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Review

Genotoxicity evaluation of fried meat: A comprehensive review

J. Sanz-Serrano[a], A. López de Cerain[a,b,*], R. Garayoa[c], A. Azqueta[a,b], A. Vettorazzi[a,b]

[a] Universidad de Navarra, School of Pharmacy and Nutrition, Department of Pharmacology and Toxicology, Irunlarrea 1, E-31008, Pamplona, Spain
[b] IdiSNA, Navarra Institute for Health Research, Spain
[c] Universidad de Navarra, School of Pharmacy and Nutrition, Department of Nutrition, Food Science and Physiology, Irunlarrea 1, E-31008, Pamplona, Spain

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ABSTRACT

Some years ago, the IARC published the carcinogenic potential of processed and red meat. It is known that frying meat can produce genotoxic substances. A systematic review of the literature was conducted to evaluate *in vitro* and *in vivo* genotoxicity of fried meat. A total of 31 scientific articles were retrieved and analyzed. The meat extraction methods have been grouped into 6 types based on their similarity to an initially described method or on the general methodology used (solid-liquid extraction or others). The *in vitro* mutagenic results have been summarised by type of meat studied (beef, pork, others), cooking conditions (method, time and temperature), extraction method, and test used, with or without S9. Most articles assessed the mutagenicity of the extracts using the Ames test. Meat extracts were consistently positive in strains TA98/TA1538 with metabolic activation. In the *in vitro* studies with meat from restaurants, positive results were always found with variations in the number of His^+ revertants between samples or between restaurants. The few *in vivo* studies retrieved show evidence of induced DNA damage in colon cells and chromosome aberrations in bone marrow cells after daily treatment with fried red meat for 4 weeks or longer.

1. Introduction

Meat is one of the most important sources of energy, protein, and essential micronutrients. Meat usually needs to be cooked in some way, by means of chemical or physical processes, before ingestion. In particular, cooking aims to achieve better digestibility, palatability, and conservation of meat, but it is also known to produce some undesirable substances related to genotoxic and carcinogenic processes (IARC, 2018). Indeed, processed meat has recently been classified as carcinogenic to humans (Group 1), while red meat has been classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (IARC, 2018). Unfortunately, the specific mechanisms underlying these effects are still unknown, although different mechanistic hypotheses have been proposed (Domingo and Nadal, 2017; Cascella et al., 2018).

In general, global average meat consumption (total per capita) in 2011 showed major differences between high-consuming (from 208 g/day in Europe to 318 g/day in Oceania) and low-consuming regions (from 51 g/day in Africa to 86 g/day in Asia) (IARC, 2018). In some cases, these values are notably higher than the upper limits recommended for maintaining good health (from 100 to 500 g/wk) (Godfray et al., 2018). In fact, it has been suggested that high levels of meat consumption may contribute to the burden of chronic diseases such as colorectal cancer (CRC), coronary heart disease, and type 2 diabetes (Aune et al., 2009; Micha et al., 2010; IARC, 2018; Bechthold et al., 2019). One of the most important associations is based on epidemiological studies linking the consumption of red and processed meat to CRC and other types of cancer (IARC, 2018).

Moreover, scientific evidence has revealed the presence of several known genotoxic compounds in uncooked and cooked meat. Limited but interesting evidence exists of the direct link between cancer and intrinsic molecules of raw meat such as the heme group or N-glycolylneuraminic acid (Neu5Gc) (Cascella et al., 2018). Furthermore, it was established several decades ago that heat-treated meat may contain known carcinogenic compounds. For example, heterocyclic aromatic amines (HAAs) and polycyclic aromatic hydrocarbons (PAHs) appear at mild and high temperatures, respectively. Some HAAs (IQ-type) are produced by the Maillard reaction at normal cooking temperatures ($< 300\,^{\circ}C$); other HAAs (non-IQ-type) and PAHs are formed at much higher temperatures ($> 300\,^{\circ}C$) as a consequence of pyrolysis (for a review, see Koszucka and Nowak, 2018). Among the different cooking procedures, frying can produce an appreciable level of these mutagenic and carcinogenic compounds, such as HAAs and PAHs (IARC, 2018). Heat conduction has a direct impact in the formation of the crust, the

* Corresponding author. Universidad de Navarra, School of Pharmacy and Nutrition, Department of Pharmacology and Toxicology, Irunlarrea 1, E-31008, Pamplona, Spain.
E-mail address: acerain@unav.es (A. López de Cerain).

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outer part of the meat, where mutagen concentration reaches its highest levels (Dolara et al., 1979). Also, N-nitroso compounds (NOCs) are carcinogens that are formed both endogenously and when meat is cured (Weisburger and Raineri, 1975; Spiegelhalder et al., 1976). Finally, environmental contaminants already present in raw meat that are also susceptible to modification by cooking may also contribute to the carcinogenicity of meat (Domingo and Nadal, 2016). Unfortunately, the mechanisms that link meat to carcinogenesis are still not clear and more studies will be needed to elucidate this issue (Domingo and Nadal, 2017; Cascella et al., 2018).

Genotoxicity testing is an important step for the risk assessment of substances in food and feed. As stated by the European Food Safety Authority (EFSA), the main purpose of genotoxicity testing is: "i) to identify substances which could cause heritable damage in humans; ii) to predict potential genotoxic carcinogens in cases where carcinogenicity data are not available; and iii) to contribute to understanding of the mechanism of action of chemical carcinogens" (EFSA, 2011). A complete genotoxicity evaluation includes *in vitro* and *in vivo* tests that aim to detect compounds that induce genetic damage by different mechanisms (ICH S2R1, 2012). A stepwise battery approach is therefore recommended, as no single test is capable of detecting all genotoxic mechanisms relevant in mutagenesis. Different *in vitro* analyses are selected to cover different genetic endpoints such as gene mutations, structural chromosome aberrations, and aneuploidy. Both the World Health Organization (WHO) and the EFSA have established specific guidelines for the risk assessment of chemicals in food (Table 1). Negative results in the *in vitro* test battery are usually sufficient to discard genotoxic potential, unless there are reasons for special concern. One or more positive *in vitro* test results, however, indicate that further testing is required. In this case, the assays to be conducted are normally selected on a case-by-case basis (WHO, 2009; EFSA, 2011).

At present, there is a need to shed light on the biological impact of meat ingestion in humans. Thus, the epidemiologic evidence provided by the IARC report (IARC, 2018) must be supplemented with experimental approaches in order to gain insight into the causative effect of such factors. As previously indicated, a great deal of genotoxicity evidence exists for single compounds (HAAs, HAPs, acrylamide, etc.) known to be present in meat. Although the aforementioned studies are important to understanding the contribution of specific compounds to the overall carcinogenic effect, it is also important to study the food as a whole and in the most comparable conditions to human exposure. Meat may be considered as a matrix that provides a mixture of different genotoxic compounds. Indeed, current guidelines for the genotoxicity assessment of chemical mixtures clearly indicate that "if a mixture contains 1 or more chemical substances that are individually assessed to be genotoxic *in vivo* via a relevant route of administration, the mixture raises concern for genotoxicity" (EFSA, 2019). Thus, this approach is especially relevant to meat. The different molecules present in cooked meat may interact with each other and analyzing food extracts as a mixture that represents compounds to which humans may be exposed may thus increase our understanding of the carcinogenic risk of meat consumption. Furthermore, frying is commonly used in daily household cooking tasks and mass catering companies, and, is a cooking method that produces some of the highest levels of mutagens.

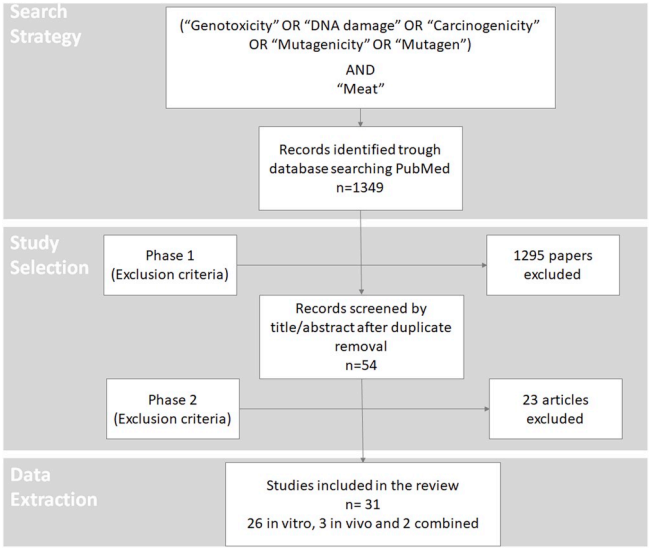

Fig. 1. Search strategy and outcome of the systematic review in Pubmed.

Therefore, the aim of this study was to carry out a systematic search of scientific articles assessing the genotoxicity of fried meat extracts *in vitro* and *in vivo*. Moreover, from the retrieved articles, we analyzed the different methods used for extracting the genotoxic compounds from meat.

2. Material and methods

2.1. Search strategy and data source

A systematic literature search in Pubmed was carried out, including all articles published up to January 1, 2019. The review objective was to identify the genotoxic effects, evaluated *in vitro* and *in vivo*, of fried meat extracts. The search was carried out using the following keywords: "(Genotoxicity OR DNA damage OR carcinogenicity OR mutagenicity OR mutagen) AND meat". Fig. 1 shows the search flowchart.

2.2. Study selection

The selection of the scientific articles retrieved from the search was conducted in 2 phases: Phase 1 (screening titles and abstracts) and Phase 2 (examination of entire article). Data were gathered as interpreted by the original publication; thus, the authors of this review did not alter the interpretation of the authors.

Phase 1. The selection process was conducted by screening titles and abstracts of all retrieved studies using the following exclusion criteria:

Table 1
Strategies and Assays Recommended by WHO and EFSA for Genotoxicity Testing of Chemicals in Food. For the following references: OECD 471 (OECD, 1997); OECD 474 (OECD, 2016a); OECD 487 (OECD, 2016c); OECD 488 (OECD, 2013); OECD 489 (OECD, 2016b).

	WHO (2009)	EFSA (2011)
In vitro	Gene mutation test in bacteria AND One or two tests in mammalian cells detecting point mutations or chromosome damage	Ames test (OECD 471) AND Micronuclei test (OECD 487)
In vivo	One or two tests detecting DNA damage (DNA adducts or Comet assay) and/or point mutations in transgenic mice and/ or chromosome aberrations and/or micronuclei	Erythrocyte micronuclei test (OECD 474) AND/OR Comet assay (OECD 489) AND/OR Transgenic rodent assay (OECD 488)

1. Articles dealing with human epidemiological, intervention, or clinical trials.
2. Articles focusing on the analytical development of techniques for food-mutagen detection.
3. Articles with experimental designs aimed exclusively at looking for the inhibitory effect of any substance on food-mutagen formation, without information on "control" groups (i.e. cooked/processed meat without adding the inhibitory substance).
4. Articles assessing the genotoxicity of specific or pure compounds known to be present in fried or otherwise cooked meat (HAPs, HAAs, ferric citrate, etc.), obtained either commercially or from extracts.
5. Articles assessing the genotoxicity of commercial beef extracts, such as Bacto Beef Extracts.
6. Studies dealing with fish; only studies evaluating meat from mammals or fowls were included.
7. Studies in which meat is cooked by procedures other than frying (pan-fried, deep-fried).
8. *In vivo* studies in which genotoxicity of meat was measured in the urine or feces of laboratory animals fed with meat or meat extracts.
9. Studies dealing with carcinogenicity endpoints.

If the title and abstract screening did not identify precise information concerning the defined exclusion criteria, the article was labelled as "not clear" and included in Phase 2 (full document analysis) for further screening.

Phase 2. Articles that potentially met eligibility criteria (or that were not clearly excluded) in Phase 1 were retrieved and the full texts were reviewed. More specifically, methods, results and principal conclusions in the full article were screened.

At this stage, some articles were rejected for the following reasons:

1. The article met some of the exclusion criteria defined in step 1.
2. The cooking procedure was still not clear.

2.3. Data extraction

Articles retrieved after applying the 2-phase selection strategy were assessed for data extraction.

First, the different methods used to prepare the meat extracts were retrieved and grouped chronologically by procedure similarity. The basic steps of each group of methods are also shown (Fig. 2). Reference to each group of extraction methods has been included in the different tables that describe the genotoxicity evaluation from both *in vitro* and *in vivo* studies.

For *in vitro* studies, information was collected in 4 tables. Tables 2–4 include beef, pork, and other animal meats, respectively, cooked in laboratory-controlled conditions. Table 5 includes *in vitro* analysis of food fried in commercial establishments such as restaurants and fast food stalls. Table 6 includes all *in vivo* tests evaluating the genotoxicity of meat extracts.

3. Results and discussion

A total of 31 articles were retrieved from the systematic search. More specifically, the search obtained 26 *in vitro* studies, 3 *in vivo* studies and 2 studies in which both *in vitro* and in *vivo* experiments were performed (Fig. 1).

3.1. Extraction methods

As a first step and due to the fact that the extraction method may influence the recovery of the genotoxic compounds, an analysis of the different methods used in the retrieved articles was carried out. For that purpose, the main extraction methods used in the different articles were grouped into 6 different types (A to F), depending on the method on which they were based (Fig. 2). All the methods were grouped based on similarity to an initial method (Commoner et al., 1978 [method A]; Felton et al., 1981 [method B]; Bjeldanes et al., 1982b [method C]; Kasai et al., 1979 [method D]) or the general methodology used (solid-liquid extraction [method E] and other [method F]).

This classification was also used for the data extraction of the different experiments (Tables 2 to 6).

In 1978, Commoner et al. (method A) established the basic method for recovering bases (Commoner et al., 1978). Felton and colleagues showed that extracted mutagens were presumed to be organic bases, as acid and neutral fractions failed to show any mutagenic activity (method B) (Felton et al., 1981). In terms of specific known mutagens in the extracted fraction, the methods used by Commoner et al. (1978) and Miller and Buchanan (1983) were presumed to detect B[a]P and/or volatile nitrosamines. However, the authors found that these compounds were not present in the mutagenic fraction obtained from ground beef or fried bacon when methods A or B were used, respectively.

A year later, the latter author and colleagues (Bjeldanes et al., 1982b) (method C) compared the efficiency of 4 different extraction methods, including the 2 cited previously. A new procedure using an XAD-2 resin was found to be the quickest and most efficient method for recovering the mutagens present in meat. The main extraction methods (A, B, C, D) appear to show substantially similar thin-layer chromatography and high-pressure liquid chromatography profiles. The authors therefore concluded that the same mixtures of compounds were isolated but varying in the quantitative recovery of each method (Bjeldanes et al., 1982b). Using similar procedures, other studies have demonstrated that different heterocyclic aromatic amines (HAAs) are indeed extracted and present in the mutagenic fraction obtained from fried ground beef (Felton et al., 1984; Knize et al., 1985) or pork (Overvik et al., 1989).

HAAs were also extracted using other similar methods (method D) (Kasai et al., 1979) that employ silica-based columns instead of XAD-2 columns. As in the case of method C, when compared to methods A or B, method D proved to be the most efficient (Wang et al., 1982). Moreover, the authors also stated that, contrary to the methods that used ammonium ions (such as method A), method D does not lead to the formation of mutagens that are artefacts.

Procedures clustered in Group E include solid/liquid extraction methods that are based on mixing meat with polar or non-polar solvents, removing solids and directly evaluating the genotoxicity of the supernatants. Within this group, Overvik et al. (1984) and Nilsson et al. (1986) used a similar method (using chloroform and methanol as extraction solvents), while Pourazrang et al. (2002) followed a method previously used by Ohta et al. (1990), a method that uses ethyl acetate (but with prior ammonium sulfamate treatment) and that has been shown to extract N-nitroso compounds (NOCs).

A sixth group (group F) includes a variety of other extraction methods that could not be grouped anywhere else. For instance, Stavric et al. (1993, 1995) used a procedure based on liquid-liquid extraction of the sample at different pH values. The authors considered that the method was able to extract HAAs because, after spiking a commercial bouillon sample with a 500 ppb of [^{14}C] ([2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ), they were able to recover 95% of [^{14}C]. Unfortunately, the full details of the experiment were not shown. Finally, 3 of the most recent articles followed a different approach to that of the majority of researchers. Martin et al. (2002) and Pfau et al. (2006) followed a tandem extraction method that was specifically validated for HAA quantification by HPLC-UV (Gross, 1990). Darwish et al. (2015), however, used an extraction method that used a silica gel chromatography column (Darwish et al., 2015) and was specifically validated for B[a]P quantification by HPLC-FLD.

Overall, the presence or absence of different known mutagenic compounds (B[a]P, NOCs, HAAs) in the different experiments makes it difficult to attribute the genotoxic potential of the fried meat evaluated

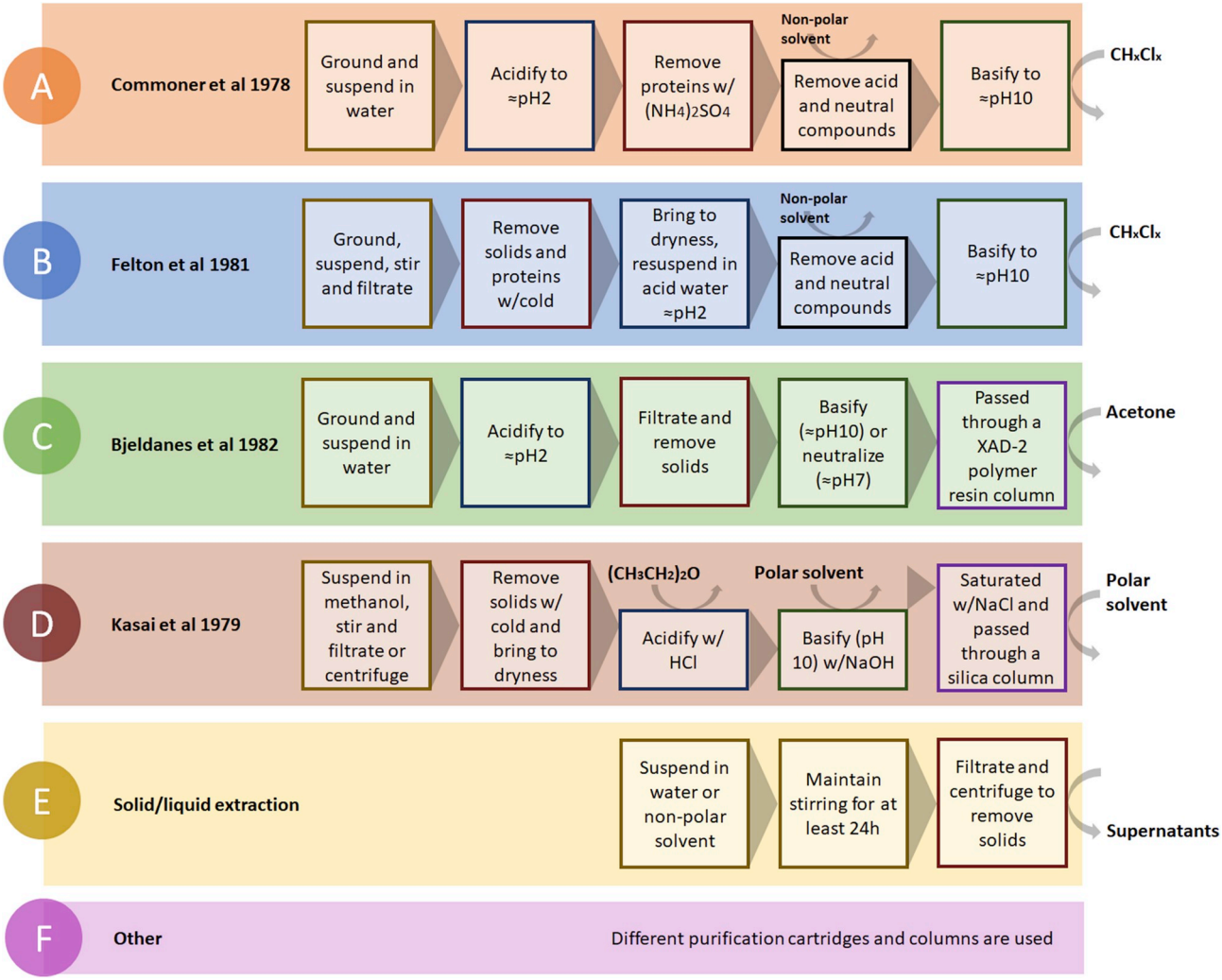

Fig. 2. Non-exhaustive description of the 6 main extraction methods used in all papers retrieved. Only the basic steps are described. A, B, C, and D generally have an extra final step, which consists of evaporating the nonpolar solvent to dryness and dissolving it in a small volume of dimethyl sulfoxide or ethanol.

to a single compound or to a specific mixture. Moreover, although some attempts have been made to characterize the extracts, all the studies have unfortunately focused on a single compound (or family) and it is therefore unclear whether all the methods used are able to extract all possible mutagens.

3.2. *In vitro* genotoxicity evaluation

In relation to articles dealing with *in vitro* genotoxicity evaluation, Tables 2–5 summarize all relevant data from each article retrieved from the systematic search. Each of the tables contains the different assays performed for meat cooked under controlled conditions for the different types of meat such as beef (Table 2), pork (Table 3), and other meats (Table 4), or from samples prepared in catering kitchens or restaurants (Table 5). The different experimental systems and test conditions were extracted for each assay.

In general, ground beef was clearly the most widely assessed type of meat out of all bovine meat. In contrast, many different variations of pork have been tested, such as sausages, lean ground pork, and bacon. Aside from the latter 2, mutton was found to be the most evaluated meat. In the case of samples prepared in catering kitchens, the origin of the hamburgers, sausages and hot dogs was unfortunately not specified and it is therefore unclear whether the meat was beef, pork, or a mixture of both.

The Ames test was used in all the studies showing results of *in vitro* genotoxicity assays, and only approximately 15% of the articles included results of other assays, such as the SOS umu test (Whong et al., 1986), the single cell gel electrophoresis assay (Martin et al., 2002; Taj and Nagarajan, 1994), the mammalian cell gene mutation test (Gocke et al., 1982), and the sister chromatid exchange test (Gocke et al., 1982).

Regarding the Ames test, OECD guideline 471 for the testing of chemicals, "Bacterial reverse mutation test" (OECD, 1997), recommends a battery of 5 different strains in order to detect agents that induce different kinds of DNA mutation. Most of the studies reviewed used only 1 or 2 strains and the most frequently used were those detecting frameshift mutations (*Salmonella typhimurium* TA1538/TA98 and TA1537/TA97). Other strains able to detect base substitutions were used in only a few studies: *Salmonella typhimurium* TA1535/TA100 and TA102 (Tables 2–5). TA1538 and TA98 have a mutation in the *hisD* gene of the histidine operon, coding for histidinol dehydrogenase, and detect frameshift mutations; TA1537 and TA97 have different mutations in *hisC* and *hisD* genes and also detect frameshift mutations with different specificity; TA1535 and TA100 have a mutation in the *hisG* gene coding for the first enzyme of the histidine biosynthesis and detect base-pair substitutions, primarily in GC pairs. Moreover, TA98, TA97a, and TA100 have the plasmid pKM101, which increases spontaneous and chemically induced mutagenesis by enhancing an error-prone DNA repair present in *Salmonella typhimurium*, thus increasing the sensitivity of those strains. TA102 has a different mutation in the *hisG* gene and

Table 2
In vitro evaluation of beef extracts fried under controlled conditions in a laboratory.

Paper/Assay	Extraction method[a]	Meat type	Cooking method	Cooking times (min)	Cooking temperatures (°C)	Result	
						S9-	S9+
Bacterial gene mutation assay							
Salmonella typhimurium TA98							
Spingarn and Weisburger (1979)	A	Sirloin beef	Fried in a frying pan seated on a hot plate	5, 10, 15, 20	NS	–	+
Felton et al. (1981)	A	Ground beef	Fried on a gas heated stainless-steel griddle	NS	200	–	+
Felton et al. (1981)	B	Ground beef	Fried on a gas heated stainless-steel griddle	NS	200	–	+
Iwaoka et al. (1981)	A	Ground beef	Cooked in an electric fry pan	5	190	–	+
Wang et al. (1982)	B	Ground beef	Fried without oil in a conventional frying pan	10	190		+
Wang et al. (1982)	D	Ground beef	Fried without oil in a conventional frying pan	5, 10, 15	190		+
Whong et al. (1986)	C	Beef	Fried with a hot plate	NS	350		+
Laser Reutersward et al. (1987)	C	Beef	Pan-fried on a Teflon coated plate, no fat	3	150–200	–	+
Laser Reutersward et al. (1987)	C	Bovine heart	Pan-fried on a Teflon coated plate, no fat	3	150–200	–	+
Laser Reutersward et al. (1987)	C	Bovine tongue	Pan-fried on a Teflon coated plate, no fat	3	150–200	–	+
Laser Reutersward et al. (1987)	C	Bovine liver	Pan-fried on a Teflon coated plate, no fat	3	150–200	–	–
Laser Reutersward et al. (1987)	C	Bovine kidney	Pan-fried on a Teflon coated plate, no fat	3	150–200	–	–
Ong et al. (1989)	C	Beef	Fried on a hot plate	NS	230		+
Vikse and Joner (1993)	B	Beef	Fried in a frying pan lightly greased with maize oil	2 × 6	250		+
Augustsson et al. (1999)	C	Ground beef	Frying pan	2 × (2–4)	150–225		+
Augustsson et al. (1999)	C	Minute beef	Frying pan	2x(1.5–2)	150–225		+
Perez et al. (2002)	C	Ground beef	Frying pan with and without oil or butter	10, 20, 30	170–180	–	+
Darwish et al. (2015)	F	Lean ground beef	Pan-fried in vegetable oil	2 × 8	180		–
Salmonella typhimurium TA100							
Spingarn and Weisburger (1979)	A	Sirloin beef	Fried in a frying pan seated on a hot plate	5, 10, 20	NS	–	–
Felton et al. (1981)	A	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	–
Felton et al. (1981)	B	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	–
Salmonella typhimurium TA1535							
Felton et al. (1981)	A	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	–
Felton et al. (1981)	B	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	–
Salmonella typhimurium TA1537							
Felton et al. (1981)	A	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	+
Felton et al. (1981)	B	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	+
Salmonella typhimurium TA1538							
Commoner et al. (1978)	A	Lean ground beef	Cooked in an electrically heated home hamburger cooking appliance	1.5, 3, 5.5	NS	–	+
Dolara et al. (1979)	A	Lean ground beef	Cooked in an electrically heated home hamburguer appliance or electric frypan	3–5.5	190, 210	–	+
Pariza et al. (1979)	A	Lean ground beef	Fried, no especifica nada mas	2, 4, 6, 8, 10, 14, 20	143, 191, 210		+
Felton et al. (1981)	A	Ground beef	Fried on a gas heated stainless steel griddle	NS	200	–	+
Felton et al. (1981)	B	Ground beef	Fried on a gas heated stainless steel griddle	2, 5, 8	200	–	+
Iwaoka et al. (1981)	A	Ground beef	Cooked in an electric fry pan	5	190	–	+
Bjeldanes et al. (1982a)	B	Ground beef	When normal procedures required cooking oil, corn oil was used, most samples were cooked in pans	12	150–300		+
Bjeldanes et al. (1982a)	B	Round steak	When normal procedures required cooking oil, corn oil was used, most samples were cooked in pans	2.5	190		–
Bjeldanes et al. (1982a)	B	Round steak	When normal procedures required cooking oil, corn oil was used, most samples were cooked in pans	2 × 6	200, 250		+
Knize et al. (1985)	C	Lean ground beef	Fried	2 × 6	200, 250, 300		+
Knize et al. (1985)	C	High-fat ground beef	Fried	2 × 6	200, 250, 300		+

(*continued on next page*)

Table 2 (*continued*)

Paper/Assay	Extraction method[a]	Meat type	Cooking method	Cooking times (min)	Cooking temperatures (°C)	Result	
						S9-	S9+
Barrington et al. (1990)	D	Beef	Flat iron skillet with frying medium: margarine, butter and oil	2 × (5,10)	140–200		+
Martin et al. (2002)	F	Lean ground beef	Fried without any additional oil or fat in a pan	NS	NS	–	+
Salmonella typhimurium YG1019							
Martin et al. (2002)	F	Lean ground beef	Fried without any additional oil or fat in a pan	NS	NS	–	+
Others							
SOS/umu assay							
Salmonella typhimurium TA1535 pSK1002							
Whong et al. (1986)	C	Beef	Fried with a hot plate	NS	350		+
Single cell gel electrophoresis (comet) assay (strand breaks)							
MCL-5 cells							
Martin et al. (2002)	F	Lean ground beef	Fried without any additional oil or fat in a pan	NS	NS	+	

NS: Non-specified.
Positive (+) indicates at least one of the conditions was found to be positive.
Cooking times: 2x indicates "for each side"; two numbers separated by a "-" indicate some intermediate times have been also assessed; two numbers separated by a " " indicate only those times have been assessed with no intermediates.

[a] For summarised information on extraction methods A-F see Fig. 2.

detects base-pair substitutions, primarily in AT pairs (Table 7) (Maron and Ames, 1983).

The different results obtained in the Ames test with extracts obtained from cooked samples of each type of meat are explained below.

Beef. Results in TA1538/TA98 and TA1537 were all positive only with metabolic activation by Aroclor-1254-treated rat liver (S9) fraction and negative in TA1535/TA100 (Table 2). Thus, extracts from fried beef were able to induce frameshift mutations. These results are also in accordance with the fact that HAAs need to be bioactivated to cause genotoxicity. Specifically, HAAs undergo oxidation at the exocyclic amine group by cytochrome P450 enzymes to form the genotoxic N-hydroxylated-HAA metabolites that, in turn, can react with DNA or undergo further metabolism to produce unstable esters that adduct to DNA (Koszucka and Nowak, 2018).

Only 3 exceptions to this rule were observed: the extracts of fried bovine liver or kidney, fried for 3 min were negative (Laser Reuterswärd et al., 1987); the extracts of lean ground beef fried for 8 min each side were also negative (Darwish et al., 2015), and round steak fried for 2.5 min at 190 °C, followed by 7 min at 107 °C on each side, were also negative (Bjeldanes at al., 1982a). Meat composition and cooking or experimental conditions may account for these differences. The most important group of HAAs (IQ type, IQ being 2-amino-3-methyl-3H-imidazo[4,5-f]quinolone) is mainly created by the Maillard reaction, whilst non-IQ HAAs are obtained from pyrolyzed amino acids and proteins (at > 300 °C). The Maillard reaction occurs between free amino groups and carbonyl compounds, commonly reducing sugars, at ordinary cooking times and temperatures (> 100 °C). Furthermore, creatinine seems to be essential in the process to form the group 2-aminoimidazo, which is responsible for the mutagenicity of IQ-type HAAs (Skog et al., 1998; Koszucka and Nowak, 2018).

Laser Reuterswärd et al. studied the mutagenic activity of several bovine tissues (meat, heart, tongue, liver, and kidney) because they have different concentrations of creatine, monosaccharides and free amino acids, all of which are precursors of the mutagenic IQ-type HAAs (Laser Reuterswärd et al., 1987). The 5 tissues had a relatively similar water, fat, and protein content, but creatine/creatinine levels were very different in meat, heart, and tongue, compared to liver and kidney: 33, 25, 19, 2.2 and 2.3 μmol/g wet tissue, respectively. Liver contained a much higher content of glucose and more free amino acids than the other bovine tissues (183 μmol/g wet tissue vs 0.45–8.2 μmol/g wet tissue and 101 μmol/g wet tissue vs 30–71 μmol/g wet tissue, respectively). The authors concluded that, of the 3 groups of precursors, the creatine/creatinine content seemed to be the most important factor in the production of mutagenicity in fried patties of meat, tongue, heart, liver, and kidney, fried at 150 °C, 175 °C, or 200 °C for 3 min.

It has been previously demonstrated that time and temperature of cooking are 2 factors that independently increase the mutagenic potency of meat cooked samples (Pariza et al., 1979, Perez et al., 2002). In 1979, Pariza et al. demonstrated that uniformly prepared hamburgers pan-fried under carefully controlled conditions of temperature and time produced very low mutagenic activity at 143 °C at different frying times (between 4 and 20 min). In contrast, frying at 191 °C or 210 °C for up to 10 min resulted in the generation of considerably higher levels of mutagenic activity. Furthermore, in 2002, Pérez et al. observed that uniformly prepared hamburgers fried at 170°C-180 °C for 10–30 min produced highly significant mutagenic activity. According to these studies, it may be assumed that 2.5 min at 190 °C followed by 7 min at 107 °C on each side, or 8 min at 180 °C, probably does not provide sufficient time and temperature to observe a positive mutagenic response in round steak beef (Bjeldanes et al., 1982a) or lean ground beef (Darwish et al., 2015), respectively. Experimental conditions may also have an effect on the negative data that have been retrieved from this study. The objective of the study by Darwish et al. was to compare the mutagenicity of meat patties prepared using different cooking methods (boiling, pan-frying, and charcoal grilling) (Darwish et al., 2015). To this end, meat extracts were tested in the TA 98 strain with metabolic activation and

Table 3
In vitro evaluation of pork extracts fried under controlled conditions in a laboratory.

Paper/Assay	Extraction method[a]	Meat type	Cooking method	Cooking times (min)	Cooking temperatures (°C)	Result	
						S9-	S9+
Bacterial gene mutation assay							
Salmonella typhimurium TA97a							
Pourazrang et al. (2002)	E	Nitrated sausage	Fried	2 × 3	175	–	–
Pourazrang et al. (2002)	E	Non-nitrated sausage	Fried	2 × 3	175	–	–
Salmonella typhimurium TA98							
Berg et al. (1990)	C	Lean ground pork	Broiled in a pan	2–25	200		+
Overvik et al. (1987)	B	Lean pork	Fried in a frying pan with fat (9 different fats)	10, 10.5	200–250		+
Miller and Buchanan (1983)	B	Nitrite-free bacon	Fried in an electric skillet	12	200	–	+
Miller and Buchanan (1983)	B	Nitrite-free bacon	Fried in an electric skillet	2–10	171		+
Miller and Buchanan (1983)	B	Nitrite-free bacon	Fried in an electric skillet	6	25–225		+
Miller and Buchanan (1983)	B	Nitrite-treated bacon	Fried in an electric skillet	12	200		+
Pourazrang et al. (2002)	E	Nitrated sausage	Fried	2 × 3	175	–	–
Pourazrang et al. (2002)	E	Non-nitrated sausage	Fried	2 × 3	175	–	–
Nilsson et al. (1986)	E	Pork	Broiled without fat and frying with 9 different fats	10	200, 250		+
Vikse and Joner (1993)	B	Pork	Fried in a frying pan without any additives, lightly greased with maize oil	2 × 6	250		+
Augustsson et al. (1999)	C	Pork belly	Pan-fried	2 × (2,4)	150–225		+
Augustsson et al. (1999)	C	Pork chops	Pan-fried	2 × 6	150–225		+
Augustsson et al. (1999)	C	Sausage	Pan-fried	2 × 2	150–225		–
Gocke et al. (1982)	A	Sausages (pork)	Fried in pan at temperatures of the fat	2–40	150–230	–	+
Whong et al. (1986)	C	Shredded pork	Fried with a hot plate	NS	350		+
Perez et al. (2002)	C	Frankfurters	Frying pan with and without oil or butter	10	170–180	–	+
Salmonella typhimurium TA100							
Pourazrang et al. (2002)	E	Nitrated sausage	Fried	2 × 3	175	–	+
Pourazrang et al. (2002)	E	Non-nitrated sausage	Fried	2 × 3	175	–	–
Gocke et al. (1982)	A	Sausages (pork)	Fried in pan with fat	40	150–230	–	+
Salmonella typhimurium TA102							
Pourazrang et al. (2002)	E	Nitrated sausage	Fried	2 × 3	175	–	–
Pourazrang et al. (2002)	E	Non-nitrated sausage	Fried	2 × 3	175	–	–
Salmonella typhimurium TA1535							
Gocke et al. (1982)	A	Sausages (pork)	Fried in pan with fat	40	150–230	–	–
Salmonella typhimurium TA1537							
Gocke et al. (1982)	A	Sausages (pork)	Fried in pan with fat	40	150–230	–	+
Salmonella typhimurium TA1538							
Bjeldanes et al. (1982a)	B	Bacon	With or without corn oil, most samples were cooked in pans	2 × (7, 10)	225		+
Bjeldanes et al. (1982a)	B	Bacon	With or without corn oil, most samples were cooked in pans	2 × 6	200, 250, 280		+
Bjeldanes et al. (1982a)	B	Ham	With or without corn oil, most samples were cooked in pans	2 × (3, 5, 10)	275		+
Bjeldanes et al. (1982a)	B	Ham	With or without corn oil, most samples were cooked in pans	2 × 6	200, 250, 280		+
Bjeldanes et al. (1982a)	B	Pork chops	With or without corn oil, most samples were cooked in pans	2 × (3, 6)	275		+
Bjeldanes et al. (1982a)	B	Pork chops	With or without corn oil, most samples were cooked in pans	2 × 6	200, 250, 280		+
Bjeldanes et al. (1982a)	B	Sausages	With or without corn oil, most samples were cooked in pans	2 × 6	200, 250, 280		+
Gocke et al. (1982)	A	Sausages (pork)	With or without corn oil, most samples were cooked in pans	40	150–230	–	+
Salmonella typhimurium YG1019							
Pfau et al. (2006)	F	Pork	Fried on frying plate	2 × 6	200		+
Others							

(*continued on next page*)

Table 3 (*continued*)

Paper/Assay	Extraction method[a]	Meat type	Cooking method	Cooking times (min)	Cooking temperatures (°C)	Result	
						S9-	S9+
SOS/umu assay							
Salmonella typhimurium TA1535 Psk1002							
Whong et al. (1986)	C	Shredded pork	Fried with a hot plate	NS	350		+
Gene mutation assay (6-Thioguanine resistance) V79 cells							
Gocke et al. (1982)	A	Sausages (pork)	Fried in pan at temperatures of the fat	40	150–230	–	–
Sister chromatid exchange assay V79 cells							
Gocke et al. (1982)	A	Sausages (pork)	Fried in pan at temperatures of the fat	40	150–230	–	±

NS: Non-specified.
± Inconclusive.
Positive (+) indicates at least one of the conditions was found to be positive.
Cooking times: 2x indicates "for each side"; two numbers separated by a "-" indicate some intermediate times have been also assessed; two numbers separated by a "," indicate only those times have been assessed with no intermediates.
[a] For summarised information on extraction methods A-F see Fig. 2.

the quantity of B(a)P was also compared. The mutagenicity of pan-fried samples was lower than that of grilled samples and higher than that of boiled samples, and this was correlated with the quantity of B(a)P. Ames results were presented as His^+ revertants at only 1 concentration and according to the results presented by the authors, pan-fried samples did not even double the number of revertants in the controls (DMSO or raw-meat samples) (20 His^+ vs less than 40 His^+), and the differences were not found to be statistically significant. The authors considered this a negative result but it may rather be an unclear result.

TA1537 and YG1019 were used by Felton et al. (1981) and Martin et al. (2002), respectively, and positive results with S9 were obtained in both cases. Furthermore, 1 author showed positive results in an SOS-umu assay with *Salmonella typhimurium* TA1535 Psk1002 with S9 (Whong et al., 1986). Moreover, although very few articles included base-pair substitution strains TA100 and TA1535, it appears that these strains are not capable of detecting mutagenicity of beef extracts. Few experiments have been performed with TA102, a strain that detects substitutions at AT base pairs. Thus, 4 frameshift sensitive strains (TA1538/TA98, TA1537, and YG1019) were clearly and consistently positive in many of the experiments. It is important to emphasize that no author has observed mutagenicity without metabolic activation. This is in accordance with the fact that HAAs are pro-mutagens that need to be bioactivated to be mutagenic (Koszucka and Nowak, 2018). With respect to other assays, only Martin et al., 2002 studied DNA damage by the SCGE (comet) assay in MCF-5 cells, with positive results, although details of cooking conditions are not provided.

Pork. TA98 and TA1538 appear to be consistently positive, depending on metabolic activation (Table 3). Only 1 sample of sausages showed no mutagenicity with S9 in an article; the authors stated that an explanation might be that the sausage contained only 44% meat, thus affecting transmission of heat and formation of mutagens (Augustsson et al., 1999). Furthermore, Perez et al. (2002) found a lower level of mutagenic activity in TA98 with S9 in hamburgers than in sausages, and attributed the difference to the lower content of protein and the higher content of fat in sausages. The lower level of proteins is directly related to the products of the Maillard reaction and a high content of fat may dilute its precursors (Perez et al., 2002). In a study designed to evaluate the inhibition of mutagenic NOC formation using vitamin E and C, Pourazrang et al. (2002) also found negative results in both nitrated and non-nitrated sausages in TA97a, TA98, and TA102. Nitrated sausages in combination with S9 were found to be positive only in TA100. Comparing these results with those of other authors, the cooking conditions were milder with regard to frying time (2 × 3 min) and temperature (175 °C). The mutagenicity detected appears to be attributable to NOC formation, as the authors used an extraction method for these compounds and indicated that mutagenicity was not detectable in sausages not treated with nitrite (Pourazrang et al., 2002). This may appear to contradict the study by Miller and Buchanan (1983), in which no differences in the mutagenicity of nitrite-treated and nitrite-free bacon were observed in TA98. However, the authors stated that the mutagenicity they detected was not attributable to the presence of nitrosamines based on the fact that i) nitrosamines are considered base-pair mutagens, and thus, not detectable in TA98, and ii) the extraction method they used did not include a liquid pre-incubation normally needed for the extraction of nitrosamines. This information thus indicates the importance of the extraction method and the need to use different strains of *Salmonella typhimurium* for the correct interpretation of the results. Sausages in combination with S9 were also found to be positive in TA100 but negative in TA1535 (Gocke et al., 1982); however, in this case, they were also positive with TA98 and TA1537 with S9. As well as the Ames test, a SOS-umu assay was positive in *Salmonella typhimurium* TA100 Psk1002; the 6-thioguanine resistant cell test in V79 cell line was negative; and the sister chromatid exchange test was inconclusive .

Other meats. The other types of meat were found to be positive in strains TA98/TA1538, TA100, and TA100NR with metabolic activation

Table 4
In vitro evaluation of non-beef and non-pork animal extracts fried under controlled conditions in a laboratory.

Paper/Assay	Extraction method[a]	Meat type	Cooking method	Cooking times (min)	Cooking temperatures (°C)	Result	
						S9-	S9+
Bacterial gene mutation assay							
Salmonella typhimurium TA98							
Vikse and Joner (1993)	B	Deer	Fried in a frying pan lightly greased with maize oil	2 × 6	250		+
Vikse and Joner (1993)	B	Goat	Fried in a frying pan lightly greased with maize oil	2 × 6	250		+
Sflomos et al. (1989)	B	Ground lamb	Cooked in a griddle	2, 4, 6, 8, 10, 12	100–300		+
Vikse and Joner (1993)	B	Grouse	Cooked in a griddle	2 × 6	250		+
Vikse and Joner (1993)	B	Hare	Cooked in a griddle	2 × 6	250		+
Vikse and Joner (1993)	B	Hen	Cooked in a griddle	2 × 6	250		+
Vikse and Joner (1993)	B	Horse	Cooked in a griddle	2 × 6	250		+
Augustsson et al. (1999)	C	Meatballs	Pan-fried	NS	150–225		+
Vikse and Joner (1993)	B	Minced elk	Pan-fried	2 × 6	250		+
Vikse and Joner (1993)	B	Mutton	Pan-fried	2 × 6	250		+
Taj and Nagarajan 1994	E	Mutton	Deep fried	20	150–200	+	+
Taj and Nagarajan (1994)	C	Mutton	Deep fried	20	150–200	+	+
Vikse and Joner (1993)	B	Pheasant	Pan-fried	2 × 6	250		+
Vikse and Joner (1993)	B	Rabbit	Pan-fried	2 × 6	250		+
Vikse and Joner (1993)	B	Reindeer	Pan-fried	2 × 6	250		+
Vikse and Joner (1993)	B	Roe-deer	Pan-fried	2 × 6	250		+
Salmonella typhimurium TA100							
Sflomos et al. (1989)	B	Ground lamb	Cooked in a griddle	2, 4, 6, 8, 10, 12	100–300		–
Taj and Nagarajan (1994)	E	Mutton	Deep fried	20	150–200	+	+
Taj and Nagarajan (1994)	C	Mutton	Deep fried	20	150–200	+	+
Salmonella typhimurium TA100NR							
Taj and Nagarajan (1994)	E	Mutton	Deep fried	20	150–200	+	+
Taj and Nagarajan (1994)	C	Mutton	Deep fried	20	150–200	+	+
Salmonella typhimurium TA1535							
Sflomos et al. (1989)	B	Ground lamb	Cooked in a griddle	2, 4, 6, 8, 10, 12	100–300		–
Salmonella typhimurium TA1538							
Bjeldanes et al. (1982a)	B	Chicken	With or without corn oil, when needed, most samples were cooked in pans	25 (10 + 15)	103		+
Bjeldanes et al. (1982a)	B	Chicken	Deep fried	12	101		+
Sflomos et al. (1989)	B	Ground lamb	Cooked in a griddle	2, 4, 6, 8, 10, 12	100–300		+
Barrington et al. (1990)	B	Lamb	Flat iron skillet with frying medium: margarine, butter and oil	2 × (2.5, 5)	140–200		+
Single cell gel electrophoresis (comet) assay (strand breaks)							
Cultured human lymphocytes							
Taj and Nagarajan (1994)	E	Mutton	Deep fried	20	150–200	+	+
Taj and Nagarajan (1994)	C	Mutton	Deep fried	20	150–200	–	+

Positive (+) indicates at least one of the conditions was found to be positive.
Cooking times: 2x indicates "for each side"; two numbers separated by a "-" indicate some intermediate times have been also assessed; two numbers separated by a "," indicate only those times have been assessed with no intermediates.
[a] For summarised information on extraction methods (EM) A-F see Fig. 2.

(Table 4). Taj and Nagarajan et al. (1994) found positive results in TA98, TA100, and TA100NR, also without metabolic activation. These results do not agree with the rest of the scientific evidence, as bioactivation is always needed to observe genotoxic results in the case of PAH, HAAs, or NOCs. However, it should be noted that the mutton was salted and sun-dried for a week prior to deep-frying. This process may give rise to different mutagens or to a mixture of PAHs, HAAs, and NOCs, thereby producing a different genotoxic response. Indeed, interactions among NOCs, heme iron, and HAAs have been described (IARC, 2018). The authors did not discuss these findings (Taj and Nagarajan, 1994). In TA1535 and TA100 strains, which are capable of detecting base substitutions mutagens, extracts from ground lamb fried at different times and temperatures gave negative results (Sflomos et al., 1989). With respect to other assays, Taj and Nagarajan et al. (1994) studied DNA damage using the SCGE (comet) assay in cultured human lymphocytes and found positive results both with and without metabolic activation. Again, these positive results in the absence of metabolic activation were not discussed.

Commercial. Of all the articles that included the *in vitro* analysis of fried meat, 5 out of 26 articles assessed meat cooked in restaurants or catering companies. Furthermore, the mutagenicity of commercial fried meat has been evaluated only in TA1538/TA98 (Table 5). It is well known that controlled experimental assays are an essential part of any research. Nonetheless, real conditions in samples that may potentially be eaten by the general population must be also assessed, thus ensuring a quality assessment of the real impact on our society. Most of the studies gave positive results, indicating that the general population may be exposed to mutagenic compounds.

Spingarn and Weisburger, 1979 found significant mutagenic activity in conventionally cooked meat obtained from the local franchises of 2 US national fast-food chains, but the number of samples analyzed and the proportion of positive samples are not provided in the article. Also, Gocke et al. (1982) stated that fried sausages obtained from local bratwurst stands gave similar values of His^+ revertants than "edible" sausages prepared in the laboratory, but no information on the number of samples analyzed is given in the article. Stavric et al. (1995) studied 16 samples of hamburgers and 14 samples of hot dogs, randomly obtained from commercial fast food establishments or street vendors. A high proportion of samples were positive (15/16 hamburgers and 8/14 hot dogs), with a very wide range of His^+ revertants per gram, even for

Table 5
In Vitro evaluation of fried meat extracts obtained from a catering service or restaurant.

Paper/Assay	Extraction method [a]	Meat type	Cooking method	Result [b]
Bacterial gene mutation assay				
Salmonella typhimurium TA98				
Spingarn and Weisburger (1979)	A	Hamburgers	Pan-fried	+ (NS)
Gocke et al. (1982)	A	Sausages	NS	+ (NS)
Stavric et al. (1995)	F	Hamburgers	Hot plate	+ (15/16)
Stavric et al. (1995)	F	Hot-dogs	Hot plate	+ (8/14)
Salmonella typhimurium TA1538				
Pariza et al. (1979)	A	Hamburgers	Grilling (frying)	+ (6/8)
Bjeldanes et al. (1982a)	B	Hamburgers	Grilled	+ (19/21)

NS: Non-specified
[a] *For summarised information on extraction methods A-F see Figure 2*
[b] *Number of positive samples /total number of samples studied*

the same type of product obtained from the same outlet at different times. According to the authors, this indicates a possible inconsistency in cooking procedures during the preparation of these products. Pariza et al. (1979) studied cooked hamburgers from 4 restaurants that prepared them by grilling (frying). They obtained samples from each of the restaurants at 2 different times separated by a week. Results were generally reproducible in each restaurant after a time interval of 1 week, but mutagenic activity varied widely between samples from the different restaurants, 1 of which produced a negative result. Bjeldanes et al. (1982a) studied commercially cooked hamburgers obtained from 7 fast food restaurants (4 samples per visit/3 visits per restaurant in a period of 9 weeks). A very high proportion of positive results was obtained (19/21) but mutagenic activity varied widely between the restaurants and also within the restaurants. Of the 7 restaurants sampled, 3 provided hamburgers with consistently low mutagenic activity, 2 with consistently high mutagenic activity, and 2 showed considerable variation between sampling times. Results could not be quantitatively linked to frying conditions (time/temperature), although qualitatively, the authors associated a higher mutagen content with more severe cooking conditions, which may vary between restaurants.

No assays other than the Ames test were found to be used in testing genotoxic activity of fried meat extracts from catering services or restaurants.

A major difficulty encountered in this review was the extraction of information on cooking methods, either due to a lack of information or a lack of harmonization of cooking concepts. For example, many authors did not specify whether any kind of fat was added for frying. As mutagen formation depends directly on temperature and cooking time, all the details regarding food handling need to be clearly specified. Adding fat to a fryer affects the degree of mutagen formation, as fat is an effective heat-transferring agent (Overvik et al., 1987). Nevertheless, this effect is less obvious at lower temperatures (200 °C) and may even display lower mutagenic activity than samples without added fat (Nilsson et al., 1986).

As explained above, data gathered from tests other than Ames are limited, insufficient, and thus inconclusive. Very little is known about the *in vitro* genotoxicity of meat extracts other than mutagenicity in *Salmonella typhimurium* strains. Other *in vitro* assays are needed to elucidate different types of damage that compounds generated during cooking conditions may produce. As explained above, *in vitro* assays are essential to discard genotoxic compounds, but several *in vitro* approaches must be used to cover different genetic endpoints, as each test focuses on a specific endpoint (gene mutations, both structural and numerical chromosome aberrations and DNA lesions). Both WHO and EFSA guidelines are clear about the need to perform a gene mutation test in bacteria in combination with at least 1 test that detects chromosome damage as the first step in the risk assessment of chemicals in food.

3.3. *In vivo* genotoxicity evaluation

Although many articles dealing with the *in vitro* evaluation of different types of meat were found, only 5 articles focusing on the *in vivo* assessment of fried meat genotoxicity were selected; these studies are shown in chronological order in Table 6. As has already been mentioned, the international WHO and EFSA guidelines establish the need to perform *in vivo* assessment when clear mutagenicity is shown *in vitro*. This review shows the scarce evidence of fried meat *in vivo* genotoxicity.

The main types of meat evaluated were pork sausages, mutton, red meat, and chicken, alone or mixed with standard pellet diet (Table 6). It is important to take into account that, in the case of *in vivo* assays, there is no particular need to produce extracts in order to expose cells to mutagens. Therefore, most of the assays reviewed used oral administration, and meat was mixed with standard pellet diet. Where extracts were used, the route of administration was intravenous or intraperitoneal. Only 2 authors performed more than 1 test.

Two micronucleus tests were performed by Gocke et al. (1982) and Taj and Nagarajan (1994), although experimental conditions vary considerably. Standard pellet diet mixed with salted and sun-dried mutton that had been deep-fried at 150–200 °C and given orally to Wistar rats for 1–2 months was found to be positive for bone marrow erythrocytes (Taj and Nagarajan, 1994). Extracts of pork sausages that had been fried in a pan with fat for 40 min at 150-230 °C, and given as a single intraperitoneal bolus to NMRI mice, however, were negative for bone marrow erythrocytes (Gocke et al., 1982). In addition, Taj and Nagarajan (1994), under the same experimental conditions described above, evaluated chromosome aberrations in bone marrow cells and strand breaks in hepatocytes and lymphocytes, and found positive responses in both tests. Those authors also found that, after 1 month of interrupting diet with fried mutton, the level of chromosome aberrations, MN and DNA strand breaks decreased but did not reach the levels of untreated control animals. These results indicate that daily exposure to fried meat may damage DNA and have some effects at the chromosome level. The other *in vivo* assays performed by Gocke et al. (1982) were negative or inconclusive, but it must be noted that exposure conditions are very different with a single IP administration of sausage extracts.

Positive results were found in the comet assay when exposing rats or pigs to 4 weeks of hot-plate cooked red meat (Toden et al., 2006; Belobrajdic et al., 2012). Despite the fact that exposure conditions were not exactly the same, the results indicate that colon cells of animals exposed to fried red meat for 4 weeks show DNA strand breaks. Among the compounds that may be responsible for this effect, the authors

Table 6
In Vivo evaluation of meat fried under controlled conditions in a laboratory.

Paper	Assay	Species	Tissue	EM[a]	Route of admin. (duration/ time till death)	Meat type	Cooking method	Cooking times (min)	Cooking Temp. (°C)	Result
Gocke et al. (1982)	Host mediated assay	NMRI mice	Liver	A	Intravenous (one bolus - 1h)	Sausages (pork)	Pan-fried w/ fat	NS	NS	±
	Mammalian spot test	C57BL mice	Whole animal	A	Intraperitoneal (bolus - 2-5 weeks postnatal)	Sausages (pork)	Pan-fried w/ fat	NS	NS	-
	Micronucleus assay	NMRI mice	Bone marrow	A	Intraperitoneal (one bolus-30h)	Sausages (pork)	Pan-fried w/ fat	40 min	150-230°C	-
	Sex-linked recessive lethal assay	Drosophila melanogaster Berlin K and Basc tester strain	Adults and larvae	A	Oral and injected for adults, and oral for larvae (NS)	Sausages (pork)	Pan-fried w/ fat	40 min	150-230°C	-
Taj and Nagarajan (1994)	Chromosome aberrations	Wistar rats	Rat bone marrow erythrocytes	NA	Oral (1m, 2m, or 2m+1 without - NS)	10 and 20% mutton in standard pellet diet	Deep fried	20 min	150-200°C	+
	Micronucleus test	Wistar rats	Rat bone marrow erythrocytes	NA	Oral (1m, 2m, or 2m+1 without - NS)	10 and 20% mutton in standard pellet diet	Deep fried	20 min	150-200°C	+
	Single cell gel electrophoresis assay	Wistar rats	Hepatocytes and Lymphocytes	NA	Oral (1m, 2m, or 2m+1 without - NS)	10 and 20% mutton in standard pellet diet	Deep fried	20 min	150-200°C	+
Toden et al. (2006)	Single cell gel electrophoresis assay	Sprague Dawley rats	Colon	NA	Oral (4 weeks- NS)	Red meat (25%) in standard pellet diet	Hot-plate	NS	150	+
	Single cell gel electrophoresis assay	Sprague Dawley rats	Colon	NA	Oral (4 weeks - NS)	Red meat or chicken (15, 25, 35%) diet, with or without starch	Hot-plate	NS	150	+
Winter et al. (2011)	O6-MeG quantification	CBJ57 male mice	Colon	NA	Oral (4weeks - NS)	Diets of 15% or 30% protein as red meat	Hot-plate	NS	Medium temp.	+
Belobrajdic et al. (2012)	Single cell gel electrophoresis assay	Large white pigs	Colon	NA	Oral (4 weeks −2/day)	Cooked red meat (300g/kg bw), with or without arabinoxylans	Hot-plate			±

NS: Non-specified
NA: Non-applicable
± Inconclusive
Positive (+) indicates at least one of the conditions was found to be positive
Cooking times: two numbers separated by a "-" indicate some intermediate times have been also assessed
[a] For summarised information on EM (Extraction methods) A-F see Fig. 2.

Table 7
Genotypes of the *Salmonella typhimurium* strains used for mutagenesis testing with the Ames test (adapted from Maron and Ames, 1983).

Strain	Mutation detected	pKM101 (R factor)	Histidine mutation		
			hisD	hisC	hisG
TA97	Frameshift	+	hisD6610		
TA98	Frameshift	+	hisD3052		
TA100	Substitutions (GC)	+			hisG46
TA102	Substitutions (AT)	+			hisG428
TA1535	Substitutions (GC)	–			hisG46
TA1537	Frameshift	–		hisC3076	
TA1538	Frameshift	–	hisD3052		

mention HAAs, nitrosamines, and heme iron present in red meat (Toden et al., 2006). Moreover, diets of 15% or 30% protein such as red meat cooked on a gas hot plate increased the number of DNA adduct O^6-MethylGuanine in cells of the distal colon extracted from mice treated for 4 weeks (Winter et al., 2011).

In conclusion, although the number of *in vivo* studies is low, evidence exists to show induction of DNA damage in colon cells and chromosome aberrations in bone marrow cells of animals after oral daily treatment with a diet containing fried red meat for 4 weeks or longer.

4. General conclusions and recommendations

Overall, this systematic search shows that few articles (31 studies) have evaluated the genotoxicity of fried meat. Moreover, very few studies have been carried out recently. Most of the studies used the Ames test and consistent positive results with TA98/TA1538 (with S9) have been found in all types of meat, but especially in beef. In general, negative results are associated with short cooking times and low temperatures or with the use of TA strains detecting base-pair mutations, although the number of studies using strains TA100 or TA1535 is comparatively very low. The positive results obtained *in vitro* in TA98/1538 in controlled laboratory conditions correlate with the few studies that evaluate food samples prepared under real catering conditions (all of them tested in TA98/TA1538 strains), and with positive results obtained in the few *in vivo* genotoxicity studies carried out to date. However, some limitations and knowledge gaps have been detected in this review process and are indicated below:

- There is a lack of harmonization in the description of cooking procedures. This aspect is important in order to give recommendations for healthy ways of cooking meat.
- It is unclear whether the different extraction methods used for genotoxicity testing are able to extract all relevant mutagens present in the extract. Some validations have been made in some cases, but only for some individual compounds. This will be interesting for correlating the presence of specific mutagens with the genotoxic response.
- Although many Ames tests have been done, very few studies have used the 5 OECD recommended strains and, thus, not all the extracts have been characterized for different types of DNA mutations.
- Data on in *vitro tests* other than the Ames test and *in vivo* testing are limited or insufficient. Performing these follow-up tests in accordance with current guidelines may provide interesting mechanistic information.

Overall, given that some meat consumption is needed for a healthy diet and that the evidence suggests that fried meat extracts are genotoxic, it would be advisable to carry out a risk-benefit analysis (EFSA, 2010) of meat consumption. All of the abovementioned steps may help provide more specific recommendations on meat consumption and the healthiest way of preparing it, and thus provide important insights for the risk-benefit analysis of meat consumption.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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