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Food Safety

With the world's growing population, the provision of a safe, nutritious and wholesome food supply for all has become a major challenge (1). To achieve this, effective risk management based on sound science and unbiased information is required by all stakeholders, including the food industry, governments and consumers themselves. In addition, the globalization of the food supply requires the harmonization of policies and standards based on a common understanding of food safety among authorities in countries around the world. With some 280 chapters, the *Encyclopedia of Food Safety* provides unbiased and concise overviews which form in total a comprehensive coverage of a broad range of food safety topics, which may be grouped under the following general categories (1):

1. History and basic sciences that support food safety,
2. Foodborne diseases, including surveillance and investigation,
3. Foodborne hazards, including microbiological and chemical agents,
4. Substances added to food, both directly and indirectly,
5. Food technologies, including the latest developments,
6. Food commodities, including their potential hazards and controls,
7. Food safety management systems, including their elements and the roles of stakeholders.

The Encyclopedia provides a platform for experts from the field of food safety and related fields, such as nutrition, food science and technology and environment, to share and learn from state-of-the art expertise with the rest of the food safety community (1).

Plastic plays a significant and growing role in modern day society, delivering many benefits, particularly in food safety and preservation, and can help to reduce food waste (2).

Advances in packaging can not only reduce losses but also improve food quality and safety. To offer the best food protection the FAO suggests that a packaging solution could include more, but better packaging, rather than less packaging.

A novel approach for food safety, which integrates a statistical study and subjective discussion, was adopted to review the recent advances in the environment and food safety (3).

Here, a scientometric-based statistical study was conducted based on 4904 publications collected from the Web of Science Core Collection database. It was found that the research on the environment and food safety grew steadily from 2001 to 2020. The statistical analysis of most-cited papers, titles, abstracts, keywords, and research areas revealed that the research on the environment and food safety was diverse and multidisciplinary.

In addition to the scientometric study, strategies to protect the environment and ensure the safety of food were critically discussed, followed by a discussion on the emerging research topics, including emerging contaminates, e.g., microplastics, rapid detection of contaminants, e.g., biosensors, and environmentally friendly food packaging materials, e.g., biodegradable polymers (3).

1.1 Food Security

Plastic pollution arising from food systems is driving policies for reduction, removal, reuse and recycling (4).

A systematic scoping review was used to describe the extent, range and nature of published evidence since 2000 on seven major plastic types used at any point within food systems.

The majority of the publications focus on the agricultural production; relatively fewer publications relate to retail, household and food waste disposal plastics (4).

Some plastic types were more frequently linked to certain outcomes than others: Poly(ethylene) and poly(propylene) (PP) were frequently explored in relation to effects on the nutrient or bacterial content of crops and food (n = 445 and 274, respectively), but only one study considered the effects of PP (commonly used in infant feeding bottles) on the presence of plastics or associated chemicals in the human body.

Among the studies that looked at plastics and chemicals in the human body, as well as human health states and/or physiological changes, the most commonly explored types were unspecified plastics, miscellaneous plastics and poly(ethylene terephthalate) (PET). Despite finding evidence of each major plastic type in our sample, a lack of chemical specification of the plastic exposures was very common (4).

1.2 Migration of Substances from Packaging

The usage of new food packaging materials has increased the number of occurring hazards due to the migration from packaging material to the packaged food (5). Although polymers have mainly monopolized the interest of migration testing and experimentation, recent studies have revealed that migration also occurs from traditional materials generally considered to be safe, such as paper, carton, wood, ceramic, and metal. The regulations and the directives of the EU tend to become stricter in this respect.

The emphasis is on reaching a consensus in terms of food simulants and testing conditions for migration studies. Furthermore, the list of hazardous monomers, oligomers, and additives continues to be augmented in order to ensure that the consumer safety is in current agreement with the hazard analysis critical control points (HACCP), which is continuously gaining ground (5).

Food and beverages can be very aggressive chemical milieu and may interact strongly with materials that they touch (6). Whenever food is placed in contact with another substance, there is a risk that chemicals from the contact material may migrate into the food. These chemicals may be harmful if ingested in large quantities, or impart a taint or odor to the food, thereby negatively affecting the food quality. Food packaging is the most obvious example of a food contact material. As the demand for prepackaged foods increases, so might the potential risk to consumers from the release of chemicals into the food product.

Chemical Migration and Food Contact Materials reviews the latest controls and research in this field and how they can be used to ensure that food is safe to eat.

In a monograph, the regulation and quality control of chemical migration into food are discussed (6). Then, the latest developments in areas such as exposure estimation and analysis of food contact materials are reviewed. Finally, specific chapters on major food contact materials and packaging types are presented, such as recycled plastics, metals, paper and board, multilayer packaging and intelligent packaging.

The large number of synthetic materials that are used for the manufacture of packages makes the evaluation of the food package interactions complicated (7).

Different parameters, such as the nature of the food of interest, the type of food package contact, the time and temperature of contact, the packaging materials used, the properties of the migrating substances, as well as the amount of potential migrants contained in the packaging materials, can drastically affect the migration rate and the extent of migration.

Due to the extreme variety of foods used, several food simulants have been suggested and applied for testing the migration phenomenon under various laboratory conditions (7). The use of many of those simulants is defined by national and international legislation. The main migration phenomena, which are related to the most commonly used packaging materials, have been detailed (7). In the study, it was clearly demonstrated that the complexity of the migration phenomena requires more research to establish internationally accepted risk management procedures and standardized testing methods.

1.2.1 Modeling of Migration

The potential for the use of migration modeling for studying polyolefin packaging materials, such as low-density and high-density poly(ethylene) and poly(propylene), was summarized and demonstrated with practical examples (8).

For these polymers, an upper limit of migration into foodstuffs can be predicted with a high degree of statistical confidence. The only analytical information needed for modeling in such cases is the initial concentration of the migrant in the polymer matrix.

For polyolefins of unknown origin or newly developed materials with new properties, a quick experimental method has been described for obtaining the characteristic matrix parameter needed for the process of migration modeling.

For easy handling of both the experimental results and the diffusion model, a user-friendly software has been developed. An additional aim of the described method is the determination of the migrant partition between polymer and food or food simulant and the specific contribution of the migrant molecular structure on the diffusion coefficient. For migration modeling of packaging materials with multilayer structures, a numerical solution of the diffusion equation is described. This procedure has also been applied for modeling the migration into solid or high-viscosity foodstuffs (8).

The permeation through and diffusion/migration from high-density poly(ethylene), poly(butylene), poly(propylene) and crosslinked poly(ethylene) films was investigated experimentally with three different methods to determine diffusion coefficients in these polyolefins for a series of additives, their degradation products and other organic substances in the 20°C to 60°C temperature range (9).

The experimental methods used were dynamic permeation through additive-free polymer films, kinetic desorption from additivated films into water and kinetic migration from additivated into additive-free polymer films. It was found that in general the temperature dependence of the obtained diffusion coefficients was well represented by the Arrhenius law.

Some of these results also suggested that the contact of the polyolefins with water had an influence on the magnitude of the diffusion coefficients and on their apparent activation energy of diffusion (9).

The migration of phthalate from PET bottles containing non-alcoholic beer was done by performing an adaptive neuro-fuzzy inference system analysis (10).

The data showed that the storage temperature, contact surface and storage period correlate with the rate of migration. The migration of phthalate increases with storage duration gradually and reduces under different temperatures and contact surface. Moreover, increased temperature and storage duration resulted in an increase in migration level ranging from 0.6 $\mu g\, l^{-1}$ to 2.9 $\mu g\, l^{-1}$ (10).

The study used an adaptive neuro-fuzzy inference system analysis architecture, which consists of three inputs (temperature, surface and storage period), Gaussian-bell membership functions for each input variable and one output layer, which represent the migration level. The validation and training models showed an excellent match between the experimental and predicted values of adaptive neuro-fuzzy inference system analysis.

The analysis of the model showed that adaptive neuro-fuzzy inference system analysis is a powerful tool for predicting phthalate migration from bottles containing non-alcoholic beer (10).

1.2.2 Sample Pretreatment Methods

An excessive absorption of migrating substances from food contact materials can affect human health. Thus, it is essential to analyze the migration of contaminants from food contact materials (11).

However, comprehensive analysis has been challenged by low concentration of migrating substances and manifold and complex matrix interference of food contact materials. Therefore, appropriate sample pretreatment methods should be applied before instrumental detection, which is essential to improve the analytical efficiency, sensitivity, and reliability.

The development of sample pretreatment methods for the analysis of migrating substances from food contact materials has been reviewed in the past decade (11). To extract volatile and semi-volatile substances, headspace extraction, headspace solid phase microextraction, and a purge and trap technique are discussed here.

For non-volatile substances, solid-liquid extraction and field-assisted extraction are usually used to extract them from food contact materials; while liquid-liquid extraction, solid phase extraction, and their corresponding microextraction techniques play important roles in the enrichment process (11).

Also, new progress in the development of sample pretreatment methods of food contact materials was summarized, covering new devices, specific adsorbents, and sample preparation methods for rapid detection (11).

1.2.3 Special Chemicals

1.2.3.1 Styrene

Poly(styrene) (PS) is extensively used in diverse forms for packaging of many food products such as meat, dairy and bakery products. There is a potential migration of styrene monomer from PS packages into the foods which are in contact with them (12).

The representative styrene migration from PS packaging material into the corresponding foods was detailed. Also, the addressed parameters affecting the styrene migration were discussed. The analytical methods for detecting the styrene monomer in food products and PS packaging materials was also covered in the study. The possible safety and health issues related to the styrene monomer migration were also covered.

The quality of PS packaging materials in terms of their styrene monomer residue level and the storage conditions of foods can greatly affect styrene migration. Also, the food characteristics, such as fat content and pH, can significantly affect the migration of styrene. Although styrene is considered a nontoxic compound, its migration into foods can downgrade sensorial properties as well as result in health problems. In some cases, the presence of styrene in foods can cause carcinogenic, hematological, cytogenetic, and neurotoxic issues (12).

A sensitive, accurate and fast headspace-solid phase microextraction-gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS/MS) method was developed and validated for the determination of styrene in various food matrices. The mean recovery ranged from 90% to 116% with a relative standard deviation of =11% (13).

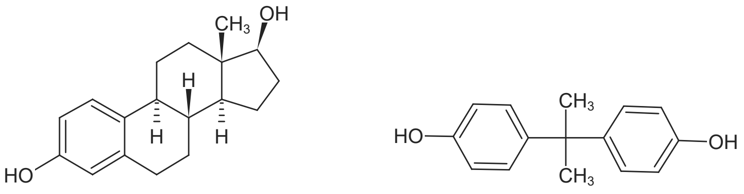
This method was used for the determination of the concentration of styrene in 23 foodstuffs packed in PS containers, as well as the levels of styrene migrating into various foods (water, milk, cheese or cream) from 14 tableware or kitchenware articles made of styrene plastics. All samples were collected from the Greek market in 2020.

The concentrations of styrene in the packaged foods ranged from 0.4 to 160 $ng\, g^{-1}$, with the highest concentration found in a meat product packed in a foamed PS tray. It is worth noting that 56% of PS packaged dairy products and desserts had a styrene concentration of higher than 10 $ng\, g^{-1}$. Particularly high levels of styrene that have not previously been reported, of up to 46 $ng\,g^{-1}$, were found in dairy products for children. The highest level of styrene migration from tableware or kitchenware articles, 89 $ng\,g^{-1}$, was observed when disposable cups from foamed PS were filled with milk at 70°C for 2 *h* (13).

1.2.3.2 Bisphenol A

Bisphenol A is mainly used in the production of poly(carbonate), a material with high durability and strength. This chemical is also used in the production of epoxy resins, applied in the coating of metal surfaces in contact with food. Moreover, it is employed in thermal paper (14).

Bisphenol A has been widely reported by the media, being the aim of diverse scientific studies, since it is a xenoestrogen with a chemical structure identical to that of *β*-estradiol, which allows it to interact with human estrogen receptors. These compounds are shown in Figure 1.1.



β-Estradiol

Bisphenol A

Figure 1.1 Xenoestrogens.

Bisphenol A monitoring is essential to avoid a potential health risk of the population. The European Union has recently updated the specific migration limit for bisphenol A at 0.05 $mg\,kg^{-1}$ of food.

Regarding the levels of migration determined in different types of food contact materials, it was found that, despite the great majority of studies showing positive samples, none of them exceeded the value of specific migration limit established by the European Union at the time of the studies (14).

1.2.3.3 Benzophenone

Benzophenone may be present in cartonboard food-packaging materials as a residue from UV-cured inks and lacquers used to print on the packaging (15). It may also be present if the cartonboard is made from recycled fibers recovered from printed materials.

A method has been developed to test for benzophenone in cartonboard packaging materials and to test the migration levels in foodstuffs.

Here, the packaging material is extracted with a solvent containing d10-benzophenone as the internal standard. Foods are extracted with solvent containing d10-benzophenone and the extract is defatted using hexane. The extracts are analyzed by gas chromatography (GC)-mass spectroscopy (MS). d10-Benzophenone is the deuterium labeled benzophenone. It is shown in Figure 1.2.

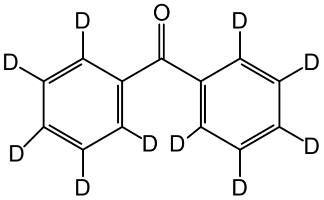


Figure 1.2 d10-Benzophenone.

For the analysis of food, the limit of detection was 0.01 $mg\,kg^{-1}$ and the limit of quantification was 0.05 $mg\,kg^{-1}$. The calibration was linear from 0.05 $mg\,kg^{-1}$ to 20 $mg\,kg^{-1}$. The method for food analysis was validated in-house and it also returned satisfactory results in a blind check-sample exercise organized by an independent laboratory.

The methods were applied to the analysis of 350 retail samples that used printed cartonboard packaging. A total of 207 (59%) packaging samples had no significant benzophenone (<0.05 $mg\,dm^{-2}$). Seven (2%) were in the range 0.05 $mg\,dm^{-2}$ to 0.2 $mg\,dm^{-2}$, 60 (17%) were from 0.2 $mg\,dm^{-2}$ to 0.8 $mg\,dm^{-2}$ and 76 (22%) were from 0.8 $mg\,dm^{-2}$ to 3.3 $mg\,dm^{-2}$.

A total of 71 samples were then selected at random from the 143 packaging samples that contained benzophenone, and the food itself was analyzed. Benzophenone was detected in 51 (72%) of the foods. Two food samples (3%) were in the range 0.01 $mg\,kg^{-1}$ to 0.05 $mg\,kg^{-1}$. A total of 29 (41%) were from 0.05 $mg\,kg^{-1}$ to 0.5 $mg\,kg^{-1}$, 17 (24%) were from 0.5 $mg\,kg^{-1}$ to 5 $mg\,kg^{-1}$ and three (4%) food samples exceeded 5 $mg\,kg^{-1}$. The highest level of benzophenone in food was 7.3 $mg\,kg^{-1}$ for a high-fat chocolate confectionery product packaged in direct contact with cartonboard, with room temperature storage conditions and with a high contact area:food mass ratio. When the mass fraction of the migration of benzophenone was calculated for the different contact and storage regimes involved, the attenuation effects of indirect contact and of low temperature storage were cumulative. Thus, there was a sixfold reduction in migration for indirect contact compared with direct contact, a sixfold reduction for chilled/frozen storage compared with ambient storage, and 40-fold reduction for the two contact conditions combined (15).

1.2.3.4 Perfluorochemicals

Perfluorochemicals are widely used in the manufacturing and processing of a vast array of consumer goods, including electrical wiring, clothing, household and automotive products. Furthermore, relatively small quantities of perfluorochemicals are also used in the manufacturing of food contact substances that represent potential sources of oral exposure to these chemicals (16).

The most recognizable products to consumers are the uses of perfluorochemicals in non-stick coatings (poly(tetrafluoroethylene) (PTFE)) for cookware and also their use in paper coatings for oil and moisture resistance. Recent epidemiology studies have demonstrated the presence of two particular perfluorochemicals, perfluorooctane sulfonate and perfluorooctanoic acid, in human serum at very low part per billion levels. These perfluorochemicals are biopersistent and are the subject of numerous studies investigating the many possible sources of human exposure. Among the various uses of these two chemicals, perfluorooctane sulfonate is a residual impurity in some paper coatings used for food contact and perfluorooctanoic acid is a processing aid in the manufacture of PTFE used for many purposes, including non-stick cookware.

Very little information is available on the types of perfluorochemicals that have the potential to migrate from perfluoro coatings into food. One obstacle to studying the migration is the difficulty in measuring perfluorochemicals by routine conventional analytical techniques such as GC/MS or LC-UV. Many perfluorochemicals used in food contact substances are not detectable by these conventional methods. As liquid chromatography-mass spectrometry (LC/MS) develops into a routine analytical technique, potential migrants from perfluoro coatings can be more easily characterized. In a study, data is presented on the types of perfluorochemicals that are used in food packaging and cookware. Additionally, research is presented on the migration or potential for migration of these chemicals into foods or food-simulating liquids.

The results from migration tests show $mg\,kg^{-1}$ amounts of perfluoro paper additives/coatings transfer to food oil. Analysis of PTFE cookware shows residual amounts of perfluorooctanoic acid in the low $\mu g\,kg^{-1}$ range. Perfluorooctanoic acid is present in microwave popcorn bag paper in amounts as high as 300 $\mu g\,kg^{-1}$ (16).

1.2.4 Safety of Recycled HDPE and PP

An analytical protocol was set up and successfully applied to study the food safety of recycled high-density poly(ethylene) (HDPE) and PP crates (17). A worst-case scenario was applied that focused not only on overall migration and specific migration of accepted starting materials but also on migratable degradation products of polymers and additives that may be formed during mechanical recycling.

The analytical protocol was set up to cover a wide variety of possible migrants. Identification and semi-quantification were possible for almost all migrants that increased significantly with increasing mechanical recycling steps for both the HDPE and PP crates.

It was concluded that the analytical protocol was suitable to study the influence of (multiple) recycling on the food safety of plastic materials. The protocol can be applied to other plastic foodcontact materials and provides valuable information on the food safety of the recycling process and the resulting recycled foodcontact materials in addition to challenge testing (17).

1.3 Food Safety and Hygiene

Food hygiene is the conditions and measures necessary to certify the safety of food from production to consumption. Food can become contaminated at any point during slaughtering or harvesting, processing, storage, distribution, transportation and preparation.

All conditions and measures that are required during production, processing, storage, distribution and preparation of food to ensure that it is safe, wholesome and fit for human consumption were defined in 1984 by the World Health Organization (WHO).

A lack of requisite food hygiene can lead to foodborne diseases and death of the consumer. Foodborne illness has been associated with improper storage or reheating (50%), food stored inappropriately (45%) and cross contamination (39%). Proper food preparation can prevent most foodborne diseases.

More than 200 known diseases can be transmitted through food (18). Some of these diseases are collected in Table 1.1.

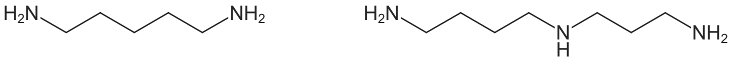
Table 1.1 Known diseases (19).

Disease or Agent	
Bacterial	Bacterial
Bacillus cereus	Botulism, foodborne
Brucella spp.	*Campylobacter* spp.
Clostridium perfringens	*Escherichia coli*
Listeria monocytogenes	*Salmonella typhi*
Salmonella, nontyphoidal	*Shigella* spp.
Staphylococcus	*Vibrio cholerae*
Vibrio vulnificus	*Yersinia enterocolitica*
Parasitic	Parasitic
Cryptosporidium parvum	*Cyclospora cayetanensis*
Giardia lamblia	*Toxoplasma gondii*
Trichinella spiralis	
Viral	Viral
Norwalk-like viruses	Rotavirus
Astrovirus	Hepatitis A

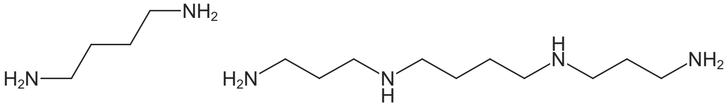
Hazard analysis and critical control points is a systematic preventive approach to food safety from biological, chemical, and physical hazards in production processes that can cause the finished product to be unsafe and designs measures to reduce these risks to a safe level. Food hygiene and safety usually refer to contamination with microorganisms or microbes.

1.3.1 Sensors for Amine Detection

Biogenic amines are good indicators of food freshness because they are products of microbial fermentation (20). In the process of food spoilage, microbes break down amino acids via deaminization to generate ammonia, and via decarboxylation to generate biogenic amines such as cadaverine, putrescine, spermidine, spermine, and others. These amines are shown in Figure 1.3.



Cadaverine Spermidine



Putrescine Spermine

Figure 1.3 Biogenic amines.

These biogenic amines not only signal food spoilage, but also have an adverse impact on human health and physiological functions. Thus, monitoring biogenic amines in food is important both because the chemical species can have toxic effects, and because they signify food spoilage by microbes. When compared to time-temperature indicators, which only respond to temperature changes, a system detecting the presence of biogenic amines offers a more direct method of monitoring food safety and hygiene.

Aggregation-induced emission (AIE) active chemosensors exhibit a change for UV-vis absorption and become non-luminescent upon protonation (20). Upon deprotonation, the chemosensors revert to their original absorption and emission. This deprotonation process can be triggered in the presence of amines, and specifically, biogenic amines. So, the chemosensors can detect amine species, e.g., biogenic amines produced during food fermentation, quickly and with a high sensitivity.

As chemicals, 1,2-dihydroquinoxaline derivates can be used (20, 21).

Some derivates are shown in Table 1.2 and Figure 1.4.

Table 1.2 1,2-Dihydroquinoxaline derivates.

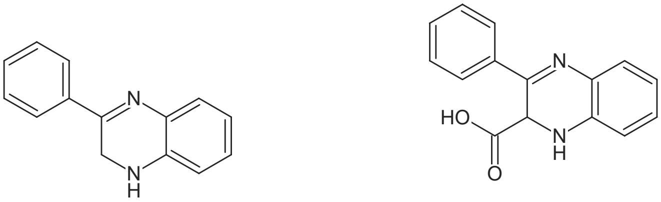
Compound
3-Methyl-2-prop-2-enyl-1,2-dihydroquinoxaline
3-Methoxy-6-(trifluoromethyl)-1,2-dihydroquinoxaline
2-Phenyl-1,2-dihydroquinoxaline
3-Phenyl-1,2-dihydroquinoxaline
3-Phenyl-1,2-dihydroquinoxaline-2-carboxylic acid
3-Methyl-2-propan-2-yl-1,2-dihydroquinoxaline
5-[6-[4-(Trifluoromethyl)phenyl]pyrimidin-4-yl]oxy-1,2-dihydroquinoxaline
5-Bromo-3-[4-(trifluoromethyl)phenyl]-1,2-dihydroquinoxaline
3-[Chloro(phenyl)methyl]-1,2-dihydroquinoxaline
3-(1H-Indol-3-yl)-1,2-dihydroquinoxaline
6-Chloro-1,2-dihydroquinoxaline
3-(3-Nitrophenyl)-1,2-dihydroquinoxaline
Ethyl 7-amino-3-ethoxy-6-(trifluoromethyl)-1,2-dihydroquinoxaline-2-carboxylate
8-Amino-2-ethyl-5-(hydroxymethyl)-1,2-dihydroquinoxaline-6-carboxylic acid
2,3-Dimethyl-1,2-dihydroquinoxaline
2-Chloro-3-(5-chloro-1H-pyrazol-3-yl)-1,2-dihydroquinoxaline
2-Bromo-5-[6-[4-(trifluoromethyl)phenyl]pyrimidin-4-yl]oxy-1,2-dihydroquinoxaline

1.4 Impact of Microplastics on Humans and the Environment

Micro- and nanoplastics have the potential to be transferred between trophic levels and, therefore, the risk characterization and the assessment of dietary exposure to them constitutes a current challenge for food safety alongside the study of the role of plastics as vectors of other contaminants and pathogenic microorganisms (22).

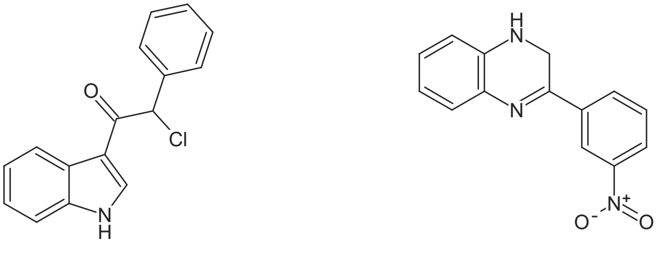
3-Methyl-2-prop-2-enyl-
1,2-dihydroquinoxaline

2-Phenyl-
1,2-dihydroquinoxaline



3-Phenyl-
3-Phenyl-1,2-dihydroquinoxaline

3-Phenyl-1,2-dihydroquinoxaline-
2-carboxylic acid



3-(1H-Indol-3-yl)-
1,2-dihydroquinoxaline

3-(3-Nitrophenyl)-
1,2-dihydroquinoxaline

6-Chloro-1,2-dihydroquinoxaline

2,3-Dimethyl-
1,2-dihydroquinoxaline

Figure 1.4 1,2-Dihydroquinoxaline derivates.

The risk posed by microplastics to humans and the environment, has become a hot topic (23). The concern is focused not only on the effect of microplastics as such but also on additives and chemical contaminants absorbed by microplastics that may be released and negatively affect animals and environmental health. Despite several works having been written on this topic, a number of knowledge gaps still should be filled to enable a correct risk assessment of this important issue. For example, the relevance of microplastics for food safety has not yet been fully established and scientific results aimed at establishing a possible health risk for contaminants associated with microplastics are rather controversial. The risk assessment of microplastics in foodstuff is still at a very early stage and very few studies on the monitoring of microplastics in foodstuff and their effects on human health are available. Additionally, it is difficult to compare results from different studies as methodologies and study designs are not uniform. For this reason, it is not always possible to reach some definitive conclusion (23).

Studies have shown high concentrations of chemical contaminants that adsorb microplastics from the surrounding environment (24).

The compounds are listed in Table 1.3.

Table 1.3 Contaminants in microplastics (24).

Compound	Amount in pallets/[$ng\,g^{-1}$]
Polycyclic aromatic hydrocarbons	52.1 – 17,023.6
Polychlorinated biphenyl	0.9 – 2285.8
Organochlorine pesticides	0.4 – 13,488.7
UV filters	0 – 37,740.3
Brominated diphenyl ethers	0 – 180.58
Organophosphorus flame retardants	20.0 – 378.0
Compound	**Amount in fragments/[$ng\,g^{-1}$]**
Polycyclic aromatic hydrocarbons	35.1 – 8725.8
Polychlorinated biphenyl	1.6 – 772.5
Organochlorine pesticides	0.4 – 3778.8
UV filters	3.7 – 2169.3
Brominated diphenyl ethers	0.06 – 3923.9
Organophosphorus flame retardants	22.6 – 7013.9

1.4.1 *Polychlorinated Biphenyl Compounds*

Details of polychlorinated biphenyl compounds are shown in Table 1.4 and in Figure 1.5

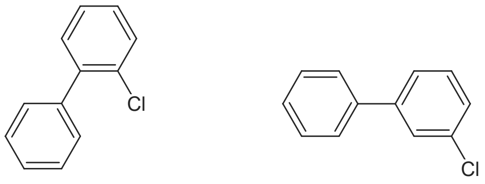
Table 1.4 Polychlorinated biphenyl compounds.

Abbreviation	Name
PCB 1	2-Chlorobiphenyl
PCB 2	3-Chlorobiphenyl
PCB 3	4-Chlorobiphenyl
PCB 5	2,3-Dichlorobiphenyl
PCB 7	2,4-Dichlorobiphenyl
PCB 11	3,3′-Dichlorobiphenyl
PCB 12	3,4-Dichlorobiphenyl
PCB 14	3,5-Dichlorobiphenyl
PCB 15	4,4′-Dichlorobiphenyl
PCB 18	2,2′,5-Trichlorobiphenyl
PCB 28	2,4,4′-Trichlorobiphenyl
PCB 29	2,4,5-Trichlorobiphenyl
PCB 31	2,4′,5-Trichlorobiphenyl
PCB 52	2,2′,5,5′-Tetrachlorobiphenyl
PCB 77	3,3′,4,4′-Tetrachlorobiphenyl
PCB 101	2,2′,4,5,5′-Pentachlorobiphenyl
PCB 105	2,3,3′,4,4′-Pentachlorobiphenyl
PCB 138	2,2′,3,4,4′,5′-Hexachlorobiphenyl
PCB 149	2,2′,3,4′,5′,6-Hexachlorobiphenyl
PCB 153	2,2′,4,4′,5,5′-Hexachlorobiphenyl
PCB 170	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl
PCB 180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl
PCB 209	Decachlorobiphenyl

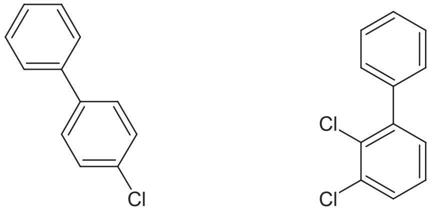
1.4.2 *Polycyclic Aromatic Hydrocarbon Compounds*

Fossil fuels and other organic materials formed during incomplete burning can produce and release a complex cluster of emerging pollutants into the environment known as polycyclic aromatic hydrocarbons (25, 26).

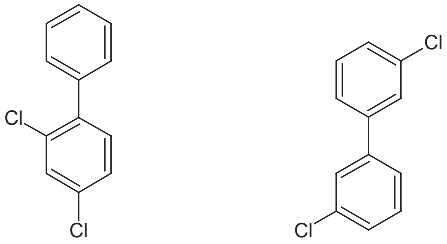
Also, human activities have been multiplying the waste and effluents generated in the environment containing different polycyclic aromatic hydrocarbons. All sorts of contaminants including poly-



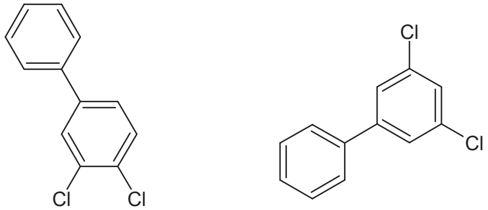
2-Chlorobiphenyl 3-Chlorobiphenyl



4-Chlorobiphenyl 2,3-Dichlorobiphenyl



2,4-Dichlorobiphenyl 3,3′-Dichlorobiphenyl

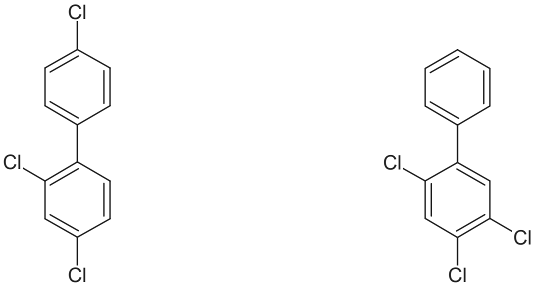


3,4-Dichlorobiphenyl 3,5-Dichlorobiphenyl

Figure 1.5 Polychlorinated biphenyl compounds.

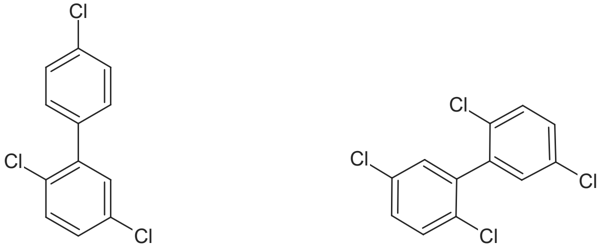
4,4′-Dichlorobiphenyl

2,2′,5-Trichlorobiphenyl



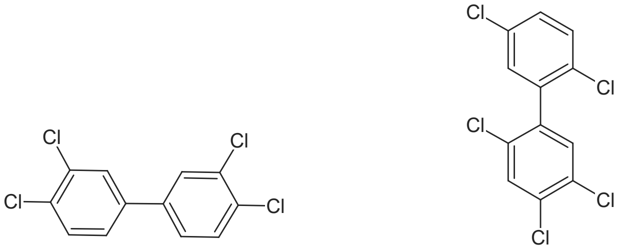
2,4,4′-Trichlorobiphenyl

2,4,5-Trichlorobiphenyl



2,4′,5-Trichlorobiphenyl

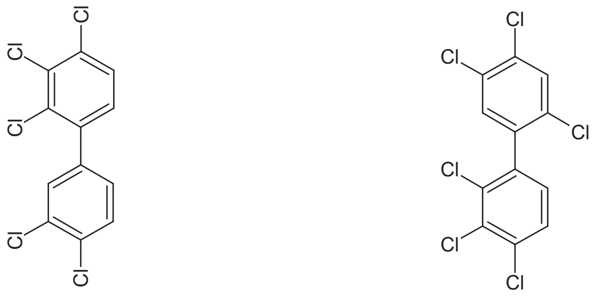
2,2′,5,5′-Tetrachlorobiphenyl



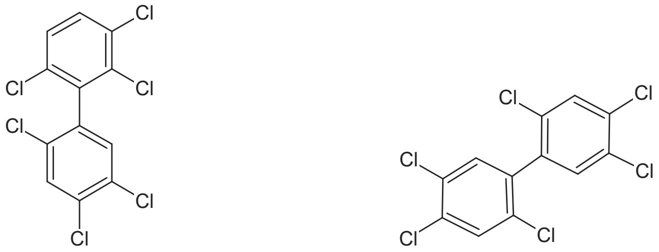
3,3′,4,4′-Tetrachlorobiphenyl

2,2′,4,5,5′-Pentachlorobiphenyl

Figure 1.5 (cont) Polychlorinated biphenyl compounds.



2,3,3′,4,4′-Pentachlorobiphenyl 2,2′,3,4,4′,5′-Hexachlorobiphenyl



2,2′,3,4′,5′,6-Hexachlorobiphenyl 2,2′,4,4′,5,5′-Hexachlorobiphenyl

2,2′,3,3′,4,4′,5-Heptachlorobiphenyl 2,2′,3,4,4′,5,5′-Heptachlorobiphenyl

Figure 1.5 (cont) Polychlorinated biphenyl compounds.

cyclic aromatic hydrocarbons ultimately find their way into the aquatic ecosystem and thereby deplete the quality and standards of the aquatic habitats directly or indirectly (26).

Details of polycyclic aromatic hydrocarbon compounds are shown in Table 1.5 and in Figure 1.6

Table 1.5 Polycyclic aromatic hydrocarbons.

Name	Name
Benz[a]anthracene	Benzo[b]fluoranthene
Benzo[j]fluoranthene	Benzo[k]fluoranthene
Benzo[a]pyrene	Chrysene
Dibenz[a,h]acridine	Dibenz[a,j]acridine
Dibenz[a,h]anthracene	7H-Dibenz[c,g]carbazole
Dibenzo[a,e]pyrene	Dibenzo[a,h]pyrene
Dibenzo[a,i]pyrene	Indeno[1,2,3-cd]pyrene

Benz[a]anthracene is produced during incomplete combustion of organic matter. It is one of the carcinogenic constituents of tobacco smoke (27).

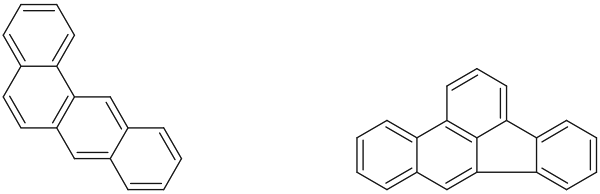
Also, benzo[j]fluoranthene is present in fossil fuels and is released during the incomplete combustion of organic matter. It has been traced in the smoke of cigarettes, exhaust from gasoline engines, emissions from the combustion of various types of coal and emissions from oil heating, as well as an impurity in some oils such as soybean oil (28).

The primary sources of dibenzopyrenes in the environment are combustion of wood and coal (29), gasoline and diesel exhaust (30), and tires (31). Dibenzo[a,l]pyrene is a constituent of tobacco smoke.

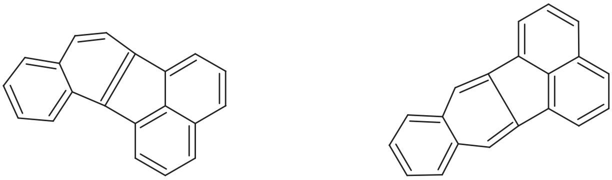
1.4.3 Organochlorine Pesticides

Details of organochlorine pesticides are shown in Table 1.6 and in Figure 1.7

Fungal contamination of animal feed is often unavoidable and is a serious concern given that many of these contaminants include toxic metabolites known as mycotoxins (32). Mycotoxin contamination can occur in a crop growing in the field, or contamination may be introduced during harvesting, storage and/or processing of the animal feed for use in raising monogastric and ruminant animals.

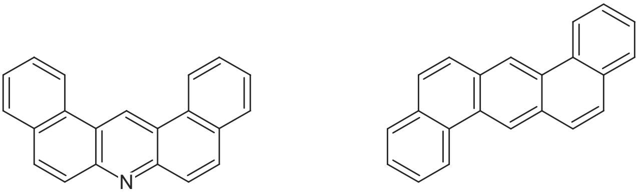


Benz[a]anthracene Benzo[b]fluoranthene



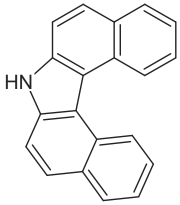
Benzo[j]fluoranthene Benzo[k]fluoranthene

Benzo[a]pyrene Chrysene

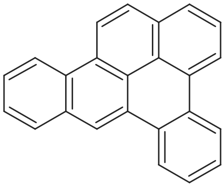


Dibenz[a,j]acridine Dibenz[a,h]anthracene

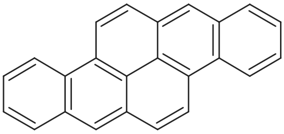
Figure 1.6 Polycyclic aromatic hydrocarbons.



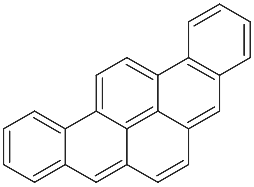
7H-Dibenz[c,g]carbazole



Dibenzo[a,e]pyrene



Dibenzo[a,h]pyrene

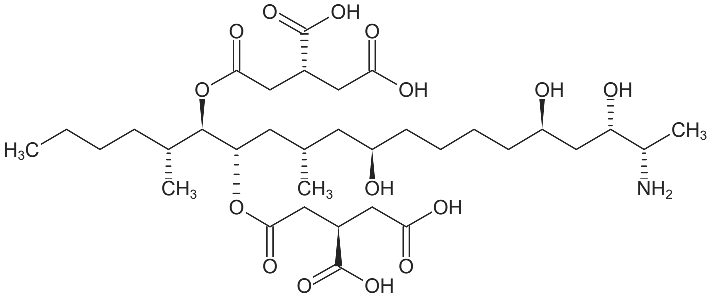


Dibenzo[a,i]pyrene

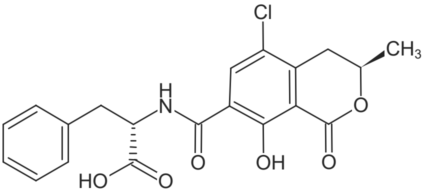
Figure 1.6 (cont) Polycyclic aromatic hydrocarbons.

Table 1.6 Organochlorine pesticides.

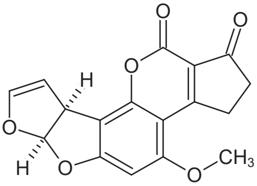
Name	Name
Aflatoxin	Fumonisin
Ochratoxin	Zearalenone



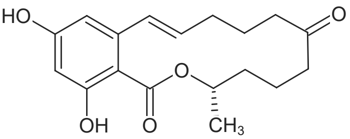
Fumonisin



Ochratoxin A



Aflatoxin



Zearalenone

Figure 1.7 Organochlorine pesticides.

Mycotoxins are fairly stable compounds often found in animal feed for monogastric and ruminant animals, and they are a known cause of a wide variety of deleterious effects in these animals. Pesticides are other common contaminants of animal feed. Endotoxins are not natural contaminants of feedstuffs. Feedstuffs can be contaminated with endotoxins when mixed with products of animal origin.

Endotoxins are another type of toxin of bacterial origin, which are commonly found in the gastrointestinal tract of livestock; and pesticides are commonly found in the foodstuffs fed to various types of livestock, and as a result, have been known to have toxic effects on livestock (32).

Mycotoxins are known to cause toxic, teratogenic, mutagenic, and carcinogenic effects, and have been linked to a depression of the animal's immune system. Furthermore, mycotoxins can affect different organs in an animal, including urinary, digestive, nervous, reproductive, and immune systems, and as such, it makes it more difficult to establish a precise diagnosis once an animal is affected. The effects of mycotoxins depend on the level of contamination, the presence of one or more toxins, the type of animal, its age, the time of exposure, genetic makeup, and its nutritional and health status at the time of exposure to contaminated feed.

The most dangerous mycotoxins affecting poultry are aflatoxin, ochratoxin, T-2 toxin, fumonisin, and deoxynivalenol, also known as DON. These mycotoxins, along with other trichothecene mycotoxins, can also affect monogastric and ruminaNZ amimals, to greater or lesser degrees (32).

1.4.3.1 Aflatoxin

Aflatoxins are various poisonous carcinogens and mutagens that are produced by certain molds, particularly *Aspergillus* species (33). The fungi grow in soil, decaying vegetation and various staple foodstuffs and commodities such as hay, sweetcorn, wheat, millet, sorghum, cassava, rice, chili peppers, cottonseed, peanuts, tree nuts, sesame seeds, sunflower seeds, and various spices. In short, the relevant fungi grow on almost any crop or food. When such contaminated food is processed or consumed, the aflatoxins enter the general food supply. They have been found in both pet and human foods, as well as in feedstocks for agricultural animals. Animals fed contaminated food can pass aflatoxin transformation products into eggs, milk products, and meat (34).

For example, contaminated poultry feed is the suspected source of aflatoxin-contaminated chicken meat and eggs in Pakistan (35). Children are particularly affected by aflatoxin exposure, which is associated with stunted growth (36), delayed development (37), liver damage, and liver cancer.

An association between childhood stunting and aflatoxin exposure has been reported in some studies but could not be detected in all studies (38).

Adults have a higher tolerance to exposure, but are also at risk. No animal species is immune. Aflatoxins are among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M1.

Aflatoxin poisoning most commonly results from ingestion, but the most toxic aflatoxin compound, B1, can permeate the skin.

The United States Food and Drug Administration (FDA) action levels for aflatoxin present in food or feed is 20 to 300 *ppb* (39). The FDA has had occasion to declare both human and pet food recalls as a precautionary measure to prevent exposure.

The term *aflatoxin* is derived from the name of the species *Aspergillus flavus*, in which some of the compounds first were discovered. The word was coined around 1960 after its discovery as the source of Turkey X disease (40). Aflatoxins form one of the major groupings of mycotoxins, and apart from *Aspergillus flavus*, various members of the group of compounds occur in species such as *Aspergillus parasiticus*, *Aspergillus pseudocaelatus*, *Aspergillus pseudonomius*, and *Aspergillus nomius* (41).

1.4.3.2 Fumonisin

The fumonisins are a group of mycotoxins derived from *Fusarium* of the *Liseola* section (42). They have strong structural similarity to sphinganine, the backbone precursor of sphingolipids. Sphinganine is shown in Figure 1.8.

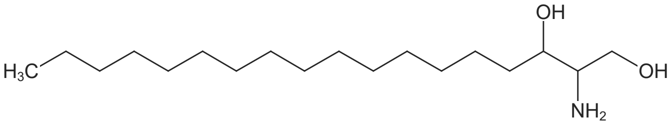


Figure 1.8 Sphinganine.

1.4.3.3 Ochratoxin

Ochratoxins are a group of mycotoxins produced by some *Aspergillus* species (mainly *A. ochraceus* and *A. carbonarius*, but also by 33% of *A. niger* industrial strains) and some *Penicillium* species, especially *P. verrucosum* (43). Ochratoxin A is the most prevalent and relevant fungal toxin of this group, while ochratoxins B and C are of lesser importance.

Ochratoxin A is known to occur in commodities such as cereals, coffee, dried fruit, and red wine. It is possibly a human carcinogen and is of special interest as it can be accumulated in the meat of animals. Exposure to ochratoxins through diet can cause acute toxicity in mammalian kidneys. Exposure to ochratoxin A has been associated with Balkan endemic nephropathy, a kidney disease with high mortality in people living near tributaries of the Danube River in Eastern Europe (44).

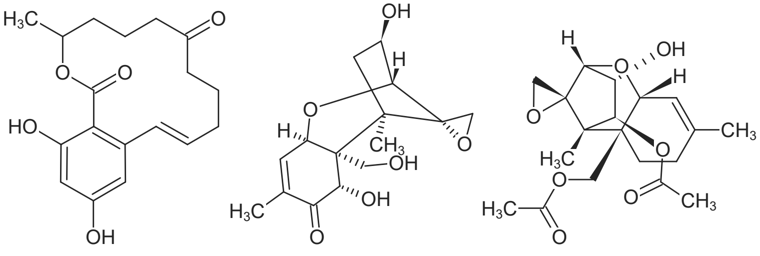
It has been suggested that carriers of alleles associated with phenylketonuria may have been protected from spontaneous abortion caused by ochratoxin exposure, providing a heterozygous advantage for the alleles despite the possibility of severe intellectual disability in the more rare instance of inheritance from both parents (43).

1.4.3.4 Zearalenone

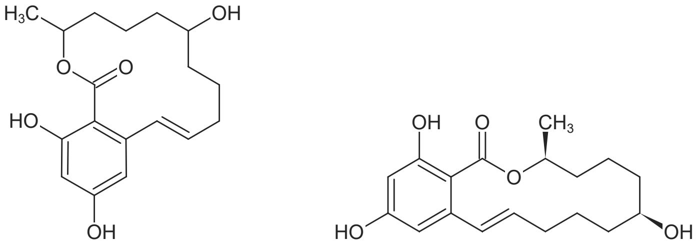
Zearalenone (ZEN), also known as RAL and F-2 mycotoxin, is a potent estrogenic metabolite produced by some *Fusarium* and *Gibberella* species (45). The chemical name of zearalenone is 6-[10-hydroxy-6-oxo-trans-1-undecenyl]-β-resorcyclic acid lactone. Specifically, the *Gibberella zeae,* the fungal species where zearalenone was initially detected, in its asexual/anamorph stage is known as *Fusarium graminearum* (46). Several *Fusarium* species produce toxic substances of considerable concern to livestock and poultry producers, namely deoxynivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol and zearalenone.

In particular, zearalenone is produced by *Fusarium graminearum, Fusarium culmorum, Fusarium cerealis, Fusarium equiseti* (47), *Fusarium verticillioides* (48), and *Fusarium incarnatum*. Zearalenone is the primary toxin that binds to estrogen receptors, causing infertility, abortion or other breeding problems, especially in swine (48). Often, zearalenone is detected together with deoxynivalenol in contaminated samples and its toxicity needs to be considered in combination with the presence of other toxins (49).

Studies in animal models suggest that zearalenone is metabolized primarily to α-zearalenol and β-zearalenol. α-Zearalenolis metabolized predominantly into β-zearalenol and, to a lesser extent, into zearalanone. Some compounds are shown in Figure 1.9.



Zearalenone Deoxynivalenol Diacetoxyscirpenol



α-Zearalenol β-Zearalenol

Figure 1.9 Estrogenic metabolites.

Zearalenone is heat-stable and is found worldwide in a number of cereal crops such as maize, barley, oats, wheat, rice, and sorghum (50–52).

Its production increases when the climate is warm with air humidity at or above twenty percent (48). The environmental pH plays also a role in the toxin's production. When temperatures fall to 15°C, alkaline soils still support ZEN production. At the preferred *Fusarium* temperature, which ranges between 25°C and 30°C, neutral pH results in the greatest toxin production (53).

1.4.3.5 Brominated Diphenyl Ethers

Details of brominated diphenyl ethers are shown in Table 1.7 and in Figure 1.10

Table 1.7 Brominated diphenyl ethers.

Abbreviation	Name
BDE 15	4,4′-Dibromodiphenyl ether
BDE 28	2,4,4′-Tribromodiphenyl ether
BDE 36	3,3′,5-Tribromodiphenyl ether
BDE 37	3,4,4′-Tribromodiphenyl ether
BDE 47	2,2′,4,4′-Tetrabromodiphenyl ether
BDE 49	2,2′,4,5′-Tetrabromodiphenyl ether
BDE 66	2,3′,4,4′-Tetrabromodiphenyl ether
BDE 71	2,3′,4′,6-Tetrabromodiphenyl ether
BDE 75	2,4,4′,6-Tetrabromodiphenyl ether
BDE 77	3,3′,4,4′-Tetrabromodiphenyl ether
BDE 85	2,3′,4,4′,6-Pentabromodiphenyl ether
BDE 99	2,2′,4,4′,5-Pentabromodiphenyl ether
BDE 100	2,2′,4,4′,6-Pentabromodiphenyl ether
BDE 119	2,3′,4,4′,6-Pentabromodiphenyl ether
BDE 126	3,3′,4,4′,5-Pentabromodiphenyl ether
BDE 138	2,2′,3,4,4′,5′-Hexabromodiphenyl ether
BDE 153	2,2′,4,4′,5,5-Hexabromodiphenyl ether
BDE 154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether
BDE 181	2,2′,3,4,4′,5,6-Heptabromodiphenyl ether
BDE 183	2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether
BDE 203	2,2′,3,4,4′,5,5′,6-Octabromodiphenyl ether
BDE 205	2,3,3′,4,4′,5,5′,6-Octabromodiphenyl ether
BDE 206	2,2′,3,3′,4,4′,5,5′,6-Nonabromodiphenyl ether
BDE 207	2,2′,3,3′,4,4′,5,6,6′-Nonabromodiphenyl ether
BDE 209	Decabromodiphenyl ether

On the other hand, plastics have additives that are intentionally added during their manufacture to give them color, or other properties, such as flexibility, termoresistance, UV resistance, and fire resistance.

Antimicrobial food packaging incorporating triclosan (5-chloro-2-(2,4-dichlorphenoxy)-phenol) was banned in the European Union in 2010 (54). In other countries, regulations on the use of triclosan in food contact materials are unclear.

In this context, compliance with the EU ban on triclosan was investigated in commercial antibacterial PP food containers sold through online sales platforms. Triclosan was present in all food containers tested. Migration studies to food simulants under different conditions (e.g., conventional and microwave heating, single and repeated use) have been performed according to Regulation 10/2011.

The highest triclosan migration levels corresponded to 95% ethanol at 40°C for 10 *d*, although migration rates varied between different containers. Furthermore, microplastics were found to leach from the surface of antibacterial food containers when exposed to microwave heating or oven heating. Therefore, microplastics containing triclosan might enter food in contact with the containers during use, posing a potential health risk for consumers (54).

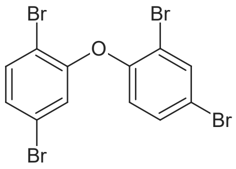
1.4.4 Carcinogens

1.4.4.1 Carcinogen Contamination

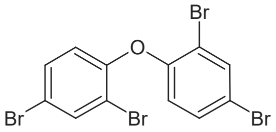
Carcinogen contamination in the food chain, for example, heavy metal ions, pesticides, acrylamide, and mycotoxins, have caused serious health problems (55). A major objective of food safety research is the identification and prevention of exposure to these carcinogens, because of their impossible-to-reverse tumorigenic effects.

However, the detection of carcinogens is difficult because of their trace-level presence in food. Thus, reliable and accurate separation and determination methods are essential to protect food safety and human health.

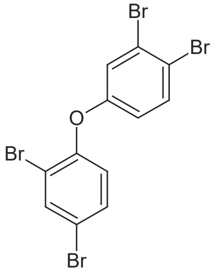
The state of the art was summarized for separation and determination methods for analyzing carcinogen contamination, especially the advances in biosensing methods. Furthermore, the application



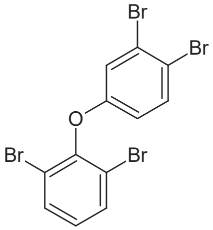
2,2′,4,5′-Tetrabromo-diphenyl ether



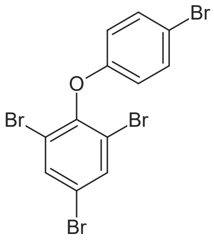
2,2′,4,4′-Tetrabromo-diphenyl ether



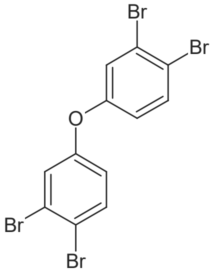
2,3′,4,4′-Tetrabromo-diphenyl ether



2,3′,4′,6-Tetrabromo-diphenyl ether

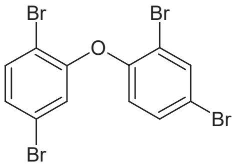


2,4,4′,6-Tetrabromo-diphenyl ether

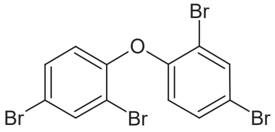


3,3′,4,4′-Tetrabromo-diphenyl ether

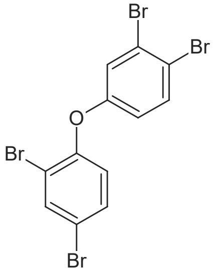
Figure 1.10 Brominated diphenyl ethers.



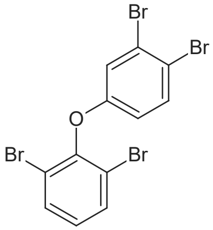
2,2′,4,5′-Tetrabromo-
diphenyl ether



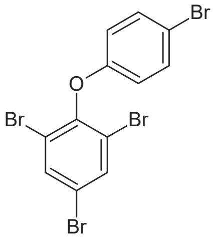
2,2′,4,4′-Tetrabromo-
diphenyl ether



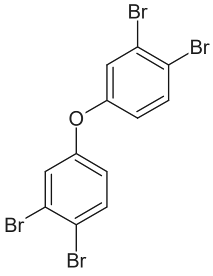
2,3′,4,4′-Tetrabromo-
diphenyl ether



2,3′,4′,6-Tetrabromo-
diphenyl ether

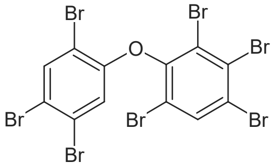


2,4,4′,6-Tetrabromo-
diphenyl ether

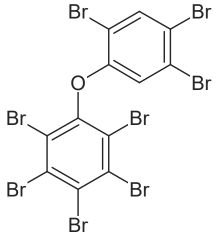


3,3′,4,4′-Tetrabromo-
diphenyl ether

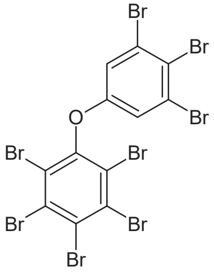
Figure 1.10 (cont) Brominated diphenyl ethers.



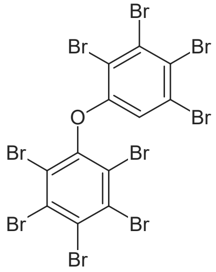
2,2′,3,4,4′,5′,6-Heptabromo-
diphenyl ether



2,2′,3,4,4′,5,5′,6-Octabromo-
diphenyl ether



2,3,3′,4,4′,5,5′,6-Octabromo-
diphenyl ether



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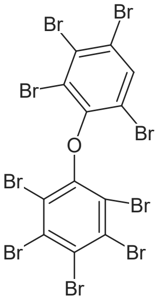
diphenyl ether



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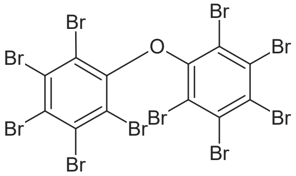
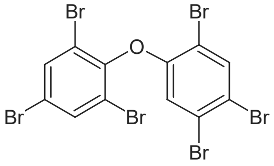
diphenyl ether

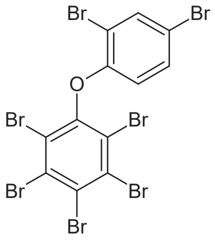


Decabromo-
diphenyl ether

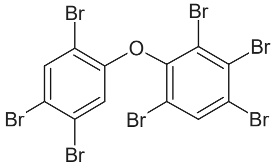
Figure 1.10 (cont) Brominated diphenyl ethers.



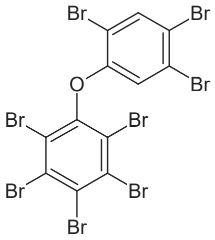
2,2′,4,4′,5,6′-Hexabromodiphenyl ether



2,2′,3,4,4′,5,6-Heptabromodiphenyl ether

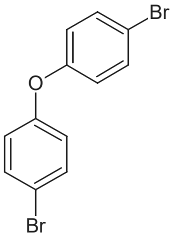


2,2′,3,4,4′,5′,6-Heptabromodiphenyl ether

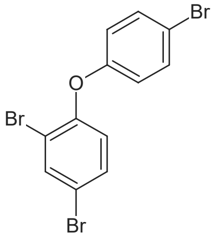


2,2′,3,4,4′,5,5′,6-Octabromodiphenyl ether

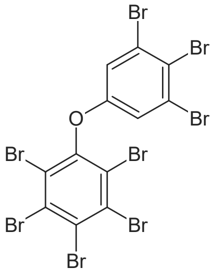
Figure 1.10 (cont) Brominated diphenyl ethers.



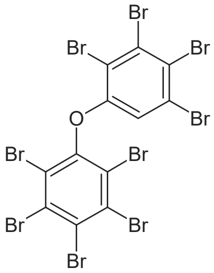
4,4′-Dibromo-
diphenyl ether



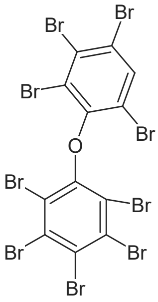
2,4,4′-Tribromo-
diphenyl ether



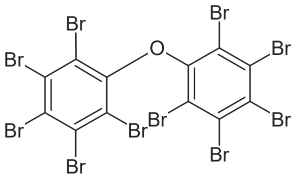
2,3,3′,4,4′,5,5′,6-Octabromo-
diphenyl ether



2,2′,3,3′,4,4′,5,5′,6-Nonabromo-
diphenyl ether



2,2′,3,3′,4,4′,5,6,6′-Nonabromo-
diphenyl ether



Decabromo-
diphenyl ether

Figure 1.10 (cont) Brominated diphenyl ethers.

of promising technology including nanomaterials, imprinted polymers, and microdevices was detailed (55).

1.4.4.2 Reduction of Carcinogens

A wide variety of foods have tested positive for the presence of acrylamide (56). Acrylamide has especially been found primarily in carbohydrate food products that have been heated or processed at high temperatures. Examples of foods that have tested positive for acrylamide include coffee, cereals, cookies, potato chips, crackers, french-fried potatoes, breads and rolls, and fried breaded meats.

In general, relatively low contents of acrylamide have been found in heated protein-rich foods, while relatively high contents of acrylamide have been found in carbohydrate-rich foods, compared to non-detectable levels in unheated and boiled foods. Reported levels of acrylamide found in various similarly processed foods include a range of 330-2,300 $\mu g\, kg^1$ in potato chips, a range of 300-1100 $\mu g\, kg^1$ in french fries, a range of 120-180 $\mu g\, kg^1$ in corn chips, and levels ranging from not detectable up to 1400 $\mu g\, kg^1$ in various breakfast cereals.

It is believed that acrylamide is formed from the presence of amino acids and reducing sugars. For example, it is believed that a reaction between free asparagine, an amino acid commonly found in raw vegetables, and free reducing sugars accounts for the majority of acrylamide found in fried food products. Asparagine is shown in Figure 1.11. Asparagine accounts for approximately 40% of the total free amino acids found in raw potatoes, approximately 18% of the total free amino acids found in high protein rye, and approximately 14% of the total free amino acids found in wheat.

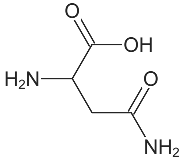


Figure 1.11 Asparagine.

The most likely route for the formation of acrylamide involves a Maillard reaction (56). The Maillard reaction is a chemical reaction between amino acids and reducing sugars that gives browned food its distinctive flavor. It is named after the French chemist Louis Camille Maillard, who first described it in 1912 while attempting to reproduce biological protein synthesis (57).

The Maillard reaction has long been recognized in food chemistry as one of the most important chemical reactions in food processing and can affect flavor, color, and the nutritional value of the food (56). The Maillard reaction requires heat, moisture, reducing sugars, and amino acids.

The first step of the Maillard reaction involves the combination of a free amino group from free amino acids or proteins, with a reducing sugar, e.g., glucose, to form Amadori or Heyns rearrangement products.

The second step involves the degradation of the Amadori or Heyns rearrangement products via different alternative routes involving deoxyosones, fission, or Strecker degradation. A complex series of reactions, including dehydration, elimination, cyclization, fission, and fragmentation, results in a pool of flavor intermediates and flavor compounds.

The third step of the Maillard reaction is characterized by the formation of brown nitrogenous polymers and copolymers.

Figure 1.12 shows the pathways for the formation of acrylamide starting with asparagine and glucose.

A process and a method for reducing the amount of acrylamide in thermally processed foods has been developed (56). This allows the production of foods with significantly reduced levels of acrylamide.

The method provides a continuous feed of peeled and sliced raw potatoes and contacting the continuous feed of raw potato slices with an aqueous solution at about 60°C for about 5 *min* to reduce the amount of the acrylamide precursors in the raw potato slices (56).

Using another method, a continuous feed of peeled and sliced raw potatoes, par-frying the raw potato slices at about 171°C to about 182°C until the moisture content is reduced to 3% to 10%, then oven-drying the par-fried slices at less than about 120°C until the moisture content is further reduced to about 1% to 2%. Also, some other methods have been detailed (56).

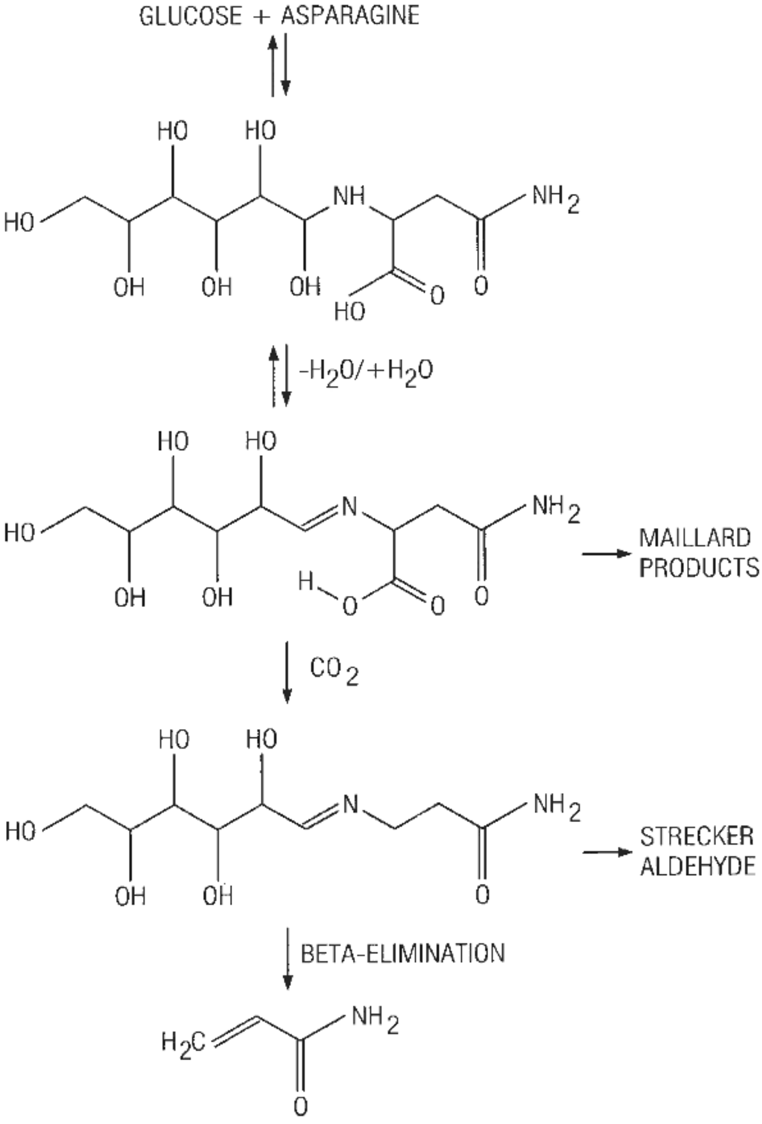


Figure 1.12 Formation of acrylamide (56).

L-Tryptophan can be applied to a foodstuff to prevent the development of mutagens and carcinogens (58). L-Tryptophan is shown in Figure 1.13. Before cooking of a foodstuff, such as hamburger, L-tryptophan is applied to the surfaces thereof to inhibit the generation of IQ-type carcinogens (58).

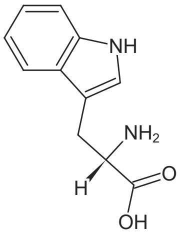


Figure 1.13 L-Tryptophan.

The L-tryptophan can be sprinkled on the surface of the foodstuff or incorporated into a sauce which is applied to the foodstuff or put into solution in water (58). The L-tryptophan is preferably applied in an amount greater than the order of magnitude of 1.0 $mg\,cm^{-2}$.

For testing, patties with a thickness of 3 *mm* and 9.6 *cm* were prepared from 50 *g* of store-bought 85% lean ground beef using a glass Petri dish cover with 1.5×9.6 *cm* as a mold. Inhibitor-treated and untreated patties were then either fried or broiled for 5 *min*.

When the concentration of added L-tryptophan was varied from 1.75 to 140 *mM* in the complete liquid reflux mode, IQ-type mutagenicity was inhibited, in a dose-dependent fashion, over a range of 15 to 100% (58).

1.4.4.3 Biosensors

Common substances in food can pose a great threat to human health, including foodborne pathogens, heavy metals, mycotoxins, pesticides, herbicides, veterinary drugs, allergens and illegal additives (59). In order to develop rapid, low-cost, portable and on-site detection methods of those contaminants and allergens to ensure food safety, gold nanoparticles of versatile shapes and morphologies, such as nanorods, nanoclusters, nanoflowers, nanostars, nanocages, nanobipyramids and nanowires, have been employed as probes because they possess extraordinary properties that can be used to design biosensors that enable the detection of various contaminants and allergens.

By means of a surface modification, gold nanoparticles can directly or indirectly sense specific targets based on different mechanisms, such as hydrogen bonds, nucleic acid hybridization, aptamer-target binding, antigen antibody recognition, enzyme inhibition, and enzyme mimicking activity. Gold nanoparticles can induce a distinct color change from red to blue when they transform from a monodispersed state to an aggregated state in liquid solution, which can be observed by the naked eye. If Raman molecules are functionalized on gold nanoparticles, their aggregation will alter the interparticle distance and induce the surface-enhanced Raman scattering that can be employed for highly sensitive detection.

Ultra-small gold nanoparticles, such as gold nanoclusters, also feature in fluorescence that enable a fluorescent readout. The formats of gold nanoparticles for food safety detection in real-world range broadly include but are not limited to films, fibers, liquid solutions, tapes, chips and lateral flow strips (59).

1.4.4.4 Anti-Carcinogens

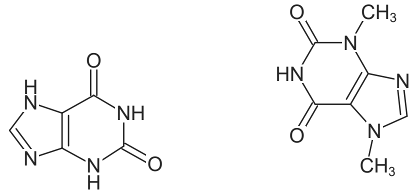
The proliferation of cancer cells by deoxyribonucleic acid synthesis is considered as a key biological index that is observed at the progression stage of carcinogenesis (60).

Therefore, substances that can inhibit such a process are expected to inhibit the progression stage of carcinogenesis, thereby preventing as well as inhibiting the development of cancer (61–63).

A variety of foods, such as coffee, tea, coke and chocolate, contain caffeine, c.f. Figure 5.4, a xanthine derivative.

Theobromine is also a xanthine derivative. Unlike caffeine, it is abundant in cacao bean or cacao bean husk. In general, cacao bean contains about 1.5%-3% of theobromine and cacao bean husk contains about 2% of theobromine. Xanthine derivates are shown in Figure 1.14.

Processed food such as chocolate, which is made from cacao bean, contains about 0.2% to 0.5% of theobromine and this content is about 7–10 times larger than that of caffeine (64).



Xanthine Theobromine

Figure 1.14 Xanthine derivates.

The general pharmacological functions of xanthine derivatives include stimulation of the central nervous system, skeletal muscle and cardiac muscle as well as relaxation of smooth muscle and coronary artery, accentuation of secretion of gastric juice, and diuresis.

By contrast, although caffeine is present in a variety of foods and has a wide scope of pharmacological activities, theobromine is only abundantly present in cacao bean and cacao bean husk, and thus the studies carried out on theobromine have not been extensive. Caffeine has been reported to have anti-carcinogenic activities; for example, a synergistic effect on therapeutic agent for gastric cancer (65), an inhibitory effect on lung cancer (66), and an inhibitory effect on 12-*o*-tetradecanoyphorbol-13-acetate-induced carcinogenesis (67, 68). 12-*o*-Tetradecanoyphorbol-13-acetate is shown in Figure 1.15.

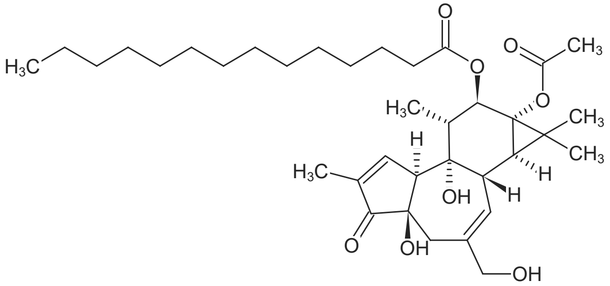


Figure 1.15 12-*o*-Tetradecanoyphorbol-13-acetate.

The isolation of theobromine from cacao bean can be done as follows (60):

Preparation 1–1: Cocoa butter was removed from cacao bean containing cacao bean husk. Then 6 parts by weight of 50% acetone solution was added to 1 part by weight of the remaining fraction (cacao mass) and agitation extraction was performed for 5 *h* at 60°C while refluxing. The extract was centrifuged for 30 *min* at 4°C at a rate of 8,000 *rpm* and the resulting supernatant was collected. The extraction was repeated once for the remnant and all the supernatant was combined. Theobromine was isolated from the extract by high-performance liquid chromatography according to the method of Kreiser.

1.5 Methods of Food Packaging

Food packaging is an essential part of modern food processing. The issues of food packaging have been described in a chapter of a monograph (69).

During recent years, there has been a continuing modernization of agricultural activity, focused on the increase of productivity and quality, which has allowed a continuous development and the capacity to adjust the offer to the evolution of consumer habits and preferences (70, 71). Within this context, there has been special interest in the incorporation of quality, food health and safety standards, the strengthening of local zoo and phytosanitary heritage, environmental concern, corporate social responsibility, technological innovation and market management. All of which are instruments that favor production differentiation. Fresh exportable products present high perishability, which dramatically affects their quality when arriving at distant markets, thus reducing their value. Therefore, to extend their lifespan and maintain food safety, the quality of fresh products to be exported is a constant challenge to the industry.

Basically, two problems must be faced when seeking to keep fruits and vegetables fresh. First, they are alive and many chemical and biochemical reactions are occurring. Some reactions, if not controlled, may rapidly lead to the aging of vegetables and the loss of freshness. Secondly, the risk of microbiological contamination must be delayed as long as possible, since it compromises food safety, as well as the product final quality. Microbiological growth may cause serious alterations such as smell, funny taste and/or alteration in color and texture of vegetables (72).

Berries are particularly highly perishable and present important post-harvest physiological activity. As a consequence, they have short ripening and aging periods, which makes their commercialization a real challenge. They're susceptible to developing post-harvest diseases, to the point that some symptoms can be observed only 12 hours after being kept at room temperature, especially if they are wet. In general, the lifespan of berries is limited due to diseases caused by fungi, particularly *Botrytis cinerea* (70).

Studies in the USA showed that annual loss of fresh berries through the commercialization chain can rise to 40% of national production, which causes many millions of dollars in loss, even though that country is known for its high technology and for presenting different means for refrigerated transport. *Botrytis cinerea* is the agent that causes the gray mold rot, which infects more than 200 different vegetal species, producing serious economic loss before and after picking. This pathogen may attack the crop in any of its developing states and can infect any part of the plant.

Due to the considerable impact of this pathogen and to the economic consequences on important crops such as grapes, tomatoes, strawberries, raspberries, cranberries, among others, there have been an important number of studies on the biology of this fungus, the interactions in which it participates and on possible methods to control it (73).

Although berries may be of a high quality, they have a short lifespan, mainly because of fungal growth (*Botrytis*), which causes an important loss due to product rejection and the poor quality image in the target markets. On the other hand, berries are exported in packages not in accordance with international market trends. Considering international trends, it is essential to develop recyclable and/or active biodegradable packaging with antimicrobial capacity, allowing for an increase in the lifespan of berries, as well as their quality and safety, at a competitive price, while taking into consideration the packaging traditionally used by the industry nowadays. All this is done without significantly modifying the present packaging production line or their common use form.

Controlling physiological processes and the growth of microorganisms is the key to the preservation and long lifespan of fruits and vegetables. That way, packaging systems and low temperatures are technologies that contribute to the extension of the lifespan, quality and safety of these products.

Fresh fruits are mostly exported using modified atmosphere packaging (MAP) technology in order to reduce metabolic processes and bioburden. Usually, entire coverage for pallets is used to contain the modified atmosphere. These conditions, however, are complex in practice, expensive, and may be affected by external factors, such as blackouts or leaks, often making them a nonviable process to maintain for periods over 30-35 days, as in the case of blueberries. The main difficulty of this technology informed by some berry exporting companies is the variation in the gaseous mixtures in different pallets, making the homogenization of the exported load a difficult task (70).

At present, one of the main requirements in the food industry is the development of noninvasive procedures that may cause minimum alteration in food physicochemical and sensory characteristics. In this context, active packages have emerged as an excellent alternative to complement these requirements, since they extend the lifespan of food, minimize additive incorporation and improve food safety with minimum alteration of its characteristics, providing the chance to develop new products.

Besides which, active packages can be combined with other technologies, adding a synergic effect, increasing the quality and lifespan of the product. Not long ago, packaging had a merely passive function, which consisted of separating the product from the environment, acting as a shield against physical and/or mechanical effects, chemical reactions and the invasion of microorganisms, as well as preventing migration of their own compounds into food, with minimal interaction with the food, and therefore becoming an inert shield. In the last few years, a dramatic step forward has occurred in the area of food packaging concerning new packaging techniques.

From a traditional system focused on the search for the maximum inertia between package and food, there has been a turn to the development of a kind of packaging called active packaging; that is, packages which interact with the food in order to improve its properties. Thus, active packaging can be defined as a system in which food, packaging and environment act positively to increase the lifespan or improve some property of the food. This has eventually led to the concept of packaging as an emerging technology in food preservation (70).

However, in recent years, with the demand for fresh and better quality products, the concept of packaging has changed to offer active protection to the product. Thus, active packaging can be defined as a system in which food, packaging and the environment act positively to increase the lifespan or improve the sole property of the food (74).

The packaging can be called active when it performs a desired role, as well as providing an inert shield against external conditions. The word *desired* is important for this definition, because it makes a clear difference between desired and non-desired effects. The most important active packaging concepts are: antimicrobial packaging; edible covering; oxygen, ethylene, odor and taste absorbers; moisture regulators; carbon dioxide, antimicrobial materials, antioxidant and flavor releasers. Their applications are many and their use is clearly growing. The active packaging system has been used for years in Japan and in recent years its use has been growing in Europe and the United States. Consequently, active packaging technology is an emerging promising area in food technology, which may contribute several preservation benefits in a broad variety of products.

The current concern as to how to improve and guarantee safety in processed food has raised great interest in the use of active antimicrobial packaging. Lately, prominent food packaging research teams worldwide along with leading companies in the development of packaging are expending significant effort to develop active packages with antimicrobial capacity. Nevertheless, many patented developments are targeted to the use of compounds whose use in food packaging is conditioned, since the legislation in many countries forbids such compounds in direct contact with the food, as is the case of silver ions, for instance.

Within the concept of active packaging, antimicrobial packaging is defined as packaging which incorporates active antimicrobial agents instead of the same substances added directly on the food. The concept of antimicrobial packaging assumes the gradual release of the antimicrobial agent from the packaging to the food, inhibiting or delaying microorganism growth on its surface (75).

As the microbiological contamination of most food occurs mainly on the surface due to the processing and subsequent manipulation, efforts have been made to improve safety, delaying food deterioration through the use of antimicrobial materials applied with aerosols or by immersion. However, direct superficial application of antibacterial substances on food has limited benefits, since active substances are neutralized upon contact, or are rapidly diffused from the surface to the interior of food. On the other hand, incorporation of a bactericidal or bacteriostatic substance in food formulations may result in partial inactivation of the active substances by constituents of the product itself, and a limited effect on the surface microflora can be expected (76).

Therefore, the use of active packaging which contains antimicrobial agents could be more efficient, due to the slow migration of agents from the packaging material to the surface of the product, helping to keep high concentrations where they are more necessary. If an antimicrobial agent can migrate from the packaging for a long period of time, its activity can also be extended to the stages of transportation and storage during product distribution. From the point of view of food security, antimicrobial substances used in developing active packaging must be approved to be in contact with the food, and must, therefore, be considered *generally considered as safe* by the FDA. In this regard, it is important to highlight that antimicrobial materials must be incorporated into food packaging in such concentrations so that the released concentration is allowed by the regulation.

The most commonly used food packaging antimicrobial agents are: sorbic acid, propionic acid, potassium sorbate, benzoic acid, sodium benzoate and citric acid (76). Bacteriocins, such as nisin and pediocin, have also been used (77), besides other compounds, among which are enzymes, such as peroxidase and lysozyme, the latter used to inhibit microbial growth of Gram-positive bacteria (78); natural poly(saccharide)s with antimicrobial properties such as chitosan (79); phenolic compounds extracted from plants; chelating agents such as ethylenediamine tetraacetic acid (EDTA), and metals such as silver ions. Ethylenediamine tetraacetic acid is shown in Figure 1.16.

The triclosan compound (2,4,4′-trichloro-2′-hydroxydiphenyl ether), c.f. Figure 6.1, was approved for food contact use in the European Union by the Scientific Committee on Food (SCF, 2000) with a limit of 5 *mg/kg* of food. Antimicrobial agents in packaging

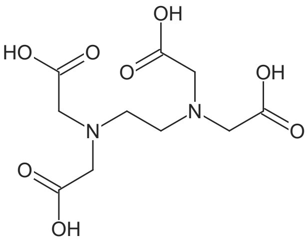


Figure 1.16 Ethylenediaminetetraacetic acid.

are able to control microbiological contamination by use of three mechanisms: reducing microorganism growth rate, increasing the lag phase, or inactivation by contact (76). Antimicrobial materials can act by inhibiting essential metabolic routes, or by intervening in the microorganism's reproductive genetics, while other compounds modify the functioning of the cell membrane or the cell wall structure, as is the case in lysozyme, which destroys the cell wall without modifying metabolic routes, while lactoferrin and EDTA act as essential cation chelating agents (80). Considering that most antimicrobial agents have different action mechanisms, it is thought that mixing different kinds of antimicrobial agents could increase antimicrobial activity through synergistic reactions, provided that they don't interfere with each other. Therefore, it is desirable to experiment with combinations of several antimicrobial agents to extend their activity in a wider range of microorganisms, maximizing the effectiveness and safety of packaging systems with antimicrobial agents. The method employed to incorporate the active agent with antimicrobial capacity into the packaging can be achieved in different ways.

In some cases, the antimicrobial agent is incorporated as a coating in the internal part of the film which will be in direct contact with the food. Also, the antimicrobial agent can be incorporated into the polymer matrix itself, in which case, the agent diffuses through all the amorphous zones of the polymer, or through the holes and empty spaces between polymeric chains. Undoubtedly, incorporation into the polymeric matrix requires studies on the subject that define the effective concentration levels of antimicrobial agents, kinetics of the agent diffusion, distribution of the agent in the matrix and the effective action kinetics on microorganisms.

On the other hand, the methodology to incorporate the active agent into the plastic film limits the possibilities to use antimicrobial agents. Thus, when the agent is incorporated into the polymeric matrix by using extrusion, its resistance to high temperatures and high pressure becomes essential. Active packaging technology is an emerging promising area in food technology that can contribute several benefits to the preservation of a broad range of products. Within the concept of active packaging, antimicrobial packaging is defined as packaging that incorporates active antimicrobial agents in its structure, avoiding the addition of these substances directly on the food. The concept of antimicrobial packaging consists of the gradual release of the antimicrobial agent from the package to the food, inhibiting or delaying microorganism growth on the food surface. Active packaging application for preserving food has been used for extending the lifespan and increasing the quality of various products such as carrots, broccoli, strawberries, cheese, garlic, among others.

At present, there is great international interest in the use of active packaging related to the food industry, among which is packaging with antimicrobial capacity. Nonetheless, most research studies are focused on the incorporation of antimicrobial agents in non-recyclable or non-biodegradable plastic packaging, or on edible coatings applied directly on the food. The globalization of international trade opens clear possibilities for the strengthening of production development and commercialization of fruit production, particularly those species recently incorporated or grown, that may become relevant in world markets, not as much due to their volume, but due to some special attributes, whether it is quality, opportunity, environmental issues or others.

In this way, quality and sustainability issues represent relatively recent concepts, which have, however, gained prominence in the list of consumer's concerns, and in the minds of authorities, producers and academicians around the world, along with other actors in society.

This new reality, which has gradually started taking over the markets and commercialization of production has involved developing two basic regulatory categories:

1. Those related to product quality, where regulations seek to control harmful effects, both in consumer health and the environment, of the importing country, and
2. Those related to processes, where the regulations include requirements that seek to develop production processes that are harmless to the environment and the safety of workers in the importing countries.

Added to the above is the requirement of processing techniques that can guarantee the harmlessness of the products.

Although this phenomenon has opened new niche markets, such as green markets, ecological markets, environmental seals, quality certifications, among others, it is also true that it is generating new demands for industrial, agricultural, forestry and fishing production, which forces it to promote a strategy based on quality in order to broaden the access to markets and to improve competitive conditions through technological innovation and new investments. Today, one of the most serious environmental concerns is the amount of waste produced and stored as a consequence of packaging and boxing used. Most parts of the materials used by packaging industries come from fossil fuels, which are known to have low degradability. On account of this, food packaging materials, as any other short-term packaging material, represent a serious problem worldwide (81). Thus, it is thought that 25% of world waste comes from packaging. The current global consumption of plastic is more than 200 million tons, with an annual growth of around 5%, which represents the biggest application field of crude oil (82).

In the food packaging industry, to remain competitive and to offer better plastic solutions means being able to deal with some key elements, such as the approval of food contact material by the FDA and waste management, within most of the legislations. Growing environmental awareness demands environmentally friendly use attributes from packaging and processes. As a consequence, recyclability and/or biodegradability of packaging materials are not only functional requirements, but also important environmental attributes. The recycling of solid waste is a common practice in countries committed to sustainable development.

The European Parliament and Council Directive 94/62/EC, of 20 Dec. 1994, requires that a minimum of 15% plastic from packaging and packaging waste be recycled by the end of 2005, while Directive 2004/12/EC of the European Parliament and Council, of 11 Feb. 2004, establishes that a minimum of 22.5% must be recycled by the end of 2011 (83). At present there is great interest in recyclable materials, which are those materials reused by the industry in order to minimize environmental waste or even biodegradable materials, which are defined as those materials that degrade due to the action of microorganisms naturally present in the environment such as bacteria, fungi and algae (84).

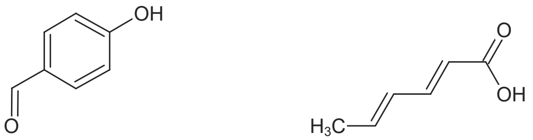
Therefore, a great effort to extend the lifespan of food and improve food quality as well as reduce packaging waste has encouraged the exploration of new packaging materials. In view of the background analyzed, it becomes evident that there is enough evidence to support the possibility of using antimicrobial agents in food packaging, especially for dried fruits, in order to improve safety and to extend the product lifespan.

Even though the use of ecologically friendly material is a worldwide demand there are no publications where the application of antimicrobial agents in industrial-level packaging is reported, especially regarding ways of attaching the agent to the packaging and, least of all, the use of packaging that is not only antimicrobial, but environmentally friendly as well. That is the challenge of the present project: to extend the lifespan of berries through the incorporation of an antimicrobial agent to an ecologically friendly material. Although there are only a few patents on antimicrobial food packaging, there are patents concerning active packaging with antioxidant capacity, not antimicrobial, however they present microencapsulation of active agents, i.e., their incorporation into the food itself, in pharmaceutical and cosmetic products.

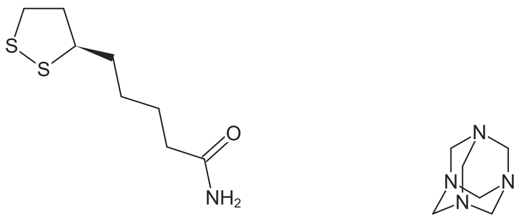
In particular, the US patent (85) shows active packaging and/or active packaging closing in different formats, made of metal and/or synthetic and/or associated natural materials where at least one element and/or component of the packaging contains and distributes one or several food additives and supplements and/or ingredients or processing aids used to produce food and cosmetics. The additive can fulfill antimicrobial functions regarding the packaged product.

Materials can be made through cryogenic grinding, along with the substance that generates singlet oxygen, ammonium stearate or other surfactant material with properties as soup and a polymeric resin to form a consistent concentrate as a thin homogeneous powder. The concentrate is added to large batches of polymer which are then processed by conventional equipment to create plastic films, cloth, etc.

A preparation method for bioactive packaging materials made of synthetic polymers or polymeric materials from natural sources has been shown (86). It covers the packaging material with a polymeric coating which contains immobilized preservatives, where the application of a solution, or a polymer dispersion with preservatives in the packaging material is by means of spray and/or coating and/or sinking the packaging material in such solution or dispersion and/or fold or carving printing followed by evaporation of the solvent under room or increased temperature, and the preservative is a microbicide and/or bacteriostatic preservative and/or fungicides; antibiotics and/or silver compounds and/or phenol derivatives and/or bacteriocins and/or esters of *p*-hydroxybenzoic acid and/or fatty acid monoglycerides and/or benzoic acid and/or sorbic acid and/or chitosan and/or 2,4,4-trichloro-2-hydroxydiphenylether and/or lysozyme and/or nisin and/or hexamethylenetetramine. Some of these compounds are shown in Figure 1.17.



p-Hydroxybenzoic acid　　Sorbic acid



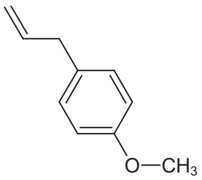
Lysozyme　　Hexamethylenetetramine

Figure 1.17 Bacteriocins.

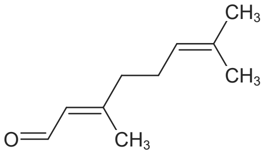
An antimicrobial packaging material for food materials may contain between 0.05% to 1.5% weight of an essential oil. The oil can be selected from linalool and/or methyl chavicol, but also one or more from citral, generaniol, methyl cinnamate, methyl eugenol, 1,8-cineol, *trans-α*-bergamot, carvacrol and thymol, c.f. Figure 1.18. These compounds are mixed with one or more selected polymers of ethylene vinyl alcohol copolymer, polyacrylates, which include copolymers of ethyl acrylate, methyl methacrylate, ionomers, nylons and other hydrophilic polymers, or polymers with functional groups capable of partly anchoring the additives, and the mixture covers the food contact layer of a film of food grade packaging or is incorporated into a film of food grade packaging. A binding agent, such as poly(ethylene glycol), is added to the mixture to improve volatile oil retention in the polymer during the process. This material has no regulatory limitations and in the mentioned concentrations, it does not create detectable unwanted flavors.

In recent years, there has been great interest in the development of active packaging with antimicrobial capacity, to exercise this function on the food surface for as long as the food is in direct contact with the packaging. The packaging materials with antimicrobial characteristics can be divided into two major groups: those which possess antimicrobial agents that must migrate to the surface of the packaging to exercise their function and that come into direct contact with the food, and those which do not need to migrate to exercise their function, Various films with antimicrobial characteristics have been developed, mainly in Japan. Thus, Dupont has introduced MicroFree™, based on the incorporation of three kinds of silver or copper salts on a support of inorganic powder thermosetting agents and which can therefore be introduced into the polymeric resin itself during the extrusion process. At present, three kinds of MicroFree™ are being offered: Z-200 (silver on a zinc support), T-558 (silver, copper oxide and zinc silicate on a titanium dioxide support), and B-558 (silver, copper oxide and zinc silicate on a barium sulfate support), which have proved to have a beneficial effect on *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. These agents, in spite of being approved by the Environmental Protection Agency, have not been approved by the FDA for direct contact with food.

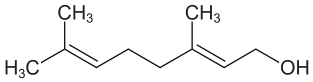
Metallic ions, copper and silver, as well as quaternary ammonium



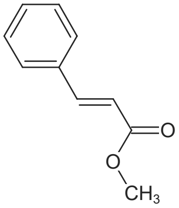
Methyl chavicol



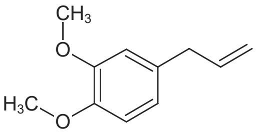
Citral



Generaniol



Methyl cinnamate



Methyl eugenol

Figure 1.18 Oil compounds.

salts and other compounds, are allowed in Japan, but not in the USA as safe antimicrobial agents. For their application in food packaging, these agents not only must be safe, but they must also migrate with difficulty towards the food. Despite the fact that copper ions can destroy microorganisms and viruses and copper is essential for living things as an enzyme constituent, it is also considered toxic and, therefore, according to US regulations is not allowed for direct contact with food.

In Japan, the regulations for the use of metallic ions are not as strict as they are in the US, and for that reason there has been plenty of work on the incorporation of silver ions, inert supports such as synthetic zeolites in many cases, such as Apacider A™, in which the silver ions chemically and physically bind with calcium phosphate on a zeolite support. Nevertheless, this agent presents problems in its incorporation as extrusion additive, requiring a large number of surfactants that must be added both during the extrusion process, to thereby enhance its dispersion in the plastic matrix and its migration towards the food service. The Japanese companies Shinanen New Ceramics along with Mitsubishi, developed Zeomic™, in which silver ions were incorporated on a zeolite support. Although they have a broad range of action that shows good performance in the extrusion process, the use of ions are still restricted due to their incompatibility with direct food contact. Other compounds have been proposed and their antimicrobial capacity has been evaluated.

For example, antimicrobial Microban™, i.e., 5-chloro-2-(2,4 dichlorophenoxy)phenol or triclosan, can be found in the market, and it is widely used in polyolefins with applications in toys, garbage bags, kitchen cutting boards, among others. However, it has to be mentioned that Microban is not accepted by US regulations as a material for direct contact with food, and it is even being questioned by the US Environmental Protection Agency (EPA). The Japanese company Sumitomo developed a thermoplastic resin for food packaging films, incorporating benomyl, one of the systemic fungicides with the broadest range of action; however, it is highly improbable that US regulating authorities will accept benomyl for direct contact with food. Some other structures with antimicrobial characteristics refer to the formation of chlorine dioxide inside the packaging. Benomyl is shown in Figure 1.19.

This invention by the Southwest Research Institute and Bertrand

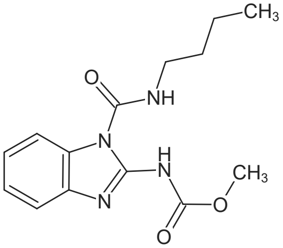


Figure 1.19 Benomyl.

Technologies, Inc., has led to the packaging system called Microatmosphere™, where the use of chlorine dioxide for food packaging has been accepted in the US. Many efforts are being made in the search for active agents from natural sources with antimicrobial capacity, many of which are being tested for their effectiveness *in-vitro* in laboratory experiments; however, there is a lack of technological development towards the ability of applying these agents in food packaging processes, and that is what will be addressed by this patent by means of the extrusion process or coating. The incorporation of antimicrobial agents both inside the polymer matrix and in the form of coating must meet the international legislation concerning packaging materials to be in direct contact with food. For instance, the US legislation defines a food additive as any substance which under use conditions may become part of the food, so all those components which intentionally or non-intentionally, may pass to the food from the packaging material must be previously admitted as food additive, except for substances recognized as GRAS (title 21 of the Federal Regulations). Therefore, in this case the antimicrobial materials to be incorporated into the structure must be approved for their use in direct contact with food by the FDA, or they must be GRAS substances, as is the case of natural origin substances (plant extracts such as thymol, carvacrol, linalool, among others).

In the case of using antimicrobial materials approved by the FDA, the legislation requires levels of maximum global migration of the extractable substances from the packaging material. These regulations and requirements concerning food packaging material are included in the form of positive lists in the Code of Federal Regulations (C.F.R.), in parts 174-179. The regulations are subdivided into sections for polymers, adhesives, coatings, adjuvants, among others, thus establishing the additive identity, safety conditions for its use and, in the case of polymer resins, they specify limits of all extractable substances of the finished article.

The use of plastic packaging with a high permeability to oxygen is being considered, as required by American legislation, in order to avoid anaerobic bacterial growth. The European Union has a very strict regulation on the necessary characteristics of packaging that is in direct contact with food, so that packaging must be ruled by Directive 2005/79/EC of 18 Nov. 2005, which rules all aspects concerning articles and materials which are in direct contact with food, and where the limits of maximum global migration (i.e., total amount of components that can pass from the packaging into the food, regardless of their nature) and specific migration (maximum amount of a specific component that can pass from the packaging into the food, subject which has a particular interest from the toxicological or sensorial point of view) for the starting substances and additives used when manufacturing plastic materials for food. Nonetheless, European legislation in Regulation 1935/2004 (87) authorizes two kinds of packaging: one that reacts *intelligently* on the product by indicating if the product has expired or is degraded, and another one that reacts *actively* by introducing beneficial chemical modifications in food, which increases its storage time.

This regulation is consistent with the latest developments in the food-packaging innovation area. In any case, the incorporation of antimicrobial agents will be performed according to and following international legislation. Food packaging can become the most evident waste source produced by the public. This has raised environmental concerns, leading to a strengthening of European legislation and boxing regulation, in order to reduce the amount of packaging waste, since solid waste recycling is a common practice in countries committed to sustainable development. The European Parliament and Council Directive 94/62/EC of 20 Dec. 1994, required that a minimum of 15% plastic from packaging and packaging waste be recycled by the end of 2005, whereas Directive 2004/12/EC of the European Parliament and Council of 11 Feb. 2004, stated that a minimum of 22.5% must be recycled by the end of 2011.

The reduction of the impact on the environment caused by traditional packaging waste is addressed. Whereas berries are currently commercialized in primary packaging made of non-biodegradable polymeric materials which produce environmental contamination after its use, changing that kind of packaging to recyclable and/or biodegradable packaging, which is in better accordance with international regulation requirements 2005/20/EC and consumer requirements internationally, will lead to the fulfillment of such requirements.

There is still a need to consolidate or improve the presence of food stuff in highly demanding markets, a goal that can be achieved as long as the food, besides being competitive and attractive, is safe, good quality and with a lifespan appropriate to the distance it is sought to bridge. Thus, the development of food preserving technologies is required in which the packaging systems play an essential role in extending the lifespan and increasing the quality and safety of the food, thereby incorporating added value to the product. To be a leader in the food industry, it is not only necessary to be a leader in the areas of production and exporting, but also in the innovation and development of packaging systems capable of safeguardong the quality of food, with special attention given to consumer requirements worldwide.

Thus, one of the requirements in developed countries, where consumers are highly aware of the environmental damage that packaging waste produces, is linked to environmentally friendly packaging, an area in which biodegradable and/or recyclable packaging plays a very important part. It is possible to develop totally recyclable or biodegradable packaging with antimicrobial capacity to inhibit microbiological growth on the surface of the packaged product, in order to be able to offer safer food, with better quality and a longer lifespan, thus reducing the losses accrued due to the rejection of fresh refrigerated fruits.

This innovation will also allow reaching new markets which increasingly require environmentally friendly packaging. The packaging incorporates a proactive and positive aptitude on the packaged food, combining the antimicrobial activity with the reduction of the environmental impact. Thus, a solution that can be easily adopted by the industry can be obtained (70, 71).

1.6 Recycling Safety

The European Food Safety Authority (EFSA) panel on Food Contact Materials, Enzymes and Processing Aids (CEP) assessed the safety of the recycling process for circular plastics (EU register number RECYC228) (88), which uses the Starlinger iV+ technology (89).

The input is hot caustic washed and dried PET flakes mainly originating from collected post-consumer PET containers, with no more than 5% PET from non-food consumer applications. The flakes are dried and crystallized in a first reactor, then extruded into pellets. These pellets are crystallized, preheated and treated in a solid-state polycondensation (SSP) reactor. Having examined the challenge test provided, the panel concluded that the drying and crystallization, extrusion and crystallization and SSP are critical in determining the decontamination efficiency of the process.

The operating parameters to control the performance of these critical steps are temperature, air flow and residence time for the drying and crystallization step, and temperature, pressure and residence time for the extrusion and crystallization step as well as the SSP step. It was demonstrated that this recycling process is able to ensure that the level of migration of potential unknown contaminants into food is below the conservatively modeled migration of 0.1 $\mu g\, kg^{-1}$ food. Therefore, the panel concluded that the recycled PET obtained from this process is not a safety concern when used at up to 100% for the manufacture of materials and articles for contact with all types of foodstuffs for long-term storage at room temperature, with or without hotfill. The final articles made of this recycled PET are not intended to be used in microwave and conventional ovens and such uses are not covered by this evaluation (88).

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