammary Fibroadenomas in the Aging Female Fischer 344 Rat: Relevance to Human Health Risk Assessment. Prepared for The Acrylamide Monomer Producers Group. The K.S. Crump Group, Inc., Ruston, LA. December 14, 1999.

26. Crump, KS. The biological role of acrylamide-induced thyroid follicular cell tumors in the aging F344 rat to human health outcomes. Prepared for the Polyelectrolyte Producers Group. The K.S. Crump Group, Inc. Ruston, LA. November 2000.

27. USEPA, Assessment of thyroid follicular cell tumors. EPA/630/R97/002. Risk Assessment Forum, USEPA, Washington DC, March 1998.

28. Collins, JJ, Swaen, GMH, Marsh, GM, Utidjian, HMD, Caporossi, JC, Lucas, LJ. Mortality patterns among workers exposed to acrylamide. *J. Occup. Med.* 31:614-617, 1989.

29. Marsh, GM, Lucas, LJ, Youk, AO, Schall, LC. Mortality patterns among workers exposed to acrylamide: 1994 follow up. *Occup. Eniron. Med.* 56:181-190, 1999.

30. Segerback, D, Calleman, CJ, Schroeder, JL, Costa, LG, Faustman, EM. Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of ^{14C}-acrylamide. *Carcinogenesis* 16:1161-1165, 1995.

31. Calleman, CJ. The metabolism and pharmacokinetics of acrylamide: Implications for mechanisms of toxicity and human risk estimation. *Drug Metab. Rev.* 28:527-590, 1996.

32. Sumner, SCJ, Fennel, TR, Moore, TA, Chanas, B, Gonzalez, F, Ghanayem, BI. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol.* 12:1110-1116, 1999.

33. Parent RA, Paust DE, Schrimf, MK, Talaat, RE, Doane RA, Caravello HE, Lee SJ, Sharp, DE. Metabolism and distribution of [2,3-^{14}C] acrolein in Sprague-Dawley rats. II. Identification of urinary and fecal metabolites. *Toxicol. Sciences* 43: 110-120, 1998.

34. Van Buren BL, Lowengart, G. Reaction of DNA with glycidaldehyde. Isolation and identification of a deoxyguanosine reaction product. *J. Biol. Chem*. 252: 5370-5371, 1977.

35. Gomes, R, Meek, ME, Eggleton, M. Acrolein, Concise International Document 43, World Health Organization, Geneva, 2002.

36. Jurch, GR, Tatum, JH. Degradation of D-glucose with acetic acid and methylamine. *Carbohyd. Res.* 15:233-239, 1970.

37. Maillard, LC. Action of amino acids on sugars. *Compt. Rend*. 154:66-88, 1912.

38. T. H. Parliament, M. C. Morello and R. J. McGorrin, Eds., *Thermally Generated Flavors, Maillard, Microwave and Extrusion Processes*, ACS Symposium Series 543, American Chemical Society, Washington, DC, (1994).

39. Ames, BN, McCann, J, Yamasaki, E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mut. Res*. 31:347-364, 1975.

40. McCann, J, Choi, E, Yamasaki, E, Ames, BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. *Proc. Nat. Acad Sci.* 72**:**5135-5139, 1975.

41. Sugimura, T, Kawachi, T, Nagao, M, Yahagi, T, Seino, Y, Okamoto, T, Shudo, K, Kosuge, T, Tsuji, K, Wakabayashi, K, Iitaka, Y, Itai, A. Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. *Proc. Jpn. Acad.* 53:58-61, 1977.

42. Yamamoto, T, Tsuji, K, Kosuge, T, Okamoto, T, Shudo, K, Takeda, K, Iitaka, Y, Yamaguchi, K, Seino, Y, Yahagi, T, Nagao, M, Sugimura, T. Isolation and structure determination of mutagenic substances in L-glutamic acid pyrolysate. *Proc. Jpn. Acad.* 54B:248-250, 1978.

43. Matsukura, N, Kawachi, T, Morino, K, Ohgaki, H, Sugimura, T. Takayama, S. Carcinogenicity in mice of mutagenic compounds from a tryptophan pyrolysate. *Science.* 213:346-347, 1981.

44. Takayama, S, Masuda, M, Mogami, M, Ohgaki, H, Sato, S, Sugimura, T. Induction of cancer in the intestine, liver and various other organs of rats by feeding mutagens from glutamic acid pyrolysate. *Gann.* 75:207-213, 1984.

45. Yoshida, D, Matsumoto, T, Yoshimura, R, Matsuzaki, T. Mutagenicity of amino-a-carbolines in pyrolysis products of soybean globulin. *Biochem. Biophys. Res. Comm.* 96:915-920, 1978.

46. Matsumoto, T, Yoshida, D, Tomita, H. Determination of mutagens, amino-a-carbolines in grilled foods and cigarette smoke condensate. *Cancer Lett*. 12:105-110, 1981.

47. Spingarn, NE, Garvie, CT. Formation of mutagens in sugar-ammonia model systems. *J. Agr. Food Chem.* 27:1319-1321, 1979.

48. Springarn, NE, Slocum, LA, Weisburger, JH. Formation of mutagens in cooked foods. II. Foods with high starch content. *Cancer Lett.* 9:7-12, 1980.

49. J. H. Weisbuger and N.E. Spingarn "Mutagens as a function of mode of cooking of meats", *Naturally Occurring Carcinogens-Mutagens and Modulators of Carcinogenesis*, EC Miller et al. (Eds.) Japan Sci. Soc. Press, Tokyo/University Park Press, Baltimore pp 179-184, (1979).

50. Kasai, H, Yamaizumi, Z, Wakabayashi, K, Nagao, M, Sugimura, T, Yokoyama, S, Miyazawa, T, Spingarn, NE, Weisburger JH, Nishimura, S. Potent novel mutagens produced by broiling fish under normal conditions. *Proc. Japan Acad*. 56 ser B:278-283, 1980.

51. Spingarn, NE, Kasai, H, Vuolo, LL, Nishumura, S, Yamaizumi, Z, Sugimura, T, Matsushima, T, Weisburger, JH. Formation of mutagens in cooked foods. III. Isolation of a potent mutagen from fried beef. *Cancer Lett*. 177-183, 1980.

52. Kasai, H, Yamaizumi, Z, Shiomi, T, Yokayama, S, Miyazawa, T, Wakabayashi, K, Nagao, M, Sugimura, T, Nishumura, S. Structure of a potent mutagen isolated from fried beef. *Chem. Letters.* 485-488, 1981.

53. Jagerstad, M, Olsson, K, Grivas, S, Negishi, C, Wakabayashi, K, Tsuda, M, Sato S, Sugimura, T. Formation of 2-amino-3,8-dimethylimidazoquinoxaline in a model system by heating creatinine, glycine and glucose. *Mut. Res*. 126:239-244, 1984.

54. H. Omura, N. Jahan, K. Shinohara and H. Murakami, "Formation of mutagens by the Maillard reaction", in The Maillard Reaction in Foods and Nutrition , G. R. Waller and M. S. Feather, eds. ACS Symposium Series 215, American Chemical Society, Washington DC (1983).

55. Garcia-Villanova, B, Guerra-Hernandez, E, Martinez-Gomez, E, Montilla, J. Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *J. Agr. Food Chem.* 41:1254-

1255, 1993.

56. Stofberg, J, Grundschober, F. Consumption ratio and food predominance of flavoring materials. *Perfumer & Flavorist*. 12:27-68, 1987 .

57. Oruna-Concha, MJ, Duckham, SC, Ames, JM. Comparison of volatile compounds isolated from the skin and flesh of four cultivars after baking. *J. Agr. Food Chem.* 49:2414-2421, 2001.

58. Riha III, WE, Izzo, HV, Zhang, J, Ho, C-T. Non-enzymatic deamidation of food proteins. *Critical Reviews in Food Science and Nutrition.* 36:225-255, 1996.

59. Eppendorfer, WH, Bille, SW. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower as influenced by nitrogen fertilization and phosphorus and potassium deficiency. *J. Sci. Food Agric.* 71:449-458, 1996.

60. Murcia, MA, Lopez-Ayerra, B, Martinez-Tome, M, Garcia-Carmona, F. Effect of industrial processing on amino acid content of broccoli. *J. Sci. Food Agric.* 81:1299-1305, 2001.

61. Anonymous, "Fatty acid and amino acid profiles of selected peanut cultivars and breeding lines", *J. Food Comp. Anal.* 11, 100-111, 1998.

62. Mottram, DS, Wedzicha, BL, Dodson, AT. Acrylamide is formed in the Maillard reaction. *Nature* 419: 448-449, 2002.

63. Stadler, RH, Blank, I, Varga, N, Robert, F, Hau, J, Guy, PA, Robert, M-C, Riediker, S. Acrylamide from Maillard reaction products. *Nature* 419: 449, 2002.

64. "German researchers see cancer risk in coffee", Reuters, August 27, 2002

65. Muller, D."Learning on acrylamide formation in potato based foods", CIAA Report, September 17, 2002

66. "Concentration of acrylamide measured in various foods (incomplete list up to August 20, 2002)", Office Federal de la Sante Publique, 2002.

67. Tricker, AR, Preussmann, R. Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. Mutation Research 259: 277-289, 1991.

68. Howard JW, Fazio, T. Analytical methodology and reported finding of polycyclic aromatic hydrocarbons in foods. J. Assoc. Off. Anal. Chem. 63: 1077-1140, 1980.

69. Block, G, Patterson, B, Subar, A. Fruit, vegetables, and cancer prevention: A review of the epidemological evidence. *Nutr. Cancer.* 18:1-29, 1992.

70. Gold, LS, Slone, TH, Stern, BR, Manley, NB, Ames BA. Rodent Carcinogens: Setting Priorities. *Science* 258: 261-265 (1992). This article may be accessed on the internet at http://potency.berkeley.edu/text/science.html

71. Scientific Committee of the Norwegian Food Control Authority. Risk assessment of acrylamide intake from foods with special emphasis on cancer risk. June 2002.

72. Adamson, RH, Thorgeirsson, UP, Sugimura, T. Extrapolation of heterocyclic amine carcinogenesis data from rodents and nonhuman primates to humans. *Archives Toxicol (Supplement)* 18: 303-318, 1996

73. Anonymous. New tests confirm acrylamide in American foods. Center for Science in the Public Interest press release, June 25, 2002.

74. Proposition 65 Advisory 02-III, McKenna&Cuneo, L.L.P., March 20, 2002 (http://www.mckenacuneo.com/articles/article_detail.cfm?514