Food Standards Agency Project A05009

The Safety of Irradiated Foods: A literature review

Technical Report

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1. INTRODUCTION

1.1 Background and Rationale

“Never in the history of man has any method of food processing invoked so much comment, induced so great a controversy, nor required such an expenditure of funds before its use could be permitted even on a limited basis as has the use of ionising energy” (Goldblith 1970).

The work presented in this review is based on a Food Standards Agency funded project (A05009). Prior to this, no previous review had been commissioned by the Agency. The researcher completed a PhD on food irradiation at the University of Reading in 1995 so many of the papers published until that date had already been reviewed (a copy of this previous literature review is appended). This highlighted a number of areas of concern, particularly among consumers regarding its use on a commercial basis. Despite numerous reports assuring the safety of irradiated food, the public remains unconvinced and commercialisation of the process relies on public confidence (Jukes 1990). The concerns expressed by consumers are founded in five main areas, which are outlined below and are discussed in detail later in the text.

1.2 Perceived Concerns

The use of food irradiation remains an emotive issue, despite over 100 years of research on the subject. Consumer resistance to food irradiation is founded in five main areas, four of which are concerned with the ‘wholesomeness’ of irradiated foods. A 'wholesome' food is defined as one which is free of harmful, toxic chemicals, does not pose microbiological risks and has satisfactory nutritional qualities (Urbain 1986). The United Nations definition also includes the provision that no induced radioactivity of the food occurs. There is some controversy between the terms 'wholesome' and 'safe for human consumption' (Webb and Lang 1987).

The five main areas of concern identified were:

1. Fear of Induced Radioactivity

2. Effect on Food Nutrients
3. Toxicity Effects
4. Microbiological Effects
5. Detection of Irradiated Foods

The effect on food nutrients was not, on the whole, considered as part of this review as there is no real concern in terms of the safety of irradiated foods. Effects on nutrients may be mentioned in the main review as some of the papers assessed evaluated the effects on nutrients as well as investigating other aspects of the irradiation process. Another area that has not been addressed, for the same reason, is the effect of irradiation on the organoleptic properties of the foods. As stated above, this may be mentioned where changes in the sensory properties may be linked to other, more serious changes in the food.

This review will consider the other three concerns – toxicity and microbial effects as well as the detection of irradiated foods. Major developments have occurred, particularly in detection technologies, in the last ten years.

1.3 Safety Criteria

Takeguchi (2002) identified the criteria that should be considered when determining the safety of irradiated food. U.S. Congress defined safety as: “reasonable certainty that no harm will result from the proposed use of an additive. It does not- and cannot- require proof beyond any possible doubt that no harm will result under any conceivable circumstance.” (p. 759).

For irradiated foods, Takeguchi (2002) suggested that the following criteria and factors should be considered:

- The specific organism or target at which the irradiation process is aimed. Insects are more sensitive than microorganisms, which in turn are more sensitive than toxins or enzymes
- An evaluation of the type and amount of radiolytic products

- Recognition that the food being irradiated is made up of individual cells and that what happens inside each cell is not necessarily the same as that which would happen if the cellular components are irradiated separately

Takeguchi goes on to state that:
"The irradiation process followed in any given location should be accompanied by written procedure that maximises the advantage of the process, minimises the unwanted effects and provides adequate directions to produce a safe product consistently under current good manufacturing (and good irradiation) conditions." (p. 759).

1.4 Aim and Objectives

The aim of this review is to investigate the current literature in respect of the safety of the irradiation process, the safety of irradiated food and the safety of the irradiation process for food packaging. This will be achieved with the following objectives:

1. Collate all the relevant publications and unpublished data (where appropriate) regarding the safety of irradiated food
2. Critically evaluate the publications for methodology, data analysis and conclusions drawn
3. Produce an objective review of the publications to date with unbiased, rationalised conclusions

2. SOURCES AND TYPES OF REFERENCES

A number of resources were used to collate all relevant references.

2.1 Direct contact with relevant organisations

It was already known that a number of organisations exist that investigate food irradiation. Contact was made with the International Atomic Energy Agency (IAEA), the Food and Drug Administration in the USA (FDA), the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), bodies that have all previously commissioned research on the subject and published their findings. Contact was also attempted with members of groups that have now closed or ceased to operate in the original format, such as the International Consultative Group on Food Irradiation (ICGFI).

2.2 Electronic Resources

Through the UWIC library, a number of electronic search engines were used. These included Athens, which gives access to a number of sites, including the ISI Web of Knowledge and Science Direct; Scopus, a large database covering technical, medical and social sciences (mainly peer reviewed articles) and HighWire Press (a division of the Stanford University Libraries), which produces the online versions of high-impact, peer-reviewed journals and other scholarly content. This latter resource also contains a large number of consumer oriented publications.

2.3 Existing Databases

Two existing databases were identified, with the assistance of the project team at the Agency. These were the United States Department of Agriculture Food Irradiation Database (http://www.nal.usda.gov/fnic/foodirad/intro.html) and the online version of the Bibliographie zur Bestrahlung von Lebensmitteln (Bibliography on Irradiation of Foods, http://www.bfa-ernaehrung.de/BFELEMISTW/SF). The USDA database consists of reports, articles, books and book chapters relevant to research and development of irradiated food from 1947 to 1997. The overall focus was to record all factual

information and results of experiments sponsored by the US Government, with special emphasis on unpublished or difficult to obtain research reports. The second database, is an information service supported by ZADI (Zentralstelle füt Agrardokumentation und Information), the German Centre for Documentation and Information in Agriculture.

While both these databases were used as reference sources, it was found that the US database in particular was not user-friendly. It cited that it had "special emphasis on unpublished or difficult to obtain research reports". I found, however, that without knowing the actual report number, it was impossible to find this unpublished work. There is no way to access the entire database to browse the entirety of its content – specific search terms must be input. The German database was more user-friendly and a number of references were sourced.

2.4 Types of Reference

A wide range of reference types was assessed during the course of this review. They range from the peer-reviewed, academic papers, to articles in consumer publications expressing personal opinions. The purpose of using different types of references was to gain an overall impression of the status of irradiated food in legislature, the scientific community and in the minds of the public. When analysing the data contained in the publication, the type of reference was taken into account. It is generally acknowledged that data from peer reviewed sources is more robust than from other sources, so this was a factor in the data analysis section of the project. No account was taken of the impact factor of different academic publications.

3. DATABASE CONSTRUCTION

The database used for this project is Reference Manager (version 11), which is produced by Adept Scientific (Letchworth, Hertfordshire, http://www.adeptscience.co.uk/) and is a bibliographic reference management software package that also has the capability to directly search the internet, create bibliographies for documents and allow publication of databases on the web. There is also the option to customise each database to allow for flexibility of use. As part of this project, the researcher undertook a one-day training course on the software programme and has customised the database used in this project. The optional fields that may be added were assigned to account for the type of information stored in each reference – microbiological, radiological or toxicological safety, or whether the publication related to the irradiation of packaging or to the safety of the process itself. An option for a 'general' article was also included to account for those papers concerning organoleptic, nutritional or consumer aspects.

Each reference is assigned an identification number that cannot be duplicated within the database. The database does not reassign previously used numbers if a reference has been deleted, so that if different versions of the same database are in use, there can be no opportunity for confusing different references. This is the reason that the numbering in the database exceeds the number of actual references present by approximately 760.

4. THE REFERENCES

4.1 Frequency of References

The number of references as a function of time will allow us to gain an insight into activity in the field of food irradiation research in general. As can be seen from the database, the time period ranges from 1927 – 2007 and, while it is obvious that the earliest years yielded very few publications, it is interesting to determine the status up until 2006 (2007 will not be included as a complete year cannot be input into the analysis). While it is accepted that the database is not exhaustive, it does represent the majority of publications in the public domain and, therefore, available to most researchers. Figure 1 shows the frequency of published papers, reports, legislation and other articles over time.

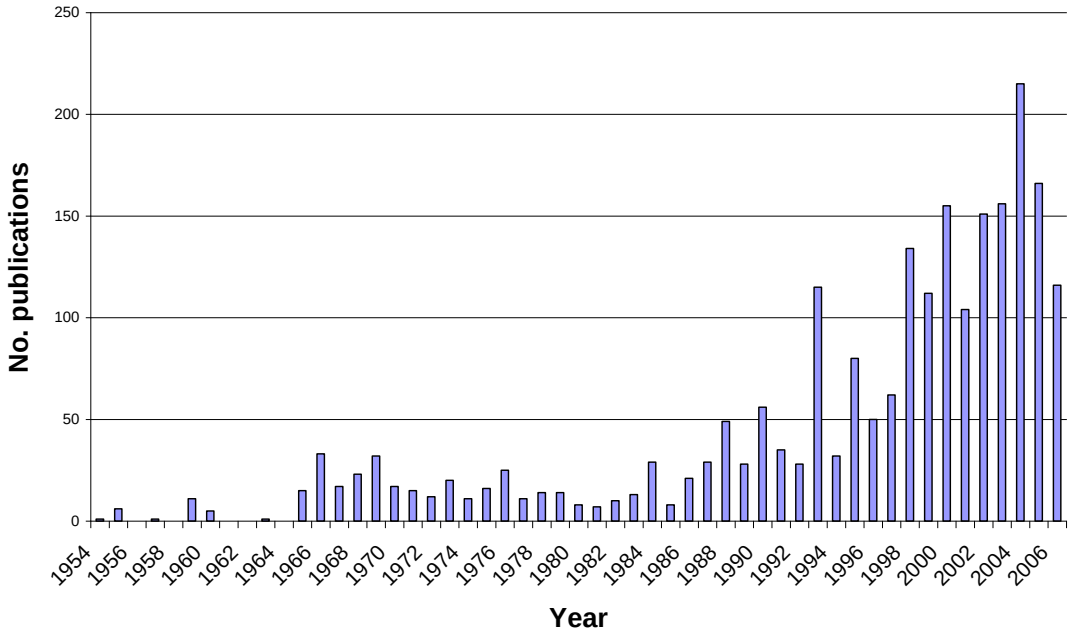

Figure 1. The frequency of publications relating to food irradiation from 1957 – 2006 (one paper was published in 1927).

The publications dated from 1990 onwards (1990 was the first year that the number exceeded 50) were further analysed in terms of the focus of the paper (the fields in the database labelled general or review article (including nutritional and sensory changes), microbiological, radiological, toxicological,

process). The reason for this analysis is to determine whether the focus of research in food irradiation has changed over the past 16 years. In some cases, the paper was assigned to more than one category (for example, if the publication dealt with the microbiological and toxicological effects of irradiation of a specific commodity) or was assigned to a category with very little information being available. This is due to the fact that approximately 10% of the total number of publications in the database were actually on file and it proved impossible to obtain copies of each publication. Table 1 below shows the percentage of publications for each year in each of the categories assigned.

YEAR	CATEGORY ASSIGNED						TOTAL
	G	M	NS	T	P	D	
1990	27	4	6	5	0	20	**62**
1991	11	12	2	5	2	6	**38**
1992	9	3	2	7	1	7	**29**
1993	32	16	13	6	1	50	**118**
1994	11	10	5	1	0	8	**35**
1995	21	18	13	5	2	31	**90**
1996	14	11	4	1	2	18	**50**
1997	14	18	5	6	1	22	**66**
1998	34	24	21	5	9	33	**126**
1999	32	21	10	10	12	27	**112**
2000	52	31	19	11	12	33	**158**
2001	29	20	17	13	4	19	**102**
2002	33	30	28	12	4	34	**141**
2003	29	33	34	15	4	27	**142**
2004	47	45	48	13	13	23	**199**
2005	28	51	19	12	4	21	**135**
2006	24	35	26	13	9	16	**123**
TOTAL	**447**	**382**	**272**	**140**	**80**	**405**	**1726**

Key:

G General or review article, including nutritional or sensory changes
M Microbiological
NS Nutrition / sensory

T Toxicological
P Process
D Detection of irradiated foods

Table 1. A breakdown of the focus of publications on food irradiation between 1990 and 2006.

It can be seen that the focus of the majority of publications, with the exception of general or review papers, has been detection and microbiological aspects of irradiated food. There are comparatively few publications regarding toxicological issues (140 out of 1726) or packaging issues (80 publications). Of the publications available so far for 2007 (three, to 12 January), two are focussed on detection of irradiated food while the third is concerned with changes to rheological properties following radiation processing.

4.2 Analysis of References

Each reference obtained (hard or electronic copy) was assessed to determine whether it was an original research article that contained raw data. If this was the case, the publication was analysed. It was not possible to analyse general or review articles as there was not enough detail about actual data presented in the majority of these. The following criteria were used in order to analyse the original research papers:

The Methodology used (so comparisons could be made with other, similar publications). The details recorded here include a reference to the type of paper (as in Table 1 above), the type of radiation source used (usually gamma or electron beam, but UV was also included where there are direct comparisons with ionising radiation), the overall dose(s) applied, the average dose rate, the ambient temperature and humidity and sample details. It is noticeable that not all authors quote all of these parameters. Each parameter is important as each can affect the results obtained. Without access to this information, it can be difficult to directly compare different data sets.

The data analysis techniques employed (the type of statistics used and the rationale). The method in which the authors presented the results was

recorded as well as any specific calculations applied to the data. The statistical tests employed were also recorded and the validity of each was assessed by Dr Keith Morris, the School's statistical expert. Again, it is a feature of some of the older publications that no statistical analysis at all was applied. This is less common in the more recent papers.

The validity of conclusions drawn by the authors. On the basis of the results presented, the confidence that we can have in the conclusions drawn was assessed. This was based upon comparisons with other researchers' data and, in some cases, the choice of language used by the authors. The latter measure was more important for the earlier publications as publishing guidelines have become more stringent in recent years and authors are less likely to make forceful statements or claims.

5. THE AREAS OF CONCERN

Once the references were analysed, they were subdivided into the categories defined previously and the following emerged as areas of current concern. The effect of irradiation on the nutritional properties of the food were largely discounted as, for the majority of the population in the UK and other first world countries, this will not have a significant impact on public health. An analysis of the effect of food irradiation on the nutritional status of food targeted at under-or malnourished individuals or populations is beyond the remit of this project. In 1999 the World Health Organisation approved the use of high dose irradiation and stated that there were no issues regarding the safety or wholesomeness of irradiated food on the grounds of:

- Radiation chemistry
- Nutrition (with the exception of thiamine, which has been shown to be sensitive to radiation although WHO stated that it was unlikely that irradiated foods would comprise a large enough proportion of the diet for this to be a problem)
- Microbiological considerations (where it was concluded that high dose irradiation was equivalent to thermal processing as both produce shelf-stable, microbiologically safe foods)
- Toxicological considerations (this aspect will be discussed in more depth in section 5.2)
- Minimum and maximum dose absorbed by the food (it was stated that these could be accurately defined assuming proper monitoring and recording at the irradiation facility is undertaken).

The need for further validation was identified for new packaging materials, using existing methodology to determine a damage-dose response relationship. The overall conclusion stated:
“food irradiated at any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. ….Accordingly, irradiated foods are deemed wholesome throughout the technologically useful

dose range from below 10 kGy to envisioned doses above 10 kGy." (FAO/IAEA/WHO 1999, p.161).

Each of the areas of concern is now investigated in detail. Some publications may appear in more than one section as the work contained is relevant to more than one area. In each case, the conclusions from the WHO assessment of high dose irradiation are also included.

5.1 Microbiological Safety

From the wide range of publications collated during the course of this study, it is clear that the microbiological aspects have received most attention from researchers. Historical consumer concerns are centred on two microbiological issues:

1. The induction of radiation resistance in human pathogens
2. A change in the spoilage pattern that the food undergoes, meaning that consumers may not recognise an irradiated food as spoiled, and there is the possibility of pathogen contamination at a level sufficient to cause food poisoning.

There was no firm evidence to support either of these concerns. This section will look at the studies that have been carried out on different microorganisms, the different matrices used during irradiation (food systems, model systems etc.) and studies where general shelf life issues are addressed.

5.2.1 Specific Microorganisms

5.2.1.1 *Listeria* sp.

The majority of papers published about specific microorganisms involve *Listeria* sp., usually *L. monocytogenes*. The following authors have all published research regarding the effects of irradiation on *Listeria* sp. and the main findings of each paper are included:

Patterson (1989). The doses used for the destruction of Salmonella from poultry will also destroy *Listeria*.

Lewis and Corry (1991). The proportion of carcases contaminated with *L. monocytogenes* and the number of *Listeria* was lower in irradiated than unirradiated raw chicken.

Grant and Patterson (1995). Irradiation leads to heat sensitisation of *L. monocytogenes*, which lasted for up to 2 weeks.

Thayer and Boyd (1999). Irradiation in air was significantly more lethal to *L. monocytogenes* than irradiation under modified atmospheres or vacuum.

Thayer and Boyd (2000). The safety of ground turkey was not compromised by contamination with *L. monocytogenes* following irradiation.

Clardy *et al.* (2002). When sandwiches containing *L. monocytogenes* were irradiated at a dose of 3.9 kGy, there was a 5 log reduction and numbers decreased further on storage at 4°C.

Savvaidis *et al.* (2002). The level of L. monocytogenes on artificially inoculated trout was reduced by 2 log cycles after 2 kGy and subsequent growth at 4°C was suppressed for up to 18 days.

Niemira *et al.* (2002a). The D_{10} value for *L. monocytogenes* varies considerably between different types of vegetable. The D_{10} value also increased significantly with decreasing temperature.

Sommers *et al.* (2003). Addition of compounds such as sodium diacetate and potassium lactate to beef bologna had an observable effect on the D_{10} value and on subsequent growth of the organism during storage at 4°C. The D_{10} value is reduced and onset of growth is delayed by the presence of both compounds.

Niemira *et al.* (2003a). Doses of less than 1 kGy are effective at reducing the numbers of *L. monocytogenes* on endive by 4 log cycles and subsequent growth is prevented, without any adverse sensory effects.

Foong *et al.* (2004). The type of meat product can affect the radiation resistance of *L. monocytogenes* (by a factor of approximately 0.5 kGy). There was no growth of survivors after doses of 2 kGy and storage of ready to eat meats at 4°C for five weeks. Survivors were able to grow when the meats were stored at 10°C.

Chen *et al.* (2004). There is a synergism between pediocin and irradiation. Concentrations of pediocin of 6000 AU and doses of 2.3 kGy or more were able to inhibit pathogen growth for 12 weeks at 4 or 10°C on frankfurters.
Mendonca *et al.* (2004). Starvation of *L. monocytogenes* significantly increases radiation resistance.
Romero *et al.* (2005). Vitamin E does not increase the radiation resistance of *L. monocytogenes* in ground turkey meat.
Sommers and Boyd (2005). When irradiating multi-component foods (tortilla, cheese and luncheon meat wrap) the reduction in pathogen levels was limited by the higher radiation resistance of the organism on the meat component.
Bari *et al.* (2005). 1 kGy of radiation is sufficient to reduce the levels of *L. monocytogenes* on fresh vegetables by between 4.14 and 5.25 log cycles, without compromising the sensory properties, over a 7 day period.
Badr (2005). Doses of 1 and 2 kGy significantly reduced the levels of *L. monocytogenes* and the organism was effectively eliminated at 3 kGy in raw beef sausage.
Foley *et al.* (2005). Acid adaptation of *L. monocytogenes* in a seafood salad did not significantly increase its radiation resistance.
Zhu *et al.* (2005). Addition of 2% sodium lactate and 0.1% sodium diacetate during irradiation at 1 kGy were effective at suppressing growth of *L. monocytogenes* for 6 weeks at 4°C and ensuring safety of ready to eat meat.
Dhokane *et al.* (2006). A dose of 1 kGy irradiation of packaged, minimally processed carrot and cucumber gave a 3 log reduction of *L. monocytogenes* and 4 log reduction of *Salmonella* Typhimurium. Neither organism could be recovered after 8 days storage at 10°C.
Caillet *et al.* (2006a). *L. monocytogenes* was more sensitive to irradiation on ready to use carrots in the presence of antimicrobial compounds (*trans*-cinnamaldehyde was the most effective in air while winter savoury and Chinese cinnamon were effective under modified atmospheres only).
Caillet *et al.* (2006b). This paper reinforces the data presented above when *L. innocua* was used.
Robertson *et al.* (2006). 2 kGy X-ray irradiation reduced *L. monocytogenes* on smoked mullet to undetectable levels although 1.5 kGy also reduced the

levels by 1 log cycle at 3°C over 60 days and 1.7 log cycles at 10°C over 17 days.
Mintier and Foley (2006). Either gamma or electron beam irradiation at 0.51 or 1.15 kGy effectively reduced the levels of *L. monocytogenes* on romaine lettuce with no increase in number during the storage period.
Caillet and Lacroix (2006). A combination of irradiation with oregano essential oil, a synergistic effect was observed in relation to the levels of intracellular ATP. Murein composition was also significantly affected by the two treatments.
Bari *et al.* (2006). Irradiation of ground pork at 3 kGy completely inactivated *L. monocytogenes* when the pork was stored at 4, 0 or -18°C. The results of the sensory analysis were inconclusive.

Four of these papers highlight possible concerns with the effect of irradiation on *Listeria* sp. The issue of multi-component foods is considered in more detail in the discussion. The problem of increased radiation resistance following starvation of the cells has little application in food irradiation. It may be more significant in decontamination of the food processing environment but irradiation has not been proposed as a feasible technology for this as yet. It is possible that the organism may display increased resistance to other antimicrobial treatments following starvation but that issue is beyond the remit of this report. The issue of increased resistance at low temperatures for produce is more important as reduced temperature is often used to minimise irradiation-induced organoleptic changes. The temperature effect was not seen with meat products. There is, however, enough evidence to suggest that more research is required in this area. The same is true for the effect of modified atmospheres on resistance of the organism as MAP is a commonly used for chilled raw and ready to eat products, with which *Listeria* sp. are typically associated.

5.2.1.2. *E. coli*

Many of the papers cited here are concerned primarily with *E. coli* O157. There is no current evidence to suggest that non-EHEC strains are more or less resistant to the effects of ionising radiation.

Bánáti *et al.* (1993). Irradiation of *E. coli* on chicken breast meat showed increased resistance compared with irradiation on microbiological media. There was also some evidence of a tailing effect.

Thayer and Boyd (1993). *E. coli* O157 was significantly more resistant to gamma irradiation in lean minced beef compared with chicken. No verotoxin was detected from artificially contaminated ground lean beef following irradiation at 1.5 kGy and temperature abuse at 35°C for 20 hours.

Fielding *et al.* (1994). *E. coli* was more sensitive to electron beam irradiation at pH 4.13 and 4 compared with higher pH values. The growth of the irradiated bacteria at these pH values was also inhibited.

Fielding *et al.* (1997). Further work demonstrated that when the pH was modified with acetic acid, there was a much more pronounced reduction at pH 4.6 compared with hydrochloric acid. These studies were performed in nutrient broth, not in a real food system.

Buchanan *et al.* (1998). Radiation resistance of *E. coli* O157 in apple juice was increased when the organism was allowed to become acid adapted prior to irradiation. It was shown, however, that a dose of 1.8 kGy to apple juice should achieve a 5 log reduction of the organism.

López-González *et al.* (1999). Higher D_{10} values were obtained for *E. coli* O157:H7 when irradiated in beef patties at -15°C compared with 5°C. Packaging also had an effect, with a Saran/polyester/polyethylene bag giving the highest D_{10} value when electron beam irradiated at -15°C. In general, gamma irradiation resulted in higher D_{10} values, possibly due to the lower dose rate.

Rajkowski and Thayer (2000). D_{10} values for *E. coli* O157:H7 on sprouts were 0.34 (meat isolate) and 0.30 (vegetable isolate).

Niemira *et al.* (2002b). The type of lettuce significantly affects the radiation resistance of *E. coli* O157:H7.

Thayer *et al.* (2003). A dose of 2 kGy will effectively decontaminate alfalfa sprouts from *E. coli* O157:H7.

Bari *et al.* (2003). Dry heat and irradiation (2.5 kGy) was effective at reducing the levels of *E. coli* O157:H7 on alfalfa, radish and mung bean seeds.

Rajkowski *et al.* (2003). The radiation dose required to inactivate *E. coli* O157:H7 was higher than previously indicated (D_{10} = 1.1 kGy on broccoli sprouts).

Chiasson *et al.* (2004). Addition of carvacrol to minced beef increased the radiation sensitivity of *E. coli* but the addition of ascorbic acid reduced radiosensitivity. MAP and carvacrol increased the radiosensitivity of the organism by 2.7 times.

Foley *et al.* (2004). A combination of irradiation and chlorination reduced levels of *E. coli* on fresh cilantro (coriander) by more than 7 log cycles (compared with 1 log cycle for chlorination alone and 6.7 log cycles for 1.05 kGy irradiation).

Wang *et al.* (2004). The D_{10} values for *E. coli* O157:H7 in apple cider were between 0.25 and 0.34 while the normal flora ranged between 0.24 and 0.59. This demonstrates that the doses used for reduction of the normal flora (with the exception of yeasts), will also reduce the levels of the pathogen.

Bari *et al.* (2004). *E. coli* O157:H7 on radish and mung bean sprouts can be effectively reduced by irradiation at 1.5 or 2 kGy.

Badr (2005). Doses of 1 and 2 kGy significantly reduced the levels of *E. coli* O157 and the organism was effectively eliminated at 3 kGy in raw beef sausage.

Arthur *et al.* (2005). A 4 log reduction of *E. coli* O157:H7 on beef flanks was effected by 1 kGy electron beam irradiation.

Caillet *et al.* (2005). The combination of gamma irradiation and oregano essential oil had a significant effect on the murein composition of *E. coli* O157:H7 although some muropeptides were not affected. A significant reduction was observed when the two treatments were combined.

Chiasson *et al.* (2005). This study reported similar results to the 2004 paper.

Mayer-Miebach *et al.* (2005). Non-pathogenic *E. coli* demonstrated increased resistance when irradiated in ground turkey or when frozen, compared with irradiation in nutrient broth.

Edwards and Fung (2006). Irradiation has the potential for being used as an online treatment for the decontamination from *E. coli* O15:H7 on beef carcasses in commercial abattoirs. As the treatment would be surface only, this part of the carcass is generally removed so the resulting meat would not have to be labelled as irradiated (under US law).

There do not appear to be any major concerns regarding the use of irradiation for the decontamination of products contaminated with *E. coli*. The only issue appears to be the varying radiation resistances reported for different products, even within the same commodity, for example lettuce.

5.2.1.3 *Salmonella*

Mackey and Derrick (1982). *Salmonella typhimurium* was more able to recover from sublethal gamma irradiation than sublethal heat treatment.

Szczawińska *et al.* (1991). There is evidence that the growth of some strains of Salmonella post-irradiation is better than on unirradiated meat at 10 and 20°C. This highlights the requirement for adequate chilling and prevention pf post-process contamination.

Serrano *et al.* (1997). *Salmonella enteritidis* of animal origin is significantly more resistant than type cultures. Mild heating prior to irradiation did not reduce the D_{10} value but a dose of 1.5 kGy should reduce the incidence of the organism in shell and liquid eggs by 4 log cycles.

Chung *et al.* (2000). Doses of 1.5 and 3 kGy were effective at reducing the level of *Salmonella* Typhimurium on beef steaks by up to 5.3 log cycles. The organism was not recovered during the course of the experimental period (8 days).

Rajkowski and Thayer (2000). D_{10} values for *Salmonella* on sprouts were 0.54 (meat isolate) and 0.46 (vegetable isolate). *Salmonella* could not be recovered from sprouts naturally contaminated with *Salmonella* after 0.5 kGy irradiation.

Rajkowski *et al.* (2003). The radiation dose required to inactivate *Salmonella* was higher than previously indicated (D_{10} = 0.74 kGy on broccoli sprouts).

Jakabi *et al.* (2003). *Salmonella* Enteritidis and Infantis in oysters were reduced by 5-6 log cycles after 3 kGy gamma irradiation. The oysters were not killed.
Niemira *et al.* (2003b). When *Salmonella* was inoculated into concentrated orange juice, significant variability was seen in the radiation and freezing resistance. The frozen irradiated (2 kGy) samples showed a reduction of 3.3 log after 14 days, compared with 1.2 log for the unirradiated control.
Thayer *et al.* (2003). A dose of 2 kGy will effectively decontaminate alfalfa sprouts from *Salmonella*.
Sherry *et al.* (2004). Different serovars of *Salmonella* display very different radiation resistances. Five groupings were identified with D_{10} values ranging between 0.36 and 0.65.
Chiasson *et al.* (2004). Addition of carvacrol to minced beef increased the radiation sensitivity of *Salmonella* Typhi but the addition of ascorbic acid reduced radiosensitivity. MAP and carvacrol increased the radiosensitivity of the organism by 9.9 times.
Bari *et al.* (2004). *Salmonella* on radish and mung bean sprouts can be effectively reduced by irradiation at 1.5 or 2 kGy.
Chiasson *et al.* (2005). This study reported similar results to the 2004 paper.
Niemira and Solomon (2005). This study showed that some *Salmonella* serovars in biofilms can be more sensitive to the effects of irradiation than planktonic bacteria. Other serovars are not more sensitive.
Talbot *et al.* (2006). These authors identified irradiation as a potential treatment in the slaughterhouse and during production for the reduction of multi-drug resistant *Salmonella* from ground beef.

These studies also show that different serovars of the same genus can display very different behaviour under the same conditions. It is imperative that the exact serovars is known or a worst case scenario must be predicted.

5.2.1.4 Other organisms

Kamat *et al.* (1997). A dose of 1 kGy (gamma irradiation) at -40°C was effective at reducing the levels of *Yersinia enterocolitica* in raw pork meat without any subsequent growth during chill storage.

Sommers and Novak (2002). Different *Y. enterocolitica* serotypes display different radiation resistances, but this is not linked to the presence of the virulence plasmid that encodes for host immune suppression factors.
Lambert and Maxcy (1984). Irradiation of *Campylobacter jejuni* at -30°C led to increased resistance compared to higher temperatures. The physiological status of the organism did not affect resistance.
Chung *et al.* (2000). The levels of *Pseudomonas fluorescens* in beef steaks were effectively reduced by irradiation at 1.5 and 3 kGy. Survivors were, however, able to grow 2 days after being irradiated at either dose using electron beam irradiation. No growth was seen after gamma irradiation.
Jakabi *et al.* (2003). *Vibrio parahaemolyticus* in oysters were reduced by 6 log cycles after 3 kGy gamma irradiation. The oysters were not killed.
Grant *et al.* (1993). Irradiation at 3-4 kGy reduced the numbers of both *Staphylococcus aureus* and *Bacillus cereus* in roast beef and gravy. Growth and subsequent toxin formation following irradiation were delayed. Toxins were produced by both organisms at 22°C but not at 15°C.
Lamb *et al.* (2002). When *S. aureus* was inoculated onto ready to eat sandwiches and irradiated at 5.9 kGy, there was no growth over a period of 13 days. Irradiation at 3.85 kGy did allow growth but this was significantly lower than the unirradiated control.
Valero *et al.* (2006). Irradiation causes heat sensitisation of spores of *B. cereus*. The authors propose a model for the prediction of this effect.
Kiss *et al.* (1978). Comparisons of the salt and radiation sensitivity of *Clostridium botulinum* types A, B and E indicate that those strains that are salt tolerant tend to be radiation sensitive and *vice versa*. Sensitisation to radiation by salt was apparent in the decline phase of the death curve but not in the shoulder phase.
Lim *et al.* (2003). Irradiation of *Cl. botulinum* type E spores in the presence of 0.5% sodium chloride leads to increased sensitivity. This is not the case for sodium thioglycollate. Also, pre-freezing of spores at -75°C for 30 days increased sensitivity.
Setlow (2006). A systematic study of the relationship between core water content and radiation resistance for *Bacillus subtilis* is required. It is thought

that the small, acid soluble proteins are not involved in the resistance mechanism. The role of dipicolinic acid is not clear.
Husman *et al.* (2004). Ionising (gamma) irradiation can readily inactivate bacteriophage MS2 in water and low protein calicivirus stocks. This effect was not observed at higher protein concentrations. Less inactivation of the caliciviruses was observed. The doses used to kill bacteria may not be sufficient to inactivate foodborne viruses.

As expected, bacterial endospores are resistant to irradiation. There is, however, very little information regarding the effect of food irradiation on viruses or protozoa.

It is also known that there are a number of radiation resistant bacteria, such as *Deinococcus radiodurans* (Minton 1994). Collins *et al.* (2000) isolated a radiation resistant bacterium from irradiated pork and it has been named as *Hymenobacter actinosclerus* sp. nov. It is a Gram negative bacillus, aerobic and oxidase and catalase-positive. Its relevance to food safety is not known.

5.2.2 Specific Food Commodities

A significant number of studies have been carried out in food commodities, examining the natural flora of the food rather than artificially contaminating it with pathogens or spoilage organisms. It has generally been found that irradiation is suitable for extending the shelf life of meat products such as chicken, pork, beef, rabbit and comminuted products. The decontamination of seafood such as prawns (shrimp) and kwamegi (a type of fish) has been found to be successful. Irradiation is not suitable for viral decontamination of clams as the shellfish dies at doses that are sublethal to the viruses (Harewood *et al.* 1994).

Fresh produce has become a more common vehicle of foodborne illness in recent years and much work has been performed on the effectiveness of irradiation on a range of produce. It has been found that irradiation can be suitable and will significantly reduce levels of bacteria but that there is a great deal of variation between different food products and even different varieties

of the same commodity, for example lettuce. Of particular interest to the USA is the success of irradiation in decontamination of ‘sprouts’, a food eaten extensively for perceived health benefits.

There are however, issues with changes to the sensory properties of products following irradiation. Although this is not a safety issue, it does limit the dose of radiation to which any food commodity can be subjected. There are also significant differences in radiation resistance under different gaseous atmospheres. Irradiation of poultry meat under vacuum or carbon dioxide was more lethal than in air (Patterson 1988). Combination treatments, or hurdle technology, have been identified as providing a solution to these organoleptic changes but thorough investigation of each combination must be made to assure product safety.

5.2 Toxicological Safety

One of the main historical issues identified regarding the toxicological safety of irradiated foods lay in the inability to detect a unique radiolytic product (URP). This was seen as a problem as it did not allow easy detection of irradiated foods. This problem has now been largely overcome and this review will only briefly discuss detection methods as, in themselves they do not present a risk to food safety. The absence of a URP was seen by proponents of food irradiation as a positive feature as, if we could not detect anything produced by irradiation that was not produced by any other, accepted process, then food irradiation could be said not to present any unique hazards to the consumer. Analysis of the effects of radiolytic products, whether unique or not, has also been a subject of debate.

5.2.1 Feeding Trials

It was recognised in the 1970s that traditional animal feeding trials presented problems when attempting to address the issue, due to the problems with administering the radiolytic component in a pure form as irradiation can produce a multitude of products and to determine the safe limit for human consumption based on the no-effect-level (NEL) of each component (usually 1% of the NEL). At this time, the concept of ‘chemiclearance’ for determining

the toxicological safety of irradiated foods was adopted. This refers to the prediction of types and amounts of URPs that will be formed in foods at a given dose under specified conditions. It relates the average radiation dose absorbed by a food and the amount of a person's diet that will be irradiated (Shea 2000). Also at this time, a large number of trials were performed to assess the mutagenic potential of irradiated foods and the outcome of these was negative. These were some of the arguments used by the Joint Expert Committee on Food Irradiation when it stated that irradiation of any food up to an overall average dose of 10 kGy did not result in any toxicological hazard (Elias 1989).

A report compiled as part of a class project (Masters in Public Health Program at the University of Texas Medical Branch, Galveston, Texas) identified a number of concerns regarding the impact of irradiated food on health (Ashley et al. 2004). Among these was the criticism of the design and execution of a number of *in vitro* studies into toxicological safety. These studies used food juices, extracts and digests in mutagenic studies using cells of mammalian (including human), bacterial and vegetable origin and largely produced negative effects. Some possible chromosome changes and cytotoxic effects were reported but, as food contains many compounds that may interfere with the tests, the results were not deemed significant. The same authors were also concerned that, when the WHO published its report into the wholesomeness of foods irradiated at doses of above 10 kGy (FAO/IAEA/WHO 1999) five peer reviewed publications, all of which were feeding trials (four on rodents and one on monkeys) were disregarded. All these trials report toxicological effects. The FAO/IAEA/WHO report assessed the validity of the large number of feeding trials that have been performed on irradiated food. A wide range of foods were used in these studies, many of which were performed by the US Army and both long and short term studies were carried out. In 1986 the US FDA reviewed over 400 studies and stated that only 5 of the studies reviewed were:
"considered to have been properly conducted, fully adequate by 1980 standards, and capable of standing alone to support the safety of irradiated foods" (FAO/IAEA/WHO 1999).

The study group responsible for the FAO/IAEA/WHO report (1999), however, included some of the trials rejected by the FDA in its own evaluation in order to gain a wider perspective. The studies were subdivided as follows and the conclusions drawn by the study group are outlined:

- *Subchronic studies* (safety and nutritional adequacy of a variety of dietary item and complete diets, performed on rats, mice, dogs, pigs, quails and chickens). Very few adverse effects were found and are linked with nutritional adequacy and not unique to irradiation
- *Carcinogenicity and chronic toxicity studies* (two year carcinogenicity bioassays and multi-generation reproductive toxicology evaluations on rats, mice, pigs, dogs and monkeys). No irradiation induced increase in tumours or changes in reproductive function in rats or mice were noted. One study noted an unusual heart lesion in mice but this was not reproducible in other studies. There was no evidence of any pathological abnormalities in dogs, although the duration of the study was too short to determine carcinogenicity. Likewise, there was no evidence of adverse findings in male monkeys (there were problems in rejection of the irradiated diet by female monkeys on grounds of palatability). Additionally, there was no evidence of induction of testicular tumours in mice and dogs as a result of consuming irradiated as opposed to thermally treated chicken.
- *Reproduction and teratology studies* (rats, mice, pigs, dogs and hamsters). There was no evidence of any differences in growth, feed consumption, reproduction, haematology, urinary and organ histopathology between an irradiated or autoclaved diet in rats. Nor were there any differences between feed consumption, growth, haematological and biochemical parameters in pigs over three generations. When these pigs were sacrificed and used to produce ham products that were fed to rats, some of which was irradiated, there were no observed treatment effects in feed consumption, growth, mortality, haematology, biochemistry of blood or urine, organ weights, histopathology or tumour incidence. In a long term toxicity study of rats,

there was reported decreased weight gain among females of the F_3 generation but the cohort of animals used was small so caution must be exercised when interpreting these results. Overall, it was concluded that there were no observable trends between irradiated and control animals in terms of reproductive and teratological end points.

- *Mutagenicity studies* (tests for changes in the chromosomes). Although both in vitro and in vivo studies have been performed, the data from the latter are deemed more important. It was concluded that 2-dodecylcyclobutanone (see below) displayed some cytotoxic effects in an *in vitro* study and an indication of a weakly positive effect in rats, although the study group questioned this finding. Other studies in *Drosophila* (fruit fly) and Chinese hamsters found no adverse effects.
- *Human clinical studies*. There have been relatively few trials performed on humans, the majority being carried out by the US Army. The subjects were assessed by clinical examination and for cardiac performance, haematological, hepatic and renal function. All studies have been short term (up to two periods of 15 days separated by control and washout intervals). No clinical abnormalities were discovered up to one year following the trials. One note of interest, however, is that it was not possible to conduct a double-blind trial with a placebo as a control as the volunteers were easily able to distinguish between the irradiated and control foods. The studies cannot give any evidence of long term nutritional deficiencies or carcinogenic effects due to the short duration.

Perhaps the best known human feeding trial is that performed by Bhaskaram and Sadasivan (1975) where fifteen malnourished children (suffering from kwashiorkor) were fed a diet containing irradiated wheat. The authors concluded that there was an increase in polyploidy and abnormal cells during the course of the trial. When the irradiated diet was discontinued, the abnormal cells reverted to a basal level. There is no mention of any statistical analysis (the sample size was too small to allow any robust statistics to be performed, there being three groups of only five children in each group)

although statistical significance is quoted. It would not be possible to replicate this trial in the 21st Century due to ethical considerations. Another trial involving healthy adults did not, initially, indicate any increase in chromosomal aberrations but upon reanalysis of the data by Louria (2001), increases were demonstrated.

In addition to the reports quoted in the FAO/IAEA/WHO publication, DeRouchey *et al.* (2003) determined that, when nursery pigs were fed a diet solely of irradiated food, the growth was lower than that of pigs fed a diet where selected ingredients were irradiated (n=40 or 48 per treatment as the trial was performed twice, 880 animals in total). They also analysed the effect of feeding weaning pigs a diet of animal plasma (irradiated or unirradiated) or no animal plasma (n=30 per treatment, 330 animals in total). It was found that those pigs fed on irradiated animal plasma were statistically significantly heavier than either other group. Data were analysed as randomised complete block designs and statistical differences were reported when $P \leq 0.05$, with statistical tendencies reported at $P \leq 0.1$. The overall conclusion of this study was that, while the irradiation process will reduce the bacterial load of diets fed to nursery pigs, the mechanism relating to the differences in growth rates depending on whether the whole diet or only components of it are irradiated is unclear. The authors suggest that further research is needed.

Ashley *et al.* (2004) have questioned the exclusion of a number of the feeding trials from the evaluation, suggesting that bias in the favour of irradiation has been introduced. They also question the validity of the trials that were included, stating that there is insufficient evidence presented regarding the levels of radiolytic products ingested by the animals and humans. They conclude that there is no widespread acceptance of the results of these feeding trials.

5.2.2 Unique Radiolytic Products (URPs)

As outlined above, for many years, there was no evidence of the existence of URPs. In recent years, however, it has been determined that irradiation of foods containing high levels of fat (triglycerides) can give rise to elevated

levels of compounds called alkyl-cyclobutanones, a phenomenon unique to irradiated foods. The specific components produced include:

- 2-dodecylcyclobutanone (2-DCB) from palmitic acid
- 2-tetradecylcyclobutanone (2-TCB) from stearic acid
- 2-tetradecenylcyclobutanone (2-TDCB) from oleic acid (Delincée *et al.* 2002).

These compounds have the ability to enter the bloodstream by permeating the intestinal barrier, and accumulating in adipose tissue.

Lee *et al.* (2000) determined that irradiation induced the formation of hydrocarbons and 2-alkylcyclobutanones in irradiated perilla seeds (n=3). Analysis by GC-MS showed that the effect was dose dependent and that there was a variety of hydrocarbons formed (predominantly 8-heptadecene and 1,7-hexadecadiene from oleic acid and 6,9-heptadecadiene and 1,7,10-hexadecatriene from linoleic acid). 2-(5'-tetradecenyl) cyclobutanone, a 2-alkylcyclobutanone, was found at the highest concentration. None of these compounds was found in unirradiated perilla seeds. Statistical analysis of the results was not reported. Kim *et al.* (2004) found an increased level of gamma radiation-induced hydrocarbons (pentadecane and 1-tetradecene) and 2-alkylcyclobutanones in dried squid (*Todarodes pacificus*) irradiated at doses in excess of 0.5 kGy (2.5 kGy h^{-1}). As before, these compounds were not found in unirradiated squid. Details regarding the number of replicates and the statistical analysis of results were not provided.

Horatovich *et al.* (2005) assessed the effect of irradiation on a number of different food commodities (ewe's cheese100 kGy), liquid egg (0.5, 1, 3 5 kGy), avocado (0.1, 0.5, 1 kGy) and poultry meat (0.5, 1, 3, 10 kGy)). This study determined the effect of post-irradiation storage and found that, over a period of time (up to 28 days), the levels of the 2-alkylcyclobutanones decreased. All of these experiments were performed in triplicate but the authors make no mention of statistical analysis of the results but the protocol used conformed to EN 1785 (Foodstuffs – Detection of irradiated foods

containing fat – Gas chromatographic / Mass spectrometric analysis of 2-Alkylcyclobutanones).

Studies on the genotoxic potential of these compounds have been undertaken by Delincée and Pool-Zobel (1998), Raul *et al.* (2002), Delincée *et al.* (2002) and Knoll *et al.* (2006). Delincée and Pool-Zobel (1998) studied the genotoxic (50 cells scored on each slide) effects of 2-DCB *in vitro* using rat and human colon cells. The study determined that 2-DCB at levels of 0.3 – 1.25 mg/ml induced DNA strand breaks as well as a cytotoxic (n=3) effect that was concentration related. The later study by Delincée *et al.* (2002), however, found no indication of any cytotoxic (n=3) or genotoxic (50 cells scored on each slide) effects on human colon tumour cell lines caused by 2-TCB at the highest concentration tested (400 µm) after 30 minutes. Only after 1-2 days was any cytotoxic effect observed. Neither study by Delincée and co-workers mentions any details of statistical analysis other than an indication of the mean and standard error of the mean.

Further *in vivo* research by Raul *et al.* (2002) fed male Wistar rats, daily, either a solution of highly pure 2-tetradecyl-cyclobutanone (2-tDCB) or 2-(tetradec-5'-enyl)-cyclobutanone (2-tDeCB) (0.005% in 1% ethanol), and injected them with a known carcinogen (azoxymethane (AOM)) at weeks 3 and 4. Control mice were fed on 1% ethanol only. After 3 months, there were no significant differences in the total number of preneoplastic lesions in the colon of AOM controls and 2-ACB-treated animals. After 6 months, however, the total number of tumours in the colon was threefold higher in the 2-ACB-treated animals than in the AOM controls. Medium and larger tumours were detected only in 2-ACB-treated animals. This demonstrates that a compound found exclusively in irradiated dietary fats may promote colon carcinogenesis in animals treated with a chemical carcinogen. It does also suggest that the 2ACBs alone do not initiate colon carcinogenesis. It must, however, be stated that the amount of 2-ACB consumed was much higher than that which a human would consume in a diet containing irradiated food. This work was supported by that of Knoll *et al.* (2006) who investigated the effects of 2-DCB on a cell line (LT97) of human colon adenoma cells, primary human epithelial

cells and on HT29clone19A cells (differentiated human colon tumour cell line). This latter study found that the 2-DCB was cytotoxic to the adenoma and epithelial cells, an effect that was dependent on both time and dose, but not on the colon tumour cell line. There was also DNA damage in the adenoma cells but not the differentiated human colon tumour cell line. The authors concluded:
"These findings provide additional evidence that this compound may be regarded as a possible risk factor for processes in colon carcinogenesis."

The number of animals used in the Raul *et al.* (2002) study was 12 in each of two test (2-tDCB and 2-tDeCB) and one control group (36 animals in total) and this allowed statistics to be performed and to assess significant differences (one way ANOVA and student's t-test). Knoll's study used between 3 and 6 replicates and quotes statistical significance at both $P \leq 0.05$ and $P \leq 0.01$ for both one way ANOVA (with Dunnett's multiple comparison test) and two way ANOVA (with Bonferroni's multiple comparison test) (Knoll *et al.* 2006).

Kim *et al.* (2001) also studied the effect of consumption of gamma-irradiated fats on plasma lipid concentrations and hepatic cholesterol metabolism in Sprague-Dawley rats (a total of 61 male rats were used in the study, n=8). The authors determined significant differences by one way ANOVA and a Duncan multiple-range test was performed if differences were identified at $\alpha=0.05$ This study discovered no significant changes in plasma and liver lipid metabolism in test (fed on AIN-76 semi synthetic diet irradiated at 5 kGy) compared with control rats (fed the same diet, but unirradiated). These authors also discuss the lack of evidence linking radiolytic products of fat with irradiation doses of less than 10 kGy.

Karam and Simic (1990) evaluate the effect of irradiation on the formation of ortho-tyrosine by radiation and organic solvents in chicken tissue. Yields of tyrosine were calculated but no statistics were presented. The authors concluded that o-tyrosine is produced in chicken during irradiation and that the formation follows a linear response.

5.2.3 Mycotoxins

While it is now recognised that the irradiation process can induce the formation of potentially harmful URPs, there is also evidence linking the prevalence of mycotoxins in foods that have been irradiated. Blank *et al.* (1992) irradiated cheddar cheese, inoculated with spore suspensions of *Aspergillus ochraceus* (inoculum levels of $6x10^1$ or $6x10^2$) or *Penicillium cyclopium* (inoculum levels of $5x10^1$ or $5x10^2$), with electron beam irradiation (10 MeV, 0, 0.21, 0.52 and 1.15 kGy) to determine the minimum dose required for spore inactivation and the extension of shelf life achieved. The cheese was subsequently stored at either 10 or 15°C. They found that, at the lower inoculum levels, no mould growth was observed after 1.15 kGy, when the cheese was stored at either temperature. Mould growth was delayed significantly by irradiation at 0.21 and 0.52 kGy compared with the unirradiated control. When the higher inoculum level was used (0 and 1.15 kGy only), growth of *P. cyclopium* only was observed. Inoculum level also affected the minimum dose required for spore inactivation and survival curves (n=3). The authors concluded that irradiation can enhance the shelf life of vacuum packaged cheddar cheese but that storage temperature is critical if complete destruction of all mould spores is not achieved. While the authors quote significant differences ($P \leq 0.01$), no mention is made of the statistical tests applied.

In 1991 Aziz *et al.* studied the effect of gamma radiation (0, 1, 2 and 3 kGy, 0.8 kGy h^{-1}) on tomato paste and juice at a variety of different water activities (0.98, 0.95, 0.90 and 0.88, altered using sodium chloride) inoculated with *Alternaria alternata* and incubated at 15 or 25°C. It was found that, as the water activity decreased, the growth of the organism in tomato juice reduced with increasing dose. There was no growth at water activities of 0.95 or 0.90 after 3 kGy when incubated at either temperature. The production of the mycotoxin tenuazonic acid (TZA) also followed the same pattern with none being detected after 3 kGy in all conditions of temperature and water activity (juice, n=2) while tomato paste showed positive results except at a water

activity of 0.90 at 2 and 3 kGy (15°C). The study shows that combination of irradiation dose, water activity and temperature of incubation must be considered when inhibiting the production of this mycotoxin. As before, no mention is made of the statistical tests applied.

Aziz *et al.* (1997) determined the effect of gamma irradiation (0, 2, 4, 6 and 8 kGy, 98 Gy min^{-1}) on *Fusarium* mycotoxins (zearalenone, deoxynivalenol and T-2 toxin) in wheat (n=40), flour (n=40) and bread (n=20). The data were analysed using the multiple range test and significance was reported at $P \leq 0.05$. It was shown that irradiation reduced the levels of *Fusarium* sp. in wheat and flour ($P \leq 0.01$) immediately after 4 kGy irradiation and that, at this dose, the amount of mycotoxin produced was also significantly reduced. Growth was completely inhibited in both commodities after a dose of 6 kGy while toxin was not detected only after 8 kGy. Gamma irradiation greatly reduced the natural occurrence of the mycotoxins in bread at 8 kGy although further work is required on the chemistry and technological changes that may occur as a result of irradiation. Kottapalli *et al.* (2003) evaluated hot water (45, 50, 55 and 60°C for 0, 5, 10 and 15 minutes) and electron beam irradiation (2.3 to 11.3 kGy, triplicate experiments) to reduce *Fusarium* infection (FI) and germinative energy (GE) in malting barley. Data were analysed using analysis of variance and significance was reported where $P \leq 0.05$. Doses in excess of 4 kGy reduced FI with a slight increase in germination observed at 6 and 8 kGy. Doses in excess of 10 kGy led to a significant decrease in germination. The authors concluded that both hot water and irradiation have potential to reduce FI with little effect on GE.

Further work by Aziz *et al.* (2002) investigated the effects of gamma irradiation and maize lipids on the production of aflatoxin B_1 from maize artificially inoculated with *Aspergillus flavus* (n=6). Data were analysed as above. Doses of 0, 0.5, 1, 1.5, 2, 2.5 and 3 kGy were used (200 Gy min^{-1}at room temperature). The results show that survival of *A. flavus* was significantly reduced at doses of 1 kGy and above and that no growth was observed after 45 days at 3 kGy. This was observed for both full fat and

defatted maize. Similarly, the levels of aflatoxin produced were lower in irradiated maize than unirradiated, with the defatted maize displaying significantly lower levels than unirradiated or irradiated full fat maize. It is also apparent that there is an increase in fungal lipase activity at low doses of irradiation (1 and 2 kGy), although more research is needed to determine how the free fatty acids may affect aflatoxin production. Aquino *et al.* (2005) also considered *A. flavus* contamination of maize. Maize grains (preirradiated at 20 kGy and inoculated *A. flavus* spores) were gamma irradiated at doses of 2, 5 and 10 kGy (n=5, 4.74 kGy h^{-1}) to determine the level of *A. flavus* present after 15 days and the levels of aflatoxin B^1 and B^2. Data were analysed using the Student and the Tukey test and significance reported at P≤0.05 and 0.01. It was found that all irradiation doses significantly reduced the number of *A. flavus* and the level of aflatoxin present. No aflatoxin was detected after 10 kGy. Significantly lower levels of B_1 were observed after 2 kGy compared with 5 kGy (possible water activity effects) and there was no difference between 2 and 5 kGy for B_2.

Refai *et al.* (2003) determined that gamma irradiation of spice paste used to make basterma (an Egyptian cured meat product), pepper, garlic, fenugreek, coriander and capsicum at 5 kGy (64 Gy min^{-1} at ambient temperature) rendered them free from moulds and aflatoxins (samples taken 2 weeks after irradiation). The samples were, however, contaminated with moulds when no irradiation or doses of 1 or 3 kGy were used. The paper makes no mention of statistical analysis although a sample size of n=10 is quoted for mould counts before processing (± SE) and n=40 for counts after processing. The authors also noted that the quality of the product throughout the whole production process must be considered, not just at the post-irradiation stage.

All the trials show evidence that irradiation can reduce the levels of mycotoxin present in foods.

5.2.4 Allergens

Food irradiation has also been shown to reduce allergenic properties of some foods. Byun *et al.* (2000) investigated the effect of gamma irradiation at a

dose of 0, 1, 3, 5, 7 or 10 kGy (10 kGy h^{-1}) on the antigenicity of shrimp allergen (a heat stable protein, HSP) against both mouse and human immunoglobulin E, from people with immediate hypersensitivity to cooked shrimp (n=15, data analysed by least-squares means and the Duncan multiple range test). They discovered that the amount of HSP present reduced with increasing irradiation dose and the ability of human IgE to bind to irradiated HSP was also reduced. This is probably due to conformational changes in the allergenic protein meaning that the modified protein was not recognised as antigenic to humans. The authors concluded that gamma irradiation, at levels currently permitted, can reduce the antigenicity of shrimp allergens. Similar results were obtained by Lee *et al.* (2001) when considering milk proteins, another of the twelve major sensible allergens (n=15, data analysed as by Byun *et al.* 2000). The milk proteins used were bovine α-casein (ACA) and β-lactoglobulin (BLG) and were tested against the sera of human patients diagnosed as having IgE-mediated bovine milk allergy (n=20). Rabbit polyclonal antibodies produced against the two milk proteins were also tested. The samples were gamma irradiated at doses of 0, 3, 5 and 10 kGy (10 kGy h^{-1}). The results show that, as before, the binding capacity of the IgE to the irradiated proteins was reduced, the reduction being dose dependent. The results also demonstrate that the solubility of the proteins was reduced following irradiation, due to structural modification. Overall, the study supports the theory of reduced allergenicity of milk allergens by gamma irradiation.

These results are not surprising as it has been known for a number of years that ionising radiation can affect the structure of proteins. Yamamoto (1992) reviewed these effects on amino acids and enzymes in both liquid and solid state. It was concluded that the effects of irradiation were more pronounced in solid than in aqueous phase. The mechanisms were also elucidated (peptide bond breakage, aggregation and effects of the radiolytic products of water) so it may be possible to predict the effects that may occur when different allergens are irradiated. Poms and Anklam (2004) reviewed the effects of irradiation on food allergens and concluded that:
“Proteins that have been exposed to irradiation present distinct structural modification caused by aggregation, fragmentation, and amino acid

modification which affect the solubility of proteins, their tertiary and secondary structure, and their immunoreactivity." (Poms and Anklam 2004, p. 1470).
In addition to milk and shrimp, these effects were also observed in hen's eggs (Lee *et al.* 2005a, b), but not in celery (Jankiewicz *et al.* 1997). In wheat, the allergenicity of the protein gliadin was increased by irradiation (Leszczynska *et al.* 2003).

5.2.5 Effects on other food compounds

Modi *et al.* (1990) determined that gamma irradiation at 8 kGy (9.4 or 12.2 kGy h^{-1}) completely inactivated staphylococcal enterotoxin A in gelatine phosphate buffer but in minced meat (a 15% slurry, inoculated with purified enterotoxin) up to 37% of the toxin remained (n=3). In fact, up to 26% of the toxin could still be detected after 23.7 kGy. This demonstrates that the presence of food components exerts a protective effect although it is interesting to note that these authors discovered that the protective effect of mince was lower at a concentration of 50% than 30%. The data were presented as the percentage toxin remaining of the initial level and no statistical analysis is presented. This study, however, reinforces the fact that irradiation cannot be used on raw materials of poor quality as, although the microorganisms themselves may be destroyed or inactivated, their metabolites, including some toxins may remain active.

A number of studies have been performed on the effect of gamma irradiation on furan and one on acrylamide levels in foods. Both these compounds are considered as possible human carcinogens. Fan (2005a) investigated the formation of furan from carbohydrates and ascorbic acid following irradiation. Solutions of glucose, fructose and sucrose (50 mg mL^{-1}) and malic, citric and ascorbic acid (5 mg mL^{-1}) were irradiated at doses of 0, 2.5 or 5 kGy (0.091 kGy min^{-1}) at 5°C (n=4). The effect of pH (3, 4, 5, 6, 7 and 8) was also investigated (n=4). Data were analysed by least significant difference test using the general linear model. Significant differences were quoted when $P \leq 0.05$). They determined that there was negligible furan formation in malic or citric acids at any pH value. Ascorbic acid, however, gave rise to the highest

levels (20 ng mL^{-1} at 5 kGy). Lower levels were produced in fructose and sucrose with very little found after irradiation of glucose. The prevalent pH during irradiation had significant effects with the amount of furan produced decreasing with increasing pH. In all cases, more furan was produced at pH 3 than at any other pH, an effect most prominent in ascorbic acid. The study concludes that irradiation does induce the formation of furan (although heat treatment has the same effect, producing similar amounts). It was also evident that both pH and substrate concentration affected the levels of furan produced. The same author also investigated the production of furan in gamma irradiated (0.091 kGy min^{-1}) and heat treated fruit juice (apple and orange) (Fan 2005b). The juices were irradiated at doses of 0, 1, 2, 3, 4 or 5 kGy to determine the amount of furan produced by irradiation or spiked d_4-furan (deuterated furan) and irradiated at doses of 0, 1, 2, 3 or 4 kGy to determine degradation of this compound (n-4). The data were analysed as described in Fan (2005a). As before, it was shown that the level of furan increased linearly with increasing irradiation dose. This increase continued for 3 days following irradiation. Conversely, the level of d_4-furan decreased on irradiation. Again, thermal processing of both juices also gave similar results (orange juice submerged in boiling water for 5 minutes gave comparable levels to irradiation at 3.5 kGy).

Fan and Mastovska (2006) also considered whether ionising radiation could reduce levels of furan and acrylamide in foods (water, sausages, frankfurters, canned infant sweet potatoes, canola oil and potato chips)(n=2). Data were analysed as previously (Fan 2005a and b). It was found that the food matrix had a considerable impact on the amounts of furan present, with the compound being very sensitive to irradiation in water and meat products but with levels increasing in carbohydrate and ascorbic acid rich foods (as would be expected, considering Fan's earlier work). Acrylamide was also sensitive when irradiated in water but there was no significant reduction in the amounts in potato chips, even at doses of 10 kGy. The authors concluded that in foods containing high levels of furan or acrylamide, irradiation at doses of 10 kGy will only partially reduce the levels. It was also identified that the water content of the food is a major contributing factor.

Jipa *et al.* (2005) studied the effect of gamma irradiation at 0, 9.6, 26 and 84 kGy (0.4 kGy h-*1* at room temperature) on the chemiluminescence (CL) of crude gluten from wheat. The results demonstrate an increase in peroxy radicals and hydroperoxides and demonstrated the suitability of CL to study oxidation effects in grains exposed to such treatments. This paper, however, provided scant experimental details or rationale for the work. No indication of the sample size, in terms of number of replicates, or details of statistical analysis were presented.

5.3 Detection of Irradiated Food

Methods of detection can be based upon a number of different parameters. There have been considerable advances in the detection of irradiated food in recent years. This review will concentrate on those that are aimed at detection of URPs that are of public health concern. Other methods will, however, be discussed but it must be remembered that the actual identification of irradiated foods does not, in itself have any bearing on food safety. The detection methods are included in this report as it is a legal requirement that foods containing an irradiated ingredient are labelled as such (Anon. 1999). As Delincée (1993) states:

“Although, in principle, the administrative control of facilities licensed for food irradiation, and compulsory labelling of treated foods as proposed by Codex Alimentarius (ref.), supported by an international inventory of facilities (ref.), should provide a reliable control of irradiated food, it seems desirable to have an additional means of detection …….. by analysing the food itself.” (p. 352).

There are a range of detection methods, each based upon analysis of different parameters. Some of these methods are covered in European Standard protocols (Delincée 2002a). Each type of method will be discussed in terms of the principle of analysis with reference to the validity and sensitivity of each method in real foodstuffs, where available. The detection of 2-ACBs is covered separately under 5.3.7.

5.3.1 Photostimulated luminescence (PSL) and thermoluminescence (TL)

These techniques were developed to identify components of irradiated food based upon changes in the luminescence of non-degradable components under different conditions. It is routinely used for the detection of shell-on prawns and other seafood. Fu *et al.* (2005) used luminescence to detect irradiated milk powder (3% moisture). The powder was gamma irradiated at doses of up to 9 kGy (1.6 kGy h^{-1}) and the resulting luminescence was detected by an ultra-weak luminescence analyser. This type of luminescence is produced as light emission from biological systems following chemiexcitation. The study identified a peak that was not present in unirradiated product. There are limitations to the process and sensitivity and reproducibility need to be improved. There is potential, however, that the peak could act as a marker for irradiated foods. Engin (2007) has evaluated TL to assay the dust collected from irradiated black pepper (n=5). The study concluded that TL is the best method for determining irradiated black peppers. The technique does, however, require specialist equipment and for the majority of users, samples are sent to an external laboratory for analysis (for example, in the UK, a number of food manufacturers send samples to the Scottish Universities Environmental Research Centre (SUERC)).

5.3.2 DEFT/APC Method

This methods assesses the microbiological quality of a food (the APC, or aerobic plate count), in relation to the quality of the food before processing, by the DEFT (direct epifluorescent filter technique) method, by which the number of viable and non-viable, or non-culturable cells present in the food sample can be assessed. If the APC is found to be significantly lower than the DEFT count, then this is taken as an indication that the food may have been irradiated. There are a number of potential flaws in this technique however. It has been shown that food preservation treatments other than irradiation can give a positive result using this test, leading to false positives. As the method is based upon the number of microorganisms that have the ability to form a colony on an agar plate, it is logical to assume that storage of the food following any preservative treatment could, potentially allow these microorganisms to multiply and lead to false negative results. The method

was further refined by Campden and Chorleywood Food Research Association (CCFRA 1995). Their report concluded that the DEFT/APC method could identify irradiated (5 kGy and above) meat, poultry, fish and seafood that was subsequently stored either chilled (detection possible for up to 15 days for meat, poultry and fish and 6 days for seafood) or frozen (detection possible for up to 8 weeks). Herbs and spices could also generally be identified as irradiated using this method. The high pressure treatment of minced beef and heat treatment of parsley can lead to false positive results. Other foods tested were not affected. The authors concluded that, while the technique shows potential, more work is required to overcome potential problems with false positives and negatives.

5.3.3 Electron Spin Resonance Spectroscopy

This is the technique that the European Reference protocol (EN 1786) is based upon but it was not developed with the detection of foods containing on small fragments of bone in mind. Marchioni *et al.* (2005a, 2005b) modified the method so that detection of food containing low levels of bone-containing ingredients was possible. The modification consists of a purification stage that uses enzymatic hydrolysis followed by ESR. This resulted in a method with excellent sensitivity that was also capable of detecting two different types of irradiated ingredients (bone containing MRM and spices, the latter being detected by TL). The authors conclude that the technique developed could enhance the current EN 1786 protocol and complete the official protocols for detecting irradiated food.

Miyahara *et al.* (2004) investigated the application of ESR to foods containing sugars and concluded that it could be applied to dried fruits irradiated at 1 kGy, a dose typically used for insect disinfestations. Delincée and Soika (2002) reported an improvement in ESR detection using a refined method to detect irradiated food that contained cellulose (de Jesus *et al.* 1999). It was found that the sensitivity of ESR could be improved for the detection of irradiated (electron beam) strawberries and papayas (0.5 kGy after 2-3 weeks storage). The same was not true of spices.

5.3.4 Immuoassay

Tyreman *et al.* (2004) have developed a technique based upon immunoassay, a technology characterised by high specificity and sensitivity. The theory behind the test, which was developed for prawns, is that a DNA base becomes modified during irradiation (dihydrothymidine) and this is identified by an enzyme linked immunosorbent assay (ELISA) technique. The study concluded that the immunoassay developed was simple to prepare, sensitive and reliable and has the features deemed desirable for routine food testing. Among its advantages are the ability of the technique to be used on crude homogenates, obviating the need for DNA extraction. Furthermore, the authors state that:

"This assay is a simple, cheap and sensitive addition to the methods currently available for the detection of irradiated foods."

5.3.5 DNA Comet Assay

The comet assay has been used for a number of years to detect levels of DNA damage from a variety of cells. It has application to the detection of irradiated food as it is known that ionising radiation can cause both single and double strand breaks in the DNA of the food cells and of contaminating microorganisms. Delincée (1998) evaluated the technique to determine DNA fragmentation in grapefruits. The same author later applied the comet assay to frozen hamburgers (2002b). Delincée concluded that the technique could prove a useful screening test and that suspect samples could be further analyse using officially validated methods. This is necessary as other preservation techniques can lead to false positive results. Later work, however, identified some limitations of the process (Delincée *et al.* 2003). When the technique was applied to various seeds (electron beam irradiated at 10 MeV, 0 – 5 kGy), the comet assay proved useful for the identification of buckwheat, linseed, melon, nigella, poppy, sesame and sunflower seeds. There were, however, false positive results from fennel, fenugreek, millet and mustard seeds. The paper does not include details of the number of replicates tested, nor any statistical analysis performed. The authors still regard the comet assay a useful pre-screening technique but recognise its limitations.

Jo and Kwon (2006) assessed the suitability of this method to detect irradiation in kiwi fruit, compared with ESR. The fruit were subjected to gamma irradiation at doses of up to 2 kGy (n=3, 4°C, dose rate not known), whilst packed in LDPE. They determined that both methods could be used to detect irradiation of the fruit after six weeks (the shelf life of the fruit). It was noted, however, that the comet assay was more successful on the seeds while ESR could be used on both the seeds and the flesh of the fruit. As recommended by Delincée (2002a), Jo and Kwon (2006) also state that the comet assay should be verified by further analysis, such as ESR.

Techniques based upon DNA analysis generally require extraction of the DNA from the target cell prior to analysis. Giacomazzi *et al.* (2005) compared three methods for bacterial DNA extraction from cold-smoked salmon. The study showed that a DNA extraction kit (Qiagen DNeasy tissue kit) was superior to a direct DNA extraction using chemical and physical techniques, or the Pitcher method. It was also discovered, however, that physical treatments such as irradiation and freezing hamper DNA extraction. This leads us to the conclusion that caution should be exercised when using molecular techniques for bacterial analysis from irradiated foods, as false negatives may arise.

5.3.6 Other methods

Buchalla and Begley (2006) investigated the use of liquid-chromatography-mass-spectrometry (LC-MS) with atmospheric-pressure chemical ionisation (APCI) as a means of detecting low molecular weight irradiation products from polyethylene terephthalate. Samples of the film were gamma irradiated at doses of 0, 25 and 50 kGy (6 kGy $h^{-1,}$ ambient temperature). No details were give regarding the number of replicated or the statistical tests applied. The authors identified a unique irradiation product, not previously described using LC-MS. The technique has the potential for detecting low molecular weight irradiation products, even from the most radiation resistant polymers.

Dogan *et al.* (2007) assessed the potential of mid-Fourier Transform Infrared (FTIR) spectroscopy for the characterisation of irradiated hazelnut (*Corylus*

avellana L.). The nut samples were gamma irradiated at doses of 0, 1.5, 3.5 and 10 kGy (n=8, dose rate not known) and the data were analysed using the *t*-test. Significance was reported at $P<0.05$, 0.01 and 0.001. The study showed that high and low doses of irradiation caused molecular changes to the nuts. These changes could be detected with FTIR spectroscopy. The authors concluded that the technique could be successfully used to detect irradiated foods and it has the advantage that it can be used for dried, liquid, solid and fresh foods, so it is a more versatile protocol than others available.

Electron Paramagnetic Resonance (EPR) spectroscopy has also been applied to the detection of irradiated bones of meat and fish products, as well as to shells from molluscs (Sin *et al.* 2005). This is a non-destructive technique that relies upon the production of free radicals in the food and can be used for foods that contain hard, dry components. The study used bone and shell taken from meat and molluscs, irradiated at doses of 0, 1, 3 and 5 kGy (3.57 Gy min^{-1} at 20°C). A dose response equation and regression coefficient was generated for each sample. It was determined that the EPR signal was stable for the meat and shell samples but not the fish samples and that all blind samples were correctly identified as irradiated or unirradiated.

It is well known that electron beam irradiation has very low penetrating power and that complex food shapes can lead to variations in the dose absorbed by different parts of the same food item. Kim *et al.* (2006) developed and validated a methodology for dose calculation under these circumstances. The authors irradiated food phantoms (apples) and measured absorbed dose at all points colorimetrically. The dose distribution, on a 3-D basis was then calculated by Monte Carlo methods. They concluded that positioning of the food was critical to ensure an even dose distribution. This has considerable implications for processors of complex foods to ensure that all items receive comparable, uniform doses.

5.3.7 Detection of 2-ACBs

Horatovich *et al.* (2006) determined that, by replacing the EI (electronic impact) ionisation with an isobutene CI (chemical ionisation) in gas chromatography / mass spectrometry (GC/MS), a more sensitive and specific detection of the most abundant 2-ACBs in food could be achieved. The authors state that they can now perform an analysis of virtually any irradiated food to detect 2-ACBs with conventional laboratory equipment in a standard food quality control laboratory. Sin *et al.* (2006) applied pentafluorophenyl hydrazine to GC/MS to the detection of irradiated chicken, pork and mangoes (1, 3, 5 kGy 3.57 kGy min^{-1}, 20°C). The statistical significance of the results was determined using the students-*t* test and reported where $P \leq 0.05$ ($n=5$). The 2-ACBs were detected in none of the unirradiated samples and in all but two of the irradiated samples. The false negative results (one chicken and one pork at 1 kGy) were probably due to interference from endogenous substances around similar retention times. It was concluded that the method is suitable for the detection of irradiated meat samples at low doses of irradiation and shows better signal-to-noise ratios than the EN 1785 method.

Obana *et al.* (2005) developed a technique to detect irradiated meat and fish (beef, pork, chicken and salmon) using accelerated solvent extraction with hot, pressurised ethyl acetate, followed by GC/MS analysis of 2-DCB and 2-TCB. The statistics performed correlated the 2-ACBs with fatty acids formed as a result of the irradiation process (gamma irradiation, 0.7 – 7 kGy at 15 kGy h^{-1} at -19°C). Both 2-ACBs were detected at all irradiation doses and there was a good dose-response relationship, though it appeared that there was more production of 2-TCB than 2-DCB at frozen temperatures.

5.3.8 Detection of other compounds

Miyahara *et al.* (2002) identified a range of hydrocarbons produced by the irradiation of fatty acid methyl esters (to mimic fatty foods). The esters were irradiated in hexane at doses of 0.74 to 10 kGy with dose rates varying from 10 to 500 Gy h^{-1} and temperatures of between -40 and 20°C. Using capillary gas chromatography and mass spectrometry, they determined that the range

of radiolytic products formed was affected by dose, temperature, substrate concentration, oxygen concentration and dose rate effects were also observed. It is not only actual food commodities that are irradiated. Leth *et al.* (2006) assessed a number of methods to determine whether herbal food supplements had been irradiated. The supplements (106 products) were obtained from importers and none were labelled as irradiated. The samples were tested by the DEFT/APC method and, if the difference between the counts was in excess of 4 log cycles, the samples were sent to SUERC for TL and PSL analysis. Samples were then irradiated at a dose of 1 kGy (type of irradiation, dose rate and temperature not reported). It was found that the DEFT/APC method gave a large number of false positive results. The PSL only identified 7 of the 15 irradiated samples and the TL failed to identify 10% of samples. It is clear then, that for this type of product, there is currently no suitable European standard protocol to detect the presence of irradiated components.

The studies discussed highlight the need for robust design of experimental protocols so that confounding factors are not overlooked. It also emphasises the point that the detection of irradiated food can also be affected by these factors and this must be considered when evaluating data, as all experimental conditions must be reported.

5.4 Effect of Irradiation on Food Packaging

Irradiation is already in common use for the decontamination of food packaging and of medical devices. It is important, therefore, to know the effect of ionising radiation on the physical and chemical status of food packaging materials. This is made even more important when we consider that food irradiation can be used as a terminal, post-packaging decontamination technique. This adds complexity to the issue as the packaging material is in physical contact with the food during application of ionising radiation. As well as ensuring that the mechanical properties of the food package retain their integrity, the issue of migration of components of the packaging materials (in general, synthesised polymers) must be addressed.

Buchalla *et al.* (1992) reviewed the literature available for the effects of ionising radiation on polymers for the Institut für Sozialmedizin und Epidemiologie des Bundesgesundheitsamtes (Institute for Social Medicine and Epidemiology in Berlin, Germany). This review presented the data available on a wide range of materials used in the packaging of foods and pharmaceutical and healthcare items. The data presented includes the formation of gaseous and volatile radiolysis products, changes in global migration from the packaging, taint and odour problems, changes in the mechanical properties, changes in gas permeability and any apparent degradation of the polymers. They concluded that the polyethylenes (LDPE and HDPE) and polypropylene (including the variety of additives) are well investigated while the other polymers (polyvinyl chloride, polystyrene, polyethylene terephthalate, polyamides etc.) are less well understood. The authors conclude that the effects of irradiation are very much dependent upon the structure of the polymer, its processing history and the conditions prevalent during the irradiation process.

Goulas *et al.* (1995) evaluated the effect of gamma irradiation on migration behaviour of plasticizers (dioctyladipate (DOA) and acetyltributylcitrate (ATBC)) from food grade films (PVC and PVDC/PVC). The films were irradiated at doses of 4 and 9 kGy, with or without contact with olive oil (8-10°C, 0.6 and 1.3 kGy h^{-1} respectively, n=3). Unirradiated samples subjected to thermal treatments (20°C, 94 h; 50°C, 30 or 60 min.; 80°C, 30 or 60 min.) were also prepared. The results show that, although the plasticizers did migrate into the olive oil, there were no significant differences (although no statistical analysis is mentioned) in the migrated amounts of either plasticizer between irradiated and unirradiated samples, at either 4 or 9 kGy. The study concludes that the doses used do not affect the migration properties of PVC or PVDC/PVC films. Higher doses may, however, increase the levels of migration.

More recently, Goulas *et al.* (2002) studied the effect of gamma irradiation on the physicochemical (infrared spectra, migration into aqueous and fatty food

simulants) and mechanical (gas (oxygen and carbon dioxide) and water vapour permeability and mechanical strength) properties of monolayer flexible plastics packaging materials. The films used, ethylene vinyl acetate (EVA), HDPE, LDPE, polystyrene (PS), bi-axially oriented polypropylene (BOPP) and Ionomer, were irradiated at 0, 5, 10 and 30 kGy (0.7 kGy h^{-1}, temperature not stated). The results show that irradiation did not induce any statistically significant changes in the permeability of all films to gas or water vapour (n=3, results reported as mean ± SD) or IR analysis. The mechanical properties tested (tensile strength, Young's modulus, % elongation at break) also showed no significant change after 5 or 10 kGy (n=5, results presented ± SD). Conversely, at 30 kGy, HDPE, Ionomer and BOPP demonstrated decreased tensile strength, while LDPE and Ionomer showed a decrease in the % elongation at break. Young's modulus was unaffected, as were the EVA and PS films under all conditions. In terms of the migration characteristics, there was no significant difference when the films were irradiated in contact with distilled water, nor did irradiation at 5 or 10 kGy in 3% aqueous acetic acid (except Ionomer, which demonstrated decreased permeability). At 30 kGy, however, BOPP demonstrated an increase in migration values while Ionomer showed a decrease. There were no changes in the other films in acetic acid. In iso-octane, BOPP also showed an increase in migration at 30 kGy while HDPE and Ionomer showed a decrease. The authors conclude that the Ionomer film studied did not meet the EU requirements for migration characteristics. The other statistically significant changes observed were all at the highest irradiation dose, which will not affect the behaviour of films used for in-pack irradiation. It may, however, have implications for the irradiation of packaging materials prior to contact with food commodities.

Komolprasert *et al.* (2003) compared the effects of gamma and electron beam irradiation on semi-rigid amorphous polyethylene terephthalate copolymers (two were used with differing amounts of 1,4- cyclohexane dimethanol (CHDM), diethylene glycol and ethylene glycol). Each polymer (n=3-5) was irradiated with gamma (5 kGy at 0.05 kGy min^{-1}, 25 and 50 kGy at 0.1 kGy min^{-1}) and electron beam (5 kGy at 5 kGy s^{-1}, 25 and 50 kGy at 10 kGy s^{-1}). Data were analysed using one-way ANOVA and Bonferroni multiple mean

comparison *t*-test. The results show that no unique chemicals were derived from the irradiated compared with the unirradiated samples, nor did the higher doses significantly affect migration. The levels of acetaldehyde were significantly increased, which may result in sensory changes in the packaged food, but there was no increase in non-volatile migration into food. Chytiri *et al.* (2006) determined that, when a multi-layer packaging film, containing a layer of different percentages of recycled LDPE was gamma irradiated at doses of 5, 10, 30 and 60 kGy, there was no significant effect on a range of mechanical properties. Irradiation at 60 kGy did induce small changes in mechanical properties ($P \leq 0.05$), independent of the presence of recycled material (n=10 statistical analysis not identified). This demonstrates the potential for the reuse of packaging material for irradiated foods, although this study did use high quality pre-consumer scrap.

Studies also show that the type of packaging used during the irradiation process can affect the development of off-odours, lipid oxidation and volatile production in foods (for example Nam *et al.* 2003). There is evidence, therefore, of adverse changes to packaging materials but only at doses in excess of those used for the irradiation of pre-packaged foods (> 10 kGy).

5.5 The safety of the Irradiation Process

While it is recognised that 'radiation' is a hazard to human health, it is widely acknowledged that the risk of any incident from a radiation facility is minimal. There have been accidents relating to the approximately 170 gamma irradiation facilities worldwide (though not necessarily food irradiation facilities, nor in the UK). Strictly enforced and proven measures are in place to minimise any potential human contact. These measures include the storage of radioactive sources for gamma irradiation (^{60}Co and ^{137}Cs) underwater when not in use. Personnel are also excluded from the vicinity during use and screening is also in place to ensure safety. Electron beam and X-ray radiation do not use any radioactive source and the equipment can be 'switched off' when not in use, making it safe. The radiation industry is considered to have a strong safety record and that all accidents reported in the last 30 years have

been the result of safety systems being bypassed and proper control measures not being followed (United States General Accounting Office 2000).

Irradiation also produces the toxic gas, ozone. This is produced by electrical discharge into air, which occurs during electron beam irradiation. There are occupational exposure standards for both the UK and the US. The current Occupational Exposure Standard (OES) for ozone in the UK is 0.2ppm, averaged over a fifteen-minute period (Anon 1998). In the US, the National Ambient Air Quality Standards set the upper limit at 0.12 ppm h^{-1}. Irradiation facilities must monitor this and are not permitted to operate in the US if this limit is exceeded (Smith and Pillai 2004).

It must be remembered that such concerns are not generally expressed over facilities irradiating medical devices, possibly because irradiation for this purpose is a less emotive issue. The author has not found any evidence of risks to food, environmental or personnel safety linked with the process of food irradiation.

6. DISCUSSION

The World Health Organisation stated:
"food irradiated at any dose appropriate to achieve the intended technological objective is both safe to consume and nutritionally adequate. ….Accordingly, irradiated foods are deemed wholesome throughout the technologically useful dose range from below 10 kGy to envisioned doses above 10 kGy." (FAO/IAEA/WHO 1999, p.161).

This review has, however, raised some concerns over some aspects of food irradiation.
The production of unique radiolytic products, in particular 2 ACBs in fatty foods. These compounds have been shown to be potentially carcinogenic and there appears to be strong evidence for their presence in irradiated foods. The efficacy of the feeding trials has been highlighted as an area of concern and the WHO, in the report cited above, dismissed a number of these trials as being invalid due to experimental deficiencies. Ashley *et al.* (2004) question this decision and propose that the data should be considered. These authors also state that the potential increase in the number of cases of cancer in the population, as a result of consumption of these compounds from irradiated food, may go unrecognised as the actual percentage increase would be very small, even though it may represent a considerable number of people.

There is also some evidence for the potential for chromosome damage in people. While the India study was not very well designed and a small number of children were used, the fact remains that polyploidy was observed. The same results were observed in the Chinese study following reanalysis of the data. This is of particular concern for under or malnourished individuals. Louria (2001) suggests that a meticulous study should be conducted, whereby adults and children from different ethnic groups are fed a diet containing irradiated foods and that their chromosomes should then be subjected to analysis after 2-3 months. If abnormalities are discovered, then there is cause for concern. In today's political and ethical climate, it seems unlikely that such a study would be given approval by any Local Research Ethics Committee (LREC).

While this project was not concerned with the effect of food irradiation on the organoleptic properties of the food, it was apparent that many authors discuss the effect of irradiation on the parameters on concern particularly the microbiological status of an irradiated food in relation to the sensory properties. There would be little merit in irradiating a food at a dose that will achieve microbiological stability to the detriment of its organoleptic properties. Louria (2001) also states that the reduction in vitamin content of the food can pose real problems to those who are not adequately nourished.

It was noted during the course of this review, that many of the publications dated before approximately 1990 made no mention of the number of replicates used in each experiment (with the exception of feeding trials) nor was the method of statistical analysis given. The exact conditions under which the irradiation was performed were also poorly described in many cases. This was less apparent in later publications as the quality of academic, peer reviewed papers has improved. It is also now recognised that very small changes in environmental conditions can have a considerable impact on radiation resistance of microorganisms or production of radiolytic products. It is recommended, therefore, that all such information is provided in any future publications. It is also recognised that there are still many gaps in our knowledge where food irradiation is concerned. Many of these are discussed in section 7. The gaps in the knowledge identified above cannot be assumed to be equivalent to the future research needs. A risk assessment of each must be performed in order to ascertain those that present a negligible or low risk to the consumer and, as a result, are a low priority for research funding, and those that represent a higher or significant risk to public health. These should, by their nature, be given precedence in terms of future calls for research.

7. FUTURE RESEARCH NEEDS

In the absence of a full risk assessment and in light of the data discussed in the review, the following knowledge gaps can be considered as representing a higher risk:

The role of 2-ACBs in colon carcinogenesis (Raul *et al.* 2002, Ashley *et al.* 2004). Systematic and comprehensive animal bioassays are required, each following exact protocols so that direct comparisons between data sets can be made. There is also the potential for the use of biomarkers in the determination of food mutagens. While no single biomarker exists, there is a panel that reflects gene-environment interactions may be predictive of risk and this needs to be determined. This will assist in the analysis of low dose exposures and low risk populations. Biomarkers can also be used to determine hypotheses regarding etiological relationships where the exact mutagen is not known. (Goldman and Shields 2003)

The observed chromosome changes, although from dubious trials, could be further researched. This could be taken further by using the technologies afforded us by genomics, for example transcriptomics will tell us if any of these chromosomal changes could lead to over or underexpression of different proteins. Metabolomics will highlight the range of metabolites that are produced when certain irradiated foods are consumed.

The evidence shown to date for a reduction in allergenicity and antigenicity of food allergens is promising. With increasing numbers of food allergies and heightened public awareness, this could be a potential area for future research that provides a positive aspect of the process.

Although this review was not concerned with the nutritional aspects of food irradiation, it is recognised that for a large proportion of the world's population, this may be an area of particular concern. Approval of new food products for irradiation and increased doses must be based on a risk based assessment of the benefits as opposed to the potential detrimental effects. This is especially important for third world countries due to the high level of foodborne illness

(Ashley *et al.* 2004). DeRouchey *et al.* (2004) postulated that there may be deactivation of unknown anti-nutritional factor by irradiation of diet components compared with whole diet. This is an area that should be addressed.

“Studies in man may be essential for the proper carrying out of risk management with irradiated foods along the lines suggested for the safety evaluation of novel foods despite the difficulties associated with human epidemiological studies on a large sample of volunteers over prolonged periods” (Elias 1989).
While it is logical and makes scientific and epidemiological sense for humans to be used for feeding trials, there are ethical issues that would require attention. It may be that other countries, for example the USA, would not have such issues and that feeding trials could take place there. It would not, however, overcome some of the issues raised regarding the importance of ethnicity and diet type when conducting these trials.

In terms of food packaging, it is recommended that, in this era of sustainability, an evaluation of recycled materials for food packaging should be carried out (Buchalla and Begley 2006). This is vital before approval be given for their use as the history of these recycled materials may not be known and could have some bearing on the volatiles produced during the irradiation process or their subsequent mechanical or permeability properties. This could have a direct impact on food safety.

Smith and Pillai (2004) suggest that specific pathogen reduction protocols be devised and approved so that all future research in this area is conducted using robust methodology and the data sets can be directly compared. There are, however, limitations to this. Different radiation facilities operate at different dose rates and this has been shown to affect resistance of microorganisms. The inherent differences between gamma and electron beam would also need to accounted for in protocols but his is not an insurmountable problem.

It appears that very little research has been performed on the inactivation kinetics of foodborne viruses or protozoan parasites. As the importance of these two groups of microorganisms in terms of foodborne illness is high, further work in this area is required. This should follow development of the protocols mentioned above. It should also take other factors into account that have been mention in this review but have also been identified by others as areas for future consideration. These include radiosensitisation of microorganisms (or microbial stress conditions) by components of the food and by the application of novel combination processes. The organoleptic attributes of food limit the radiation dose that can be applied. This can have an obvious effect on food safety. Linked with this is the irradiation of multi-component foods, which has been highlighted as a problem. It is logical to assume that the radiation dose required should be targeted at the most resistant organisms but this may lead to organoleptic changes in some components of the food that are more susceptible.

It is also important that the information contained within this database is accessible on a needs basis. Work has also been considered regarding the transfer of all the information contained in the Reference Manager database, along with notes made on approximately 16% of the publications contained therein (104 publications are still on request and, should these be obtained, notes will also be made on these), into a customised Access database. The reason for this is that the majority of people will not be able to view the Reference Manager database due to licence restrictions. The majority of computer users can, however, view Access files as this programme is a standard component of Microsoft Office.

8. CONCLUSION

The United States General Accounting Office produced a report in 2000 that stated:

“Scientific studies conducted by public and private researchers worldwide over the past 50 years support the benefits of food irradiation while indicating minimal potential risks.” (p. 4).

The main conclusion of this review is that some areas would benefit from further investigation.

It must be recognised that the behaviour of microorganisms and the production of URPs is influenced considerably by the matrix in which the irradiation takes place and the prevailing environmental conditions. It may not be possible to accurately predict behaviour without laboratory trials being performed. The extent to which all new food commodities must be subject to these trials must be a decision based upon risk assessment.

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